6 **This is a post peer-review version**

7 **Title**: Evolutionary stasis and lability in thermal physiology in a group of tropical lizards.

8

9 **Authors**: Martha M. Muñoz*[1], Maureen A. Stimola*[2], Adam C. Algar[3], Asa Conover[4],

10 Anthony Rodriguez[5], Miguel A. Landestoy[6], George S. Bakken[7], and Jonathan B. Losos[1]

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

Abstract

24

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

Introduction

41

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

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 (e.g., Sinervo et al. 2010; Chen et al. 2011). 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 (Williams et al. 2008).

Behaviour and physiology can interact to determine how organisms interact with

their thermal environments (Stevenson 1985). Organisms that thermoregulate limit

exposure to suboptimal temperatures – a phenomenon commonly referred to as the

'Bogert effect' (Bogert 1949; Huey et al. 2003). 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 (Sarmiento 1986; Ghalambor et al.

2006), thermoregulation should 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 (Schwartz 1989; Glor et al. 2003). Previous work by

Hertz and Huey (1981) found similar body temperatures and heat tolerance among three

cybotoid species and provided ecological data suggesting 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

3

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

4

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. marcanoi*, 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 1km^2; 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 × 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 *lmorigin* 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*

9

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

10

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˚C) and cold-acclimated *A. cybotes* (mean = 10.6˚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°C,

283 whereas at Loma de Toro T_e (high elevation), it ranged from 10.9 – 18.1°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

412

413 **Works Cited**

414 Adams, D.C. 2013. Comparing evolutionary rates for different phenotypic traits on a

415 phylogeny using likelihood. *Syst. Biol.* 62, 181—192.

416

417 Adolph, S.C., and Porter, W.P. 1993. Temperature, activity, and lizard life histories. *Am.*

418 *Nat.* 142, 273—295.

419

420 Angilletta Jr, M.J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*.

421 Oxford, UK: Oxford University Press.

422

423 Angilletta Jr, M.J., Huey, R.B., and Frazier, M.R. 2010. Thermodynamic effects on

424 organismal performance: Is hotter better? *Physiol. Biochem. Zool.* 83, 197—206.

425

426 Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F., and Chown,

427 S.L. 2013. Heat freezes niche evolution. *Ecol. Lett.* 16, 1206—1219.

428

429 Bakken, G.S. 1992. Measurement and application of operative and standard operative

430 temperatures in ecology. *Am. Zool.* 32, 194—216.

431

432 Bakken, G.S., and Angilletta, M.J. 2013. How to avoid errors when quantifying thermal

433 environments. *Funct. Ecol.* in press

434

435 Bakken, G.S., and Gates, D.M. 1975. Heat-transfer analysis of animals: some

436   implications for field ecology, physiology, and evolution. In *Perspectives of Biophysical*

437   *Ecology* (eds D.M. Gates and R.B. Schmerl), pp. 255—290. New York: Springer-Verlag.

438

439   Barrett, R.D.H., Paccard, A., Healy, T.M., Bergek, S., Schulte, P.M., Schluter, D., and

440   Rogers, S.M. 2010. Rapid evolution of cold tolerance in stickleback. *Proc. R. Soc. B* 278,

441   233—238.

442

443   Bennett, A.F. 1987. Evolution of the control of body temperature: Is warmer better?, In

444   *Comparative Physiology: Life in the water and on land* (eds P. Dejours, L. Bolis, C.R.

445   Taylor, and E.R. Weibel), pp. 421—431. Padova: Liviana Press.

446

447   Blomberg, S.P., Garland Jr., T., and Ives, A.R. 2003. Testing for phylogenetic

448   signal in comparative data: behvioural traits are more labile. *Evolution* 57, 717—745.

449

450   Bogert, C.M. 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* 3,

451   195—211.

452

453   Burnham, K.P., and Anderson, D.R. 2002. *Model selection and multimode inference: a*

454   *practical information-theoretic approach.* New York: Springer-Verlag.

455

456   Butler, M. A., and King, A.A. 2004. Phylogenetic comparative analysis: a modeling

457   approach for adaptive evolution. *Amer. Nat.* 164, 683—695.

