Received: 4 April 2020 | Revised: 14 August 2020 | Accepted: 19 August 2020

DOI: 10.1111/jbi.13984

RESEARCH PAPER

Journal of Biogeography | WILEY

Weak phylogenetic and climatic signals in plant heat tolerance

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Funding information
Garden Club of America; Division of Environmental Biology, Grant/Award Number: DEB-1350125

Handling Editor: Simon Scheiter

Abstract

Aim: High heat tolerance is a potential way for plants to maintain performance under high temperatures that can be acted upon by environmental filters to influence community assembly. Plant heat tolerances are phenotypically plastic and thus common garden experiments are needed to test if species from hotter environments have consistently higher heat tolerance than species from colder environments. Past studies that have measured heat tolerance from species grown in common gardens have found conflicting relationships between species' climatic origins and their heat tolerance, possibly due to phylogenetic non-independence of study species. In this study, we test the hypothesis that phylogenetic structure can help to explain variation in heat tolerance in order to resolve the conflicting relationships between climate and plant heat tolerance.

Location: Fairchild Tropical Botanic Garden and the Gifford Arboretum, Miami, FL, USA.

Taxon: Pteridophytes, Gymnosperms and Angiosperms.

Methods: We tested for phylogenetic signal in the photosynthetic heat tolerance of 123 species of ferns, gymnosperms, magnoliids, monocots and eudicots by calculating Blomberg's K. Phylogenetic independent contrasts of heat tolerance and climatic distributions for >100 species were used to test the hypothesis that climate can predict variation in heat tolerance.

Results: Species' heat tolerances were not phylogenetically conserved according to Blomberg's K, but we found significant differences in the heat tolerance of ferns, gymnosperms, magnoliids, monocots and eudicots. When controlling for phylogenetic non-independence, we found a significant, but weak relationship between the mean maximum temperature of the warmest month of species' climatic distributions and their photosynthetic heat tolerance.

Main conclusions: We conclude that phylogeny and climate are weak predictors of photosynthetic heat tolerance. However, differences among the groups we studied suggest that the variation in heat tolerance may be better explained by differences in microenvironment, thermoregulatory traits and leaf temperatures.

KEYWORDS

climate change, common garden, community assembly, ecophysiology, environmental filter, photosynthesis, phylogenetic conservation

1 | INTRODUCTION

High temperatures may act as environmental filters that prevent some species from establishing in a given community, thereby influencing community composition, structure and function. Plant heat tolerance is the maintenance of essential plant functions at high temperatures that contribute to the productivity of a genotype, and as such, is a potentially useful trait for understanding the role of high-temperature filters in community assembly (Hall, 1992; Porch & Hall, 2013). Photosynthesis may be particularly useful for understanding the temperature filters on community assembly because it is highly temperature sensitive, is essential for most plants, and affects individual productivity. The photosynthetic heat tolerance (PHT) is commonly assessed by measuring chlorophyll *a* fluorescence, which is associated with photosystem II (PSII) photochemistry. High temperatures can damage PSII photochemistry by causing enzymes in chloroplast thylakoid membranes to disassociate and denature (Wahid et al., 2007). This damage is usually measured using initial fluorescence (F_0) or the maximum quantum yield (F_V/F_M), and is used to calculate PHT.

Consistent with the hypothesis that high temperatures act as an environmental filter, communities in hotter climates tend to contain plant species with higher PHT than communities from cold climates (Feeley et al., 2020; O'Sullivan et al., 2017; Smillie & Nott, 1979). Similarly, plant species from hotter environments tend to have higher PHT (Daas et al., 2008; Drake et al., 2018; Havaux & Tardy, 1996; Mooney & Billings, 1961). However, PHT is phenotypically plastic and can acclimate to new growing temperatures (Daas et al., 2008; Drake et al., 2018; Havaux & Tardy, 1996; Valladares & Pearcy, 1997); consequently, apparent differences in heat tolerance across coarse climatic gradients may reflect acclimation rather than community assembly processes.

Common garden experiments control for environmental variation so that the role of climatic filters on species' physiological tolerances can be assessed. Knight and Ackerly (2002, 2003) grew congeneric plant species from contrasting thermal environments in a common garden, but observed no difference in their PHT (Knight & Ackerly, 2002, 2003). This lack of difference in these species' heat tolerance may have been caused by phenotypic plasticity and by the lower temperature of the common garden environment compared to field sites (Knight & Ackerly, 2002, 2003). The lower observed heat tolerances in the common garden may have been caused by lower production of heat shock proteins (Wahid et al., 2007), isoprenoids (Logan & Monson, 1999; Taylor et al., 2019), photoprotective pigments (Krause et al., 2015; Zsófi et al., 2009), or membrane-fortifying solutes and fatty acids (Hüve et al., 2006; Zhu et al., 2018) known to contribute to high PHT. In contrast, Zhu et al. (2018) found that species from warmer climates had higher PHT irrespective of common garden cooling or heating experiments, supporting the hypothesis that climate acts as a filter on PHT.

These contrasting results may be resolved by incorporating the phylogenetic relatedness among species. Climatic distributions are thought to be highly conserved among closely related species, which can help explain many macroecological patterns, such as the latitudinal biodiversity gradient in plants (Kerkhoff et al., 2014). If climatic tolerances are predictive of physiological tolerances, then PHT should exhibit phylogenetic conservation and be correlated with climatic tolerance (Godoy et al., 2011). Indeed, phylogenetic conservation of heat tolerance could explain why the PHT of the closely related species studied by Knight and Ackerly (2002) did not differ, while phylogenetic non-independence could have resulted in variation in PHT that Zhu et al. (2018) attributed to climate (Felsenstein, 1985).

Phylogenetic analysis of functional trait data is commonly used to study species' niches and patterns of community assembly (e.g. Cavender-Bares et al., 2006). Phylogenetic approaches also have the potential to explain the high interspecific variation in PHT observed within and among communities (Feeley et al., 2020; O'Sullivan et al., 2017). This variation currently hampers our ability to understand how climate influences fundamental aspects of ecological processes like community assembly and may provide a physiological basis for predicting species-level responses to climate change.

In this study, we tested the hypothesis that variation in PHT can be explained by species' climatic distributions and phylogeny. Specifically, we quantified the effects of temperature on the maximum quantum yield of photosystem II (PSII) photochemistry for 123 plant species from 74 families grown in a common environment. We used this novel dataset (the largest of its type yet assembled) to construct a phylogeny from a trimmed mega-tree to test if PHT is phylogenetically conserved. To further understand how the thermal limits of PSII photochemistry differ among plant lineages, we tested for differences in heat tolerance among the fern, gymnosperm, basal angiosperm, monocot and eudicot lineages. Lastly, we tested if climate acts as an environmental filter by comparing species' PHT and climatic tolerance while controlling for relatedness using phylogenetically independent contrasts (PICs).

