Drivers of thermal tolerance breadth of plants across contrasting biomes: do mean or seasonality in climate indices matter more?

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Author Contributions

VFB, AMC, SKCJ, ABN, LABR and AL designed the study, PAA, RVG, VGB and AMC collated and analysed the data. All authors contributed to intellectual conceptualisation, data collection, interpretation of results, and writing. All authors meet all criteria for authorship.

Data availability statement

Data will be made available on the Dryad Digital Repository on acceptance of publication.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

Abstract

1. The Climate Variability Hypothesis (CVH) predicts that species from environments with more variable temperatures should have wide thermal tolerance breadth. We addressed this question in plants and asked which local climate predictors (including precipitation, mean and extreme temperatures and thermal variability) best predict species physiological thermal limits.
2. Measures of low ($T_{crit\text{-}cold}$) and high ($T_{crit\text{-}hot}$) photosystem II thermal tolerance thresholds were used to determine thermal tolerance breadth (TTB), along with ice nucleation temperature ($T_{nucleation}$, freezing tolerance) of 69 plant species sampled from three contrasting biomes: alpine, desert and wet temperate.
3. Thermal tolerance metrics $T_{crit\text{-}cold}$, $T_{nucleation}$, $T_{crit\text{-}hot}$ and TTB all differed among biomes. Notably, desert species had the most cold and heat tolerant leaves, and therefore the widest TTB, whereas species in alpine and temperate biomes had similar TTB. For plants in all biomes, TTB exceeded the thermal range of their local climate.
4. Overall, two Principal Component axes of local climate drivers explained substantial variation in all tolerance metrics. Extreme hot, dry climates improved freezing and heat tolerance. High thermal variability and low minimum temperatures also improved freezing tolerance, but were unrelated to heat tolerance or TTB. Species explained a significant amount of variation among all metrics, but this was not due to phylogenetic relatedness. A small portion of the variation in physiological thermal thresholds was explained by growth form. Unexplained variation could be due to microclimate-driven plasticity, leaf traits or thermoregulatory mechanisms.
5. **Synthesis**. Our results provide some support for the climate variability hypothesis in plants: photosynthetic thermal tolerance breadth was greatest in more thermally variable biomes.

This relationship was largely driven by cold tolerance, with variation in heat tolerance explained better by mean and extreme temperatures. Therefore, we conclude that, the CVH alone is not sufficient to explain variation in plant thermal tolerance, with many aspects of climate being potentially important drivers.

Introduction

Desert and alpine plant species live at the thermal limits of biological processes. Unlike those in temperate regions, plants living in extreme environments are exposed to both very low and very high temperatures and thus to a wide thermal range and highly variable thermal regimes, both among days and seasons. Given the important influence of temperature in shaping species distributions (Garcia-Pichel et al., 2013, Tattersall et al., 2012), the innate thermal tolerance thresholds (lowest and highest temperature that a species can withstand) and thermal tolerance breadths (range of temperature between upper and lower thermal tolerance thresholds) of species might be expected to reflect the temperature variation characterising their environment. The climate variability hypothesis (CVH; Janzen, 1967) predicts that species with *wide* thermal tolerance ranges are likely to evolve in environments with *variable* temperatures compared to thermal specialists with *narrow* tolerance ranges that evolved in thermally *stable* environments. This hypothesis is a cornerstone of thermal ecology, but to date has been primarily explored in animals (Addo-Bediako et al., 2000, Calosi et al., 2010, Compton et al., 2007, Baudier et al., 2018).

Cold and heat tolerance thresholds are rarely studied concomitantly. In natural systems, cold tolerance studies predominate in alpine environments where plants can withstand very low temperatures and tolerate extracellular ice formation and the resulting cytoplasmic dehydration (Sakai and Larcher, 1987, Larcher, 2003). Heat tolerance studies have historically been primarily focussed on warm and desert environments (with notable exceptions, e.g., Buchner et al., 2015).

In a range of desert species, heat tolerance has been associated with native microhabitat conditions (Curtis et al., 2016), although such differences are sometimes less marked under common garden conditions (Downton et al., 1984, Knight and Ackerly, 2002). Despite great progress, a recent systematic review revealed that less than 5% of more than 3000 studies examining thermal tolerance of plants considered both heat and cold tolerance thresholds together (Geange et al., 2021). This focus on either cold or heat tolerance overlooks the fact that plants in extreme biomes can experience both cold and heat stress. In addition, unlike in the animal kingdom, we lack an understanding of plant thermal tolerance breadth (TTB) based on concomitant measurements of heat and cold tolerance. The magnitude of TTB and the ecological drivers thereof are almost entirely unknown for plants.

Until recently, most research has focussed on agricultural species (Geange et al., 2021), limiting our understanding of plant thermal tolerance in a wider, ecological context. Temperature increases with climate change have motivated an increase in studies on plant heat tolerance, particularly in relation to climate variability and the occurrence of unseasonal extreme events (Rummukainen, 2012, Seneviratne et al., 2012). However, field sampling studies rarely encompass multiple biomes or a broad range of thermal environments across landscapes. There remains a critical gap in our understanding of how plants in extreme *vs* benign environments tolerate both heat and cold stress *in situ*, which limits our capacity to predict where the most thermally sensitive species occur.

Differing patterns of climate among contrasting biomes provide an opportunity to explore the role of climate variability in upper and lower thermal thresholds and on thermal tolerance breadth. Of the few studies that have compared thermal thresholds among biomes, Smillie and Nott (1979) found that heat tolerance was lower in alpine species, followed by temperate and tropical species. In a rare study considering both upper and lower thermal thresholds together, Cunningham and Read (2006) found that, in common garden conditions, temperate origin species have lower heat

72 and higher cold tolerance than tropical species. Meta-analytical studies across a wide range of
73 species and biomes suggest that heat tolerance decreases and cold tolerance increases with latitude;
74 however, these shifts in tolerance do not keep pace with an observed latitudinal temperature
75 gradient (Lancaster and Humphreys, 2020). Further, predictors of thermal tolerance variation with
76 latitude may include mean maximum temperature of the warmest month and mean annual
77 temperature (O'Sullivan et al., 2017, Lancaster and Humphreys, 2020), but threshold-based
78 predictors (e.g. number of days over 35˚C per year; Maher et al., 2019) are suggested to be drivers
79 of heat tolerance (Mora et al., 2015). Given that water availability influences plant heat tolerance
80 (Curtis et al., 2016, Marchin et al., 2022) and freezing tolerance (Sierra-Almeida et al., 2016),
81 precipitation parameters are also likely to affect thermal thresholds. Further, upper and lower
82 thermal thresholds can vary in relation to the methods used (Lancaster and Humphreys, 2020,
83 PerezFeeley et al., 2021), impairing the capacity for large-scale meta-analyses to capture such
84 sources of variation and limiting their power for generalisable conclusions. Finally, although we
85 have evidence that climate variables influence plant heat and cold thermal tolerance thresholds
86 independently, the climate predictors of thermal tolerance breadth, are virtually unknown. If we
87 can reveal general patterns underlying variation in, and the climate drivers of, physiological
88 thermal tolerance thresholds among co-occurring plant species within and among biomes, we can
89 strengthen predictions of which and where species are likely to be most vulnerable to changing
90 thermal conditions.

91 Here we sought to determine the climate drivers of leaf-level physiological thermal tolerance
92 thresholds and thermal tolerance breadth of plant species in extreme and benign environments in
93 the context of the climate variability hypothesis. We used chlorophyll fluorescence to determine
94 the low and high photosystem II (PSII) thermal tolerance thresholds and TTB of 22 alpine, 24
95 desert and 23 wet temperate rainforest species (temperate hereafter) during the growing season.

