Integrative and Comparative Biology

Integrative and Comparative Biology, pp. 1–11
doi:10.1093/icb/icz031

Society for Integrative and Comparative Biology

SYMPOSIUM

Infection Outcomes are Robust to Thermal Variability in a Bumble Bee Host–Parasite System

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From the symposium "The world is not flat: Accounting for the dynamic nature of the environment as we move beyond static experimental manipulations" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2019 at Tampa, Florida.

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Synopsis Climate change-related increases in thermal variability and rapid temperature shifts will affect organisms in multiple ways, including imposing physiological stress. Furthermore, the effects of temperature may alter the outcome of biotic interactions, such as those with pathogens and parasites. In the context of host–parasite interactions, the beneficial acclimation hypothesis posits that shifts away from acclimation or optimum performance temperatures will impose physiological stress on hosts and will affect their ability to resist parasite infection. We investigated the beneficial acclimation hypothesis in a bumble bee–trypanosome parasite system. Freshly emerged adult worker bumble bees, *Bombus impatiens*, were acclimated to 21, 26, or 29°C. They were subsequently experimentally exposed to the parasite, *Crithidia bombi*, and placed in a performance temperature that was the same as the acclimation temperature (constant) or one of the other temperatures (mismatched). Prevalence of parasite transmission was checked 4 and 6 days post-parasite exposure, and infection intensity in the gut was quantified at 8 days post-exposure. Parasite strain, host colony, and host size had significant effects on transmission prevalence and infection load. However, neither transmission nor infection intensity were significantly different between constant and mismatched thermal regimes. Furthermore, acclimation temperature, performance temperature, and the interaction of acclimation and performance temperatures had no significant effects on infection outcomes. These results, counter to predictions of the beneficial acclimation hypothesis, suggest that infection outcomes in this host–parasite system are robust to thermal variation within typically experienced ranges. This could be a consequence of adaptation to commonly experienced natural thermal regimes or a result of individual and colony level heterothermy in bumble bees. However, thermal variability may still have a detrimental effect on more sensitive stages or species, or when extreme climatic events push temperatures outside of the normally experienced range.

Introduction

Ongoing climate change manifests in a variety of ways (Easterling et al. 2000), including changes in thermal variability. Significant shifts in temperatures are predicted to become more frequent, of greater amplitude, and more rapid. Thus, organisms will be challenged by a more frequently fluctuating thermal environment and will be more likely to encounter sub-optimal conditions (Parmesan 2006; Vasseur et al. 2014). The thermal environment can dramatically influence physiology and behavior (Vogt 1986; Weidenmüller et al. 2002; Seidl et al. 2005; Pörtner and Farrell 2008; Woodard 2017), and the rate at which temperature shifts occur can influence critical limits of organisms (Oyen and Dillon 2018). Greater thermal variability, in terms of both amplitude and frequency, will increase the probability that an individual experiences significant thermal shifts within their lifetime. Shifts between past acclimation and current performance temperatures, independent of direction, may reduce individual function and ultimately fitness, with outcomes determined by the extent of plasticity in thermal physiology (Gunderson et al. 2017). There is evidence for

intra- and inter-specific variation in the plasticity to cope with these thermal challenges (Nowakowski et al. 2018).

In addition to potentially decreased general performance, temperature changes can disrupt biotic interactions between organisms, particularly when the interacting organisms differ in their abilities to thermally acclimate (Rohr et al. 2018). There may be consequences for disease transmission dynamics and host–parasite interactions that could alter infection outcomes, and host and parasite fitness (Thomas and Blanford 2003; Altizer et al. 2013; Elderd and Reilly 2014; Sternberg and Thomas 2014). Additionally, infection by parasites may influence a host's thermal tolerance and thereby affect its susceptibility to shifts in temperature/climate (Greenspan et al. 2017). Climate warming may not always be detrimental to hosts if the risk of parasitism is reduced (Gehman et al. 2018), and the effects of temperature shifts on the outcome of infection may be difficult to predict due to effects on both host and parasite biology (Roberts et al. 2018). However, the predominant view is that increased thermal variability and elevated temperatures will negatively affect hosts when interacting with parasites (Cohen et al. 2017; Nowakowski et al. 2018; Rohr et al. 2018).

