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LETTER

Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances

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Abstract

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has caused the greatest known wildlife pandemic, infecting over 500 amphibian species. It remains unclear why some host species decline from disease-related mortality whereas others persist. We introduce a conceptual model that predicts that infection risk in ectotherms will decrease as the difference between host and pathogen environmental tolerances (i.e. tolerance mismatch) increases. We test this prediction using both local-scale data from Costa Rica and global analyses of over 11 000 *Bd* infection assays. We find that infection prevalence decreases with increasing thermal tolerance mismatch and with increasing host tolerance of habitat modification. The relationship between environmental tolerance mismatches and *Bd* infection prevalence is generalisable across multiple amphibian families and spatial scales, and the magnitude of the tolerance mismatch effect depends on environmental context. These findings may help explain patterns of amphibian declines driven by a global wildlife pandemic.

Keywords

Amphibian, *Batrachochytrium dendrobatidis*, biodiversity, CT_{max}, disease, ectotherm, habitat loss, susceptibility, thermal tolerance, traits.

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INTRODUCTION

Emerging infectious diseases have contributed to population declines in multiple, divergent animal taxa (Daszak *et al.* 2000). Of these disease-related declines, most are attributed to host-generalist fungal pathogens (Fisher *et al.* 2012; Eskew & Todd 2013). The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has caused one of the largest wildlife pandemics, infecting over 500 amphibian species with near 100% mortality in some outbreaks (Lips *et al.* 2006; Vredenburg *et al.* 2010; Olson *et al.* 2013). However, not all amphibian species are highly susceptible to *Bd* infection and resultant disease; some species and populations persist after epizootics whereas others are driven locally extinct (Lips *et al.* 2006; Vredenburg *et al.* 2010; Scheele *et al.* 2014). Variation in *Bd* infection prevalence has been linked to both extrinsic and intrinsic factors, including regional climate, local forest canopy cover, host life history and host endemism (Bielby *et al.* 2008; Smith *et al.* 2009; Becker & Zamudio 2011; Murray *et al.* 2011; Puschendorf *et al.* 2011; Liu *et al.* 2013; Raffel *et al.* 2013; Catenazzi *et al.* 2014). However, a unifying framework that integrates host, pathogen and environmental characteristics is needed to better explain patterns of infection prevalence among environments and host species. Understanding variation in host susceptibility to fungal pathogens may be critical in predicting and preventing future biodiversity declines in an era of global pathogen transport.

Here, we introduce and test an eco-epidemiological model that describes how a mismatch in environmental tolerances between an ectotherm host and its pathogen can decrease infection risk and how the environment can modify the strength of this relationship (Fig. 1). The conceptual model builds on first principles of disease ecology that characterise disease as a state that emerges from the interaction of a conducive host, pathogen and environment (the disease triangle; Scholthof 2007). We integrate thermal physiology of amphibian hosts and the *Bd* pathogen with previous research that has highlighted the fundamental role of regional and local temperatures in modifying infection risk (Woodhams *et al.* 2003; Richards-Zawacki 2010; Becker & Zamudio 2011; Liu *et al.* 2013; James *et al.* 2015). For instance, species distribution models based on bioclimatic data suggest that environmental suitability for *Bd* and probability of host infection are high in regions with temperatures near the growth optimum of *Bd* (Murray *et al.* 2011, 2013; Liu *et al.* 2013; Menéndez-Guerrero & Graham 2013). Studies have also shown that variation in canopy cover can explain local patterns of infection or host population persistence (Raffel *et al.* 2010; Becker & Zamudio 2011; Puschendorf *et al.* 2011; Scheele *et al.* 2015); dense forest cover reduces sub-canopy temperatures within a region, creating cool local conditions favourable for *Bd* growth.

The primary prediction of the model is that species-level infection risk will decrease as the degree of mismatch in host

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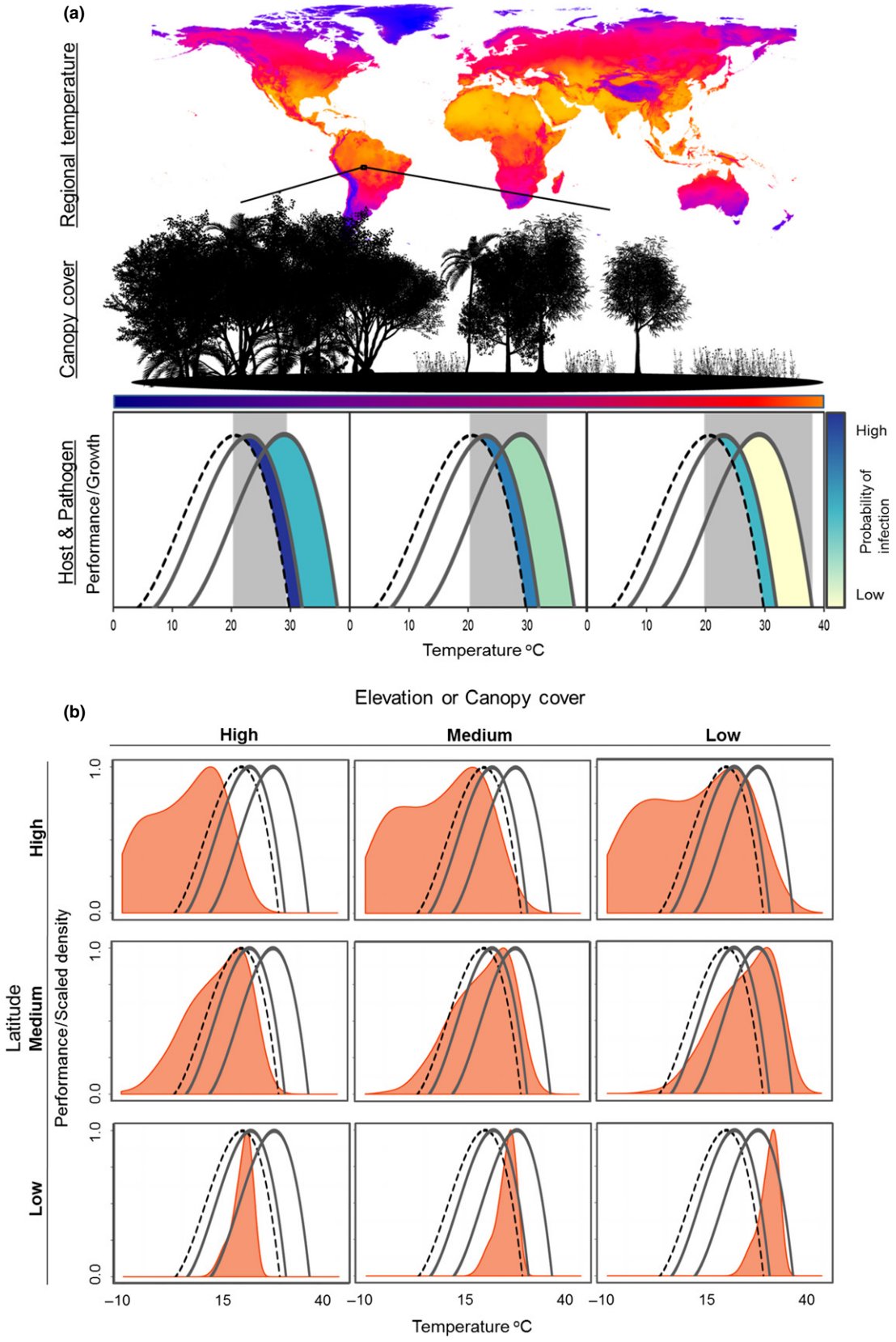

