A functional analysis of the contribution of climatic niche divergence to adaptive radiation

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Abstract

Analyses of evolutionary radiations have traditionally focused on traits related to resource acquisition, habitat use, and mate choice. Nonetheless, incorporation of climatic niche divergence has become more common in recent years, driven by the development of correlative ecological niche models (ENMs) that allow niche characterization based solely on geo-referenced locality data. However, ENMs require no data on organismal traits that link climatic conditions to performance, and thus have little to say about how organisms phenotypically adapt to different climatic conditions, what the performance consequences of phenotypic divergence are, and how phenotypic divergence influences resource partitioning within communities of co-occurring species. Here, we conduct a functional (*i.e.*, physiological) analysis of climatic niche divergence within Greater Antillean *Anolis* radiations. We find that physiological divergence occurred repeatedly within these radiations, that there are performance consequences of physiological divergence within natural habitats, and that physiological divergence facilitates species co-existence within species-rich communities. We also use phylogenetic methods to compare rates of evolution of ecologically relevant physiological and morphological traits, and find that physiology has evolved either more slowly or as fast (but not faster) than morphology. Our analysis demonstrates the benefit of a functional approach to understanding macroevolutionary patterns of climatic niche divergence.

Introduction

During adaptive radiations, the proliferation of species is accompanied by adaptive phenotypic diversification, leading to a pattern in which multiple closely related but ecologically distinct species coexist (Simpson 1953; Schluter 2000). The vast majority of research on adaptive radiation, and evolutionary radiation in general, has focused on morphological traits directly linked to resource acquisition, structural habitat use, and mating display (Schluter 2000; Seehausen et al. 2008; Fleishman et al. 2009; Arnegard et al. 2010). Classic examples include divergence in jaw morphology in African cichlids (Meyer 1993) and bill shape in Darwin's finches (Grant 1999) to specialize on different food sources, and body shape divergence in Caribbean *Anolis* lizards to specialize on different structural microhabitats (*e.g.*, twigs, trunks and grasses) (Williams 1983; Losos 2009).

In contrast, adaptation to aspects of the climatic environment, such as temperature and water availability, have traditionally received much less attention in studies of evolutionary radiations, particularly in animals (Schluter 2000). This has been changing in recent years due to the development of global scale climate databases and correlative ecological niche models (ENMs) that allow one to estimate parameters of a species' abiotic ecological niche based on geo-referenced species locality data (Peterson et al. 2011). Due largely to their relative ease of use and the fact that they facilitate the incorporation of ecologically intractable or understudied species into ecological and evolutionary analyses, ENMs are now commonly used to calculate rates and frequencies of climatic niche divergence and to infer adaptive diversification (Knouft et al. 2006; Warren et al. 2008; Quintero and Wiens 2013; Lawing et al. 2016).

70 However, ENMs also have several disadvantages that limit the inferences that can
71 be drawn from their application (Guisan and Thuiller 2005; Kearney and Porter 2009;
72 Buckley et al. 2010; Araújo and Peterson 2012; Alvarado-Serrano and Knowles 2014;
73 Warren et al. 2014). For example, they provide no information about what phenotypic
74 traits, if any, have evolved to facilitate the occupation of different climatic niches. In
75 other words, functional analyses using the ENM approach alone are not possible
76 (Buckley et al. 2010). In addition, the climatic data used in ENMs are typically collected
77 at scales much larger than those at which organisms experience and utilize their habitats
78 (Potter et al. 2013). Thus, allopatric populations from broadly different climates could
79 actually experience very similar abiotic conditions because of differences in microhabitat
80 use (Bogert 1949; Huey et al. 2003); conversely, different species that are broadly
81 sympatric could actually experience very different abiotic conditions because of
82 differential microhabitat use (Kaspari et al. 2015).
83 When species adapt to different climates, the phenotypes most likely to diverge
84 are physiological traits related to tolerance for, and sensitivity to, abiotic conditions
85 (Hochachka and Somero 2002). For example, physiological variation is extensive across
86 environmental gradients (Levin et al. 2001; Hochachka and Somero 2002; Angilletta
87 2009; Sunday et al. 2011). Physiological divergence can also lead to reproductive
88 isolation between populations (Matute et al. 2009; Keller and Seehausen 2012) and
89 facilitate fine-scale resource partitioning among co-occurring taxa (Rand 1964),
90 characteristics that can hypothetically facilitate adaptive radiation (Schluter 2000).
91 Indeed, the evolution of thermal physiology is hypothesized to play an important role in
92 the radiation of a number of groups including bolitoglossine salamanders (Wake 1987;

Kozak and Wiens 2007), *Liolaemus* lizards (Labra et al. 2009; Bonino et al. 2011; Pincheira-Donoso 2011), *Drosophila* flies (Kellermann et al. 2009) and *Petrolisthes* crabs (Stillman and Somero 2000). Still, a comprehensive understanding of the contribution of physiological adaptation to the process of adaptive radiation is currently lacking.

Greater Antillean *Anolis* lizards are a textbook example of adaptive radiation, best known for the repeated morphological evolution of distinct perch type (*i.e.*, structural microhabitat) specialists, known as ecomorphs (Williams 1983; Losos et al. 1998; Mahler et al. 2013). Nonetheless, morphological specialization cannot fully explain the Greater Antillean radiation because most cladogenic events are not associated with ecomorphological divergence (Losos 2009). Furthermore, many *Anolis* communities support more than one species of a given ecomorph (Williams 1983; Losos et al. 2003), and thus morphological specialization cannot fully explain the diversity of *Anolis* communities.

Climatic niche divergence is proposed to be a major contributor to the species richness of Greater Antillean anoles based primarily on body temperature and ENM studies (Ruibal 1961; Rand 1964; Heatwole et al. 1969; Williams 1972; Huey and Webster 1976; Losos et al. 2003; Knouft et al. 2006; Hertz et al. 2013; Algar and Mahler 2015). However, we know relatively little about how the occupation of different thermal niches has been facilitated by physiological evolution during this radiation (Muñoz et al. 2014), and the extent to which physiological divergence has facilitated the co-existence of species within diverse anole communities. For example, what components of physiology evolve during adaptive radiations? Additionally, what are the geographical patterns of physiological divergence? In other words, are physiologically divergent

species geographically separated, or do they occur sympatrically? The latter scenario would indicate that physiological divergence can promote the maintenance of high community-level diversity by facilitating species co-existence. Finally, how does the tempo of evolution of physiological traits compare to that of morphological traits important to divergence along other ecological axes (*e.g.*, the structural niche axis)?

