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Disease and thermal acclimation in a more variable and unpredictable climate

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Global climate change is shifting the distribution of infectious diseases of humans and wildlife with potential adverse consequences for disease control[1–4]. As well as increasing mean temperatures, climate change is expected to increase climate variability[5,6], making climate less predictable. However, few empirical or theoretical studies have considered the effects of climate variability or predictability on disease, despite it being likely that hosts and parasites will have differential responses to climatic shifts[6,7]. Here we present a theoretical framework for how temperature variation and its predictability influence disease risk by affecting host and parasite acclimation responses. Laboratory experiments conducted in 80 independent incubators, and field data on disease-associated frog declines in Latin America[6], support the framework and provide evidence that unpredictable temperature fluctuations, on both monthly and diurnal timescales, decrease frog resistance to the pathogenic chytrid fungus *Batrachochytrium dendrobatidis*. Furthermore, the pattern of temperature-dependent growth of the fungus on frogs was opposite to the pattern of growth in culture, emphasizing the importance of accounting for the host–parasite interaction when predicting climate-dependent disease dynamics. If similar acclimation responses influence other host–parasite systems, as seems likely, then present models, which generally ignore small-scale temporal variability in climate[7], might provide poor predictions for climate effects on disease.

Changes in mean temperature and precipitation can dramatically influence disease outcomes, particularly for parasites with ectothermic hosts and vectors[2,3,8]. As well as affecting mean temperature and precipitation, climate change is expected to alter the variability of these factors[5,6,9,10]. However, few studies have investigated the effects of climatic variability on parasitism[7,11–13] or on species interactions in general[14,15]. Predictive disease models typically use monthly or annual means in climatic factors, ignoring their ubiquitous shorter-term fluctuations[7,16]. This approach can miss important biological effects caused by nonlinear responses of parasites and hosts to temperature[16]. Furthermore, climate–disease models are typically parameterized using data from constant-temperature experiments, ignoring potentially important parasite and host acclimation responses to temperature shifts. An acclimation response, a form of phenotypic plasticity, is a reversible change of a physiological trait in response to an environmental change[17]. Acclimation responses to temperature are pervasive and have important effects on ectotherm physiology, including the immune system[17–19]. Yet the degree to which acclimation responses to climatic variability affect host–parasite interactions is unknown[19].

We propose two primary hypotheses to predict how thermal acclimation responses will influence parasitism. First, we hypothesize that unpredictable temperature shifts will increase parasite growth rates on or in hosts, because parasites probably acclimate to temperature shifts more quickly than their hosts owing to their smaller sizes and thus faster tissue-specific metabolisms[20]. This might allow parasites to exploit periods of suboptimal host immunity following unpredictable temperature shifts[6,19]. Second, we hypothesize that both hosts and parasites can anticipate and acclimate to predictable (for example, diurnal or seasonal) temperature fluctuations[17], such that both will perform better (that is, have higher parasite infectivity and host resistance) when temperature is predictable than when it is unpredictable. Here, infectivity refers to a parasite's intrinsic rate of population growth on a host in the absence of host resistance; host resistance refers to the effectiveness of the host immune system and/or behavioural mechanisms at reducing parasite growth.

Based on these two hypotheses, we developed a theoretical framework (illustrated in Fig. 1) to provide qualitative predictions for how the timescale and predictability of temperature variation should influence the population growth rate of a microparasite in or on an individual host. We first assumed that both parasite infectivity and host resistance increase through time following a shift in temperature until they reach an asymptotic, fully acclimated condition (Supplementary Methods and Fig. S1). We also assumed that parasites acclimate to new temperatures faster than their hosts (Supplementary Methods). Based on these assumptions, we predicted peak parasite growth rates when temperature shifts occur at an intermediate frequency, that is, when there is sufficient time for acclimation of parasite infectivity but not for host resistance before the next temperature shift (Fig. 1 and Supplementary Fig. S1). Longer time periods between temperature shifts (infrequent shifts) are predicted to reduce pathogen growth rates (Fig. 1 and Supplementary Fig. S1). On very short timescales (that is, time between shifts approaching 0), neither host nor parasite has time to acclimate to each new temperature (Fig. 1 and Supplementary Fig. S1). In the absence of parasite and host acclimation responses, the effects of temperature variability on parasite population growth should depend only on the relative magnitudes and breadths of the thermal performance curves for parasite infectivity and host resistance. Increased predictability of temperature fluctuations might benefit either the parasite or host, depending on their relative abilities to anticipate and acclimate to predictable temperatures (Fig. 1).