and pathogen thermal tolerances increases because host species with high tolerances are able to access thermal niche space that is detrimental to the pathogen (Fig. 1; hereafter, the 'tolerance mismatch hypothesis'). However, a possible alternative expectation is that infection prevalence may increase with increasing degree of mismatch in host and

Figure 1 (a) An eco-epidemiological model describing variable host susceptibility to infection. Temperature gradients modify overall infection risk at regional scales (mediated by latitude and elevation) and local scales (mediated by canopy cover) (Raffel *et al*. 2010; Becker & Zamudio 2011; Liu *et al*. 2013). Warm colours indicate high temperatures and cool colours indicate low temperatures. Relative infection risk among host species, however, depends on the degree of mismatch in thermal tolerances between pathogen and hosts. The dashed lines represent the temperature-dependent growth rate of *Batrachochytrium dendrobatidis* (*Bd*) (Piotrowski *et al*. 2004), and solid lines represent performance curves for hypothetical host species with different critical thermal maxima (Whitehead *et al*. 1989). The filled area under the curves highlight the degree of mismatch between host and pathogen thermal tolerances, and the colour indicates the relative probability of infection. A slight mismatch limits a host's access to thermal niche space that is unsuitable to the pathogen whereas a greater mismatch allows for a greater range of operative body temperatures that can inhibit pathogen growth. Grey shading behind the curves represents a plausible range of daily air temperatures under different levels of canopy cover for a low elevation site in the tropics (Robinson *et al*. 2013). (b) The nine panels illustrate how environmental temperature varies with latitude and elevation or canopy cover and thus likely modifies the degree to which mismatches in host and pathogen thermal tolerances determine infection risk. In all panels, the filled distributions represent scaled density plots of maximum daily temperatures throughout the year. Latitude affects the mean, maximum and range of environmental temperatures that are available whereas elevation and canopy cover shift the distributions of environmental temperatures. The dashed lines represent the thermal performance curves of *Bd* and the solid lines represent thermal performance curves of two hypothetical amphibian hosts that differ in their thermal tolerances. The total available environmental space that could enable hosts to avoid or abate disease can be found by integrating the area under the environmental temperature density curve that is outside the thermal performance curve of *Bd*, yet within the thermal performance curve of a given amphibian host. This figure illustrates cases where available environmental space may limit opportunities for effective disease abatement or avoidance (e.g. low latitude, high elevation/canopy cover) as well as cases where there is environmental space unsuitable for the pathogen that cannot be accessed by the host with a lower thermal tolerance, but which is available to the host with a greater thermal tolerance (e.g. medium latitude, low elevation/canopy cover). These panels highlight how the degree of mismatch in host and pathogen environmental tolerances determine the extent to which hosts can exploit thermal opportunities for abating disease.

pathogen thermal tolerances, if host species with high thermal tolerances are able to limit and sustain subclinical infections whereas species with low thermal tolerances quickly succumb to disease (and therefore disappear from sampled populations, lowering observed infection prevalence). In either scenario, we expect that the importance of thermal tolerance mismatches in altering infection prevalence will depend on ambient temperatures because ambient temperatures both affect environmental suitability for *Bd* and influence host body temperatures. Assuming that many amphibian hosts are capable of behavioural thermoregulation above ambient temperatures under a broad range of climate conditions (given sufficiently high thermal tolerances; Tracy *et al*. 2010), we would expect the magnitude of the relationship between tolerance mismatch and infection prevalence to generally decrease with increasing regional temperatures, as environmental suitability decreases for *Bd* (Fig. 2). At local scales, we expect warmer, open canopy sites to be associated with lower overall infection risk than cooler, closed canopy sites that are typically more suitable for *Bd* growth (Fig. 2; Raffel *et al*. 2010; Becker & Zamudio 2011).

We define the mismatch in host and pathogen thermal tolerances using species' upper thermal tolerances (i.e. critical thermal maxima – CT_{max}), because this physiological parameter is both widely reported and because it delimits the amount of thermal niche space available to the host but unavailable to the pathogen (Fig. 1). The upper thermal tolerance for *Bd* is approximately 30 °C (Piotrowski *et al*. 2004), with only slight variation (~ 2 °C) in thermal profiles among isolates (Stevenson *et al*. 2013); in contrast, amphibian CT_{max} typically ranges between 30 and 42 °C across species (Sunday *et al*. 2014). It is possible that a mismatch in host and pathogen lower thermal tolerances (i.e. CT_{min}) could also influence infection risk at low temperatures. However, many amphibian species have a CT_{min} above that of *Bd* (Piotrowski *et al*. 2004; Sunday *et al*. 2014), suggesting that mismatches in upper thermal tolerances are generally more relevant to infection risk. To abate infection, host species do not need to achieve body temperatures at or near their CT_{max}; however, the greater a host species' CT_{max} is relative to that of *Bd*, the more likely that host can achieve body temperatures that enhance immune response (see below) or that are otherwise detrimental to pathogen growth without themselves succumbing to thermal stress (Rohr *et al*. 2013).

