Thermal niche evolution across replicated *Anolis* lizard adaptive radiations

Alex R. Gunderson[†‡*], D. Luke Mahler[§], and Manuel Leal[¶]

†Romberg Tiburon Center and Department of Biology, San Francisco State University, 3150 Paradise Dr., Tiburon, California 94920 and ‡Department of Integrative Biology, University of California, Berkeley, California 94720-3140

*Corresponding author: e-mail: alexrgunderson@gmail.com

§Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, M5S 3B2 CA

¶ Division of Biological Sciences, University of Missouri, 105 Tucker Hall, Columbia, MO 65211.

Running title: Physiology of adaptive radiation

Keywords: *Anolis*, thermal biology, physiology, thermal tolerance, thermal performance, evolutionary rates, sympatry, species richness

Data accessibility: If accepted, data herein will be archived at Dryad (http://datadryad.org)

Abstract

How evolutionary and ecological forces interact to promote the maintenance of biodiversity is a fundamental biological question. Here, we investigate this question by focusing on physiological evolution along the thermal niche axis in replicated radiations of *Anolis* lizards, groups that are best known for resource partitioning based on morphological evolution. We find repeated divergence in thermal physiology within these radiations, and that this divergence has performance consequences within natural habitats. In addition, morphologically similar species that co-occur invariably differ in their thermal physiology, providing evidence that physiological divergence facilitates species co-existence within these communities. Despite their ecological importance, phylogenetic comparative analyses suggest that thermal niche traits have evolved more slowly than key morphological traits related to the structural niche. Phylogenetic analyses also reveal that physiological (*i.e.*, fundamental) thermal niche evolution is correlated with realized thermal niche evolution based on geo-referenced locality data, but the latter incompletely predicts variation in the former. We provide comprehensive evidence for the importance of physiological divergence within *Anolis* adaptive radiations, including the complementary roles of physiological and morphological divergence in maintaining community-level diversity. We recommend greater integration of fundamental niche traits into analyses of climatic niche evolution, as they facilitate a more complete understanding of the phenotypic and ecological consequences of climatic divergence.

53 *Introduction*

54 A mechanistic understanding of how divergent phenotypic evolution facilitates species coexistence is a
55 fundamental problem in ecology and evolution [1]. This problem comes into particular focus in the study
56 of adaptive radiation, a process for which a hallmark is the repeated evolution of niche differences that
57 allow closely related species to partition resources and stably co-occur [2-4]. Traditionally, most research
58 on trait evolution during adaptive radiation has focused on morphological traits associated with dietary
59 resource acquisition or structural habitat use, due to the clear role such adaptations play in mediating
60 competition and facilitating coexistence [3, 5-7]. Physiological adaptation to abiotic conditions has
61 received comparatively less attention in adaptive radiation studies, particularly in animals [3, 8]. Such
62 adaptations are more typically studied for their role in generating divergence among geographically
63 disjunct lineages experiencing different climates [9, 10]. However, physiological divergence can also play
64 an important role in adaptive radiation by facilitating fine-scale partitioning of the local climatic niche
65 [11], providing an additional resource axis along which a radiating clade may diversify [12, 13]. Indeed,
66 such abiotic niche divergence has been hypothesized to underlie many impressive radiations, including
67 bolitoglossine salamanders [14, 15], *Liolaemus* lizards [16-18], *Drosophila* flies [19], *Petrolisthes* crabs
68 [20] and Lake Whitefish [21].

69 One of the most common tools applied in recent investigations of abiotic niche divergence is
70 correlative ecological niche modeling (ENM), which estimates species' realized niches (*i.e.*, the range of
71 conditions that species occupy) using information about species occurrences and broad-scale climate
72 conditions [22-26]. Though correlative ENMs are powerful for addressing many questions, they also have
73 characteristics that limit the inferences that can be made from their application [27-32]. With respect to
74 adaptive radiations, one limitation is that correlative ENMs are based on geo-referenced species locality
75 data and therefore provide no information about phenotypic traits (physiological or otherwise) linked to
76 performance [28]. Performance-based traits relate to the fundamental niche (broadly, the range of
77 conditions a species could occupy). Knowledge of performance-based traits is critical for understanding
78 how species are able to occupy the habitats that they do with respect to both abiotic factors and other

species. ENMs may also be limited in what they can tell us about the role of abiotic niche divergence in coexistence, as they cannot provide fine-scale information about micro-environmental niche use or abiotic tolerances. Such factors can be of paramount importance for coexistence in radiating clades, as species with identical or largely overlapping ranges may be able to partition resources via adaptation to different thermal microenvironments [11, 33].

Here, we investigate the contribution of thermal niche divergence to the replicated *Anolis* lizard adaptive radiations of Puerto Rico and Jamaica by analyzing patterns and performance consequences of physiological divergence across 16 species (Fig. 1). Greater Antillean *Anolis* are best known for morphological diversification along the fundamental structural niche axis [34-36], but morphological evolution cannot fully explain the patterns of divergence and community composition observed across the Greater Antilles [37]. Divergence in the thermal niche is also proposed to be an important component of *Anolis* diversification based primarily on realized niche estimates from natural history and ENM studies [12, 13, 22, 38-43]. However, we still know little about how physiology evolves during realized niche divergence [44] and the extent to which physiological evolution facilitates species co-existence.

We first use data from the Puerto Rican *cristatellus* species group to investigate repeated divergence in thermal physiology and performance between species that occupy distinct thermal environments. Next, we integrate these data with measurements of operative thermal conditions in Puerto Rico to explore the performance consequences of physiological divergence. We then consider spatial patterns of physiological variation to evaluate the contribution of physiological evolution to fine-scale niche partitioning and community-level species co-existence across Puerto Rico and Jamaica. Finally, we conduct phylogenetic analyses of rates of physiological and morphological evolution to compare diversification patterns between traits associated with the fundamental thermal niche and the fundamental structural niche, and we employ phylogenetic methods to estimate the strength of the evolutionary association between fundamental and realized thermal niche divergence. For the latter, we estimate realized thermal niche characteristics of each species from geo-referenced bioclimatic data commonly used in correlative ENM analyses.

Methods

We measured physiological traits for 304 individuals across 16 species from three islands (Fig. 1; see Supplementary Table 1 for species information). Throughout, we focus primarily on the eight Puerto Rican members of the *cristatellus* group, for two reasons. First, phylogenetic and natural history data indicate that these species diversified *in situ* and form four sister species pairs [45-47], with each pair representing an independent divergence in realized thermal niche based on the degree to which they occupy open versus shaded perches (which throughout we refer to as "warm niche" and "cool niche" species, respectively)[12, 40, 48, 49]. Second, we have detailed measurements of operative thermal environments in Puerto Rico to investigate performance consequences of physiological divergence. Experiments in Puerto Rico were conducted May 20 – June 20 2011, October 8-21 2011, and May 13-28 2012 at the Mata de Plátano field station. On Jamaica, we sampled five of the six extant endemic species, plus the introduced *A. sagrei*. As with the Puerto Rican species, the endemic Jamaican anoles evolved *in situ* from a single colonization event [50]. Experiments in Jamaica occurred from February 25 – March 13 2013 at Green Castle Estates, a privately owned farm and nature preserve in St. Mary Parish. Prior to experiments, lizards were kept individually in plastic cages (18 x 11 x 15 cm) with a wooden dowel perch on an approximately 12L:12D light schedule. Lizards were watered daily and fed crickets or Phoenix worms every other day. The exception was *A. acutus* from St. Croix, which were brought to Durham, NC and housed following Gunderson and Leal [51].