Rapid fluctuations that shift hosts away from temperatures to which they have been acclimated have been identified as a potential driver of changes in host susceptibility (Fedorka et al. 2016; Cohen et al. 2017). When temperature changes occur for both hosts and parasites, differences in their abilities to acclimate or adapt will be important. For example, it is predicted that broader thermal limits of parasites or faster acclimation or adaptation, due to larger population sizes and shorter generation times, will lead to detrimental consequences for hosts (Cohen et al. 2017). Furthermore, it has been suggested that temperature shifts, independent of direction, have the potential to influence the outcome of infection (Raffel et al. 2013; Altman et al. 2016). Specifically, thermal acclimation responses and the consequences of energetic stresses imposed on hosts by thermal shifts may influence physiological performance and resistance to infection, or conversely parasite infectivity (Paull et al. 2015; Altman et al. 2016). There are multiple hypotheses for how mean temperature and temperature variation may influence organismal performance and consequently infection outcomes (Altman et al. 2016). Here, we focus on the *beneficial acclimation hypothesis* (Leroi et al. 1994; Altman et al. 2016). In the case of infection, the beneficial acclimation hypothesis proposes that a host individual acclimated to a certain temperature will be more resistant to infection (increased performance) at that temperature, relative to individuals acclimated to other temperatures.

Ectothermic or facultatively endothermic heterotherms may be less able to buffer against temperature changes resulting from thermal variability (Deutsch et al. 2008). Yet, with the exception of amphibian-disease systems (Rohr et al. 2004; Rohr and Palmer 2013; Raffel et al. 2015), studies of how host organisms in these categories, including insects, fare in response to the multiple ecological stresses of temperature shifts and infectious disease have been lacking (Kaunisto et al. 2016). The study of thermal shifts and consequences for infection is particularly pertinent for temperate organisms (Vasseur et al. 2014), including those that provide vital ecosystem services, such as bee pollinators. Several bumble bee species are in decline worldwide (Cameron et al. 2011; Brown et al. 2016; IUCN 2018), with bees threatened by exposure to multiple risk factors including climate change, habitat loss, agro-chemical exposure, and infectious diseases (Potts et al. 2010; Vanbergen and the Insect Pollinators Initiative 2013; Goulson et al. 2015). Interactions between these stressors are likely to generate greater than additive detrimental effects on individuals and populations (Vanbergen and the Insect Pollinators Initiative 2013). Under the beneficial acclimation hypothesis, variability in the thermal environment resulting from ongoing climate change could have detrimental consequences for bees infected with pathogenic parasites.

Climate change has been suggested to affect bumble bee populations through southern range contractions or shifts in elevation and the failure to track warming at northern limits (Kerr et al. 2015). Climate change linked temperature changes can affect floral availability for queens emerging from hibernation, with a potential consequence of decoupled mutualisms (Miller-Struttmann et al. 2015). Also, shifts in the thermal environment may directly influence the physiology of generally cold-adapted bumble bees, with susceptibility to these changes plausibly being species or caste specific (Woodard 2017). The thermal physiology of heterothermic bumble bees is a fascinating and important avenue of further study (Oyen et al. 2016). Bumble bees can regulate temperature on both the individual and colony level, such as the increasing of thoracic temperature to enhance foraging (Heinrich 1972, 1975, 1976) and fanning to cool developing brood at high temperatures (Heinrich 1974; Weidenmüller et al. 2002). Despite these impressive

thermoregulatory capacities, bumble bees will still experience high and low temperatures under normal conditions (Heinrich 1976). Furthermore, their regulatory abilities are constrained and, even when they are efficacious, they will likely impose both behavioral and physiological costs (Heinrich 1972; Vogt 1986). This means that when bumble bees are faced with temperature extremes and fluctuations away from acclimated temperatures, there are likely to be tradeoffs between energy invested in regulating temperature and other traits, which may include immunity and defenses against parasites and disease.

Bumble bees and their well-studied gut-infecting trypanosome parasite *Crithidia bombi* (Sadd and Barribeau 2013) offer an excellent opportunity to test the beneficial acclimation hypothesis, and study how thermal shifts influence host–parasite interactions. *Crithidia bombi* is transmitted via feces within colonies or between colonies during foraging events (Durrer and Schmid-Hempel 1994; Otterstatter and Thomson 2007). Infection has a number of documented effects, including reductions of foraging ability (Otterstatter and Thomson 2006; Gegear et al. 2007), worker longevity (Brown et al. 2000), queen hibernation (Fauser et al. 2017), colony foundation (Brown et al. 2003), and colony fitness (Brown et al. 2003; Yourth et al. 2008). Of interest for the study of infection by this parasite against a backdrop of environmental variation is that infection and virulence can be context dependent (Brown et al. 2000, 2003; Logan et al. 2005). Although there are strong host and parasite genetic components governing infection dynamics (Barribeau et al. 2014), simple environmental changes, such as nutrition, can alter these infection outcomes (Sadd 2011). However, there have been no studies directly assessing how shifts in the thermal environment may influence infection outcomes.

