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8

9 **Authors:** Martha M. Muñoz*¹, Maureen A. Stimola*², Adam C. Algar³, Asa Conover⁴,
10 Anthony Rodriguez⁵, Miguel A. Landestoy⁶, George S. Bakken⁷, and Jonathan B. Losos¹

11 *denotes equal contribution
12

13 **Addresses:**

14 1 Department of Organismic and Evolutionary Biology and Museum of Comparative
15 Zoology, Harvard University, Cambridge, MA, 02138 USA

16 2 Department of Ecology, Evolution and Environmental Biology, Columbia University,
17 1200 Amsterdam Avenue, NY, 10027 USA

18 3 School of Geography, University of Nottingham, Sir Clive Granger Building,
19 University Park, Nottingham NG7 2RD, United Kingdom

20 4 Stuyvestant High School, 345 Chambers Street, New York, NY, 10282 USA

21 5 Department of Wildlife, Fish, & Conservation Biology, University of California, Davis,
22 One Shields Avenue, Davis, CA, 95616 USA

23 6 Sociedad Ornitológica de la Hispaniola, Gustavo Mejía Ricart 119 B, Apto. 401,
24 Galerías Residencial, Santo Domingo, República Dominicana

25 7 Department of Biology, Indiana State University, Terre Haute, IN, 47809 USA
26

27 **Key words:** thermoregulation, thermal physiology, physiological evolution, *Anolis*
28 lizards, Bogert effect

24 **Abstract**

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

40

41 **Introduction**

42 Rising temperatures present unique challenges for tropical ectotherms, which
43 already generally function near their upper thermal limits: even small temperature
44 increases can have disproportionately large negative consequences for these organisms
45 (Deutsch et al. 2008; Huey et al. 2009). Studies assessing tropical ectotherms'
46 vulnerability to climate warming have traditionally focused on predicting where warming

47 will have the most pronounced effects on organismal fitness by correlating physiological
48 traits with environmental data and using these relationships to infer where range shifts
49 and local extinctions will occur (e.g., Sinervo et al. 2010; Chen et al. 2011). However, the
50 evolutionary potential of populations to respond to novel selective pressures imposed by
51 rising temperatures is an equally important and comparatively unexplored aspect of
52 response to climate warming (Williams et al. 2008).

53 Behaviour and physiology can interact to determine how organisms interact with
54 their thermal environments (Stevenson 1985). Organisms that thermoregulate limit
55 exposure to suboptimal temperatures – a phenomenon commonly referred to as the
56 ‘Bogert effect’ (Bogert 1949; Huey et al. 2003). Physiological traits that behavioural
57 thermoregulation can shield from selection should evolve less than traits that cannot be so
58 easily buffered and thus are exposed to stronger selection. Because many environments
59 are more thermally complex in the day than at night (Sarmiento 1986; Ghalambor et al.
60 2006), thermoregulation should be more effective at shielding diurnal organisms from
61 selection on upper than lower physiological limits and, consequently, tolerance to cold
62 should evolve faster than tolerance to heat.

63 In this study we compare rates of physiological evolution in the cybotoids, a
64 tropical clade of *Anolis* lizards from the Caribbean island of Hispaniola whose members
65 differ extensively in thermal habitat (Schwartz 1989; Glor et al. 2003). Previous work by
66 Hertz and Huey (1981) found similar body temperatures and heat tolerance among three
67 cybotoid species and provided ecological data suggesting they are good thermoregulators.
68 Our study expands on this work in terms of populations, species, and physiological traits
69 examined. The cybotoid clade is unique among Caribbean anoles because its species are

70 found from sea level to almost 3,000 meters (Henderson and Powell 2009); as a result,
71 the environments they experience pose different selective pressures and provide the
72 opportunity for behavioural and physiological adaptation to different thermal extremes.

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

81

82 **Materials and Methods**

83 *Study Organisms and Study Sites*

84 The cybotoid anoles are a clade of nine species from Hispaniola commonly found
85 on trunks or near the ground (Schwartz 1989). Species occupy nearly all available
86 climatic environments from xeric semi-deserts to high elevation mountains, which have
87 been occupied independently by two different lineages (Glor et al. 2003; Wollenberg et
88 al. 2013). Our sampling was conducted in June and July 2011 and focused on the seven
89 cybotoids found in the Dominican Republic (Fig. 1). The other two species are *A.*
90 *breslini*, which is restricted to northwestern Haiti and is ecologically quite similar to *A.*
91 *whitemani* (Williams 1963), and *A. haetianus*, which is found only in Western Haiti and
92 is likely synonymous with *A. cybotes* (Glor et al. 2003). Where possible, we sampled

93 several populations that, together, encompassed most of a species' altitudinal range (Fig.
94 1; Table 1). *Anolis cybotes* is found nearly island-wide; we sampled it at three elevations
95 in each of the two principal mountain chains, the Sierra de Baoruco (SB) and Cordillera
96 Central (CC). In the SB we sampled the mid-elevation species, *A. strahmi* and *A.*
97 *whitemani*, and the high elevation species, *A. armouri*. In the CC, we sampled the mid-
98 elevation species *A. marcanoii*, and the high elevation species, *A. shrevei*.

99 We gathered climatic measurements for each locality by extracting all
100 temperature variables (bio 1 – bio 11; Supp. Table 1) from environmental layers available
101 in the WorldClim dataset (resolved to approximately 1km²; Hijmans et al. 2005). These
102 variables summarize seasonal and annual temperature trends. To account for collinearity
103 among thermal variables, we reduced data dimensionality using a principal components
104 (PC) analysis on the correlation matrix.

105

106 *Measurement of physiological performance indices*

107 We measured field body temperature, T_b , during one continuous 13-hour period
108 (0600 – 1900 hr) at 13 localities from 20 June to 31 July 2011. Due to logistical
109 constraints, one locality (*A. shrevei* – 1950 m) was sampled from 0600 - 13:45, but
110 results for that population are consistent with those from other populations (Table 1).
111 Following established methods (Huey 1974; Hertz 1992), we walked slowly through each
112 habitat and used a standard noose to capture adult male lizards, which are more
113 conspicuous and easier to sample than females. For every lizard, we recorded core
114 temperature (T_b) to the nearest 0.1°C using a thermocouple (Type T, Copper-Constantan)
115 inserted ~1cm into the cloaca and connected to a temperature logger (HH603A, Omega).

116 Each lizard was measured only once. T_b generally correlates closely with the optimal
117 performance temperature (T_{opt} , the temperature at which organisms maximally perform a
118 function such as running) in many diurnal lizards, including tropical anoles (Huey et al.
119 2012).

