Parasite intensity is driven by temperature in a wild passerine 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 of 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 when average temperature was high in the previous summer. In this Mediterranean study system, parasite intensity thus appears to be primarily driven by ambient temperature during spring and summer. This suggests a major role for thermal tolerance at various stages in the blowfly life cycle, with implications for a possible northwards range shift for these parasites as climate keeps warming up. Finally, given that ambient temperature increases throughout the breeding season and that blowfly infestation has well-established consequences for survival and recruitment of blue tits, these results also suggest temporal variation in the selection that parasites may impose on their hosts, and in particular on host phenology.

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 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 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 parasites and disease need to be supported by detailed empirical knowledge acquired regionally, and in relevant host-parasite systems (Hernandez et al. 2013, Roiz et al. 2014).

Amongst parasites and pathogens, invertebrates spending part of their life cycle as free-living (*i.e.* away from their host) are likely most affected by weather and climate (Hernandez et al. 2013, Rose et al. 2014, Charlier et al. 2016, Ogden and Lindsay 2016). In those ectoparasites developmental time, activity rates, and survival often display bell-shaped responses, *i.e.* highest at intermediate temperature and moisture values (reviewed in Ogden and Lindsay 2016). Local weather fluctuations may therefore result in either increased or decreased parasite intensities depending on the current position of populations relative to these optima (Stromberg 1997, Elderd and Reilly 2014, Eads and Hoogland 2016). In the longer run parasites could respond by means of adaptive evolution under directional climate change. In addition, invertebrates 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 a 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 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 was 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 in press, Heeb et al. 1996, Remeš and Krist 2005, Kleindorfer and Dudaniec 2009, Moreno et al. 2009), yet the relative contributions of these 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 (Bennett and Whitworth in press, Owen and Ash 1955, Møller et al. 1990, 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 and old nest materials (Matyukhin and Krivosheina 2008). The host spectrum of *Protocalliphora* is quite large, although avian hole-nesters seem to be especially exposed to this ectoparasite (Owen and Ash 1955, Jamriska et al. 2010). Nest-box studies showed that bird nestlings developing in nests with more *Protocalliphora* larvae have lower body condition, reduced growth or smaller body size (Merino and Potti 1996, Hurtrez-Boussès et al. 1997, Charmantier et al. 2004), decreased resting time (Simon et al. 2005) and lower physiological performance (Simon et al. 2004, Thomas et al. 2007). In addition, *Protocalliphora* abundance was found positively related to bacterial loads measured on blue tit (*Cyanistes caeruleus*) 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 avian reproductive effort as reflected in increased feeding rates by males or increased investment into nest-sanitation behaviour by females (Hurtrez-Bousses et al. 1998, Bańbura et al. 2004). On the island of Corsica, blue tit nestlings (*Cyanistes caeruleus*) are exposed to the highest *Protocalliphora* loads reported 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 interannual variability in mean parasite intensities

relates to temperature and rainfall during the summer, autumn, winter and spring preceding each breeding season.

Methods

Study sites and monitoring

Data from uniquely ringed female breeders were obtained for years 1997-2014 from seven study plots situated in two valleys on the island of Corsica (plots Avapessa AVA, Arinelle ARI, Feliceto FEL, Filagna FIL, Grassa GRA and Muro MUR in the Muro valley; plot Pirio PIR 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 AVA, FEL, and MUR. The evergreen oak *Q. ilex* was the dominant tree species in the other four study plots where blue tit broods were smaller and reared later in the season (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 cm²).

Following basic protocols (Blondel et al. 2006) boxes were visited at least once a week to check the progress of each avian breeding attempt, the initiation and progress of nest construction, the first egg-laying dates expressed in Julian dates, and the number of eggs and 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, nest thickness is an appropriate proxy for nest volume, *i.e.* habitat size for nest ectoparasites.