We also determined ice nucleation temperatures to examine freezing tolerance in all species. To examine the relationships between climate variability and physiological thresholds, we extracted long-term, locally downscaled climate parameters for each sampling locality. Aim 1 was to determine how the thermal tolerance thresholds and TTB of a broad range of plant species differed among the contrasting biomes. We hypothesised that thermal thresholds would be skewed towards the extremes of the biomes; colder threshold temperatures in the alpine and hotter in the desert, with temperate species having milder cold and heat tolerance thresholds. Aim 2 was to test whether species from more the thermally variable alpine and desert biomes would have greater tolerance to both heat and cold, and thus wider thermal tolerance breadth than temperate biome species, in accordance with the CVH. Aim 3 was to assess whether variation in thermal tolerance thresholds and TTB could be explained by local climate variables, and whether the relationships were linear or not. We hypothesised that climate extremes and variability would have a stronger association with thermal tolerance thresholds and TTB than mean climate values, supporting the CVH.

Materials and Methods

Study locations

Sites used in this study were designated to one of three biomes in New South Wales, Australia: alpine (Monaro Ngarigo, Ngunnawal, Wiradjuri and Walgalu Country, Kosciuszko National Park), desert (Ngemba and Barkindji Country, Gundabooka National Park), and temperate (wet temperate rainforests of Dharawal, Wadi Wadi and Yuin Country in the Illawarra Region: Royal National Park, Illawarra State Conservation Area and Bass Point Conservation Area). Sampling sites incorporated a range of vegetation types sampled in each biome (mean climatic details from sites are inset in Fig. 1).

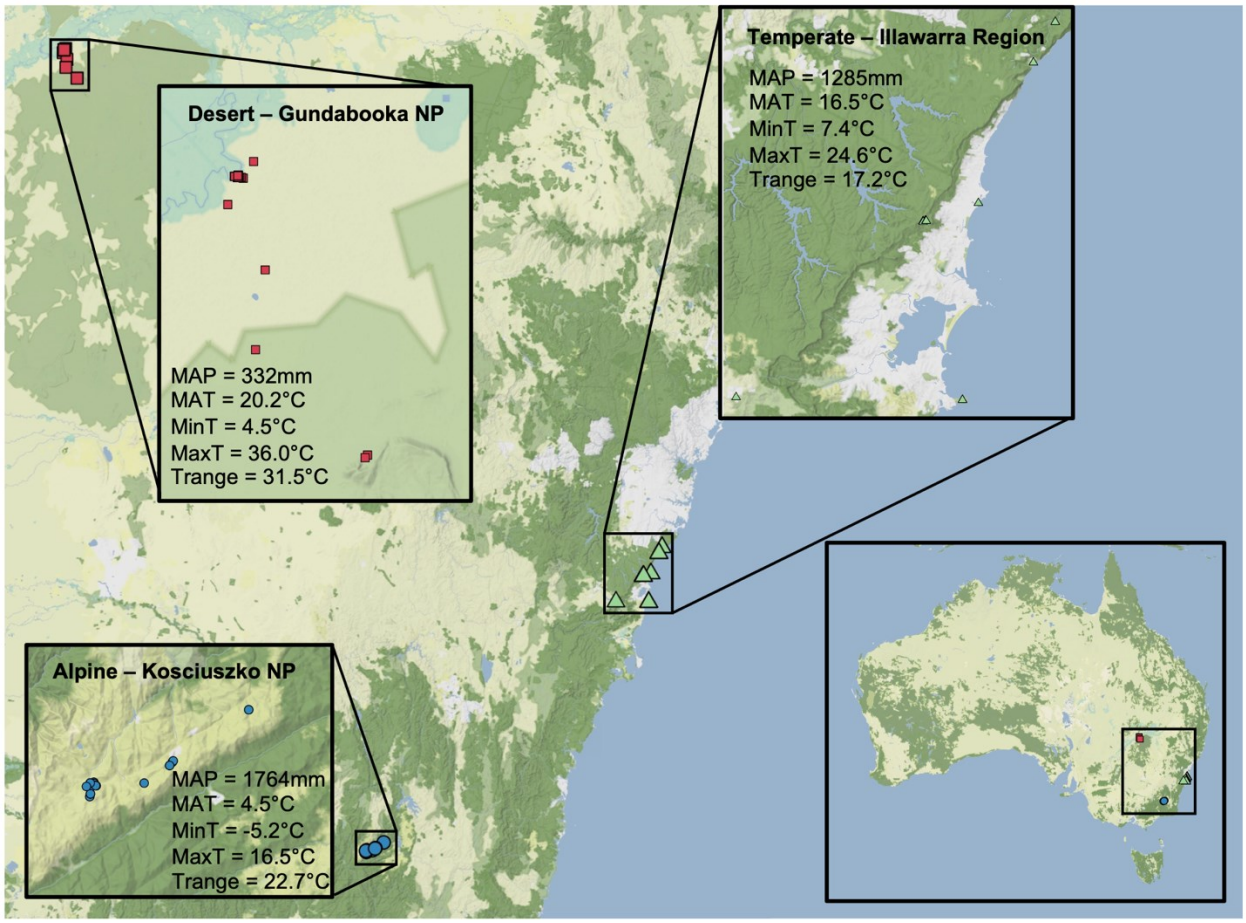


Fig. 1. Locations of study sites across three biomes in NSW, Australia. Desert sites (red) in Gundabooka National Park (GNP), wet temperate rainforest sites (green) in the Illawarra Region (temperate), and alpine sites (blue) in Kosciuszko (KNP). GNP sites spanned open eucalypt woodland, mulga and chenopod shrublands between elevations of 100 to 224 m a.s.l. KNP sites encompassed feldmark, herbfields and sphagnum bog vegetation formations between 1792 and 1855 m a.s.l. In the temperate environments, collections were conducted across the Illawarra, including the tall open forest and rainforest communities between 100 to 400 m. a.s.l. MAP = mean annual precipitation, MAT = mean annual temperature, MinT = mean minimum temperature of the coldest month, MaxT = mean maximum temperature of the warmest month, T_{range} = MaxT - MinT).

Species selection

A total of 69 species (22 alpine, 24 desert and 23 temperate species) were selected to encompass a representative sample of growth forms and plant families and where possible, included

133 congeneric and/or con-familial species between two or more biomes (Table S1). We included
134 community dominants and a range of less abundant species to represent a cross section of species
135 occupying each biome. Species replicates were sampled across days within each sampling period
136 to incorporate daily variation in thermal tolerance thresholds.

137 ***Leaf sample collection***

138 Mature, fully expanded leaves were harvested from five to seven individuals per species. For
139 shrubs and trees, small branches were collected from the northern facing side of the plant at a
140 height of 1-1.5 m. For smaller growth forms, entire individuals or rosettes were collected. Samples
141 were kept in cool sealed plastic bags with moistened paper towel until they were processed
142 (approximately 1-2 h after collection). In the field laboratory, leaves (size ranging 0.25 cm^2 to
143 5 cm^2) were cut (or combined) to fit an approximate area of 1 cm^2 and kept on wet florist foam in
144 humid dark containers until thermal tolerance assays were performed. Measurements occurred
145 during January 2020, March 2020 and December 2020 for the desert, temperate and alpine biomes,
146 respectively. Sampling periods were selected to capture peak growing season in each region.

