Parasite intensity is driven by temperature in a wild bird

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Abstract

Increasing awareness that parasitism is an essential component of nearly all aspects of ecosystem functioning, as well as a driver of biodiversity, has led to rising interest in the consequences of climate change in terms of parasitism and disease spread. Yet empirical knowledge on the extent and ways in which climatic factors affect parasite prevalence and intensities remains scarce. In an 18-year, multi-site, correlative study we investigated the contributions of weather variables and other factors to spatio-temporal variation in infestation by blowfly parasitic larvae (*Protocalliphora* spp.) in nests of Corsican blue tits (*Cyanistes caeruleus*). We found that ambient temperature during the nestling stage is strongly and positively related to parasite load (number of parasites per chick), both across broods when controlling for year, and across years. In addition, annual mean parasite load also increased with minimal spring temperature, and decreased with increasing average temperature in the previous summer. There was no indication of a dependence of parasite dynamics on host dynamics in this system, likely due in part to the wide host range of blowflies that do not solely rely on blue tit hosts. This suggests a major effect of temperature during the blowfly life cycle, with potential implications for blowfly – host interactions across their geographical range as climate keeps warming up. Finally, given that ambient temperature increases throughout the breeding season and that blowflies negatively affect survival and recruitment of blue tits, these results also mean that parasites, along with caterpillar availability, can drive selection for breeding date in this system.

Introduction

As global climate is very likely to keep warming up, it is of growing importance to understand how populations and communities respond to variations in temperature and precipitation. While milder, wetter winters are expected for Northern Europe, in the Mediterranean most of the expected climatic change will likely translate into warmer, drier summers (IPCC 2013). Increasing awareness that parasitism is an essential component of nearly all aspects of ecosystem functioning, as well as a driver of biodiversity (Hudson et al. 2006), has led to a rising interest in the consequences of climate change in terms of parasitism and disease spread (Harvell et al. 2002, Brooks and Hoberg 2007). Attempts were therefore made to predict the direction of change in disease prevalence in response to climate warming (e.g. Møller et al. 2013). However, it seems that no single scenario is to be expected given the dynamic nature of host-parasite interactions, the huge variation in parasite life histories, and the complexity of their effects at multiple levels within ecosystems (Mas-Coma et al. 2009, Rohr et al. 2011, Altizer et al. 2013). Therefore, predictions of the effects of climate change on infectious diseases need to be supported by detailed empirical knowledge acquired regionally in well-studied host-parasite systems (Hernandez et al. 2013, Roiz et al. 2014).

Understanding the population dynamics of parasites in relation to that of their hosts has been a central focus in disease ecology. In comparison, the direct influence of abiotic factors on parasites remains little studied, despite the fact that many parasites have in their life cycles at least one outside-host stage, during which they are exposed to environmental variability. In particular, ectoparasites that spend a significant part of their life cycle as free-living (*i.e.* away from their host) are most likely to be affected by weather conditions and climate changes (Hernandez et al. 2013, Rose et al. 2014, Charlier et al. 2016, Ogden and Lindsay 2016). For example developmental time, activity levels, and survival of ectoparasites often display bell-shaped responses to climate, *i.e.* are highest at intermediate temperature and moisture values (reviewed in Ogden and Lindsay 2016). Local weather fluctuations may therefore result in either increased or decreased ectoparasite intensities depending on the current position of populations relative to these optima (Stromberg 1997, Elderd and Reilly 2014, Eads and Hoogland 2016). In addition, invertebrates (including ectoparasites) may be differently affected by climatic variability depending on their biology, and in particular on their ability to take refuge in micro-habitats that can buffer adverse climatic conditions (e.g. Roiz et al. 2014, Ogden and Lindsay 2016).

Within nesting cavities, birds are in contact with invertebrates, including nest-dwelling ectoparasites that use avian nest material as habitat (Loye and Zuk 1991, Christe et

al. 1994, Clayton and Moore 1997). The fitness costs of infestation as well as the fitness benefits of host defense traits are well documented in a wide range of bird species (e.g. Møller et al. 1990, Eeva and Nurmi 1994, Cantarero et al. 2013). This contrasts with the currently limited understanding of the factors driving the large temporal and spatial variation often found in ectoparasite intensities (Hurtrez-Boussès et al. 1999, Heeb et al. 2000, Dudaniec et al. 2007, Moreno et al. 2009). It has been suggested that nest ectoparasites might be influenced by weather, nest size or composition, or interactions with other invertebrate species within the nest microhabitat (Bennett and Whitworth 1991, Heeb et al. 1996, Remeš and Krist 2005, Kleindorfer and Dudaniec 2009, Moreno et al. 2009), yet the contributions of these factors relative to host factors remain unclear.

Among the best-studied nest ectoparasites in free-ranging birds are the haematophagous larvae of *Protocalliphora* blowflies that feed on bird nestlings' blood (Owen and Ash 1955, Møller et al. 1990, Bennett and Whitworth 1991, Eeva and Nurmi 1994, Hurtrez-Boussès et al. 1997, Dawson et al. 2005, Remeš and Krist 2005, Cantarero et al. 2013). The first eggs deposited by adult *Protocalliphora* blowflies hatch from the beginning of the bird nestling stage onwards, and develop into three larval stages before pupating. Adult blowflies are free-living and likely overwinter in litter or in old nest materials (Matyukhin and Krivosheina 2008). The reported host spectrum of *Protocalliphora* is large amongst hole-nesting birds (Owen and Ash 1955, Jamriska et al. 2010). In blue tits (*Cyanistes caeruleus*), *Protocalliphora* blowflies have well-established detrimental effects on nestling growth and survival, resting time, aerobic capacity at fledging, and eventually post-fledging survival and recruitment (Merino and Potti 1996, Hurtrez-Boussès et al. 1997, Charmantier et al. 2004, Simon et al. 2004, 2005, Thomas et al. 2007). In addition, *Protocalliphora* abundance is positively related to bacterial loads measured on nestlings, suggesting higher risks of bacterial infection in nests heavily infested by *Protocalliphora* (Mennerat et al. 2009). *Protocalliphora* do not parasitize adult birds, but indirectly affect parental effort as reflected in increased feeding rates or increased investment into nest-sanitation behaviour (Hurtrez-Boussès et al. 2000, Bańbura et al. 2004). On the island of Corsica, blue tit nestlings are exposed to the highest *Protocalliphora* loads reported so far in European study sites, while other types of nest ectoparasites are rarely found (Hurtrez-Boussès et al. 1997, 1999, Mennerat et al. 2008).

