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Effects of Experimental Variation in Temperature on Larval Densities of Parasitic *Protocalliphora* (Diptera: Calliphoridae) in Nests of Tree Swallows (Passeriformes: Hirundinidae)

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ABSTRACT Larval *Protocalliphora* Hough are obligate hematophagous parasites of nestling birds. While there is much interest in their effects on birds, little is known about the factors influencing their population sizes within nests. We experimentally increased temperature at nests of tree swallows (*Tachycineta bicolor* Vieillot) on two study areas and compared densities of larval *Protocalliphora* with control nests. We found that number of *Protocalliphora* varied in a curvilinear fashion with temperature, with population sizes highest in nests around 25°C and decreasing at both higher and lower temperatures. After controlling for temperature, we found differences between study areas in number of *Protocalliphora* in nests, and we hypothesize that these results are caused by differences in the size and dispersion of nests between the two areas. There was no seasonal variation in number of *Protocalliphora* in nests, but numbers increased significantly with the number of hosts in a nest, presumably because more resources were available for exploitation as host brood size increased. The cell-mediated immune responses of nestling tree swallows was positively correlated with number of *Protocalliphora*, and we suggest that these results are the consequence of the immune systems of nestlings in heavily parasitized nests being more sensitive to challenges by novel antigens or mitogens. To our knowledge, our study is the first to show variation in densities of larval *Protocalliphora* as a consequence of experimental manipulation of temperature and provides insight into important factors affecting population sizes of parasites in avian nests.

KEY WORDS population density, *Protocalliphora*, tree swallows, *Tachycineta bicolor*

BLOW FLIES OF THE genus *Protocalliphora* Hough (Diptera: Calliphoridae) are a holarctic group of species found commonly in the boreal forest and other areas (Sabrosky et al. 1989). Adult flies are thought to feed on natural sugars such as nectar, whereas larval stages are obligate hematophagous parasites of nidicolous birds (Bennett and Whitworth 1991). Adult flies oviposit in nests or directly on birds soon after hosts hatch. Blow fly eggs hatch within 24–48 h, and the larvae pass through three instars, taking a number of blood meals from their avian hosts. The duration of these feeding stages ranges from 7 to 15 d and is followed by a short nonfeeding prepupal stage of 1–4 d (Sabrosky et al. 1989). The pupal stage can range from 9 to 36 d depending on ambient temperature, and it is thought that only adults overwinter (Sabrosky et al. 1989).

In the past two decades, there has been a considerable amount of interest in the effects of parasites on host behavior and fitness, and a number of studies have

shown that infestations by *Protocalliphora* can be deleterious for host birds, e.g., (Pinkowski 1977, Roby et al. 1992, Whitworth and Bennett 1992, Merino and Potti 1995, Hurtrez-Boussès et al. 1997a, b). While the studies of Bennett (1957) and Whitworth (1971, 1976) have provided much of our knowledge of the basic biology of *Protocalliphora* (see also Sabrosky et al. 1989, Bennett and Whitworth 1991, 1992, Whitworth and Bennett 1992), we know relatively little about the life history and population dynamics of any species of *Protocalliphora*. Most manipulative studies by ornithologists have been concerned with reducing or increasing levels of infestation and documenting the subsequent consequences for host birds. However, the factors that determine natural levels of infestation by *Protocalliphora* are essentially unknown.

Insects are poikilotherms and, therefore, ambient temperature is the most important factor influencing their growth and development (Sharpe and Demichele 1977). For most species, there is probably an optimal temperature where development proceeds quickly with minimal mortality; as ambient temperature deviates away from optimal, rates of growth and development are reduced, whereas mortality may increase (Sehnal 1991). In addition, development and survival of insects in temperate areas may be affected

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by daily cycles of temperature variation that correspond closely with photoperiod. While some species of insects develop rapidly under conditions with a thermoperiod, others perform better at constant temperatures (references in Beck 1991). Within the genus *Protocalliphora*, it is known that time spent in both the prepupal and pupal stages is reduced as ambient temperatures increase (Hori et al. 1990, Bennett and Whitworth 1991); extremely high temperature causes deformities or is lethal. Many authors have speculated that levels of infestation are reduced during years with cool and wet weather during the host's breeding season (Wittmann and Beason 1992, Merino and Potti 1996, Hurtrez-Boussès et al. 1999, Germaine and Germaine 2002). In addition, Hurtrez-Boussès et al. (1999) hypothesized that high levels of infestation of Corsican blue tits (*Parus caeruleus* Linnaeus) was caused by high ambient temperatures, whereas Whitworth (1976) showed that cliff swallow (*Petrochelidon pyrrhonota* Vieillot) nests that contained many feathers could maintain higher levels of infestation, presumably because these nest were warmer. Nonetheless, to our knowledge, no experimental studies have directly manipulated temperature and examined its effects on levels of infestation by *Protocalliphora*. Here, we show how manipulating the microclimate of nest sites of tree swallows (*Tachycineta bicolor* Vieillot) subsequently influences levels of parasitism. We test how numbers of *Protocalliphora* vary with both mean temperature of nests sites, as well as the degree of variation in nest temperature. We also test whether levels of infestation are affected by the number of hosts in individual nests, their ability to mount an immune response, and timing of breeding by hosts.