Drawing on recent studies, we suggest several non-mutually exclusive mechanisms that could underlie the role of host thermal tolerance in the tolerance mismatch conceptual model: (1) Complete avoidance of infection could be achieved by species that occupy local habitats that are thermally unsuitable for *Bd* (Catenazzi *et al*. 2014). (2) Infected individuals may rid themselves of *Bd* by inducing behavioural fevers (Richards-Zawacki 2010; Rowley & Alford 2013); however, interspecific variation in host thermal tolerances places constraints on the ability of some species to warm themselves to temperatures that inhibit *Bd* growth. (3) Antifungal activity of commensal bacteria living on amphibian skin is also temperature dependent (Daskin *et al*. 2014). Host species able to thermoregulate at temperatures favourable to these bacteria may benefit from improved function of defensive microbial symbionts. (4) Immune function is impaired at low temperatures and is expected to increase near ectotherm hosts' thermal optima (Raffel *et al*. 2006; Rollins-Smith *et al*. 2011); thus, host species with high thermal tolerances (and assumed corresponding optima) may be better able to maximise immune function at body temperatures that are also costly to *Bd*, thereby lowering infection risk or disease severity compared to species with low thermal tolerances (Rohr *et al*. 2013).

Although temperature is a primary driver of *Bd* infection distribution and severity, mismatches in host and pathogen tolerances for other environmental variables could also affect disease risk (e.g. Stockwell *et al*. 2015). We tested the potential applicability of the tolerance mismatch model to other environmental gradients by determining whether a composite metric of environmental tolerance – host sensitivity to habitat modification (SHM) – also predicts *Bd* infection prevalence. Species with low SHM are able to tolerate conversion of natural habitat to human land uses (Materials and Methods); therefore, SHM likely encompasses multiple tolerance axes,

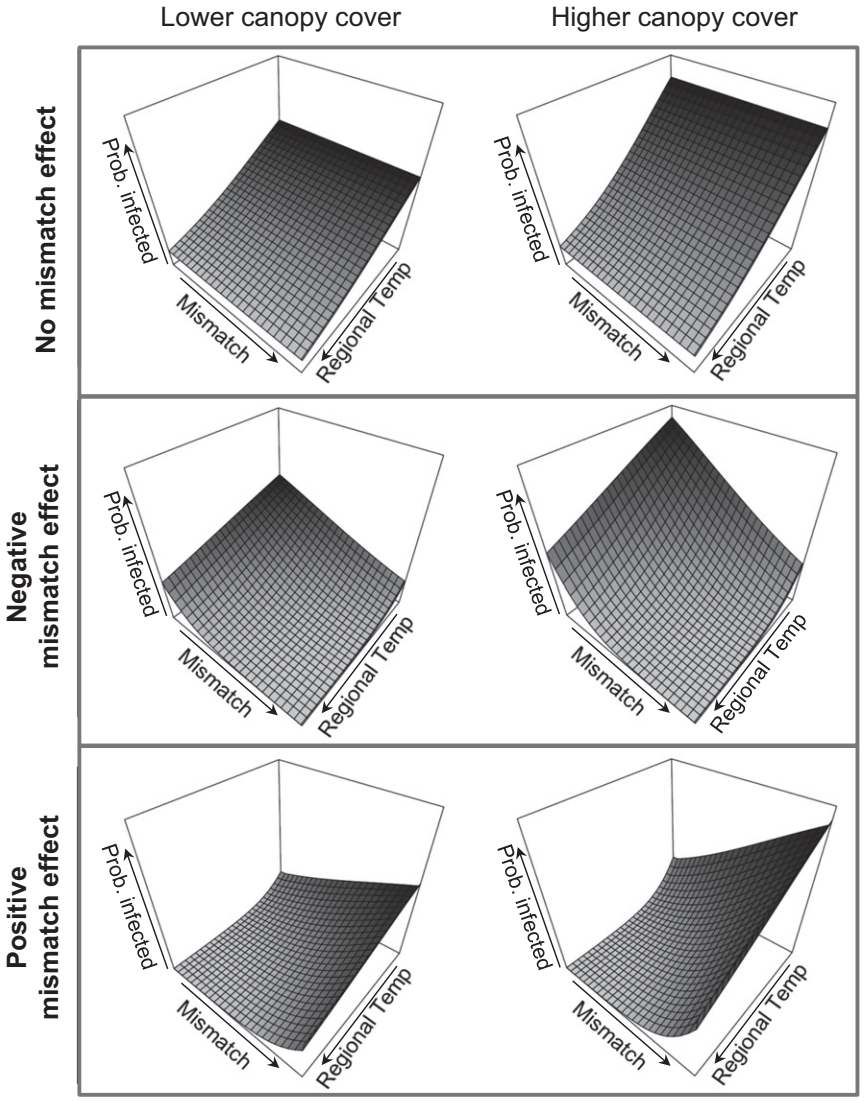

Figure 2 Hypothetical response surfaces showing predicted relationships among infection probability, mismatch in host and pathogen thermal tolerances ('mismatch'), and the thermal environment (regional temperatures and local canopy cover). Under a scenario with 'no mismatch effect', we would expect regional temperature and canopy cover to be the primary drivers of infection probability, with little or no change in infection probability with increasing mismatch in host and pathogen thermal tolerances. The 'negative mismatch effect' panel represents the tolerance mismatch hypothesis: infection probability is expected to decrease with increasing mismatch in host and pathogen thermal tolerances under most/all environmental conditions, as host species are able to access thermal niche space unavailable to the pathogen. We also expect that the magnitude of this effect will depend on environmental context. For example, infection probability may be highest for species with narrow mismatches that occur in regions with cool temperatures favourable for *Bd* growth; within a given region, infection probability is generally expected to be lower in areas of low canopy cover (warmer local temperatures) than in areas of high canopy cover (cooler local temperatures). The 'positive mismatch effect' panel represents an alternative expectation in which infection probability increases with increasing degree of mismatch in host and pathogen thermal tolerances, as species with narrow tolerance mismatches are less able to tolerate infection and quickly succumb to disease. This scenario predicts a lower probability of *observing* infection in host species most susceptible to disease (i.e. those with the narrowest tolerance mismatches).

which may include thermal tolerance, desiccation tolerance and/or other abiotic stress tolerances. We expected that infection prevalence would decrease with increasing host tolerance to habitat modification, as hosts become increasingly able to exploit disturbed, open canopy habitats or forest microhabitats that are suboptimal for pathogen growth.