2 | MATERIALS AND METHODS

2.1 | Site and species selection

This study was conducted using the living collections of Fairchild Tropical Botanic Garden (FTBG; 25.677 N, 80.275 W) and the University of Miami's John C. Gifford Arboretum (UM; 25.724 N, 80.281 W) in Coral Gables, Florida, USA. Both sites are located within 6 km of each other, experience identical monsoonal subtropical climates characterized by a mean annual temperature (MAT) of 24.1°C, and mean total annual rainfall of roughly 130 cm (Hijmans et al., 2005). Together, the two sites provide access to an ecologically and evolutionary diverse collection of living plants from throughout the global tropics (Perez et al., 2019).

We selected study species by randomly drawing families represented within the combined collections of both sites. Within a given family, we opted to sample species that were abundant within the living collections and mature individuals that were growing in areas

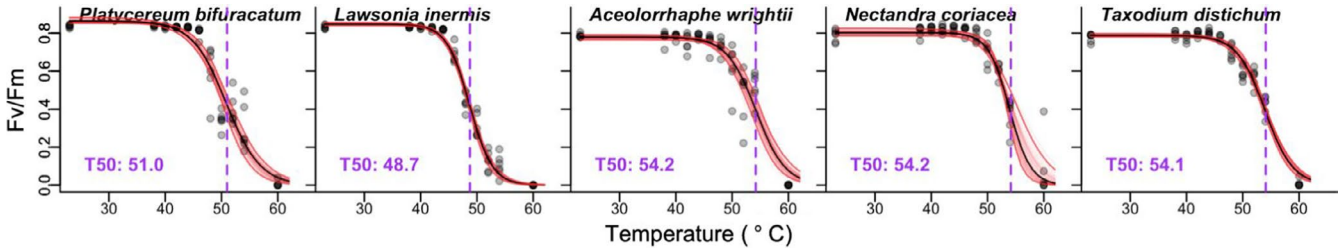

FIGURE 1 The maximum quantum yield of PSII (F_V/F_M) modelled as a function of temperature to calculate the T_{50} heat tolerance for five species representing each major taxonomic group in the present study. Red shaded lines show the bootstrapped fit for the 'nls' model and the vertical line represents the estimate of T_{50}. Shaded points indicate overlapping and non-overlapping values of F_V/F_M recorded at each treatment temperature

with direct sun exposure. Differences in light exposure can affect heat tolerance (Valladares & Pearcy, 1997) so we quantified an illumination index for each species following the 'Clark and Clark' method (1992; Jennings et al., 1999). This method categorizes the level of direct sunlight received by a plant canopy (ranges from 1 = 'no direct light' to 5 = 'crown fully exposed to direct light') and was selected because of its reasonably high repeatability among few observers. Our final dataset consisted of up to four species per family and included several different growth habits.

2.2 | Heat tolerance

PHT is often measured with F_0 or F_V/F_M. F_0 signals changes in the number of open reaction centres of PSII, but is subject to error when measured during stress treatments (e.g. heating) that alter the optical properties of leaves (Baker, 2008). F_V/F_M, on the other hand, provides a relative index of PSII function (where $F_V = F_M - F_0$; F_M = closed reaction centres) that is not biased by confounding effects of changing leaf optical properties during stress treatments (Baker, 2008). Although PHTs estimated with F_0 and F_V/F_M are usually correlated, we assessed PHT using F_V/F_M.

To quantify PHT, leaves of up to 12 individual study species were collected in the mornings and brought to nearby laboratory facilities at University of Miami. Depending on the size of the leaf, between 1 and 66 leaves (leaflets were used if leaves were compound) per individual were collected and used in for measuring heat tolerance. In order to minimize the possibility of down-regulation in PHT, we collected leaves during the hottest months (June through October) of 2018. The mean daytime temperature for this period was 28.2°C and the mean maximum temperature was 31.6°C (https://w2.weather.gov/climate/index.php?wfo=mfl, accessed 7 July 2019).

Once in the laboratory, the leaves were cut into ca. 1.9-cm diameter disks. Many of the species that we sampled had irregular leaf sizes, shapes and thicknesses that prevented uniform discs. When necessary, thick leaves were cut into ~3 cm^2 squares, narrow leaves were cut into ~3 cm^2 rectangles and whole leaves were used for some species with small leaves. In some cases, for example, for species with compound leaves and very small leaflets, the leaflets on one half of a rachis were removed and remainder of the leaf area cut to 3 cm^2. The total leaf area that we subjected to heat treatment was similar for all species except *Pinus elliottii* which had fascicles trimmed to ~4 cm in length.

One layer of Miracloth was placed on the abaxial surface of the leaf disks and three layers of Miracloth were placed on the adaxial leaf surface to prevent anaerobiosis during heat treatments (Krause et al., 2010). Miracloth-enclosed leaves were then placed into waterproof plastic bags with the air removed and they were submerged in water baths maintained at room temperature (~23°C), 38, 40, 42, 44, 46, 48, 50, 52, 54 or 60°C with circulating heaters. Immediately following 15-min of heat treatment, leaf pieces were removed from the water and bags and were placed into Petri dishes lined with moist paper towels where they were allowed to recover for 24 hr at room temperature under low light (~1 μmol photons m^{-2} s^{-1}). Following recovery, leaf pieces were dark-acclimated for 20 min before we measured their maximum quantum yield with an OS30p$^+$ handheld fluorometer (Opti-Science, Hudson, NH USA). We then calculated the temperature that causes a 50% reduction in F_v/F_m (T_{50}) as:

$$\text{heat tolerance } (T_{50}) = \frac{\log\left(\frac{\theta_a}{x} - \theta_b\right)}{\theta_c}$$

where θ_a is the asymptote of the heat treatment-response variable relationship, θ_b is a constant, x represents 50% reduction in F_v/F_m compared to control treatments and θ_c is the decay parameter. These 'θ' parameters were optimized and fit to the temperature-response variable relationship using the nls function in R's 'stats' package following

$$y = \frac{\theta_a}{1 + e^{-(\theta_b + \theta_c T)}}$$

where T is the heat treatment temperature (R Core Team, 2020). We generated bootstrapped means and 95% confidence level estimates of T_{50} by reiterating the 'nls' model 100 times while randomly resampling data with replacement before each iteration for each species (Figure 1). The bootstrapped mean PHT for each species was used in all subsequent analyses. The same approach was also used to calculate the critical temperature (T_{crit}) PHT for each species, which corresponds to the temperature that causes the initial decrease in F_V/F_M. Results of analyses using T_{crit} are reported in the Supporting Information.