Because nests were not visited every day at the end of the incubation stage, the onset of hatching was calculated based on the physical development of the nestlings within a few 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 by using the wing plumage colors or, for previously ringed birds, from the monitoring records (cf. Blondel et al. 2006). The age of female breeders may contribute to variation in parasite loads by affecting their sanitation behaviour. Breeding attempts for which female age could not be identified 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 informs on regional climatic variation (see also Grosbois et al. 2006). We averaged the daily minimum and maximum ambient temperature to obtain a measure of daily average ambient temperature. Based on this, 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 interannual 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

Nests were collected 15 days post-hatching, stored in hermetic plastic bags, and replaced by similar amounts of new nest material, mainly moss. In the laboratory, *Protocalliphora* larvae and pupae were sorted out of the nest material and counted. *Protocalliphora* larvae develop into three successive larval stages before pupating. 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 (1996), Heeb et al. (2000) and Mennerat et al. (2008; 2009). In some study years (e.g. Mennerat et al. 2008; 2009), nests were enclosed in cotton bags to facilitate their collection (without: 274 nests; with: 261 nests). Cotton bags were first inserted under blue tit nests around hatching time, so that the blue tit parents could habituate to the presence of the bag. A few days later the edge of the bag was pulled up to reach the same height as that of the nest.

Statistical analysis

This study includes data from 535 broods covering an 18-year period (Table 1). Our study only focused on first-clutch broods for which the contents of the nests were not manipulated, nests that were not experimentally treated against *Protocalliphora*, and nests for which there

170 was no evidence of predation. We used the mean number of *Protocalliphora* larvae per
171 nestling (*i.e.* the number of larvae found in a nest divided by the number of chicks present at
172 time of sampling) as a measure of parasite load. All analyses were performed in the statistical
173 programming environment *R 3.2.2* (http://r-project.org). Model validation was performed by
174 visual inspection of residuals.
175
176 *Within-year variation in parasite intensity*
177 To investigate the relation between parasite intensity and current biotic or abiotic factors, we
178 used linear mixed-effect models with log-transformed parasite load as a dependent variable
179 (*lmer* from the lme4 package). We applied forward, AIC-based model selection starting with a
180 set of models with nest thickness, cotton bag treatment (with *vs* without a cotton bag), egg-
181 laying date (in Julian dates), and weather during the nestling stage (average ambient
182 temperature and rainfall) as explanatory variables. Female age was also included because nest
183 sanitation behaviour (exclusively performed by females), and thus parasite intensities, may be
184 affected by breeding experience (Banbura et al. 2001). Year (n=18), study site (n=7), nestbox
185 identity (n=243), and female identity (n=385) were included as random effect factors.
186
187 *Interannual variation in parasite intensity*
188 We further investigated the interannual variation in mean parasite load in relation to weather
189 during the summer, autumn, winter and spring preceding each breeding period. The study
190 sites are located in two distinct valleys (Table 1) that differ markedly in a range of factors
191 (Blondel et al. 2006). We used linear models with mean (yearly average, calculated for each
192 valley) *Protocalliphora* abundance per chick as a dependent variable. We applied forward,
193 AIC-based model selection with an initial set of explanatory models with valley as a factor (to
194 account for potential valley-specific relations between parasite intensities and weather) and
195 yearly mean values of egg laying-date, ambient temperature during the nestling period,
196 minimum, maximum, and average ambient temperature and total rainfall during each three-
197 month period (*i.e.* season) preceding the breeding season when parasite intensities were
198 sampled. Interactions between valley and all other covariates were also included.
199
200 **Results**
201 *Within-year variation in parasite intensity*
202 The final model for brood parasite intensity included only ambient temperature during the
203 nestling stage and cotton bag treatment as explanatory variables, and its fit was further