Materials and Methods

We studied tree swallows breeding in artificial nest boxes from May to August 2003 in the vicinity of Prince George, British Columbia, Canada (53° N, 123° W), on two different study areas. The two areas (Steward and Hydro sites) were ≈35 km apart and both consisted of open agricultural areas mixed with small patches of coniferous and deciduous trees and small wetlands. Tree swallows arrived on both sites in early to mid-May and began laying eggs in late May and early June. At the Steward site, nests boxes with a floor area of 230 cm² were mounted ≈30 m apart on fence posts. At the Hydro site, boxes (floor area of 475 cm²) were mounted on electrical poles ≈800 m apart.

Beginning in mid-May, we visited nest boxes every 1–3 d, which allowed us to determine clutch initiation date, clutch size, and hatching date of swallows. Nests were paired according to hatching date and brood size, and each member of the pair was randomly allocated to either the treatment or control group. At treatment nests, we placed a small air-activated heating pad underneath the nesting material. These pads contained a mixture of charcoal, iron powder, vermiculite, salt, sawdust, and moisture, and when exposed to the air, produced elevated temperatures for 2 d because of the oxidation of the iron powder. Heating

pads were replaced with new ones every 2 d so that nests in the treatment group had elevated temperatures when young were between 4 and 16 d old. Control nests were visited at the same frequency, but we installed pads in which all iron powder had already been oxidized; therefore, no heat was produced. At a subsample of nests ($n = 32$), we used data loggers to document the temperature of the nest directly above the heat pads but below the nesting material. Temperature readings were recorded every 2 min during the period when nestlings were between 4 and 16 d old. We subsequently calculated the mean temperature, as well as the average daily coefficient of variation in temperature, for each nest. When nestlings were 12 d old, we assessed their T cell-mediated immune response (CMI) by injecting 30 μl of 1 mg/ml phytohaemagglutinin-P (Sigma, St. Louis, MO) in phosphate-buffered saline intradermally in the right wing web. We measured the thickness of the wing web with a thickness gauge (nearest 0.01 mm) four times just before and 24 h (± 1.5 min SE) after injection, and the means were subsequently used in analyses. CMI was the difference in thickness of the wing web before and after injection of the mitogen (Smits et al. 1999, 2001).

Tree swallows fledge between 18 and 22 d of age, so we visited nests at 23–24 d after hatch to collect nests. Nesting material was carefully removed from each box and stored in bags at room temperature for 14 d. The nesting material was then carefully teased apart to remove all adult, puparia, and pupae of *Protocalliphora* spp. We quantified *Protocalliphora* only at nests where young survived to at least 12 d old. It is unlikely that larvae would have survived in nests that failed before 12 d; therefore, including these nests in analyses would potentially bias estimates of prevalence and intensity. Most nest failure was caused by predation and inclement weather. We quantified levels of infestation by *Protocalliphora* at 20 tree swallow nests where temperatures were experimentally raised (10 at each site) and at 17 control nests (9 at the Hydro site and 8 at the Steward site).

To investigate whether manipulating microclimate of nests had consequences for parasite load, we first tested whether our treatment had the desired effect. We used analysis of variance (ANOVA) to determine if the mean temperature of the heated boxes differed significantly from control boxes and whether the temperature of heated boxes differed between areas (Steward versus Hydro sites). For our analyses of population sizes of *Protocalliphora*, we used the total number of puparia and pupae recovered from each nest as our dependent variable in analyses, because this would represent the minimum number of individual *Protocalliphora* in nests. To meet assumptions of normality, we transformed these data ($\ln + 1$) before analysis. To examine relationships between numbers of *Protocalliphora* in nests and temperature variables, we used a combination of Pearson's correlation analysis and nonlinear regression. From this latter analysis, we calculated residuals and used these in analysis of covariance (ANCOVA) to test how numbers of *Pro-*