To test predictions of this theoretical framework, we conducted three controlled-temperature experiments on the Cuban

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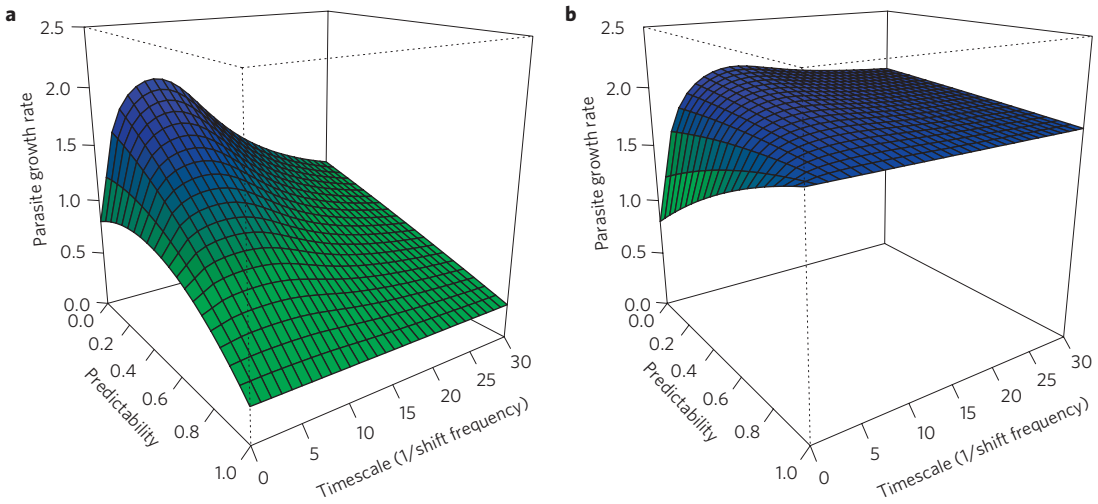

Figure 1 | Graphical representation of the temperature variability hypothesis. Graphs show qualitative theoretical predictions for microparasite population growth ($G(t)$) when temperature shifts occur at different frequencies or different levels of predictability, ranging from $B = 0$ (completely unpredictable) to $B = 1$ (completely predictable). **a**, Predictions when the host's ability to anticipate and respond to predictable temperatures (for example, constant or diurnal) is high relative to that of the parasite ($r(t_\infty) = 0.9$). **b**, Predictions when the host's ability to respond to predictable temperatures is low relative to that of the parasite ($r(t_\infty) = 0.5$). The modelling methods used to generate these graphs are available in the Supplementary Methods.

treefrog (*Osteopilus septentrionalis*) and the pathogenic chytrid fungus (*B. dendrobatidis*), using up to 80 separately controlled incubators (Supplementary Fig. S2) to ensure independent replication of temperature treatments. The Cuban treefrog was selected as a model host because it is susceptible to *B. dendrobatidis* infection, is widely available in Florida where this work was conducted and is found in the tropics, a region with widespread amphibian declines. The first two experiments compared how amphibian–*B. dendrobatidis* interactions respond to a constant temperature versus an unpredictable temperature shift, whereas the third experiment compared predictable versus unpredictable temperature shifts. *B. dendrobatidis* infects amphibian skin and has been implicated in hundreds of amphibian declines and extinctions worldwide, making it a greater threat to global biodiversity than any other known pathogen[6,21]. It has a generation time of four to ten days depending on temperature[22] and, relative to its amphibian hosts, it is smaller in size, faster in metabolism and has fewer cells and processes to adjust following a temperature shift[20,23]. Hence, *B. dendrobatidis* probably acclimates to a temperature shift more quickly than amphibians, for which acclimation of the immune system is thought to take three to six weeks[19]. Given this biology, we predict that *B. dendrobatidis* growth should be greater on frogs following an unpredictable temperature shift than on frogs already acclimated to the new temperature.

