PROCEEDINGS OF THE ROYAL SOCIETY B

Research

CrossMark
click for updates

Author for correspondence:
Martha M. Muñoz
e-mail: mmunoz@oeb.harvard.edu

†These authors contributed equally to this study.

Royal Society Publishing

Evolutionary stasis and lability in thermal physiology in a group of tropical lizards

Martha M. Muñoz[1],[†], Maureen A. Stimola[2],[†], Adam C. Algar[3], Asa Conover[4], Anthony J. Rodriguez[5], Miguel A. Landestoy[6], George S. Bakken[7] and Jonathan B. Losos[1]

[1]Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA
[2]Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam Avenue, NY 10027, USA
[3]School of Geography, University of Nottingham, Sir Clive Granger Building, University Park, Nottingham NG7 2RD, UK
[4]Stuyvestant High School, 345 Chambers Street, New York, NY 10282, USA
[5]Department of Wildlife, Fish and Conservation Biology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
[6]Sociedad Ornitológica de la Hispaniola, Gustavo Mejía Ricart 119 B, Apto. 401, Galerías Residencial, Santo Domingo, Dominican Republic
[7]Department of Biology, Indiana State University, Terre Haute, IN 47809, USA

Understanding how quickly physiological traits evolve is a topic of great interest, particularly in the context of how organisms can adapt in response to climate warming. Adjustment to novel thermal habitats may occur either through behavioural adjustments, physiological adaptation or both. Here, we test whether rates of evolution differ among physiological traits in the cybotoids, a clade of tropical *Anolis* lizards distributed in markedly different thermal environments on the Caribbean island of Hispaniola. We find that cold tolerance evolves considerably faster than heat tolerance, a difference that results because behavioural thermoregulation more effectively shields these organisms from selection on upper than lower temperature tolerances. Specifically, because lizards in very different environments behaviourally thermoregulate during the day to similar body temperatures, divergent selection on body temperature and heat tolerance is precluded, whereas night-time temperatures can only be partially buffered by behaviour, thereby exposing organisms to selection on cold tolerance. We discuss how exposure to selection on physiology influences divergence among tropical organisms and its implications for adaptive evolutionary response to climate warming.

1. Introduction

Rising temperatures present unique challenges for tropical ectotherms, which already generally function near their upper thermal limits: even small temperature increases can have disproportionately large negative consequences for these organisms [1,2]. Studies assessing tropical ectotherms' vulnerability to climate warming have traditionally focused on predicting where warming will have the most pronounced effects on organismal fitness by correlating physiological traits with environmental data and using these relationships to infer where range shifts and local extinctions will occur [3,4]. However, the evolutionary potential of populations to respond to novel selective pressures imposed by rising temperatures is an equally important and comparatively unexplored aspect of response to climate warming [5].

Behaviour and physiology determine how organisms interact with their thermal environments [6]. Organisms that thermoregulate limit exposure to suboptimal temperatures—a phenomenon commonly referred to as the 'Bogert effect' [7,8]. Physiological traits that behavioural thermoregulation can shield from selection should evolve less than traits that cannot be so easily buffered, and thus are exposed to stronger selection. Because many environments are more thermally complex in the day than at night [9,10], thermoregulation should
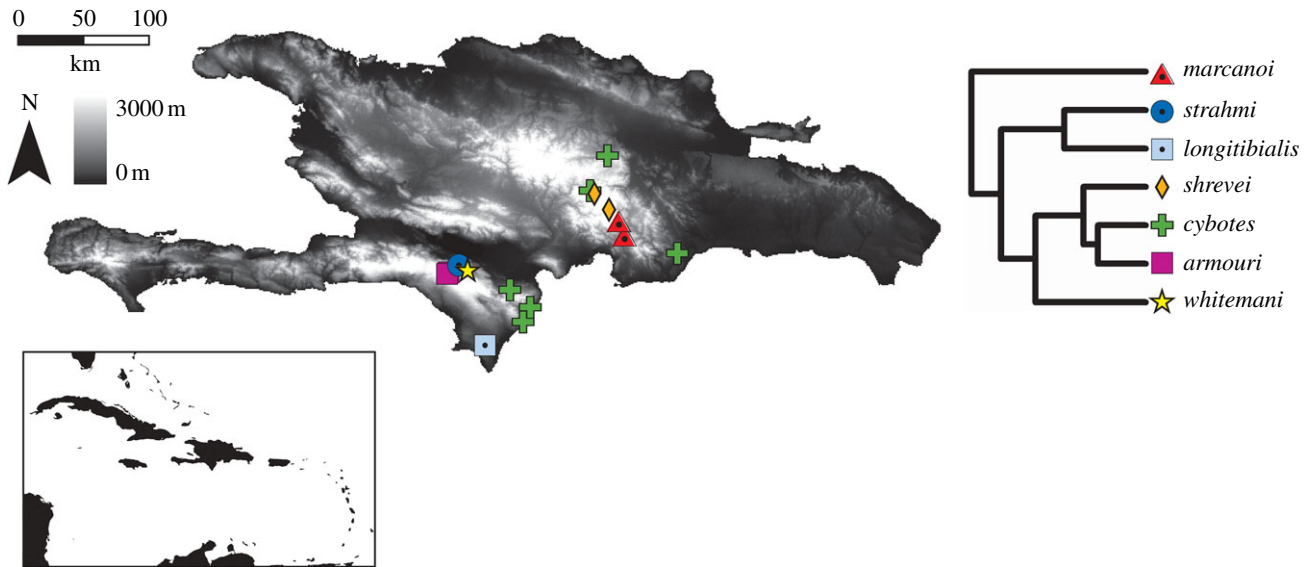
Figure 1. Map showing altitudinal variation and the localities for each cybotoid population sampled in this study. The map inset shows the location of Hispaniola with respect to the other islands in the Caribbean basin. Greyscale indicates elevation and range from dark (low) to light (high) elevation. Species are denoted in different colours and shapes in the phylogeny generated based on Mahler *et al.* [16] (see Material and methods).

be more effective at shielding diurnal organisms from selection on upper than lower physiological limits and, consequently, tolerance to cold should evolve faster than tolerance to heat.