The objective of this study is to understand how temperature shifts imposed on the bumble bee host influence interactions with the gut parasite *C. bombi*, particularly relating to parasite transmission and host resistance. The overarching beneficial acclimation hypothesis proposes that shifts away from acclimation or optimum performance temperatures will impose physiological stress on hosts and will affect their ability to resist parasite infection (Altman et al. 2016). Specifically, we predict that bees acclimated to one temperature, experimentally exposed to *C. bombi*, and then immediately shifted to a distinct temperature for performance will have higher transmission prevalence and infection intensities than bees that are exposed and returned to their acclimation temperature.

Materials and methods

General bumble bee and *Crithidia* maintenance

Four commercial bumble bee colonies (*Bombus impatiens*) sourced from Koppert Biological Systems (Koppert Biological Systems, Howell, MI, USA) were transferred to custom observation hives (Pomeroy and Plowright 1980) and maintained in the laboratory at 26 ± 1.5°C under red light illumination. Original queens and a random subset of workers were screened for common gut parasites, and all colonies were deemed parasite-free. Colonies were provided with honey bee-collected pollen (Brushy Mountain Bee Farm, NC, USA) three times a week and sugar water (1 g cane sugar:1 mL boiled tap water with 0.1% cream of tartar to promote sucrose hydrolysis) *ad libitum*. Newly-emerged, callow worker adult bees were isolated from these colonies and held individually with sugar water provided *ad libitum* after being placed into the experimental thermal regime treatments (see below).

Two strains of *C. bombi* previously isolated from wild bumble bee populations were used. Strain AK 08.052 (lab specific ID) was isolated from Alaska in 2008 and is hereafter referred to as strain AK. Strain IL 16.075 was isolated in Central Illinois in 2016 and is hereafter referred to as strain IL. These strains were derived from single parasite cells, confirmed as *C. bombi*, and are maintained in a frozen strain bank at −80°C, following previous methods (Salathé et al. 2012). In order to have viable *C. bombi* cells available for experimental exposures, strain stocks were thawed weekly to inoculate fresh FP-FB media and cultured at 27°C and 3% CO_2 (Salathé et al. 2012).

Thermal regimes and experimental parasite exposures

Isolated adult worker bees were allowed to acclimate for 1 week following emergence in their individual holding containers at one of three temperature treatments (21, 26, or 29°C). The 21 and 29°C temperature treatments were administered via incubators, while the 26°C treatment bees were kept in the main colony room. Other conditions of relative humidity (40–50%) and lighting were identical between incubators and the main colony room. Accuracy of the administered temperatures was confirmed by checking thermal traces from ibutton dataloggers (Maxim Integrated, San Jose, CA, USA). The range of 21–29°C was chosen because these values are near the lower and upper ends of average experienced summer temperatures for bees in Central Illinois based on the average daily maximum and minimum

temperatures in July as reported by the Illinois State Water Survey.

After 7 days at the acclimation temperature, bees were experimentally exposed to *C. bombi*. *Crithidia bombi* cell densities of *in vitro* cultures (3–4 days following their initiation) were quantified using Fast-Read 102® chambers (Immune Systems, UK) and then, immediately before experimental parasite exposures took place, diluted with sugar water to give a final concentration of 10,000 cells/10 µL of sugar water solution. Before exposure, bees were isolated in vials for 2–3 h without sugar water, and then presented with a 10 µL inoculum of either the AK or IL strain of *C. bombi*. Bees were observed until they extended their proboscis into the inoculum, and consumption was considered complete once the inoculum was no longer visible. Any bees that did not consume the inoculum within 30 min were removed from the experiment. After inoculation was confirmed visually, bees were returned to a box with sugar water provided *ad libitum* and placed back at either their acclimation temperature ("constant") or at one of the other temperature treatments ("mismatched"). This gave nine combinations of acclimation and performance temperatures (Fig. 1).