Here, we take a functional approach to investigate the physiological and ecological consequences of climatic niche divergence in West Indian *Anolis* lizards. Our analyses focus primarily on the *cristatellus* species group from Puerto Rico, but we also conduct analyses that include data from other Puerto Rican anoles as well as species from St. Croix and Jamaica (Fig. 1). First, we use data from the *cristatellus* species group to investigate divergence in thermal physiology between species that occupy distinct thermal niches. Next, we integrate these data with measurements of operative thermal environments in Puerto Rico to explore the performance consequences of this physiological variation. We then consider geographical patterns of physiological trait divergence to evaluate the contribution of thermal adaptation to niche partitioning and community-level species co-existence across Puerto Rico and Jamaica. Finally, we conduct phylogenetically-based analyses of phenotypic rates of evolution with physiological and morphological traits to compare macro-evolutionary patterns of diversification between traits associated with divergence along the thermal and structural niche axes.

Methods

STUDY SPECIES

139 We measured physiological traits for a total of 304 individuals representing 16 species of
140 anole from the islands of Puerto Rico, St. Croix (U.S. Virgin Islands), and Jamaica (Fig.
141 1; see Supplementary Table 1 for sample sizes and natural history information for each
142 species). Nine species are from Puerto Rico, eight of which are members of the
143 *cristatellus* species group that diverged *in situ* on the greater Puerto Rico bank following
144 a single colonization event (Jackman et al. 1999; Losos 2009). According to recent
145 phylogenetic analyses these species most likely fall into four sister-species pairs
146 (Nicholson et al. 2005; Mahler et al. 2010; Gamble et al. 2014) (Fig. 1). Each species
147 within a given pair occupies a different thermal niche (Rand 1964; Huey and Webster
148 1976; Hertz 1992b, a). We sampled six species from Jamaica, including five of the six
149 extant endemic species on the island, plus the introduced *A. sagrei*. As with the Puerto
150 Rican *cristatellus* group, all endemic Jamaican species evolved *in situ* as the result of a
151 single colonization event (Jackman et al. 1999). Throughout, we focus primarily on the
152 Puerto Rican *cristatellus* radiation because of the fortuitous pattern of species
153 relationships (*i.e.*, four sister species pairs with different thermal niches) and because we
154 have detailed measurements of operative thermal environments in Puerto Rico with
155 which to investigate performance consequences of physiological divergence.
156 Experiments in Puerto Rico were conducted May 20 – June 20 2011, October 8-
157 21 2011, and May 13-28 2012 at the Mata de Plátano field station in the Arecibo
158 municipality (18.414037 N; 66.728582 W; elevation 150 m). Most lizard collection
159 occurred at and around Mata de Platano (*A. cristatellus*, *A. krugi*, *A. pulchellus*, and *A.*
160 *culvieri*) and El Verde Research Station (18.322416 N; 65.818052 W, elevation 350 m;
161 *A. gundlachi*, *A. evermanni*, and *A. stratulus*) along the north coast. Two species occur

162 only on the southwest coast (*A. poncensis* and *A. cooki*), and were collected at Bosque
163 Estatal de Boquerón (17.936091 N; 67.192854 W, elevation 5 m) and Bosque Estatal de
164 Guánica (17.963301 N; 66.889752 W, elevation 5 m), respectively. In Jamaica, animal
165 collection and experiments occurred from February 25 – March 13 2013 at Green Castle
166 Estates, a privately owned 1600 square acre farm and nature preserve in St. Mary Parish
167 (18.287927 N; 76.807951 W, elevation 50 m). Prior to experiments, lizards were kept
168 individually in plastic cages (18 x 11 x 15 cm) with a wooden dowel for a perch on an
169 approximately 12L:12D light schedule. Lizards were watered daily and fed crickets or
170 Phoenix worms every other day. The only exception was *A. acutus* from St. Croix
171 (captured at 17.753274 N; 64.866563 W, elevation 172 m), which were brought back to
172 Durham, NC and housed following Gunderson and Leal (2012).
173
174 **HEAT TOLERANCE**
175 Heat tolerance (CT_{max}) was measured following Leal and Gunderson (2012). Briefly, the
176 tip of a thermocouple probe (~1.5 m, 40 gauge) was placed inside the cloaca and secured
177 to the tail with surgical tape so that body temperature could be measured continuously
178 during the experiment with a model HH603A Type T (sensitivity $\pm$ 0.1°C) digital
179 thermometer (Omega, Stamford, CT, USA). The lizard was loosely tethered to a piece of
180 cardboard (29L, 21W cm) with dental floss looped around the waist and body
181 temperatures were raised by placing lizards under a 150w double-mirror incandescent
182 light bulb. Lizards were flipped onto their backs at one-degree intervals starting at 34°C
183 to test righting ability. If the lizard righted itself, it was placed back under the light bulb
184 and warmed to the next temperature. If not, the trial was terminated. CT_{max} was recorded

185 as the last temperature at which the lizard righted itself + 0.5°C (Leal and Gunderson
186 2012). Mean warming rates during experiments were very similar among species, ranging
187 from 1.8 to 2.6°C/min (Fig. S1). All lizards were captured three days prior to CT_{max}
188 measurement. We modeled variation in CT_{max} among species using Analysis of Variance
189 (ANOVA), with separate analyses for the species on Puerto Rico and Jamaica. Data were
190 log-transformed prior to analysis. Planned orthogonal contrasts were applied to test *a*
191 *priori* predictions about divergence in CT_{max} between sister species with different thermal
192 niches (in Puerto Rico) and divergence among sympatric, morphologically similar
193 species (Puerto Rico and Jamaica).
194
195 **SPRINT PERFORMANCE**
196 Temperature-dependent sprint speeds were measured for thirteen species (*A. cuvieri*, *A.*
197 *acutus*, and *A. valencienni* were excluded) following Gunderson and Leal (2012). Briefly,
198 lizards were filmed at high speed (120 frames/s; Sony© Handycam, Tokyo, Japan) while
199 sprinting up a 2 m wooden racetrack marked with a line every 12.5 cm. The ramp was set
200 at a 37° angle as anoles will jump instead of run on a horizontal surface (A. Gunderson,
201 personal observation). Videos were watched frame-by-frame to determine speed. Lizards
202 were run 2-4 times at each temperature (two runs were the minimum, additional runs
203 were added if the lizard stopped or jumped off of the track during a run). Runs for a
204 lizard at a given temperature were a minimum of 40 minutes apart. Speed for a lizard at a
205 given temperature was taken as the fastest 25 cm run at that temperature (Huey 1983;
206 Losos 1990). In Puerto Rico, lizards were run at five body temperatures in the following
207 randomized order: 32, 22, 27, 17, and 35°C. In Jamaica, lizards were run at the same

208 temperatures in the same order, except the 17°C temperature was excluded. All
209 individuals were captured one day prior to the start of trials, and each temperature
210 treatment was administered on a different day over five straight days (four days for
211 Jamaican lizards). Body temperatures were achieved by placing lizards into a calibrated
212 chilling-heating incubator (Cole-Parmer©, Vernon Hills, IL, USA) for 30 min prior to a
213 run (Gunderson and Leal 2012).