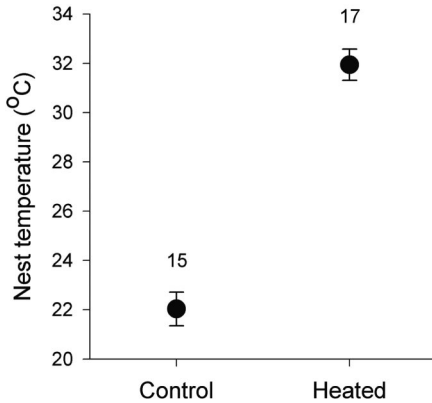


Fig. 1. Differences in temperature of nests of tree swallows that were experimentally heated and control nests.

protocalliphora varied between areas as well as with brood size and hatching date (see Results). Area was used as a main effect, whereas hatching date and brood size were used as covariates. To study whether the ability of nestlings to mount an immune response could influence population sizes of *Protocalliphora*, we used Pearson’s correlation to test for relationships between CMI and number of *Protocalliphora* corrected for temperature. Statistical analyses were performed using SPSS (Norusis 2000). All tests were two-tailed, and results were considered significant at the $P < 0.05$ level. Values are presented as means \pm SE.

Results

Prevalence of infestation by *Protocalliphora* was high in our study, with *Protocalliphora* being detected in 33 of the 37 nests (89.2%). Among infested nests, the average number of *Protocalliphora* was 50.1 ± 8.6 (range, 1–178). We detected *P. bennetti* Whitworth, *P. braueri* Hendel, *P. occidentalis* Whitworth, and *P. rugosa* Whitworth in a sample of 30 nests where species were identified. *P. rugosa* was the most commonly occurring species (25/30 nests), and *P. braueri* was found in only a single nest, whereas prevalence of the other two species was intermediate (*P. bennetti* in 12/30 nests, *P. occidentalis* in 15/30 nests). Multispecies infestations were common, with 17 nests having two species of *Protocalliphora*, and three nests harbored three species.

Installation of heating pads in treatment nests significantly raised temperatures of the nests ($F = 114.03$; $df = 1,28$; $P < 0.0001$; Fig. 1), and this effect was consistent between areas; there were no differences in nest temperature between the Hydro and Steward sites ($F = 2.20$; $df = 1,28$; $P = 0.15$), nor was the interaction significant ($F = 0.10$; $df = 1,28$; $P = 0.76$). Despite this disparity in temperature, our initial analyses showed no significant differences in number of *Protocalliphora* between heated and control nests ($F = 1.20$; $df = 1,35$; $P = 0.28$), nor was there any correlation between number of *Protocalliphora* and average nest temperatures ($r = -0.08$, $n = 32$, $P = 0.67$). However,

Table 1. Results from a quadratic regression ($F = 5.24$; $df = 2,29$; $P = 0.01$) between mean nest temperature and number of *Protocalliphora* in nests of tree swallows

Variable	β	SE	T	P
Temperature	1.413	0.450	3.14	0.0038
Temperature ²	-1.028	0.009	-3.20	0.0033
Constant	-14.123	5.719	-2.47	0.0197

when we analyzed data separately for treatment and control nests, there was some suggestion that number of *Protocalliphora* declined with temperature at heated nests ($r = -0.54$, $n = 17$, $P = 0.03$) and increased with temperature at control nests ($r = 0.42$, $n = 15$, $P = 0.11$), although these latter results were not significant. Nonetheless, these results suggested that the relationship between number of *Protocalliphora* and average nest temperature was curvilinear, and a quadratic equation fit to these data confirmed such a relationship (Table 1; Fig. 2). There was a significant negative correlation between the average temperature of a nest and the coefficient of variation ($r = -0.81$, $n = 32$, $P < 0.0001$), suggesting that one consequence of heating nests boxes is that the degree of variation in temperatures was reduced, probably because nest temperature at heated nests did not decline during the night to the same extent as control nests. Regardless, the degree of variation in temperature in nests did not seem to be important in determining levels of infestation, because we could not find any significant relationship, linear or curvilinear, between the coefficient of variation for temperature and number of *Protocalliphora* (all $P > 0.35$).