Heat tolerance (CT_{max}) was measured following Leal and Gunderson [52]. Briefly, lizards were warmed under a heat lamp while monitoring their body temperatures with a wire thermocouple probe placed inside the cloaca. Lizards were flipped onto their backs at one-degree intervals starting at 34°C, and CT_{max} was recorded as the temperature at which they lost righting ability. Warming rates were very similar among species, ranging from 1.8 to 2.6°C/min (Fig. S1). All lizards were captured three days prior to CT_{max} measurement. We modeled variation in log-transformed CT_{max} among species using analysis of

variance (ANOVA). Planned orthogonal contrasts were applied to test *a priori* predictions about divergence in CT_{max} between sister species (in Puerto Rico) and divergence among sympatric, morphologically similar species (Puerto Rico and Jamaica).

Temperature-dependent sprint speeds were measured for thirteen species following Gunderson and Leal [51]. Briefly, we analyzing high-speed video (120 frames/s) of lizards running up a 2 m wooden racetrack set at a 37° angle and marked with a line every 12.5 cm. Lizards were run 2-4 times at each temperature (two runs minimum, additional runs were added if the lizard stopped or jumped off of the track during a trial). Speed for a lizard at a given temperature was taken as the fastest 25 cm interval at that temperature [53, 54]. Puerto Rican lizards were run at five body temperatures in the following randomized order: 32, 22, 27, 17, and 35°C. Conditions were the same for Jamaican lizards, except the 17°C temperature was excluded. All individuals were captured one day prior to the start of trials, and each temperature treatment was administered on a different day over five straight days (four days for Jamaican lizards). No animals were used for both heat tolerance and sprint performance. Target body temperatures were achieved by placing lizards into a calibrated chilling-heating incubator (Cole-Parmer©, Vernon Hills, IL, USA) for 30 min prior to a run [51].

The body temperature of maximum performance (the "optimal temperature," T_{opt}) was estimated for each individual [sensu 55]. Thermal performance curves are non-linear [56], and we initially fit two different non-linear models to the sprint data for each individual: a second order polynomial and a modified Gaussian function ($P(T) = e^{-(\frac{T-T_{opt}}{2 \bullet \sigma})^2}$) commonly used to fit performance curves for ectotherms [57], including *Anolis* sprinting [51, 58]. The Gaussian model provided relatively poor fits to the data (much higher residual standard errors than the second-order polynomial model (Fig. S2)). Therefore, T_{opt} estimates from the second-order polynomial models were used in analyses. T_{opt} estimates were constrained such that T_{opt} had to occur within the range of experimental temperatures. This is a conservative assumption that likely leads us to underestimate divergence in thermal physiology between species (see Results). T_{opt} data were heavily skewed (see Results), so we compared T_{opt} among species by

assessing overlap in bootstrapped 95% confidence intervals (999 bootstrap replicates using the adjusted bootstrap percentile method due to the skew in our data [59]). Analyses were conducted with the "boot" and "boot.ci" functions, respectively, from the "boot" package in R.

We calculated the expected physiological performance of Puerto Rican species under natural warm (open xeric forest) and cool (shaded mesic forest) operative thermal regimes found on Puerto Rico [60]. Operative temperature distributions provide a quantitative description of a thermal environment integrating the physical features of the organism [61], representing the distribution of available body temperatures in a habitat. Operative temperature distributions came from a previous study [51] and were measured with copper lizard models [49] during the peak breeding season (summer) for Puerto Rican anoles [62]. Mean operative temperature in the mesic forest is significantly cooler than that in the xeric forest, by 4.5°C [51].

We estimated two performance metrics under the different operative environments: 1) relative sprint performance capacity [51, 58, 61], and 2) probability of overheating (the percentage of operative temperature observations over $CT_{\max}$). For relative performance capacity, we generated species-level thermal performance curves using our sprint speed and $CT_{\max}$ data. The curves were a combination of two functions fit to the data: one describing performance below T_{opt}, and the other describing performance from T_{opt} to $CT_{\max}$ [51, 57, 58]. The former function was a second-order polynomial fit to the sprint data, and the latter function took the form: $P(T) = 1.0 - \left(\frac{T - T_{opt}}{CT_{\max} - T_{opt}} \right)^2$ [51, 58]. Curves were scaled to relative performance by setting sprint speed at T_{opt} to 1.0 (see Fig. S3 for fitted curves). Performance in each habitat was calculated by applying the curves to operative temperatures [51, 58, 61].

To compare rates of evolution of thermal physiology to ecomorphological traits involved in fundamental structural niche partitioning, 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 and morphological data of Mahler *et al*. [46] for these analyses. Shape data (head length, femur length, and toepad width) were size-corrected using phylogenetic regression in phytools [63] prior to analysis. We used the approach of Adams [64] to conduct pairwise rate comparisons for all combinations of these five traits. We used likelihood ratio tests to compare a model in which the two traits could 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 rate variation is represented in terms of relative change in proportion to the trait mean; this is essential for comparison of traits measured using different units [64]). For these analyses, we co-estimated among-trait covariances (similar results were obtained with covariances fixed at zero).

To test for an association between fundamental and realized thermal niche estimates, we conducted a phylogenetically-controlled correlation test between CT_{max} and geo-referenced Worldclim temperature data for each species based on collection localities. We used the first principal component axis from the *Anolis* climate temperature dataset of Algar and Mahler (2015), in which a phylogenetic principal component analysis (PCA) was used to condense the 11 Worldclim variables related to temperature into PCA axes (Supplementary Table S2). The first PC axis loaded positively for all variables related to temperature (e.g., mean annual temperature, mean maximum temperature; Supplementary Table S2), and can therefore be considered a composite estimate of habitat temperature.

Results

All Puerto Rican sister species pairs have diverged in at least one physiological trait (T_{opt}, CT_{max}, or both), and in the predicted direction in all cases (Fig. 2). T_{opt} differed between the *A. evermanni*/*A. stratulus* and *A. cristatellus*/*A. cooki* species pairs (non-overlapping bootstrapped 95% CIs), but not between the *A. poncensis*/*A. gundlachi* or *A. pulchellus*/*A. krugi* species pairs (Fig. 2A). We note that the T_{opt} of *A. cooki* and *A. stratulus* were likely underestimated because most individuals had a T_{opt} of 35°C solely because that was our maximum test temperature. CT_{max} differed among Puerto Rican species ($F_{7,72} = 42.74$; $P < 0.001$), with significant divergence in three of the four species pairs: *A. evermanni*/*A. stratulus*, *A.*

205 *poncensis*/*A. gundlachi*, and *A. pulchellus*/*A. krugi* (all $P < 0.001$), but not in the *A. cristatellus*/*A. cooki*
206 species pair ($P = 0.490$; Fig. 2B).