Checks for transmitting parasite cells and quantification of infection intensity

Fecal samples were collected from individuals 4 and 6 days post-parasite exposure, and the presence or absence of transmitting *C. bombi* cells in the feces was determined with a phase contrast microscope at 400× magnification. Eight days post-exposure, individual bees were frozen and stored at −20°C. Individuals were later thawed, their guts dissected and homogenized in 100 µL of ringer saline solution and stored at −20°C until DNA extraction. For all bees, forewings were removed, and the radial cell length was measured as a proxy for body size (Müller et al. 1996; Schmid-Hempel and Schmid-Hempel 1996). DNA extraction was performed on homogenized guts using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. DNA sample quality was verified using a µDrop plate in a MultiSkan GO plate reader (ThermoFisher, Waltham, MA, USA). Infection intensity was quantified using qPCR (Ulrich et al. 2011) using a QuantStudio3 Real-Time PCR Machine (ThermoFisher). Parasite infection intensity, based on cell number derived from a standard curve of DNA extracted from known *Crithidia* cell numbers, was normalized to the relative copies of the *B. impatiens*5 C-actin gene to account for differential DNA extraction efficiencies between samples (Palmer-Young et al. 2018). Each DNA sample was run in duplicate, and any duplicates that had a calculated coefficient of variation above 0.20 were rerun and averaged across replicates after omitting any outlier values (according to Palmer-Young et al. 2018).

Statistical analyses

All analyses were performed in R 3.5.0 for Mac OS X (R Core Team 2018). All maximal models included body size (as determined by wing radial cell measurements), parasite strain (AK or IL), and host colony (A, B, C, or D) as main effects, and the interaction between parasite strain and host colony. In addition, one set of models testing for effects of specific acclimation and performance temperatures or their combination included these thermal environment terms and their interaction. A further set of models, testing for the effect of a mismatch between acclimation and performance temperatures, combined acclimation and performance temperatures into a single variable and coded them as constant (same temperatures) or mismatched (different temperatures). Transmission 4 and 6 days post-parasite exposure, as evidenced by shedding of parasite cells in the feces, was analyzed with generalized linear models with a binomial error structure and a logit-link function using the *lme4* package (Bates et al. 2015). On day 4 post-parasite exposure, 160 bees gave feces samples that were screened for *C. bombi* presence, while on day 6 post-exposure 163 bees gave feces samples for screening. An approach with a separate model for both days was favored over a single model including day as a main effect and individual identity as a random effect due to model convergence problems with the latter, as a consequence of independent random subsets of bees not giving feces samples on a given day. DNA was extracted from the guts of 193 bees 8 days after parasite exposure. Standardized infection intensities were obtained for 191 bees after two bees with low quality DNA measurements were removed. Sample numbers, in parentheses, were distributed as follows across acclimation/performance temperatures: 21/21°C (23), 21/26°C (24), 21/29°C (22), 26/21°C (22), 26/26°C (21), 26/29°C (20), 29/21°C (22), 29/26°C (21), and 29/29°C (16). Standardized infection intensities of these samples were log-transformed ($\log[y+1]$) to meet model assumptions and fitted with a linear model. This approach was taken because model diagnostics showed that

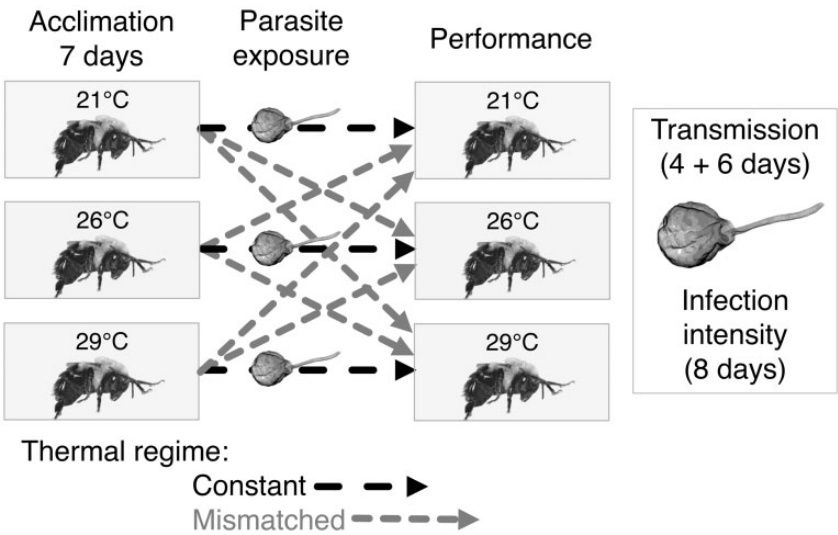

Fig. 1 Schematic representation of beneficial acclimation methods. Age controlled workers were acclimated to one of three acclimation temperatures for 7 days, after which they were exposed to one of two strains of *Crithidia bombi*. After exposure, bees were either returned to the temperature they were acclimated to (long dash black arrows, constant), or assigned to one of the two other performance temperatures (short dash gray arrows, mismatched). (Bee image by Ben Sadd and *C. bombi* SEM image by ETH Zurich, Boris Baer.)

generalized linear models with either negative binomial or quasi-poisson error structures produced poor fits. Maximal models were simplified by sequentially eliminating non-significant terms through likelihood ratio tests, and nested models were compared and selected using AICc (Burnham and Anderson 2002). The package *emmeans* (Lenth 2018) was used to calculate estimated marginal means and their confidence intervals for levels of model terms.