In this study, we compare rates of physiological evolution in the cybotoids, a tropical clade of *Anolis* lizards from the Caribbean island of Hispaniola whose members differ extensively in thermal habitat [11,12]. Previous work by Hertz & Huey [13] found similar body temperatures and heat tolerance among three cybotoid species and provided ecological data suggesting that they are good thermoregulators. Our study expands on this work in terms of populations, species and physiological traits examined. The cybotoid clade is unique among Caribbean anoles because its species are found from sea level to almost 3000 m [14]; as a result, the environments they experience pose different selective pressures and provide the opportunity for behavioural and physiological adaptation to different thermal extremes.

We first present a comparative analysis of three ecologically important physiological traits—cold tolerance (CT_{min}), body temperature (T_b), and heat tolerance (CT_{max})—across thermal environments. We then compare rates of evolution among these traits using a likelihood-based approach and test whether behavioural thermoregulation can limit exposure to extreme temperatures using field estimates of basking site selection and night-time temperatures. Finally, we discuss the role of behaviour in setting the pace of physiological evolution in tropical ectotherms and how behaviour influences adaptive evolutionary potential in physiological traits.

2. Material and methods

(a) Study organisms and study sites

The cybotoid anoles are a clade of nine species from Hispaniola commonly found on trunks or near the ground [11]. Species occupy nearly all available climatic environments from xeric semi-deserts to high elevation mountains, which have been occupied independently by two different lineages [12,15]. Our sampling was conducted in June and July 2011 and focused on the seven cybotoids

found in the Dominican Republic (figure 1). The other two species are *Anolis breslini*, which is restricted to northwestern Haiti and is ecologically quite similar to *Anolis whitemani* [17], and *Anolis haetianus*, which is found only in western Haiti and is probably synonymous with *Anolis cybotes* [12]. Where possible, we sampled several populations that, together, encompassed most of a species' altitudinal range (figure 1 and table 1). *Anolis cybotes* is found nearly island-wide; we sampled it at three elevations in each of the two principal mountain chains, the Sierra de Baoruco (SB) and Cordillera Central (CC). In the SB, we sampled the mid-elevation species, *Anolis strahmi* and *A. whitemani*, and the high elevation species, *Anolis armouri*. In the CC, we sampled the mid-elevation species, *Anolis marcanoi*, and the high elevation species, *Anolis shrevei*.

We gathered climatic measurements for each locality by extracting all temperature variables (bio 1—bio 11; electronic supplementary material, table S1) from environmental layers available in the WorldClim dataset (resolved to approx. 1 km²; [18]). These variables summarize seasonal and annual temperature trends. To account for collinearity among thermal variables, we reduced data dimensionality using a principal components (PCs) analysis on the correlation matrix.

(b) Measurement of physiological performance indices

We measured field body temperature, T_b, during one continuous 13 h period (06.00–19.00) at 13 localities from 20 June to 31 July 2011. Owing to logistical constraints, one locality (*A. shrevei*—1950 m) was sampled from 06.00 to 13.45, but results for that population are consistent with those from other populations (table 1). Following established methods [19,20], we walked slowly through each habitat and used a standard noose to capture adult male lizards, which are more conspicuous and easier to sample than females. For every lizard, we recorded core temperature (T_b) to the nearest 0.1°C using a thermocouple (Type T, Copper-Constantan) inserted approximately 1 cm into the cloaca and connected to a temperature logger (HH603A, Omega). Each lizard was measured only once. T_b generally correlates closely with the optimal performance temperature (T_{opt}, the temperature at which organisms maximally perform a function, for example running) in many diurnal lizards, including tropical anoles [21].

For each lizard captured, we recorded the time, weather conditions (sunny, mixed, or overcast skies) and 'basking status'

Table 1. Locality name, species sampled, and altitude (m) are given. (Mean critical thermal minimum (CT_{min}), body temperature (T_b), midday body temperature (midday T_b), and critical thermal maximum (CT_{max}) are given for each population. Units for physiological metrics are °C ± 1 s.e.m. and sample size is given in parentheses. For *A. cybotes*, the mountain chain corresponding to the sampling locality—Cordillera Central (CC) or the Sierra de Baoruco (SB)—is also given.)

species	locality	alt (m)	CT_{min} (°C)	T_b (°C)	CT_{max} (°C)
wild-measured					
A. cybotes (SB)	Los Patos	45	11.3 ± 0.4 (16)	30.1 ± 0.3 (45)	39.5 ± 0.1 (20)
A. cybotes (CC)	San Cristóbal	56	11.4 ± 0.2 (16)	29.4 ± 0.5 (53)	39.2 ± 0.2 (16)
A. longitibialis	Jaragua	105	12.8 ± 0.2 (18)	28.9 ± 0.2 (101)	38.5 ± 0.3 (18)
A. whitemani	Puerto Escondido	411	12.2 ± 0.4 (15)	27.9 ± 1.1 (17)	38.8 ± 0.3 (15)
A. strahmi	Camino Aguacate	454	11.3 ± 0.5 (6)	26.3 ± 0.7 (7)	39.2 ± 0.2 (6)
A. marcanoi	Ocoa	458	12.6 ± 0.3 (9)	—	38.2 ± 0.3 (9)
A. cybotes (CC)	Jarabacoa	690	10.7 ± 0.2 (18)	29.0 ± 0.6 (39)	40.3 ± 0.2 (18)
A. cybotes (SB)	Guayuyal	727	10.8 ± 0.4 (15)	26.6 ± 0.4 (53)	38.7 ± 0.2 (15)
A. marcanoi	La Horma	879	11.3 ± 0.1 (16)	29.1 ± 0.6 (48)	38.7 ± 0.2 (16)
A. cybotes (CC)	Constanza	1390	10.0 ± 0.5 (11)	29.2 ± 0.5 (10)	39.5 ± 0.6 (11)
A. cybotes (SB)	La Hoz	1395	8.7 ± 0.4 (9)	28.6 ± 0.9 (11)	38.9 ± 0.2 (9)
A. shrevei	Valle Nuevo—low	1950	9.6 ± 0.6 (9)	28.0 ± 1.0 (10)	39.9 ± 0.3 (9)
A. armouri	Loma de Toro—low	2020	8.2 ± 0.6 (9)	—	39.3 ± 0.3 (9)
A. armouri	Loma de Toro—high	2318	7.2 ± 0.4 (12)	25.9 ± 0.7 (21)	—
A. shrevei	Valle Nuevo—high	2450	6.2 ± 0.3 (11)	27.4 ± 1.0 (20)	40.4 ± 0.3 (11)
cold acclimatized					
A. cybotes	Los Patos	45	10.6 ± 0.3 (19)	—	—
A. armouri	Loma de Toro—high	2318	7.2 ± 0.3 (18)	—	—

(basking in the full or filtered sun, perching in the shade under sunny or mixed skies or in the shade under overcast skies) following Hertz [20]. For a statistical analysis of basking site choice, we removed observations for lizards captured in overcast conditions, because these lizards did not have the opportunity to choose between perching in the sun or in the shade. We tested for weather-dependent basking choice using a logistic regression model such that individuals' basking behaviour (perching in shade = 0, perching in sun = 1) was evaluated as a function of elevation, weather (sunny or mixed skies) and an elevation × weather interaction. An effect of elevation alone would indicate that certain basking behaviours are more likely to occur at different elevations, whereas a significant interaction would indicate that active lizards differ in how they exploit weather conditions at different elevations.