We evaluated the relationship between host environmental tolerances and infection prevalence by combining global analyses and a local case study in Costa Rica. The aims of our global analyses were twofold. First, we tested the tolerance mismatch hypothesis by determining whether the inclusion of host tolerances (thermal tolerance mismatch or SHM) and their interaction with thermal environment better predicted infection prevalence compared with models that did not account for host tolerances. Because temperature unequivocally plays a role in *Bd* infection (Woodhams *et al.* 2003), we focused on extrinsic variables that characterise both regional temperature, which varies along latitudinal and elevational gradients, and canopy cover, which modifies temperatures locally by reducing direct solar insolation (see Fig. 1b; Pringle

et al. 2003; Raffel *et al*. 2010; Liu *et al*. 2013). Second, we compared the relative importance of tolerance variables to other intrinsic variables that have been proposed as determinants of disease susceptibility among amphibians, including larval habitat, range size and body size (Lips *et al*. 2003; Bielby *et al*. 2008; Smith *et al*. 2009; Bancroft *et al*. 2011). At the local scale we expected infection prevalence would again decrease with increasing mismatch in host and pathogen thermal tolerances because greater thermal tolerances should allow hosts to exploit microhabitats unsuitable for *Bd*, such as sunflecks and tree-fall gaps.

MATERIALS AND METHODS

Global data set of infection records

To analyse global patterns of infection, we compiled results of *Bd* assays from the *Bd*-Maps online database (http://www.bd-maps.net), accessed on 20 February 2015. We excluded records from studies of captive individuals and any for which the accuracy of sample coordinates was classified as 'vague', 'region' or 'country centroid', keeping only records where coordinates reflected site-specific sample locations (Fig. S1). We compiled infection status data for species for which we had corresponding data on CT_{max} or SHM (see below).

Species traits and environmental tolerances

For global analyses, we obtained amphibian CT_{max} data from a published database (Sunday *et al*. 2014). All CT_{max} values reflected loss of motor function as the experimental endpoint after slowly ramping body temperatures. We excluded CT_{max} values from experiments where individuals were acclimated at cold temperatures (5–10 °C), because these acclimation temperatures are near the CT_{min} of some species and bias downward estimates of CT_{max} (Fig. S2). Amphibian CT_{max} is not strongly associated with latitude or elevation (Sunday *et al*. 2014), reducing potential for biases in CT_{max} associated with biogeographic gradients. To quantify species SHM, we extracted data from 41 published field surveys that reported species relative abundances in natural habitats and adjacent altered habitats (i.e. sites within the same landscape; detailed methods in Thompson *et al*. 2016). We then classified species as natural habitat specialists, generalists, or disturbed habitat specialists using a multinomial model (Chazdon *et al*. 2011). The model classifies a species as a natural habitat specialist, for example, if two thirds or more of individuals are recorded from natural habitat. Using only species that were classified with confidence (at $P = 0.005$; Chazdon *et al*. 2011), we calculated the natural log of the ratio of species relative abundance in natural habitat and altered habitat (i.e. an effect size) as a quantitative index of species SHM. Larval habitat, range size and maximum body size (snout-to-vent length [SVL]) were obtained from the IUCN Red List database (http://www.iucnredlist.org) and species accounts on AmphibiaWeb (http://amphibiaweb.org). For the global analyses, we classified species into three larval habitat types: (1) lentic and aquatic generalists, (2) lotic and (3) terrestrial (e.g. direct developers). For the Costa Rica analysis, we classified larval habitat as either terrestrial or aquatic so that each category was represented by a sufficient number of species in the smaller, local-scale data set. We calculated range sizes (km^2) from IUCN range maps. Other demographic variables, such as clutch size, have been correlated with *Bd*-related declines (Bielby *et al*. 2008). However, we did not include clutch size because there is no obvious mechanism linking clutch size to *Bd* infection prevalence in adult amphibians, even though this trait may influence a species' propensity to decline as a result of disease.

Regional temperatures and local canopy cover

We considered multiple regional temperature variables in our global analyses that represent different measures of the density distributions shown in Fig. 1b; these included mean maximum temperature of the warmest month and mean annual temperature, which we extracted at a spatial resolution of 30 arc seconds (~ 1 km^2) from the WorldClim data set (Hijmans *et al*. 2005) in ArcGIS for each *Bd* sample location. We also calculated the standard deviation of mean maximum monthly temperatures to characterise annual temperature variation (i.e. seasonality). Other extrinsic variables, such as elevation and latitude, have been correlated with *Bd* prevalence in the literature, but these are almost certainly surrogates for regional temperature variation, which directly affects pathogen physiology and infection risk (Fig. 1b; Austin 2007; James *et al*. 2015). Because canopy cover modifies local temperatures by reducing solar insolation (Fig. 1b), we characterised local canopy cover using a high-resolution (30 m) global forest cover data set developed from Landsat data covering 2000–2012 (Hansen *et al*. 2013); we calculated the average canopy cover within 500 m circular buffers centred on each locality using the 'raster' package (Hijmans 2015) in R (version 3.1.2, R Core Team 2014).