In an 18-year, multi-site, correlative study, we investigated how *Protocalliphora* infestation intensities varied both within and across years, with the main objective to understand the relative contributions of climatic and other factors to spatio-temporal variation in parasite intensities. We more specifically explored within-year variability in relation to host

life history, nest characteristics and weather during the nestling stage, while accounting for spatial variation. We also explored how inter-annual variability in mean parasite intensities relates to host dynamics and life history, as well as to temperatures and rainfall during the summer, autumn, winter and spring preceding each breeding season.

Methods

Study sites and monitoring

Data from blue tit broods and uniquely ringed female breeders were obtained for years 1997-2014 from seven study plots located in two valleys on the island of Corsica: plots Avapessa, Arinelle, Feliceto, Filagna, Grassa and Muro in the Regino valley, and plot Pirio in the Fango valley) (Blondel 1985, Lambrechts et al. 2004). The broad-leaved deciduous oak *Quercus humilis* favouring the production of earlier and larger blue tit broods was the dominant tree species in plots Avapessa, Feliceto, and Muro. The evergreen oak *Q. ilex* was the dominant tree species in the other four study plots where blue tit broods are smaller and occur later in the season than in deciduous habitats (Lambrechts et al. 2004, Blondel et al. 2006). Breeding blue tits used either wood-concrete Schwegler B1 boxes (Schorndorf, Germany) or concrete boxes of similar dimensions (nest-chamber size of *ca.* 113 cm^2).

Following basic protocols (Blondel et al. 2006) boxes were visited at least once a week to check the initiation and progress of nest construction, and determine the egg-laying date, number of eggs, and number of nestlings in the nest. Nest thickness, *i.e.* the vertical distance between the bottom floor and the top of the external nest wall (Hurtrez-Boussès et al. 1999), was measured either shortly before or during the egg-laying period. Given that all nestboxes in this study have similar internal diameters (12 cm), nest thickness is an appropriate proxy for nest volume, *i.e.* habitat size for nest ectoparasites.

Because nests were visited weekly at the end of the incubation stage, the onset of hatching was calculated based on the physical development of the nestlings at 0 to 6 days after hatching (Descamps et al. 2002). When nestlings were between nine and 15 days old, adult breeders were trapped inside the nestbox. The age of the female parent (yearling *vs* older) was determined either from the monitoring records for previously ringed birds, or by comparing the colour of the alula and primary wing coverts to that of greater wing coverts (Blondel et al. 2006). Parental female age may contribute to variation in parasite loads because nest sanitation behaviour (exclusively performed by females) may be affected by breeding experience (Hurtrez-Boussès et al. 2000, Banbura et al. 2001). Breeding attempts for which females could not be caught were not considered in this study.

Meteorological data

Records of daily minimum temperature (°C), daily maximum temperature (°C), and daily rainfall (mm) were obtained from the meteorological station of Calvi in Corsica. This weather station is situated at a maximum of 20 km from each of the study plots and thus gives reliable information on regional meteorological variation (see also Grosbois et al. 2006). We averaged the daily minimum and maximum ambient temperature to estimate daily average ambient temperature. For each nest during the two weeks following hatching we calculated the average ambient temperature and the average amount of rainfall. This period corresponds to the time when *Protocalliphora* larvae develop in blue tit nests by intermittently feeding on nestling blood, while spending the rest of the time hidden amongst nest materials (Hurtrez-Boussès et al. 1999).

To explore the effect of inter-annual climatic variation on mean *Protocalliphora* intensities we used meteorological archives of minimal, maximal and average monthly temperatures, as well as monthly rainfall. We calculated average temperature and total rainfall over three-months periods corresponding to summer (June-August), autumn (September-November), winter (December-February), and spring (March-May) preceding each breeding season.

Protocalliphora abundance

Previous studies (Hurtrez-Boussès et al. 1999) have shown that two blowfly species coexist in Corsican blue tit populations : *Protocalliphora azurea* (Fallén 1817) and *Protocalliphora falcozi* (Séguy 1928). Since it is impossible to morphologically distinguish between the two species at larval and pupal stages, we kept them pooled as *Protocalliphora.* Nests were collected 15 days post-hatching (*i.e.* one week before fledging), stored in hermetic plastic bags, and replaced in the nestbox by similar amounts of new nest material, mainly moss. In the laboratory, *Protocalliphora* larvae and pupae were carefully sorted out of the nest material and counted. Our counts included the total number of second-stage larvae, third-stage larvae and pupae (excluding first-stage larvae that are difficult to detect due to their small size), following the protocols presented in Hurtrez-Boussès et al. (1999), Heeb et al. (2000) and Mennerat et al. (2008; 2009). In some study years and sites (e.g. Mennerat et al. 2008; 2009), some nests were enclosed in cotton bags to facilitate their collection (without bag: 274 nests; with bag: 261 nests). Cotton bags were first inserted under blue tit nests around hatching time,