We measured the critical thermal minimum (CT_{min}) and maximum (CT_{max}), which refer to the low and high temperatures at which an organism loses locomotor function; these are widely used for measuring the tolerance limits of performance in ectotherms [22,23]. CT_{min} and CT_{max} are estimated as the lower and upper temperatures at which a lizard fails to right itself when flipped onto its back [22]. After capture, we gave adult male lizards a 24 h rest period in a large, insulated ice chest (Coleman) in which temperature was maintained near 23°C at all sites. To measure core temperature during the tolerance experiments, an Omega temperature probe (Type T, 36 gauge) was placed approximately 1 cm into the cloaca of each lizard and secured to the base of the tail using a small piece of surgical tape, ensuring that tail movement was uncompromised. The temperature probe was connected to a digital temperature logger (HH147U, Omega). The lizard was placed into a perforated plastic container where it could move freely. After the lizard attained ambient temperature, the container was moved to an insulated icebox coated with a layer of crushed ice. Because

the rate of temperature change during a tolerance experiment can alter an organism's performance [24], we reduced body temperature at a constant rate of approximately 1°C min^{-1} for all lizards. To conduct the experiment, we reduced body temperature to 14°C, at which point we flipped the lizard onto its back using a pair of blunt tweezers and stimulated it to flip itself back over by gently probing the base of its tail and pressing its thighs. If the lizard flipped over after 15 s, we then lowered core temperature 0.5°C and repeated this procedure, continuing until a temperature was reached at which the lizard failed to right itself in the allotted time. CT_{min} was recorded as the temperature at which the righting response was lost.

Animals were given 24 h to rest in the ice chest before CT_{max} trials. The method for estimating CT_{max} was similar to that of CT_{min} except that a 100 W light bulb was suspended approximately 30 cm above the Tupperware container. We placed lizards in the Tupperware container and increased their core temperature at a rate of approximately 1°C min^{-1} by exposing them to the heat source. We began flipping lizards when they began to cool through panting (i.e. the 'panting threshold'; [25]) following the procedure described above, and recorded the temperature at which the righting response was lost as CT_{max}.

Estimation of CT_{min} and CT_{max} is potentially confounded by the rate of temperature change, body size and starting conditions [24,26,27]. We performed linear regressions with mean population CT_{min} and CT_{max} as the dependent variables against the population means for rate of temperature change, initial experimental temperature and body mass (see the electronic supplementary material, table S2). We conducted separate analyses for each pair of dependent and independent variables, and each regression was weighted by the variance in CT_{min} or CT_{max}. Because none of these models were statistically significant (see the electronic supplementary material, table S3), we used raw CT_{min} and CT_{max} values in subsequent analyses.

We compared physiological traits to thermal habitat (three PC variables, see Results) using population means and independent contrasts. We calculated standardized independent contrasts (scaled by the expected variance) for the weighted species means of each physiological trait (CT_{min}, T_b and CT_{max}) and each of the thermal habitat variables (PC I, PC II and PC III) using the *pic* function in the APE package [28,29] in R [30]. Although they are not properties of the organisms, environmental traits may reflect underlying ecological traits [31,32]. We used the time-calibrated, majority rule consensus tree of Mahler *et al.* [16], with the topology generated using Bayesian maximum clade credibility [33]. This consensus tree contains 187 of approximately 375 recognized species of anoles (all but 19 species of Caribbean anoles), including all the species used in this study (figure 1). We used regression through the origin to compare the contrasts for physiological traits with the contrasts for thermal environment traits using the *lmorigin* function in APE [29].

(c) Measuring rates of physiological trait evolution

To ensure comparability among traits, we used the *fitContinuous* function in the GEIGER package [34] in R to fit three different models of evolution to each physiological trait. These models were: (i) Brownian Motion, a random walk; (ii) Ornstein–Uhlenbeck, a random walk in which characters tend to return to a single optimum; and (iii) Early Burst, in which the overall rate of evolution exponentially slows through time [35–38]. We calculated the Akaike information criterion corrected for small sample size (AICc; [39]) for each model and compared the fits by examining the Akaike weights [40].

We used Adams' [41] method to evaluate whether the rate of evolutionary change varied among physiological traits. This method compares a model that allows rates to vary among traits to one in which the rates are constrained to be equal using a likelihood ratio test and AICc. To account for intraspecific measurement error, we incorporated the standard error of the mean in our estimation of rates of evolution. We used the APE library [42,43] and new code supplied by Adams [41] in R.

(d) Cold-acclimatization trials

Because of the large differences in CT_{min} we discovered among populations (see Results), we conducted an experiment to assess how short-term acclimatization influences variation in this trait. In June 2013, we collected adult male lizards from two populations differing greatly in thermal environment— *A. armouri* (Loma de Toro, elevation = 2318 m; $n = 18$) and *A. cybotes* (Los Patos, elevation = 45 m; $n = 19$). Kolbe *et al.* [44] found that a two-week acclimatization at 22.5°C was sufficient to elicit a strong plastic response in CT_{min} in a lowland population of *Anolis cristatellus* from Puerto Rico without inducing severe thermal stress. We maintained lizards at 19.4°C (range = 17.4°C–21.9°C) for three weeks and measured CT_{min} following the procedure described above.

(e) Night-time temperature measurement

We measured night-time operative temperature (T_e) in the same two, thermally contrasting, localities. T_e refers to an organism's equilibrium temperature in the absence of metabolic heating or evaporative cooling (*sensu* [45]), which we estimated using replicas made of electroformed copper. These models mimic the thermal properties of a thermoconforming lizard (e.g. colour, shape and size; [46]). We embedded iBUTTON data loggers (DS1921K Maxim) into copper models shaped using a mould of *A. cybotes*—this new generation of copper models permits automated temperature recording (for details of their construction, see [47]). Methods for calibrating the copper models are given in the electronic supplementary material. We launched these

models in Los Patos (12 on trees, 11 on rocks and 11 under rocks; 5–7 June 2013) and in Loma de Toro (11 each on trees, on rocks and under rocks; 14–16 June 2013) with the devices set to automatically record T_e at 10 min intervals. We randomly selected perches, orientation and height for model placement on trees following Hertz [20]. We also recorded sleep site selection for lizards at each of these localities during the experimental period.