2.3 | Phylogenetic analysis

To test if the T_{50} of F_V/F_M was phylogenetically conserved, we used the phylomatic function in the 'brranching' R package (Ooms & Chamberlain, 2019) to generate a phylogeny for the study species from the trimmed R20120829 mega-tree (Gastauer & Meira-Neto, 2016), and assigned fossil-calibrated branch lengths using the ph_bladj function in the 'phylocomr' R package (Ooms & Chamberlain, 2018). We then tested for phylogenetic signal in PHT by calculating Blomberg's K (Blomberg et al., 2003; Swenson, 2014). Values of Blomberg's K equal to 1 indicate that trait variation across a phylogeny is indistinguishable from Brownian motion. Values of $K > 1$ indicate that phylogeny predicts more trait variation than expected given Brownian motion due to trait conservation (Blomberg et al., 2003; Losos, 2008; Swenson, 2014). When $K < 1$, phylogeny predicts less trait variation than expected under Brownian motion due to phylogenetic trait convergence. A null distribution of K was created to test if our observed K was significantly different from 1 using the 'phylosig' command in the 'phytools' R package (Revell, 2012).

We used a two-way Kruskal–Wallis test to assess differences in T_{50} among the ferns, gymnosperms, magnoliids, monocots, or eudicots within our dataset. This two-way test included an interaction term between group and crown illumination (to account for the potential effects of shading on T_{50}). The Kruskal–Wallis test was followed by post-hoc pairwise comparisons of heat tolerance for each group using a Wilcoxon rank sum tests with a Holm-Bonferroni correction.

2.4 | Phylogenetically independent contrasts

Species' climatic tolerances were determined by accessing species occurrence data as available through the Global Biodiversity Information Facility using the 'spocc' R package (GBIF; http://www.gbif.org/, accessed July 7, 2019; Chamberlain, 2020). Occurrence records were screened and records with obvious geo-referencing errors (e.g. coordinates within oceans) and duplicate entries were excluded from our dataset. We next extracted the BIO1 (annual mean temperature), BIO5 (mean maximum temperature of the warmest month), BIO12 (total annual precipitation) and BIO18 (precipitation of warmest quarter) from the WorldClim database (2.5 arc-minute resolution) at each' occurrence location (Hijmans et al., 2005). These latter two variables were chosen because previous research has indicated that plant heat tolerance can be positively correlated with average and extreme temperatures and negatively correlated with water availability (Curtis et al., 2016). To minimize the influence of outliers potentially caused by geo-referencing or taxonomic errors, all occurrence records falling outside the 95% quantiles for any of these climate layers were excluded from our final dataset. Only species with ≥15 records in the remaining data were used in subsequent analyses. We then calculated the mean and upper 97.5 quantile of each climatic variable (BIO1, BIO5, BIO12, BIO18) for each species.

We performed phylogenetically independent contrasts on both PHTs and geographically based climatic tolerance to control for any phylogenetic non-independence in these traits. Non-independence of data was accounted for with contrasts that are computed throughout a phylogenetic tree (determined with same procedure as above) as the difference between two daughter nodes standardized by the square root of their sum of branch lengths (Felsenstein, 1985). Phylogenetic contrasts were performed using the 'pic' function in the 'ape' R package (Paradis & Schliep, 2018) which results in $n - 1$ contrasts where 'n' is the number of species in the phylogeny. Our final dataset of mean climatic variables across species' geographical distributions and species' heat tolerance contained observations for 103 of our original 123 species. We addressed the hypothesis that climate acts as an environmental filter on species' occurrence using ordinary least squares regression with a y-intercept set to zero to test if the PICs of climatic tolerance and T_{50} were correlated.

Lastly, to account for a potential bias in our study that could have resulted from decreases in heat tolerance caused by acclimation to the lower temperature of our sample site (FTBG & UM) compared to species' native climates, we subsampled our species to include just those for which the site is near or above the upper limits of their native climatic distributions. We assumed that individuals of species sampled at the hot portions of their species' climatic distributions were least likely to down-regulate their heat tolerance (which if unaccounted for could potentially obscure any relationship between climate and observed heat tolerance). To select these species, we calculated the upper 97.5th quantile of the mean annual temperature (BIO1) and mean maximum temperature of the warmest month (BIO5) from each species' cleaned occurrence data (see above). These high-temperature thresholds were then compared to the mean annual and mean maximum temperatures at FTBG and UM. Species with high-temperature thresholds that were less than our site's mean and maximum temperature were then used to test the hypothesis that heat tolerance are correlated to climate. PICs were re-performed with this subset of species to standardize variation among the nodes of our phylogenetic tree needed to account for phylogenetic non-independence.

3 | RESULTS

The T_{50} we observed ranged from 47.5°C (*Bourreria havanensis*, Boraginaceae; *Zanthoxylum coriaceum*, Rutaceae) to 56.7°C (*Agathis macrophylla*, Araucariaceae) and averaged 51.5°C (2.1 *SD*). No clear taxonomic designation was found for the fern *Deparia japonica* (Arthyriaceae), which prevented us from including it in our calculation of Blomberg's K or in the PICs. We detected a significant, but weak phylogenetic signal in T_{50} ($K = 0.22$; $p = 0.018$) indicating that PHT varied more among relatives than what would be expected given Brownian motion (Figure 2). Blomberg's K's were also calculated for the species' climatic tolerances and are reported in Table 1. Blomberg's K for mean BIO5, BIO12 and BIO18 exhibited similarly weak phylogenetic signals ($K < 1$, $p < 0.05$; Table 1), and no signal