To examine how other factors may have affected the number of *Protocalliphora*, we used the residuals from the quadratic regression with mean temperature. After controlling for the effects of temperature, ANCOVA suggested that infestations were heavier on the Hydro site (67.5 ± 12.4 *Protocalliphora* per nest) than the Steward site (20.6 ± 6.7 ; $F = 4.02$; $df = 1,24$; $P =$

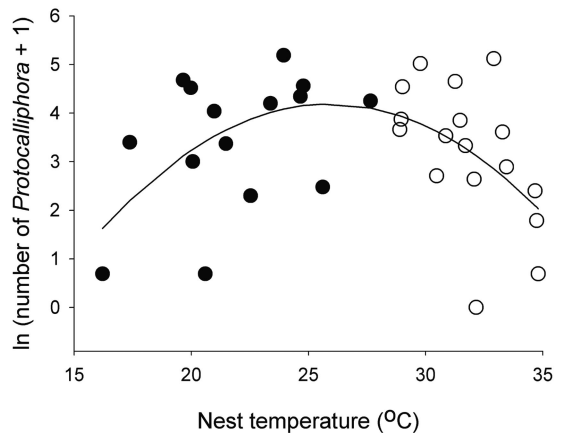


Fig. 2. Relationship between number of *Protocalliphora* infesting nests of tree swallows and nest temperature. ●, control nests; ○, heated nests. Note that number of *Protocalliphora* is plotted on a logarithmic scale.

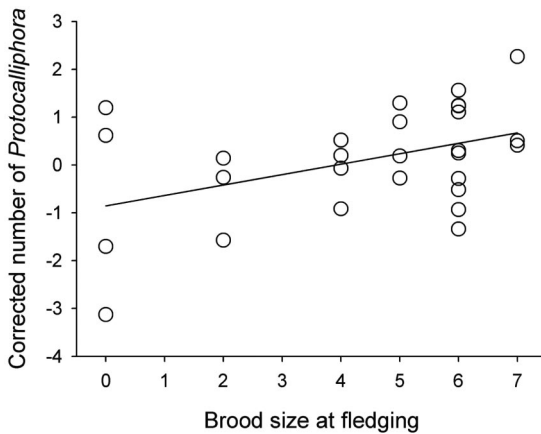


Fig. 3. Increase in number of *Protocalliphora* with increasing brood size of tree swallows. Number of *Protocalliphora* is corrected for temperature (see Results).

0.05) and that numbers of *Protocalliphora* increased with brood size ($F = 4.07$; $df = 1,24$; $P = 0.05$; Fig. 3), but there was no effect of hatching date on infestation levels ($F = 0.26$, $df = 1,24$; $P = 0.62$). Finally, we found a significant positive correlation between cell-mediated immune responses of nestling tree swallows and number of *Protocalliphora* ($r = 0.38$, $n = 29$, $P = 0.04$; Fig. 4).

Discussion

Our results show that populations of *Protocalliphora* in nests of tree swallows varied in a curvilinear fashion with nest temperature (Fig. 2). To our knowledge, our study is the first to report the effects of experimental variation in temperature on infestation rates by *Protocalliphora* in free-living birds. Bennett and Whitworth (1991) reported nest temperatures of 39–40°C in a number of bird species in Algonquin Park, On-

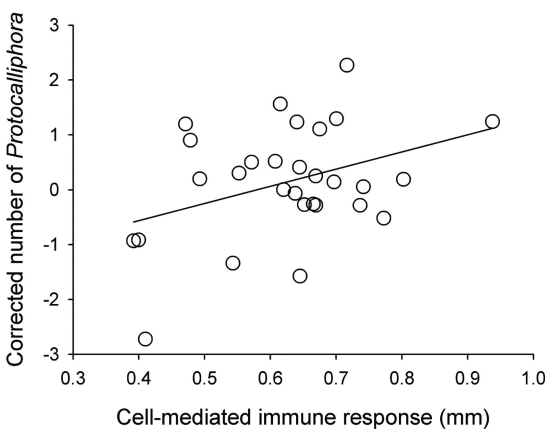


Fig. 4. Relationship between number of *Protocalliphora* in nests and cell-mediated immune response of nestling tree swallows. Number of *Protocalliphora* is corrected for temperature (see Results).

tario, and these values clearly exceed what we report here (Fig. 1). However, we suspect that temperatures were measured directly beneath the nestlings and so may have been measuring the body temperatures of the hosts as opposed to temperature of the nesting substrate. Indeed, larvae spend part of their life cycle attached to nestlings and experience these elevated temperatures regularly. However, because they are generally intermittent feeders, migrating into the nesting material when not actively feeding (Bennett and Whitworth 1991), they are probably not exposed to such elevated temperatures continuously.