458

459 Chen, I.C., Hill, J.K., Ohlemüller, R., Roy, D.B., and Thomas, C.D. 2011. Rapid range

460 shifts of species associated with high levels of climate warming. *Science* 333, 1024—

461 1026.

462

463 Chown, S.L., Jumbam, K.R., Sørensen, J.G., and Terblanche, J.S. 2009. Phenotypic

464 variance, plasticity and heritability estimates of critical thermal limits depend on

465 methodological context. *Funct. Ecol.* 23, 133—140.

466

467 Cowles, R.B. and Bogert, C.M. 1944. A preliminary study of the thermal requirements of

468 desert reptiles. *B. Am. Mus. Nat. Hist.* 83, 265—296.

469

470 Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak,

471 D.C., and Martin P.R. 2008. Impacts of climate warming on terrestrial ectotherms across

472 latitude. *Proc. Natl. Acad. Sci. USA* 105, 6668—6672.

473

474 Donaldson, L.R., and Olson, P.R. 1957. Development of rainbow trout brood stock by

475 selective breeding. *Trans. Am. Fish. Soc.* 85, 93—101.

476

477 Drummond, A.J., and Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by

478 sampling trees. *BMC Evol. Biol.* 7, 214.

479

480 Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125, 1—15.

481

482   Freckleton, R.P., and Harvey, P.H. 2006. Detecting non-Brownian trait evolution in

483   adaptive radiations. *PLoS Biology* 4, e373.

484

485   Garland, Jr., T., Harvey, P.H., and Ives, A.R. 1992. Procedures for the analysis of

486   comparative data using phylogenetically independent contrasts. *Syst. Bio.* 41, 18—32.

487

488   Gaston, K.J., and Spicer, J.I. 1998. Do upper thermal tolerances differ in geographically

489   separated populations of the beachflea *Orchestia gammarelllus* (Custacea: Amphipoda)?

490   *J. Exp. Mar. Biol. Ecol.* 229, 265—276.

491

492   Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J., and Wang, G. 2006. Are

493   mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.*

494   46, 5—17.

495

496   Gilchrist, G.W., and Huey, R.B. 1999. The direct response of *Drosophila melanogaster*

497   to selection on knock-down temperature. *Heredity* 83, 15—29.

498

499   Glor, R.E., Kolbe, J.J., Powell, R., Larson, A., and Losos, J.B. 2003. Phylogenetic

500   analysis of ecological and morphological diversification in Hispaniolan trunk-ground

501   anoles (*Anolis cybotes* group). *Evolution* 57, 2383—2397.

502

503   Grant, B.W., and Dunham, A.E. 1988. Thermally imposed time constraints on the activity

504   of the desert lizard *Sceloporus merriami*. *Ecology* 69, 167—176.

505

506   Gunderson, A.R., and Leal, M. 2012. Geographic variation in vulnerability to climate

507   warming in a tropical Caribbean lizard. *Funct. Ecol.* 26, 783—793.

508

509   Hamilton, W.J., III. 1973. *Life's color code*. New York: McGraw Hill.
510

511   Hansen, T.F. 1997. Stabilizing selection and the comparative analysis of adaptation.

512   *Evolution* 51, 1341—1351.

513

514   Harmon, L. J., Weir, J.T., Brock, C., Glor, R.E., and Challenger, W. 2008. GEIGER:

515   investigating evolutionary radiations. *Bioinformatics* 24, 129—131.

516

517   Henderson, R.W. and Powell, R. 2009. *Natural History of West Indian Reptiles and*

518   *Amphibians*. Gainesville: University Press of Florida.

519

520   Hertz, P.E. 1992. Temperature regulation in Puerto Rican *Anolis* lizards: a field test using

521   null hypotheses. *Ecology* 73, 1405—1417.

522

523   Hertz, P.E., Arce-Hernandez, A., Ramirez-Vazquez, J., Tirado-Rivera, W., and Vazquez-

524   Vives, L. 1979. Geographical variation of heat sensitivity and water loss rates in the

525   tropical lizard *Anolis gundlachi*. *Comp. Biochem. Phys. A* 62, 947—953.

