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Cite this article: Sauer EL, Fuller RC, Richards-Zawacki CL, Sonn J, Sperry JH, Rohr JR. 2018 Variation in individual temperature preferences, not behavioural fever, affects susceptibility to chytridiomycosis in amphibians. *Proc. R. Soc. B* **285**: 20181111. http://dx.doi.org/10.1098/rspb.2018.1111

Received: 18 May 2018
Accepted: 27 July 2018

Subject Category:
Ecology

Subject Areas:
behaviour, ecology, health and disease and epidemiology

Keywords:
thermoregulation, behavioural fever, amphibian declines, *Batrachochytrium dendrobatidis*, disease ecology, thermal biology

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4200437.

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Variation in individual temperature preferences, not behavioural fever, affects susceptibility to chytridiomycosis in amphibians

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The ability of wildlife populations to mount rapid responses to novel pathogens will be critical for mitigating the impacts of disease outbreaks in a changing climate. Field studies have documented that amphibians preferring warmer temperatures are less likely to be infected with the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). However, it is unclear whether this phenomenon is driven by behavioural fever or natural variation in thermal preference. Here, we placed frogs in thermal gradients, tested for temperature preferences and measured *Bd* growth, prevalence, and the survival of infected animals. Although there was significant individual- and species-level variation in temperature preferences, we found no consistent evidence of behavioural fever across five frog species. Interestingly, for species that preferred warmer temperatures, the preferred temperatures of individuals were negatively correlated with *Bd* growth on hosts, while the opposite correlation was true for species preferring cooler temperatures. Our results suggest that variation in thermal preference, but not behavioural fever, might shape the outcomes of *Bd* infections for individuals and populations, potentially resulting in selection for individual hosts and host species whose temperature preferences minimize *Bd* growth and enhance host survival during epidemics.

1. Introduction

Increases in emerging infectious diseases over the last few decades have caused global declines in biodiversity [1,2]. Anthropogenic global climate change is predicted to influence human and wildlife disease dynamics worldwide, possibly exacerbating these disease-driven declines [3,4]. One reason that climate change might affect disease dynamics is because the infectivity and virulence of pathogens, as well as host resistance and tolerance of infection can vary with climatic conditions [5]. This is especially true for ectothermic hosts, which have only a limited ability to regulate body temperature independent of environmental temperatures and can struggle to combat stressors, such as disease, when exposed to sub-optimal temperatures [6–8]. Additionally, individual ectothermic hosts can vary in their preferred temperatures, which can affect their susceptibility to infections [9]. Hence, epidemics could select for host individuals and species that inherently prefer temperatures that facilitate tolerance and/or resistance to pathogens, a process that would occur across generations [9,10].

Hosts can also cope with pathogens using plasticity, which is a change in host physiology (e.g. acquired immunity), morphology, or behaviour during the life of the host, and thus occurs within rather than across generations. For instance, upon infection, ectothermic hosts could modify their temperature preferences

(via behavioural thermoregulation), selecting environmental temperatures that are unfavourable for the parasite, ideal for host defences, or both. Ideally, this plasticity in response to infection should be differentiated from preferred temperatures in the absence of infections. Understanding the extent to which host populations can mount rapid plastic responses to pathogens might be critical for predicting the impacts of continued widespread disease outbreaks in a changing climate.

Many ectothermic hosts exhibit a type of plasticity called behavioural fever, which is when a host increases its temperature preference (T_{pref}) in response to pathogen exposure [11–13]. Behavioural fever has most commonly been documented in response to bacterial and viral pathogens, which tend to grow well at high temperatures [14]. In these cases, behavioural fever tends to increase host immune responses, which is believed to provide a net benefit to the host despite the increased pathogen growth at the higher temperature [14]. If behavioural fever is effective against thermophilic pathogens, it might be even more effective against psychrophilic (cold-loving) pathogens because the higher temperatures might both stimulate host immunity and be directly detrimental to pathogen growth.

An example of a relatively cold-tolerant pathogen is the fungus *Batrachochytrium dendrobatidis* (*Bd*). *Bd* causes the disease chytridiomycosis, is associated with global amphibian declines [7,15], grows best in culture under cool conditions between 18°C and 22°C, and can be cleared from some hosts when held above 25°C for extended periods of time [16–19]. In fact, field studies have documented little to no *Bd* in populations associated with hot springs and relatively warm low-elevations, even when surrounding or adjacent high-elevation populations have high prevalence [20–22].