To test this first prediction, we conducted two experiments in which we acclimated Cuban treefrogs to one of two temperatures (15 °C or 25 °C) for four weeks, switched half the replicates to the other temperature and then exposed frogs to *B. dendrobatidis* or control inoculations. We then compared resistance to infection of acclimated and unacclimated frogs by measuring *B. dendrobatidis* growth on frogs two weeks following *B. dendrobatidis* exposure. We conducted separate experiments on wild-caught adults (acclimation experiment 1) and captive-reared juveniles (acclimation experiment 2), because frog size is a negative predictor of *B. dendrobatidis*-induced mortality and thus might influence thermal effects on *B. dendrobatidis* susceptibility[24]. A 10 °C shift approximates the largest change in mean temperature that can be expected from one week or month to the next in the area where frogs were collected (Supplementary Table S1). However, these experiments were designed to test whether frog acclimation increases resistance to infection, rather than to match common field conditions where sustained shifts from one constant temperature to another are rare.

As predicted, adult frogs exposed to a temperature decrease had significantly higher levels of *B. dendrobatidis* two weeks post infection than frogs already acclimated to this temperature (acclimation experiment 1, Fig. 2a and Supplementary Tables S2 and S3). However, there was no significant acclimation effect following a temperature increase (Fig. 2a and Supplementary Table S3). Juvenile frogs had a similarly significant acclimation effect following a temperature decrease, again with no significant acclimation effect following a temperature increase (acclimation experiment 2, Fig. 2b and Supplementary Tables S2 and S3). Thus, both experiments found evidence of a stronger acclimation effect following a drop in temperature than following an increase in temperature. Although acclimation experiment 1 revealed no acclimation effects on frog mortality, juvenile frogs in acclimation experiment 2 experienced higher *B. dendrobatidis*-induced mortality following a temperature decrease (Fig. 2c,d and Supplementary Tables S4 and S5). It is unclear why acclimation influenced frog survival in one experiment and not the other (Supplementary Notes and Discussion). Nevertheless, these results suggest that acclimation effects might facilitate *B. dendrobatidis*-induced amphibian population declines when month-to-month temperature variability is higher than normal, either by increasing *B. dendrobatidis* transmission through increased zoospore production, or by directly increasing juvenile frog mortality.

Consistent with this assertion was the previous finding that month-to-month variation in temperature was the best annual climatic predictor of *B. dendrobatidis*-related frog declines of Latin American *Atelopus* spp. (ref. 6). This suggests that climatic variability might indeed have exacerbated *B. dendrobatidis* outbreaks in the wild. However, this earlier analysis did not distinguish between monthly increases and decreases in temperature. Based on our finding that acclimation effects were stronger with decreases than with increases in temperature (Fig. 2b), we postulated that the original association between frog declines and monthly temperature shifts might have been driven more by monthly drops in temperature than by monthly increases in temperature. We therefore reanalysed the *Atelopus* data, testing separately for effects of monthly decreases in temperature