207 Physiological divergence influenced predicted performance in natural environments. In three of
208 four species pairs, the cool niche species had higher predicted performance than the warm niche species
209 in the shaded operative thermal environment, with mean performance advantages of 8-14% depending on
210 the species pair and time of day (Fig. 3). The lone exception was the *A. pulchellus*/*A. krugi* species pair,
211 for which both have similar performance estimates (Fig. 3G). Warm niche species tend to have a
212 performance advantage in the open operative environment, particularly during midday hours (from 10:00-
213 13:00; Fig. 3). The *A. cristatellus*/*A. cooki* species pair is the exception (Fig. 3D). However, *A. cooki*
214 midday performance is likely underestimated due to our probable underestimation of its T_{opt} (see above).

215 No species are under threat of overheating in the shaded operative environment (Fig. 4A, C, E,
216 G). However, all species have overheating risk in the open operative environment (Fig. 4B, D, F, H). For
217 three of the four sister-species pairs (*A. stratulus*/*A. evermanni*, *A. poncensis*/*A. gundlachi*, and *A.*
218 *pulchellus*/*A. krugi*), the cool niche species has a higher probability of overheating (Fig. 4B, F, H).
219 Difference in overheating probability are particularly acute during midday, when cool niche species have
220 overheating probabilities at least twice that of their warm niche counterparts (G-tests, all $P < 0.05$). The
221 *A. cristatellus*/*A. cooki* species pair, which had no divergence in CT_{max}, was the only exception (Fig. 4D).

222 Significant physiological divergence is also apparent among Jamaican species. Among species
223 mean T_{opt} ranges from 29.5 to 34.6°C (Fig. 5A) while CT_{max} ranges from 35.8-41.5°C (Fig. 5B; ANOVA,
224 $F_{3,36} = 13.6$, $P < 0.001$). Note that T_{opt} values of *A. grahami* and *A. opalinus* are likely underestimated due
225 to our upper experimental limit of 35°C (see above).

226 Across Puerto Rico and Jamaica, sympatric species that share the same ecomorphology invariably
227 differ in thermal physiology (Table 1). Thermal tolerance has evolved significantly more slowly than
228 some, but not all, ecomorphological traits (Table 2). CT_{max} evolved significantly more slowly than body

size (SVL; $P < 0.001$) and relative limb length (femur; $P = 0.029$), but did not differ from head length ($P = 0.105$) or toepad width ($P = 0.112$).

CT_{max} was significantly positively correlated with geo-referenced climatic temperatures as represented by temperature principal components axis 1 ($P = 0.006$, $r^2 = 0.45$; Fig. 6).

Discussion

Abiotic niche evolution has been invoked as an important component of many evolutionary radiations, but the functional consequences of abiotic niche divergence are generally unknown. We demonstrate that the Puerto Rican *Anolis* adaptive radiation is accompanied by physiological divergence in both heat tolerance and sensitivity to sub-lethal temperature variability. Warm-niche species (determined based on preference for open versus shaded perches) have either higher heat tolerance, higher optimal physiological temperatures, or both, compared to cool-niche species. The anoles of Jamaica also diversified physiologically, with species evolving essentially the full range of heat tolerances and optimal temperatures observed in their Puerto Rican counterparts (Fig. 5).

Repeated divergence in physiological traits under similar conditions is itself evidence of adaptation [65]. However, we provide more direct evidence of adaptation by estimating physiological performance of species in warm and cool operative environments on Puerto Rico. Cool niche species have higher predicted performance in shaded operative environments than their warm niche sister species in three of the four species pairs (Fig. 3), with the lone exception a species pair for which there was no divergence in T_{opt}. The pattern was less pronounced in the open operative environment, with warm niche species having a sizable performance advantage in only two species pairs (Fig. 3). However, the performance advantage for warm niche species becomes apparent when considering overheating risk. During midday hours in the warmer open environment, 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 exhibited no divergence in thermal tolerance (Fig. 2B).

254 The high community-level species diversity associated with *Anolis* and other adaptive radiations
255 is facilitated by fine-scale resource partitioning [3]. Sympatric anoles are known to behaviorally partition
256 thermal microhabitats [12, 13], and our analysis indicates that this is facilitated by physiological
257 divergence. When co-occurring anole species share the same fundamental structural niche (*i.e.*,
258 ecomorphological microhabitat specialization), they invariably differ in thermal physiology (Table 1).
259 This pattern is not only maintained for endemic members of the community, but also for introduced
260 species: in Jamaica, the endemic trunk-ground species *A. lineatopus* is found in sympatry with the
261 introduced trunk-ground species *A. sagrei*, and these species differ significantly in thermal tolerance
262 (Table 1). These data highlight that, even in radiations for which morphological divergence is important
263 in facilitating coexistence, physiological divergence can also play an important role, providing an
264 orthogonal axis of diversification.

265 We found that thermal tolerance evolved more slowly than some ecomorphological traits (SVL
266 and femur length), and evolved at similar rates to others (head length and toe-pad width; Table 2). In
267 general, divergence along the fundamental thermal niche axis appears to have occurred more slowly than
268 divergence along the fundamental structural niche axis, at least within the Puerto Rican and Jamaican
269 anole radiations. This result is inconsistent with a recent analysis that found that thermal niche, estimated
270 as field body temperature, evolves more quickly than morphology in Caribbean anoles [42]. Discrepancy
271 between these results may be due to the different traits analyzed and/or the different species included, or
272 the fact that that study estimated rates of absolute, rather than proportional, trait change. Regardless, our
273 results suggest that high rates of evolutionary change are not necessary for traits to be ecologically
274 important, as the relatively slow divergence that we find in anole thermal tolerance has important
275 performance consequences with respect to overheating risk in warm habitats (Fig. 4).

276 We found that evolution of the fundamental and realized thermal niche are correlated, as $CT_{\max}$
277 was significantly positively correlated with geo-referenced temperature data across species (Fig. 6).
278 Therefore, the realized thermal niches estimated in correlative ENM studies can capture some of the
279 underlying variability in the fundamental thermal niche. Nonetheless, the majority of variability in the

280 data (~55%) is left unexplained, and there are clear cases where realized and fundamental niche estimates
281 diverge. For example, *A. lineatopus* and *A. gundlachi* have very similar CT_{max} (mean CT_{max} = 36.3°C and
282 36.2°C, respectively), but occur in very different realized thermal niches (temperature PC1 = 0.14 and -
283 2.66, respectively). Conversely, *A. opalinus* and *A. pulchellus* occur in very similar realized niches
284 (temperature PC1 = -0.53 and -0.51, respectively) but have very different CT_{max} (mean CT_{max} = 37.5°C
285 and 41.3°C, respectively). These results underscore the fact that broad-scale climatic conditions are not
286 necessarily reflective of the underlying thermal biology of the organism being considered. Much of this
287 discrepancy is likely due to behavioral thermoregulation, which allows taxa in the same geographic
288 location to experience very different body temperatures and those in different geographic locations to
289 experience very similar body temperatures [33, 66].