Results

Proportion of bees transmitting parasite cells in the feces

As expected, a greater proportion of bumble bees had *Crithidia* cells in the feces 6 days post-experimental exposure (0.57) compared with 4 days post-exposure (0.31). However, there was no significant effect of acclimation temperature, performance temperature, or the interaction between them on the probability of a bee shedding parasite cells at either time point (Table 1). In addition, when acclimation and performance temperatures were combined and coded as constant or mismatched between acclimation and performance thermal environments, there was also no influence on transmission (Table 1 and Fig. 2A, B). However, there was a significant effect of parasite strain at both time points (Table 1), with strain IL transmitting in a greater proportion of bees than strain AK (Fig. 3A, B). Moreover, there was a trend for host colony differences at day 4 (Table 1A) and a significant effect of host colony on transmission 6 days post-exposure (Table 1B). At both time points, the order of colonies, ranked by increasing proportions of *Crithidia* shedding bees, was A, C, B, and D (Fig. 4A, B). Four days post-exposure, there was a marginally non-significant effect of body size (Table 1A), with body size being significant in the model fitted to the transmission data at day 6 (Table 1B). Increasing body size, as measured by the radial cell of the forewing (mm), reduced transmission at both 4 days ($\beta = -1.27$) and 6 days ($\beta = -2.58$) post-exposure.

Gut infection intensities

Patterns of quantitative parasite infection intensities in the gut of bumble bees at day 8 post-experimental exposure to *C. bombi* largely reflected the presence and absence data from fecal transmission checks, with the same terms maintained in the final model (Table 2). There was again no significant effect of acclimation temperature, performance temperature, or the interaction. Also, constant or mismatched thermal environments did not significantly differ in infection intensities (Fig. 2C). Differences between the parasite strains in transmission were repeated in infection intensities, with exposures to strain IL leading to heavier infections, relative to strain AK (Fig. 3C). Host colony also influenced infection, with the hierarchy of susceptibility mirroring that of the transmission data (Fig. 4C), and increasing body size reduced infection loads ($\beta = -3.09$).

Discussion

We found no support for the beneficial acclimation hypothesis in the infection outcomes of a bumble bee host and trypanosome parasite system. There were no significant effects of acclimation temperature, performance temperature, or the interaction of acclimation and performance temperatures on the proportion of bees transmitting parasites or established infection intensities. Furthermore, when treatments were grouped based on the relationship between acclimation and performance temperature into constant or mismatched, there was also no effect on infection outcomes. However, there were significant effects of parasite strain, host colony identity, and bee size on all measured infection parameters. The parasite strain and host colony of origin effects on infection outcomes align with previous work showing an influence of parasite and host genotypes (Sadd and Barribeau, 2013).

There is no evidence for an influence of the performance temperature imposed during the infection on either transmission or infection intensity. Within the temperature ranges tested, the infection outcomes appear to be robust to the thermal environment during infection. This is in contrast to work in other systems showing effects of temperature on host immunity and resistance to infection (Poulin 2006; Linder et al. 2008; Malek and Byers 2018). For example, *Drosophila melanogaster* showed reduced immunity and resistance to bacterial infection at 25 and 29°C, relative to 17°C (Linder et al. 2008). The full range of outcomes of changes in the thermal environment for host resistance to infection of positive, negative, or no effect have been shown, but in several other systems host resistance can be significantly altered with small realistic changes in the thermal environment (Thomas and Blanford 2003). Although bumble bee individuals can regulate thoracic temperature to some degree, abdominal temperature more closely tracks changes in the ambient temperature

Table 1 Model terms and statistics from generalized linear models with binomial error distributions fit to data on bees transmitting *C. bombi* parasite cells in their feces at A) 4 and B) 6 days after experimental exposure