147 **Thermal tolerance threshold assays**

148 The photosynthetic machinery, specifically photosystem II (PSII), embedded in the thylakoid
149 membrane has long been recognised as a thermally sensitive structure (Schreiber and Berry, 1977,
150 Berry and Bjorkman, 1980, Seemann et al., 1984). The temperature at which the PSII are disrupted
151 causes a rise in minimal chlorophyll *a* fluorescence (F_0) as a leaf is heated or cooled (Schreiber
152 and Berry, 1977, Neuner and Pramsohler, 2006). This critical temperature indicates a threshold
153 beyond which physiological and photochemical systems become increasingly impaired and
154 damage can occur if temperatures are sustained.

155 ***Lower and upper thresholds of PSII ($T_{crit\text{-}cold}$ and $T_{crit\text{-}hot}$)***

156 The critical low and high temperature thresholds of PSII ($T_{crit\text{-}cold}$ and $T_{crit\text{-}hot}$) were measured using
157 T-F_0 curves, i.e., the rise in F_0 (Neuner and Pramsohler, 2006, Arnold et al., 2021) with set heating
158 or cooling rates. One leaf per individual (or several small leaves abutting one another) was used
159 for each of the heating and cooling assays with n = 5-7 replicates per species. For each assay run,
160 45 samples were secured with double-sided tape to paper on a Peltier plate (CP-121HT; TE-
161 Technology, Inc., Michigan, USA), used to cool or heat samples at a set rate using LabView-based
162 control software (National Instruments, Texas, USA) and adapted code from TE-Technology, Inc.
163 A vacuum sealed double-glazed glass was placed on top of the leaf samples on the plate to avoid
164 water condensation, particularly at freezing temperatures, and compress samples to ensure
165 maximum leaf contact with the Peltier plate (see Arnold et al., 2021 for more details).

166 Chlorophyll fluorescence was measured with a Pulse Amplitude Modulated (PAM) chlorophyll
167 fluorescence imaging system (Maxi-Imaging-PAM; Heinz Walz GmbH, Effeltrich, Germany)
168 mounted above the Peltier plate. Leaves were first dark-adapted, and the PSII photochemistry
169 maximum quantum efficiency (F_V/F_M) measured to check starting function of leaves before
170 another dark adaption for 15min prior to assay. Circular areas of interest were created in the middle
171 of leaf samples avoiding edges for F_0 measurements using the Maxi-Imaging-PAM software.
172 During temperature ramping, F_0 was measured every 20 s with a weak blue low frequency (1 Hz)
173 pulse modulated measuring light (0.5 µmol photons m^{-2} s^{-1}). Leaf temperatures were
174 simultaneously measured with 40-gauge type-T thermocouples (OMEGA Engineering, Singapore)
175 attached to undersides of leaves and logged with a multi-channel DT85 Datataker datalogger
176 (Lontek, Australia). For hot T-F_0 measurements, samples were heated to 60 °C at a rate of 30 °C
177 h^{-1} (0.5 °C min^{-1}). For cold T-F_0 measurements, the leaves were cooled down to -20 °C or -25 °C
178 when logistically possible, at a rate of 15 °C h^{-1} (0.25 °C min^{-1}). Temperature rates were selected
179 as moderate temperature changes following Arnold et al. (2021). The starting temperature of each

heating/cooling assay depended on average field lab temperature, generally 15 °C for alpine and temperate, and 20 °C for desert measurements.

$T_{crit\text{-}cold}$ and $T_{crit\text{-}hot}$ were determined as inflection points on the T-F_0 curves, using the *segmented* R package (Muggeo, 2017) (code available from https://github.com/pieterarnold/Tcrit-extraction). For further description of T-F_0 curve see Arnold et al. (2021). $T_{crit\text{-}cold}$ and $T_{crit\text{-}hot}$ were used to represent the lower and upper bounds of the PSII thermal tolerance breadth, TTB (TTB = $T_{crit\text{-}hot}$ - $T_{crit\text{-}cold}$).

Ice nucleation temperatures

Freezing tolerance is tied to the temperatures at which ice formation occurs, the ice nucleation temperature ($T_{nucleation}$). Measuring $T_{nucleation}$ is required to understand whether temperature-dependent damage to photosynthetic machinery occurs in association with ice formation. $T_{nucleation}$ was estimated from the leaf temperature data collected during $T_{crit\text{-}cold}$ measurements, where freezing exotherms can be identified visually as small increases in temperature when leaf temperature for each sample is plotted against time. The temperature at which the exotherm began was recorded as $T_{nucleation}$ (Larcher, 2003).

Local climate variables for sampling locations

Climate data were obtained from gridded datasets at 1 km resolution based on spatial interpolations of long-term (1981-2010) conditions accessed from the CHELSA v2.1 database (Karger et al., 2017). For each species, we extracted the following local climate variables for each species at leaf collection sites: mean annual precipitation (MAP), mean annual temperature (MAT), mean minimum temperature of the coldest month (MinT), mean maximum temperature of the warmest month (MaxT), and calculated the annual thermal range (T_{range} = MaxT - MinT). Table S2 includes a complete list of species and sampling locations, which are also shown on Fig. 1.

Statistical analyses

All analyses and data visualisations were conducted in the R Environment for Statistical Computing version 4.3.1 (R Core Team, 2023). To address aims 1 and 2, we calculated summary statistics (means and standard errors) for each thermal tolerance threshold metric by biome and species to evaluate their differences. Pairwise Tukey's Honest Significant Differences were used to compare means among biomes. To address aim 3, we first generated a correlation matrix for the five key local climate variables (MAP, MAT, MinT, MaxT, T_{range}) using the R package *corrplot* (Wei and Simko, 2021). All local climate variables were moderately to strongly correlated with each other (Fig. 1A); therefore, we elected to conduct a Principal Components Analysis (PCA) with the variables. The first two Principal Component axes (PC1 and PC2, respectively) explained 99.6% of the variation among the five climate variables (Table 1), therefore we used these two axes as local climate variables in analyses.

Prior to model fitting, we assessed if the shared evolutionary history across our 69 species may also explain differences in thermal tolerance thresholds. We generated a phylogenetic tree using the R package *V.phylomaker* (Jin and Qian, 2022) and calculated the phylogenetic signal and statistical significance for $T_{crit\text{-}hot}$, $T_{crit\text{-}cold}$, $T_{nucleation}$ and TTB using four methods: Albouheif's C_{mean}, Moran's I, Blomberg's K and Pagel's λ using the *phylosignal* package (Keck et al., 2016).

We analysed our thermal tolerance threshold data using linear mixed effects regression models with correlated random effects structure using the *Almer* function from the *evolvability* R package (Bolstad et al., 2014). The *Almer* function is built on the *lmer* function from *lme4* (Bates et al., 2015) but incorporates a correlated random effects structure A, which we used to fit a phylogenetic variance-covariance matrix to account for phylogenetic relatedness among species. For each response variable ($T_{crit\text{-}hot}$, $T_{crit\text{-}cold}$, $T_{nucleation}$ and TTB), we fit four candidate models that included

226 fixed effects of both climate variables, PC1 and PC2, as either linear or second-degree (quadratic)
227 polynomial terms in combination (i.e., fixed effects in model 1 = PC1 + PC2; model 2 = PC1 +
228 $PC1^2$ + PC2; model 3 = PC1 + PC2 + $PC2^2$; model 4 = PC1 + $PC1^2$ + PC2 + $PC2^2$). Each candidate
229 model included three random effects: species (69 levels), growth form (10 levels), and a structured
230 phylogenetic matrix. Variance explained by differences among species was therefore separated
231 from phylogenetic relatedness. We calculated Akaike's Information Criterion (AIC) values for
232 each model fit by maximum likelihood to allow for model comparisons, and then calculated
233 Akaike's weights using the *MuMIn* R package (Bartoń, 2023) to identify the most parsimonious
234 model for each thermal tolerance threshold response variable. The final model was refit with
235 restricted estimate maximum likelihood and its parameters reported, including R^2_{GLMM} values
236 (Johnson, 2014) to calculate the variance explained by fixed effects (marginal R^2; mR^2) and the
237 variance explained by both fixed and random effects (conditional R^2; cR^2).