526

527 Hertz, P.E., Arima, Y., Harrison, A., Huey, R.B., Losos, J.B., and Glor, R.E. 2013.

528 Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution* 677,

529 2101—2113.

530

531 Hertz, P.E., and Huey, R.B. 1981. Compensation for altitudinal changes in the thermal

532 environment by some *Anolis* lizards on Hispaniola. *Ecology* 62, 515—521.

533

534 Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., and Jarvis, A. 2005. Very high

535 resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965—

536 1978.

537

538 Hoffmann, A.A., Chown, S.L, and Clusella-Trullas, S. 2012. Upper thermal limits in

539 terrestrial ectotherms: how constrained are they? *Functional Ecology* 27, 934—949.

540

541 Hoffmann, A.A., Sorensen, J.G., and Loeschcke, V. 2003. Adaptation of *Drosophila* to

542 temperature extremes: bringing together quantitative and molecular approaches. *J.*

543 *Therm. Biol.* 28, 175—216.

544

545 Huey, R.B. 1974. Behvioural thermoregulation in lizards: importance of associated costs.

546 *Science* 184, 1001—1003.

547

548 Huey, R.B. 2009. Evolutionary physiology of insect thermal adaptation to cold

549 environments. In *Low Temperature Biology of Insects* (eds D.L. Denlinger and R.E. Lee,

550 Jr.), Cambridge University Press, Cambridge, U.K.

551

552 Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Álvarez Pérez, H.J.,

553 and Garland, Jr., T. 2009. Why tropical forest lizards are vulnerable to climate warming.

554 *P. Roy. Soc. B-Biol. Sci.* 276, 1939—1948.

555

556 Huey, R.B., Hertz, P.E., and Sinervo, B. 2003. Behvioural drive versus behvioural inertia

557 in evolution: A null model approach. *Am. Nat.* 161, 357—366.

558

559 Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., and Williams,

560 S.E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour,

561 physiology, and adaptation. *Philos. T. Roy. Soc. B* 367, 1665—1679.

562

563 Huey, R.B. and Kingsolver, J.G. 1989. Evolution of thermal sensitivity of ectotherm

564 performance. *Trends Ecol. Evol.* 4, 131—135.

565

566 Huey, R.B. and Webster, T.P. 1976. Thermal biology of *Anolis* lizards in a complex

567 fauna: the *cristatellus* group on Puerto Rico. *Ecology* 57, 985—994.

568

569 Janzen, D.H. 1967. Why are mountain passes higher in the tropics? *Am. Nat.* 101, 233—

570 249.

571

572   Kearney, M., Shine, R., and Porter, W.P. 2009. The potential for behavioural

573   thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl.*

574   *Acad. Sci. USA* 106, 3835—3840.

575

576   Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D.,

577   Blomberg, S.P., and Webb, C.O. 2010. Picante: R tools for integrating phylogenies and

578   ecology. *Bioinformatics* 26:1463—1464.

579

580   Kolbe, J.J., VanMiddlesworth, P.S., Losin, N., Dappen, N., and Losos, J.B. 2012.

581   Climatic niche shift predicts thermal trait response in one but not both introductions of

582   the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecol. Evol.* 2, 1503—

583   1516.

584

585   Leal, M., and Gunderson, A.R. 2012. Rapid change in the thermal tolerance of a tropical

586   lizard. *Am. Nat.* 180, 815—822.

587

588   Lutterschmidt, W.I., and Hutchison, V.H. 1997. The critical thermal maximum: history

589   and critique. *Can. J. Zoolog.* 75, 1561—1574.

590

591   Mahler, D.L., Revell, L.J., Glor, R.E., and Losos, J.B. 2010. Ecological opportunity and

592   the rate of morphological evolution in the diversification of Greater Antillean anoles.

593   *Evolution* 64, 2731—2745.