214 The body temperature of maximum physiological performance (the "optimal
215 temperature," T_{opt}) was estimated for each individual (sensu Logan et al. 2014). Thermal
216 performance curves are non-linear (Angilletta 2009), and we initially fit two different
217 non-linear models to the sprint data for each individual: a second order polynomial and a
218 modified Gaussian function ($P(T) = e^{-(\frac{T-T_{opt}}{2 \bullet \sigma})^2}$) that has been commonly used to fit
219 performance curves for ectotherms (Deutsch et al. 2008), including *Anolis* sprint
220 performance (Huey et al. 2009; Gunderson and Leal 2012). The Gaussian model provided
221 relatively poor fits to the data, with residual standard errors much higher than the second-
222 order polynomial model for all individuals (Fig. S2). Therefore, we use T_{opt} estimates
223 from the second-order polynomial models for all analyses. T_{opt} estimates were
224 constrained such that T_{opt} had to occur within the range of experimental temperatures.
225 Thus, if the model predicted a T_{opt} greater than 35°C, we set that individual's T_{opt} to
226 35°C. This is a conservative assumption that likely leads us to underestimate divergence
227 in thermal physiology between species (see Results).

228 We were unable to use ANOVA for analyses of T_{opt} data because for four of our
229 species, most individuals ran fastest at the highest test temperature, yielding heavily
230 skewed distributions (see Results). Thus, we compared T_{opt} among species by assessing

overlap in 95% confidence intervals. We estimated confidence intervals based on 999 bootstrap replicates using the adjusted bootstrap percentile method due to the skew in our data (Efron 1987). Bootstrap resampling and 95% CI estimation were calculated in R using the "boot" and "boot.ci" functions, respectively, from the "boot" package.

PERFORMANCE CONSEQUENCES OF PHYSIOLOGICAL DIVERGENCE

To evaluate the performance consequences of physiological divergence between Puerto Rican sister species pairs, we calculated the expected physiological performance of each species under natural warm and cool temperature regimes found on Puerto Rico. Two primary habitat types on Puerto Rico are shaded, cool mesic forest and more open, warm xeric forest (Ewel and Whitmore 1973). All four "warm niche" species in the species pairs occur in xeric forest, and all four "cool niche" species in the species pairs occur in mesic forest. In addition, two of the four "warm niche" species (*A. pulchellus* and *A. stratulus*) can occur in mesic forest where they favor open areas (Hertz 1979 and personal observation), and one "cool niche" species (*A. cristatellus*) can occur in xeric forest where it favors shaded areas (Gunderson and Leal 2012). From a previous study (Gunderson and Leal 2012), we have a data set of operative temperature distributions from mesic and xeric forest measured with copper lizard models (Hertz 1992a) during the summer, the peak of the breeding season for Puerto Rican anoles (Gorman and Licht 1974). The operative temperature distribution is a quantitative description of a thermal environment from the organism's perspective (Hertz et al. 1993) and can be thought of as a distribution of potential body temperatures in each habitat. Mean operative temperature

253 in the mesic habitat is significantly cooler than that in the xeric habitat, by 4.5°C
254 (Gunderson and Leal 2012).
255 We estimated the physiological performance in the mesic and xeric operative
256 thermal environments using two metrics: 1) mean relative physiological performance
257 capacity (Hertz et al. 1993; Huey et al. 2009; Gunderson and Leal 2012), and 2) threat of
258 overheating, the percentage of operative temperature observations over $CT_{\max}$. To
259 estimate mean relative performance capacity, we generated a thermal performance curve
260 for each species using our sprint performance and $CT_{\max}$ data sets. Thermal performance
261 curves were a combination of two functions: one describing performance below T_{opt}, and
262 the other describing performance from T_{opt} to $CT_{\max}$ (Deutsch et al. 2008; Huey et al.
263 2009; Gunderson and Leal 2012). The former function was a second-order polynomial fit
264 to the sprint data of each species. The latter function took the form:
265 $P(T) = 1.0 - \left(\frac{T - T_{opt}}{CT_{\max} - T_{opt}} \right)^2$ (Huey et al. 2009; Gunderson and Leal 2012). Fitted curves for
266 each species can be seen in Fig. S3. The curves were scaled to represent relative
267 performance by setting sprint speed at T_{opt} to 1.0. With these curves, we calculated the
268 mean relative physiological performance capacity of each species in each habitat (Hertz
269 et al. 1993; Huey et al. 2009; Gunderson and Leal 2012). Theory predicts that relative
270 performance is expected to remain high as species adapt to different thermal
271 environments (Angilletta et al. 2006; Martin and Huey 2008).
272
273 **RATES OF EVOLUTION**
274 To compare rates of evolution of thermal niche traits to morphological traits involved in
275 structural niche partitioning (*sensu* Hertz et al. 2013), we used a phylogenetic

comparative approach. Rate of evolution of heat tolerance (CT_{max}) was compared to rates for body size (snout-vent-length, or SVL), head length, femur length, and adhesive toepad width for the 4th hindtoe. We used the maximum clade credibility phylogeny of Mahler et al. (2010) for these analyses. Morphological data are also from Mahler et al. (2010) and are described in greater detail therein. Shape data (head length, femur length, and toepad width) were size-corrected using phylogenetic regression in phytools (Revell 2012) prior to analysis. We used the phylogenetic approach described by Adams (2013) to conduct pairwise rate comparisons for all combinations of these five traits. For each comparison, we used likelihood ratio tests to compare a model in which the two traits were free to evolve at different rates to a model in which the traits were constrained to evolve at a single rate. Rates were estimated using species means of natural log-transformed values for all traits (such that variation is represented in terms of proportional change) and permitting co-estimation of among-trait covariances (similar results were obtained with covariances fixed at zero).