3. Results

(a) Thermal habitat varies markedly across Hispaniola

For this study, we visited various localities in the Dominican Republic that spanned more than 2400 m in altitude and a variety of habitats ranging from lowland scrub to montane pine forests. Not surprisingly, sites varied considerably in temperature (figure 2). In the PC analysis of the WorldClim thermal variables, we recovered three axes with eigenvalues greater than 1 that together explained 99.6% of the variation in the thermal data (see the electronic supplementary material, tables S4 and S5). PC I (hereafter 'Thermal PC I') explained 73.2% of the variation and loaded highly for mean annual temperature, mean temperatures of the wettest and driest quarters, maximum temperature of the warmest month and minimum temperature of the coldest month. PC II (14.2% variation explained; hereafter 'Range PC II') loaded highly for daily and annual temperature ranges, and PC III (12.2% variation explained; 'Seasonality PC III' axis) loaded with variables related to thermal seasonality.

(b) Analyses of thermoregulation and physiology

In approximately 164 h of field observations, we collected T_b from 435 lizards and basking site data from 381 lizards. The extent of basking in the sun varied greatly at different elevations (logistic interaction term; $\chi_1^2 = 4.07$, $p = 0.044$); lizards at higher elevation were more likely to bask, whereas those at lower elevation sought shade. Neither CT_{max} nor T_b varied significantly with any of the thermal habitat PC variables (table 2). CT_{min} was positively correlated with Thermal PC I ($r = 0.934$, $p < 0.001$), which loaded heavily with mean annual temperature, and this relationship remained significant after phylogenetic correction (table 2).

(c) Evolutionary analyses of physiology

Brownian motion was the most strongly supported model for all three traits (CT_{min}, T_b and CT_{max})—Akaike weights were more than 0.93 in all cases (see the electronic supplementary material, table S6), allowing for a comparison of evolutionary rates among traits. Likelihood ratio tests indicated that, overall, the three physiological traits evolved at different rates, although the differences were just above the significance threshold ($p = 0.06$) when intraspecific measurement error was taken into account (table 3). Pairwise comparisons showed that rates of evolution for CT_{min} were significantly higher than for CT_{max}, even when intraspecific measurement error was considered. However, differences in rates of evolution between T_b and other traits were not significant in the analysis incorporating intraspecific variation (table 3), either because rates do not actually differ, or because high variance in T_b obscures differences in rates of evolution.
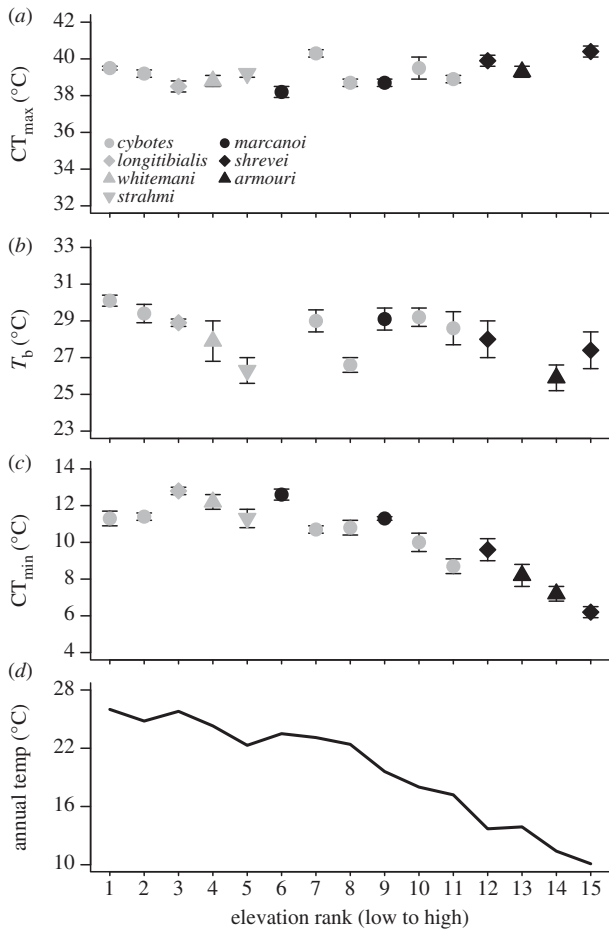
Figure 2. Population means ($\pm$ 1 s.e.m.) are given for critical (*a*) thermal maximum (CT_{max}), (*b*) body temperature (T_b), and (*c*) critical thermal minimum (CT_{min}). The mean annual temperature for each locality is provided in (*d*). The *x*-axis denotes elevation rank for each population. Species are denoted in different colours and shapes.

(d) Cold-acclimatization experiment

Mean CT_{min} was not significantly different between wild-measured (mean = 11.3°C) and cold-acclimatized *A. cybotes* (mean = 10.6°C) (unpaired *t*-test: $t = 1.53$, $p = 0.136$). Mean CT_{min} remained the same (7.2°C) between wild-measured and cold-acclimatized *A. armouri*. CT_{min} was significantly higher in *A. cybotes* than in *A. armouri* in both the wild-measured (unpaired *t*-test: $t = 7.72$, $p < 0.001$) and cold-acclimatized treatments ($t = 9.28$, $p < 0.001$).

(e) Night-time temperature experiment

Night-time operative temperatures (T_e) showed marked differences between high and low elevation (figure 3). At Los Patos (low elevation), T_e ranged from 24.6°C to 29.8°C, whereas at Loma de Toro T_e (high elevation), it ranged from 10.9°C to 18.1°C. T_e was on average, though not always, somewhat higher under rocks than on top of rocks or on trees (figure 3; electronic supplementary material, figure S1), particularly early in the evening. All lizards at Los Patos were observed sleeping on vegetation (43 observations), whereas lizards at Loma de Toro were observed sleeping on vegetation (14 out of 30) and underneath rocks (16 out of 30) in roughly equal numbers (test for differences in site selection among populations: $\chi_1^2 = 26.3$, $p < 0.001$).