Disease assays and measurement of host thermal tolerances in Costa Rica

We collected skin swabs from common amphibian species within the 1500 ha La Selva Biological Station reserve in north-eastern Costa Rica during November–March in 2007, 2008 and 2011. We sampled animals for *Bd* presence by applying a standardised number of swab strokes on the limbs, venter and dorsum of each animal (Whitfield *et al*. 2012). We extracted DNA from swabs using Qiagen DNeasy Blood and Tissue kits and measured zoospore equivalents using standard quantitative polymerase chain reaction methods (Boyle *et al*. 2004; Kerby *et al*. 2013). We ran all samples in triplicate. Additional details of methods are described in Whitfield *et al*. (2012, 2013).

We measured CT_{max} for 226 individuals of 16 frog species from eight families in Costa Rica. In 2011, we captured frogs at La Selva Biological Station reserve and transported them to an on-site shaded ambient-air laboratory. Frogs were kept in plastic containers at ambient temperatures for 2–24 h prior to CT_{max} trials. We measured CT_{max} by placing individuals in water baths and increasing water temperature from ambient (~ 25 °C) at a rate of ~ 0.5 °C/min (Catenazzi *et al*. 2014). Water temperature was measured using a thermocouple. While in water baths, we flipped frogs onto their backs every 60 s until

they exhibited loss-of-righting reflex for 5 s. We recorded the temperature at which frogs exhibited loss-of-righting reflex as the CT_{max}. Directly following the trial, we transferred frogs to ambient-temperature water baths before being released.

Statistical analyses

Some measures of regional temperature were highly correlated (Figs S3 and S4). Because the strength of temperature effects on infection prevalence may vary among different temperature measures (e.g. maximum vs. mean temperature), we conducted separate sets of analyses for each measure to evaluate robustness of our global analyses to different temperature variables. Other predictor variables exhibited weak–moderate correlations that did not meet thresholds for multicollinearity (Figs S3 and S4; Zuur *et al*. 2009). There was little overlap between species records in *Bd*-Maps for which we could obtain values of host CT_{max} and SHM, precluding inclusion of both variables in the same models. Therefore, we first analysed all infection assays from the *Bd*-Maps database for which we had information on host CT_{max}. In these analyses, we calculated 'thermal tolerance mismatch' as the difference between the CT_{max} of each host and that of *Bd*, assuming a CT_{max} of 30 °C for *Bd* (Piotrowski *et al*. 2004). We then repeated all analyses on a second set of host species from the *Bd*-Maps database for which we were able to estimate SHM. We were unable to calculate a similar SHM mismatch index because SHM for *Bd* is unknown. However, assuming a constant SHM for *Bd*, the mismatch between host and *Bd* SHM will have a 1:1 relationship with host SHM.

For the global data set, we modelled the probability of *Bd* infection in response to intrinsic and extrinsic factors by fitting generalised linear mixed models (GLMM) representing aggregated binomial regressions (McElreath 2016) in R (version 3.1.2, R Core Team 2014). We fit models using the 'glmer' function in package 'lme4' (Bates *et al*. 2014). The response consisted of the number of infected and uninfected individuals at a given sample location (1240 total sample locations) for a given species (53 total species); observations, therefore, represented species-by-site prevalence records ($n = 1645$) weighted in the model by the number of *Bd* assays conducted for each species-by-site record (11 435 total assays). We fit all models with varying intercepts among contributing studies and among species nested within families and compared models fit with competing fixed effects structures. We first fit an intercept only model and a full model with regional temperature, canopy cover, thermal tolerance mismatch, geographic range size, larval habitat and body size all included additively. We then fit models with each single predictor variable, all intrinsic variables grouped (thermal tolerance mismatch, range size, larval habitat and body size), all pairwise combinations of intrinsic variables, extrinsic variables grouped (regional temperature and canopy cover) and models that specifically represented the tolerance mismatch hypothesis (thermal tolerance mismatch, regional temperature and canopy cover). Finally, we fit models with interaction terms for intrinsic (all two-way interactions), extrinsic (two-way interactions) and the tolerance mismatch hypothesis (three-way interactions) predictor sets. A total of 25 competing models were compared in each of six model sets (each model set was constructed separately for all combinations of temperature and tolerance metrics used). We standardised all continuous predictor variables prior to analysis and ranked competing models using AICc. As a measure of model fit, we calculated conditional R^2_{GLMM} (Nakagawa & Schielzeth 2013). To evaluate relative single-variable importance, we fit all possible additive subsets of the full model and calculated the cumulative Akaike weights for models containing each variable. Separate model sets were constructed and evaluated as described above for species in the *Bd*-Maps database that had corresponding information on SHM, which included analyses of 1111 species-by-site prevalence records (at 754 unique sites) for 73 species.

To evaluate the importance of single intrinsic factors in explaining *Bd* prevalence among species at a continuous forest site in Costa Rica, we compared generalised linear models (GLMs) fit with thermal tolerance mismatch, body size (SVL), larval habitat and geographic range size as predictors. In the local-scale analysis, we did not include a random effect of species, because prevalence observations in the data set were at the species level and we did not consider interactions among variables because of small sample size ($n = 16$ species observations). As a measure of model fit, we calculated pseudo-R^2 for top-ranked GLMs based on likelihood ratios. We validated the GLMs by comparing results with models that accounted for phylogenetic relationships among species, fitting phylogenetic generalised estimating equations (GEE) with the 'compar.gee' function in package 'ape' (Paradis *et al*. 2004). We obtained branch lengths for the focal taxa from a time-calibrated amphibian phylogeny that included 2871 extant taxa (Pyron & Wiens 2011). Two of 16 species in the Costa Rica data set were not present in the tree, so we substituted branch lengths for these species with those of sympatric congeneric species (Fig. S5). We first tested for phylogenetic signal associated with thermal tolerance mismatch (i.e. species CT_{max}) by comparing a star phylogeny ($\lambda = 0$) to a λ-transformed phylogeny representing the level of divergence, given tolerance mismatch, expected under a Brownian motion model. Values of λ near zero indicate trait values are independent of phylogenetic relatedness whereas λ values near one indicate strong phylogenetic signal. Significance of the λ transformation was assessed using a likelihood ratio test. We then fit phylogenetic GEEs to each independent variable while incorporating phylogenetic correlation structure; the variance–covariance matrix was derived from a Brownian motion model of trait evolution based on the community phylogeny. A binomial distribution was specified, and species observations were weighted by the number of individuals sampled. We ranked competing models using a quasi-likelihood information criterion.