Results

PHYSIOLOGICAL DIVERGENCE BETWEEN PUERTO RICAN SPECIES PAIRS

Physiological divergence is apparent in at least one trait (T_{opt}, CT_{max}, or both) between all sister species pairs on Puerto Rico, and in all cases the pattern of divergence is in the direction predicted based on the species' thermal niches.

Significant differences in T_{opt} were observed for the *A. evermanni*/*A. stratulus* and *A. cristatellus*/*A. cooki* species pairs (*i.e.*, no overlap in 95% confidence intervals),

but not in the *A. poncensis*/*A. gundlachi* or *A. pulchellus*/*A. krugi* species pairs (Fig. 2A). However, it should be noted that our analysis almost certainly underestimates the T_{opt} of *A. cooki* and *A. stratulus*, which can be seen by looking at Fig. 2A; in both of these species, nearly all of the individuals had a T_{opt} of 35°C solely because that was our maximum test temperature.

CT_{max} differed significantly among Puerto Rican species ($F_{7,72} = 42.74$; $P < 0.001$). Planned orthogonal contrasts demonstrated significant divergence in three of the four species pairs: *A. evermanni*/*A. stratulus*, *A. poncensis*/*A. gundlachi*, and *A. pulchellus*/*A. krugi* (all $P < 0.001$), but not in the *A. cristatellus*/*A. cooki* species pair ($P = 0.490$; Fig. 2B).

PHYSIOLOGICAL PERFORMANCE IN DIFFERENT THERMAL REGIMES

For three of the four sister-species pairs, the cool niche species is predicted to have higher physiological performance capacity throughout the day within the shaded operative thermal environment, with the mean performance advantage being 8-14% depending on the species pair and time of day (Fig. 3). The lone exception occurs with the *A. pulchellus*/*A. krugi* sister species pair, for which performance estimates are similar for both species throughout the day (Fig. 3G). In the open habitat operative environment, the warm niche species tend to have a performance advantage, particularly during midday hours (from 10:00-13:00; Fig. 3). The exception in this case is the *A. cristatellus*/*A. cooki* species pair, for which *A. cristatellus* has higher predicted performance capacity early and late in the day, and both species have similar predicted performance during the midday hours (Fig. 3D). However, midday performance of *A. cooki* is likely

underestimated due to our probable underestimation of the T_{opt} of this species (see above).

None of the species are under threat of overheating in the shaded operative thermal environment (Fig. 4A, C, E, G). However, all species have a threat of overheating in the open habitat operative thermal environment (Fig. 4B, D, F, H). For three of the four sister-species pairs (*A. stratulus*/*A. evermanni*, *A. poncensis*/*A. gundlachi*, and *A. pulchellus*/*A. krugi*), the cool niche species has a higher probability of overheating throughout the day (Fig. 4B, F, H). The difference in probability of overheating is particularly acute during the midday hours, when the cool niche species in these sister-species pairs have probabilities of overheating that are at least twice that of warm niche species (all of which are significant differences; G-tests, all $P < 0.05$). The only sister-species pair for which this relationship does not hold is *A. cristatellus*/*A. cooki*, for which there was no divergence in CT_{max} (Fig. 4D).

PHYSIOLOGICAL EVOLUTION WITHIN THE JAMAICAN RADIATION

Significant differences in thermal physiology were also present among Jamaican species. Mean T_{opt} ranges from approximately 29.5 to 34.6°C (Fig. 5A; true T_{opt}s of *A. grahami* and *A. opalinus* likely underestimated due to our experimental upper measurement limit of 35°C, see above), while CT_{max} ranges from 35.8-41.5°C among species (Fig. 5B; ANOVA, $F_{3,36} = 13.6$, $P < 0.001$).

PHYSIOLOGICAL DIFFERENCES BETWEEN SYMPATRIC SPECIES

Across Puerto Rico and Jamaica, sympatric species that occupy the same structural niche (*i.e.*, are morphologically similar) invariably differ in thermal physiology (Table 1). Three of these sympatric pairs are sister species from Puerto Rico described above (*i.e.*, the trunk-crown ecomorph species *A. stratulus*/*A. evermanni*; the trunk-ground species *A. cristatellus*/*A. cooki;* the grass-bush species *A. krugi*/*A. pulchellus*). The trunk-ground species *A. cristatellus* and *A. gundlachi* also co-occur in some areas of Puerto Rico and differ in CT_{max} (P<0.001). On Jamaica, the trunk-ground anole *A. lineatopus* and trunk-crown anole *A. grahami* differ in CT_{max}, as do *A. lineatopus* and the introduced trunk-ground anole *A. sagrei* (both P<0.001; Table 1).

RATES OF PHYSIOLOGICAL AND MORPHOLOGICAL EVOLUTION

Thermal tolerance has evolved more slowly than some, but not all, morphological traits associated with ecomorphological divergence (Table 2). CT_{max} evolved significantly more slowly than body size (SVL; $P < 0.001$) and relative limb length (femur; $P = 0.029$). No differences in evolutionary rates were found between CT_{max} and either head length ($P = 0.105$) or toepad width ($P = 0.112$).

Discussion

Evolution of the climatic niche has emerged as an important component of many evolutionary radiations, but the functional consequences of climate niche divergence are generally unknown. We demonstrate here that climatic niche divergence among species in the *Anolis* adaptive radiation is accompanied by physiological divergence in both temperature tolerance and sensitivity to sub-lethal temperature variability. Species that

occupy warmer niches have either higher heat tolerance, higher optimal temperatures for physiological performance, or both, compared to species that occupy cooler thermal niches. Such physiological diversification has occurred repeatedly, as is most easily seen among four sister-species pairs present on Puerto Rico that differ in thermal niche (Fig. 2). Divergence in thermal physiology is also present on Jamaica, with species spanning essentially the full range of heat tolerances and optimal temperatures found on Puerto Rico (Fig. 5).

The repeated divergence of thermal physiology among species occupying distinct thermal niches is itself evidence of adaptation (Harvey and Pagel 1991). However, we also provide more direct evidence of the adaptive significance of physiological divergence by estimating physiological performance parameters for Puerto Rican species based on detailed measurements of warm and cool thermal environments found on the island. In shaded habitat, cool niche species have higher predicted mean performance capacities than their warm niche sister species in three of the four species pairs (Fig. 3). The only exception is the *A. krugi*/*A. pulchellus* species pair, for which there was no divergence in optimal temperature. Within the warmer open habitat the pattern was less pronounced, with warm niche species appearing to have a sizable performance advantage in only two of the species pairs (Fig. 3). The advantage of physiological divergence for warm-niche species becomes much more apparent when considering overheating risk. During midday hours in the warmer open habitat, warm niche species in three of the four species pairs had less than half the overheating risk of cool-niche species (Fig. 4). The lone exception was the *A. cristatellus*/*A. cooki* species pair, which had no divergence in

389 thermal tolerance (Fig. 2B). No species was at risk of overheating in the cooler, shaded
390 habitat (Fig. 4).