Not surprisingly, several studies suggest that *Bd* dynamics are influenced by temperature [16,19,23,24], but whether amphibians respond to *Bd* with behavioural fever in the field and laboratory remains controversial. Multiple field studies correlating amphibian body temperature and *Bd* infection have shown that individual amphibians with higher body temperatures are less likely to be infected with *Bd* relative to individuals with lower body temperatures within the same population [9,21,25]. One hypothesis for this pattern is that some but not all individuals preferred microhabitats with temperatures that were unfavourable for *Bd*, regardless of whether they were infected [9]. By contrast, other researchers have hypothesized that these field patterns were the result of amphibians intentionally moving to warmer microhabitats to resist infection (i.e. behavioural fever) [25]. Two laboratory experiments tested for *Bd*-induced behavioural fever and reported mixed results. The first experiment found no evidence of *Bd*-induced behavioural fever in toad tadpoles [26]. The second study claimed to have provided evidence for *Bd*-induced behavioural fever in adult amphibians, but it had low statistical power and consequently could not conclusively support or rule out a behavioural fever response [27].

These conflicting laboratory and field results might be partly a product of the effectiveness of pathogen defences of some host species not increasing with temperatures. For example, the thermal mismatch hypothesis predicts that host species adapted to warmer temperatures might perform more poorly than the pathogen at cool temperatures, and vice versa, creating a scenario where warm- and cool-adapted hosts most often experience outbreaks at cool and warm temperatures, respectively [24,28]. There is support for this hypothesis in the amphibian-*Bd* system [24].

Here, we attempt to address the controversy regarding whether anuran amphibians tend to adjust their preferred temperature when infected with *Bd*. Our goals were to determine if: (i) there was individual-level variation in T_{pref} within the tested species, (ii) there were correlations between T_{pref} and *Bd* growth within and among the tested species of frogs, (iii) there was any support for the thermal mismatch hypothesis, and (iv) any tested amphibian species changed their T_{pref} in response to *Bd* exposure. To accomplish these goals, we exposed five species of adult frogs (Cuban tree frogs, *Osteopilus septentrionalis*, southern toads, *Anaxyrus terrestris*, Panamanian golden frogs, *Atelopus zeteki*, northern cricket frogs, *Acris crepitans*, and American toads, *Anaxyrus americanus*) to *Bd* in thermal gradients ranging in temperature from 9°C to 34°C [29] to assess individual T_{pref} before and after *Bd* exposure. We also measured *Bd* growth on individuals over time to assess whether any variation in T_{pref} affected *Bd* growth.

2. Methods

(a) Thermoregulation experiments

Experiments were conducted at the three locations: *O. septentrionalis* and *An. terrestris* experiments took place in Tampa, FL, *An. americanus* and *Ac. crepitans* experiments took place in Champaign, IL, and *At. zeteki* experiments took place in New Orleans, LA. See the electronic supplementary material, methods for details regarding animal collection and maintenance as well as protocols regarding *Bd* exposures and measuring *Bd* growth on hosts. In each experiment, we first measured individual baseline non-infected T_{pref} in thermal gradient apparatuses. All species except for *At. zeteki* (thermal gradient range: 19°C to 38°C; see the electronic supplementary material, methods for more details and description) were in thermal gradient apparatuses that were previously shown to provide variation in temperature that is independent of moisture/humidity and which does not confound amphibian and prey temperature preferences (12°C to 33°C see the electronic supplementary material, figure S4 and methods; and Sauer *et al.* [29] for thermogradient construction and validation details). After measuring non-infected T_{pref}, individuals were split into three treatment groups with similar mean body masses and non-infected T_{pref}: (i) a sham-exposed control group that was allowed to thermoregulate, (ii) a *Bd*-exposed group that was allowed to thermoregulate, and (iii) a *Bd*-exposed non-regulating group where each individual was held at their individual preferred body temperature (*O. septentrionalis*), at the population-level temperature preference (*Ac. crepitans*, *An. americanus*, *An. terrestris*), or at acclimation temperature (*At. zeteki*) by transferring them to temperature-controlled Styrofoam incubators (electronic supplementary material, figure S6) or environmental chambers (see the electronic supplementary material, methods).

Throughout the experiment, temperature measurements were taken each day, every four hours, four times a day, between 08.00 h and 22.00 h using an infrared thermometer [30] (Micro-Epsilon ThermoMeter LS (accuracy: ±0.75%) for *At. zeteki* and an Extech® High Temperature IR Thermometer (accuracy: ±2% < 932°F) for all other species) from the centre of each animal's dorsum [30] and from the substrate adjacent to the animal, except for during feeding periods (see the electronic supplementary material, methods for details on feeding). Temperature measurements were taken for at least four days before *Bd* or sham exposure and for at least two weeks after these exposures. Experiments were conducted using multiple temporal blocks to ensure adequate sample sizes (see the electronic supplementary material, table S2 for sample sizes for each temporal block in each experiment).