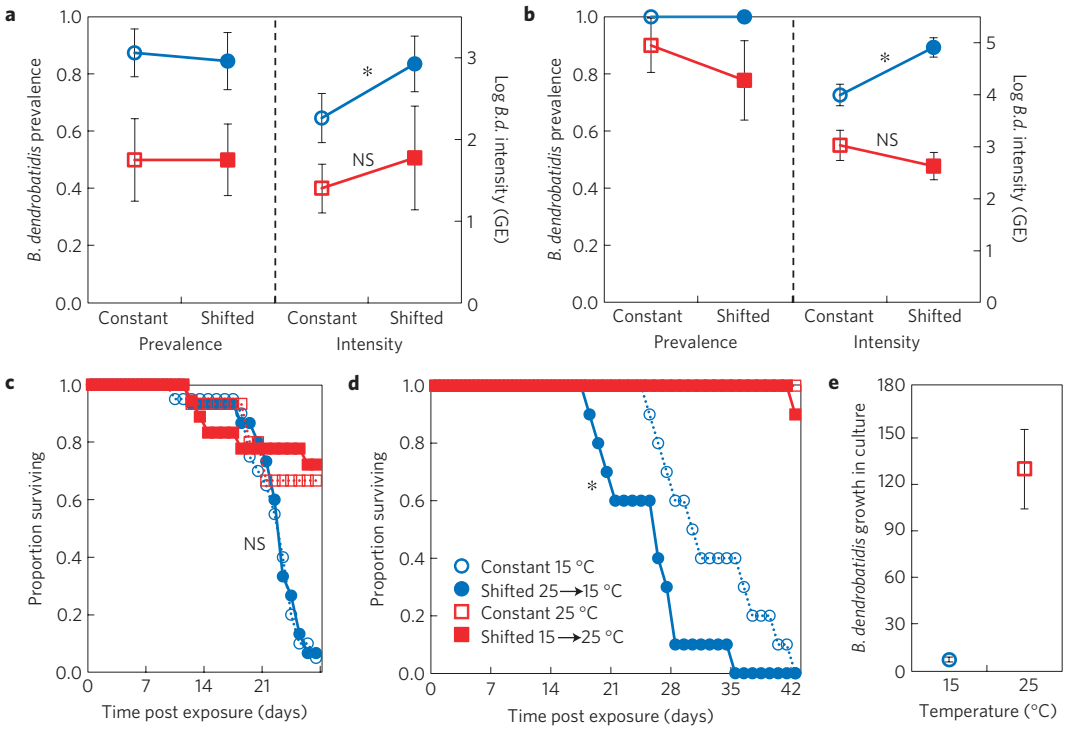

Figure 2 | Evidence for increased susceptibility to infection following a shift in temperature. Graphs **a**–**d** show the effects of exposure temperature (circles, 15 °C; squares, 25 °C) and a temperature shift (filled symbols, shifted; open symbols, constant) on *B. dendrobatidis* (*B.d.*) growth and frog mortality in the first (**a**,**c**,**e**: adult frogs) and second (**b**,**d**: juvenile frogs) acclimation experiments. **a**,**b**, Prevalence and log intensity (zoospore genome equivalents, GE) of infection two weeks post exposure for *B. dendrobatidis*-exposed frogs. *B. dendrobatidis* intensity excludes frogs with no measurable infection. **c**,**d**, Survival of *B. dendrobatidis*-infected frogs. **e**, *B. dendrobatidis* growth in culture, measured as the seven-day proportional increase in zoosporangia. Asterisks indicate significant effects of host acclimation ($P < 0.05$; NS, not significant). Means ± s.e.m.

and monthly increases in temperature (Supplementary Methods). Average monthly decreases in temperature was a better predictor of declines than average monthly increases in temperature and was a comparable predictor to overall month-to-month variation (both results based on among-model comparisons of the Akaike information criterion, corrected for sample size; Supplementary Table S6). These results suggest that much of the relationship between monthly temperature variability and *Atelopus* declines was caused by monthly decreases in temperature rather than monthly increases, as predicted based on our experimental results. This is consistent with work demonstrating that *B. dendrobatidis* outbreaks generally occur during cool seasons[25,26] and that drops in temperature trigger the release of *B. dendrobatidis* zoospores[22] and were associated with lower than expected amphibian immune parameters in the field[19].

Although greater disease susceptibility following drops in temperature might initially seem unrelated to the increases in mean temperatures associated with global climate change, a well-documented effect of climate change is increased frequency of extreme climatic events[5,10]. In this case, by increasing maximum temperatures over the course of many years, climate change might result in greater drops in temperature occurring on shorter timescales (that is, raising the ceiling so there is farther to fall). Consequently, we hypothesized that increased annual temperatures owing to global climate change might cause increases in monthly temperature variability, including increases in the severity of monthly temperature drops. Based on our laboratory and field results, such an increase in monthly temperature drops might exacerbate *B. dendrobatidis*-related frog declines. A path analysis revealed that annual temperature, which has significantly increased in the region inhabited by *Atelopus* (Supplementary Fig. S3), is a significant positive predictor of average monthly drops in temperature (Supplementary Fig. S3a). Annual temperature was also a positive predictor of the maximum monthly drop in temperature during cool seasons (Supplementary Fig. S3b), the time of year when most amphibians succumb to chytridiomycosis[25,26]. Hence, warmer annual temperatures seem to increase the severity of monthly drops in temperature (at least in this region), which have been linked both to increased *B. dendrobatidis* growth on frogs and amphibian declines.