4. Discussion

(a) Thermoregulatory behaviour influences the rate of physiological evolution

The question of how behaviour influences patterns of physiological evolution dates back to the middle of the last century [7,49] and has received renewed interest in light of concern about how ectothermic organisms can respond to climate warming [2,50]. We studied a clade of lizards whose species vary markedly in thermal environment from hot semi-deserts to cold montane environments. Despite occurring in environments that differ by as much as 15°C in mean annual temperature, field body temperature and heat tolerance were remarkably similar among populations, indicating that behavioural thermoregulation can be extraordinarily effective in limiting exposure to excessively hot or cold temperatures (i.e. the 'Bogert effect'; [8]). Our behavioural analysis demonstrates that lowland lizards were more likely to retreat to the shade under sunny conditions, whereas upland lizards were more likely to bask when the sun was out, a result in agreement with previous work on three of these species [13]. Our results are particularly striking given that other anole species exhibit markedly different body temperatures, even when they occur in sympatry [51–54], but see [55,56].

Given the ability of cybotoids to thermoregulate to approximately the same temperature throughout its range, it is not surprising that CT_{max} also shows very little interspecific variation. However, these lizards have a much more limited ability to thermoregulate at night, particularly at high elevation, where operative temperatures measured on all types of sleep sites were so low that they would incapacitate approximately 80% of lowland lizards (figure 3; electronic supplementary material, figure S1). In the absence of thermal refuges, populations have no option but to adapt physiologically. Indeed, we found that none of the lizards from high elevation experienced night-time temperatures lower than their CT_{min} (figure 3).

An alternative explanation for this finding is that differences in CT_{min} represent non-genetic effects of living in different environments. Previous studies suggest that adaptive plasticity is unlikely to account for physiological differences among populations [57]: our data support this view, as cold tolerance exhibits little acclimatization, even less so than in other anoles [44], which suggests that there is probably a genetic basis for the observed variation in CT_{min}.

The inability of thermoregulation to buffer selection on physiology during the night is an explanation for the fast rate of CT_{min} evolution in this clade (table 3; [8]). The relative stasis in CT_{max} documented here aligns with results from recent meta-analyses showing that there is less variation in heat tolerance than in cold tolerance in several ectotherm clades [58,59]. In short, behavioural thermoregulation allows cybotoid species to maintain similarly warm body temperatures during the day, but not during the night, forcing species in montane environments on Hispaniola to adapt to lower temperatures.

(b) What limits heat tolerance evolution?

Behavioural thermoregulation can help explain why CT_{max} is less variable than CT_{min} in the cybotoids, but not why the response to different environmental conditions involved behavioural, rather than physiological, change. Given that

Table 2. Results from linear regressions assessing the relationship between physiological traits (critical thermal minimum, CT_{min}; mean body temperature, T_b, and critical thermal maximum, CT_{max}) and thermal environment (PC I/mean annual temperature, PC II/temperature range and PC III/temperature seasonality) using population means (a) and independent contrasts of species means (b). (Degrees of freedom are given in parentheses. Correlations among contrasts were measured using the *cor.table* function in picante [48] in R.)

	(a) populations			(b) contrasts		
	reg. coeff.	Pearson's r	p	reg. coeff.	Pearson's r	p
CT_{min} (13,5)						
PC I/mean annual temperature	1.87	0.934	<0.001	1.56	0.973	0.001
PC II/temperature range	−0.20	0.022	0.755	0.05	0.032	0.951
PC III/seasonality	0.46	0.140	0.503	0.38	0.132	0.790
T_b (11,5)						
PC I/mean annual temperature	0.52	0.363	0.142	0.78	0.637	0.143
PC II/temperature range	−0.35	−0.296	0.341	−0.48	−0.421	0.460
PC III/seasonality	0.68	0.479	0.054	−0.73	−0.381	0.464
CT_{max} (12,5)						
PC I/mean annual temperature	−0.29	−0.618	0.110	−0.24	0.186	0.204
PC II/temperature range	−0.16	−0.228	0.300	0.13	0.881	0.672
PC III/seasonality	0.13	0.193	0.524	0.03	0.865	0.957

Table 3. Comparison of evolutionary rates for CT_{min}, T_b, and CT_{max}. (a) The full analysis of evoluationary rates (σ^2) incorporating covariation among all three traits. One test accounted for intraspecific measurement error (corrected), whereas the other did not (uncorrected). AIC_C scores for a model that allows rates to vary (observed) among traits and a model that constrains rates of evolution to be equal among traits are given (constrained), and likelihood ratio test results are also given. (b) Likelihood ratio tests for pairwise comparisons of evolutionary rates among traits. As above, the results for models that incorporate intraspecific measurement error (corrected) and for models that do not (uncorrected) are presented.

trait	σ^2	uncorrected	corrected
(a) full analysis			
CT_{min}	10.60	AIC_C (OBS) = 167.3	AIC_C (OBS) = 171.7
T_b	6.36	AIC_C (CONS) = 175.8	AIC_C (CONS) = 173.2
CT_{max}	0.78	$LRT_{d.f. = 2} = 12.56$, $p = 0.002$	$LRT_{d.f. = 2} = 5.57$, $p = 0.06$
comparison	uncorrected $LRT_{d.f. = 1}$; p	corrected $LRT_{d.f. = 1}$; p	
(b) pairwise analysis			
CT_{min} versus CT_{max}	12.40; <0.001	7.85; 0.005	
CT_{min} versus T_b	0.87; 0.350	0.32; 0.574	
CT_{max} versus T_b	6.86; 0.009	−4.70; 1.00	

time spent thermoregulating imposes a cost with regards to other activities such as foraging, predator avoidance and reproduction [19,60,61], it is unclear why selection should favour the maintenance of high body temperatures in montane habitats, instead of physiological adaptation to lower temperatures. One possibility is that behavioural modifications are easier to evolve than changes in physiological tolerances [62,63]. Given that a myriad of physiological processes (e.g. locomotion, digestion, and growth) are sensitive to temperature, the evolution of physiological tolerances may necessitate the concerted evolution of many genes (discussed in [64,65]). By contrast, shifts in basking frequency change seasonally within populations, and so behavioural shifts at different elevations may not require substantial

evolutionary change. Moreover, even if evolutionary shifts in behaviour are required, such changes may require fewer genetic changes than shifts in physiology [36].