120 For each lizard captured, we recorded the time, weather conditions (sunny, mixed,
121 or overcast skies), and “basking status” (basking in the full or filtered sun, perching in the
122 shade under sunny or mixed skies, or in the shade under overcast skies) following Hertz
123 (1992). For a statistical analysis of basking site choice, we removed observations for
124 lizards captured in overcast conditions, because these lizards did not have the opportunity
125 to choose between perching in the sun or in the shade. We tested for weather-dependent
126 basking choice using a logistic regression model such that individuals’ basking behaviour
127 (perching in shade = 0, perching in sun = 1) was evaluated as a function of elevation,
128 weather (sunny or mixed skies), and an elevation \times weather interaction. An effect of
129 elevation alone would indicate that certain basking behaviours are more likely to occur at
130 different elevations, whereas a significant interaction would indicate that active lizards
131 differ in how they exploit weather conditions at different elevations.

132 We measured the critical thermal minimum (CT_{min}) and maximum (CT_{max}), which
133 refer to the low and high temperatures at which an organism loses locomotor function;
134 these are widely used for measuring the tolerance limits of performance in ectotherms
135 (Spellerberg 1972; Lutterschmidt and Hutchison 1997). CT_{min} and CT_{max} are estimated as
136 the lower and upper temperatures at which a lizard fails to right itself when flipped onto
137 its back (Spellerberg 1972). After capture, we gave adult male lizards a 24-hour rest
138 period in a large, insulated ice chest (Coleman) in which temperature was maintained

139 near 23°C at all sites. To measure core temperature during the tolerance experiments, an
140 Omega temperature probe (Type T, 36 gauge) was placed approximately 1 cm into the
141 cloaca of each lizard and secured to the base of the tail using a small piece of surgical
142 tape, ensuring that tail movement was uncompromised. The temperature probe was
143 connected to a digital temperature logger (HH147U, Omega). The lizard was placed into
144 a perforated plastic container where it could move freely. After the lizard attained
145 ambient temperature, the container was moved to an insulated icebox coated with a layer
146 of crushed ice. Because the rate of temperature change during a tolerance experiment can
147 alter an organism's performance (Terblanche et al. 2007), we reduced body temperature
148 at a constant rate of approximately 1°C/min for all lizards. To conduct the experiment, we
149 reduced body temperature to 14°C, at which point we flipped the lizard onto its back
150 using a pair of blunt tweezers and stimulated it to flip itself back over by gently probing
151 the base of its tail and pressing its thighs. If the lizard flipped over after 15 seconds, we
152 then lowered core temperature 0.5°C and repeated this procedure, continuing until a
153 temperature was reached at which the lizard failed to right itself in the allotted time.
154 CT_{\min} was recorded as the temperature at which the righting response was lost.

155 Animals were given 24 hours to rest in the ice chest before CT_{\max} trials. The
156 method for estimating CT_{\max} was similar to that of CT_{\min} except that a 100 W light bulb
157 was suspended approximately 30 cm above the Tupperware. We placed lizards in a
158 Tupperware container and increased their core temperature at a rate of ~1°C/min by
159 exposing them to the heat source. We began flipping lizards when they began to cool
160 through panting (i.e., the 'panting threshold'; Hertz et al. 1979) following the procedure

161 described above, and recorded the temperature at which the righting response was lost as
162 CT_{max} .

163 Estimation of CT_{min} and CT_{max} is potentially confounded by the rate of
164 temperature change, body size, and starting conditions (Gaston and Spicer 1998;
165 Terblanche et al. 2007; Chown et al. 2009). We performed linear regressions with mean
166 population CT_{min} and CT_{max} as the dependent variables against the population means for
167 rate of temperature change, initial experimental temperature, and body mass (Supp. Table
168 2). We conducted separate analyses for each pair of dependent and independent variables,
169 and each regression was weighted by the variance in CT_{min} or CT_{max} . Because none of
170 these models were statistically significant (Supp. Table 3), we used raw CT_{min} and CT_{max}
171 values in subsequent analyses.

172 We compared physiological traits to thermal habitat (3 PC variables, see Results)
173 using population means and independent contrasts. We calculated standardized
174 independent contrasts (scaled by the expected variance) for the weighted species means
175 of each physiological trait (CT_{min} , T_b , and CT_{max}) and each of the thermal habitat
176 variables (PC I, PC II, PC III) using the *pic* function in the APE package (Felsenstein
177 1985; Paradis 2006) in R (R Development Core Team 2012). Although they are not
178 properties of the organisms, environmental traits may reflect underlying ecological traits
179 (Garland et al. 1992; Oufiero et al. 2011). We used the time-calibrated, majority rule
180 consensus tree of Mahler et al. (2010), with the topology generated using Bayesian
181 maximum clade credibility (Drummond and Rambaut 2007). This consensus tree
182 contains 187 of ~375 recognized species of anoles (all but 19 species of Caribbean
183 anoles), including all the species used in this study (Fig. 1). We used regression through

184 the origin to compare the contrasts for physiological traits with the contrasts for thermal
185 environment traits using the *lmargin* function in APE (Paradis 2006).

186

187 *Measuring rates of physiological trait evolution*

188 To ensure comparability among traits, we used the *fitContinuous* function in the
189 GEIGER package (Harmon et al. 2008) in R to fit three different models of evolution to
190 each physiological trait. These models were: (1) Brownian Motion a random walk; (2)
191 Ornstein-Uhlenbeck, a random walk in which characters tend to return to a single
192 optimum; and (3) Early Burst, in which the overall rate of evolution exponentially slows
193 through time (Hansen 1997; Blomberg et al. 2003; Butler and King 2004; Freckleton and
194 Harvey 2006). We calculated the Akaike Information Criterion corrected for small
195 sample size (AIC_C ; Sugihara 1978) for each model and compared the fits by examining
196 the Akaike weights (Burnham and Anderson 2002).

197 We used Adams' (2013) method to evaluate whether the rate of evolutionary
198 change varied among physiological traits. This method compares a model that allows
199 rates to vary among traits to one in which the rates are constrained to be equal using a
200 likelihood ratio test and AIC_C . To account for intraspecific measurement error, we
201 incorporated the standard error of the mean in our estimation of rates of evolution. We
202 used the APE library (Paradis et al. 2004; Paradis 2012) and new code supplied by
203 Adams (2013) in R.