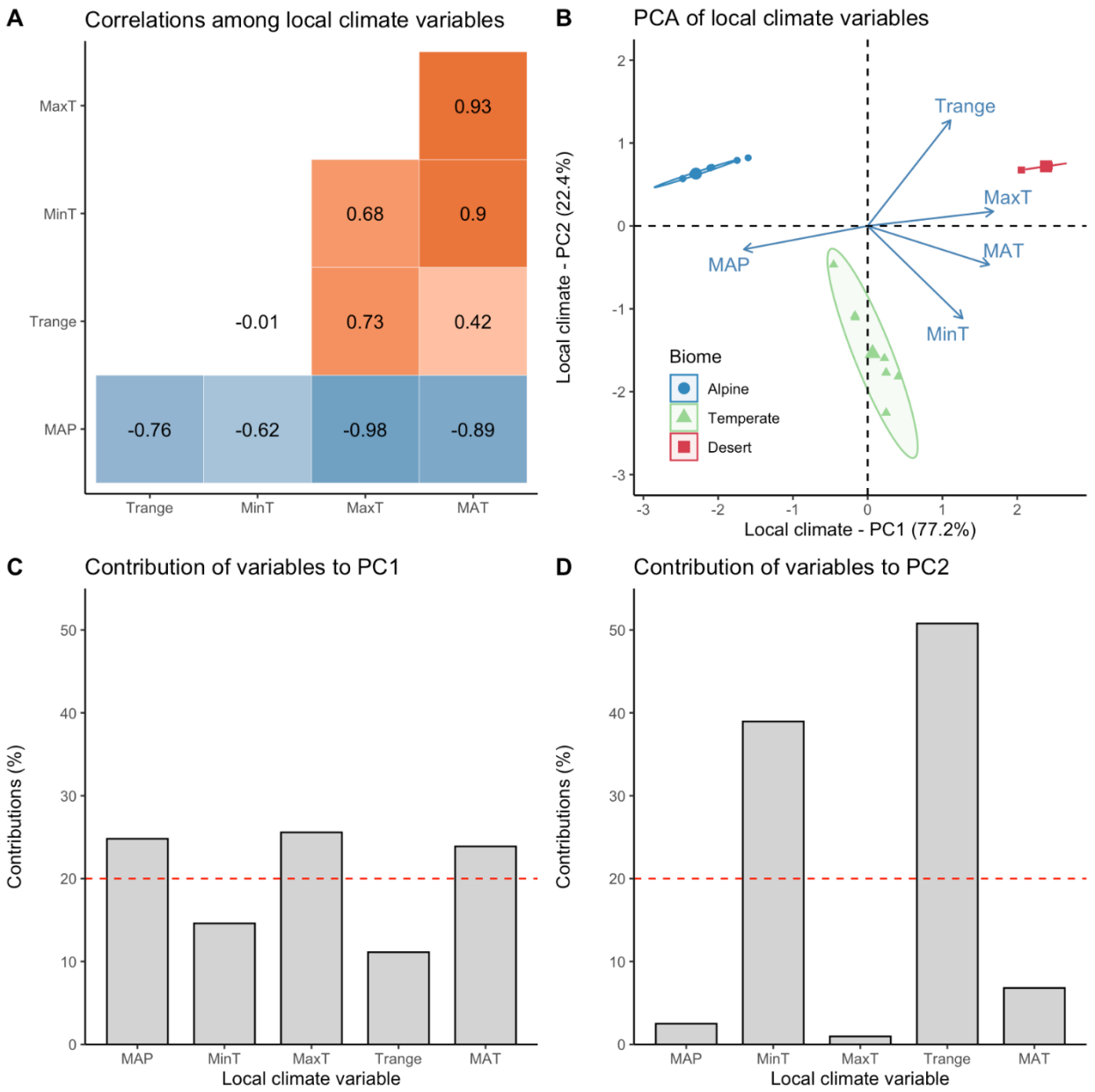
238

239 **Results**

240 **Local climate analysis**

241 Local climate variables were strongly correlated with each other (Fig. 2A) and PCA described
242 99.6% of the climate space among the three biomes along two axes (Fig. 2B). PC1 explained
243 77.2% of the variation among the five climate variables and was strongly loaded positively by
244 MAP and negatively by MAT and MaxT (Fig. 2C). Negative PC1 values can be reasonably
245 interpreted as cooler and wetter climate, while positive PC1 values indicate a hotter (mean and
246 extreme temperatures) and drier climate (Fig. 2B). PC2 explained 22.4% of the variation among
247 the five climate variables and was strongly loaded positively by T_{range} and negatively by MinT
248 (Fig. 2D). Positive PC2 values can be reasonably interpreted as a climate with colder extremes and

249 greater range in thermal extremes across the year, while negative PC2 values indicate a climate
250 with less extreme cold temperatures and greater thermal stability through the year (Fig. 2B).



251

252 **Fig. 2.** Correlations and Principal Component (PC) composition of local climate variables (MAP
253 = mean annual precipitation, MAT = mean annual temperature, MinT = mean minimum
254 temperature of the coldest month, MaxT = mean maximum temperature of the warmest month,
255 T_{range} = annual thermal range (MaxT - MinT)). (A) Correlation matrix of local climate variables,
256 (B) Principal Components Analysis biplot of PC1 and PC2 showing biome position in climate PC
257 space, contribution of individual local climate variables to (C) PC1 and (D) PC2.

Biome differences in thermal tolerance

There were clear differences among biomes for all thermal tolerance thresholds (Fig. 3A-C). Unexpectedly, desert species exhibited the most extreme cold thresholds $T_{crit\text{-}cold}$ (-13.3 ± 0.3 °C), followed by alpine (-10.9 ± 0.4 °C) and then temperate species (-8.8 ± 0.3 °C), all of which differed significantly from one another (Fig. 3A). Similar to the response of $T_{crit\text{-}cold}$, $T_{nucleation}$ values were lowest in desert species (-16.1 ± 0.4 °C), followed by alpine (-10.8 ± 0.5 °C) and warmest in temperate species (-7.5 ± 0.4 °C); again, these differed significantly from one another (Fig. 3B). Interestingly, $T_{crit\text{-}cold}$ values for alpine and temperate species were very close to their $T_{nucleation}$, whereas in desert species, ice formation occurred at markedly lower temperatures than $T_{crit\text{-}cold}$ (Fig. 3A-B). As predicted, desert species had significantly higher heat tolerance $T_{crit\text{-}hot}$ (49.3 ± 0.5 °C), followed by temperate (46.5 ± 0.4 °C) and then alpine (43.1 ± 0.4 °C) species (Fig. 3C). Thermal tolerance breadth (TTB) was significantly wider in desert species (62.5 ± 0.6 °C) than temperate (55.3 ± 0.5 °C) or alpine species (53.9 ± 0.6 °C), but alpine and temperate species had similar TTB (Fig. 3D).