594

595   Mayr, E. 1963. *Animal Species and Evolution*. Cambridge, MA: The Belknap Press of

596   Harvard University Press.

597

598   Oufiero, C.E., Gartner, G.E.A., Adolph, S.C., and Garland, Jr., T. 2011. Latitudinal and

599   climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: A

600   phylogenetic perspective. *Evolution* 65, 3590—3607.

601

602   Parmesan C. 2006. Ecological and evolutionary responses to recent climate change.

603   *Annu. Rev. Ecol. Syst. S* 37, 637—669.

604

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

606

607   Paradis E. 2012. Analyses of phylogenetics and evolution with R. New York: Springer.

608

609   Paradis E., Claude J., Strimmer K. 2004. APE: analyses of phylogenetics and evolution in

610   R language. *Bioinformatics* 20, 289—290.

611

612   R Development Core Team 2012. R: a language and environment for statistical

613   computing. Vienna, Austria: R Foundation for Statistical Computing.

614

615   Ragland, G.J., and Kinsolver, J.G. 2008. Evolution of thermotolerance in seasonal

616   environments: the effects of annual temperature variation and life-history timing in

617   *Wyeomyia smithii*. *Evolution* 62, 1345—1357.

618

619   Rand, A.S. 1964. Ecological distribution in anoline lizards of Puerto Rico. *Ecology* 45,

620   745—752.

621

622   Ruibal, R. 1961. Thermal relations of five species of tropical lizards. *Evolution* 15, 98—

623   111.

624

625   Sarmiento, G. 1986. Ecological features of climate in high tropical mountains. In *High*

626   *altitude tropical biogeography* (eds F. Vuilleumier and M. Monasterio), pp. 11—46. New

627   York: Oxford University Press.

628

629   Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B., and Charnove, E.L. 2004. Effects

630   of body size and temperature on population growth. *Am. Nat.*, 163, 429—441.

631

632   Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution*

633   50, 1766—1774.

634

635   Schwartz, A. 1989. A review of the cybotoid anoles (Reptilia: Sauria: Iguanidae) from

636   Hispaniola. *Milwaukee Public Mus. Contrib. Biol. and Geol.* 78, 1—32.

637

638   Sinervo, B., Méndez-De-La-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Cruz, M.V.-

639   S., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M.L., Meza-Lázaro,

640   R.N., Gadsden, H., Avila, L.J., Morando, M., Riva, I.J.D.L., Sepulveda, P.V., Rocha,

641   C.F.D., Ibargüengoytía, N., Puntriano, C.A., Massot, M., Lepetz, V., Oksanen, T.A.,

642   Chapple, D.A., Bauer, A.M., Branch, W.R., Clobert, J., and Sites, J.W. 2010. Erosion of

643   lizard diversity by climate change and altered thermal niches. *Science*, 328, 894—899.

644

645   Spellerberg, I.F. 1972. Temperature tolerances of southeast Australian reptiles examined

646   in relation to reptile thermoregulatory behaviour and distribution. *Oecologia* 9, 23—46.

647

648   Stevenson, R. D. 1985. The relative importance of behvioural and physiological

649   adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* 126, 362—

650   386.

651

652   Sugihara, N. 1978. Further analysis of the data by Akaike's information criterion and the

653   finite corrections. *Comm. Stat. Theory Methods* A7, 13—26.

654

655   Sunday, J.M., Bates, A.E., and Dulvy, N.K. 2010. Global analysis of thermal tolerance

656   and latitude in ectotherms. *Proc. R. Soc. B* 278, 1823—1830.

657

658   Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., and Chown, S.L. 2007.

659   Critical thermal limits depend on methodological context. *P. Roy. Soc. B-Biol. Sci.* 274,

660   2935—2942.

661

662   van Berkum, F.H. 1986. Evolutionary patterns of thermal sensitivity of sprint speed in

663   *Anolis* lizards. *Evolution* 40, 594—604.

664

665   West-Eberhard, M. 2003. *Developmental Plasticity and Evolution*. New York: Oxford

666   University Press.

667

668   Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A., and Langham, G. 2008.

669   Towards an integrated framework for assessing the vulnerability of species to climate

670   change. *PLoS Biol.* 6, 2621—2626.