204

205 *Cold-acclimation trials*

206 Because of the large differences in CT_{\min} we discovered among populations (see
207 Results), we conducted an experiment to assess how short-term acclimation influences
208 variation in this trait. In June 2013, we collected adult male lizards from two populations
209 differing greatly in thermal environment– *Anolis armouri* (Loma de Toro, elevation =
210 2318 m; n = 18) and *A. cybotes* (Los Patos, elevation = 45 m; n = 19). Kolbe et al. (2012)
211 found that a two-week acclimation at 22.5°C was sufficient to elicit a strong plastic
212 response in CT_{\min} in a lowland population of *A. cristatellus* from Puerto Rico without
213 inducing severe thermal stress. We maintained lizards at 19.4°C (range = 17.4°C –
214 21.9°C) for three weeks and measured CT_{\min} following the procedure described above.
215

216 *Night-time environmental temperature measurement*

217 We measured night-time operative temperature (T_e) in the same two, thermally-
218 contrasting, localities. T_e refers to an organism’s equilibrium temperature in the absence
219 of metabolic heating or evaporative cooling (*sensu* Bakken 1992), which we estimated
220 using replicas made of electroformed copper. These models mimic the thermal properties
221 of a thermoconforming lizard (e.g., color, shape, size; Bakken and Gates 1975). We
222 embedded iButton® data loggers (DS1921K Maxim) in copper models shaped using a
223 mold of *A. cybotes* – this new generation of copper models permits automated
224 temperature recording (for details of their construction, see Bakken and Angilletta
225 [2013]). Methods for calibrating the copper models are given in the Supplemental
226 Materials. We deployed these models (12 on trees, 11 on rocks, and 11 under rocks) in
227 Los Patos (June 5 – 7, 2013) and in Loma de Toro (11 each on trees, on rocks, and under
228 rocks) June 14 – 16, 2013 with the devices set to automatically record T_e at ten minute

229 intervals. We randomly selected perches, orientation, and height for model placement on
230 trees following Hertz (1992). In addition, we also recorded sleep site selection for lizards
231 at each of these localities during the experimental period.

232

233 **Results**

234 *Thermal habitat varies markedly across Hispaniola*

235 For this study, we visited various localities in the Dominican Republic that
236 spanned more than 2400 meters in altitude and a variety of habitats ranging from lowland
237 scrub to montane pine forests. Not surprisingly, sites varied considerably in temperature
238 (Fig. 2). In the principal component analysis of the WorldClim thermal variables, we
239 recovered three axes with eigenvalues greater than 1 that together explained 99.6% of the
240 variation in the thermal data (Supp. Table 4 & 5). PC I (hereafter ‘Thermal PC I’)
241 explained 73.2% of the variation and loaded highly for mean annual temperature, mean
242 temperatures of the wettest and driest quarters, maximum temperature of the warmest
243 month, and minimum temperature of the coldest month. PC II (14.2% variation
244 explained; hereafter ‘Range PC II’) loaded highly for daily and annual temperature
245 ranges, whereas PC III (12.2% variation explained; ‘Seasonality PC III’ axis) loaded with
246 variables related to thermal seasonality.

247

248 *Analyses of thermoregulation and physiology*

249 In approximately 164 hours of field observations, we collected T_b from 435 lizards and
250 basking site data from 381 lizards. The extent of basking in the sun varied greatly at
251 different elevations (logistic interaction term; $\chi^2 = 4.07$, $df = 1$, $p = 0.044$); lizards at

252 higher elevation were more likely to bask in the sun when it is available, whereas those at
253 lower elevation sought shade under sunny conditions. Neither CT_{max} nor T_b varied
254 significantly with any of the thermal habitat PC variables (Table 2). Tolerance to cold
255 (CT_{min} was positively correlated with Thermal PC I ($r = 0.934$, $p < 0.001$), which loaded
256 heavily with mean annual temperature, and this relationship remained significant after
257 phylogenetic correction (Table 2).

258

259 *Evolutionary analyses of physiology*

260 Brownian motion was the most strongly supported model for all three traits
261 (CT_{min} , T_b , and CT_{max}) – Akaike weights were > 0.93 in all cases (Supp. Table 6),
262 allowing for a comparison of evolutionary rates among traits. Likelihood ratio tests
263 indicated that, overall, the three physiological traits evolved at different rates, although
264 the differences were just above the significance threshold ($p = 0.06$) when intraspecific
265 measurement error was taken into account (Table 3). Pairwise comparisons showed that
266 rates of evolution for CT_{min} were significantly higher than for CT_{max} , even when
267 intraspecific measurement error was considered. However, differences in rates of
268 evolution between T_b and other traits were not significant in the analysis incorporating
269 intraspecific variation (Table 3), either because rates do not actually differ, or because
270 high variance in T_b obscures differences in rates of evolution.

271

272 *Cold acclimation experiment*

273 Mean CT_{min} was not significantly different between wild-measured (mean =
274 $11.3^{\circ}C$) and cold-acclimated *A. cybotes* (mean = $10.6^{\circ}C$) (unpaired t -test: $t = 1.53$, $p =$

275 0.136). Mean CT_{\min} remained the same (7.2°C) between wild-measured and cold-
276 acclimated *A. armouri*. CT_{\min} was significantly higher in *A. cybotes* than in *A. armouri* in
277 both the wild-measured (unpaired *t*-test: $t = 7.72$, $p < 0.001$) and cold-acclimated
278 treatments ($t = 9.28$, $p < 0.001$).

279

280 *Night-time temperature experiment*

281 Night-time operative temperatures (T_e) showed marked differences between high
282 and low elevation (Fig. 3). At Los Patos (low elevation), T_e ranged from $24.6 - 29.8^{\circ}\text{C}$,
283 whereas at Loma de Toro T_e (high elevation), it ranged from $10.9 - 18.1^{\circ}\text{C}$. T_e was on
284 average, though not always, somewhat higher under rocks than on top of rocks or on trees
285 (Fig. 3; Supp. Fig. 1), particularly early in the evening. All lizards at Los Patos were
286 observed sleeping on vegetation (43 observations), whereas lizards at Loma de Toro were
287 observed sleeping on vegetation (14/30) and underneath rocks (16/30) in roughly equal
288 numbers (test for differences in site selection among populations: $\chi^2 = 26.3$, $df = 1$, $p <$
289 0.001).

290

291 **Discussion**

292 *Thermoregulatory behaviour influences the rate of physiological evolution*

293 The question of how behaviour influences patterns of physiological evolution
294 dates back to the middle of the last century (Cowles and Bogert 1944; Bogert 1949) and
295 has received renewed interest in light of concern about how ectothermic organisms can
296 respond to climate warming (Huey et al. 2009; Kearney et al. 2009). We studied a clade
297 of lizards whose species vary markedly in thermal environment from hot semi-deserts to

298 cold montane environments. Despite occurring in environments that differ by as much as
299 15°C in mean annual temperature, field body temperature and heat tolerance were
300 remarkably similar among populations, indicating that behavioural thermoregulation can
301 be extraordinarily effective in limiting exposure to excessively hot or cold temperatures
302 (i.e. the ‘Bogert effect’; Huey et al. 2003). Our behavioural analysis demonstrates that
303 lowland lizards were more likely to retreat to the shade under sunny conditions, whereas
304 upland lizards were more likely to bask when the sun was out, a result in agreement with
305 previous work on three of these species (Hertz and Huey 1981). Our results are
306 particularly striking given that other anole species exhibit markedly different body
307 temperatures, even when they occur in sympatry (Ruibal 1961; Rand 1964; van Berkum
308 1986; Hertz et al. 2013, but see Huey and Webster 1976; Gunderson and Leal 2012).