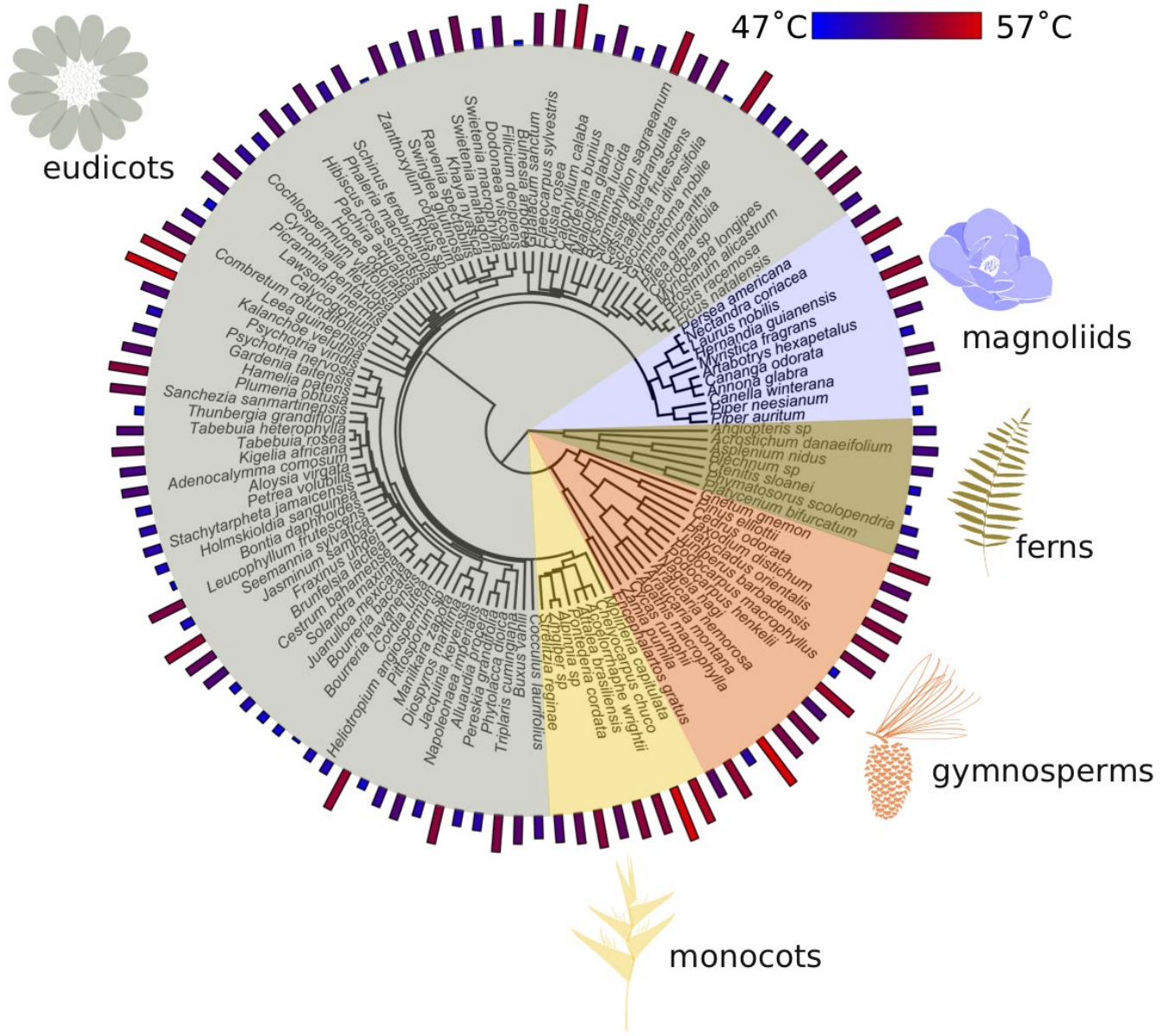

FIGURE 2 Phylogenetic tree of the T_{50} heat tolerance for 122 species used to estimate Blomberg's K (K = 0.22; $p = 0.018$). The heat tolerance for each species is depicted as a coloured bar of a different height. Low heat tolerances are depicted as small blue bars and high heat tolerances are depicted as large red bars. A colour key to T_{50} values is provided in the top right corner

was detected for BIO1. No phylogenetic signal was observed for T_{crit} (Table S2).

The Kruskal–Wallis test revealed significant differences in the T_{50} of major clades (Figure 3; Kruskal–Wallis $\chi^2 = 23.898$, $p < 0.001$, $df = 4$). Canopy illumination had no effect on T_{50} (Kruskal–Wallis $\chi^2 = 3.094$, $p = 0.542$, $df = 4$), nor did its interaction with clade (Kruskal–Wallis $\chi^2 = 28.191$, $p = 0.059$, $df = 4$). Of these clades, gymnosperms and monocots had the highest heat tolerance, ferns and eudicots had the lowest heat tolerance, and the heat tolerance of magnoliids were intermediate among the groups (Figure 3). No differences in T_{crit} were observed among the different plant clades (Supporting Information). Post-hoc pairwise Wilcoxon rank tests suggested that the T_{50} of ferns and eudicots were significantly different from gymnosperms and monocots (Table 2; Figure 3). The magnoliid group was not significantly different from any other group (Table 2; Figure 3).

We did not detect any correlation between mean BIO1 (mean annual temperature) and species' heat tolerance after the node including *Canella winterana*, was removed from the analysis on the basis of a Cook's distance >1 (Figure 4; Table 3). We observed a significant correlation between mean BIO5 (maximum temperature of the warmest month) and species' heat tolerance even after the removal of *C. winterana*. Inclusion of this outlier nearly doubles our reported r^2 value (Table 3), but ultimately explains little of the variation observed in our T_{50}. Mean BIO12 (annual precipitation) and BIO18 (precipitation of warmest quarter) were not correlated to

TABLE 1 Blomberg's K for climatic variable

Variable	BioClim name	Blomberg's K	*p*-value
Annual mean temperature	BIO1	0.209	0.102
Max temperature of warmest month	BIO5	0.235	0.016
Annual precipitation	BIO12	0.271	0.004
Precipitation of warmest quarter	BIO18	0.271	0.007

species T_{50} with or without the inclusion of *C. winterana*. The coefficients of the linear models used to test the relationships between the PICs of species' climatic tolerance and their T_{50} are summarized in Table 3. No noteworthy correlations between the PICs of the 97.5 quantiles of climate variables and the PIC of T_{50} were observed (Table S1). Similarly, we observed no relationships between PICs of mean climate variables and the PIC of T_{crit} (Table S2). We detected no correlation between the PICs of BIO5 and T_{50} after attempting to account for low T_{50} due to acclimation (Table 3 and Supporting Information). We found no correlations between climatic tolerances and T_{50} when PICs were not used.

4 | DISCUSSION

We detected a weak phylogenetic signal in T_{50} meaning sister taxa tend to have similar T_{50}, but similarity in T_{50} is less than expected under Brownian motion. This pattern of phylogenetic signal suggests heat tolerance is not phylogenetically conserved and is consistent with patterns of convergent evolution (Cavender-Bares et al., 2006; Losos, 2008). Our results suggest phylogenetic relatedness has a limited ability to predict variation in species PHT.

Despite the weak phylogenetic signal of T_{50} across our phylogeny, we did observe significant differences in heat tolerance among the five broad plant clades included in our study. These results could be an artefact of low sample sizes or differences in thermoregulatory traits (e.g. leaf size and stomatal conductance) among groups that cause leaf temperatures to be decoupled from air temperatures (Leigh et al., 2017; Michaletz et al., 2016; Nobel, 1999; Perez & Feeley, 2020). It has been proposed that heat tolerances vary according to leaf temperatures, but there is mixed support for this hypothesis (Ghouil et al., 2003; Knight & Ackerly, 2002; Leon-Garcia & Lasso, 2019; Nobel et al., 1986; Perez & Feeley, 2020). Nevertheless, large leaves tend to have higher leaf temperatures, possibly explaining why the large-leafed monocots we sampled had high T_{50} (Leigh et al., 2017; Nobel, 1999). Although, high heat tolerance has been observed in many monocots like agaves, palms, and C4 grasses, not all members of these groups exhibit large leaves (Jones, 2014; Nobel, 1988) so other explanations for the observed differences among clades are needed.