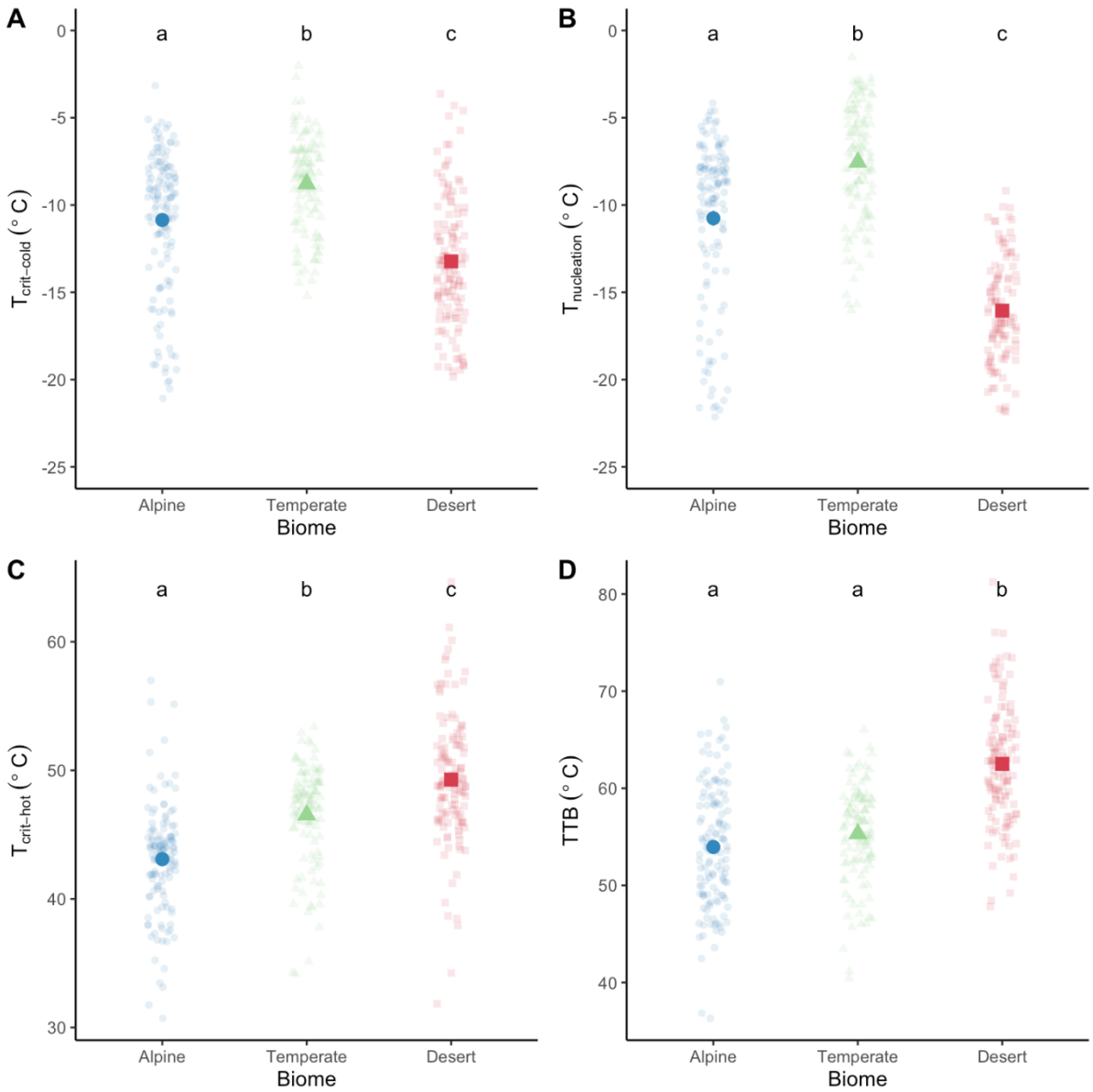


Fig. 3. Thermal tolerance thresholds and breadth across the three biomes. (A) Cold tolerance threshold ($T_{crit-cold}$), (B) ice nucleation temperature ($T_{nucleation}$), (C) heat tolerance threshold ($T_{crit-hot}$) and (D) thermal tolerance breadth (TTB = $T_{crit-hot}$ - $T_{crit-cold}$), where desert has n = 24 species, temperate has n = 23 species and alpine has n = 22 species. Small transparent points represent individual replicates and large solid points represent biome means ± SE. Lowercase letters inset in each plot denote significant differences among biomes from post-hoc Tukey's Honest Significant Differences tests within each thermal tolerance metric.

Species clearly differed in their tolerance thresholds, where the most and least tolerant species aligned with the respective characteristics of their biome. The temperate species *Oplismenus hirtellus* was the least cold tolerant ($T_{crit\text{-}cold}$ = -4.0 ºC), whereas two alpine species, *Epacris paludosa* and *Hovea montana*, were most cold tolerant ($T_{crit\text{-}cold}$ = -18.5 ºC) and the desert species *Atriplex stipitata* had the most extreme ice nucleation temperature ($T_{nucleation}$ = -20.5 °C). The alpine species *Pimelea ligustrina* was the least heat tolerant ($T_{crit\text{-}hot}$ = 38.1 ºC) and the desert species *Rhagodia spinescens* was the most heat tolerant ($T_{crit\text{-}hot}$ = 54.9 ºC). A full list of species and their individual thermal tolerance thresholds and breadth is presented in Table S1. There was considerable variation among species within biomes (Fig. 4). Within the desert biome, TTB was more similar across species (lower variability; TTB range = 56.2 – 70.3 °C; Δ14.1 °C), compared to the alpine biome, which had the greatest variation among species (TTB range = 45.9 – 63.1 °C; Δ17.2 °C), followed by the temperate biome (TTB range = 44.8 – 60.4 °C; Δ15.6 °C). The rank order of species' TTB from widest to narrowest generally reflects their biome, where 17 of the top 20 widest TTBs were all desert species (Fig. 4). By contrast, 10 of the 20 narrowest TTBs were alpine species, and temperate species had generally moderate to narrow TTB (Fig. 4).