290 We have shown that fundamental thermal niche divergence is important in the Puerto Rican and
291 Jamaican *Anolis* adaptive radiations. However, the importance of such divergence is likely not restricted
292 to these islands. For example, species of *cybotes* group trunk-ground anoles in Hispaniola have diverged
293 in thermal tolerance along an elevational gradient [44, 67], and variation in thermal physiology also
294 occurs among non-Antillean anoles [68, 69]. Water loss rates also differ among anole species and
295 populations [70-76]. In this context, our results suggest that physiological divergence may be as important
296 as morphological divergence for diversification and coexistence in this classic radiation.

297 Achieving a mechanistic understanding of how evolutionary and ecological processes interact to
298 promote the production and maintenance of biodiversity is a long-standing goal in biology. Given the
299 reality of ongoing climate change, the need to understand these processes has become immediate,
300 particularly with respect to temperature-dependent traits [77]. By focusing on physiological traits that link
301 temperature to performance, we demonstrate that the evolution of thermal physiology can facilitate
302 adaptive radiation by contributing to *in situ* performance tradeoffs and species co-existence. While our
303 trait- and performance-based analyses would not be possible using the type of broad-scale climatic data
304 used in ENMs studies alone, we find that climate-scale realized niche features correlate with fundamental

niche features. Nonetheless, climatic data provide imperfect signal with respect to the evolution of functional traits, likely due to the ability of species to behaviorally augment their effective thermal environments. We suggest that deeper insights about the contribution of climatic niche divergence to evolutionary radiations will emerge when correlative ENMs are used in tandem with experimental physiological studies within an integrative research program.

Acknowledgements

We thank Jonathan Losos for helpful comments on this manuscript, D. Steinberg for field help and the El Verde and Mata de Plátano field stations, Ridge to Reef farm, and Green Castle Estates for logistical support. Permits were provided by the Puerto Rican Departmento de Recursos Naturales y Ambientales, the St. Croix Division of Fish and Wildlife, and the Jamaican National Environment and Planning Agency. Funding was provided by NSF DDIG #1110570 to A.R.G.

References

1. Hutchinson G.E. 1959 Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist* **93**(870), 145-159.
2. Simpson G.G. 1953 *Major features of evolution*. New York, Columbia University Press.
3. Schluter D. 2000 *The ecology of adaptive radiation*, Oxford University Press.
4. McPeek M.A. 2008 The ecological dynamics of clade diversification and community assembly. *The American Naturalist* **172**(6), E270-E284.
5. Grant P.R. 1999 *Ecology and evolution of Darwin's finches*, Princeton University Press.
6. Streelman J.T., Danley P.D. 2003 The stages of vertebrate evolutionary radiation. *Trends in Ecology & Evolution* **18**(3), 126-131.
7. Meyer A. 1993 Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology & Evolution* **8**(8), 279-284.
8. Nosil P. 2012 *Ecological speciation*, Oxford University Press.
9. Chown S., Gaston K., Robinson D. 2004 Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Functional Ecology* **18**(2), 159-167.
10. Gaston K.J., Chown S.L., Calosi P., Bernardo J., Bilton D.T., Clarke A., Clusella-Trullas S., Ghalambor C.K., Konarzewski M., Peck L.S. 2009 Macrophysiology: a conceptual reunification. *The American Naturalist* **174**(5), 595-612.
11. Kaspari M., Clay N.A., Lucas J., Yanoviak S.P., Kay A. 2015 Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology* **21**(3), 1092-1102.

12. Rand A.S. 1964 Ecological distribution in anoline lizards of Puerto Rico. *Ecology* **45**, 745-752.
13. Ruibal R. 1961 Thermal relations of five species of tropical lizards. *Evolution* **15**(1), 98-111.
14. Wake D.B. 1987 Adaptive radiation of salamanders in Middle American cloud forests. *Annals of the Missouri Botanical Garden* **74**, 242-264.
15. Kozak K.H., Wiens J.J. 2007 Climatic zonation drives latitudinal variation in speciation mechanisms. *Proceedings of the Royal Society of London B: Biological Sciences* **274**(1628), 2995-3003.
16. Labra A., Pienaar J., Hansen T.F. 2009 Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. *The American Naturalist* **174**(2), 204-220.
17. Bonino M.F., Azócar D.L.M., Tulli M.J., Abdala C.S., Perotti M.G., Cruz F.B. 2011 Running in cold weather: morphology, thermal biology, and performance in the southernmost lizard clade in the world (*Liolaemus lineomaculatus* section: Liolaemini: Iguania). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **315**(8), 495-503.
18. Pincheira-Donoso D. 2011 Predictable variation of range-sizes across an extreme environmental gradient in a lizard adaptive radiation: evolutionary and ecological inferences. *PloS One* **6**(12), e28942.
19. Kellermann V., van Heerwaarden B., Sgrò C.M., Hoffmann A.A. 2009 Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* **325**(5945), 1244-1246.
20. Stillman J.H., Somero G.N. 2000 A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology* **73**(2), 200-208.
21. Dalziel A.C., Martin N., Laporte M., Guderley H., Bernatchez L. 2015 Adaptation and acclimation of aerobic exercise physiology in Lake Whitefish ecotypes (*Coregonus clupeaformis*). *Evolution* **69**(8), 2167-2186.
22. Knouft J.H., Losos J.B., Glor R.E., Kolbe J.J. 2006 Phylogenetic analysis of the evolution of the niche in lizards of the *Anolis sagrei* group. *Ecology* **87**(sp7), S29-S38.
23. Quintero I., Wiens J.J. 2013 Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecology Letters* **16**(8), 1095-1103.
24. Warren D.L., Glor R.E., Turelli M. 2008 Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* **62**(11), 2868-2883.
25. Lawing A.M., Polly P.D., Hews D.K., Martins E.P., Collar D.C., Bronstein J.L. 2016 Including Fossils in Phylogenetic Climate Reconstructions: A Deep Time Perspective on the Climatic Niche Evolution and Diversification of Spiny Lizards (*Sceloporus*). *The American Naturalist* **188**(2), 000-000.
26. Peterson A.T., Soberón J., Pearson R.G., Anderson R.P., Nakamura M., Martinez-Meyer E., Araújo M.B. 2011 *Ecological niches and geographic distributions*. Princeton, New Jersey, USA, Princeton University Press.
27. Guisan A., Thuiller W. 2005 Predicting species distribution: offering more than simple habitat models. *Ecology Letters* **8**(9), 993-1009.
28. Buckley L.B., Urban M.C., Angilletta M.J., Crozier L.G., Rissler L.J., Sears M.W. 2010 Can mechanism inform species' distribution models? *Ecology Letters* **13**(8), 1041-1054.