Osteopilus septentrionalis has previously been shown to acquire immunological resistance to *Bd* after a previous exposure and

clearance [17], so we tested whether this species could acquire the ability to exhibit a behavioural fever response to *Bd*. We exposed half of the *O. septentrionalis* to *Bd* and half to a sham inoculate, held all individuals at 23°C for 10 days, and then shifted all frogs to 30°C for 14 days for heat clearance [16]. After confirming that all individuals were uninfected, we proceeded with the T_{pref} trials previously described but with six treatments, *Bd*-naive versus *Bd*-experienced animals crossed with the three treatment groups previously described (mean $n = 6$, $N = 37$).

We were concerned that, by placing frogs into the thermal gradients immediately after *Bd* inoculations, they could quickly select a high temperature to clear the infection before it successfully established. Consequently, we conducted a separate experiment on *An. terrestris*, where individuals received *Bd* or sham exposures. We then held them at 17°C for 7 days to ensure that there was *Bd* establishment followed by considerable pathogen population growth, and then placed them into the thermogradients to test for behavioural fever as described above.

(b) Data analysis

All statistics were conducted with R 3.4.0 [31]. To test for repeatability within individuals in T_{pref} and variation in T_{pref} among individuals before infection, we conducted a one-way repeated measures ANOVA (*stats* package, *aov* function). This analysis tested whether temperature preferences of individuals varied significantly across days (main effect of day) and whether temperature preferences varied among individuals (within-individual variance, s^2). Additionally, we calculated repeatability (see the electronic supplementary material, methods for formula), the proportion of the variance explained by the individual [32].

We used a weight of evidence approach to test for behavioural fever across species (three-factor: treatment, time and species) and within species (two-factor: treatment and time) we conducted multiple repeated measures ANOVAs with individual treated as a random variable (*stats* package, *aov* function, assuming normal error distribution). For each model, we paired all pre-exposure days with each post-exposure day (time; one model for each post-exposure day) and looked for an interaction between treatment and time on ΔT_{pref} (the difference between mean pre-exposure T_{pref} of each animal and its T_{pref} at each time point). We then assessed significance using the Benjamini–Hochberg (B-H) procedure [33].

We also tested for an effect of infection intensity (log-transformed *Bd* load divided by mass of the individual) on ΔT_{pref} (difference between mean pre-exposed T_{pref} and T_{pref} during the 24 h after being swabbed) on *At. zeteki* and *An. terrestris* by conducting a linear mixed-effects model with individual as a random effect (*nlme* package, *lme* function). Individual-level *Bd* growth rates for *An. terrestris* were determined by first calculating infection intensity by dividing *Bd* loads (DNA copies) by individual mass, then log transforming infection intensity, then extracting the slope parameter from a generalized linear model of each individual's infection intensity over time (*stats* package, glm function; time in days). *Bd* growth rates for *At. zeteki* were determined by first calculating log infection intensity using the aforementioned methods then extracting the growth parameter from a logistic growth model of each individual's infection intensity over time (*bbmle* package, *mle2* function; time in weeks; see the electronic supplementary material, methods for model). Growth models for each species were chosen based on a visual examination of the shape of *Bd* load data over time. To test the influence of individual-level T_{pref} on *Bd* growth, we conducted a linear regression with the previously calculated *Bd* growth rates as the response and an individual's mean T_{pref} for the 7 days following *Bd* exposure as the predictor (*stats* package, glm function). To test for differences in *Bd* intensity (main effect of treatment) and growth (interaction between treatment and time) between regulating and non-regulating exposed treatments over time, we conducted a two-factor (treatment and time) ANOVA with individual included as a random effect (*nlme* and *stats* packages, lme function). We also ensured there was no effect of body mass on T_{pref} by conducting a one-way repeated measures ANOVA for these two species.

Additionally, we tested for reductions in *Bd* prevalence over time. To do this, we calculated prevalence for all species using animals from the *Bd*-exposed treatment and then ran a one-way ANOVA for each species separately to determine if there was a significant change in prevalence from week 1 to week 2. We also ran a two-factor (species and treatment) ANOVA for each of the two weeks followed by Tukey's *post hoc* multiple comparison tests to assess differences in prevalence between species and treatments (regulating or non-regulating) (stats package, Tukey HSD function). Tukey's *post hoc* multiple comparisons tests were also used to assess differences when a treatment had more than two levels (*multcomp* package, glht function). Finally, to test for differences in survival among treatments, we conducted a Cox-proportional hazards model (*survival* package, coxph function).