391 Our analysis of physiological performance in different thermal environments
392 assumes that animals are randomly utilizing perch sites with respect to available
393 operative temperatures. Anoles are known to behaviorally thermoregulate (Hertz et al.
394 1993), in effect modulating the thermal environment that they experience by avoiding
395 perch sites with operative temperatures that are physiologically detrimental (Huey et al.
396 2003). However, behavioral thermoregulation is costly, and animals living within
397 environments in which they do not need to make behavioral adjustments should be at a
398 substantial advantage (Huey 1974; Huey and Slatkin 1976; Sears et al. 2011; Sears and
399 Angilletta 2015). Thus, cool niche lizards in more open areas are likely to have reduced
400 space and time available for normal activity compared to the more heat-tolerant warm
401 niche species, and the same will hold true for warm-niche lizards in cooler areas
402 (Gunderson and Leal 2015, 2016).

403 The high community-level species diversity associated with many adaptive
404 radiations, including *Anolis*, is at least partially enabled by fine-scale resource
405 partitioning among constituent taxa (Schluter 2000). Sympatric anoles are known to
406 partition thermal microhabitats (Ruibal 1961; Rand 1964), and our analysis indicates that
407 this is facilitated by physiological divergence. When anole species that share the same
408 structural niche (*i.e.*, share the same or similar ecomorphology) co-occur, they invariably
409 differ in temperature-dependent physiological traits (Table 1). This pattern is not only
410 maintained for ecologically similar species that are endemic members of the community,
411 but also for introduced species: in Jamaica, the endemic trunk-ground species *A.*

412 *lineatopus* is found in sympatry with the introduced trunk-ground species *A. sagrei*, and
413 these species differ significantly in thermal tolerance (Table 1).
414 We found that physiological thermal tolerance has evolved more slowly than
415 some morphological traits (SVL and femur length), and has evolved at similar rates to
416 other morphological traits (head length and toe-pad width; Table 2). This result is at odds
417 with a recent analysis which found that divergence in thermal physiology, estimated as
418 field body temperature, occurs more quickly than morphological divergence in anoles
419 (Hertz et al. 2013). However, we note that in that study, estimated rates for thermal and
420 morphological traits are not directly comparable because they were not standardized to
421 the same scale before analysis (Adams 2013). Our results demonstrate that high rates of
422 evolutionary change are not necessary for traits to be ecologically important, as the
423 relatively slow divergence that we have observed in anole thermal tolerance has
424 important performance consequences with respect to overheating risk in warm habitats
425 (Fig. 4).
426 A critical question when assessing patterns of evolution is determining the
427 potential for observed divergence to be driven by phenotypic plasticity as opposed to
428 heritable genetic change. With respect to thermal physiology, direct evidence from anoles
429 and broader patterns from reptiles indicate that the magnitude of divergence that we have
430 observed is unlikely to be the result of acclimation to different thermal regimes. For
431 example, metabolic rates of the Puerto Rican *A. cristatellus* and *A. gundlachi*, as well as
432 *A. sagrei* introduced to Florida, do not change in response to acclimation temperature
433 (Rogowitz 1996b, a). Tests for acclimation in thermal tolerance in Greater Antillean
434 anoles have also suggested no (Leal and Gunderson 2012; Muñoz et al. 2014) or limited

435 (Kolbe et al. 2012) plasticity in this physiological trait. These results are consistent with
436 two recent meta-analyses of plasticity in thermal physiology, both of which demonstrated
437 that reptiles have among the lowest acclimation capacities of all ectotherms (Gunderson
438 and Stillman 2015; Seebacher et al. 2015). For example, among reptiles CT_{max} changes an
439 average of only 0.1°C for every 10°C change in mean body temperature (Gunderson and
440 Stillman 2015).

441 We have provided comprehensive evidence for the importance of physiological
442 divergence in the Puerto Rican and Jamaican *Anolis* adaptive radiations. However, the
443 importance of physiological divergence is likely not restricted to these islands. For
444 example, divergence in thermal tolerances have been demonstrated for trunk-ground
445 anoles of the *cybotes* group that occur along an elevational gradient in Hispaniola (Hertz
446 and Huey 1981; Muñoz et al. 2014), and variation in thermal physiology has also been
447 demonstrated among some mainland anoles (van Berkum 1986; Logan et al. 2013).
448 Physiological divergence is also not restricted to thermal traits, as there is significant
449 divergence in water loss rates among species and populations of anoles, which is often
450 correlated with thermal niche (Sexton and Heatwole 1968; Hillman and Gorman 1977;
451 Hertz et al. 1979; Hillman et al. 1979; Hertz 1980; Dmi'el et al. 1997; Gunderson et al.
452 2011). Though more work is required, the general pattern emerging is that physiological
453 divergence along the climatic niche axis may be as important as morphological
454 divergence along the structure niche axis in this classic radiation.

455 Our results highlight the benefits of a functional approach to investigating
456 climatic niche evolution. By focusing on physiological traits that link organismal
457 performance to abiotic conditions, we were able to demonstrate how phenotypes evolve

in response to climatic environments and estimate the performance consequences of observed divergence in natural habitats. In addition, our functional perspective allowed us to determine the contribution of climatic niche divergence to microhabitat partitioning and species co-existence. We suggest that the current trend to use ENMs, which contain no information about organismal phenotypes or fine-scale habitat use, should only be a first step in a more comprehensive research program to determine how and why climatic niche divergence contributes to evolutionary radiations.

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Table 1. Physiology differences between co-occurring species that occupy the same or similar structural niches (*i.e.*, share the same or similar ecomorphology). For CT_{max}, P-values are given based on one-way ANOVA with planned orthogonal contrasts. For T_{opt}, "*" indicates that the bootstrapped 95% confidence intervals did not overlap, "N.S." indicates no significant difference. "†" indicates that the species is an introduced member of the community.

Table 2. Estimated rates of evolution for heat tolerance (CT_{max}) and morphological traits related to ecomorphological divergence. Rate estimates (bold) are in the diagonal of the matrix. Upper off-diagonals contain estimates of pairwise evolutionary covariances among traits, lower off-diagonals contain P-values for likelihood ratio test comparisons of 2-rate versus 1-rate models for each trait pair. P-values less than 0.05 indicate support for a 2-rate model over a 1-rate model.