29. Alvarado-Serrano D.F., Knowles L.L. 2014 Ecological niche models in phylogeographic studies: applications, advances and precautions. *Molecular Ecology Resources* **14**(2), 233-248.
30. Kearney M., Porter W. 2009 Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology letters* **12**(4), 334-350.
31. Warren D.L., Cardillo M., Rosauer D.F., Bolnick D.I. 2014 Mistaking geography for biology: inferring processes from species distributions. *Trends in Ecology & Evolution* **29**(10), 572-580.
32. Araújo M.B., Peterson A.T. 2012 Uses and misuses of bioclimatic envelope modeling. *Ecology* **93**(7), 1527-1539.
33. Bogert C.M. 1949 Thermoregulation in reptiles, a factor in evolution. *Evolution* **3**(3), 195-211.
34. Losos J.B., Jackman T.R., Larson A., de Queiroz K., Rodríguez-Schettino L. 1998 Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**(5359), 2115-2118.
35. Williams E.E. 1983 Ecomorphs, faunas, island size, and diverse end points in island radiations of Anolis. In *Lizard ecology: studies of a model organism* (pp. 326-370. Cambridge, Harvard University Press.
36. Mahler D.L., Ingram T., Revell L.J., Losos J.B. 2013 Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* **341**(6143), 292-295.
37. Losos J.B. 2009 *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*, University of California Press.
38. Williams E.E. 1972 The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. *Evolutionary Biology* **6**, 47-89.
39. Heatwole H., Lin T.-H., Villalón E., Muñiz A., Matta A. 1969 Some aspects of the thermal ecology of Puerto Rican anoline lizards. *Journal of Herpetology* **3**, 65-77.
40. Huey R.B., Webster T.P. 1976 Thermal biology of *Anolis* lizards in a complex fauna: the *cristatellus* group on Puerto Rico. *Ecology* **57**, 985-994.
41. Losos J.B., Leal M., Glor R.E., de Queiroz K., Hertz P.E., Schettino L.R., Lara A.C., Jackman T.R., Larson A. 2003 Niche lability in the evolution of a Caribbean lizard community. *Nature* **424**(6948), 542-545.
42. Hertz P.E., Arima Y., Harrison A., Huey R.B., Losos J.B., Glor R.E. 2013 Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution* **67**(7), 2101-2113.
43. Algar A.C., Mahler D.L. 2015 Area, climate heterogeneity, and the response of climate niches to ecological opportunity in island radiations of *Anolis* lizards. *Global Ecology and Biogeography* **doi:10.1111 /geb.12327**.
44. Muñoz M.M., Stimola M.A., Algar A.C., Conover A., Rodriguez A.J., Landestoy M.A., Bakken G.S., Losos J.B. 2014 Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society B: Biological Sciences* **281**(1778), 20132433.
45. Nicholson K.E., Glor R.E., Kolbe J.J., Larson A., Blair Hedges S., Losos J.B. 2005 Mainland colonization by island lizards. *Journal of Biogeography* **32**(6), 929-938.
46. Mahler D.L., Revell L.J., Glor R.E., Losos J.B. 2010 Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* **64**(9), 2731-2745.
47. Gamble T., Geneva A.J., Glor R.E., Zarkower D. 2014 *Anolis* sex chromosomes are derived from a single ancestral pair. *Evolution* **68**(4), 1027-1041.

48. Hertz P.E. 1992 Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* **73**, 1405-1417.
49. Hertz P.E. 1992 Evaluating thermal resource partitioning by sympatric lizards *Anolis cooki* and *A. cristatellus*: a field test using null hypotheses. *Oecologia* **90**(1), 127-136.
50. Jackman T.R., Larson A., De Queiroz K., Losos J.B. 1999 Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. *Systematic Biology* **48**(2), 254-285.
51. Gunderson A.R., Leal M. 2012 Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard. *Functional Ecology* **26**(4), 783-793.
52. Leal M., Gunderson A.R. 2012 Rapid change in the thermal tolerance of a tropical lizard. *The American Naturalist* **180**(6), 815-822.
53. Huey R.B. 1983 Natural variation in body temperature and physiological performance in a lizard (*Anolis cristatellus*). In *Advances in herpetology and evolutionary biology: essays in honor of Ernest E Williams* (pp. 484-490.
54. Losos J.B. 1990 Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs* **60**, 369-388.
55. Logan M.L., Cox R.M., Calsbeek R. 2014 Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences* **111**(39), 14165-14169.
56. Angilletta M.J. 2009 *Thermal adaptation: a theoretical and empirical synthesis*, Oxford University Press.
57. Deutsch C.A., Tewksbury J.J., Huey R.B., Sheldon K.S., Ghalambor C.K., Haak D.C., Martin P.R. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* **105**(18), 6668-6672.
58. Huey R.B., Deutsch C.A., Tewksbury J.J., Vitt L.J., Hertz P.E., Pérez H.J.Á., Garland T. 2009 Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B: Biological Sciences* **276**(1664), 1939-1948.
59. Efron B. 1987 Better bootstrap confidence intervals. *Journal of the American Statistical Association* **82**(397), 171-185.
60. Ewel J.J., Whitmore J.L. 1973 Ecological life zones of Puerto Rico and US Virgin Islands. In *Forest Service Research Paper ITF-18* (U.S. Department of Agriculture.
61. Hertz P.E., Huey R.B., Stevenson R. 1993 Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *The American Naturalist* **142**, 796-818.
62. Gorman G.C., Licht P. 1974 Seasonality in ovarian cycles among tropical *Anolis* lizards. *Ecology* **55**, 360-369.
63. Revell L.J. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**(2), 217-223.
64. Adams D.C. 2013 Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. *Systematic Biology* **62**, 181-192.
65. Harvey P.H., Pagel M.D. 1991 *The comparative method in evolutionary biology*, Oxford University Press.
66. Huey R.B., Hertz P.E., Sinervo B. 2003 Behavioral drive versus behavioral inertia in evolution: a null model approach. *The American Naturalist* **161**(3), 357-366.
67. Hertz P.E., Huey R.B. 1981 Compensation for altitudinal changes in the thermal environment by some *Anolis* lizards on Hispaniola. *Ecology* **62**(3), 515-521.