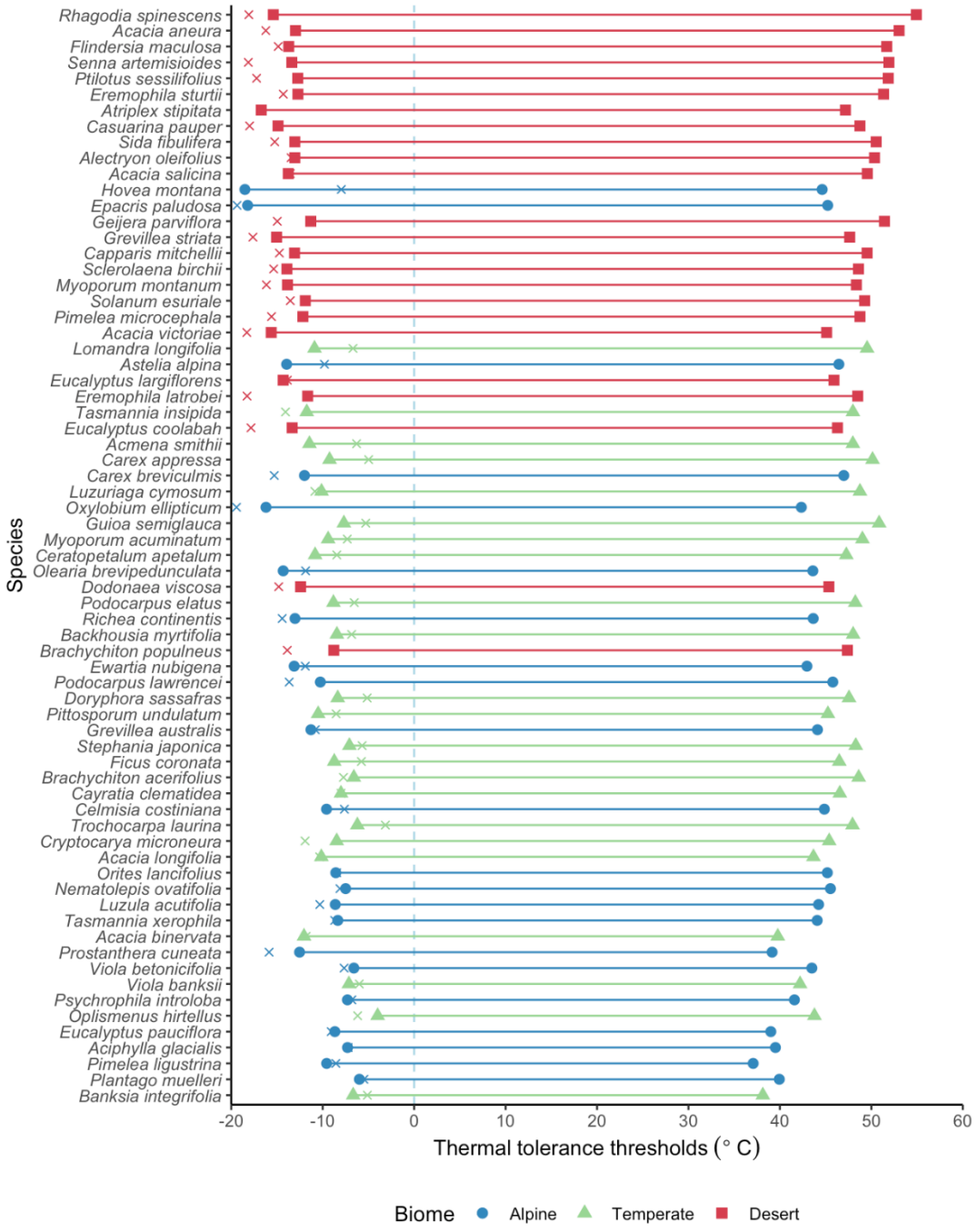


Fig. 4. Thermal tolerance breadth (TTB = $T_{crit\text{-}hot}$-$T_{crit\text{-}cold}$) for 69 species across three biomes. Species are sorted top to bottom from widest to narrowest TTB, with lines connecting the mean $T_{crit\text{-}cold}$ to the mean $T_{crit\text{-}hot}$ for each species (length of line = TTB). Red squares and lines represent desert species (*n* = 24 species), green triangles and lines represent temperate species (*n* = 23 species) and blue circles and lines represent alpine species (*n* = 22 species). Cross symbols (×) represent the mean $T_{nucleation}$ for each species. The light blue vertical dashed line represents 0 °C. Each species has *n* = 5-6 replicates for each thermal tolerance threshold value.

Consistent across all three biomes, TTB was much wider than the extreme temperature range calculated for the sampling sites; thus, most thermal tolerance thresholds extended well beyond the minimum and maximum temperatures expected for each species in their local environment. The only notable exception was for the alpine biome species, where the cold threshold was closer to the minimum temperatures representing our alpine sites (Fig. 5). Notably, TTB was wider in species from desert biomes, which are exposed to considerably greater seasonality and extremes, higher MAT, MaxT and wider T_{range} than those in alpine and temperate environments (Fig. 5).

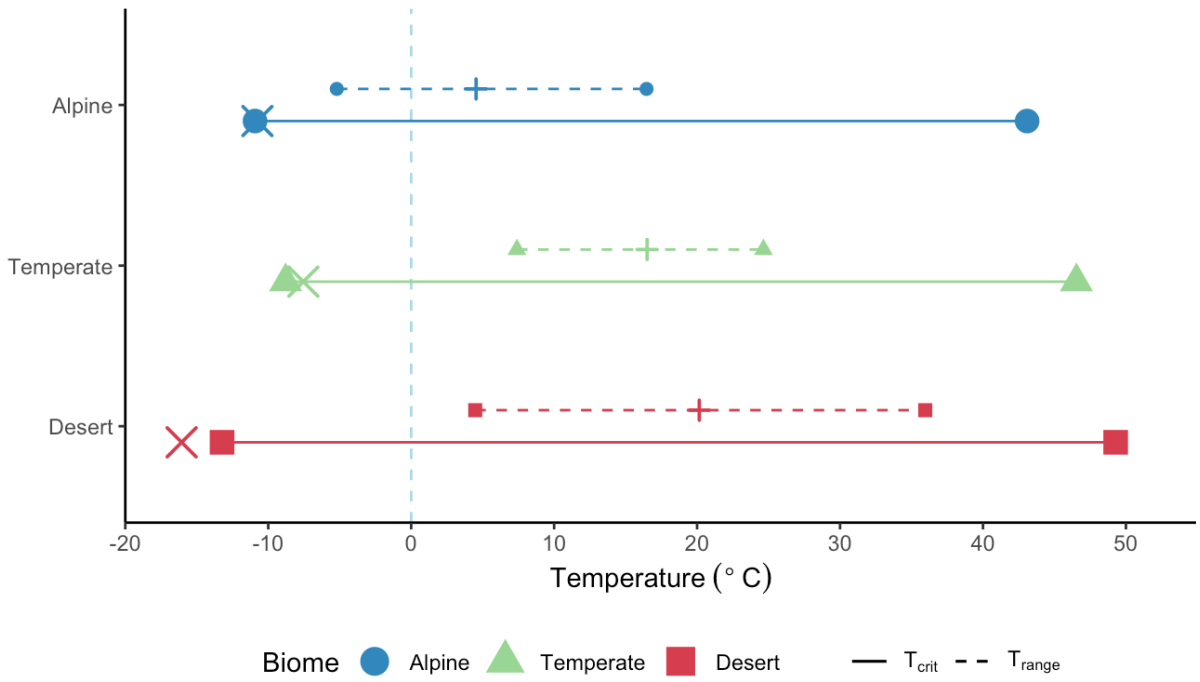


Fig. 5. Mean thermal tolerance breadth (TTB = $T_{crit\text{-}hot}$ -$T_{crit\text{-}cold}$) for the alpine, temperate and desert biomes, relative to the mean thermal range of the local environments (T_{range} = MaxT - MinT). Red squares and lines represent desert, green triangles and lines represent temperate and blue circles and lines represent alpine biomes. Cross (×) symbols represent the mean $T_{nucleation}$ and plus (+) symbols represent the mean annual temperature for each biome. Solid lines connect the mean $T_{crit\text{-}cold}$ to the mean $T_{crit\text{-}hot}$ (large symbols) for each biome (length of line = TTB). Dashed lines connect the mean MinT to the mean MaxT (small symbols) for each biome (length of line = T_{range}). The light blue vertical dashed line represents 0 °C. Each species has n = 5-6 replicates for each thermal tolerance threshold value.