Model term	X^2	df	*P*
A) 4 days post-exposure			
Body size	3.74	1	0.053
Host colony	7.00	3	0.072
Parasite strain	9.14	1	**0.003**
Host colony×parasite strain	0.74	3	0.865
Acclimation temperature[a]	1.40	2	0.497
Performance temperature[a]	1.60	2	0.448
Acclimation×performance temperature[a]	4.68	4	0.322
Mismatch treatment[a]	0.10	1	0.748
B) 6 days post-exposure			
Body size	12.31	1	**<0.001**
Host colony	11.34	3	**0.010**
Parasite strain	33.87	1	**<0.001**
Host colony×parasite strain	0.67	3	0.734
Acclimation temperature[a]	0.51	2	0.776
Performance temperature[a]	0.22	2	0.897
Acclimation×performance temperature[a]	5.59	4	0.232
Mismatch treatment[a]	0.01	1	0.924

Note: Bold terms represent terms in the final best models, with statistics of the other terms taken from before their removal.
[a]Two separate models were fitted with (1) acclimation temperature, performance temperature, and their interaction and (2) acclimation and performance temperature combined and coded as constant or mismatched under mismatch treatment.

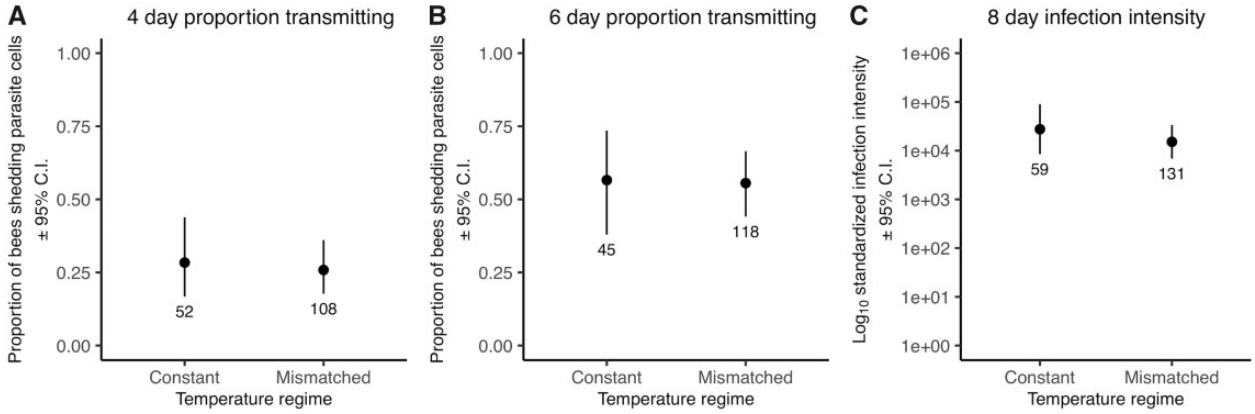

Fig. 2 Thermal regime and *C. bombi* transmission at 4 days (**A**) and 6 days (**B**) post-exposure, and infection intensity 8 days (**C**) post-exposure. The proportion of bees shedding *C. bombi* cells at day 4 (A) and day 6 (B) for bees that underwent a constant temperature treatment (same acclimation and performance temperatures) or bees that were acclimated to one temperature but then assigned to a different performance temperature (mismatched). Day 8 infection intensities were quantified using qPCR (C).

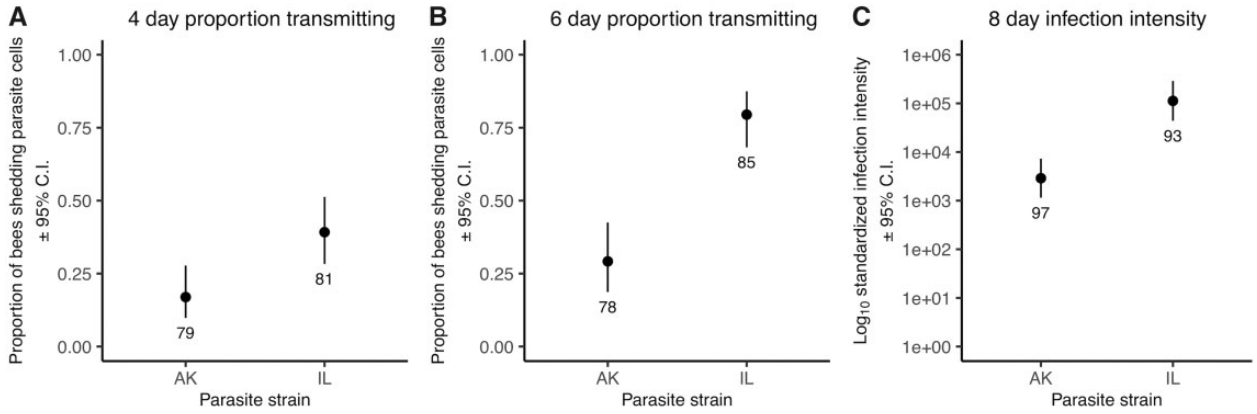

Fig. 3 Infection outcomes and parasite strain identity. The proportion of bees shedding *C. bombi* cells across the two different parasite strains used for experimental exposures at 4 days post-infection (**A**), 6 days post-infection (**B**), and the infection intensity at 8 days post-exposure (**C**).