This 'evolution along lines of least genetic resistance' (*sensu* [66]) explanation suggests that there is no inherent advantage to warmer body temperatures, but an alternative explanation for the lack of evolutionary variability in CT_{max} revolves around the fitness benefits of high temperatures. Specifically, selection may favour the maintenance of high body temperatures in cold environments because rates of biochemical reactions increase with optimal temperature [65,67,68]. Indeed, warm-adapted ectotherms generally experience higher levels of physiological performance than cold-adapted organisms [69,70]. However, if this 'hotter is better' hypothesis is true, it
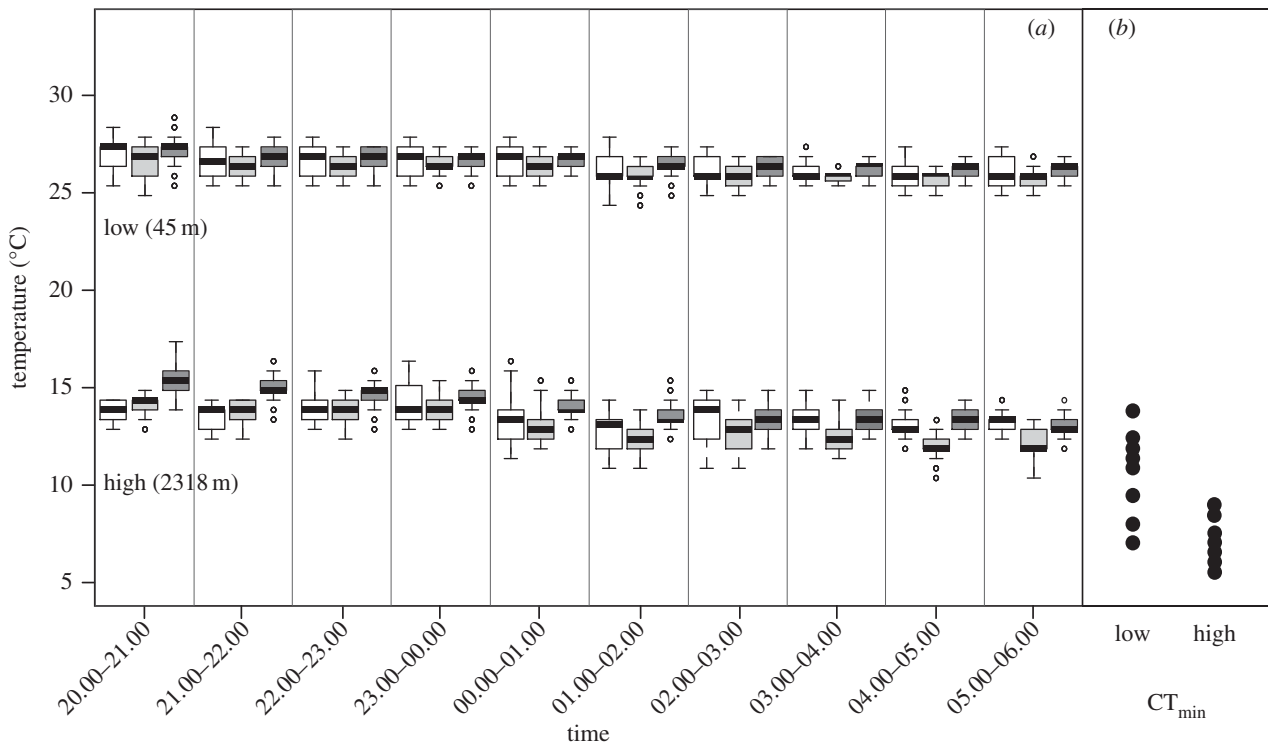
Figure 3. (a) Box plots showing the variation in operative temperatures during 1 h time blocks. Each time block summarizes temperatures collected over three consecutive nights in June 2013 at low elevation (45 m) and high elevation (2318 m). Colour denotes the type of perch where the temperature was measured as follows: on a tree, white; on top of a rock, light grey; underneath a rock, dark grey. (b) CT_{min} measured in individuals of *A. cybotes* (left) and *A. armouri* (right) from the same localities in part (a) are given.

still fails to address why low elevation populations have not evolved even higher heat tolerances.

(c) Impacts of climate change

Climate warming will probably have different effects on cybotoids from lowland and upland habitats. Warming temperatures threaten to make current ranges thermally inhospitable for many cool-adapted montane ectotherms, which may force their ranges upwards [4,71]. By contrast, it is likely that upland cybotoids will benefit, at least in the short term, from climate warming. As the climate warms, environmental temperatures will more often approximate lizards' preferred temperatures, and thus the time lizards need to spend thermoregulating should decrease and the number of hours available for other activities should increase. By contrast, higher temperatures may allow species from lower elevations to migrate upwards leading to negative interspecific interactions [2].

The challenge facing lowland cybotoids will be to avoid stressfully hot temperatures as habitats continue to warm. Many tropical lizards, particularly those near sea level, are already frequently experiencing temperatures exceeding their preferred ranges [2,3]. As warming continues, lizards in such lowland populations will eventually be unable to maintain temperatures within their preferred range for long enough periods to survive. At that point, lowland populations can only avoid local extinction by shifting their physiology to adapt to these higher environmental temperatures.

Evolutionary stasis in CT_{max} may suggest a limited ability to evolve and, thus, a heightened vulnerability to environmental warming. Some studies on *Drosophila* support the idea that heat tolerance evolution is genetically constrained, as the amount of genetic diversity for heat tolerance is limited compared with that for cold tolerance [72–74]. The observation that CT_{min} evolves readily in cybotoids and in other ectotherms [75,76] would tend to support this hypothesis. Nonetheless, it is hard to construe why diverse physiological systems would be constrained from evolving upper, but not lower tolerances. In fact, experiments on *Drosophila* [77] and salmon [78] have demonstrated that heat tolerance can increase in response to selection, although there appears to be an upper ceiling on how high heat tolerance can evolve [79]; no similar experiments have ever been conducted on vertebrates. Moreover, although cybotoid anoles show relatively little variation in heat sensitivity, some other anole clades have diversified extensively while adapting to different thermal environments [54]. Looking more broadly, other lizard species possess heat tolerances that approach 50°C (reviewed in [58,59]), suggesting that if genetic constraints exist in lizards, they are phylogenetically localized. Finding an explanation for variation among clades in physiological diversity could aid in assessing ectotherms' vulnerability to climate warming, but it is a challenge that will require integration of physiological, behavioural, and evolutionary approaches.