204 improved by adding a quadratic term for ambient temperature. Parasite load increased with
205 mean ambient temperature (linear term: $P < 10^{-4}$), with some degree of saturation at higher
206 temperatures (quadratic term: $P < 10^{-4}$; Table 2; Figure 1). In addition, parasite load was
207 higher in nests surrounded by a cotton bag than in nests collected without a cotton bag ($P =$
208 0.02).
209
210 *Interannual variation in parasite intensity*
211 Across years, mean parasite load was strongly and positively correlated with ambient
212 temperature during breeding ($P < 10^{-4}$; Figure 2), and to a lesser extent negatively correlated
213 with average temperature in the previous summer ($P = 0.009$; Figure 3) and positively
214 correlated with minimal spring temperature ($P = 0.03$; Figure 4). No other variable was
215 retained in the final model (Table 3).
216
217 **Discussion**
218 In this study we found that ambient temperature during the nestling stage is strongly related to
219 variation in parasite load, both across broods when controlling for year, and across years. In
220 addition, annual mean parasite load also increased with minimal spring temperature, and
221 decreased when average temperature was high in the previous summer. In this Mediterranean
222 study system, parasite intensity thus appears to be primarily driven by ambient temperatures
223 during spring and summer, similarly in all seven study plots. We also confirm that parasite
224 loads are higher in nests surrounded by a cotton bag compared to nests without a bag,
225 probably because these bags prevent the loss of nest-dwelling parasites, which was the main
226 argument for using them (Hurtrez-Boussès et al. 1999, Mennerat et al. 2008, 2009).
227 Our results indicate that thermal dependence is an important mechanism at various
228 stages in the life cycle of *Protocalliphora* blowflies. The main factor found to affect parasite
229 load is ambient temperature during the larval stage (*i.e.* the nestling stage of blue tit hosts). In
230 insects, temperature dependence of larval growth and survival has been extensively studied in
231 the laboratory (overview in e.g. Chown and Nicolson 2004), even though the link between
232 lab-measured thermal limits and variation in insect abundance in the field is not always
233 straightforward. Our study confirms, as observed in another host species the tree swallow
234 *Tachycineta bicolor,* that blowfly larval abundance appears driven by temperature (Dawson et
235 al. 2005). In that previous study, parasite abundance increased across the natural temperature
236 range in a curvilinear way very similar to what we report here (see Figure 1), and decreased
237 when temperatures were experimentally raised above natural levels. Taken together, these two

studies point to an optimal temperature for larval development around 23-25 °C and suggest that larval survival is significantly reduced below 20 °C. This is consistent with observations from other species within the *Calliphoridae* family (Wall et al. 2001, Lecheta et al. 2015).

We also found a negative effect of summer heat on *Protocalliphora* loads in the following spring, possibly as a consequence of increased mortality of adult 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). In the willow beetle *Chrysomela aeneicolli* the expression of heat shock protein Hsp70, indicative of physiological stress, increased in field samples along with temperature and reached high levels around 30-35 °C (Dahlhoff and Rank 2000). Furthermore, there is evidence of seasonal variation in selection at the phosphoglucose isomerase (PGI) locus inducing Hsp expression (Dahlhoff et al. 2008, Dick et al. 2013). These studies, 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 could in some years be exposed to temperatures above their thermal tolerance levels, resulting in high adult mortality. Minimal spring temperature (ranging from -4.6 °C to 3.4 °C, Figure S1) also seems to constrain parasite loads. Given that all minimal temperatures were recorded in March, *i.e.* before the reproductive period for *Protocalliphora*, this likely reflects cold-induced mortality of adults shortly after post-winter emergence. The 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, and suggest temporal variation in the selection that parasites may impose on their hosts, and in particular on host phenology (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 they main caterpillar preys (e.g. van Noordwijk et al. 1995, Visser et al. 2006), yet the present study shows that nest ectoparasites may also drive selection on laying date. *Protocalliphora* larvae are detrimental

to blue tit nestlings since they induce reduced growth, lower metabolic capacity and body condition, and eventually lower recruitment in the breeding population (Hurtrez-Boussès et al. 1997, Simon et al. 2004, Thomas et al. 2007). Prevalence is very high in Corsican populations of blue tits (Hurtrez et al 1997), yet no specific behaviour was found to either prevent these parasites from accessing the nest (Mennerat et al. 2008) or remove them (Hurtrez-Bousses et al. 1998). It appears, however, that adult blue tits can compensate for some of the harmful effects of parasites by increasing rates of chick provisioning (Hurtrez-Bousses 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 it can be inferred that parasite-mediated selection should be 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 not yet been established, nor the strength of such selection; however given that parasite loads increase with temperature and thus egg laying date, breeding phenology appears as a likely candidate, as well as parental behaviours that improve nestling growth or survival under conditions of high parasite abundance (Hurtrez-Bousses et al. 1998, Mennerat et al. 2009).