172 so that the blue tit parents could habituate to the presence of the bag. A few days later the
173 edge of the bag was pulled up to reach the same height as that of the nest.
174
175 Statistical analyses
176 This study includes data from 535 broods covering an 18-year period (Table 1). Our study
177 only focused on first-clutch broods for which the number of eggs and nestlings was not
178 manipulated, nests that were not experimentally treated against parasites, and nests for which
179 there was no evidence of predation (the main predator at these nestbox study sites is the green
180 whip snake *Hierophus viridiflavus)*. We used the mean number of *Protocalliphora* larvae per
181 nestling (*i.e.* the number of larvae and pupae found in a nest divided by the number of chicks
182 present at time of sampling) as a measure of parasite load. All analyses were performed in the
183 statistical programming environment *R 3.2.2* (http://r-project.org). Model validation was
184 performed by visual inspection of residuals.
185
186 *Within-year variation in parasite intensity*
187 To investigate the relation between parasite intensity and current biotic and abiotic factors, we
188 used linear mixed-effect models with log-transformed parasite load as a dependent variable
189 (*lmer* from the lme4 package). We applied forward, AIC-based model selection starting with a
190 set of models with nest thickness, cotton bag treatment (with *vs* without a cotton bag), egg-
191 laying date (in Julian dates), female age, and weather during the nestling stage (average
192 ambient temperature and rainfall) as explanatory variables. Year (n=18), valley (n=2), study
193 site (n=7), nestbox identity (n=243), and female identity (n=385) were included as random
194 effect factors.
195
196 *Inter-annual variation in parasite intensity*
197 We further investigated the inter-annual variation in mean parasite load in relation to weather
198 during the summer, autumn, winter and spring preceding each breeding period. The study
199 sites are located in two distinct valleys (Table 1) that differ markedly in a range of factors
200 (Blondel et al. 2006). We used linear models with mean (yearly average calculated for each
201 valley) *Protocalliphora* abundance per chick as a dependent variable. We applied forward,
202 AIC-based model selection with an initial set of explanatory models with valley as a factor (to
203 account for potential valley-specific relations between parasite intensities and weather) and
204 yearly mean values of egg laying-date, ambient temperature during the nestling period,
205 minimum, maximum, and average ambient temperature and total rainfall during each three-

month period (*i.e.* season) preceding the breeding season when parasite intensities were sampled. Two measures of host performance in the previous year (average brood size and average fledgling mass) were also included in the initial set of variables, to account for a potential relation between parasite and host dynamics. Interactions between valley and all other covariates were also included. The three explanatory variable retained in the final model were not correlated.

Results

Within-year variation in parasite intensity

The final model for brood parasite intensity included only ambient temperature during the nestling stage and cotton bag treatment as explanatory variables, and its fit was further improved by adding a quadratic term for ambient temperature. Parasite load increased with mean ambient temperature (linear term: $P < 10^{-4}$), with some degree of saturation at higher temperatures (quadratic term: $P < 10^{-4}$; Table 2A; Figure 1). In addition, parasite load was higher in nests surrounded by a cotton bag than in nests collected without a cotton bag ($P = 0.02$). Among the random effects factors, more than half of the total variance was explained by female identity and nestbox identity together (female identity: 31.7%; nestbox identity: 19.4%; Table 2B). Year, study site and valley explained 12, 7.4% and 2.4% of the variance respectively.

Inter-annual variation in parasite intensity

Across years, mean parasite load was strongly and positively correlated with ambient temperature during breeding ($P < 10^{-4}$; Figure 2), and to a lesser extent negatively correlated with average temperature in the previous summer ($P = 0.009$; Figure 3) and positively correlated with minimal spring temperature ($P = 0.03$; Figure 4). No other variable was retained in the final model (Table 3). In particular, no significant difference was detected between the two valleys. Even after excluding the year with the warmest summer (see Figure S1C), model selection resulted in the same set of variables.

Discussion

This study reveals that ambient temperature during the nestling stage is strongly related to variation in parasite load, both across broods when controlling for year, and across years. In addition, annual mean parasite load (1) increased with minimal spring temperature, and (2) decreased with increasing average temperature in the previous summer. In this Mediterranean

study system, parasite intensity thus appears to be primarily driven by ambient temperatures during spring and summer. Noticeably, as far as we could test, parasite dynamics did not relate to host dynamics. This might be explained by the fact that *Protocalliphora* blowflies have a broad host range and do not solely rely on blue tits, and that only the larval stage is parasitic. Finally, we found that both female identity and nestbox identity accounted for a relatively large amount of variance in brood parasite load. This finding that parasite loads are repeatable across females and nestboxes suggests that genetic and or maternal effects, as well as local environmental effects are important determinants of parasite abundances.

Our results suggest several ways in which ambient temperature may affect the life cycle of *Protocalliphora* blowflies. The main factor found to affect parasite load is ambient temperature during the larval stage (*i.e.* the nestling stage of blue tit hosts). In insects, temperature dependence of larval growth and survival has been studied extensively in the laboratory (overview in e.g. Chown and Nicolson 2004), yet surprisingly little in the field. Our study confirms, as observed in another host species the tree swallow *Tachycineta bicolor* (Dawson et al. 2005), that blowfly larval abundance is driven by temperature. In the tree swallow study, parasite abundance increased across the natural temperature range in a curvilinear way very similar to what we report here (see Figure 1), and decreased when temperatures were experimentally raised above natural levels (see also Castaño-Vázquez et al. 2018). As suggested in a recent study, a concomitant decrease in nest-dwelling ectoparasite loads could explain why experimental heat stress appears to have positive effects on blue tit fledglings (Andreasson et al. 2018). Our results here point to an optimal temperature for larval development around 23-25 °C and suggest that larval survival is significantly reduced below 20 °C.

We also found a negative effect of warmer summer temperatures on *Protocalliphora* loads in the following spring, possibly as a consequence of increased mortality of adult, free-living blowflies at high temperatures. While heat tolerance limits in adult *Protocalliphora* are currently unknown, in other dipteran species they appear to range from 30 °C and above (Feder et al. 1997, Berrigan et al. 2000, Chown and Nicolson 2004, Enriquez and Colinet 2017). Field studies of thermal stress in the willow beetle *Chrysomela aeneicolli*, which were carried out at a latitude close to that of our study sites, reveal that the temperatures measured on sunlit soil can approach 40 °C even when the maximum air temperature recorded by a nearby weather station did not exceed 26 °C. Considering that in our study area the maximal summer (air) temperature ranged from 34.0 up to 40.6 °C (average summer temperature 21.1 – 25.4 °C, see Figure S1), this means that adult *Protocalliphora* blowflies might in some years

be exposed to temperatures above their thermal tolerance levels, resulting in high adult mortality. This, however, remains to be tested.