3. Results

(a) Temperature preferences across individuals and species

Before *Bd* exposure, we were able to detect consistency in the T_{pref} of individuals (repeatability: $r > 0.90$ for all species; electronic supplementary material, table S1) and variation in temperature preferences among individuals (electronic supplementary material, table S1) and across species ($F_{4,158} = 6.82$, $p < 1.0 \times 10^{-4}$). *Atelopus zeteki* (mean T_{pref}: 20.8°C ± 0.65 s.e.) and *An. americanus* (21.3°C ± 0.43) preferred significantly cooler temperatures than *Ac. crepitans* (23.4°C ± 0.61) and *An. terrestris* (23.5°C ± 0.65). *Osteopilus septentrionalis* (22.5°C ± 0.70) preferred moderate temperatures and was not significantly different from any other species (figure 1). To ask whether these T_{pref} might be an artefact of differences in acclimation temperature, we tested for a correlation between acclimation temperature and species-level T_{pref} and found no trend ($t_4 = 0.60$, $p = 0.59$), but the power of this analysis is admittedly low.

(b) Behavioural fever

When we adjusted our alpha for multiple comparison tests, we found no evidence of behavioural fever after exposure to *Bd* for the omnibus test across species (interaction between treatment and time, $p <$ B-H critical value; figure 2*a*; electronic supplementary material, figure S1 and table S2). If we looked at individual species, we found no evidence of behavioural fever or shifts in T_{pref} for *An. americanus*, *An. terrestris*, or *At. zeteki* (interaction between treatment and time $p >$ adjusted threshold; figure 2*a*; electronic supplementary material, figures S1 and S2 and table S2). There were some days with significant interactions between treatment and time for *O. septentrionalis* (days 3 and 10 for the treatment group were significantly warmer; electronic supplementary material, figure S2 and table S2) and *Ac. crepitans*. For *Ac. crepitans*, the control frogs preferred significantly warmer temperatures than the *Bd*-exposed frogs, (days 6–11, 13, 17; electronic supplementary material, figure S1 and table S2), which is inconsistent with behavioural fever. Additionally, infection intensity had no effect on T_{pref} in the species where quantitative PCR was conducted (main effect of intensity on T_{pref} for *An. terrestris*: $\beta = 0.06$, $p = 0.38$ and *At. zeteki*: $\beta = 0.03$, $p = 0.44$). Despite evidence that *O. septentrionalis* can acquire immunological resistance to *Bd* after previous clearance of infections [18], previous exposure to *Bd* did not alter the T_{pref} of *O. septentrionalis* when infected

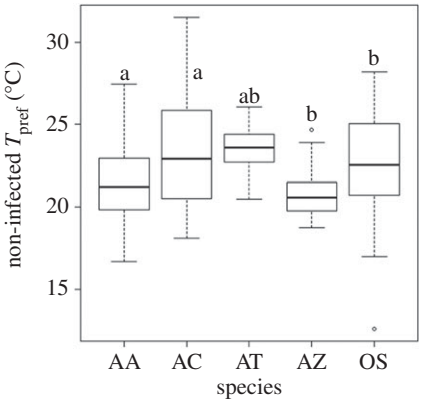

Figure 1. Temperature preferences (T_{pref}) for *Atelopus zeteki* (AZ), *Anaxyrus americanus* (AA), *Osteopilus septentrionalis* (OS), *Acris crepitans* (AC), and *Anaxyrus terrestris* (AT) prior to *Batrachochytrium dendrobatidis* exposure. Species marked with the same letter do not have significantly different T_{pref} based on a Tukey's HSD multiple comparison test ($p > 0.05$). Centre lines represent medians, boxes are first and third quartiles, and whiskers are highest and lowest points.

with *Bd* a second time (figure 2*b*; electronic supplementary material, figure S2 and table S3).

(c) *Batrachochytrium dendrobatidis* abundance and disease susceptibility

For thermoregulating *An. terrestris* and *At. zeteki*, we found that individual T_{pref} during the first week after *Bd* exposure had a significant effect on *Bd* growth rate in the thermal gradients over the course of the three week experiment. *Atelopus zeteki*, which preferred the coolest temperatures, showed a positive relationship between individual T_{pref} and *Bd* growth rate ($F_{1,11} = 4.73$, $p = 0.05$; figure 3*a*), indicating that *Bd* grew better on this species at warmer temperatures. *Anaxyrus terrestris*, which preferred the warmest temperatures, showed a negative relationship between individual T_{pref} and *Bd* growth ($F_{1,11} = 8.86$, $p = 0.01$; figure 3*b*). We also tested for an effect of mass on T_{pref} for these two species and found no effect (*At. zeteki*: $F_{1,26} = 1.02$, $p = 0.32$; *At. terrestris*: $F_{1,29} = 0.05$, $p = 0.82$). We were unable to calculate *Bd* growth rates for *O. septentrionalis* owing to low *Bd* prevalence.