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770 Table 1

Co-occurring species		Ecomorph	Physiological divergence	
			CT_{max}	T_{opt}
A. stratulus	*A. evermani*	Trunk-crown	<0.001	*
A. cristatellus	*A. cooki*	Trunk-ground	0.490	*
A. cristatellus	*A. gundlachi*	Trunk-ground	<0.001	N.S.
A. krugi	*A. pulchellus*	Grass-bush	<0.001	N.S.
A. lineatopis	*A. grahami*	Trunk-ground/Trunk-crown	<0.001	N.S.
A. lineatopis	*A. sagrei*†	Trunk-ground	<0.001	N.S.

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Table 2.

	CT_{max}	SVL	Head length	Femur length	Toepad width
CTmax	**5.80E-05**	-4.90E-05	-1.60E-05	-5.50E-05	-9.90E-06
SVL	<0.001	**2.30E-03**	8.90E-06	-1.80E-05	2.70E-05
Head length	0.105	<0.001	**2.70E-05**	1.30E-05	-6.30E-06
Femur length	0.029	<0.001	0.001	**1.40E-04**	2.20E-05
Toepad width	0.112	<0.001	0.003	0.832	**1.30E-04**

Figure 1: Phylogenetic relationships of the species included in this study, along with the islands from which they were sampled.

Figure 2: Thermal physiology of four sister-species pairs of Puerto Rican anole. Each species pair is represented in alternating grey and white regions of the plot with warm niche species in red and cool niche species in blue. (A) Optimal sprint performance temperatures (T_{opt}). (B) Heat tolerance limits (CT_{max}). "*", significant difference between members of a species pair; N.S., no significant difference between members of a species pair. See Methods for analysis details.

Figure 3: Predicted relative physiological performance of sister-species pairs of Puerto Rican *Anolis* under shaded and open habitat operative thermal environments found on Puerto Rico during morning, midday, and afternoon/evening hours. Warm niche species in red, cool niche species in blue. (A, B) *A. stratulus*/*A. evermanni*, (C, D) *A. cooki*/*A. cristatellus*, (E, F) *A. poncensis*/*A. gundlachi* (G, H) *A. pulchellus*/*A. krugi*.

Figure 4: Predicted overheating probability of sister-species pairs of Puerto Rican *Anolis* under shaded and open habitat operative thermal environments found on Puerto Rico during morning, midday, and afternoon/evening hours. Warm niche species in red, cool niche species in blue. (A, B) *A. stratulus*/*A. evermanni*, (C, D) *A. cooki*/*A. cristatellus* (E, F), *A. poncensis*/*A. gundlachi*, (G, H) *A. pulchellus*/*A. krugi*.

818 Figure 5: Thermal physiology of endemic Jamaican anoles. (A) Optimal sprinting
819 temperature (T_{opt}). *Anolis valencienni* does not have a T_{opt} estimate because this species
820 could not be induced to run. (B) Heat tolerance (CT_{max}).
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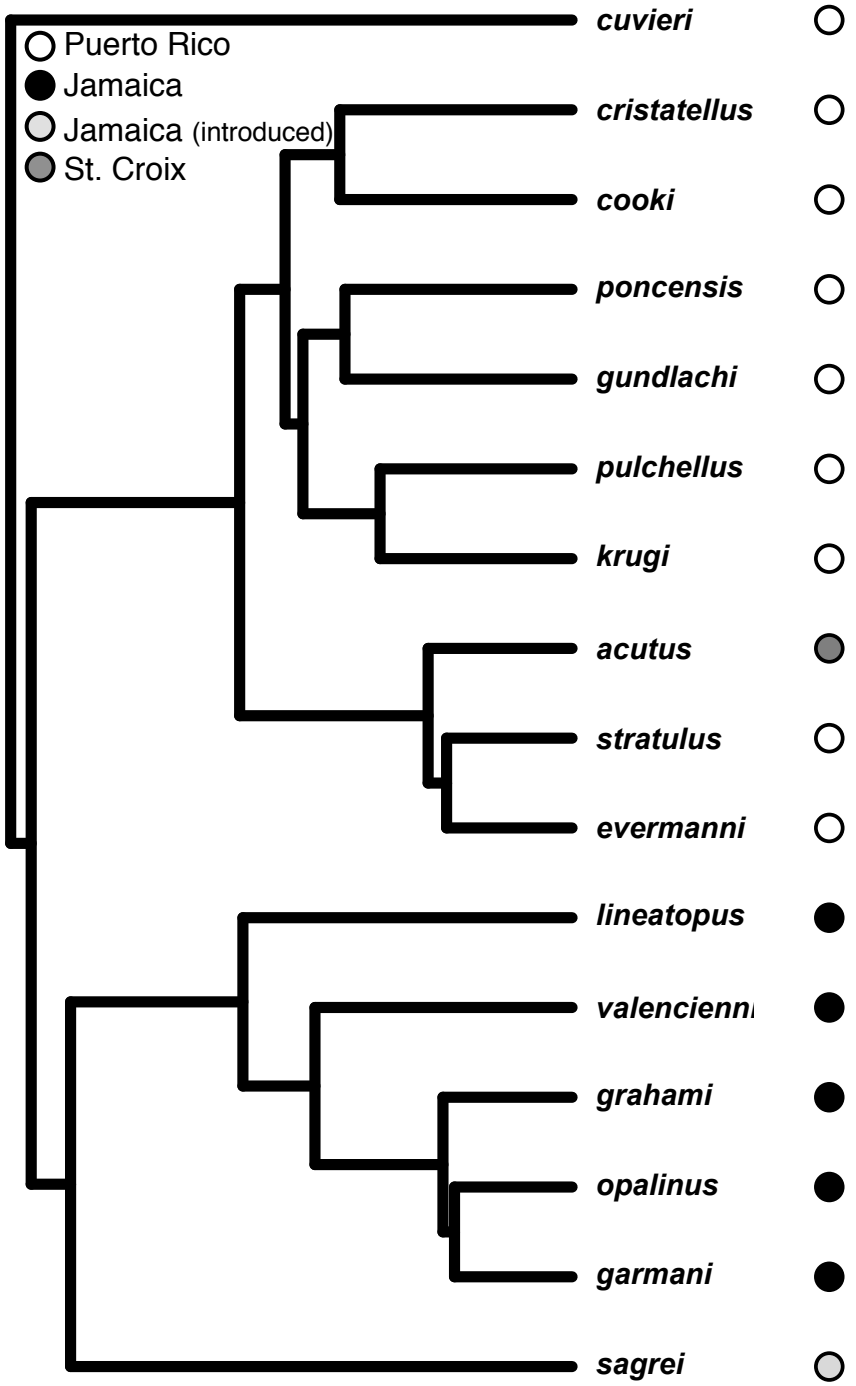
Puerto Rico
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Jamaica (introduced)
St. Croix
cuvieri
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cooki
poncensis
gundlachi
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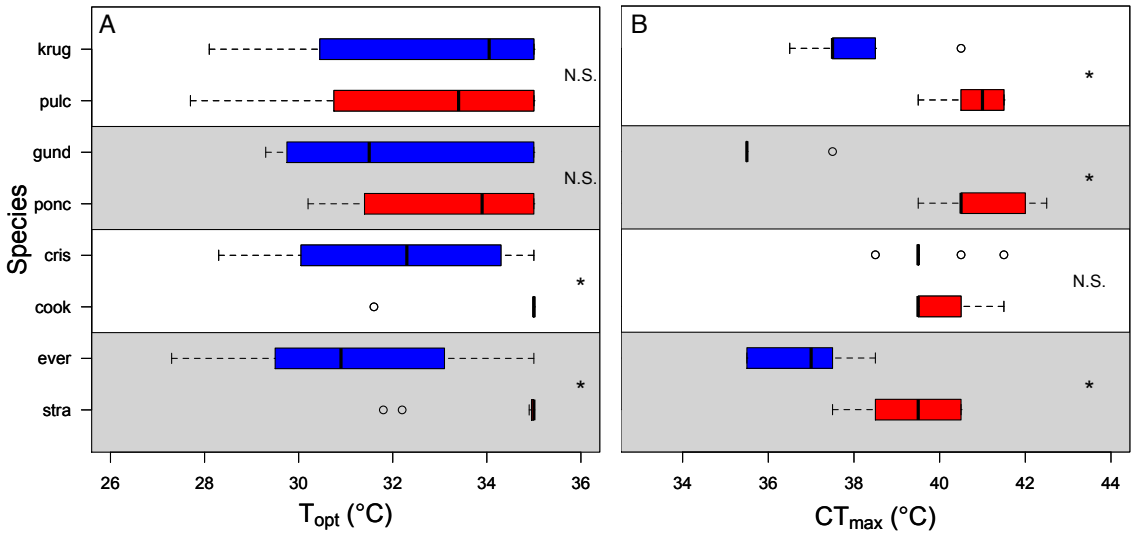
acutus
stratulus
evermanni
lineatopus
valencienni
grahami
opalinus
garmani
sagrei