Minimal spring temperature (ranging from -4.6 °C to 3.4 °C, Figure S1) also seems to influence parasite loads, while minimal winter temperature does not (ranging from -4.2 °C to 1.9 °C). Given that all minimal spring temperatures were recorded in March, this might suggest that the end of the overwintering period for adult blowflies takes place during this month after (Matyukhin and Krivosheina 2008), and that blowflies are then vulnerable to cold-induced mortality. This explanation remains however speculative, since little is currently known about the biology of blowflies in their free-living (*i.e.* adult) stage. More generally, the potential selective effect of temperature extremes on parasites in this system is an aspect that deserves further attention.

Because mean ambient temperature during the nestling stage increases linearly with egg-laying date, our results help explain why parasite loads are higher in late broods. They suggest temporal variation in the selection that parasites may impose on their hosts (Figure S2). In the focal study populations as in generally all temperate insectivorous birds, laying date is persistently under negative natural selection (Porlier et al. 2012) and the strength of selection is stronger during very warm springs (Marrot et al. 2017, 2018). This strong selection favouring early breeding females is always discussed in the context of the phenological mismatch between birds and their main caterpillar preys (e.g. van Noordwijk et al. 1995, Visser et al. 2006). The present study shows that nest ectoparasites may also be one of the drivers of selection on laying date. Despite high prevalences and intensities in Corsican populations of blue tits (Hurtrez-Boussès et al. 1997), no specific host behaviour was found to help preventing these parasites from accessing the nest (Mennerat et al. 2008) or removing them from nest material (Hurtrez-Boussès et al. 2000). It appears, however, that adult blue tits can compensate for some of the harmful effects of parasites by increasing rates of chick provisioning (Hurtrez-Boussès et al. 1998). The level of compensation depends on food availability (Simon et al. 2004), and, as a result, parasites and food interact to determine post-fledging survival and recruitment (path analysis, Thomas et al. 2007). Based on this, parasite-mediated selection is likely strongest in evergreen oak-dominated habitats, characterised by a combination of low caterpillar abundance and late breeding, and where parasite loads are highest. On the contrary in deciduous holm oak-dominated habitats, parasites might exert relatively weak selection on blue tits due to a combination of plentiful food, early breeding, and low parasite loads. The nature of traits in blue tits that could be under parasite-mediated selection has yet to be established, but breeding phenology appears as a likely candidate, as

308 well as parental behaviours that improve nestling growth or survival under conditions of high
309 parasite abundance (Hurtrez-Boussès et al. 1998, Mennerat et al. 2009). Our results also
310 reveal that the identity of the female parent accounts for a large proportion of variance in
311 parasite loads. This opens for several possible explanations, including variation in maternal
312 behaviour, but also a potential effect of host genetic factors. Since laying date is highly
313 repeatable and heritable across females in these populations (Caro et al 2009), and considering
314 our results that larval intensity increases with temperature, it is likely that a quantitative
315 genetics analysis of parasite load would reveal heritability of parasite load. Nestbox identity
316 was the second most important random effect after female identity, suggesting that other nest
317 environmental factors might have an effect, and that parasites can be one component of
318 variation in territory quality. Further investigation will be necessary to disentangle the diverse
319 influences of host behavioural, genetic and environmental factors, as well as their implications
320 in terms of selection on both hosts and parasites.

321 The apparent link between parasite loads and temperature fluctuations in this
322 Mediterranean area is relevant for host-parasite interactions at broader spatial scales.
323 Geographical distributions are expected to be limited by climate, and especially by
324 temperature (David et al. 2003, Kingsolver and Buckley 2017). In a number of insect species
325 the amplitude of thermal tolerance seems reflected in species latitudinal ranges (e.g. Calosi et
326 al. 2010) and in endotherms there is evidence that range limits relate to thermal tolerance
327 limits (e.g. Khaliq et al. 2017). *P. azurea* and *P. falcozi*, the two species present in our study
328 area, are both widespread over the Palaearctic in a wide range of host species (Wesolowski
329 2001, Matyukhin and Krivosheina 2008). *P. azurea* was reported from Spain to Scandinavia
330 (Potti 2008, Eeva et al. 2015), and *P. falcozi* mostly from Central Europe, but also Germany
331 and Corsica (Wesolowski 2001, Janoskova et al. 2010). Strikingly, of all reports from the
332 Palaearctic it is in Corsica that prevalences and intensities are the highest (Hurtrez-Boussès et
333 al. 1997). This is consistent with the idea that parasite abundances increase with minimal and
334 average spring temperatures, as both are higher on Corsica than in most other locations in
335 Europe. Furthermore *Protocalliphora* abundances relate negatively to average summer
336 temperature; this suggests that they may be limited by heat in areas located further south in
337 the Mediterranean.

338 Blowfly prevalence and intensities in Western, Central and Northern Europe seem
339 moderate at the moment: prevalence scarcely exceeds 50%, and the reported average
340 intensities remain under or around two larvae per nestling (Wesolowski 2001, Potti 2008,
341 Eeva et al. 2015). However, current projections (IPCC 2013) indicate that regardless of the

scenario, by 2100 mean spring and summer temperatures will likely have increased not only in the Mediterranean (spring: +1 °C to +5 °C; summer: +1.5 °C to +7 °C) but also in Central (spring: +1 °C to +5 °C; summer: +2 °C to +6 °C) and Northern Europe (spring: +2 °C to +5 °C; summer: +2 °C to +5 °C). Our results suggest that this might result in modified bird – blowfly interactions. A more comprehensive understanding of the relationships between climate, spring phenology, but also selection and adaptive evolution in bird hosts as well as their parasites is now needed.

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Ethical statement

Captures were performed under personal ringing permits delivered by the CRBPO (Centre de Recherches par le Baguage des Populations d'Oiseaux, English: Bird Population Ringing Research Centre) to Anne Charmantier (ringing permit number 1907), Adèle Mennerat, Philippe Perret, and Marcel Lambrechts (permit 1318). All experimental protocols were approved by the ethics committee for animal experimentation of Languedoc Roussillon (305-CEEA-LR-12066 approved in 2012) as well as by Regional Institutions (bylaw issued by the Prefecture on 15/06/2012 n° 2012167-0003).