An alternative, non-mutually exclusive explanation for the different T_{50} among clades is the macroevolutionary divergence of plant physiologies following the radiation of angiosperms. The high photosynthetic rates of the angiosperms necessitates high stomatal conductance, which reduces leaf temperature and may explain why eudicots had low T_{50} (Boyce et al., 2009; Brodribb & Feild, 2010; Brodribb et al., 2007). It has been proposed that high photosynthetic rates of angiosperms competitively excluded ancestral ferns and

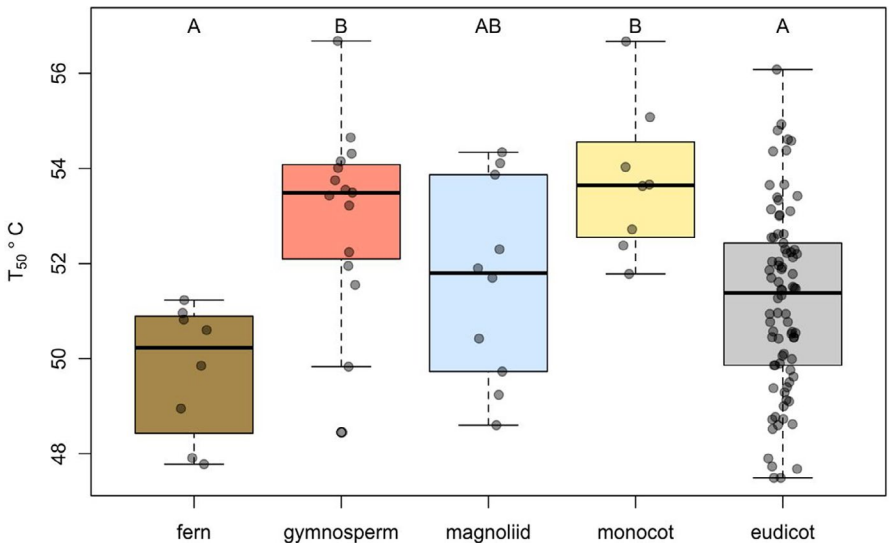

FIGURE 3 Pairwise Wilcoxon rank sum test of the heat tolerance for five different major plant clades; statistics can be found in Table 2

	Fern	Gymnosperm	Magnoliid	Monocot
Gymnosperm	0.0077	–	–	–
Magnoliid	0.831	1	–	–
Monocot	0.0016	1	0.676	–
Eudicot	0.471	0.0162	1	0.0098

TABLE 2 *p*-values for pairwise comparisons among major plant lineages using Wilcoxon rank sum tests with Holm correction

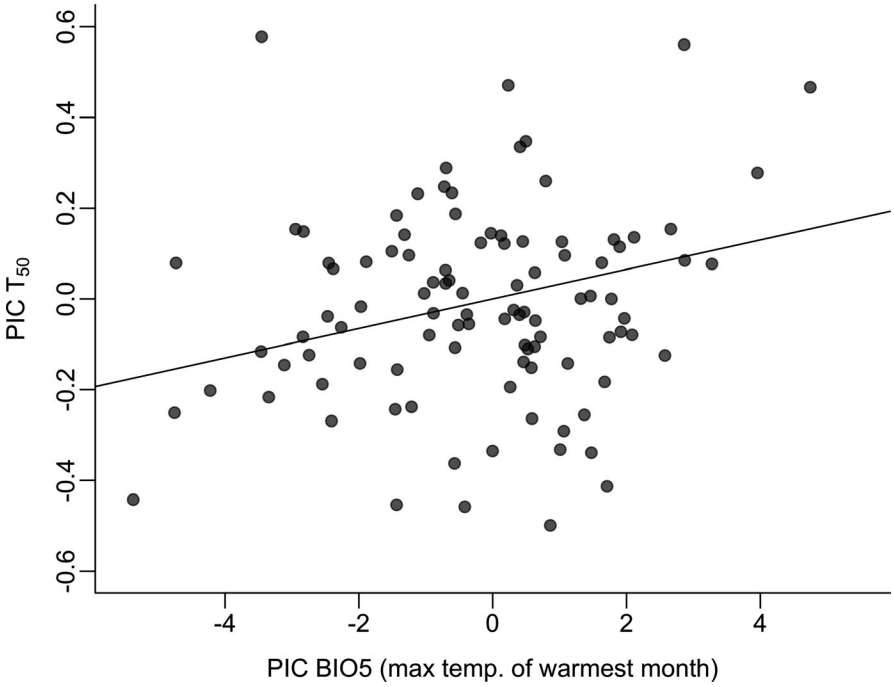

FIGURE 4 (a) The effect of the maximum temperature of the warmest month (BIO5) on species' heat tolerance after accounting for phylogenetic non-independence using phylogenetically independent contrasts (PIC). PIC $T_{50} = 0.023x + 0$; $r^2 = 0.04$, $p = 0.029$

TABLE 3 Linear model coefficients and statistics for phylogenetically independent contrasts for the mean of BIO1 (annual mean temperature), BIO5 (mean maximum temperature of the warmest month), BIO12 (total annual precipitation), BIO18 (precipitation of warmest quarter) climate variables and T50 heat tolerance

Independent variable	Dependent variable	Slope CI	*y*-intercept	r^2	*p*-value	*df*
BIO1	T50	−0.003 to 0.024	0	0.01	0.154	101
BIO5	T50	0.003–0.044	0	0.04	0.029	101
BIO12	T50	−0.001 to 0.001	0	0	0.702	101
BIO18	T50	−0.077 to 0.009	0	0	0.810	101
BIO5 (subset)	T50	−4.823 to 6.531	0	0.15	0.778	6

gymnosperms from productive habitats and relegated them to marginal shaded habitats and dry resource-limited habitats, respectively (Bond, 1989; Schneider et al., 2003; Subedi et al., 2020). Relaxed selection for high heat tolerances in the cool shaded habitats of ancestral ferns may account for the low T_{50} values for ferns in our dataset. Alternatively, high T_{50} of the gymnosperms in our study may be explained by selection for high PHT in ancestral gymnosperms growing in dry habitats. A better understanding how microhabitat and thermoregulatory traits influence leaf temperature, and how leaf temperature is coordinated with heat tolerance is needed to assess these hypotheses.