Figure 1.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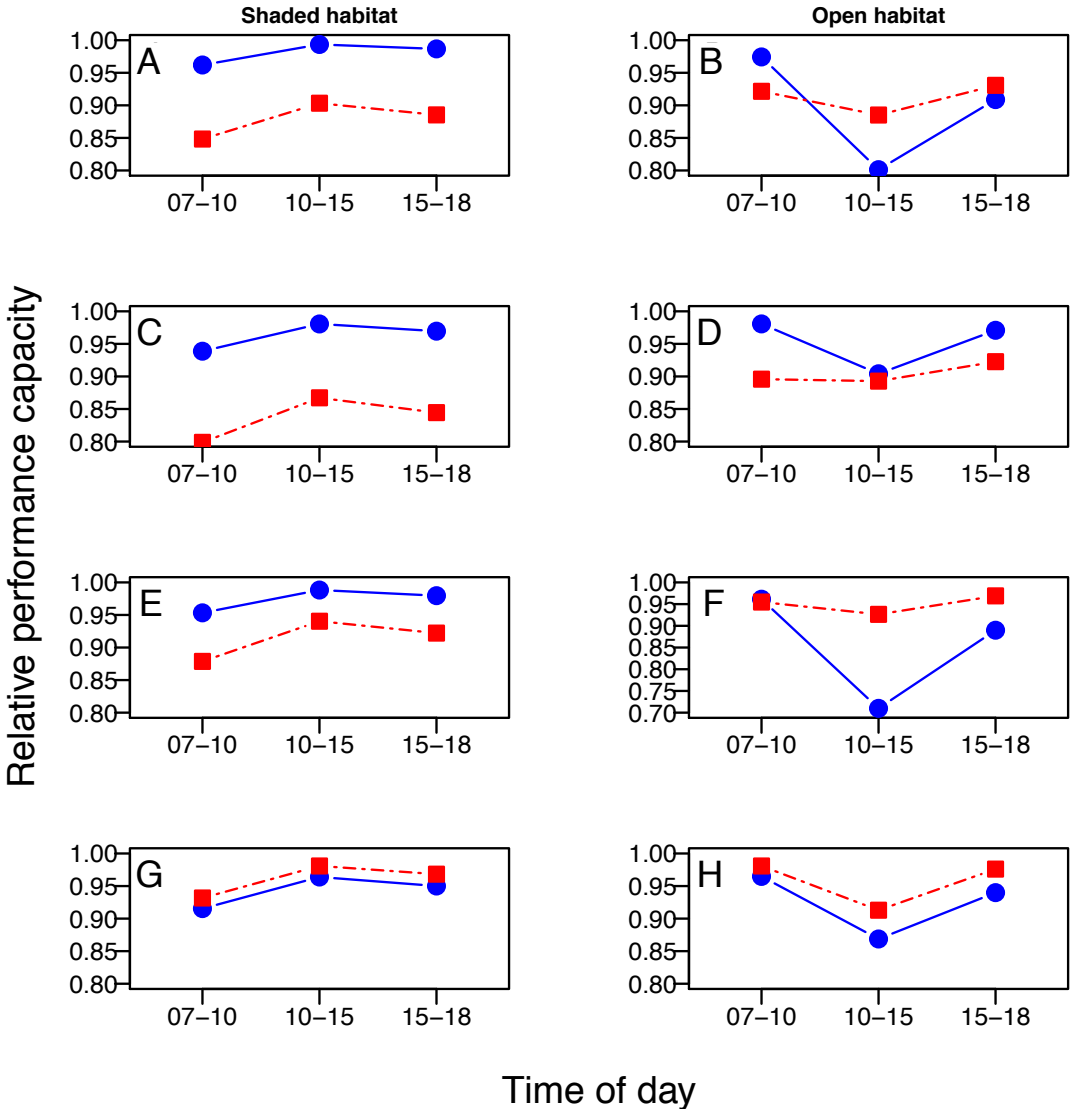
Topt (°C)
CTmax (°C)

Figure 2.