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Table 1. Yearly fluctuations in the number (mean ± SD) of *Protocalliphora* larvae per blue tit nestling on Corsica from 1997 to 2014. Sample sizes (broods) are in parentheses.

Year	Muro valley	Fango valley
1997	-	12.15 ± 7.01 (10)
1998	6.98 ± 0.84 (2)	10.38 ± 6.72 (8)
1999	6.15 ± 1.21 (2)	11.07 ± 4.53 (4)
2000	3.66 ± 2.63 (6)	13.79 ± 5.20 (5)
2001	3.07 ± 3.48 (23)	13.12 ± 5.51 (16)
2002	-	9.54 ± 3.29 (3)
2003	-	9.00 ± 3.59 (11)
2004	-	5.78 ± 4.71 (19)
2005	5.44 ± 3.65 (53)	10.23 ± 5.41 (35)
2006	-	12.44 ± 5.47 (6)
2007	3.37 ± 2.97 (56)	-
2008	5.68 ± 5.81 (52)	15.31 ± 8.58 (23)
2009	3.55 ± 2.73 (39)	11.39 ± 6.36 (33)
2010	2.94 ± 2.62 (29)	8.11 ± 4.14 (35)
2011	-	10.64 ± 6.71 (24)
2012	-	12.90 ± 7.27 (13)
2013	-	2.12 ± 1.49 (5)
2014	-	12.08 ± 10.21 (23)
Total	4.26 ± 3.93 (262)	10.70 ± 6.77 (273)

551 Table 2. Within-year variation in parasite load per chick in blue tit nests infested by
552 hematophageous *Protocalliphora* larvae, in relation to mean ambient temperature during the
553 nestling stage (*i.e.* in the period when parasite larvae develop into blue tit nests) and cotton
554 bag treatment (*i.e.* presence or absence of a cotton bag around the nest). Results from the final
555 linear mixed effects model, obtained after forward selection from an initial set of models with
556 nest thickness (*i.e.* height), cotton bag treatment, egg-laying date, female age, and average
557 ambient temperature and rainfall during the nestling stage as explanatory variables. Year,
558 valley, study site, nestbox identity, and female identity were included as random effect
559 factors.
560

A. Fixed effects	d.f.	Estimate	SE	Chisq	P
Mean ambient temperature during nesting (linear)	1	1.58	0.29	29.53	$< 10^{-4}$
Mean ambient temperature during nesting (quadratic)	1	-0.04	0.008	24.23	$< 10^{-4}$
Cotton bag treatment	1	0.22	0.09	5.56	0.02

B. Random effects	Variance	% total
Female identity (n = 385)	0.19	31.7 %
Nestbox identity (n = 243)	0.12	19.4 %
Year (n = 18)	0.07	12.0 %
Study site (n = 7)	0.04	7.4 %
Valley (n = 2)	0.01	2.4%
Residual	0.16	27.1 %

561
562

563 Table 3. Inter-annual variation in mean parasite load per chick (yearly average) in nests of
564 blue tits infested by hematophageous *Protocalliphora* larvae, in relation to yearly average
565 values of mean temperature during nesting, mean temperature in the previous summer, and
566 minimal spring temperature. Results from the final linear model, obtained after forward
567 selection from an initial set of models with valley, egg laying-date, ambient temperature
568 during the nestling period, minimum, maximum, and average ambient temperature and total
569 rainfall during each preceding season as explanatory variables, as well as interactions between
570 valley and all other covariates.

571

	d.f.	Estimate	SE	Sum of squares	P
Mean temperature during nesting	1	1.60	0.17	272.1	$< 10^{-4}$
Mean temperature in previous summer	1	-0.89	0.34	20.40	0.009
Minimal spring temperature	1	0.37	0.17	14.32	0.03