RESULTS

Host environmental tolerances as predictors of global patterns of *Bd* infection

In the global-scale model set that included thermal tolerance mismatch as a predictor variable, our analysis included 11 435 *Bd* assays from 53 species across five continents. Irrespective of the regional temperature variable considered, models that included interactions among thermal tolerance mismatch,

regional temperature and local canopy cover performed better than all other models (Akaike weight ≥ 0.987; R^2_{GLMM} = 0.66–0.70, depending on the regional temperature variable; Fig. 3, Table S1). Effect sizes from the full-additive and top-ranked models are presented in Fig. S6 and Table S2 respectively. The next best models included only the interaction between regional temperature and canopy cover and had far less support than the top models (ΔAICc = 9–35; Akaike weight ≤ 0.013; R^2_{GLMM} = 0.65–0.67; Table S1). Relative single-variable importance in these analyses was highest for thermal tolerance mismatch and regional temperature (Fig 3e). A single model fit only with thermal tolerance mismatch predicted a 60% decrease in odds of infection with each one standard deviation increase in thermal tolerance mismatch (β = −0.905, SE = 0.266; R^2_{GLMM} = 0.66; Fig. 3c). In comparison, a model fit only with regional temperature (mean maximum temperature of the warmest month) predicted a 9% decrease in odds of infection with each one standard deviation increase in temperature (β = −0.091, SE = 0.061; R^2_{GLMM} = 0.65). Although *Bd* is a host-generalist pathogen, infecting approximately 42% of species assayed, some amphibian families have been reported as under- or over-infected compared to random expectations (Olson *et al*. 2013). Further exploration of infection patterns within families showed that infection prevalence decreased with increasing thermal tolerance mismatch in most well-sampled amphibian families (Fig. S7), including families previously reported as under- or over-infected. Plethodontid salamanders were an exception to this pattern, exhibiting a weak, slightly positive relationship between thermal tolerance mismatch and infection prevalence; this result may be attributable to the low overall infection prevalence observed here (~ 0–15%) and in general across plethodontid species compared with infections in anurans or other salamanders (Rothermel *et al*. 2008).

In the global-scale analysis evaluating host SHM as a predictor variable, the data set was composed of 8749 *Bd* assays of 73 host species across six continents. Again, models that included interactions among environmental tolerance (SHM), regional temperature and canopy cover performed far better than all others (Akaike weight = 0.999; R^2_{GLMM} = 0.61–0.64, depending on temperature variable; Tables S3 and S4). A model fit only with SHM indicated that the odds of infection increased by 25% with each one standard deviation increase in sensitivity of host species to habitat modification (i.e. with decreased tolerance; β = 0.225, SE = 0.239; R^2_{GLMM} = 0.58). However, relative variable importance was lower for SHM than for extrinsic variables and was comparable to importance of other intrinsic variables (Figs S6 and S8). High Akaike weights assigned to top models – for both thermal tolerance mismatch and SHM model sets – indicate that our results are robust to different subsets of the global database and that interactions among host environmental tolerances and environmental variables generally improved model performance relative to other models.

Host thermal tolerance as a predictor of local patterns of *Bd* infection

Within the Costa Rican assemblage, the model fit with thermal tolerance mismatch was best supported and predicted a 39% decrease in the odds of infection with each one standard deviation increase in thermal tolerance mismatch (Akaike weight = 0.87; GLM β = −0.4881, SE = 0.181; pseudo-R^2 = 0.41; Fig. 4). There was a strong phylogenetic signal associated with thermal tolerance mismatch (λ = 1.23; $P < 0.001$); however, after accounting for phylogenetic relationships among species, thermal tolerance mismatch was again the best-supported variable explaining *Bd* prevalence (phylogenetic GEE β = −0.583, SE = 0.290; Table S5). In the Costa Rican assemblage, direct-developing species of the family Craugastoridae had the lowest thermal tolerances and among the highest infection prevalences.

DISCUSSION

A key finding from this work is that host tolerance – especially thermal tolerance – appears to be an important intrinsic variable explaining variation in species susceptibility to *Bd* infection. The relationship between the mismatch in host and pathogen thermal tolerances and infection prevalence is generalisable across multiple spatial scales. At the global scale, accounting for thermal tolerance mismatch considerably improved model performance compared to models that included only temperature and canopy cover as well as those fit with intrinsic variables previously identified as important determinants of infection (Bancroft *et al*. 2011; James *et al*. 2015). Moreover, the negative relationship between thermal tolerance mismatch and infection prevalence was consistent across most well-sampled amphibian families.

Environmental context modified the strength of environmental tolerance effects on *Bd* infection prevalence. The interaction between thermal tolerance mismatch and regional temperature, for example, depended on the amount of local canopy cover, but this relationship was more complex than we expected (Figs 2 and 3b). Specifically, under high canopy cover, infection prevalence decreased for host species with narrow thermal tolerance mismatches as regional temperatures decreased, possibly reflecting reduced environmental suitability for *Bd* at cold temperatures (and consequently, decreased infection risk; Liu *et al*. 2013), a scenario also illustrated in the upper left panel of Fig. 1b.