The apparent link between parasite loads and temperature fluctuations in this Mediterranean area is relevant for host-parasite interactions at broader spatial scales. Geographical distributions are expected to be limited by climate, and especially by temperature in insects (David et al. 2003, Kingsolver and Buckley 2017), where in a number of cases the amplitude of thermal tolerance seems reflected in species latitudinal ranges (e.g. Calosi et al. 2010). The parasitic larvae in the present study belong to two species, *P. azurea* and *P. falcozi*. Both species appear widespread over the Palaearctic, but whether one is more abundant than the other remains unclear (Wesolowski 2001, Matyukhin and Krivosheina 2008). *P. azurea* was reported from Spain to Scandinavia (Potti 2008, Eeva et al. 2015), and *P. falcozi* mostly from Central Europe, but also Germany and Corsica where both species coexist (Wesolowski 2001, Janoskova et al. 2010). Strikingly, of all reports from the Palaearctic it is on Corsica that prevalences and intensities are found highest (Hurtrez-Boussès et al. 1997), and this is consistent with the idea that parasite abundances increase with minimal and average spring temperatures, as both are higher on Corsica than in most other locations in Europe. Furthermore parasite loads relate negatively to average summer

temperature; this suggests that heat in areas located further south in the Mediterranean may limit their southern range.

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 Northen Europe (spring: +2 °C to +5 °C; summer: +2 °C to +5 °C). Blowfly prevalence and infestation intensities in Central and Northern Europe seem moderate at the moment (Wesolowski 2001, Eeva et al. 2015), yet our results suggest that they might increase in the future, and that the range of *Protocalliphora* spp. might shift northwards. 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.

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)

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

	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

537 Table 3. Interannual variation in mean parasite load per chick (yearly average) in nests of blue
538 tits infested by hematophageous *Protocalliphora* larvae, in relation to yearly average values
539 of mean temperature during nesting, mean temperature in the previous summer, and minimal
540 spring temperature. Results from the final linear model, obtained after forward selection from
541 an initial set of models with valley, egg laying-date, ambient temperature during the nestling
542 period, minimum, maximum, and average ambient temperature and total rainfall during each
543 preceding season as explanatory variables, as well as interactions between valley and all other
544 covariates.