671

672   Williams, E.E. 1963. *Anolis whitemani*, new species from Hispaniola (Sauria, Iguanidae).

673   *Breviora* 197, 1—8.

674

675   Wollenberg, K.C., Wang, I.J., Glor, R.E., and Losos, J.B. 2013. Determinism in the

676   diversification of Hispaniolan trunk-ground anoles (*Anolis cybotes* species complex).

677   *Evolution*, in press

678

679

Tables.

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)	Guayullal	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 Acclimated					
A. cybotes	Los Patos	45	10.6±0.3 (19)	-	-
A. armouri	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, p = 0.002	$LRT_{df=2}$ = 5.57, p = 0.06
B. Pairwise Analysis			
Comparison	Uncorrected $LRT_{df=1}$; p	Corrected $LRT_{df=1}$; p	
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 evoluationary 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 ($\pm$ 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

704

705

706

707

708
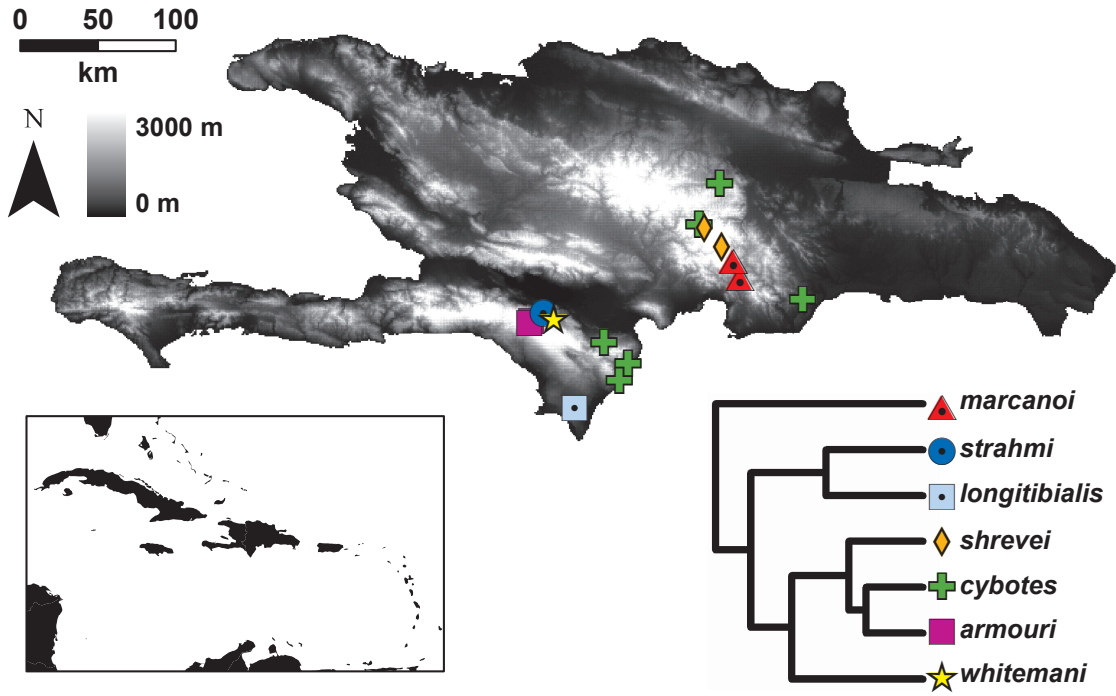
710
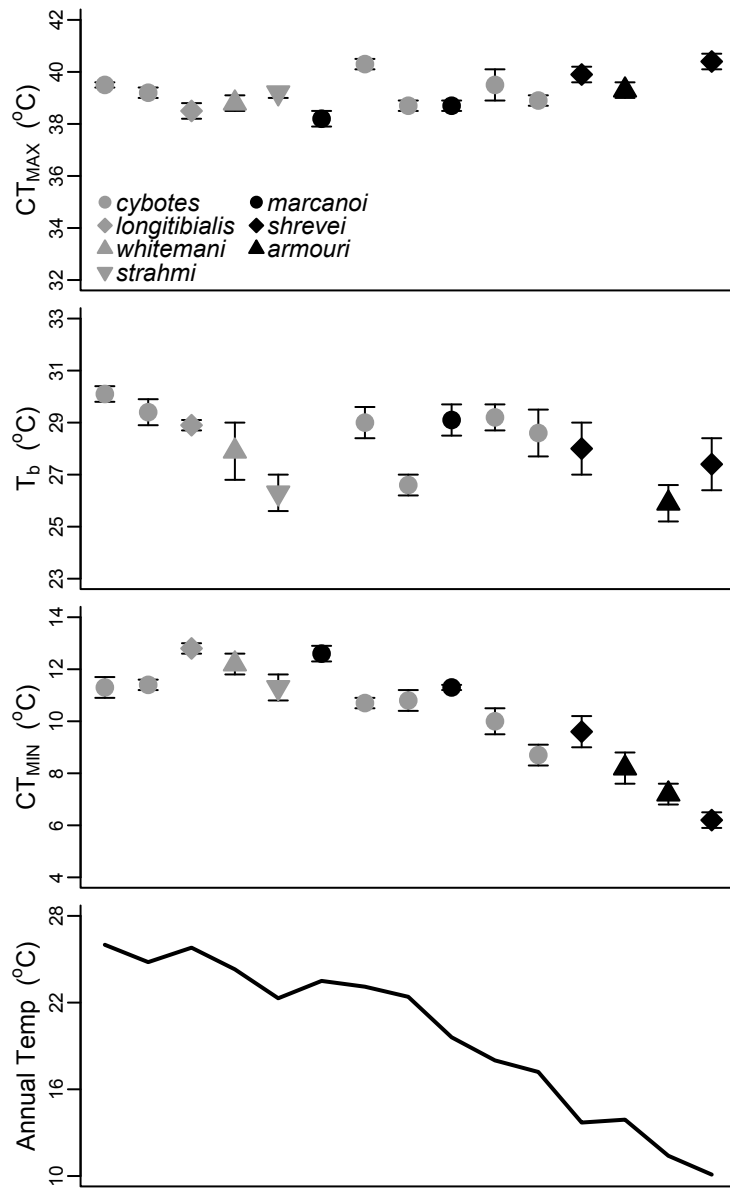
711