68. Logan M.L., Huynh R.K., Precious R.A., Calsbeek R.G. 2013 The impact of climate change measured at relevant spatial scales: new hope for tropical lizards. *Global Change Biology* **19**, 3093-3102.
69. van Berkum F.H. 1986 Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution* **40**, 594-604.
70. Gunderson A.R., Siegel J., Leal M. 2011 Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*. *Journal of Comparative Physiology B* **181**(7), 965-972.
71. Hillman S., Gorman G. 1977 Water loss, desiccation tolerance, and survival under desiccating conditions in 11 species of Caribbean *Anolis*. *Oecologia* **29**(2), 105-116.
72. Sexton O.J., Heatwole H. 1968 An experimental investigation of habitat selection and water loss in some anoline lizards. *Ecology* **49**, 762-767.
73. Hertz P., Arce-Hernandez A., Ramirez-Vazquez J., Tirado-Rivera W., Vazquez-Vives L. 1979 Geographical variation of heat sensitivity and water loss rates in the tropical lizard, *Anolis gundlachi*. *Comparative Biochemistry and Physiology Part A: Physiology* **62**(4), 947-953.
74. Hertz P.E. 1980 Responses to dehydration in *Anolis* lizards sampled along altitudinal transects. *Copeia* **1980**, 440-446.
75. Hillman S., Gorman G.C., Thomas R. 1979 Water loss in *Anolis* lizards: evidence for acclimation and intraspecific differences along a habitat gradient. *Comparative Biochemistry and Physiology Part A: Physiology* **62**(2), 491-493.
76. Dmi'el R., Perry G., Lazell J. 1997 Evaporative water loss in nine insular populations of the lizard *Anolis cristatellus* group in the British Virgin Islands. *Biotropica* **29**(1), 111-116.
77. Huey R.B., Kearney M.R., Krockenberger A., Holtum J.A., Jess M., Williams S.E. 2012 Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**(1596), 1665-1679.

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.

525

526

527
528

529

530

531

532 Table 1

co-occurring species		ecomorph	physiological trait	
			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.

533

534

535

536

537

538

539

540

541 Table 2.

542

	CT_{max}	SVL	head length	femur length	toepad width
CT_{max}	**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**

543

544

545

546

547

548

549

550

551

552

553

554

555

556

557

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*.

Figure 5: Thermal physiology of endemic Jamaican anoles. (A) Optimal sprinting temperature (T_{opt}). *Anolis valencienni* does not have a T_{opt} estimate because this species could not be induced to run. (B) Heat tolerance (CT_{max}).

Figure 6: Relationship between CT_{max} and climate temperature PC1 from Algar and Mahler (2015).

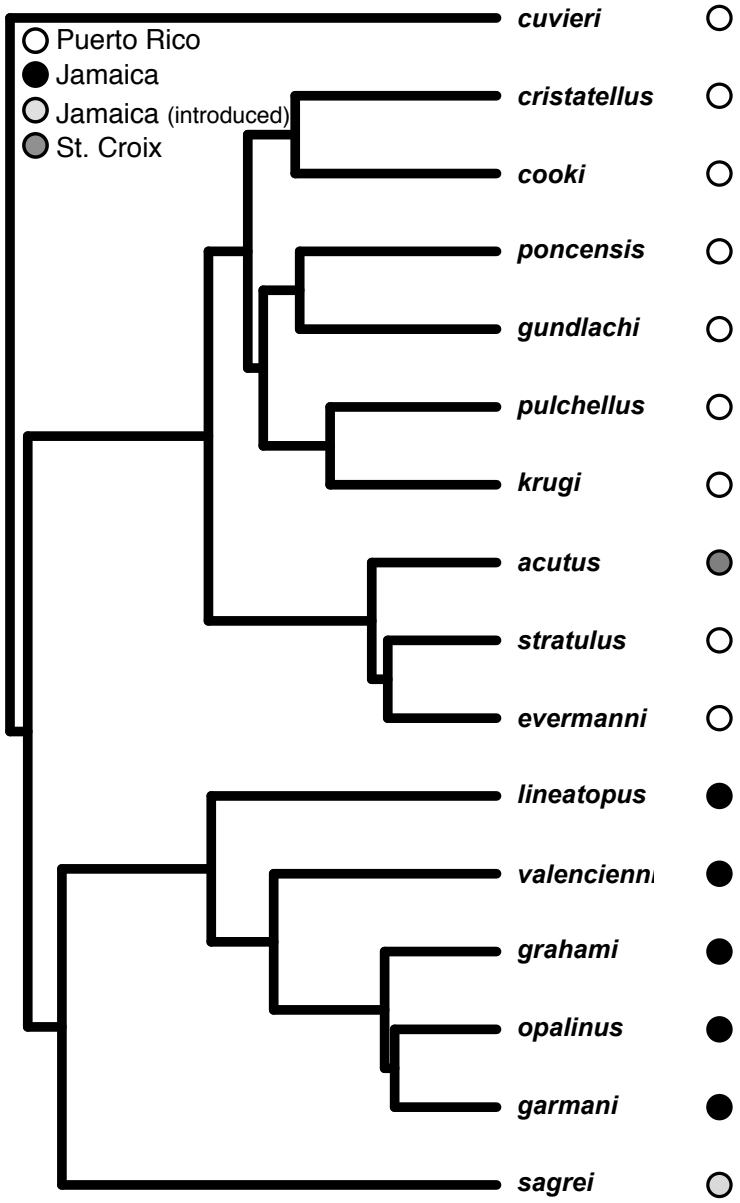

Puerto Rico
Jamaica
Jamaica (introduced)
St. Croix
cuvieri
cristatellus
cooki
poncensis
gundlachi
pulchellus
krugi
acutus
stratulus
evermanni
lineatopus
valencienni
grahami
opalinus
garmani
sagrei