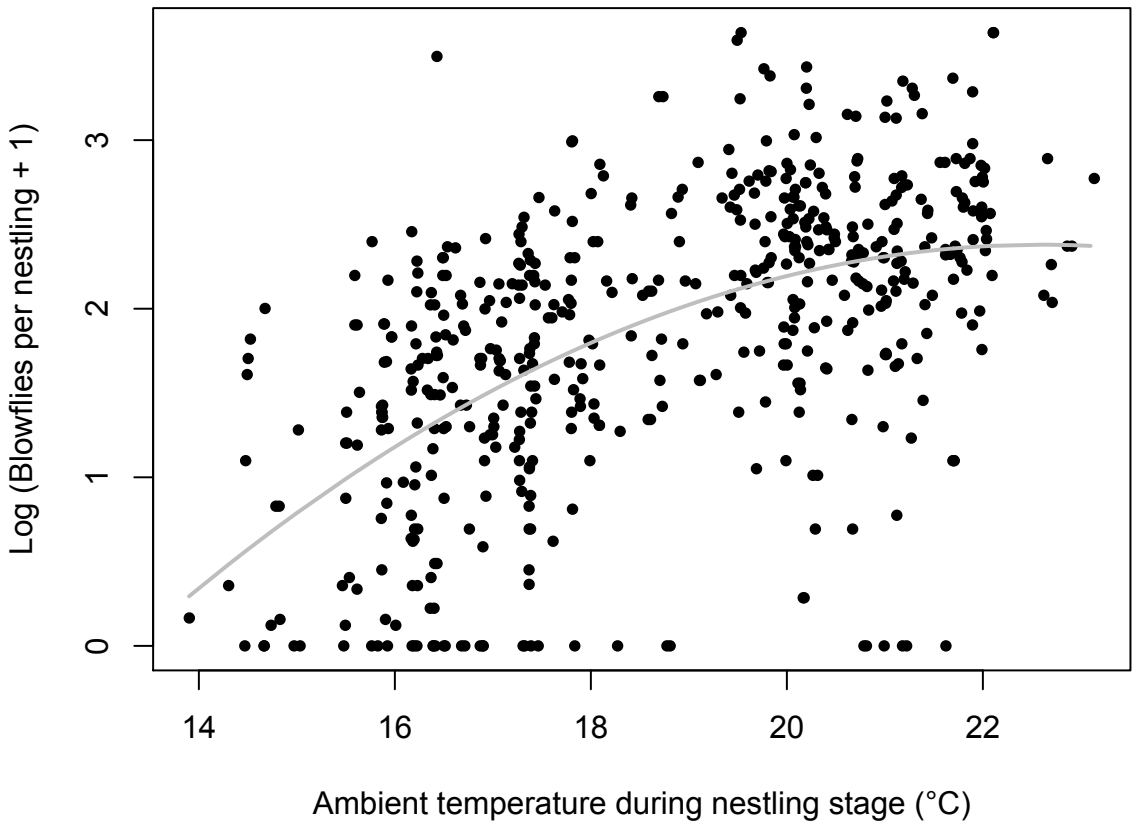
572

573

574

575 Figure 1

576



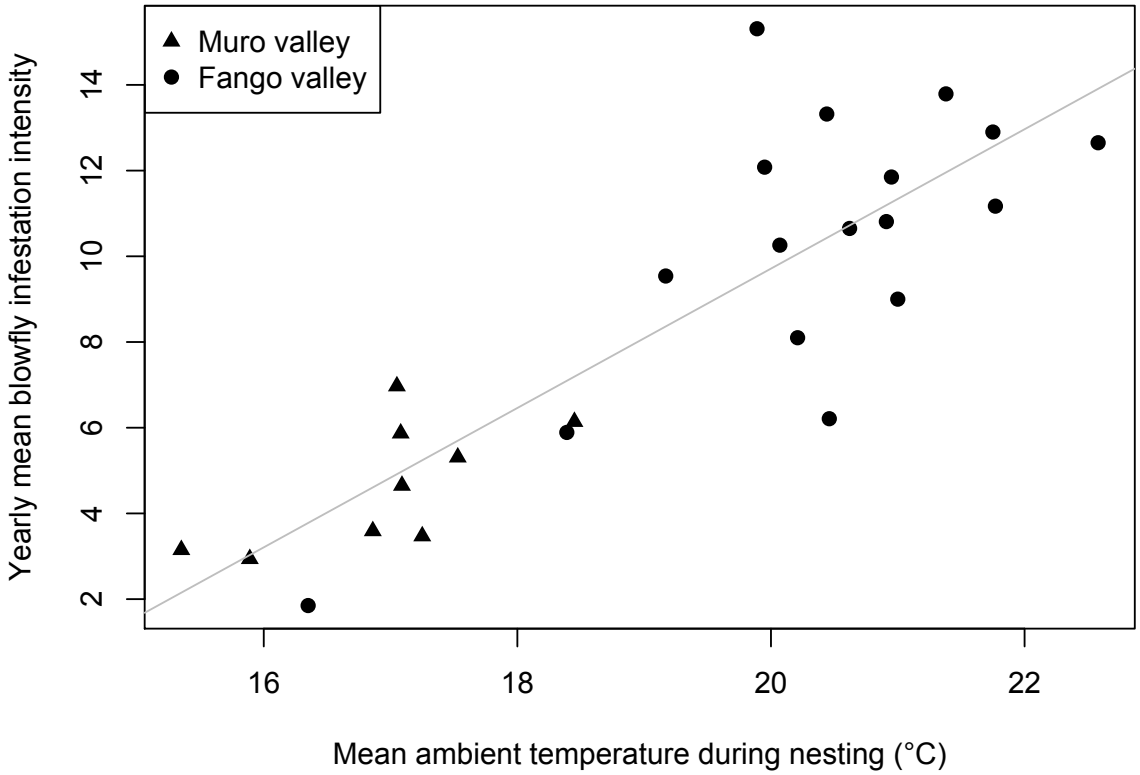
577

578 Figure 1. Across broods, parasite load increases with mean ambient temperature during the
579 nestling stage (*i.e.* during the developmental period of *Protocalliphora* larvae) in nests of blue
580 tits on Corsica. Each dot represents a brood. The grey curve shows predicted values from the
581 final model (see Table 2).