There were no detectable differences in *Bd* loads or *Bd* growth rates between regulating and non-regulating *Bd*-exposed groups (*An. terrestris* main effect of treatment: $\beta = 0.78$, d.f. = 35, $p = 0.36$; interaction between treatment and time: $\beta = -0.22$, d.f. = 63, $p = 0.58$ and *At. zeteki* main effect of treatment: $\beta = 0.68$, d.f. = 24, $p = 0.42$; interaction between treatment and time: $\beta = 0.01$, d.f. = 24, $p = 0.94$; see the electronic supplementary material, figure S3). However, there were differences in prevalence across species and within species across weeks (figure 4). Two week prevalences ranged from 100% for *At. zeteki* to 0% for *O. septentrionalis*. For *At. zeteki*, prevalence remained a constant 100% between week 1 and 2 of the experiment, whereas for *Ac. crepitans* prevalence dropped from 89% to 27% over this time period (figure 4). *Atelopus zeteki* was the only species with substantial *Bd*-induced mortality and there was no significant difference in the survival curves between regulating and non-regulating treatment groups (100% and 100% mortality and 25.1 and 20.3 mean days alive, respectively; $\beta = 0.45$, $p = 0.08$; electronic supplementary material, figure S4). The maximum mortality for any of the other species was 15% in the non-regulating *An. americanus* (electronic supplementary material, figure S4).

4. Discussion

We set out to determine if the tested species of amphibians showed any individual- or species-level variation in T_{pref}, if variation in T_{pref} among individuals or species was correlated with *Bd* growth on frogs, whether relationships between T_{pref} and *Bd* growth were consistent with the thermal mismatch hypothesis, and if any of the tested species responded to *Bd* infections by increasing their T_{pref}. We were able to detect differences in T_{pref} among individuals within a species, as well as differences in T_{pref} across species. Our methods for testing T_{pref} were identical for all species but *At. zeteki* and we found no evidence that acclimation temperature impacted species-level T_{pref}. Moreover, given that *Ac. crepitans* was acclimated to the lowest temperature and had one of the highest preferred temperatures and *At. zeteki* was acclimated to one of the higher temperatures and had the lowest preferred temperature, any undetected effect of acclimation temperature was probably small relative to any inherent species-level differences in temperature preference. We demonstrated that individual-level T_{pref} was correlated with *Bd* growth on frogs and that differences in species-level T_{pref} predicted the direction of this correlation. Though there were some effects of treatment on T_{pref} in two of the five species, we were unable to detect a significant behavioural fever response to *Bd* exposure across species. Our experimental findings suggest that previously reported field patterns correlating body temperature with *Bd* infection [9,25,34] were probably owing to standing variation in T_{pref}, where frogs that preferred warmer temperatures were less likely to be infected because of reduced *Bd* exposure and/or reduced *Bd* growth. Our study, with experiments performed across three laboratories and five species, is probably the most comprehensive test for behavioural thermoregulatory responses to *Bd* exposure in amphibian hosts.

Importantly, for each species, we demonstrated that variation among individuals in T_{pref} was greater than the variation in T_{pref} within an individual through time. That is, there was variation among individuals in their T_{pref}. Individuals often found a suitable thermal microhabitat and continuously chose that preferred temperature, even after being moved to the centre of the gradient each night. This variation among individuals represents the raw material upon which natural selection can act. Assuming that T_{pref} is heritable [35] via genetic or maternal effects [36], it stands to reason that over time a selective sweep could eliminate some of this variation, resulting in a change in average T_{pref} and a decrease in *Bd* prevalence [19]. Other disturbances that select for T_{pref} or reduce thermal microhabitat availability, such as climate change, deforestation, or disease, might also lead to population-level shifts in thermal microhabitat selection [37,38].