In our first acclimation experiment, each incubator also contained a *B. dendrobatidis* culture so we could compare temperature-dependent growth in culture to growth on frogs. In culture, *B. dendrobatidis* grew better at a constant 25 °C than 15 °C (Fig. 2e and Supplementary Table S7), opposite the pattern of *B. dendrobatidis* growth on frogs (Fig. 2a). Furthermore, *B. dendrobatidis* caused higher frog mortality at 15 °C than 25 °C, consistent with the pattern of *B. dendrobatidis* growth on frogs (Fig. 2c and Supplementary Tables S4 and S5). These results demonstrate that there was enhanced host resistance to infection at the higher temperature. Given the opposite patterns of growth in culture and on frogs, it is not surprising that previously published model projections of temperature-dependent *B. dendrobatidis* growth in the field, derived from studies of *B. dendrobatidis* growth in culture, were negatively, rather than positively, correlated with *B. dendrobatidis*-related declines of *Atelopus* frogs[27]. These findings emphasize the dangers of not accounting for both pathogen and host responses to temperature when parameterizing climate models.

The second prediction of our theoretical framework was that both host resistance and parasite infectivity would be positively associated with the predictability of temperature variation, with the outcome of infection depending on the relative abilities of host and parasite to anticipate and respond to predictable

fluctuations (Fig. 1 and Supplementary Fig. S1b). To test these predictions, we conducted a diurnal temperature experiment to compare *B. dendrobatidis* growth in culture with growth on Cuban treefrogs under two fluctuating temperature regimes: one with predictable diurnal temperatures of 15 °C (night-time) or 25 °C (daytime) and the other with unpredictable fluctuations in temperature (15 °C or 25 °C) randomized daily for each replicate. Frogs in these two treatments experienced the same mean ± standard deviation (20 ± 4–5 °C) in temperature, as well as the same absolute temperatures (Supplementary Fig. S3f,g), thereby isolating the effects of predictability on *B. dendrobatidis* infections. We chose a 10 °C shift between day and night because the average diurnal temperature range for the area inhabited by *Atelopus* spp. from 1980 to 1999 is 10.17 °C (±0.04 standard error of the mean; s.e.m.). In culture, *B. dendrobatidis* grew significantly faster with diurnal than random fluctuations (Fig. 3a). This result indicates that *B. dendrobatidis* anticipates diurnal temperature fluctuations, consistent with evidence of circadian clocks in other fungi[28] and supporting our prediction of improved parasite performance with higher predictability in temperature. However, *B. dendrobatidis* growth on frogs exhibited the opposite pattern; unlike in culture, there was greater growth with random than diurnal variation (Fig. 3b), indicating that Cuban treefrogs also responded to predictable temperature fluctuations and that the frog response overcame the fungal response. Neither pattern can be plausibly explained by differences in the frequency of temperature fluctuations between these two treatments (Supplementary Notes). Furthermore, patterns of survival matched patterns of *B. dendrobatidis* growth on frogs (Fig. 3b,c), suggesting that changes to diurnal temperature could have population-level impacts.

To further test our prediction that host resistance should be higher under constant than variable temperature conditions and to assess whether *B. dendrobatidis* growth was nonlinearly related to temperature, the diurnal temperature experiment also included three constant temperature treatments: 15 °C, 20 °C and 25 °C (the minimum, mean and maximum temperatures for the random and diurnal temperature treatments). Consistent with the two previous experiments, comparisons of the constant temperature treatments with the random temperature treatments revealed that host resistance was stronger at constant temperatures than with random fluctuations (Supplementary Notes). *B. dendrobatidis* growth was nonlinear across constant temperature treatments both in culture and on frogs, with quadratic models providing significantly better fits than linear models for the effects of temperature on both measures of *B. dendrobatidis* growth (growth in culture: $X_1^2 = 60.6$, $P < 0.001$; growth on frogs: $X_1^2 = 5.4$, $P = 0.021$). Growth in culture was higher at 20 °C and 25 °C than at 15 °C (Fig. 3a), whereas growth on frogs exhibited the opposite pattern (Fig. 3b). This indicates a strong effect of temperature on host resistance to infection. Nonlinear responses to mean temperatures can alter disease risk in variable-temperature environments, even in the absence of acclimation responses[16].