582

583 Figure 2

584



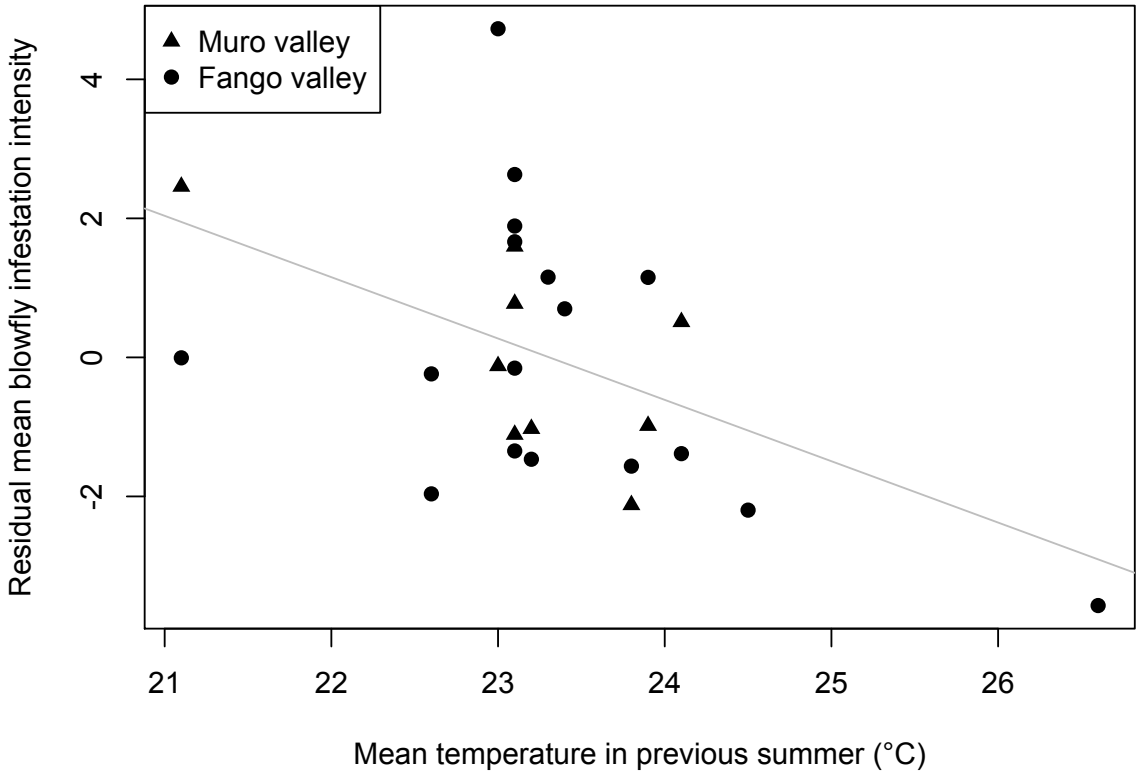
585

586 Figure 2. Across years, parasite load correlates positively with ambient temperature during the
587 nestling stage (*i.e.* during the developmental period of *Protocalliphora* larvae) in nests of blue
588 tits on Corsica. Dots represent yearly average values.

589

590 Figure 3

591



592

593 Figure 3. Across years, parasite load (corrected for ambient temperature during nesting)
594 decreases with increasing mean temperatures in the previous summer (i.e. during the adult,
595 free-living stage of *Protocalliphora* blow flies). Dots represent yearly mean values.

596

Figure 4

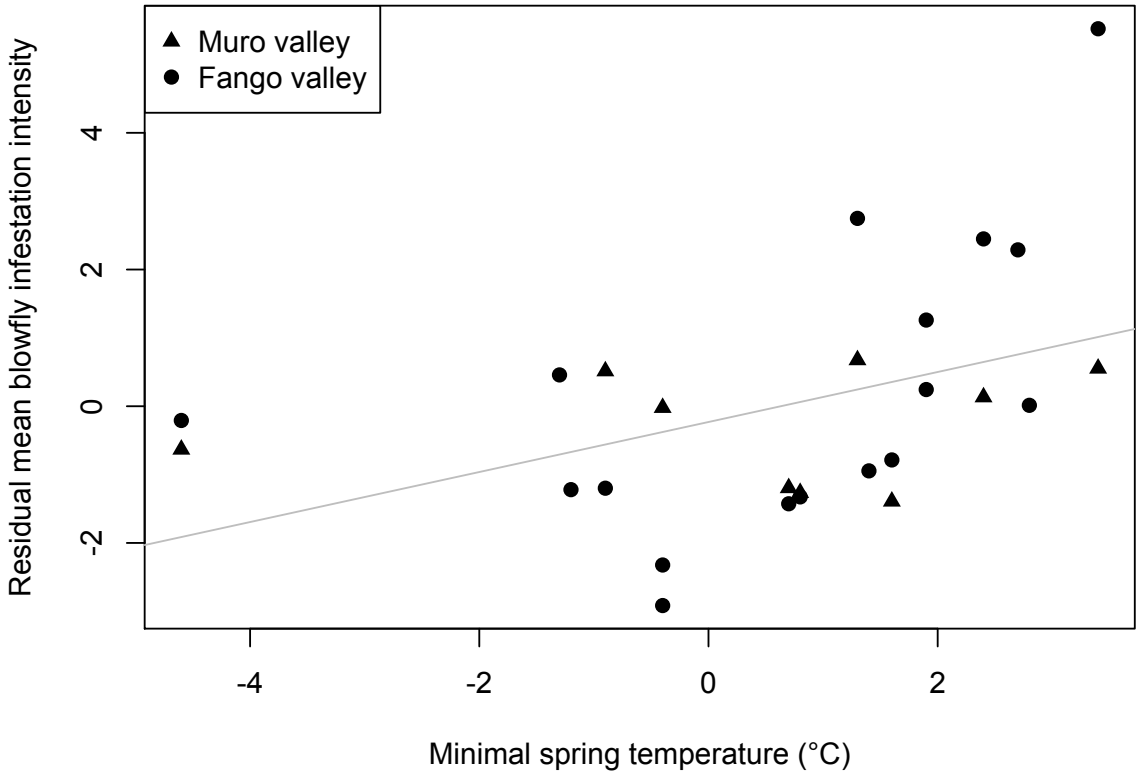
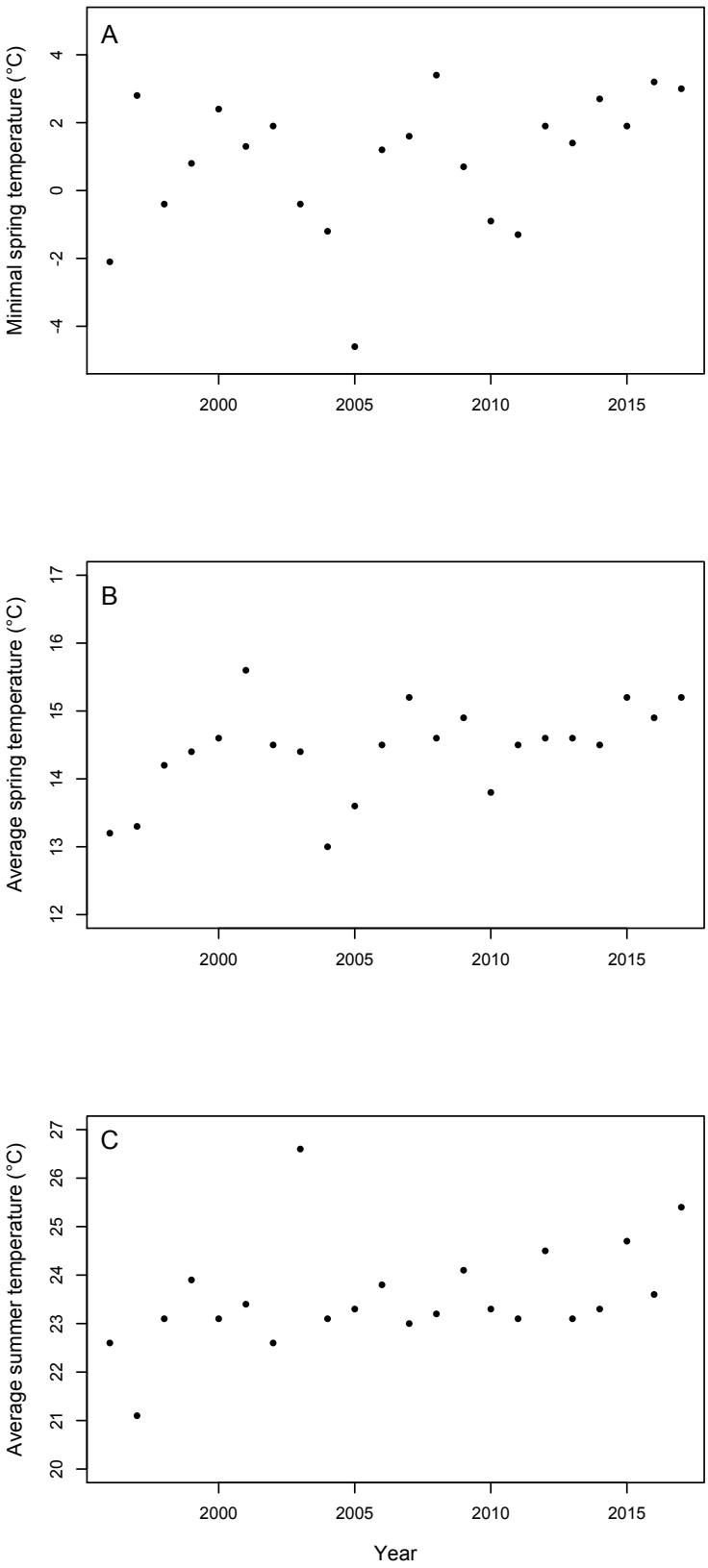