Additionally, we confirmed previous findings by detecting differences in T_{pref} among species that probably reflect their adaptations to environmental temperatures [24]. For example, *At. zeteki* was our coolest-preferring species and, not surprisingly, it is native to cool, mid-elevation sites in Central America where daily air temperatures remain in the mid to low-twenties (°C) year round [25]. By contrast, *An. terrestris* was our warmest preferring species, and it is native to warm, low elevation sites in the southeastern United States where mean temperatures in the summer reach into the high-twenties with average daily highs in the low-thirties (°C) [24]. While this study used slightly different methods to measure T_{pref} across these two species, we previously published that *At. zeteki* might prefer even cooler

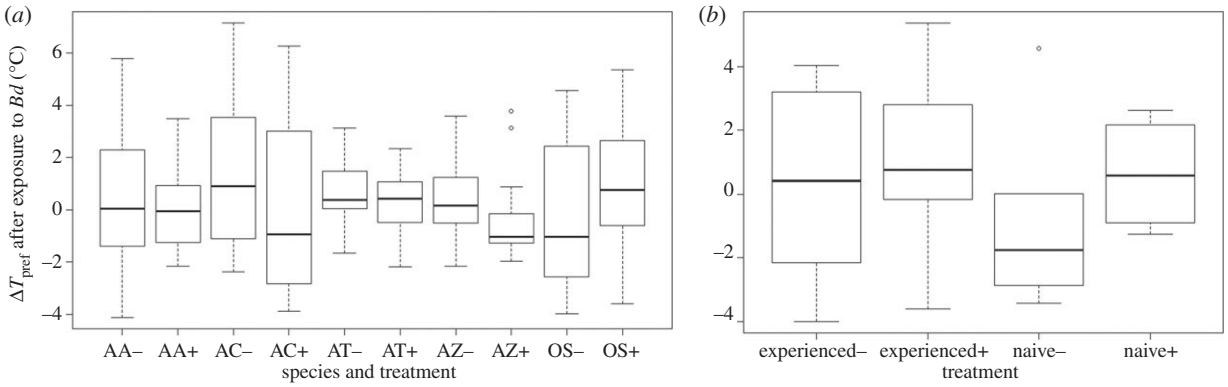

Figure 2. Change in temperature preferences (ΔT_{pref}) after exposure to *Bd* across all time points for *Atelopus zeteki* (AZ), *Anaxyrus americanus* (AA), *Osteopilus septentrionalis* (OS), *Acris crepitans* (AC), and *Anaxyrus terrestris* (AT) after frogs were (+) or were not (−) exposed to *Batrachochytrium dendrobatidis*: when all frogs were naive to *Bd* (*a*) or when half the OS were naive and half were previously exposed and cleared of *Bd* (*b*). Centre lines represent medians, boxes are upper and lower quartiles, and whiskers are highest and lowest points.

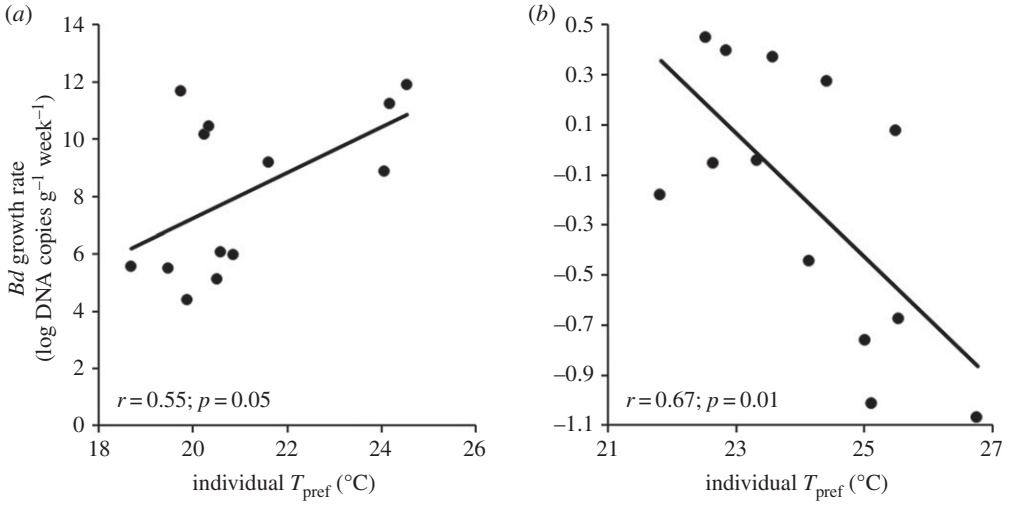

Figure 3. Relationship between individual-level temperature preference (T_{pref}) and *Batrachochytrium dendrobatidis* (*Bd*) growth on frogs for (*a*) *Atelopus zeteki* and (*b*) *Anaxyrus terrestris*. *Atelopus zeteki*, which preferred the coolest temperatures (figure 1), showed a positive relationship between *Bd* growth rate on individual hosts and host T_{pref} ($F_{1,11} = 4.73$, $p = 0.05$), indicating that *Bd* grew better on this species at warmer temperatures. By contrast, *Anaxyrus terrestris*, which preferred the warmest temperatures (figure 1), showed a negative relationship between *Bd* growth on individual hosts and host T_{pref} ($F_{1,11} = 8.86$, $p = 0.01$), indicating that *Bd* grew better on this species at cooler temperatures.

temperatures (T_{pref} 17.85 ± 0.14°C) [24] when tested using methods identical to those used for *An. terrestris* in this study. In this previous experiment, much lower minimum temperatures were available for *At. zeteki* to select (average low of 12°C compared to 19°C) than in the current experiment, which is probably why it had a lower temperature preference.