545

	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

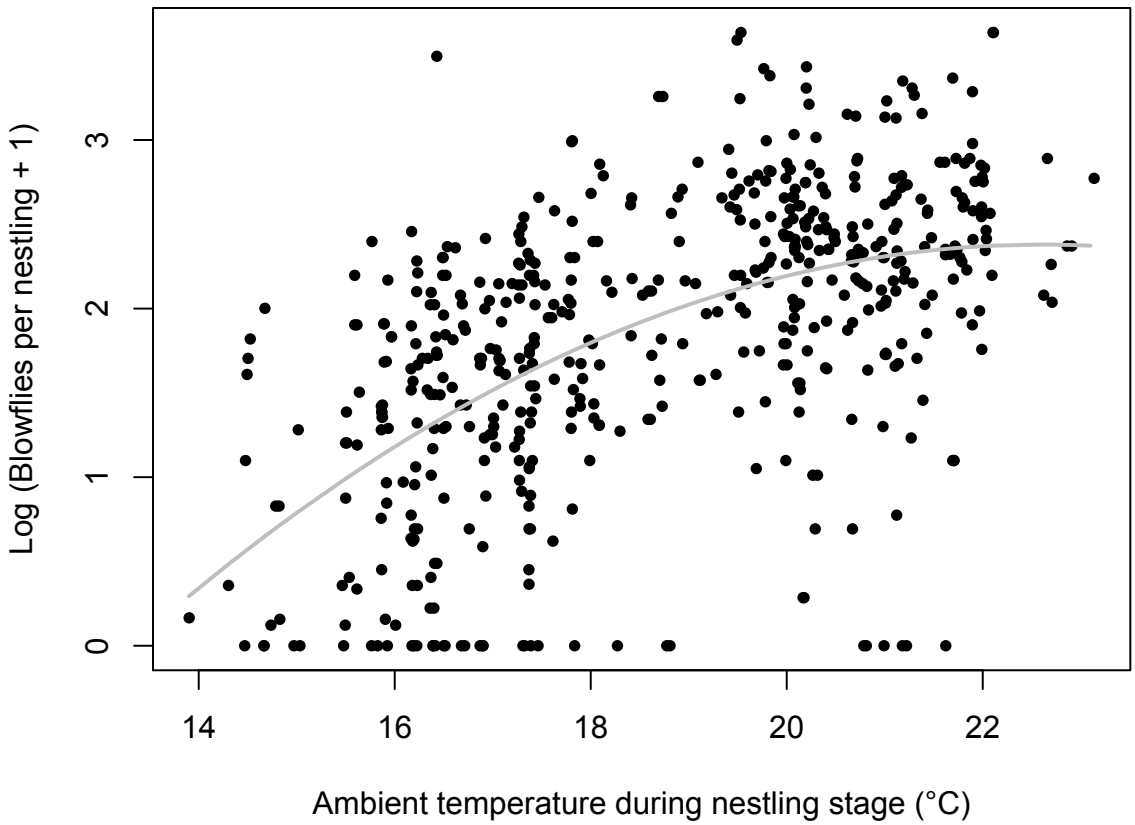
546

547

548

549 Figure 1

550



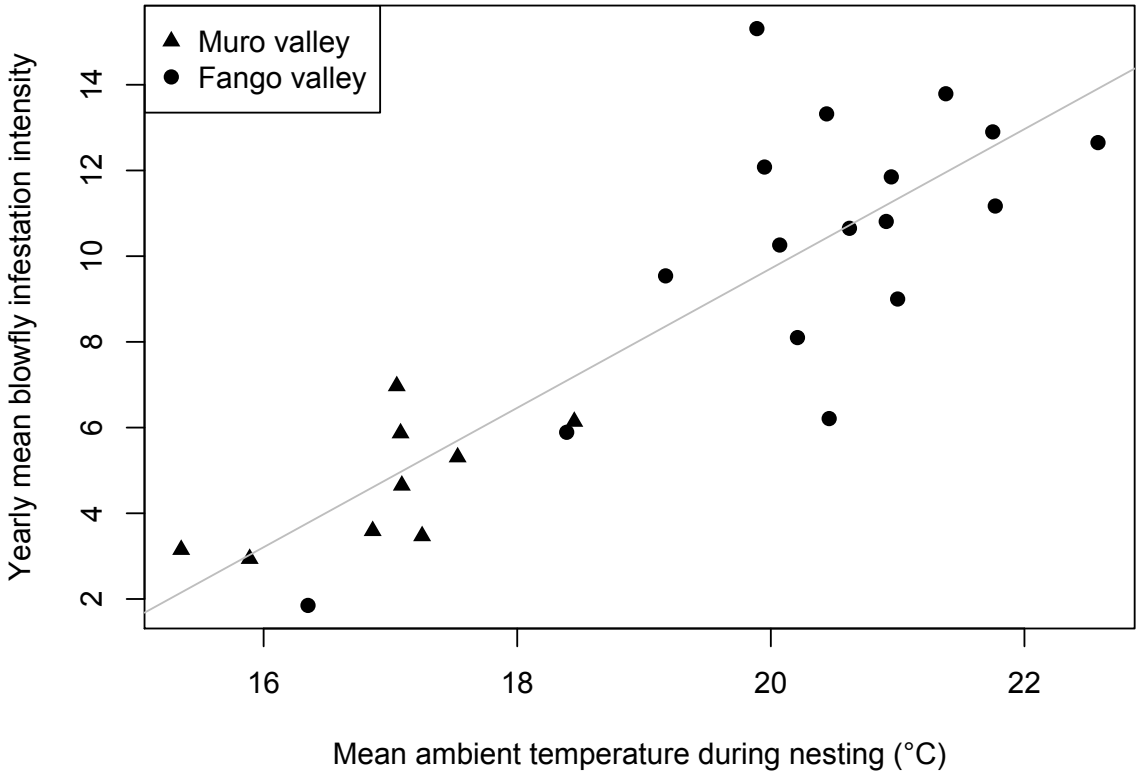
551

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