Our results suggest that variation in T_{50} is poorly coordinated with climate after accounting for phylogenetic non-independence. This refutes the hypothesis that high-temperature acts as a filter on PHT to influence community assembly. The significant correlation we observed between T_{50} and the warmest month (BIO5) corroborates the findings of other studies (Feeley et al., 2020; O'Sullivan et al., 2017; Zhu et al., 2018), but has limited biological significance given the low proportion of variance in T_{50} that is explained by climate. The weak correlation between T_{50} and mean annual temperature or maximum temperature of the warmest month is consistent with the hypothesis that cold tolerance (and not hot heat tolerance) is a better predictor of species climatic distributions (Araújo et al., 2013). Given that our study species were primarily tropical and unlikely to experience freezing temperature, their distributions in relation to high temperatures may be more due to biotic interactions than abiotic tolerances (Brown et al., 1996). However, climate is capable of predicting the edges of species' distributions where plant fitness is negatively affected (Lee-Yaw et al., 2016). If climatic extremes can predict decreases in fitness, our results indicate heat tolerance of PSII photochemistry is limited in its ability to predict species' distributions or plant productivity which we assume affects fitness. The maximum quantum yield of PSII (F_V/F_M) may be a poor predictor of productivity because it is only proportional to carbon assimilation under circumstances (i.e. low temperatures with limited photorespiration) which are unlikely to be met in the field (Baker, 2008; Brooks & Farquhar, 1985). Therefore, caution is needed when using heat tolerance of PSII to generate expectations regarding species responses or susceptibility to climate change.

Like our study, Curtis et al. (2016) measured the PHT of plants grown in a botanic garden and found that PHT and climate were

weakly correlated. If heat tolerance exhibits local adaptation, accurate local environmental data would be needed to detect any correlation with heat tolerance. The use of local climate data may explain why PHT and climate were correlated in Zhu et al.'s (2018) common garden experiment, but not Curtis et al. (2016) or in the present study. However, Curtis et al. (2016) did observe that microhabitat water availability predicted some variation in PHT, and posited that it facilitated high transpiration rates leading to reduced leaf temperatures and heat tolerance. It is possible that microhabitat and thermoregulatory traits cause leaves to experience temperatures that are not accurately represented by climate, which may explain the poor coordination between species' T_{50} and their climatic tolerances (Perez & Feeley, 2020). Indeed, microclimates may be better predictors of plant responses to climate change than macroclimate (Zellweger et al., 2020).

Phenotypic plasticity and acclimation are widely documented in the heat tolerance of PSII photochemistry, but this is unlikely to have affected our estimates of T_{50} or our results. For example, we purposely measured PHT during the hottest portion of the year to minimize any potential effect of cool-to-warm season acclimation. Furthermore, our species were primarily tropical, and tropical species are known to have limited capacities to acclimate their heat tolerance and other photosynthetic processes to high temperatures (Doughty & Goulden, 2009; Krause et al., 2010). Shade is known to decrease heat tolerance (Slot et al., 2018; Valladares & Pearcy, 1997), and we attempted to control for this by measuring fully sun-exposed leaves and canopy illumination (which had no effect on our results). Drought conditions may cause increases in heat tolerance (Gimeno et al., 2008; Valladares & Pearcy, 1997) but this is not likely to have affected our measurements given that this study was conducted in a maintained botanic garden with a monsoon climate (Perez et al., 2019). We expected that any bias in T_{50} caused by growing conditions will have been uniform across all species such that statistical relationships between heat tolerances and climate would be preserved. We also expect that any effect of acclimation or plasticity would have been minimized due to our large sample size. Lastly, we found no correlation between T_{50} and climatic tolerance even when just focusing on species without risk of acclimation of T_{50} to lower temperatures.

Climatic variables have been used as proxies for physiological tolerance and to explain important species-level and community-level responses to climate change (Brienen & et al., 2015; Clark et al., 2003; Fadrique et al., 2018). Yet, the physiological explanations for these ecological phenomena have yet to be verified and they remain poorly understood. Our results highlight the need to reconcile broad ecological phenomena with their physiological mechanisms. Instead of climate, microclimates, physiological traits and leaf temperatures show promise for understanding the sources of ecophysiological variation in plant photosynthetic heat tolerance (Perez & Feeley, 2020). We conclude that plant heat tolerances are not phylogenetically conserved and not constrained by climate.

ACKNOWLEDGMENTS

The authors would like to thank two anonymous reviewers, Drs. Carol Horvitz Nutt, Steven Oberbauer and Barbara Whitlock for advice that improved this manuscript. We thank Fairchild Tropical Botanic Garden for providing access to their collections. This research was conducted with the support of the Garden Club of America's Award in Tropical Botany to T.M.P. and US National Science Foundation (NSF) DEB-1350125 to K.J.F.

DATA AVAILABILITY STATEMENT

All heat tolerance data are available from https://github.com/tmoreperez/DPHeaT and the Open Traits Database of Plant Heat Tolerance (https://opentraits.org/datasets.html).

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REFERENCES

Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., & Chown, S. L. (2013). Heat freezes niche evolution. *Ecology Letters*, *16*(9), 1206–1219. https://doi.org/10.1111/ele.12155

Baker, N. R. (2008). Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annual Review of Plant Biology*, *59*(1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Bond, W. J. (1989). The tortoise and the hare: Ecology of angiosperm dominance and gymnosperm persistence. *Biological Journal of the Linnean Society*, *36*(3), 227–249.

Boyce, C. K., Brodribb, T. J., Feild, T. S., & Zwieniecki, M. A. (2009). Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society of Biology*, *276*(1663), 1771–1776. https://doi.org/10.1098/rspb.2008.1919

Brienen, R. J. W., Phillips, O. L., Feldpausch, T. R., Gloor, E., Baker, T. R., Lloyd, J., Lopez-Gonzalez, G., Monteagudo-Mendoza, A., Malhi, Y., Lewis, S. L., Vásquez Martinez, R., Alexiades, M., Álvarez Dávila, E., Alvarez-Loayza, P., Andrade, A., Aragão, L. E. O. C., Araujo-Murakami, A., Arets, E. J. M. M., Arroyo, L., & Zagt, R. J. (2015). Long-term decline of the Amazon carbon sink. *Nature*, *519*(7543), 344–348. https://doi.org/10.1038/nature14283

Brodribb, T. J., & Feild, T. S. (2010). Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters*, *13*(2), 175–183. https://doi.org/10.1111/j.1461-0248.2009.01410.x

Brodribb, T. J., Feild, T. S., & Jordan, G. J. (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology*, *144*(4), 1890–1898. https://doi.org/10.1104/pp.107.101352

Brooks, A., & Farquhar, G. D. (1985). Effect of temperature on the CO_2/O_2 specificity o f ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta*, *165*, 397–406. https://doi.org/10.1007/BF00392238