Although we experimentally tested for behavioural fever in both of the species that have been previously thought to respond to *Bd* exposure with fever (*At. zeteki and An. americanus*) [25,27], there was no evidence that those species or, for that matter, any of the five species exhibited a behavioural fever response to *Bd*. While our experimental results suggest that *At. zeteki* individuals which prefer warmer temperatures experience more rapid *Bd* growth, previous field studies showed that warmer *At. zeteki* were less likely to be infected with *Bd* than cooler preferring individuals in the population [25]. This inconsistency could be explained by differences in exposure given that *Bd* is considered saprophytic. In the absence of a host, *Bd* may persist better at low temperatures. If so, then *At. zeteki* which prefer warmer temperatures might have lower exposure to *Bd*. However, once exposed, *Bd* might grow faster on *At. zeteki* at higher than at lower temperatures.

We found that one species, *Ac. crepitans*, appeared to decrease preferred temperature after infection. The change in preferred temperature, however, did not appear to be beneficial to the host or pathogen as there was no difference in prevalence or survival between frogs in the regulating and non-regulating treatments. After prior exposure and heat clearance, individuals of *O. septentrionalis*, a species known to acquire immunological resistance to *Bd* [17], did not alter their thermoregulatory behaviour significantly. When we lumped the four treatments into exposed and sham-exposed, we did find that the *Bd*-exposed animals were warmer than the sham-exposed animals on day 3 and again on day 10. However, the day 3 differences were largely owing to the naive sham-exposed group sharply decreasing in temperature; there was no difference between the experienced sham-exposed and two *Bd*-exposed groups. Like the drop in

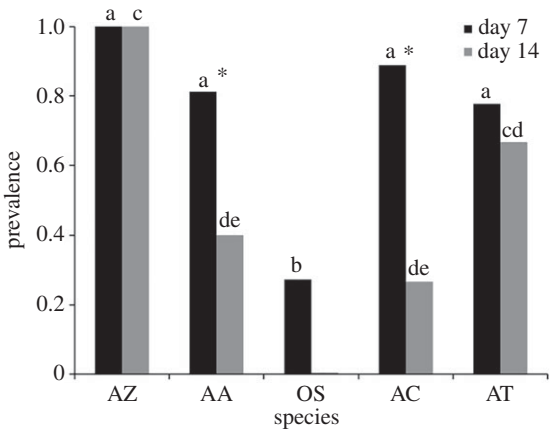

Figure 4. Prevalence of *Batrachochytrium dendrobatidis* (*Bd*) infection one and two weeks after pathogen exposure in *Atelopus zeteki* (AZ), *Anaxyrus americanus* (AA), *Osteopilus septentrionalis* (OS), *Acris crepitans* (AC), and *Anaxyrus terrestris* (AT) when they were free to roam in a temperature gradient. Within week means with different letters are significantly different from one another based on Tukey's HSD test ($p < 0.05$). Asterisks denote species that showed significant drops in prevalence from week 1 to week 2 based on an ANOVA (AA: $F_{1,22} = 6.33$, $p = 0.02$; AC: $F_{1,22} = 20.84$, $p < 0.0001$).

temperature preference observed for *Ac. crepitans*, this change in preferred temperature did not appear to be beneficial to the host or pathogen as there was no difference in prevalence or survival between frogs in the regulating and non-regulating treatments. Hence, both of these changes are possibly spurious and do not appear to be biologically significant. We also found that allowing *Bd* to grow on hosts for a week before introducing them to the thermal gradients had no effect on the likelihood of exhibiting behavioural fever.