556

557 Figure 2

558



559

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

563

Figure 3

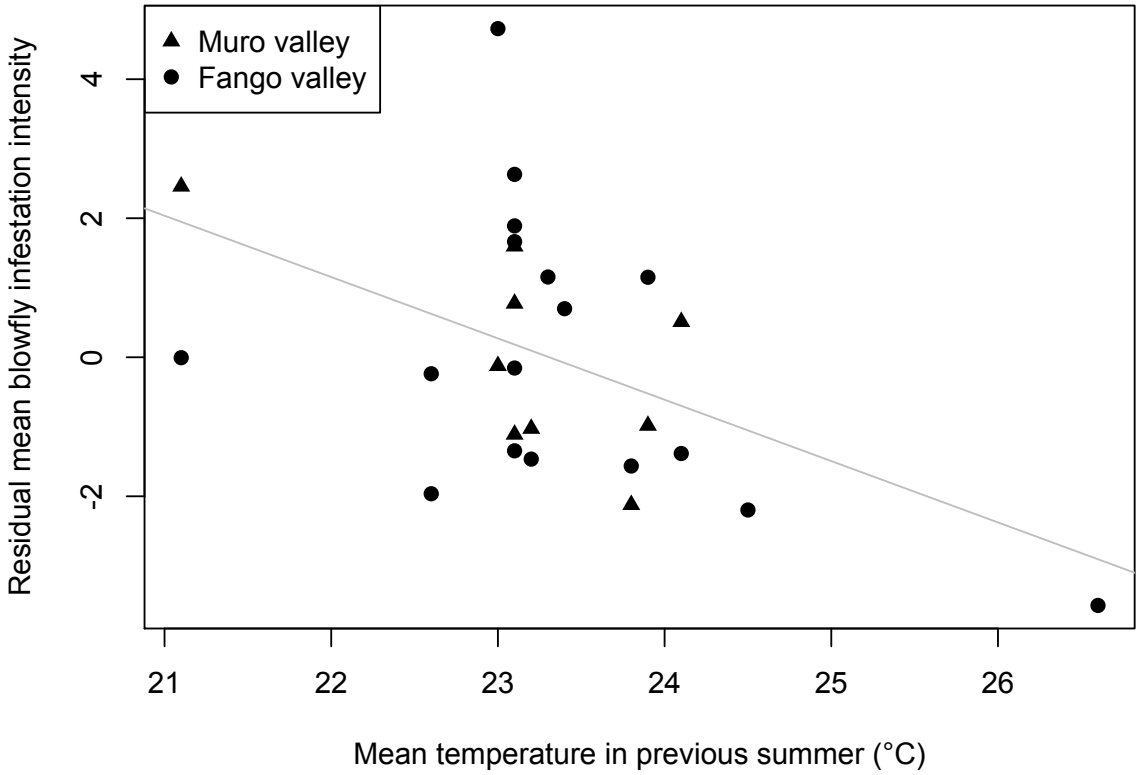
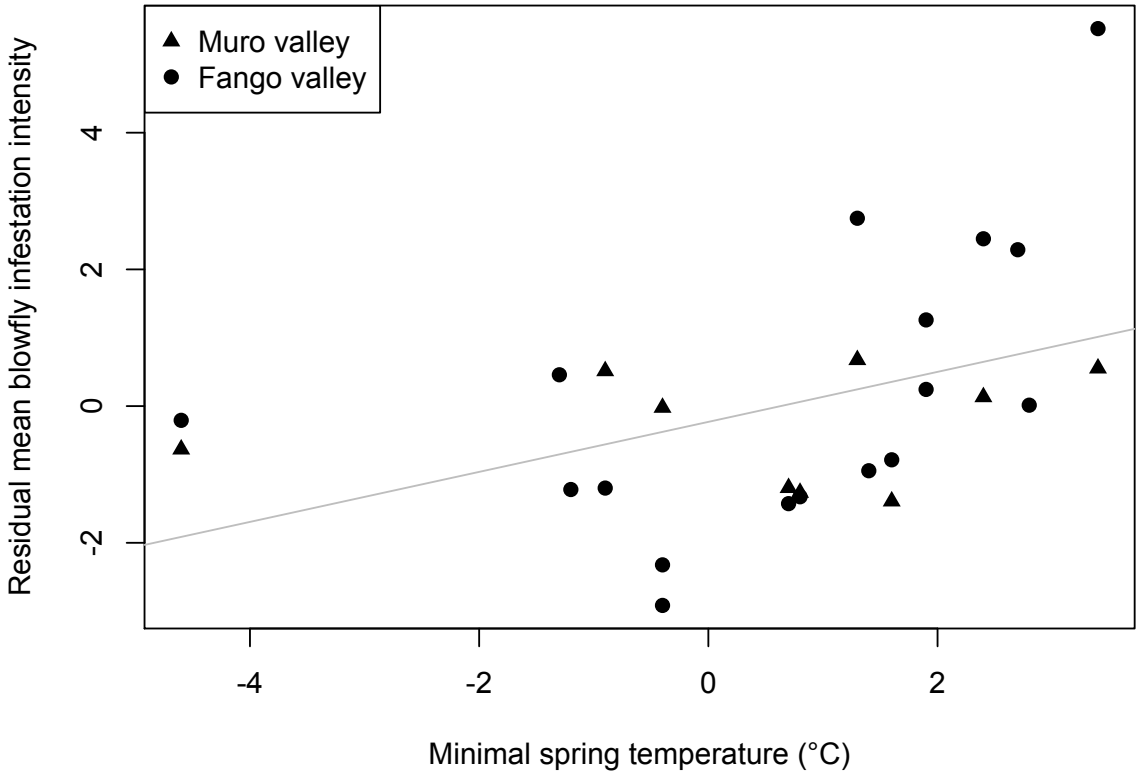