Global change has and is projected to change climate variability relative to the historic variability to which hosts evolved. This includes changes to monthly variability, diurnal temperature range and the frequency of El Niño events[5,10], all of which have been linked to *B. dendrobatidis*-related amphibian declines[6]. Global warming seems to be associated with an increase in the severity of unpredictable drops in temperature and, across daily and monthly timescales, unpredictable variation in temperature increased *B. dendrobatidis* growth on amphibians, a taxon experiencing global declines associated with disease. Thus, our results suggest that decreases in climate predictability associated with climate change could increase *B. dendrobatidis* and amphibian

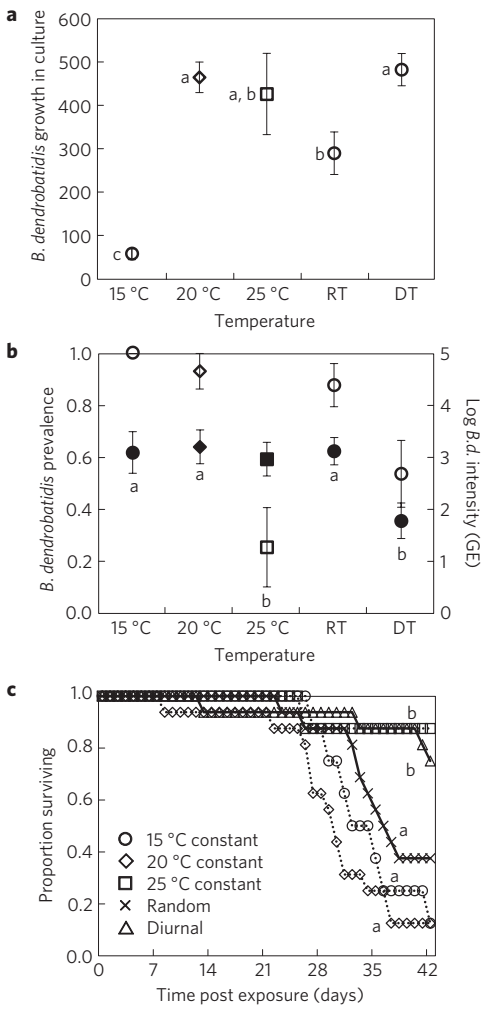

Figure 3 | Effects of random and diurnal temperature variation on *B. dendrobatidis* growth and frog mortality in the diurnal temperature experiment. RT, random temperature variation; DT, diurnal temperature variation. **a**, *B. dendrobatidis* growth in culture, measured as the seven-day proportional increase in zoosporangia. **b**, Prevalence (open symbols) and log intensity (filled symbols) of infection for *B. dendrobatidis*-exposed frogs two weeks post exposure in the diurnal temperature experiment. *B. dendrobatidis* intensity (zoospore GE) excludes frogs with no measurable infection. **c**, Survival of *B. dendrobatidis*-infected frogs in the diurnal temperature experiment (circle, 15 °C; diamondsuit, 20 °C; square, 25 °C; cross, RT; triangle, DT). Treatments labelled with the same lowercase letters were not significantly different from each other. Means ± s.e.m.

declines. Ultimately, however, a more comprehensive approach that integrates several factors, such as the nonlinearities documented here, pathogen persistence in the environment and evolutionary responses to climate change, will probably be necessary to accurately predict the effect of climate change on amphibian–*B. dendrobatidis* interactions. Future work should attempt to integrate these factors and identify the mechanisms by which alterations to diurnal temperature and climate predictability affect host–parasite interactions (see Supplementary Notes and Fig. S5 and Table S8 for potential mechanisms)[3,12].

Temperature acclimation of host resistance to parasitism is probably a widespread phenomenon. Acclimation of many physiological parameters (for example, cold hardiness) has been observed across a diversity of ectothermic taxa, including invertebrate vectors of human disease[17,29], emphasizing the importance of considering nonlinear and acclimation responses to climate change

in other host–parasite systems, particularly those of concern for human health. Parasites are ubiquitous in free-living organisms and can have dramatic effects on ecosystem function[30], so understanding how climate variation influences parasitism will be important for predicting the impacts of climate on ecosystems[3]. Given that nonlinear and acclimation responses to climate are probably pervasive, climatic variability and predictability might represent underappreciated links between climate change, disease and biodiversity losses.

Methods

Modelling methods. To help illustrate our conceptual framework for how timescale and predictability of temperature variation should influence host–parasite relationships, we modelled the geometric population growth rate of a microparasite in its host following a temperature shift, as a function of parasite and host acclimation responses. Briefly, we assumed that infectivity and resistance would each increase over time following a temperature shift, starting at a low unacclimated level immediately after the shift and increasing according to a logistic function to a higher acclimated level. We incorporated the potential ability of organisms to anticipate and adapt to predictable temperature fluctuations by adding an additional parameter (B) that allowed parasite and host to start out in a partially acclimated state following a predictable temperature shift. This parameter can be thought of as the probability that an organism correctly anticipates a temperature shift. Detailed modelling methods are available in the Supplementary Methods.