Figure 1

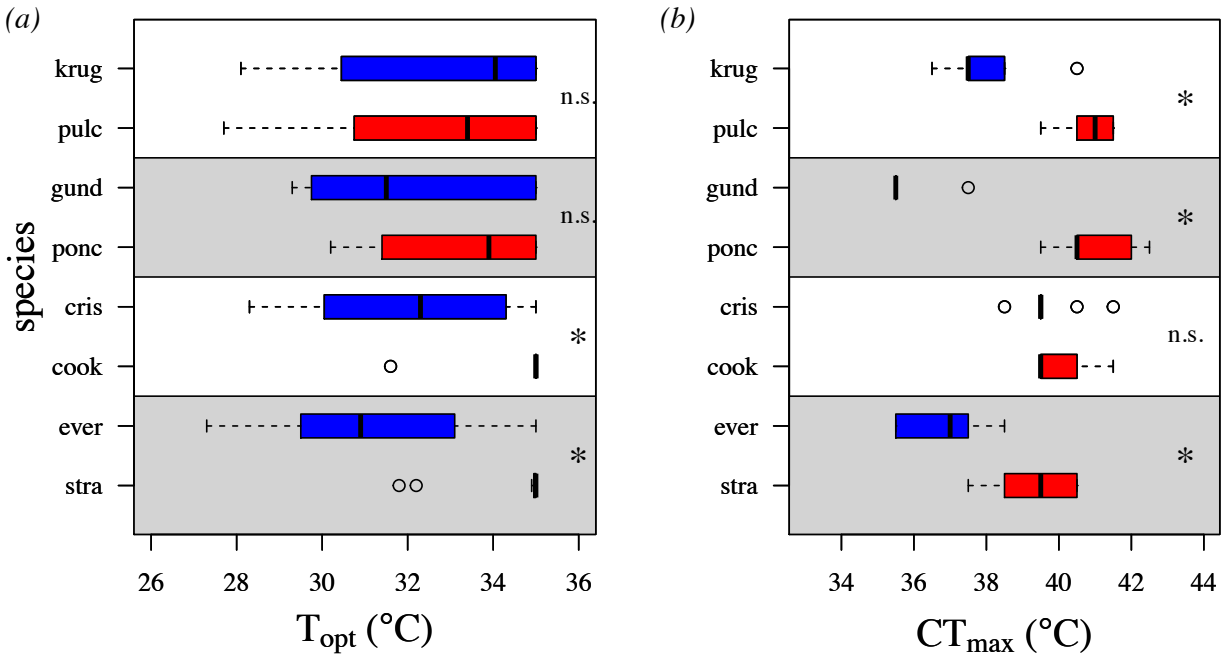

(a)
(b)
krug
pulc
gund
ponc
cris
cook
ever
stra
species
n.s.
n.s.
*
*
*
*
n.s.
*
26
28
30
32
34
36
34
36
38
40
42
44
Topt (°C)
CTmax (°C)

Figure 2.

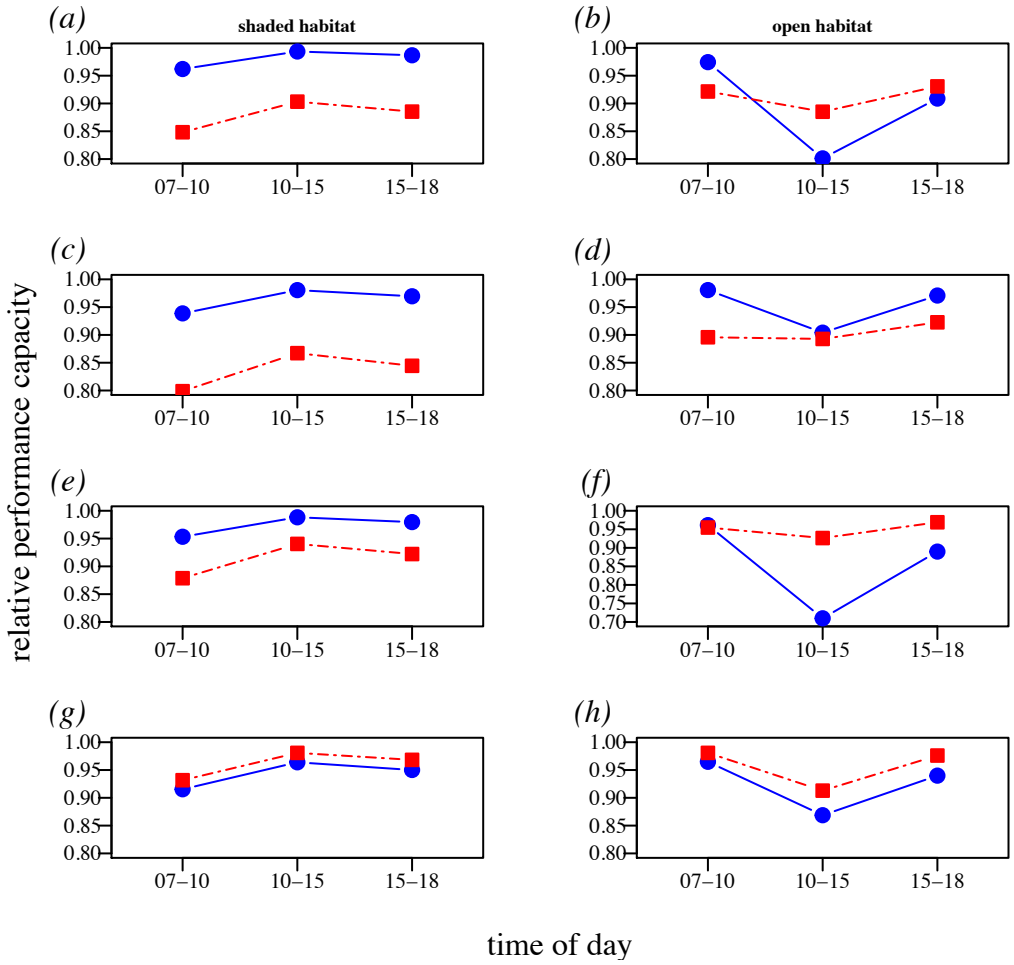

(a)
shaded habitat
(b)
open habitat
(c)
(d)
(e)
(f)
(g)
(h)
relative performance capacity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
07–10
10–15
15–18
time of day