Figure 4. Across years, parasite load (corrected for ambient temperature during nesting) increases with increasing minimal temperatures in early spring (*i.e.* during the adult, free-living stage of *Protocalliphora* blow flies). Dots represent yearly mean values.

604 Supplementary material



605

606 Figure S1. Yearly variation over the 18 years of the study in (A) minimal spring temperature,
607 (B) average spring temperature, and (C) average summer temperature.

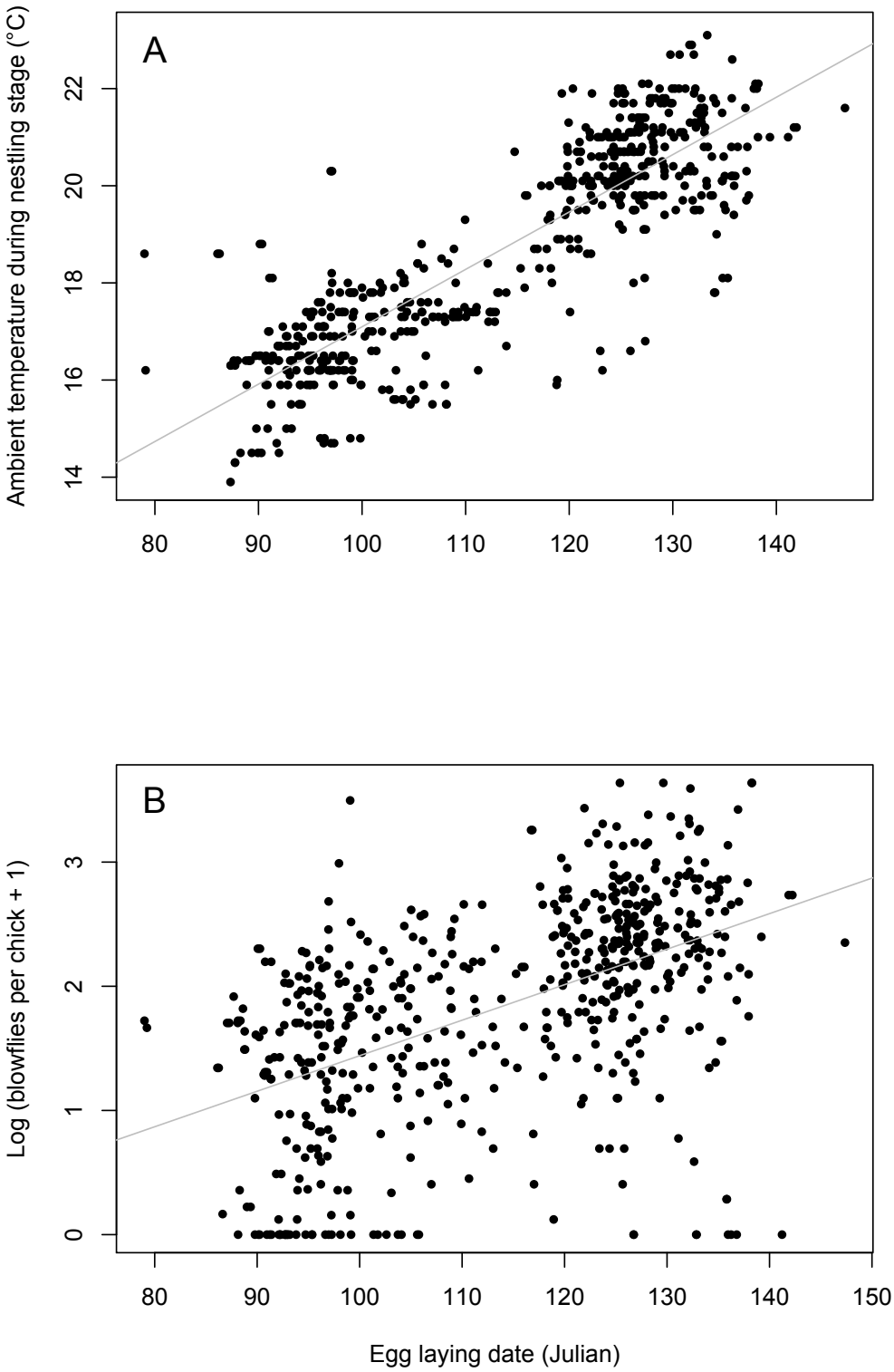


Figure S2. Both average ambient temperature (A) and parasite load (B) during the nestling stage increase with egg-laying date.