References

1. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl Acad. Sci. USA* **105**, 6668–6672. (doi:10.1073/pnas.0709472105)

2. Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Álvarez Pérez HJ, Garland Jr T. 2009 Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B* **276**, 1939–1948. (doi:10. 1098/rspb.2008.1957)

3. Sinervo B *et al*. 2010 Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894–899. (doi:10.1126/science.1184695)

4. Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD. 2011 Rapid range shifts of species associated with high levels of climate warming. *Science* **333**, 1024–1026. (doi:10.1126/science.1206432)

5. Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G. 2008 Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* **6**, 2621–2626.

6. Stevenson RD. 1985 The relative importance of behvioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**, 362–386. (doi:10.1086/284423)

7. Bogert CM. 1949 Thermoregulation in reptiles, a factor in evolution. *Evolution* **3**, 195–211. (doi:10. 2307/2405558)

8. Huey RB, Hertz PE, Sinervo B. 2003 Behvioural drive versus behvioural inertia in evolution: a null model approach. *Am. Nat.* **161**, 357–366. (doi:10. 1086/346135)

9. Sarmiento G. 1986 Ecological features of climate in high tropical mountains. In *High altitude tropical biogeography* (eds F Vuilleumier, M Monasterio), pp. 11–46. New York, NY: Oxford University Press.

10. Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. 2006 Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* **46**, 5–17. (doi:10.1093/icb/icj003)

11. Schwartz A. 1989 A review of the cybotoid anoles (Reptilia: Sauria: Iguanidae) from Hispaniola. *Milwaukee Public Mus. Contrib. Biol. and Geol.* **78**, 1–32.

12. Glor RE, Kolbe JJ, Powell R, Larson A, Losos JB. 2003 Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution* **57**, 2383–2397. (doi:10.1554/02-369)

13. Hertz PE, Huey RB. 1981 Compensation for altitudinal changes in the thermal environment by some *Anolis* lizards on Hispaniola. *Ecology* **62**, 515–521. (doi:10.2307/1937714)

14. Henderson RW, Powell R. 2009 *Natural history of West Indian reptiles and amphibians*. Gainesville, FL: University Press of Florida.

15. Wollenberg KC, Wang IJ, Glor RE, Losos JB. 2013 Determinism in the diversification of Hispaniolan trunk-ground anoles (*Anolis cybotes* species complex). *Evolution* **67**, 3175–3190. (doi:10.1111/ evo.12184)

16. Mahler DL, Revell LJ, Glor RE, Losos JB. 2010 Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* **64**, 2731–2745. (doi:10.1111/j.1558-5646.2010.01026.x)

17. Williams EE. 1963 *Anolis whitemani*, new species from Hispaniola (Sauria, Iguanidae). *Breviora* **197**, 1–8.

18. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005 Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978. (doi:10.1002/joc.1276)

19. Huey RB. 1974 Behvioural thermoregulation in lizards: importance of associated costs. *Science* **184**, 1001–1003. (doi:10.1126/science. 184.4140.1001)

20. Hertz PE. 1992 Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* **73**, 1405–1417. (doi:10.2307/1940686)

21. Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. 2012 Predicting organismal vulnerability to climate warming: roles of behaviour, physiology, and adaptation. *Phil. Trans. R. Soc. B* **367**, 1665–1679. (doi:10.1098/rstb. 2012.0005)

22. Spellerberg IF. 1972 Temperature tolerances of southeast Australian reptiles examined in relation to reptile thermoregulatory behaviour and distribution. *Oecologia* **9**, 23–46. (doi:10.1007/BF00345241)

23. Lutterschmidt WI, Hutchison VH. 1997 The critical thermal maximum: history and critique. *Can. J. Zool.* **75**, 1561–1574. (doi:10.1139/z97-783)

24. Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL. 2007 Critical thermal limits depend on methodological context. *Proc. R. Soc. B* **274**, 2935–2942. (doi:10.1098/rspb.2007.0985)

25. Hertz PE, Arce-Hernandez A, Ramirez-Vazquez J, Tirado-Rivera W, Vazquez-Vives L. 1979 Geographical variation of heat sensitivity and water loss rates in the tropical lizard *Anolis gundlachi*. *Comp. Biochem. Phys. A* **62**, 947–953. (doi:10. 1016/0300-9629(79)90033-1)

26. Gaston KJ, Spicer JI. 1998 Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarelllus* (Custacea: Amphipoda)? *J. Exp. Mar. Biol. Ecol.* **229**, 265–276. (doi:10.1016/S0022-0981(98)00057-4)

27. Chown SL, Jumbam KR, Sørensen JG, Terblanche JS. 2009 Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* **23**, 133–140. (doi:10.1111/j.1365-2435.2008.01481.x)

28. Felsenstein J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15. (doi:10. 1086/284325)

29. Paradis E. 2006 *Analysis of phylogenetics and evolution with R*. New York, NY: Springer.

30. R Development Core Team. 2012 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

31. Garland Jr T, Harvey PH, Ives AR. 1992 Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18–32. (doi:10.2307/2992503)

32. Oufiero CE, Gartner GEA, Adolph SC, Garland Jr T. 2011 Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* **65**, 3590–3607. (doi:10.1111/j.1558-5646.2011.01405.x)

33. Drummond AJ, Rambaut A. 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214. (doi:10.1186/1471-2148-7-214)

34. Harmon LJ, Weir JT, Brock C, Glor RE, Challenger W. 2008 GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131. (doi:10.1093/ bioinformatics/btm538)

35. Hansen TF. 1997 Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**, 1341–1351. (doi:10.2307/2411186)

36. Blomberg SP, Garland Jr T, Ives AR. 2003 Testing for phylogenetic signal in comparative data: behvioural traits are more labile. *Evolution* **57**, 717–745.

37. Butler MA, King AA. 2004 Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* **164**, 683–695. (doi:10.1086/ 426002)

38. Freckleton RP, Harvey PH. 2006 Detecting non-Brownian trait evolution in adaptive radiations. *PLoS Biol.* **4**, e373. (doi:10.1371/journal.pbio.0040373)

39. Sugihara N. 1978 Further analysis of the data by Akaike's information criterion and the finite corrections. *Commun. Stat. Theory Methods* **A7**, 13–26. (doi:10.1080/03610927808827599)

40. Burnham KP, Anderson DR. 2002 *Model selection and multimode inference: a practical information-theoretic approach*. New York, NY: Springer.