Figure 3

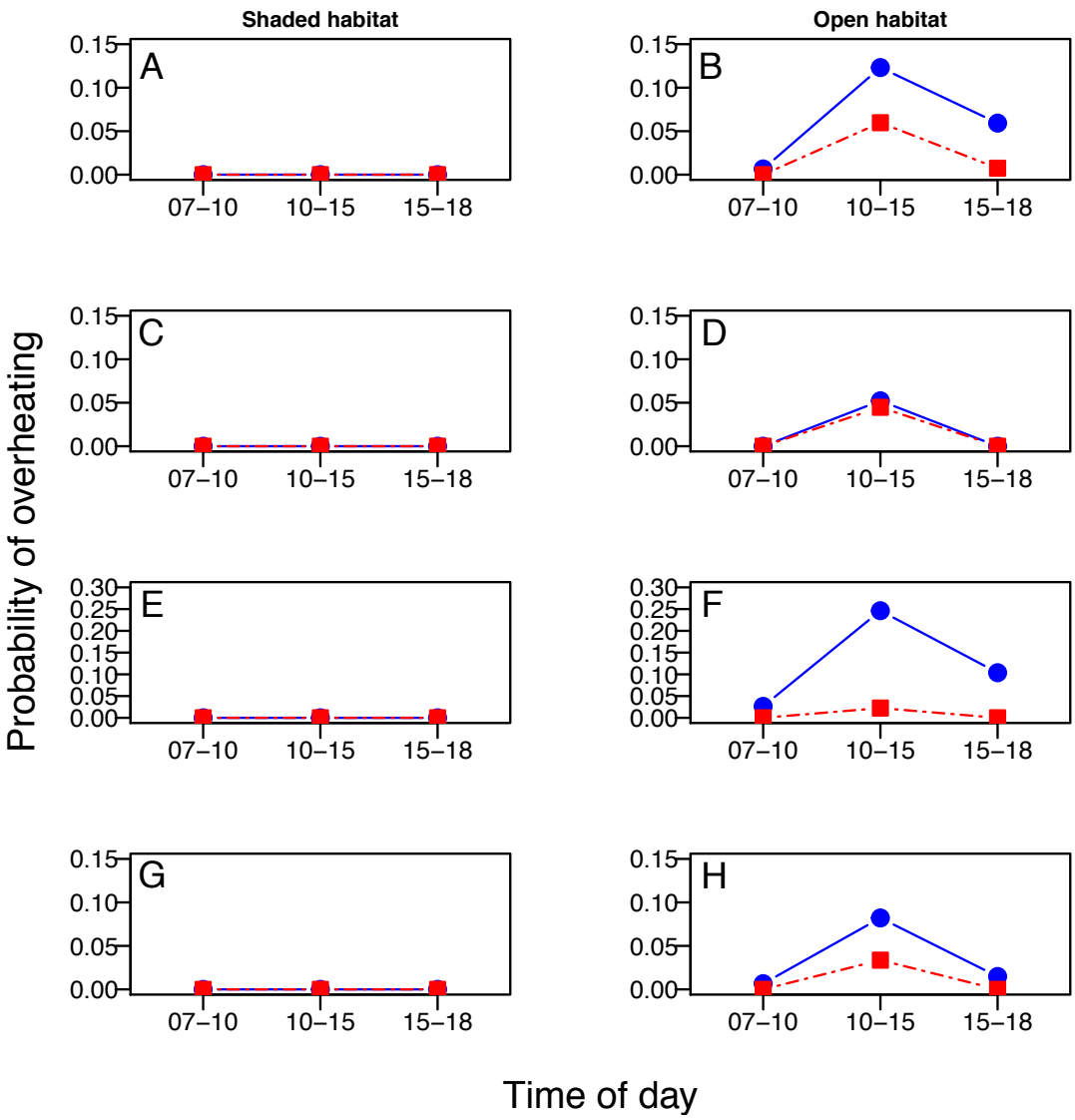
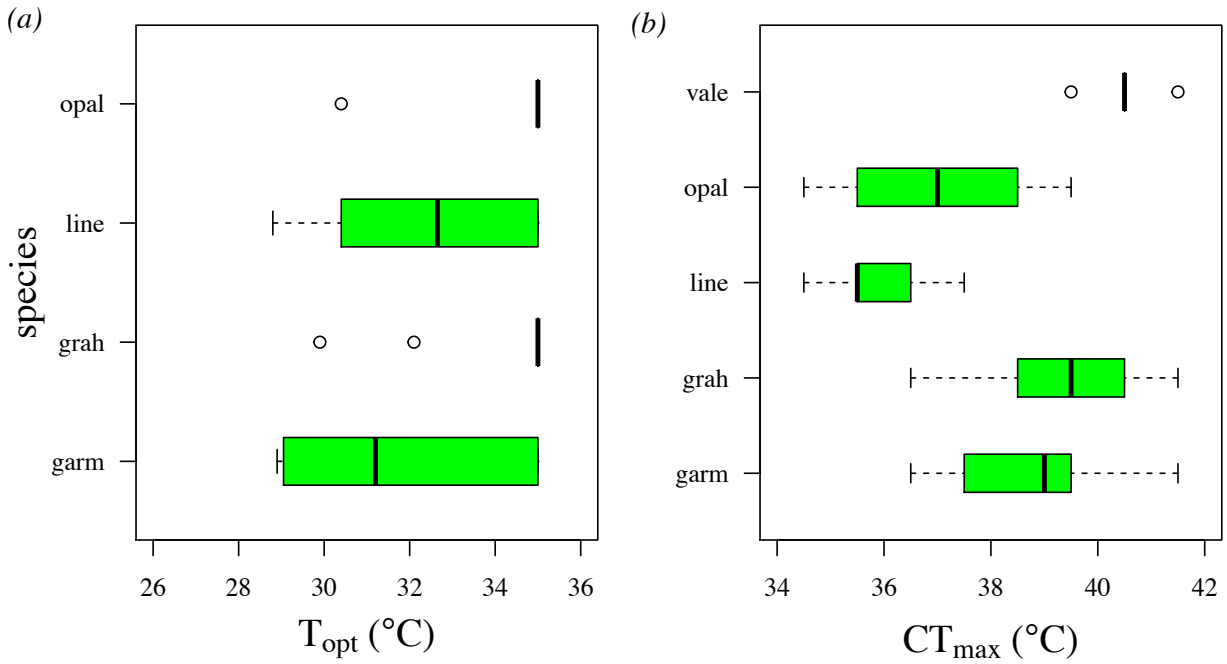

Shaded habitat
Open habitat
A
B
C
D
E
F
G
H
0.15
0.10
0.05
0.00
0.30
0.25
0.20
07–10
10–15
15–18
Probability of overheating
Time of day

Figure 4

667

668

669



670

671

672

673 Figure 5

674

675

676

677

678

679

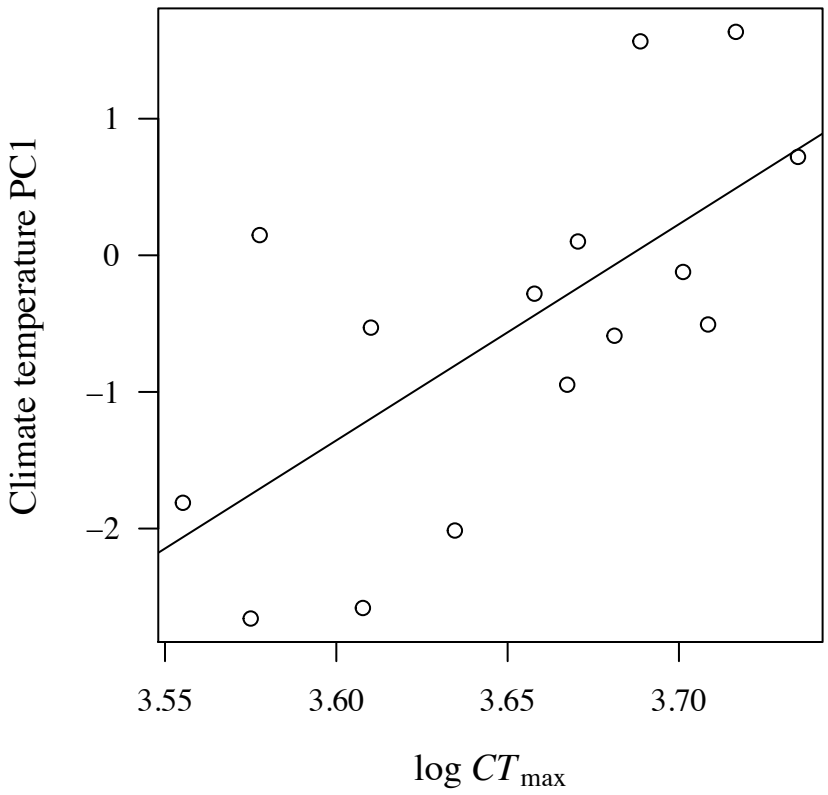


Figure 6.