309 Given the ability of cybotoids to thermoregulate to approximately the same
310 temperature throughout its range, it is not surprising that CT_{max} also shows very little
311 interspecific variation. However, these lizards have a much more limited ability to
312 thermoregulate at night, particularly at high elevation, where operative temperatures
313 measured on all types of sleep sites were so low that they would incapacitate
314 approximately 80% of lowland lizards (Fig. 3; Supp. Fig. 1). In the absence of thermal
315 refuges, populations have no option but to adapt physiologically. Indeed, we found that
316 none of the lizards from high elevation experienced night-time temperatures lower than
317 their CT_{min} (Fig. 3).

318 An alternative explanation for this finding is that differences in CT_{min} represent
319 non-genetic effects of living in different environments. Previous studies suggest that
320 adaptive plasticity is unlikely to account for physiological differences among populations

321 (Janzen 1967): our data support this view, as cold tolerance exhibits little acclimation,
322 even less so than in other anoles (Kolbe et al. 2012), which suggests that there is likely a
323 genetic basis for the observed variation in CT_{MIN} .

324 The inability of thermoregulation to buffer selection on physiology during the
325 night is an explanation for the fast rate of CT_{min} evolution in this clade (Table 3; Huey et
326 al. 2003). The relative stasis in CT_{max} documented here aligns with results from recent
327 meta-analyses showing that there is less variation in heat tolerance than in cold tolerance
328 in several ectotherm clades (Sunday et al. 2010; Araújo et al. 2013). In short,
329 behavioural thermoregulation allows cybotoid species to maintain similarly warm body
330 temperatures during the day, but not during the night, forcing species in montane
331 environments on Hispaniola to adapt to lower temperatures.

332

333 *What limits heat tolerance evolution?*

334 Behavioural thermoregulation can help explain why CT_{max} is less variable than
335 CT_{min} in the cybotoids, but not why the response to different environmental conditions
336 involved behavioural, rather than physiological, change. Given that time spent
337 thermoregulating imposes a cost with regard to its effect on other activities such as
338 foraging, predator avoidance, and reproduction (e.g., Huey 1974; Grant and Dunham
339 1988; Adolph and Porter 1993), it is unclear why selection should favour the maintenance
340 of high body temperatures in montane habitats, instead of physiological adaptation to
341 lower temperatures. One possibility is that behavioural modifications are easier to evolve
342 than changes in physiological tolerances (Mayr 1963; West-Eberhard 2003). Given that a
343 myriad of physiological processes (e.g., locomotion, digestion, growth) are sensitive to

344 temperature, the evolution of physiological tolerances may necessitate the concerted
345 evolution of many genes (discussed in Angilletta 2009; Huey 2009). In contrast, shifts in
346 basking frequency change seasonally within populations, and so behavioural shifts at
347 different elevations may not require substantial evolutionary change. Moreover, even if
348 evolutionary shifts in behaviour are required, such changes may require fewer genetic
349 changes than shifts in physiology (Blomberg et al. 2003).

350 This “evolution along lines of least genetic resistance” (*sensu* Schluter 1996)
351 explanation suggests that there is no inherent advantage to warmer body temperatures,
352 but an alternative explanation for the lack of evolutionary variability in CT_{max} revolves
353 around the fitness benefits of high temperatures. Specifically, selection may favour the
354 maintenance of high body temperatures in cold environments because rates of
355 biochemical reactions increase with optimal temperature (Bennett 1987; Huey and
356 Kingsolver 1989; Huey 2009). Indeed, warm-adapted ectotherms generally experience
357 higher levels of physiological performance than cold-adapted organisms (Savage et al.
358 2004; Angilletta et al. 2010). However, if this “hotter is better” hypothesis is true, it still
359 fails to address why low elevation populations have not evolved even higher heat
360 tolerances.

361

362 *Impacts of climate change*

363 Climate warming will likely have different effects on cybotoids from lowland and
364 upland habitats. Warming temperatures threaten to make current ranges thermally
365 inhospitable for many cool-adapted montane ectotherms, which may force their ranges
366 upward (Parmesan 2006; Chen et al. 2011). In contrast, it is likely that upland cybotoids

367 will benefit, at least in the short term, from climate warming. As the climate warms,
368 environmental temperatures will more often approximate lizards' preferred temperatures,
369 and thus the time lizards need to spend thermoregulating should decrease and the number
370 of hours available for other activities should increase; on the other hand, higher
371 temperatures may allow species from lower elevations to migrate upward, leading to
372 negative interspecific interactions (Huey et al. 2009).

373 The challenge facing lowland cybotoids will be to avoid stressfully hot
374 temperatures as habitats continue to warm. Many tropical lizards, particularly those near
375 sea level, are already frequently experiencing temperatures exceeding their preferred
376 ranges (e.g., Huey et al. 2009; Sinervo et al. 2010). As warming continues, lizards in such
377 lowland populations will eventually be unable to maintain temperatures within their
378 preferred range for long enough periods to survive. At that point, lowland populations
379 can only avoid local extinction by shifting their physiology to adapt to these higher
380 environmental temperatures.

381 Evolutionary stasis in CT_{max} may suggest a limited ability to evolve and, thus, a
382 heightened vulnerability to environmental warming. Some studies on *Drosophila* support
383 the idea that heat tolerance evolution is genetically constrained, as the amount of genetic
384 diversity for heat tolerance is limited compared to that for cold tolerance (Hoffmann et al.
385 2003, 2012; Ragland and Kingsolver 2008). The observation that CT_{min} evolves readily in
386 cybotoids and in other ectotherms (Barrett et al. 2010; Leal and Gunderson 2012) would
387 tend to support this hypothesis. Nonetheless, it is hard to construe why diverse
388 physiological systems would be constrained from evolving upper, but not lower
389 tolerances. In fact, experiments on *Drosophila* (Gilchrist and Huey 1999) and salmon

390 (Donaldson and Olson 1957) have demonstrated that heat tolerance can increase in
391 response to selection, although there appears to be an upper ceiling on how high heat
392 tolerance can evolve (Hamilton 1973); no similar experiments have ever been conducted
393 on vertebrates. Moreover, although cybotoid anoles show relatively little variation in heat
394 sensitivity, some other anole clades have diversified extensively while adapting to
395 different thermal environments (Hertz et al. 2013). Looking more broadly, other lizard
396 species possess heat tolerances that approach 50°C (reviewed in Sunday et al. 2010;
397 Araújo et al. 2013), suggesting that if genetic constraints exist in lizards, they are
398 phylogenetically localized. Finding an explanation for variation among clades in
399 physiological diversity could aid in assessing ectotherms' vulnerability to climate
400 warming, but it is a challenge that will require integration of physiological, behavioural,
401 and evolutionary approaches.

402

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412

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678

679

Tables.