41. Adams DC. 2013 Comparing evolutionary rates for different phenotypic traits on a phylogeny using likelihood. *Syst. Biol.* **62**, 181–192. (doi:10.1093/ sysbio/sys083)

42. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/ bioinformatics/btg412)

43. Paradis E. 2012 *Analyses of phylogenetics and evolution with R*. New York, NY: Springer.

44. Kolbe JJ, VanMiddlesworth PS, Losin N, Dappen N, Losos JB. 2012 Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecol. Evol.* **2**, 1503–1516. (doi:10. 1002/ece3.263)

45. Bakken GS. 1992 Measurement and application of operative and standard operative temperatures in ecology. *Am. Zool.* **32**, 194–216.

46. Bakken GS, Gates DM. 1975 Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution. In *Perspectives of biophysical ecology* (eds DM Gates, RB Schmerl), pp. 255–290. New York, NY: Springer.

47. Bakken GS, Angilletta Jr MJ. In press. How to avoid errors when quantifying thermal environments. *Funct. Ecol.* (doi:10.1111/1365-2435.12149)

48. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010 Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463–1464. (doi:10.1093/bioinformatics/btq166)

49. Cowles RB, Bogert CM. 1944 A preliminary study of the thermal requirements of desert reptiles. *Br. Am. Mus. Nat. Hist.* **83**, 265–296.

50. Kearney M, Shine R, Porter WP. 2009 The potential for behavioural thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl Acad. Sci. USA* **106**, 3835–3840. (doi:10.1073/pnas.0808913106)

51. Ruibal R. 1961 Thermal relations of five species of tropical lizards. *Evolution* **15**, 98–111. (doi:10.2307/2405846)

52. Rand AS. 1964 Ecological distribution in anoline lizards of Puerto Rico. *Ecology* **45**, 745–752. (doi:10.2307/1934922)

53. van Berkum FH. 1986 Evolutionary patterns of thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution* **40**, 594–604. (doi:10.2307/2408580)

54. Hertz PE, Arima Y, Harrison A, Huey RB, Losos JB, Glor RE. 2013 Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution* **677**, 2101–2113. (doi:10.1111/evo.12072)

55. Huey RB, Webster TP. 1976 Thermal biology of *Anolis* lizards in a complex fauna: the *cristatellus* group on Puerto Rico. *Ecology* **57**, 985–994. (doi:10.2307/1941063)

56. Gunderson AR, Leal M. 2012 Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard. *Funct. Ecol.* **26**, 783–793. (doi:10.1111/j.1365-2435.2012.01987.x)

57. Janzen DH. 1967 Why are mountain passes higher in the tropics? *Am. Nat.* **101**, 233–249. (doi:10.1086/282487)

58. Sunday JM, Bates AE, Dulvy NK. 2010 Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. B* **278**, 1823–1830. (doi:10.1098/rspb.2010.1295)

59. Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL. 2013 Heat freezes niche evolution. *Ecol. Lett.* **16**, 1206–1219. (doi:10.1111/ele.12155)

60. Grant BW, Dunham AE. 1988 Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology* **69**, 167–176. (doi:10.2307/1943171)

61. Adolph SC, Porter WP. 1993 Temperature, activity, and lizard life histories. *Am. Nat.* **142**, 273–295. (doi:10.1086/285538)

62. Mayr E. 1963 *Animal species and evolution*. Cambridge, MA: The Belknap Press of Harvard University Press.

63. West-Eberhard M. 2003 *Developmental plasticity and evolution*. New York, NY: Oxford University Press.

64. Angilletta Jr MJ. 2009 *Thermal adaptation: a theoretical and empirical synthesis*. Oxford, UK: Oxford University Press.

65. Huey RB. 2009 Evolutionary physiology of insect thermal adaptation to cold environments. In *Low temperature biology of insects* (eds DL Denlinger, RE Lee), pp. 223–241. Cambridge, UK: Cambridge University Press.

66. Schluter D. 1996 Adaptive radiation along genetic lines of least resistance. *Evolution* **50**, 1766–1774. (doi:10.2307/2410734)

67. Bennett AF. 1987 Evolution of the control of body temperature: is warmer better? In *Comparative physiology: life in the water and on land* (eds P Dejours, L Bolis, CR Taylor, ER Weibel), pp. 421–431. Padova, Italy: Liviana Press.

68. Huey RB, Kingsolver JG. 1989 Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131–135. (doi:10.1016/0169-5347(89)90211-5)

69. Savage VM, Gillooly JF, Brown JH, West GB, Charnove EL. 2004 Effects of body size and temperature on population growth. *Am. Nat* **163**, 429–441. (doi:10.1086/381872)

70. Angilletta Jr MJ, Huey RB, Frazier MR. 2010 Thermodynamic effects on organismal performance: is hotter better? *Physiol. Biochem. Zool.* **83**, 197–206.

71. Parmesan C. 2006 Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Syst.* **37**, 637–669. (doi:10.1146/annurev.ecolsys.37.091305.110100)

72. Hoffmann AA, Sorensen JG, Loeschcke V. 2003 Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* **28**, 175–216. (doi:10.1016/S0306-4565(02)00057-8)

73. Hoffmann AA, Chown SL, Clusella-Trullas S. 2012 Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* **27**, 934–949. (doi:10.1111/j.1365-2435.2012.02036.x)

74. Ragland GJ, Kinsolver JG. 2008 Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life-history timing in *Wyeomyia smithii*. *Evolution* **62**, 1345–1357. (doi:10.1111/j.1558-5646.2008.00367.x)

75. Barrett RDH, Paccard A, Healy TM, Bergek S, Schulte PM, Schluter D, Rogers SM. 2010 Rapid evolution of cold tolerance in stickleback. *Proc. R. Soc. B* **278**, 233–238. (doi:10.1098/rspb.2010.0923)

76. Leal M, Gunderson AR. 2012 Rapid change in the thermal tolerance of a tropical lizard. *Am. Nat.* **180**, 815–822. (doi:10.1086/668077)

77. Gilchrist GW, Huey RB. 1999 The direct response of *Drosophila melanogaster* to selection on knock-down temperature. *Heredity* **83**, 15–29. (doi:10.1038/sj.hdy.6885330)

78. Donaldson LR, Olson PR. 1957 Development of rainbow trout brood stock by selective breeding. *Trans. Am. Fish. Soc.* **85**, 93–101. (doi:10.1577/1548-8659(1955)85[93:DORTBS]2.0.CO;2)

79. Hamilton III WJ. 1973 *Life's color code*. New York, NY: McGraw Hill.