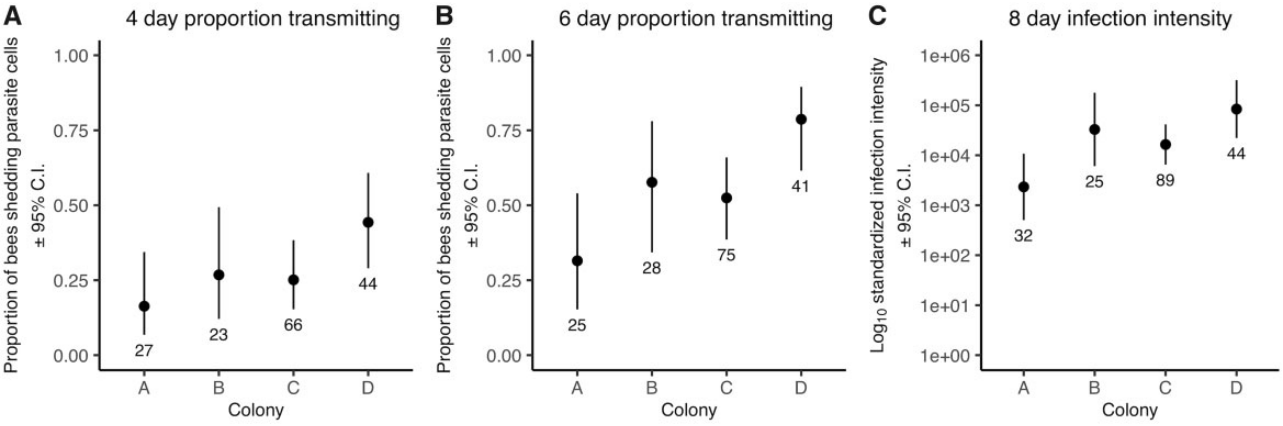

Fig. 4 Infection outcomes by colony identity. The proportion of bees shedding *C. bombi* cells across different host colonies of origin at 4 days post-infection (**A**), 6 days post-infection (**B**), and the infection intensity at 8 days post-exposure (**C**).

(Heinrich and Vogt 1993). This means that different performance temperatures were not just experienced by hosts but also by the parasites, which suggests there was no direct effect on parasites of temperature. The lack of an effect of performance temperature on infection implies that the thermal breadth of *B. impatiens* for parasite resistance at least spans the imposed temperatures. It also suggests that physiological stress (Paull et al. 2015), which could have affected resistance, did not vary across any of the imposed temperatures. However, it is possible that bumble bee hosts maintain immunity against parasites even in the face of temperature-induced physiological stress, but at a cost to other traits, such as longevity. This possibility cannot be discounted, and further studies that track survival following infection would be required to test for this. On the basis of this study, however, immediate temperature within the tested range would not add to the previously documented variation in trypanosome infection outcomes (Sadd and Barribeau 2013) and disease dynamics due to direct within-host effects.

For the beneficial acclimation hypothesis, it is not the performance temperature *per se* that matters, but rather the relationship between acclimation and performance temperatures. Neither acclimation temperature alone, nor the interaction between acclimation and performance temperatures influenced host resistance to infection, and thus there was no support for the beneficial acclimation hypothesis in relation to parasite resistance at the temperatures tested. These results are in contrast to work in other host–parasite systems showing that acclimation and performance temperatures interact to determine infection outcomes (Paull et al. 2015; Altman et al. 2016; Rohr et al. 2018). The absence of any such effects in this study could have been the result of the acclimation period being too short to allow for changes to take place, although the period of 1 week represents a substantial portion of the 4-week adult life-span of bumble bee workers in the field (Alford 1975). It is

Table 2 Model terms and statistics from a linear model fit to standardized gut parasite infection intensities (log-transformed to meet model assumptions) 8 days after experimental exposure to *C. bombi*