Species	Locality	Alt (m)	CT _{MIN} (°C)	T _B (°C)	CT _{MAX} (°C)
Wild-Measured					
<i>A. cybotes</i> (SB)	Los Patos	45	11.3±0.4 (16)	30.1±0.3 (45)	39.5±0.1 (20)
<i>A. cybotes</i> (CC)	San Cristóbal	56	11.4±0.2 (16)	29.4±0.5 (53)	39.2±0.2 (16)
<i>A. longitibialis</i>	Jaragua	105	12.8±0.2 (18)	28.9±0.2 (101)	38.5±0.3 (18)
<i>A. whitemani</i>	Puerto Escondido	411	12.2±0.4 (15)	27.9±1.1 (17)	38.8±0.3 (15)
<i>A. strahmi</i>	Camino Aguacate	454	11.3±0.5 (6)	26.3±0.7 (7)	39.2±0.2 (6)
<i>A. marcanoii</i>	Ocoa	458	12.6±0.3 (9)	-	38.2±0.3 (9)
<i>A. cybotes</i> (CC)	Jarabacoa	690	10.7±0.2 (18)	29.0±0.6 (39)	40.3±0.2 (18)
<i>A. cybotes</i> (SB)	Guayullal	727	10.8±0.4 (15)	26.6±0.4 (53)	38.7±0.2 (15)
<i>A. marcanoii</i>	La Horma	879	11.3±0.1 (16)	29.1±0.6 (48)	38.7±0.2 (16)
<i>A. cybotes</i> (CC)	Constanza	1390	10.0±0.5 (11)	29.2±0.5 (10)	39.5±0.6 (11)
<i>A. cybotes</i> (SB)	La Hoz	1395	8.7±0.4 (9)	28.6±0.9 (11)	38.9±0.2 (9)
<i>A. shrevei</i>	Valle Nuevo - Low	1950	9.6±0.6 (9)	28.0±1.0 (10)	39.9±0.3 (9)
<i>A. armouri</i>	Loma de Toro - Low	2020	8.2±0.6 (9)	-	39.3±0.3 (9)
<i>A. armouri</i>	Loma de Toro - High	2318	7.2±0.4 (12)	25.9±0.7 (21)	-
<i>A. shrevei</i>	Valle Nuevo - High	2450	6.2±0.3 (11)	27.4±1.0 (20)	40.4±0.3 (11)
Cold Acclimated					
<i>A. cybotes</i>	Los Patos	45	10.6±0.3 (19)	-	-
<i>A. armouri</i>	Loma de Toro - High	2318	7.2±0.3 (18)	-	-

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 TB), and critical thermal maximum (CT_{MAX}) are given for each population. Units for physiological metrics are °C ± 1 SEM 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.

(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 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 and significant results are highlighted in bold. Correlations among contrasts were measured using the *cor.table* function in picante (Kembel et al. 2010) in R.

A. Full Analysis			
Trait	σ^2	Uncorrected	Corrected
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 _{df=2} = 12.56, ρ = 0.002	LRT _{df=2} = 5.57, ρ = 0.06
B. Pairwise Analysis			
Comparison	Uncorrected LRT _{df=1} ; ρ	Corrected LRT _{df=1} ; ρ	
CT _{MIN} vs CT _{MAX}	12.40; < 0.001	7.85; 0.005	
CT _{MIN} vs T _B	0.87; 0.350	0.32; 0.574	
CT _{MAX} vs T _B	6.86; 0.009	-4.70; 1.00	

Table 3. Comparison of evolutionary rates for CT_{MIN}, T_B, and CT_{MAX}. (A) Presents the full analysis of evolutionary rates (σ^2) incorporating covariation among all three traits. One test accounted for intraspecific measurement error (Corrected), while 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) Presents 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.

680 Figure Legends.

681 Figure 1. Map showing altitudinal variation and the localities for each cybotoid
682 population sampled in this study. The map inset shows the location of Hispaniola with
683 respect to the other islands in the Caribbean basin. Grayscale indicates elevation and
684 range from dark (low) to light (high) elevation. Species are denoted in different color
685 triangles in the phylogeny generated based on Mahler et al. (2010) (See Methods).

686

687 Figure 2. Population means (± 1 SEM) are given for critical thermal maximum (CT_{MAX}),
688 body temperature (T_b), and critical thermal minimum (CT_{MIN}). The mean annual
689 temperature for each locality is provided in the bottom panel. The x-axis denotes
690 elevation rank for each population. Species are denoted in different colours and shapes.

691

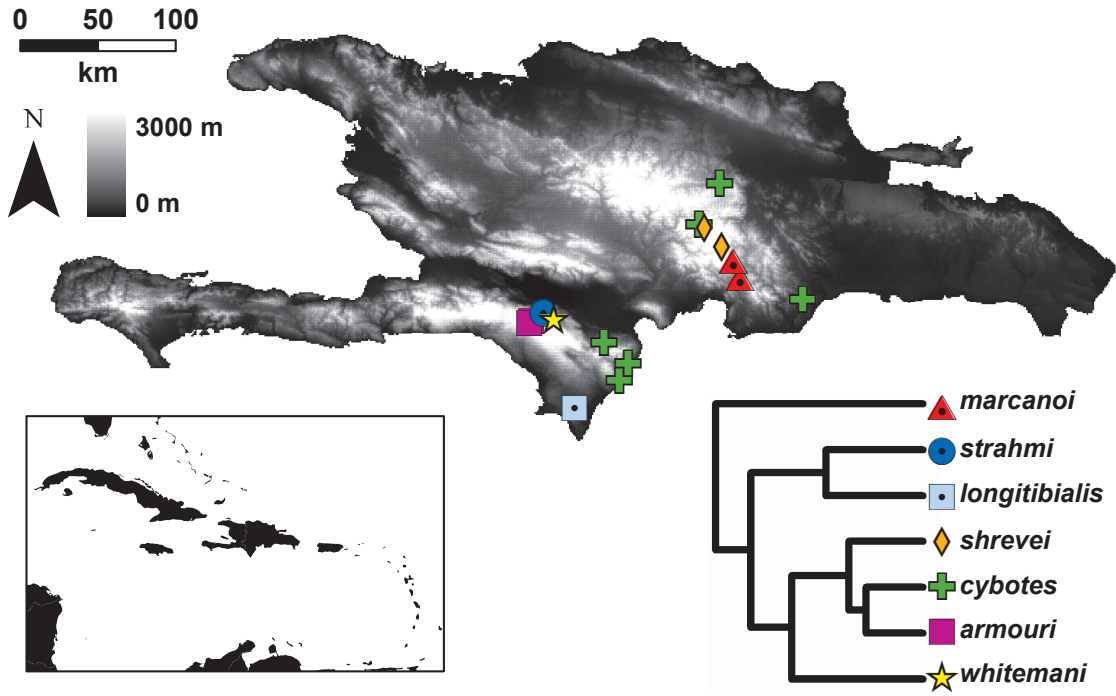
692 Figure 3. (A) Box plots showing the variation in operative temperatures during 1-hour
693 time blocks. Each time block summarizes temperatures collected over three consecutive
694 nights in June 2013 at low elevation (45 m) and high elevation (2318 m). Color denotes
695 the type of perch where the temperature was measured as follows: on a tree – white; on
696 top of a rock – light gray; underneath a rock – dark gray. (B) CT_{min} measured in
697 individuals of *A. cybotes* (left) and *A. armouri* (right) from the same localities in part (A)
698 are given.