Shaded habitat
Open habitat
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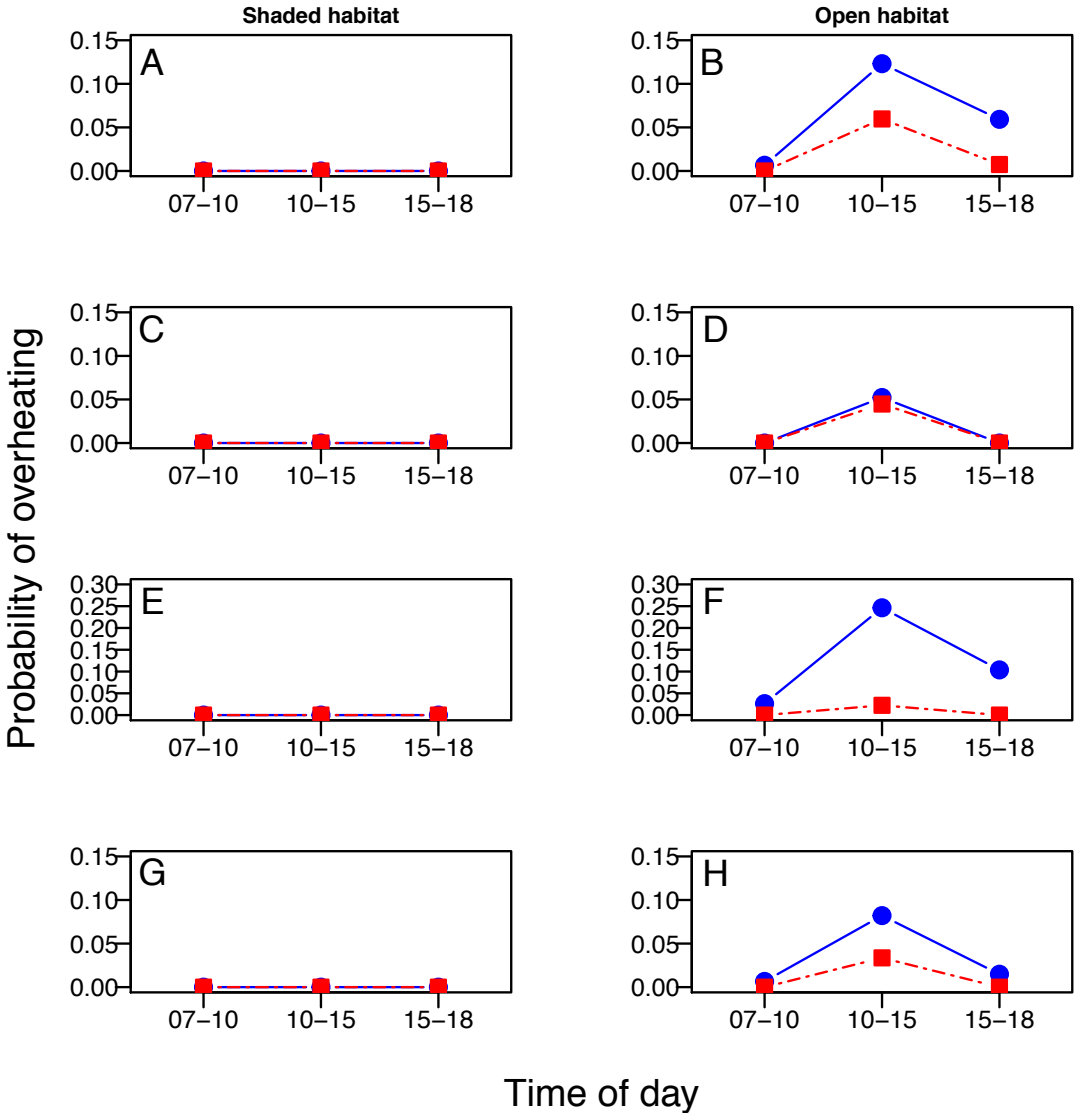
Relative performance capacity
Time of day

Figure 3


Shaded habitat
Open habitat
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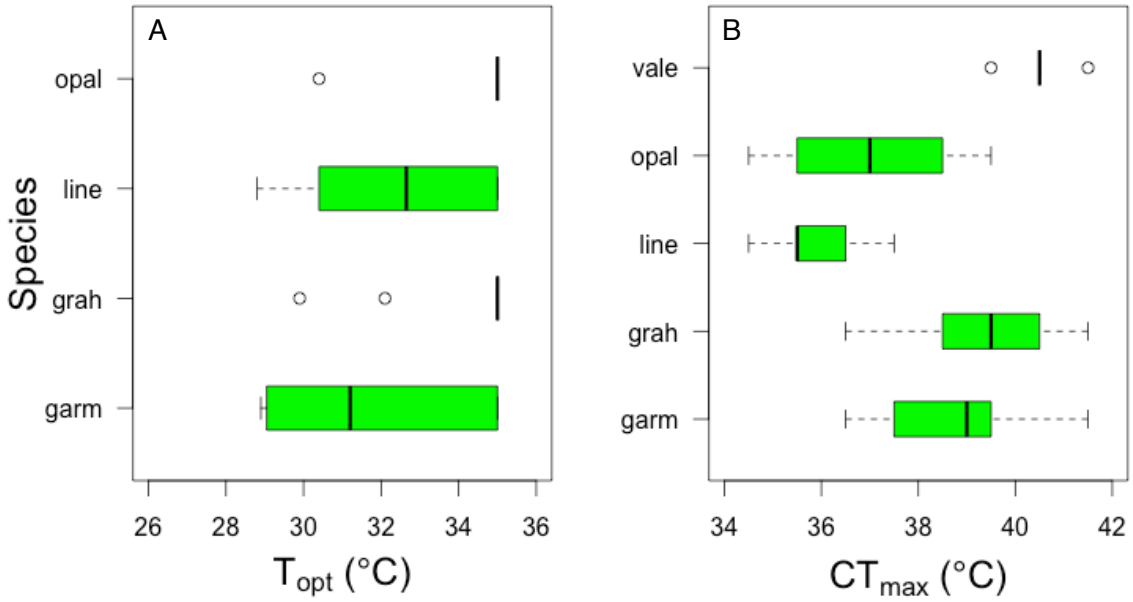
15–18
Probability of overheating
Time of day

Figure 4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T_{opt} (°C)
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CT_{max} (°C)

Figure 5