One of the most notable results of the global analysis was that mean infection prevalence decreased steeply with increasing thermal tolerance mismatch under most environmental contexts (Fig. 3), supporting the inference that thermal tolerance mismatches allow species to avoid or abate infection by accessing thermal niche space that enhances immune function and/or is detrimental to the pathogen. We did not find strong support for the alternative expectation of increased *Bd* prevalence with increasing thermal tolerance mismatch (Fig. 2). However, we cannot rule out the possibility that this relationship could arise under some circumstances, which may have contributed to unexplained variation in the global data set and the weak, positive relationship between tolerance mismatch and prevalence observed for plethodontid salamanders (Fig. S7). Instead, the analysis generally supports the conclusion that under many environmental contexts, amphibians with high thermal tolerances are able to avoid or clear infections, a phenomenon that likely contributes to the observed

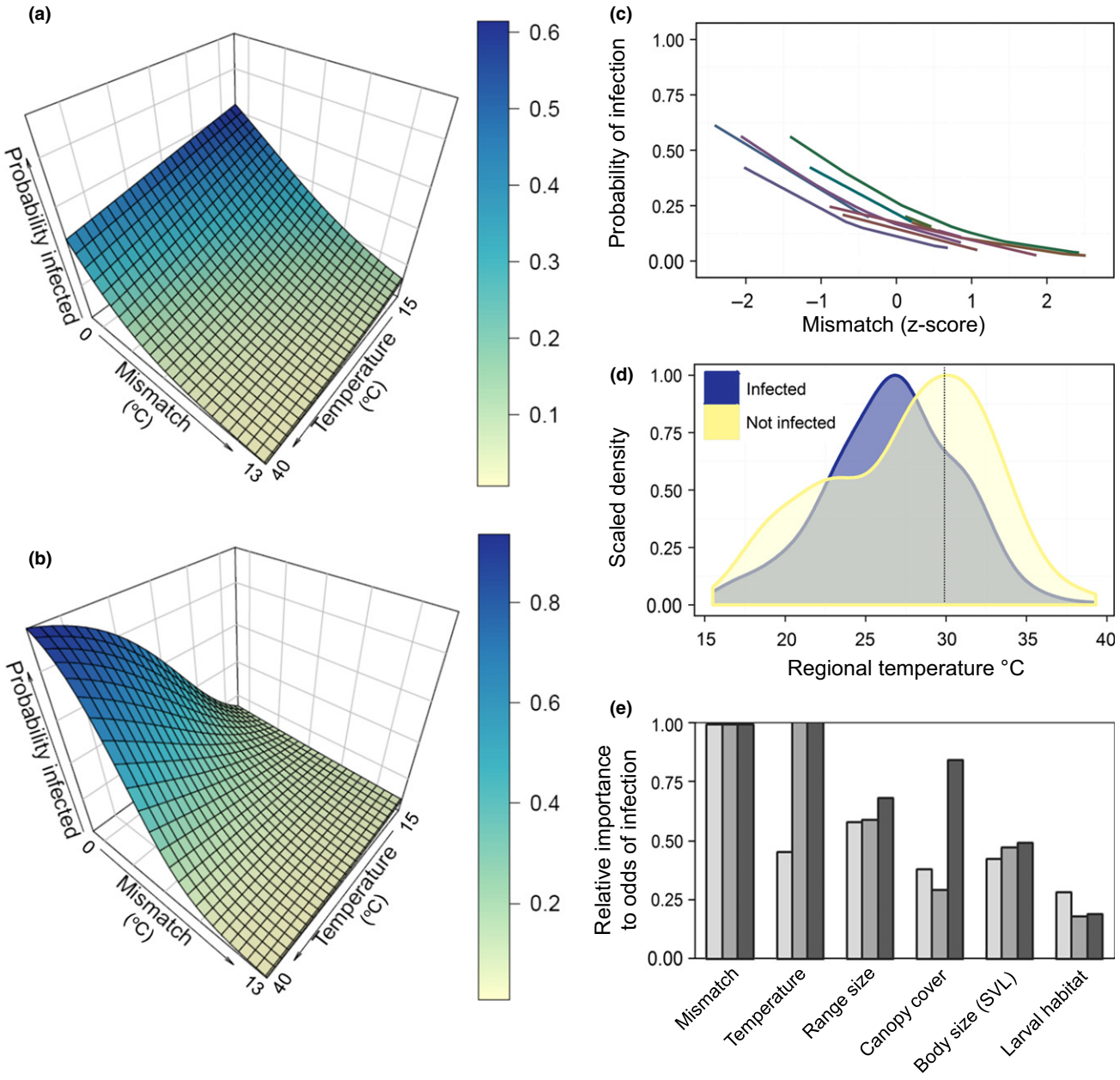

Figure 3 Effects of thermal tolerance mismatch and environmental context on infection risk from a global-scale analysis. Predicted probability of *Batrachochytrium dendrobatidis* (*Bd*) infection as a function of thermal tolerance mismatch ('Mismatch') and mean maximum temperature of the warmest month ('Temperature') in areas with low (a; 30%) and high (b; 90%) canopy cover. Thermal tolerance mismatch was calculated as the difference between the amphibian host's and *Bd*'s upper thermal limits. Estimates were generated from a generalised linear mixed model (an aggregated binomial regression) that included the interactions among three predictor variables: thermal tolerance mismatch, regional temperature and canopy cover (model 'Tolerance mismatch B' in Table S1). The model included varying intercepts among studies and among species nested within families. (c) Predicted probability of infection in relation to thermal tolerance mismatch for different amphibian families included as varying intercepts. (d) Density plot of mean maximum temperature of the warmest month grouped by infected and non-infected records from *Bd*-Maps (vertical line represents upper thermal limit for *Bd* growth in culture; Piotrowski *et al.* 2004). (e) The relative variable importance to the odds of infection calculated as cumulative Akaike weights of models containing each variable from all possible additive models. Separate bars are shown for model sets that included mean maximum temperature of the warmest month (light grey), mean annual temperature (medium grey), or the standard deviation of monthly maximum temperatures (dark grey).

variation in species susceptibility to declines from the global chytridiomycosis pandemic (Vredenburg *et al.* 2010; Scheele *et al.* 2014).