699

700 Supplemental Figure 1. Operative temperatures measured at high elevation (2318 m) and
701 low elevation (45 m) on each type of perch: trees (green); on top of rocks (gray); and
702 under rocks (blue).

703 Figure 1

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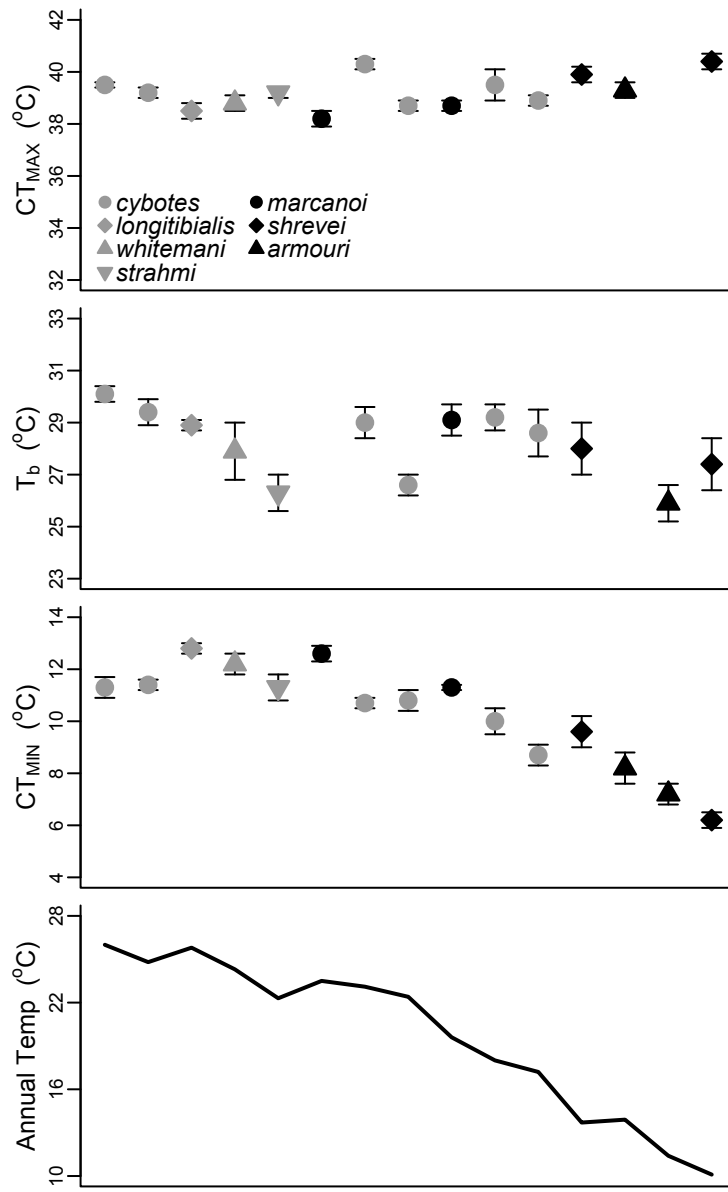
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709 Figure 2

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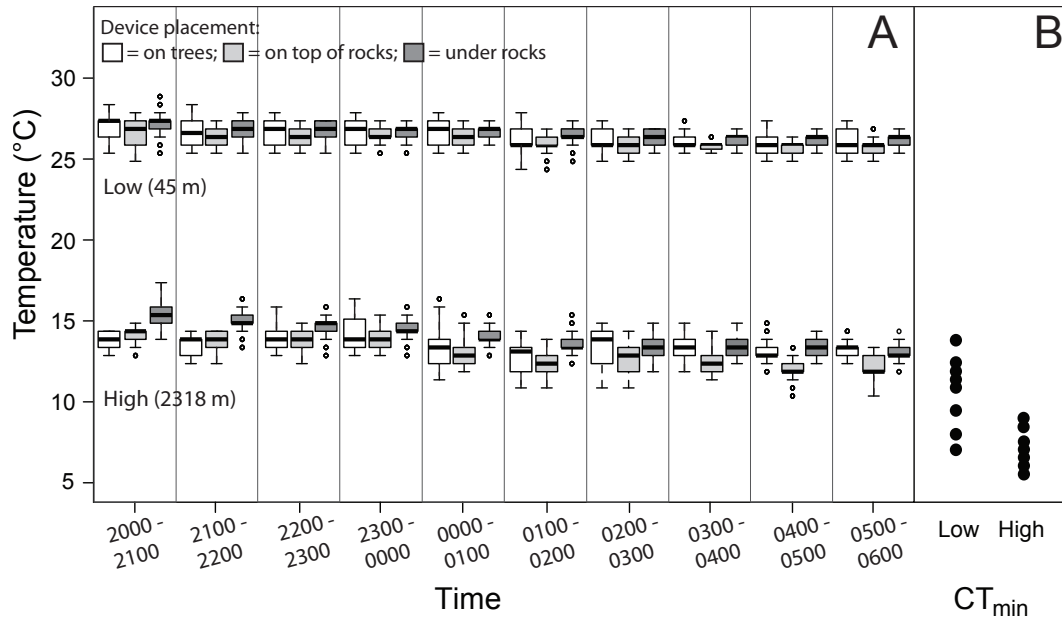
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715 Figure 3

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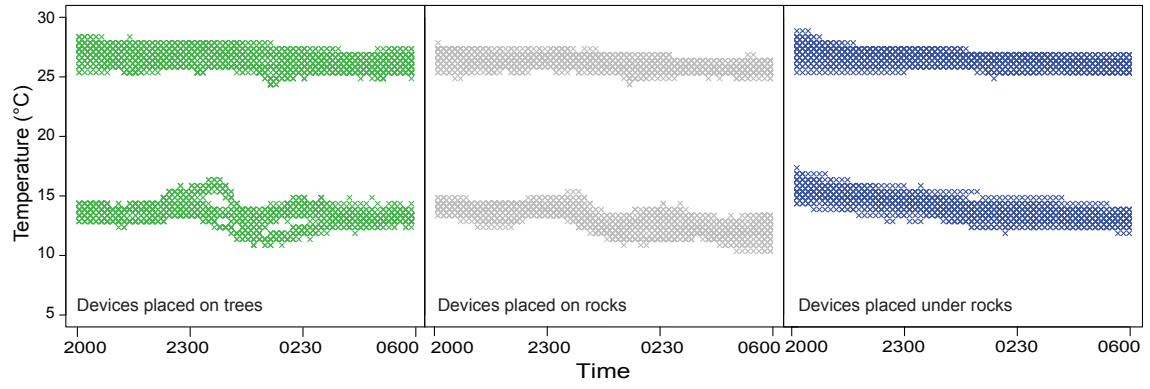