Brown, J. H., Stevens, G. C., & Kaufman, D. M. (1996). The geographic range size: Size, shape, boundaries, and internal structure. *Annual Review of Ecology and Systematics*, *27*(1), 597–623. https://doi.org/10.1146/annurev.ecolsys.27.1.597

Cavender-Bares, J., Keen, A., & Miles, B. (2006). Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology*, *87*, 109–122. https://doi.org/10.1890/0012-9658(2006)87[109:PSOFPC]2.0.CO;2

Chamberlain, S. (2020). spocc: Interface to species occurrence data sources. R package version 1.1.0. Retrieved from https://CRAN.R-project.org/package=spocc

Clark, D. A., Piper, S. C., Keeling, C. D., & Clark, D. B. (2003). Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984-2000. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(10), 5852–5857. https://doi.org/10.1073/pnas.0935903100

Curtis, E. M., Gollan, J., Murray, B. R., & Leigh, A. (2016). Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants. *Journal of Biogeography*, *43*(6), 1156–1165. https://doi.org/10.1111/jbi.12713

Daas, C., Montpied, P., Hanchi, B., & Dreyer, E. (2008). Responses of photosynthesis to high temperatures in oak saplings assessed by chlorophyll-a fluorescence: Inter-specific diversity and temperature-induced plasticity. *Annals of Forest Science*, *65*(3), 305. https://doi.org/10.1051/forest:2008002

Doughty, C. E., & Goulden, M. L. (2009). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences*, *114*(1), 1–12. https://doi.org/10.1029/2007JG000632

Drake, J. E., Tjoelker, M. G., Vårhammar, A., Medlyn, B. E., Reich, P. B., Leigh, A., Pfautsch, S., Blackman, C. J., López, R., Aspinwall, M. J., Crous, K. Y., Duursma, R. A., Kumarathunge, D., De Kauwe, M. G., Jiang, M., Nicotra, A. B., Tissue, D. T., Choat, B., Atkin, O. K., & Barton, C. V. M. (2018). Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Global Change Biology*, *24*(6), 2390–2402. https://doi.org/10.1111/gcb.14037

Fadrique, B., Báez, S., Duque, Á., Malizia, A., Blundo, C., Carilla, J., Osinaga-Acosta, O., Malizia, L., Silman, M., Farfán-Ríos, W., Malhi, Y., Young, K. R., Cuesta C., F., Homeier, J., Peralvo, M., Pinto, E., Jadan, O., Aguirre, N., Aguirre, Z., & Feeley, K. J. (2018). Widespread but heterogeneous responses of Andean forests to climate change. *Nature*, *564*(7735), 207–212. https://doi.org/10.1038/s41586-018-0715-9

Feeley, K., Martinez-Villa, J., Perez, T., Silva Duque, A., Triviño Gonzalez, D., & Duque, A. (2020). The thermal tolerances, distributions, and performances of tropical montane tree species. *Frontiers in Forests and Global Change*, *3*(March), 1–11. https://doi.org/10.3389/ffgc.2020.00025

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*(1), 1–15. https://doi.org/10.1086/284325

Gastauer, M., & Meira-Neto, J. A. A. (2016). An enhanced calibration of a recently released megatree for the analysis of phylogenetic diversity. *Brazilian Journal of Biology*, *76*(3), 619–628. https://doi.org/10.1590/1519-6984.20814

Ghouil, H., Montpied, P., Epron, D., Ksontini, M., Hanchi, B., & Dreyer, E. (2003). Thermal optima of photosynthetic functions and thermostability of photochemistry in cork oak seedlings. *Tree Physiology*, 1031–1039. https://doi.org/10.1093/treephys/23.15.1031

Gimeno, T. E., Pias, B., Lemos-Filho, J. P., & Valladares, F. (2008). Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. *Tree Physiology*, *29*, 87–98. https://doi.org/10.1093/treephys/tpn007

Godoy, O., De Lemos-filho, J. P., & Valladares, F. (2011). Invasive species can handle higher leaf temperature under water stress than Mediterranean natives Author's personal copy. *Environmental and Experimental Botany*, *71*, 207–214. https://doi.org/10.1016/j.envexpbot.2010.12.001

Hall, A. E. (1992). Breeding for heat tolerance. In J. Janick (Ed.), *Plant breeding reviews* (Vol. *10*, pp. 129–168). John Wiley & Sonc Inc.

Havaux, M., & Tardy, F. (1996). Temperature-dependent adjustment of the thermal stability of photosystem II in vivo: Possible involvement of xanthophyll-cycle pigments. *Planta*, *198*, 324–333. https://doi.org/10.1007/BF00620047

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978. https://doi.org/10.1002/joc.1276

Hüve, K., Bichele, I., Tobias, M., & Niinemets, Ü. (2006). Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. *Plant, Cell and Environment*, *29*(2), 212–228. https://doi.org/10.1111/j.1365-3040.2005.01414.x

Jennings, S. B., Brown, N. D., & Sheil, D. (1999). Assessing forest canopies and understorey illumination: Canopy closure, canopy cover and other measures. *Forestry*, *72*(1), 59–73. https://doi.org/10.1093/forestry/72.1.59

Jones, H. G. (2014). *Plants and microclimate: A quantitative approach to environmental plant physiology* (3rd ed.). Cambridge University Press.

Kerkhoff, A. J., Moriarty, P. E., & Weiser, M. D. (2014). The latitudinal species richness gradient in New World woody angiosperms is consistent with the tropical conservatism hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(22), 8125–8130. https://doi.org/10.1073/pnas.1308932111

Knight, C. A., & Ackerly, D. D. (2002). An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. *Oecologia*, *130*(4), 505–514. https://doi.org/10.1007/s00442-001-0841-0

Knight, C. A., & Ackerly, D. D. (2003). Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: Congeneric species from desert and coastal environments. *New Phytologist*, *160*(2), 337–347. https://doi.org/10.1046/j.1469-8137.2003.00880.x

Krause, G. H., Winter, K., Krause, B., Jahns, P., García, M., Aranda, J., & Virgo, A. (2010). High-temperature tolerance of a tropical tree, Ficus insipida: Methodological reassessment and climate change considerations. *Functional Plant Biology*, *37*(9), 890. https://doi.org/10.1071/FP10034

Krause, G. H., Winter, K., Krause, B., & Virgo, A. (2015). Light-stimulated heat tolerance in leaves of two neotropical tree species, Ficus insipida and Calophyllum longifolium. *Functional Plant Biology*, *42*, 42–51. https://doi.org/10.1071/FP14095

Lee-Yaw, J. A., Kharouba, H. M., Bontrager, M., Mahony, C., Csergő, A. M., Noreen, A. M. E., Li, Q., Schuster, R., & Angert, A. L. (2016). A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. *Ecology Letters*, *19*(6), 710–722. https://doi.org/10.1111/ele.12604