At the local scale, thermal tolerance mismatch was again a better predictor of infection prevalence than other intrinsic host characteristics, even though these species were sampled under relatively uniform environmental conditions at the same site (Fig. 4, Table S5). At a given site within a region, the ability of host species to exploit warm microhabitats, an ability constrained by thermal tolerance, may be a more

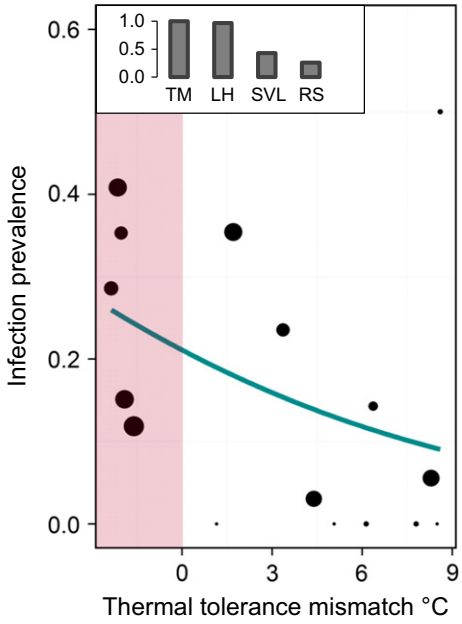

Figure 4 Relationship between *Batrachochytrium dendrobatidis* (*Bd*) infection prevalence and thermal tolerance mismatch in 16 amphibian species at La Selva Biological Station in Costa Rica. Thermal tolerance mismatch was calculated as the difference between the amphibian host's and *Bd*'s upper thermal limits. Trend line indicates the mean relationship between infection prevalence and thermal tolerance mismatch, weighted by sample size for each species. Point size is proportional to the number of individuals assayed of each species. Shaded area represents temperatures at which *Bd* is known to grow in culture. Inset shows the relative variable importance to odds of infection of thermal tolerance mismatch (TM), larval habitat (LH), mean snout–vent length (SVL) and geographic range size (RS).

important driver of variable infection risk among hosts than their use of aquatic habitats (Catenazzi *et al*. 2014), a life-history trait previously linked to elevated infection risk (Kriger & Hero 2007; James *et al*. 2015). In Costa Rica, the highest infection rates were found in the direct-developing family Craugastoridae, which contains species with the lowest thermal tolerances. Terrestrial frogs from the family Craugastoridae are among the most rapidly declining amphibian species in northeastern Costa Rica (Whitfield *et al*. 2007) and declines are likely attributable, in part, to the group's sensitivity to seasonal *Bd* outbreaks (Whitfield *et al*. 2012).

Multiple mechanisms likely underlie the observed effect of tolerance mismatches, including complete avoidance of infection through habitat selection (Catenazzi *et al*. 2014), clearance of infection through behavioural fever (Richards-Zawacki 2010; Rowley & Alford 2013), temperature-dependent metabolite production by the skin microbiome (Daskin *et al*. 2014) and temperature-dependent immune function (Raffel *et al*. 2006; Rollins-Smith *et al*. 2011). Although our study provides evidence for the importance of thermal tolerance mismatches in infection dynamics, experimental work is required to disentangle the relative importance of potential mechanisms underlying this phenomenon. Low host thermal tolerances could place constraints on the efficacy of any temperature-dependent mechanism, and it is possible that multiple mechanisms operate synergistically to influence infection probability and severity in the amphibian–*Bd* system.

Although we focused primarily on the mismatch between host and pathogen thermal tolerances, our analyses of SHM suggest that the tolerance mismatch hypothesis may extend to other environmental gradients. Species that are highly tolerant of habitat modification had lower infection prevalence; these host species are habitat generalists that may have broad ecological niches along multiple environmental gradients, including salinity, temperature and precipitation. Precipitation, for example, has also been identified as a predictor of the global distribution of *Bd* (James *et al*. 2015), and it is possible that species-specific tolerances to drought and desiccation could allow some host species to occupy environmental space unsuitable for *Bd* (Puschendorf *et al*. 2011). Unfortunately, there is currently little information on interspecific differences in desiccation tolerances with which to evaluate a similar tolerance mismatch model. The present work, however, builds on numerous studies that demonstrate the unambiguous role of temperature as a determinant of *Bd* infection risk in amphibians (Woodhams *et al*. 2003; Raffel *et al*. 2010; Liu *et al*. 2013; Menendez-Guerrero & Graham 2013), providing a foundation for understanding how host thermal tolerances can restrict opportunities for avoiding or abating disease.

Our results show how the tolerance mismatch model can be applied to the amphibian–*Bd* system to explain patterns of infection prevalence across multiple spatial scales, families and environmental contexts. Because temperature, in particular, often affects disease dynamics in ectothermic hosts, it is possible that the tolerance mismatch model may apply more broadly to host–pathogen interactions in many ectotherm groups. For instance, when desert locusts (*Schistocerca gregaria*) were experimentally infected with the host-generalist entomopathogenic fungus *Metarhizium anisopliae*, locusts that were allowed to thermoregulate at temperatures above the pathogen's thermal limit had greater survival and inhibited pathogen growth (Elliot *et al*. 2002). Whether other insect hosts can thermoregulate at temperatures that inhibit the growth of *M. anisopliae*, however, likely depends on the degree to which their thermal tolerances exceed that of the pathogen. Similarly, variation in host thermal tolerances may contribute to observed variation among host fishes in infection prevalence of the fungus-like generalist pathogen *Aphanomyces invadans*, outbreaks of which often occur when environmental temperatures decrease (Lilley *et al*. 2002). To date, the tolerance mismatch hypothesis, as it pertains to multiple host species, has yet to be examined in other ectotherm groups. Our analyses of *Bd* infection prevalence among many amphibian species build on case studies that focused on infection dynamics in single insect host species (Elliot *et al*. 2002; Higes *et al*. 2010). Taken together, these findings suggest that the mismatch between host and pathogen environmental tolerances, primarily mediated by temperature, may constitute a fundamental mechanism affecting susceptibility to disease in ectotherms.

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AUTHOR CONTRIBUTIONS

AJN conceived and designed study, compiled data, analysed data and drafted manuscript. SMW conceived and designed study, collected data, conducted laboratory work and edited manuscript. EAE compiled data, helped analyse data and helped write manuscript. MET and JPR compiled data, contributed to analyses and edited manuscript. BLC collected field data and edited manuscript. JLK and MAD directed Costa Rica work, helped fund laboratory work and edited manuscript. BDT conceived and designed study, directed global analyses and helped write manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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