318 **Local climate drivers of variation in thermal tolerance thresholds and breadth**

319 We investigated the relationships between the Principal Components of climate variables at the
320 local site of plant sampling and each of the thermal tolerance thresholds. $T_{crit\text{-}cold}$ had a significant
321 non-linear relationship with PC1 but no significant relationship with PC2 (Table 1, Fig. 6A, B).
322 Cold tolerance was greatest in plants from both ends of the PC1 axis: the extreme cold and wet
323 (alpine) as well as the hot and dry (desert), with less cold tolerant plants in between (temperate).
324 Ice nucleation temperature had a significant linear relationship with PC1 and significant non-linear
325 relationship with PC2 (Table 1, Fig. 6C, D); $T_{nucleation}$ was greatest in plants from the hottest and
326 driest climate, which worsened in increasingly cool and wet climates (Fig. 6C). Desert species had
327 consistently greater freezing tolerance, which drove the $T_{nucleation}$ relationship with PC1 to be more
328 linear than it was for $T_{crit\text{-}cold}$ (Fig. 6A, C). Freezing tolerance was worse in plants from thermally
329 stable and less extreme climates (temperate) but increased sharply toward climates with colder
330 extremes and greater range in thermal extremes across the year (Fig. 6D). Species from
331 environments with greater seasonality therefore froze at lower temperatures than species in
332 environments where temperature is more stable (Fig. 6D).

333 Heat tolerance had a significant linear relationship with PC1 but not PC2 (Table 1); $T_{crit\text{-}hot}$ was
334 highest in plants from hot, dry climate, and declined significantly as climate become increasingly
335 cool and wet (Fig. 6E), but it was unrelated to PC2 (thermal range and minimum temperature; Fig.
336 6F). Thermal tolerance breadth had a significant non-linear relationship with PC1 but was not
337 associated with PC2 (Table 1, Fig. 6G-H); TTB was highest in hot, dry climates and it declined
338 non-linearly as climates became cooler and wetter, where TTB was not different between alpine
339 and temperate species (Fig. 3D and 6G). For all thermal tolerance thresholds, among-species
340 differences explained a substantial proportion of variance ($T_{crit\text{-}cold}$ = 27.7%, $T_{nucleation}$ = 46.5%,
341 $T_{crit\text{-}hot}$ = 24.7%, TTB = 26.8%). Among-species differences were not attributable to phylogenetic

342 relatedness. Only $T_{crit\text{-}cold}$ was non-zero with phylogeny, explaining just 0.1% of variance
343 (Table 1), and all four phylogenetic signal metrics consistently identified no evidence of
344 phylogenetic signal in the thermal tolerance thresholds or breadth (Table S4). Plant growth form
345 explained additional variance, which was greatest for TTB ($T_{crit\text{-}cold}$ = 8.9%, $T_{nucleation}$ = 4.0%, $T_{crit\text{-}hot}$ = 1.6%, TTB = 9.6%).

347

348 **Table 1.** Model output summaries for Principal Components (PC1 and PC2; see Fig. 2) of local
349 climate drivers of physiological thermal thresholds.

				$T_{crit\text{-}cold}$ (°C)			
Fixed	**Coefficient ± SE**	***t-value***	***p-value***	**Random**	**Coefficient**	***R^2*** *	***R^2***
Intercept	-10.586 ± 1.241	-8.529	**<0.001**	Growth form	1.042	mR^2	0.206
PC1	-16.605 ± 6.603	-2.515	**0.012**	Species	1.836	cR^2	0.498
PC2	-36.351 ± 15.670	-2.320	**0.021**	Phylogeny	0.092	cR^2 - mR^2	0.292
$PC2^2$	0.349 ± 0.785	0.445	0.657	Residual	2.772		
				$T_{nucleation}$ (°C)			
Fixed	**Coefficient ± SE**	***t-value***	***p-value***	**Random**	**Coefficient**	***R^2***	***R^2***
Intercept	-11.504 ± 0.484	-23.745	**<0.001**	Growth form	0.762	mR^2	0.444
PC1	-0.970 ± 0.200	-4.840	**<0.001**	Species	2.595	cR^2	0.725
PC2	-49.776 ± 7.001	-7.110	**<0.001**	Phylogeny	0.000	cR^2 - mR^2	0.281
$PC2^2$	-15.997 ± 6.578	-2.432	**0.016**	Residual	2.674		
				$T_{crit\text{-}hot}$ (°C)			
Fixed	**Coefficient ± SE**	***t-value***	***p-value***	**Random**	**Coefficient**	***R^2***	***R^2***
Intercept	46.371 ± 0.427	108.578	**<0.001**	Growth form	0.568	mR^2	0.251
PC1	1.319 ± 0.186	7.110	**<0.001**	Species	2.231	cR^2	0.448
PC2	-0.172 ± 0.330	-0.520	0.603	Phylogeny	0.000	cR^2 - mR^2	0.197
				Residual	3.855		
				TTB (°C)			
Fixed	**Coefficient ± SE**	***t-value***	***p-value***	**Random**	**Coefficient**	***R^2***	***R^2***
Intercept	57.461 ± 0.894	64.268	**<0.001**	Growth form	1.906	mR^2	0.276
PC1	65.669 ± 10.560	6.218	**<0.001**	Species	3.187	cR^2	0.548
$PC1^2$	48.643 ± 24.509	1.985	**0.048**	Phylogeny	0.000	cR^2 - mR^2	0.272
PC2	-1.070 ± 1.223	-0.875	0.382	Residual	4.788		

350 *mR^2 represents the variance explained by all fixed effects and cR^2 - mR^2 represents the variance
351 explained by all random effects.