Acclimation experiments. Two replicated temperature experiments were conducted at the University of South Florida (USF) to test whether thermal acclimation of adult or juvenile frogs makes them more resistant to *B. dendrobatidis* infection. acclimation experiment 1 (September–November 2008) used wild-caught adult *O. septentrionalis*. Acclimation experiment 2 (March–May 2011) used lab-reared juvenile *O. septentrionalis*. In both experiments, frogs were acclimated to target temperatures of either 15 °C or 25 °C for four weeks. At the start of week five, half the incubators were switched to the other temperature and one frog per incubator was exposed to *B. dendrobatidis* while a second frog received a control inoculation of sterile broth. To ensure that temperature-shift effects on *B. dendrobatidis* growth could be caused only by acclimation effects on host resistance, rather than effects on parasite infectivity, all *B. dendrobatidis* inoculates were acclimated to the second (exposure) temperature for a week before frog exposure. Mortality was recorded for four (acclimation experiment 1) or six (acclimation experiment 2) weeks post exposure, using 80 and 40 replicate incubators, respectively (Supplementary Fig. S2). Further details are provided in the Supplementary Methods.

Diurnal temperature experiment. This experiment was conducted at USF in March–June 2009 to test whether diurnal or random daily temperature variation influences frog resistance to *B. dendrobatidis* infection. Frogs were held at one of three constant temperatures (15 °C ($n = 8$), 25 °C ($n = 8$), 20 °C ($n = 16$)) or one of two variable temperature treatments ($n = 16$): diurnal temperature (day 25 °C, night 15 °C) and random daily temperature (15 °C or 25 °C selected at random each day). These two variable-temperature regimes were selected so that frogs would experience the same variances, mean (20 °C), maximum (25 °C) and minimum (15 °C) temperatures, differing only in the predictability of temperature shifts. After four weeks, one frog per incubator was exposed to *B. dendrobatidis* while a second frog received a control inoculation. Mortality was recorded for six weeks post exposure. Further details are provided in the Supplementary Methods.

Experimental conditions. Replication of temperature treatments was achieved by using individual custom-built incubators placed in an environmental chamber set on a 12-h light cycle (Supplementary Methods and Fig. S1). In each incubator, frogs were maintained individually in vented plastic containers (350 ml in experiment 1; 700 ml in experiment 2) on autoclaved soil (125 ml per container) collected from the USF botanical gardens and kept wet by adding deionized water as needed. Frogs were fed five to ten crickets (10–15 mm) once per week. To measure *B. dendrobatidis* growth in culture, each incubator in acclimation experiment 1 and the diurnal temperature experiment also contained a sealed broth culture of *B. dendrobatidis* (Supplementary Methods). The mass of each frog was recorded at the end of each experiment, for use as a covariate in subsequent analyses.

***B. dendrobatidis* quantification on frogs.** *B. dendrobatidis* infection was assessed by swabbing each frog at 14 and 28 days post exposure or on the day a frog died. The swab was passed over the underside of each hindlimb ten times from hip to knee and 15 times from ankle to toe and frozen for later processing. To prevent cross-contamination with *B. dendrobatidis* DNA, the vinyl gloves used to handle each frog were rinsed sequentially in 10% bleach, 1% Novaqua and deionized water before swabbing the next frog. *B. dendrobatidis* DNA on swabs was quantified using quantitative polymerase chain reaction (Supplementary Methods).

Statistical analyses. *B. dendrobatidis* growth in culture was analysed using generalized linear models with gamma errors, and *B. dendrobatidis* infection levels were analysed using zero-inflated negative binomial generalized linear models. P-values for multiple comparisons in the diurnal temperature experiment were corrected to control for the false discovery rate (see Supplementary Methods for further details).

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Author contributions

T.R.R. and J.R.R. should be considered joint first authors of this work. T.R.R. conducted mathematical modelling and statistical analyses. T.R.R. and J.R.R. conceived the experiments and obtained financial support. J.R.R. compiled field data. All authors assisted with writing the manuscript and with design and execution of the experiments.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to T.R.R.

Competing financial interests

The authors declare no competing financial interests.