Figure 3. Across years, parasite load (corrected for ambient temperature during nesting) decreases with increasing mean temperatures in the previous summer (i.e. during the adult, free-living stage of *Protocalliphora* blow flies). Dots represent yearly mean values.

571 Figure 4

572



573

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

577

Supplementary material

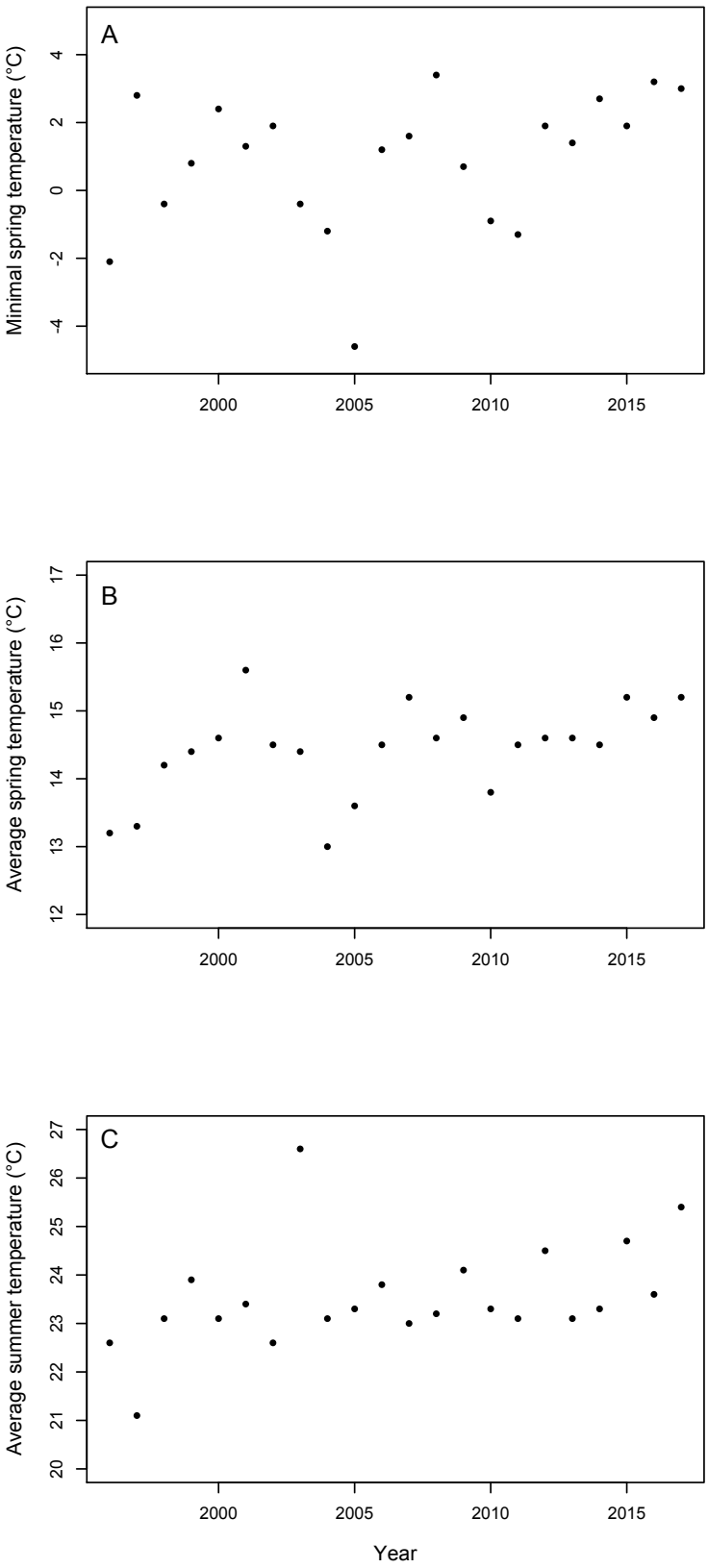


Figure S1. Yearly variation over the 18 years of the study in (A) minimal spring temperature, (B) average spring temperature, and (C) average summer temperature.