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719 Supp Figure 1

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Species	Coordinates	Alt (m)	bio 1	bio 2	bio 3	bio 4	bio 5	bio 6	bio 7	bio 8	bio 9	bio 10	bio 11
<i>A. cybotes</i> (SB)	17.96, -71.19	45	26.0	9.9	0.76	1.03	32.2	19.3	12.9	26.8	24.6	27.2	24.5
<i>A. cybotes</i> (CC)	18.43, -70.13	56	24.8	10.1	0.77	0.94	30.9	17.9	13.0	25.7	23.6	25.9	23.4
<i>A. longitibialis</i>	17.80, -71.45	105	25.8	9.9	0.73	1.10	32.3	18.9	13.4	26.9	24.5	27.2	24.3
<i>A. whitemani</i>	18.32, -71.57	411	24.3	12.3	0.76	1.13	31.9	15.9	16.0	25.3	22.7	25.6	22.7
<i>A. strahmi</i>	18.35, -71.63	454	22.3	12.9	0.77	1.18	30.0	13.3	16.7	23.3	20.6	23.6	20.6
<i>A. marcanoii</i>	18.55, -70.50	458	23.5	11.1	0.75	1.13	30.6	15.9	14.7	24.5	21.8	24.7	21.8
<i>A. cybotes</i> (CC)	19.10, -70.61	690	23.1	10.8	0.76	1.01	29.6	15.5	14.1	24.1	21.6	24.2	21.6
<i>A. cybotes</i> (SB)	18.06, -71.14	727	22.4	11.7	0.71	1.48	30.3	14.0	16.3	21.9	20.5	24.0	20.2
<i>A. marcanoii</i>	18.65, -70.54	879	19.6	13.3	0.77	1.20	27.7	10.5	17.2	20.6	18.1	20.9	17.8
<i>A. cybotes</i> (CC)	18.86, -70.73	1390	18.0	12.4	0.77	1.12	25.4	9.3	16.1	19.0	16.4	19.2	16.4
<i>A. cybotes</i> (SB)	18.18, -71.28	1395	17.2	13.0	0.76	1.29	25.1	8.2	16.9	17.7	15.5	18.6	15.3
<i>A. shrevei</i>	18.84, -70.70	1950	13.7	12.3	0.75	1.28	21.4	5.1	16.3	14.7	12.0	15.1	11.9
<i>A. armouri</i>	18.30, -71.70	2020	13.9	11.8	0.75	1.16	21.1	5.4	15.7	14.9	12.2	15.2	12.2
<i>A. armouri</i>	18.29, -71.71	2318	11.4	11.4	0.74	1.23	18.5	3.1	15.4	12.5	9.6	12.7	9.6
<i>A. shrevei</i>	18.73, -70.60	2450	10.1	11.7	0.74	1.35	17.5	1.8	15.7	11.4	8.4	11.6	8.2

Supplemental Table 1. Coordinates and altitude are given for each population sampled. Mountain chain for *Anolis cybotes* is given as SB (Sierra de Baoruco, West) and CC (Cordillera Central, East). Thermal variables were extracted from the WorldClim database (Hijmans et al. 2005) and are given for each of the sampling localities, with codes (bio 1 – bio 11) as defined in Supp. Table 1. Bioclimatic variables extracted from the WorldClim database are as follows – bio 1: Mean Annual Temperature (MAT); bio 2: mean diurnal range; bio 3: isothermality; bio 4: temperature seasonality; bio 5: maximum temperature of the warmest month; bio 6: minimum temperature of the coldest month; bio 7: annual temperature range; bio 8: mean temperature of the wettest quarter; bio 9: mean temperature of the driest quarter; bio 10: mean temperature of the warmest quarter; bio 11: mean temperature of the coldest quarter. All values are in °C, except for bio 2 and bio 7.

Species	Locality	CT _{MIN}	Cooling Rate (°C/min)	Body Mass (g)	CT _{MAX}	Heating Rate (°C/min)	Body Mass (g)
		Start Temp. (°C)			Start Temp. (°C)		
<i>A. cybotes</i> (W)	Los Patos	26.7±0.9 (20)	0.99±0.01 (16)	7.42± 1.23 (19)	27.8±0.3	0.86±0.00	7.15±1.25
<i>A. cybotes</i> (E)	San Cristóbal	24.8±0.3 (16)	0.92±0.00	6.74±1.60	26.5±0.6	0.90±0.01	6.34±1.20
<i>A. longitibialis</i>	Jaragua	26.8±2.7 (18)	1.00±0.01	7.48±0.37	31.1±0.7	0.82±0.01	7.42±0.37
<i>A. whitemani</i>	Puerto Escondido	27.0±0.2 (15)	0.94±0.00	4.66±0.54	29.1±0.4	0.92±0.00	4.66±0.54
<i>A. strahmi</i>	Camino Aguacate	27.3±0.9 (6)	0.93±0.01	6.92±0.75	29.6±0.4	0.99±0.01	6.92±0.75
<i>A. marcanoii</i>	Ocoa	26.9±0.1 (9)	0.90±0.00	3.42±0.84	28.4±0.3	0.98±0.00	3.42±0.84
<i>A. cybotes</i> (E)	Jarabacoa	29.2±0.5 (18)	1.04±0.00	6.52±0.65	29.5±0.2	0.88±0.01	6.16±0.53
<i>A. cybotes</i> (W)	Guayullal	24.9±1.1 (18)	0.96±0.01 (16)	9.07± 1.37 (16)	28.2±0.3	0.86±0.01 (15)	8.45±1.62
<i>A. marcanoii</i>	La Horma	25.7±0.5 (16)	0.87±0.01	4.22±0.32	28.9±0.3	0.86±0.00	4.22±0.32
<i>A. cybotes</i> (E)	Constanza	24.6±0.5 (11)	0.97±0.01	4.95±1.11	24.8±0.3	0.98±0.01	4.65±1.15
<i>A. cybotes</i> (W)	La Hoz	27.7±0.3 (9)	0.92±0.00	6.84±2.25	30.8±0.1	0.86±0.00 (8)	6.02±3.24
<i>A. shrevei</i>	Valle Nuevo - Low	24.6±0.4 (9)	0.79±0.01	4.62±0.33	26.8±0.4	0.99±0.00	4.31±0.36
<i>A. armouri</i>	Loma de Toro - Low	24.4±0.8 (9)	0.94±0.02	5.82±1.29	26.8±1.1	0.98±0.00	5.23±1.81
<i>A. armouri</i>	Loma de Toro - High	29.7±0.2 (12)	0.93±0.00	6.43±1.86	-	-	-
<i>A. shrevei</i>	Valle Nuevo - High	26.6±0.8 (11)	0.84±0.01	4.79±0.07	28.5±0.1	0.76±0.01	4.46±0.07

Supplemental Table 2. Mean start temperature (°C), rate of temperature change (°C/min), and lizard body mass (g) are given for each population for CT_{MIN} and CT_{MAX} experiments. Values are mean ±1 SEM and sample sizes are given in parentheses. Only sample sizes that differ from the first column are given in other columns.