Levels of infestation generally increased with temperature in control nests, and declined in heated nests, with the highest levels of infestation occurring in nests with a temperature of $\approx 25^{\circ}\text{C}$ (Fig. 2). While our study cannot distinguish whether infestations were high at this temperature because adult flies oviposited more in these nests or whether larval survival was higher, results from other studies suggest the latter may be responsible. While heated nests in our study were warmer than control nests (Fig. 1), Gold and Dahlsten (1989) thought that adult female *Protocalliphora* locate host nests by odor and not by heat or CO_2 emission. Similarly, adult females apparently begin laying eggs in host nests as soon as host eggs have hatched (Gold and Dahlsten 1989), and we did not begin manipulating microclimate until chicks were 4 d old, by which time eggs had probably already been laid by female flies. While more research is needed to elucidate the mechanism(s) by which female *Protocalliphora* locate hosts, some authors have suggested that the probability of infestation is relatively consistent among nests within a particular area (Pinkowski 1977). Reduced levels of infestation at relatively low temperatures is probably a simple consequence of reductions in growth and/or delays in morphogenesis (Sehnal 1991). It may be that many first- or second-instar larvae that were present in nests with low temperatures were unable to complete the transition to third instar and subsequently to puparia. Temperatures higher than optimal are known to cause disruptions in enzymatic activities of insects, such as halting normal protein synthesis (Denlinger et al. 1991). Temperature may also have profound influences on the predators and parasites of insects (Thomas and Blanford 2003).

We also showed that there were significant differences between our two study areas in number of *Protocalliphora* in nests, with the Hydro study area having infestations over three times as intense as those on the Steward site. Although the two areas are in close proximity and have similar habitat, there are a number of differences between the areas that may explain our results. First, nesting boxes at the Hydro site were larger than those at the Steward site. Gold and Dahlsten (1989) found that the number of *Protocalliphora* increased in nests of two species of chickadees as volume of nesting material increased. Whitworth (1976) suggested that larger nests would have less competition for resting space and could harbor larger numbers of individual larvae. In addition, larvae

would be less exposed to the toxic effects of their own and the hosts' feces in larger nests. Some species of adult birds will also eat larvae that infest their nests (Hori et al. 1990), and so larger nests would provide larvae with more hiding spaces from probing adults (Gold and Dahlsten 1989). Second, because nests were far more dispersed at the Hydro site, the number of potential hosts on this area would be much less than the Steward site, and each nest may have received eggs from multiple female *Protocalliphora*. Larger numbers of *Protocalliphora* on the Hydro site may also be unrelated to size and dispersion of nest boxes; populations of gravid females may simply have been inherently larger at the Hydro site.

Previous studies have shown that number of *Protocalliphora* in nests can vary positively (Pinkowski 1977, Wittmann and Beason 1992, Merino and Potti 1995), negatively (Hori and Iwasa 1988), or not at all (Bortolotti 1985, Hurtrez-Boussès et al. 1999, Wesolowski 2001) with the timing of host breeding during the season. After controlling for effects of nest temperature, we could find no relationship between intensity of infestations and hatching dates of tree swallows in our study, but we did find that intensity increased with brood size (Fig. 3). Many other studies have reported similar relationships with brood size (e.g., Pinkowski 1977, Roby et al. 1992, Hurtrez-Boussès et al. 1999, Wesolowski 2001, Dawson 2004). It seems likely that increased levels of parasitism with number of hosts is related simply to there being more resources available for exploitation in nests with larger broods. Finally, we showed that the cell-mediated immune response of nestlings was positively correlated with the number of *Protocalliphora* in nests (Fig. 4). Clearly, a robust immune response by hosts is unlikely to enhance populations of *Protocalliphora*. Instead, we suggest that because of the continual damage to their integument and the possibility of infection by bacteria during regular blood meals taken by larvae (see Warren 1994), the immune systems of nestlings in heavily parasitized nests were activated and more sensitive to challenges by novel antigens or mitogens, such as phytohaemagglutinin.

In conclusion, our study provides the first experimental data for the effects of variation in host nest temperature on infestation rates by *Protocalliphora* in a population of wild birds. More work needs to be done on how rates of larval growth, emergence of adults, and the subsequent reproductive performance of adult flies are affected by temperature. There is increasing interest in the effects of temperature on insects (e.g., Clarke 2003, Sinclair et al. 2003, Thomas and Blanford 2003) and in how ecological systems will respond to possible changes in climate that are currently being projected. A number of studies have used long-term data sets to show significant changes in laying date of birds in response to changes in climate (Crick et al. 1997, Brown et al. 1999, Dunn and Winkler 1999, Visser et al. 2003, but see Hussell 2003); however, the effects of increased temperatures on other aspects of avian life histories, and their parasites, are generally unknown. Bennett and Whitworth (1991) have shown

that adult *Protocalliphora* become more active at higher temperatures, and this study provides evidence that nest temperatures in the range of 25°C is optimal for heavy infestations in tree swallows (Fig. 2). Climate change may therefore ultimately affect a range of life history traits of birds, including infestations by ectoparasites such as *Protocalliphora*.

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