712

713

714

715   Figure 3

716
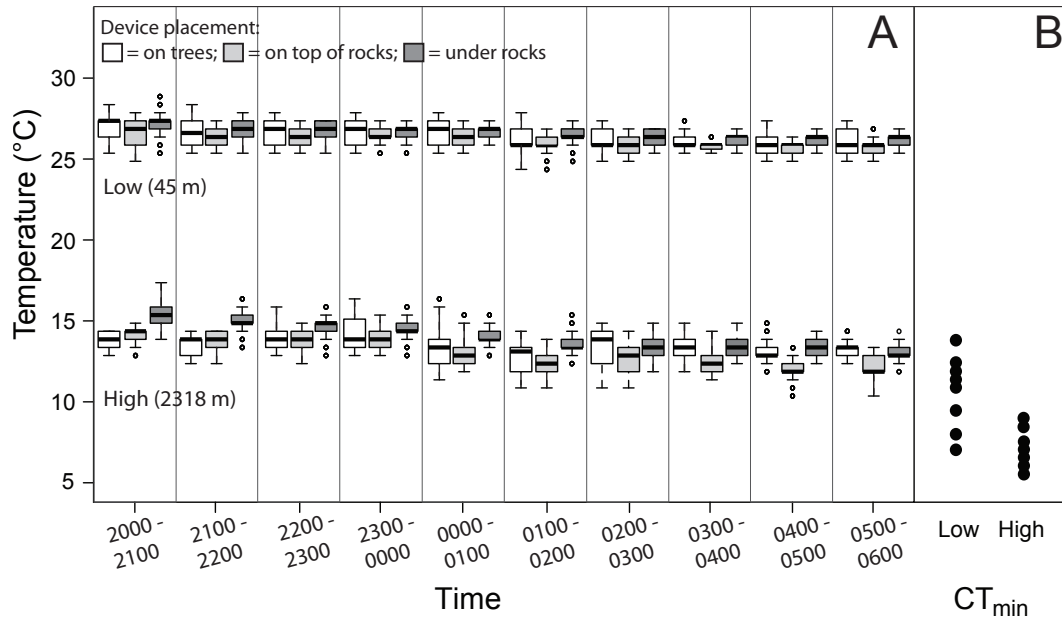
717

718

719   Supp Figure 1

720
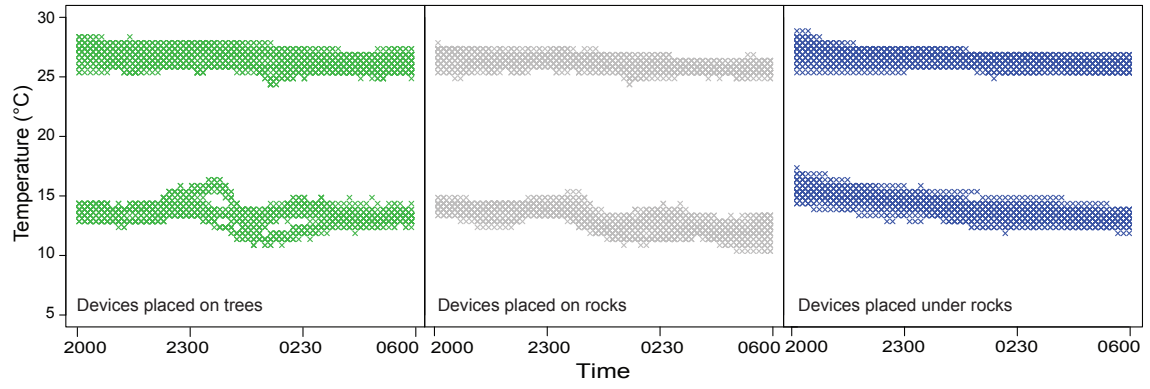
721

722

723

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
A. cybotes (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
A. cybotes (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
A. longitibialis	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
A. whitemani	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
A. strahmi	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
A. marcanoi	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
A. cybotes (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
A. cybotes (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
A. marcanoi	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
A. cybotes (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
A. cybotes (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
A. shrevei	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
A. armouri	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
A. armouri	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
A. shrevei	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$_{MIN}$			CT$_{MAX}$		
		Start Temp. (ºC)	Cooling Rate (ºC/min)	Body Mass (g)	Start Temp. (ºC)	Heating Rate (ºC/min)	Body Mass (g)
A. cybotes (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
A. cybotes (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
A. longitibialis	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
A. whitemani	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
A. strahmi	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
A. marcanoi	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
A. cybotes (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
A. cybotes (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
A. marcanoi	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
A. cybotes (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
A. cybotes (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
A. shrevei	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
A. armouri	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
A. armouri	Loma de Toro - High	29.7±0.2 (12)	0.93±0.00	6.43±1.86	-	-	-
A. shrevei	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	t	Pearson's r	p
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
A. cybotes (SB)	Los Patos	0.75	-1.18	0.37
A. cybotes (CC)	San Cristóbal	0.44	-1.18	1.05
A. longitibialis	Jaragua	0.87	-1.17	-0.95
A. whitemani	Puerto Escondido	0.98	1.00	0.44
A. strahmi	Camino Aguacate	0.64	1.50	0.83
A. marcanoi	Ocoa	0.56	-0.14	-0.08
A. cybotes (CC)	Jarabacoa	0.28	-0.61	0.61
A. cybotes (SB)	Guayullal	0.76	0.75	-2.50
A. marcanoi	La Horma	0.15	1.69	0.87
A. cybotes (CC)	Constanza	-0.47	0.66	1.01
A. cybotes (SB)	La Hoz	-0.40	1.30	0.25
A. shrevei	Valle Nuevo - Low	-1.29	0.42	-0.13
A. armouri	Loma de Toro - Low	-1.44	-0.18	0.16
A. armouri	Loma de Toro - High	-2.02	-0.64	-0.42
A. shrevei	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 estimated 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.