A. CT _{MIN}	Reg. Coeff.	df	<i>t</i>	Pearson's <i>r</i>	<i>p</i>
Start Temperature	-0.21	13	-0.57	-0.107	0.578
Mass	-0.03	13	0.09	0.057	0.927
Cooling Rate	12.87	13	1.46	0.357	0.168
B. CT _{MAX}					
Start Temperature	-0.09	12	-0.84	-0.276	0.416
Mass	0.01	12	0.10	-0.107	0.924
Heating Rate	-0.97	12	-0.42	-0.134	0.682

Supplemental Table 3: Summary of results from linear regression assessing the relationship between mean population (A) critical thermal minimum, CT_{MIN}, and (B) critical thermal maximum, CT_{MAX}, and several variables - start temperature, body mass, and rate of temperature change. Regressions were weighted by the variance in CT_{MIN} and CT_{MIN}.

Variable	PC I	PC II	PC III
MAT (bio 1)	0.97	-0.25	0.09
Mean Diurnal Range (bio 2)	-0.29	0.95	0.09
Isothermality (bio 3)	0.03	0.10	0.99
Temperature Seasonality (bio 4)	-0.32	0.57	-0.74
Maximum Temperature of Warmest Month (bio 5)	0.99	-0.13	0.05
Minimum Temperature of Coldest Month (bio 6)	0.93	-0.36	0.08
Temperature Annual Range (bio 7)	-0.32	0.93	-0.15
Mean Temperature of Wettest Quarter (bio 8)	0.95	-0.28	0.15
Mean Temperature of Driest Quarter (bio 9)	0.96	-0.26	0.11
Mean Temperature of Warmest Quarter (bio 10)	0.97	-0.24	0.06
Mean Temperature of Coldest Quarter (bio 11)	0.96	-0.27	0.12
Eigenvalue	8.1	1.6	1.3
Percent Variance Explained	73.2	14.2	12.2

Supplemental Table 4. Summary of loading, eigenvalues, and variance explained in the principal components (PC) analysis on the thermal variables (bio 1 – bio 11) for all populations sampled in this study. Loadings greater than 0.70 are highlighted in bold text.

Population	Locality	PC I – Mean Annual Temperature	PC II – Temperature Range	PC III – Temperature Seasonality
<i>A. cybotes</i> (SB)	Los Patos	0.75	-1.18	0.37
<i>A. cybotes</i> (CC)	San Cristóbal	0.44	-1.18	1.05
<i>A. longitibialis</i>	Jaragua	0.87	-1.17	-0.95
<i>A. whitemani</i>	Puerto Escondido	0.98	1.00	0.44
<i>A. strahmi</i>	Camino Aguacate	0.64	1.50	0.83
<i>A. marcanoii</i>	Ocoa	0.56	-0.14	-0.08
<i>A. cybotes</i> (CC)	Jarabacoa	0.28	-0.61	0.61
<i>A. cybotes</i> (SB)	Guayullal	0.76	0.75	-2.50
<i>A. marcanoii</i>	La Horma	0.15	1.69	0.87
<i>A. cybotes</i> (CC)	Constanza	-0.47	0.66	1.01
<i>A. cybotes</i> (SB)	La Hoz	-0.40	1.30	0.25
<i>A. shrevei</i>	Valle Nuevo - Low	-1.29	0.42	-0.13
<i>A. armouri</i>	Loma de Toro - Low	-1.44	-0.18	0.16
<i>A. armouri</i>	Loma de Toro - High	-2.02	-0.64	-0.42
<i>A. shrevei</i>	Valle Nuevo - High	-2.16	-0.32	-0.69

Supplemental Table 5. PC scores for first three PC axes are given for each population. Factor loadings for each axis is given in Supp. Table 4.

Trait	lnL			AIC _C weight		
	BM	OU	EB	BM	OU	EB
CT _{MIN}	-14.99	-14.38	-14.99	0.98	0.01	0.01
T _B	-13.20	-11.06	-13.20	0.94	0.05	0.01
CT _{MAX}	-5.86	-5.49	-5.86	0.98	0.01	0.01

Supplemental Table 6. Results of model fitting tests showing likelihood estimates and AIC_C weights for the Brownian Motion (BM), Orenstein-Uhlenbeck (OU), and Early Burst (EB) models of evolution. Bold indicates the best-supported model.

Supplementary Methods

Model Calibration

We assessed the accuracy of the copper models with embedded Thermochrons by equilibrating models at three biologically-relevant temperatures alongside a thermometer using a setup similar to Angilletta and Krochmal (2003). To this end we placed 28 models (as many as would fit) in a large incubator at the Concord Field Station, Harvard University in Bedford, MA and allowed them to acclimate over the course of an hour to three temperatures – 12, 23, and 32°C – after which temperature was recorded. On average, models deviated 0.13°C from the set incubator temperature, and the magnitude of deviation was consistently low across temperatures (Table 1).

Table 1. Accuracy of copper models (n = 28) was estimated by calculating the difference between the set temperature and model temperature.

Incubator Temperature (°C)	Model Temperature (Mean °C ± 1sd)	Mean deviance from incubator temperature
12	11.45 ± 0.21	-0.55
23	23.61 ± 0.39	0.61
32	32.32 ± 0.31	0.32

We measured the difference in equilibrium temperatures between copper models (T_e) and a live lizard (T_B) by exposing both to different temperatures, radiation, and convective conditions (reviewed in Dzialowski 2005). One live adult male *Anolis armouri* lizard and one copper lizard model were tethered to a cardboard panel using dental floss and a thermocouple (Type T, Omega) was inserted into the cloaca of each and secured using medical tape. The panel was exposed to different thermal conditions in fifteen minute intervals including: in the sun and shade on a sunny day (air temperature [T_A] = 31.1°C), outdoors on a breezy overcast day (T_A = 22°C), indoors in an air-conditioned room (T_A = 18.9°C), and indoors in a cold chamber (T_A = 4°C). To avoid thermal stress body temperatures (T_B) were not allowed to drop below 10°C or go above 35°C. T_e and T_B were automatically recorded every minute. The correlation between T_B and T_e was strong (0.889) and the coefficient of this relationship was close to 1 (coeff. = 1.03). This slope was offset by 0.86°C such that $T_B = 1.03(T_e) + 0.86$. Thus, to estimate T_B from T_e we corrected all copper model temperatures by adding 0.86°C.

Works Cited

- Angilletta, M.J., and A.R. Krochmal. 2003. The Thermochron: A truly miniature and inexpensive temperature logger. *Herpetological Review*, 34(1): 31—32.
- Dzialowski, E.M. 2005. Use of operative temperature and standard operative temperature models in thermal biology. *Journal of Thermal Biology*, 30: 317—334.