Our results suggest that previous field associations between host temperatures and *Bd* abundance were probably a result of the pre-existing variation in T_{pref}, rather than a change in thermoregulatory behaviour in response to infection. That is, frogs which already preferred warmer temperatures were less likely to be infected because their warmer temperatures caused them to either experience reduced *Bd* growth or avoid *Bd* exposure altogether. These results do not suggest that amphibians are incapable of behavioural fever, only that the species of anurans we tested did not respond to *Bd* with a behavioural fever response. In contrast to fungi, viral and bacterial pathogens have been shown to induce behavioural fevers in amphibians [39,40] as well as other ectothermic vertebrate and invertebrate hosts [11,12]. Additionally, our study controlled for moisture to avoid confounding T_{pref} with moisture preference. Thus, we cannot draw any conclusions about amphibians attempting to resist *Bd* infection by 'drying-out', a strategy that could be as effective at as behavioural fever [41].

We demonstrated that differences in species-level T_{pref} could predict the direction of the correlation between T_{pref} and *Bd* growth. The coolest preferring species (*At. zeteki*) had high *Bd* growth rates at relatively warm body temperatures, whereas the warmest preferring species (*An. terrestris*), had high *Bd* growth rates at relatively cool body temperatures. This result is consistent with the thermal mismatch hypothesis, which suggests that cool- and warm-adapted hosts might be more susceptible to disease outbreaks at abnormally warm and cool temperatures, respectively. This is hypothesized to occur because pathogens generally have wider thermal tolerances than their hosts [42], allowing them to outperform hosts under thermal mismatch conditions [24]. In addition to documenting temperature-dependent species-level variation in *Bd* susceptibility, our data also show that variation in T_{pref} among individuals can drive individual-level variation in disease susceptibility within a species. While field evidence showing variation in susceptibility and prevalence of *Bd* can be driven by variation in environmental temperature across individuals [9,25] and populations [21,43], there are very few studies that experimentally test how individual T_{pref} can drive differences in disease susceptibility within a population for this or any host–pathogen system.

In summary, none of the five host species tested exhibited a clear behavioural fever response to *Bd* infection but there were differences in individual-level T_{pref} that affected *Bd* growth. Additionally, we found species-level differences in the direction of the effect of individual-level T_{pref} on *Bd* growth that were consistent with the thermal mismatch hypothesis [24]. These results suggest that variation in T_{pref} within a population might be vital to buffer a species or populations against extirpation when a temperature-sensitive pathogen sweeps through an environment. Variation in T_{pref} might be more easily maintained in an ectothermic population when there are a wide variety of thermal microhabitats available. Thus, degradation of the thermal environment and microhabitat availability might reduce the ability of a species or population to buffer against temperature sensitive pathogens.

Ethics. *Atelopus zeteki* were obtained and used with permission from the Maryland Zoo, *An. terrestris* and *O. septentrionalis* were collected under permit with the Florida Fish and Wildlife Conservation Commission, and *An. americanus* and *Ac. crepitans* were collected under permit with the Illinois Department of Natural Resources. Experimental methods were approved by the Tulane, University of South Florida, and University of Illinois International Animal Care and Use Committees (protocols 0430R, 14112, and W IS00000548, respectfully).

Data accessibility. Data available from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.643r37b [44].

Authors' contributions. E.L.S., C.L.R.-Z., J.H.S. and J.R.R. conceived ideas and designed experiments, E.L.S. and J.R.R. oversaw experiments in Tampa, FL, C.L.R.-Z. and J.S. oversaw experiments in New Orleans, LA, J.H.S. and R.C.F. oversaw experiments in Champaign, IL, E.L.S. and J.R.R. conducted statistical analyses, and E.L.S. and J.R.R. wrote the paper with comments and edits from R.C.F., C.L.R.-Z. and J.H.S. All authors agreed to submission of the manuscript and accept the responsibility for the accuracy and integrity of the manuscript.

Competing interests. We declare we have no competing interests.

Funding. Funds were provided by grants to J.R.R. from the Department of the Army, South Florida Caribbean Cooperative Ecosystems Studies Unit, National Science Foundation (EF-1241889 and DEB-1518681), the National Institutes of Health (R01GM109499, R01TW010286-01), the US Department of Agriculture (2009-35102-0543) and the US Environmental Protection Agency (CAREER 83518801), and by grants to C.L.R.-Z. from the National Science Foundation (1649443) and Louisiana Board of Regents (LEQSF (2011-14)-RD-A-26).

Acknowledgements. We thank the Maryland Zoo for providing us with the *At. zeteki* used in the experiments. We thank G. Addison, L. Brannelly, K. Medina, J. Nguyen, M. Robak, X. Sonn and A. Styf for their assistance in all aspects of the laboratory work and maintenance of experiments. In addition, we thank D. Civitello, J. Cohen, C. Haggerty, D. Jones, K. Nguyen, C. Ramsay, S. Rumschlag, K. Surbaugh for their helpful comments on the manuscript.

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