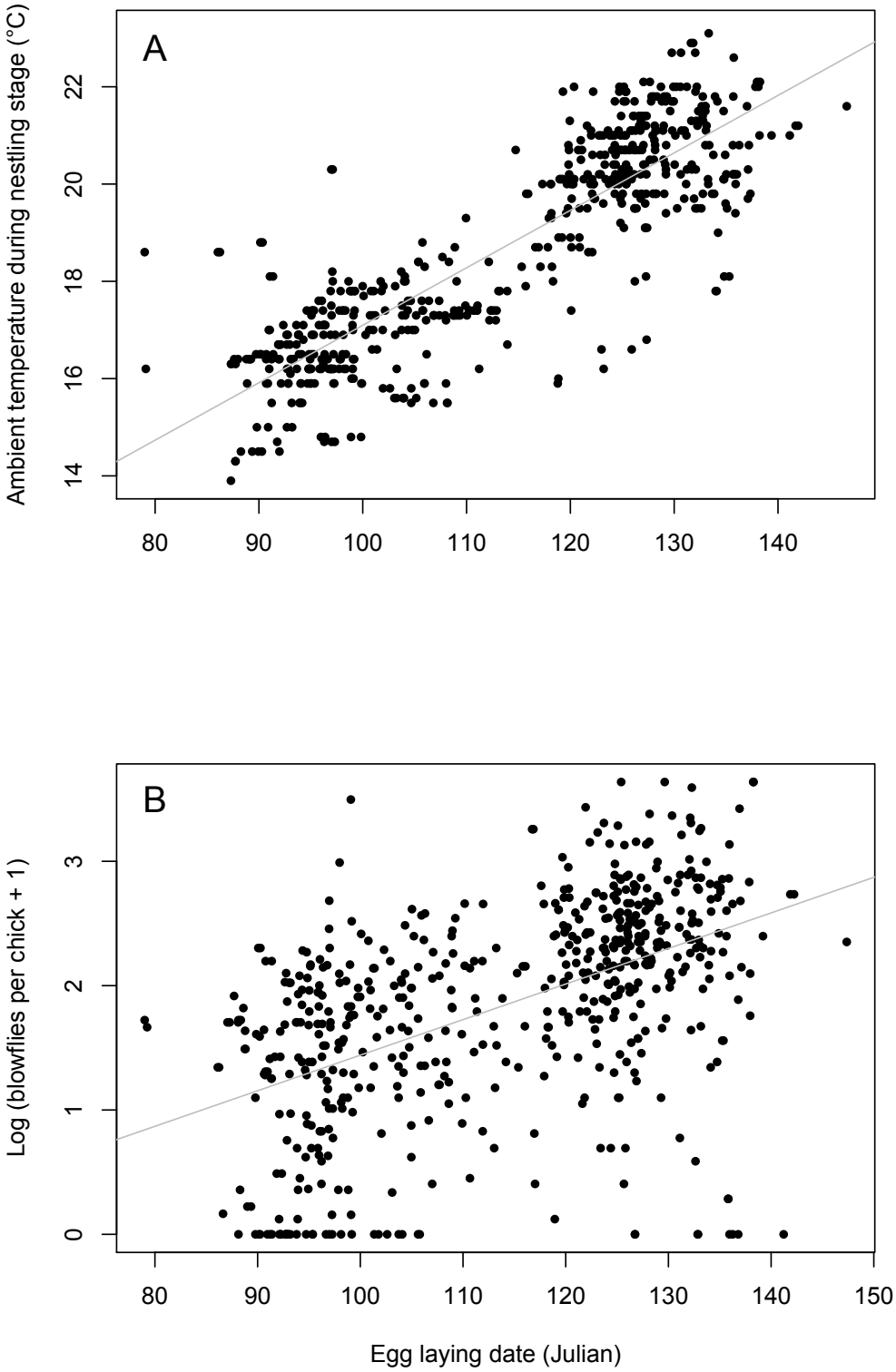


Figure S2. Both average ambient temperature (A) and parasite load (B) during the nestling stage increase with egg-laying date.