Model term	Sum of squares	*F*	df	*P*
Body size	149.6	7.88	1	**0.006**
Host colony	243.4	4.27	3	**0.006**
Parasite strain	631.9	33.29	1	**<0.001**
Host colony×parasite strain	10.9	0.19	3	0.905
Acclimation temperature[a]	4.0	0.10	2	0.901
Performance temperature[a]	19.3	0.50	2	0.607
Acclimation×performance temperature[a]	94.9	1.22	4	0.304
Mismatch treatment[a]	14.4	0.76	1	0.385
Residuals	3511.8		185	

Note: Bold terms represent terms in the final best models, with statistics of the other terms taken from before their removal.
[a]Two separate models were fitted with (1) acclimation temperature, performance temperature, and their interaction and (2) acclimation and performance temperature combined and coded as constant or mismatched under mismatch treatment.

more likely that the thermal breadth of bumble bee workers covers the tested ranges, allowing them to maintain performance even upon a shift in temperature. The tested range of shifts and temperatures is within those that would be experienced by *B. impatiens* bumble bee workers in the field, especially given their individual thermoregulation during foraging (Heinrich 1972, 1975, 1976). Additionally, the colony-level thermoregulation may mean that workers inside the nest are often exposed to warmer than ambient temperatures (Weidenmüller et al. 2002). Weak specialization within bumble bee colonies and considerable switching between in-nest and out of nest activities (Jandt et al. 2009) will also affect the naturally experienced thermal ranges and shifts. Thus, the absence of any influence of the different thermal regimes could be the result of adaptations to deal with the experimentally imposed shifts being present in this species. *Bombus impatiens* is considered to have a stable population and is found distributed across a relatively broad range of thermal environments, with native populations extending from southern Canada to southern Florida (Cameron et al. 2011). Species with more limited distributions are expected to have more narrow thermal optima (Perotti et al. 2018), so less widespread species and those adapted to on-average cooler environments may be more susceptible to deterioration in performance as a result of the temperatures and thermal shifts used in this study.

Even though this study found no evidence for temperature-related effects on infection outcomes, the consequences of acclimation to abiotic environments followed by a rapid switch may be more pronounced in natural colonies, under more extreme thermal divergence, or in other castes or species. It is important to note that adult bees in this experiment were maintained in isolation under relatively benign conditions. This is in contrast to field conditions, where worker bumble bees will potentially have greater nutritional stress and additional demands on their resources. These demands include the performance of energetically expensive colony-level thermoregulation (Vogt 1986) and foraging (Heinrich 1972), the latter of which has been shown to negatively impact bumble bee immune function (König and Schmid-Hempel 1995; Doums and Schmid-Hempel 2000). These additional stressors could alter susceptibility to thermal variability and parasite infection dynamics.

As highlighted above, this study utilized temperatures within the expected range of ambient temperatures experienced by bumble bee workers in the field. Thermal stress (Paull et al. 2015) may be imposed at more extreme temperatures outside of the normal range, such as those experienced during heatwaves, prolonged periods of temperatures above the long-term average (Rasmont and Iserbyt 2012). Warm days are expected to increase in frequency and intensity (Frich et al. 2002) and heatwaves are predicted to become more frequent, more intense, and longer (Meehl and Tebaldi 2004; Lau and Nath 2012; Perkins et al. 2012; Mazdiyasni and AghaKouchak 2015). Shifts in temperature in and out of these extraordinary extremes may be more likely to perturb performance, including resistance to infection. However, we know surprisingly little about mechanisms underlying responses to extreme climatic events, including heatwaves, and their interactions with other abiotic and biotic stressors (Van de Pol et al. 2017), making this a critical avenue for further study.

Using parasite infection as a measure of performance, this study tested the effects of previously and currently experienced temperatures and addresses the beneficial acclimation hypothesis in an important pollinator insect. No changes in parasite transmission or infection intensity across thermal regimes, yet effects of parasite genotype and host colony, reflect the robustness of host–parasite interactions within the bumble bee–trypanosome system to thermal perturbation within the temperatures tested. However, further work is required to demarcate the breadth of this thermal robustness, including

assessments of larger temperature deviations or extremes, and make predictions about how ongoing temporal and spatial changes in thermal environments will influence host–parasite dynamics and bumble bee health.

Acknowledgments

The authors thank Sadd laboratory members for contributions to maintenance of bumble bee colonies and discussion of the manuscript. We also thank the two anonymous reviewers who provided comments that improved the manuscript.

Funding

This work was supported by the National Institutes of Health from the National Institute of General Medical Sciences [grant 1R15GM129681-01 to B.M.S.], the United States Department of Agriculture [grant 2017-67013-26536 to B.M.S.], and a Phi Sigma Biological Society Weigel grant to K.B.T.

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