Leigh, A., Sevanto, S., Close, J. D., & Nicotra, A. B. (2017). The influence of leaf size and shape on leaf thermal dynamics: Does theory hold up under natural conditions? *Plant, Cell and Environment*, *40*, 237–248. https://doi.org/10.1111/pce.12857

Leon-garcia, I. V., & Lasso, E. (2019). High heat tolerance in plants from the Andean highlands: Implications for paramos in a warmer world. *PLoS One*, *10*(14), e0224218. https://doi.org/10.1371/journal.pone.0224218

Logan, B. A., & Monson, R. K. (1999). Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. *Plant Physiology*, *120*(3), 821–826. https://doi.org/10.1104/pp.120.3.821

Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, *11*(10), 995–1003. https://doi.org/10.1111/j.1461-0248.2008.01229.x

Michaletz, S. T., Weiser, M. D., Mcdowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2016). The energetic and carbon economic origins of leaf thermoregulation. *Nature Plants*, *2*(9), 1–8. https://doi.org/10.1038/nplants.2016.129

Mooney, H. A., & Billings, W. D. (1961). Comparative physiological ecology of arctic and alpine populations of oxyria digyna. *Ecological Monographs*, *31*(1), 1–29. https://doi.org/10.2307/1950744

Nobel, P. S. (1988). *Environmnetal biolgy of agaves and cacti*. Cambridge University Press.

Nobel, P. S. (1999). *Physicochemical and environmental plant physiology* (2nd ed.). Academic Press.

Nobel, S., Geller, G. N., Kee, S. C., & Zimmerman, A. D. (1986). Temperatures and thermal tolerances for cacti exposed to high temperatures near the soil surface. *Plant, Cell and Environment*, *9*, 279–287.

Ooms, J., & Chamberlain, S. (2018). phylocomr: Interface to "Phylocom". R package version 0.1.2.

Ooms, J., & Chamberlain, S. (2019). phylocomr: Interface to 'Phylocom'. R package version 0.3.2. Retrieved from https://CRAN.R-project.org/package=phylocomr

O'sullivan, O. S., Heskel, M. A., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., Zhu, L., Egerton, J. J. G., Bloomfield, K. J., Creek, D., Bahar, N. H. A., Griffin, K. L., Hurry, V., Meir, P., Turnbull, M. H., & Atkin, O. K. (2017). Thermal limits of leaf metabolism across biomes. *Global Change Biology*, *23*(1), 209–223. https://doi.org/10.1111/gcb.13477

Paradis, E., & Schliep, K. (2018). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*: 526–528.

Perez, T. M., & Feeley, K. J. (2020). Photosynthetic heat tolerances and extreme leaf temperatures. *Functional Ecology*, https://doi.org/10.1111/1365-2435.13658

Perez, T. M., Valverde-Barrantes, O., Bravo, C., Taylor, T. C., Fadrique, B., Hogan, J. A., Pardo, C. J., Stroud, J. T., Baraloto, C., & Feeley, K. J. (2019). Botanic gardens are an untapped resource for studying the functional ecology of tropical plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1763), 20170390. https://doi.org/10.1098/rstb.2017.0390

Porch, T. G., & Hall, A. E. (2013). Heat tolerance. In C. Kole, & B. C. K. Viswavidyalaya (Eds.), *Genomics and breeding for climate resilient crops: Vol. 2 target traits* (pp. 167–202). Springer-Verlag. https://doi.org/10.1007/978-3-642-37048-9

R Core Team. 2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Schneider, H., Schuettpetz, E., Pryer, K. M., Cranfill, R., Magallón, S., & Lupia, R. (2003). Ferns diversified in the shadow of angiosperms. *Nature*, *428*, 553–557. https://doi.org/10.1029/2001GB001442

Slot, M., Krause, G. H., Krause, B., Hernández, G. G., & Winter, K. (2018). Photosynthetic heat tolerance of shade and sun leaves of three tropical tree species. *Photosynthesis Research*, *141*(1), 119–130. https://doi.org/10.1007/s11120-018-0563-3

Smillie, R. M., & Nott, R. (1979). Heat Injury in leaves of alpine, temperate and tropical plants. *Australian Journal of Plant Physiology*, *6*(1), 135–141. https://doi.org/10.1071/PP9790135

Subedi, S. C., Bhattarai, K. R., Perez, T. M., & Sah, J. P. (2020). Frontiers of Biogeography Gymnosperm species richness patterns along the elevational gradient and its comparison with other plant taxonomic groups in the Himalayas. *Frontiers of Biogeography*, *12*(1), 1–14. https://doi.org/10.21425/F5FBG44232

Swenson, N. G. (2014). *Functional and phylogenetic ecology*. Springer. https://doi.org/10.1007/978-1-4614-9542-0

Taylor, T. C., Smith, M. N., Slot, M., & Feeley, K. J. (2019). The capacity to emit isoprene differentiates the photosynthetic temperature responses of tropical plant species. *Plant, Cell & Environment*, *42*(8), 2448–2457. https://doi.org/10.1111/pce.13564

Valladares, F., & Pearcy, R. W. (1997). Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll Heteromeles arbutifolia. *Plant, Cell and Environment*, *20*(1), 25–36. https://doi.org/10.1046/j.1365-3040.1997.d01-8.x

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, *61*(3), 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Zellweger, F., De Frenne, P., Lenoir, J., Vangansbeke, P., Verheyen, K., Bernhardt-Römermann, M., Baeten, L., Hédl, R., Berki, I., Brunet, J., Van Calster, H., Chudomelová, M., Decocq, G., Dirnböck, T., & Tomasz Durak, D. C. (2020). Forest microclimate dynamics drive plant responses to warming. *Science*, *775*(May), 772–775.

Zhu, L., Bloomfield, K. J., Hocart, C. H., Egerton, J. J. G., O'Sullivan, O. S., Penillard, A., Weerasinghe, L. K., & Atkin, O. K. (2018). Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. *Plant Cell and Environment*, *41*(6), 1251–1262. https://doi.org/10.1111/pce.13133

Zsófi, Z., Váradi, G., Bálo, B., Marschall, M., Nagy, Z., & Dulai, S. (2009). Heat acclimation of grapevine leaf photosynthesis: Mezo- and macroclimatic aspects. *Functional Plant Biology*, *36*(4), 310–322. https://doi.org/10.1071/FP08200

BIOSKETCH

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Author contributions: T.M.P. conceived the ideas; T.M.P. collected the data; T.M.P. analysed the data; and T.M.P. and K.J.F. wrote the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Perez TM, Feeley KJ. Weak phylogenetic and climatic signals in plant heat tolerance. *J. Biogeogr.*2021;48:91–100. https://doi.org/10.1111/jbi.13984