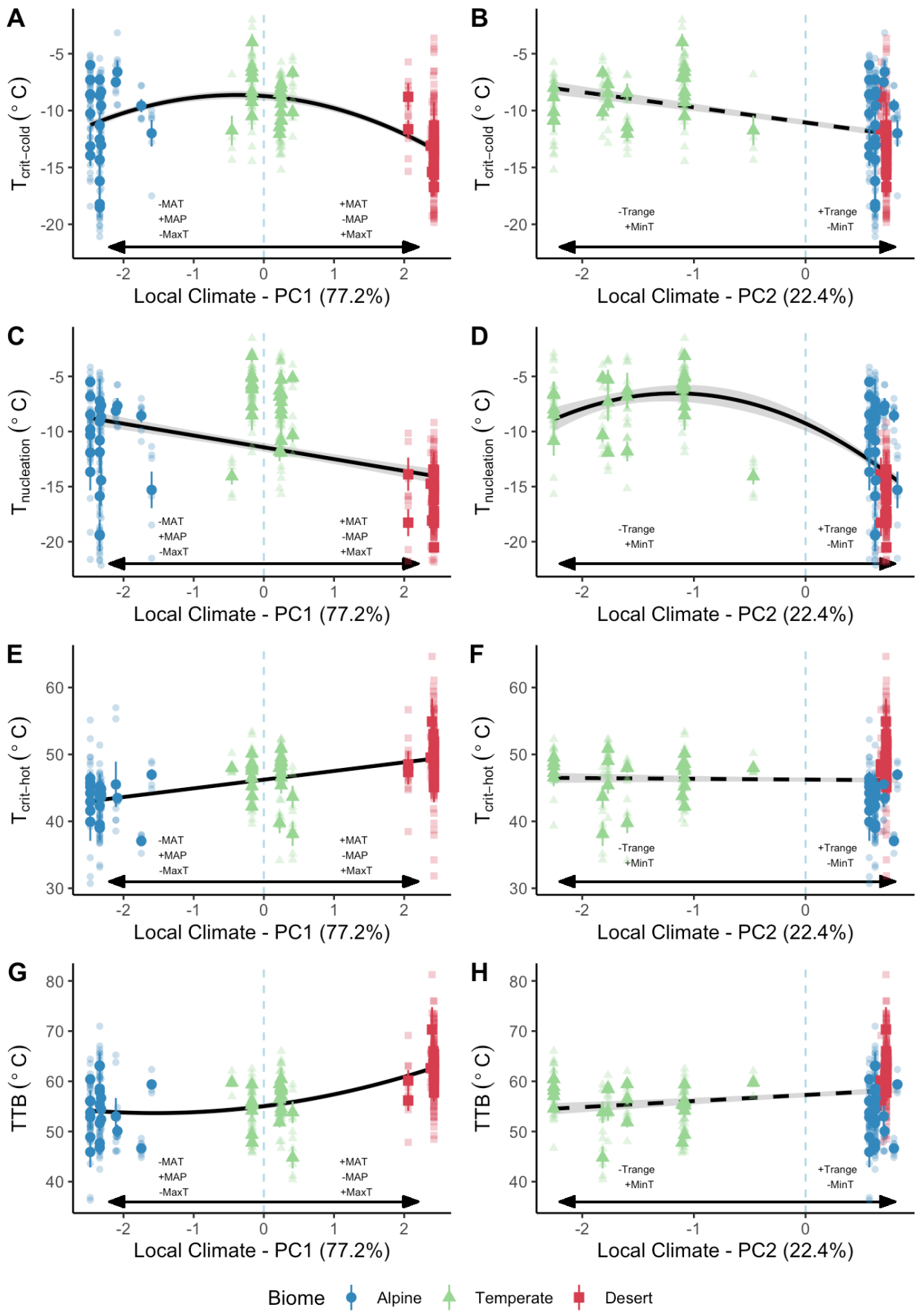

A
B
C
D
E
F
G
H
$T_{crit-cold}$ (° C)
$T_{nucleation}$ (° C)
$T_{crit-hot}$ (° C)
TTB (° C)
-MAT
+MAP
-MaxT
+MAT
-MAP
+MaxT
-Trange
+MinT
+Trange
-MinT
Local Climate - PC1 (77.2%)
Local Climate - PC2 (22.4%)
Biome
Alpine
Temperate
Desert

Fig. 6. Local climate variables (Principal Components, PC1 and PC2; see Fig. 2) that predict variation in thermal tolerance thresholds across all biomes. Regressions between (A) $T_{crit\text{-}cold}$ and PC1, (B) $T_{crit\text{-}cold}$ and PC2, (C) $T_{nucleation}$ and PC1, (D) $T_{nucleation}$ and PC2, (E) $T_{crit\text{-}hot}$ and PC1, (F) $T_{crit\text{-}hot}$ and PC2, (G) TTB and PC1 and (H) TTB and PC2. Arrows and annotations indicate climate variable loadings on PC1 and PC2 axes: positive PC1 values = hotter and drier climate (+MAT, -MAP, +MaxT); negative PC1 values = cooler and wetter climate (-MAT, +MAP, -MaxT). Positive PC2 values = greater range in thermal extremes across the year and colder extremes (+T_{range}, -MinT) negative PC2 values = greater annual thermal stability and less extreme cold temperatures (-T_{range}, +MinT), see Fig. 2 and Table S3. Large solid points are species-level means ± SE and small transparent points are individual replicates. Points are coloured by biome and regressions are predicted model fits ± SE from the best models reported in Table 1, where solid regression lines represent significant relationships.

Discussion

The climate variability hypothesis predicts that species will evolve wider thermal tolerance breadths in environments with more variable temperatures and be selected for thermal specialisation (narrow TTB) in thermally stable environments, a concept largely supported in animals (i.e., Compton et al., 2007, Sunday et al., 2011). In plants, a recent study found that the TTB of alpine species in temperate latitudes was not generally wider than the TTB of tropical latitude species (though not all these measurements were conducted concomitantly or with the same methods) (Sklenář et al., 2023). Other studies exploring CVH in plants have investigated phenotypic plasticity increase with latitude (Molina-Montenegro and Naya, 2012), species distribution range with seasonality across elevation (Mumladze et al., 2017) and photosynthetic strategy with environmental temperature variability (Perez et al., 2023). Here we explored the CVH for plants by measuring photosynthetic heat and cold tolerance thresholds concurrently to determine TTB of species growing in two extreme and more thermally variable (desert and alpine), one benign and more thermally stable (temperate).

380 Based on the CVH framework, we hypothesised that the thermal tolerance thresholds of alpine and
381 desert species would be skewed to their respective habitat extremes (aim 1). Also, we expected
382 that these skewed tolerances in the thermally variable, extreme environments would drive wider
383 TTB than the more stable, benign temperate environment (aim 2). We found that variation in TTB
384 in the more variable and extreme desert and alpine environments was indeed wider than temperate
385 species, with desert TTB values skewed towards higher temperatures, alpine TTB values skewed
386 towards colder temperatures and temperate TTB sitting in between. However, the mean TTB of
387 alpine and temperate species were similar to one another, whereas desert species had both greater
388 heat and cold thresholds. Finally, we assessed whether climate extremes and variability, rather
389 than means, explained more variation in thermal tolerance and TTB (aim 3). We found that mean
390 and maximum temperatures and precipitation (PC1 axis) had a stronger relationship with TTB than
391 thermal variability and cold extremes (PC2 axis), which provides only partial support the CVH.
392 When we explored the climate drivers of thermal tolerance thresholds, we found that thermal range
393 (within PC2), a proxy for climate variability, was only a strong driver for freezing tolerance
394 ($T_{nucleation}$). Below we explore our findings in terms of physiology and the ecological implications
395 of these results for species persistence in a changing climate.

396 **TTB well exceeds thermal ranges in each biome and varies within biomes**

397 Thermal tolerance breadth values of PSII reported here are notably wide; wider than the thermal
398 ranges in each environment and larger than measures of TTB in many other organisms. For
399 instance, across a range of marine and terrestrial ectotherm taxa, the widest recorded TTB was
400 60 °C in terrestrial arthropods (Sunday et al., 2011), while the widest TTB in our dataset was 70.3
401 °C in the desert species *Rhagodia spinescens*, with several other desert species also exceeding 60
402 °C. Within each biome, we found species with a range of wide and narrow TTB (Fig. 4). For
403 example, several alpine species across growth forms had relatively narrow TTB, such as the snow

404 bed species *Psychrophila introloba* (48.9 °C TTB) and the tree line species *Eucalyptus pauciflora*
405 subsp. *niphophila* (47.7 °C TTB). The narrow TTB of some alpine species was mainly driven by
406 their low heat tolerance ($T_{crit\text{-}hot} \approx 40$ °C), whereas other co-occurring species, *Hovea montana* and
407 *Epacris paludosa* had TTB values similar to those of many desert species (~63 °C), driven by
408 extreme cold tolerance (around -18 °C). This within-biome variation in TTB might be due to
409 variation among species microclimatic niche, which can override broad-scale climate drivers of
410 physiological tolerance (Curtis et al., 2016, Curtis et al., 2019, Aparecido et al., 2020).

411 Although thermal seasonality was greater in alpine than temperate regions sampled here, the
412 average TTB for these two biomes was remarkably similar (Fig. 3D). Temperate species appeared
413 to tolerate colder temperatures than expected, and it is possible that their relatively wide thermal
414 tolerance is a legacy of exposure to climate extremes through geological time (Byrne et al., 2008).
415 The climate legacy effect on thermal tolerance is suggested to be stronger for cold than heat
416 tolerances across both animal and plant taxa (Bennett et al., 2021) and might explain why some
417 temperate and desert species have greater freezing tolerance than expected by their current climate
418 parameters. Although metrics based on air temperatures for our alpine biome (e.g., MAT, thermal
419 range) confirm that thermal range and seasonality are large in this biome, mountain plants spend
420 4-5 months under the insulating cover of snow, which stabilises temperatures (Briceño et al.,
421 2014). This dampening of climate extremes might contribute to the similarity between alpine and
422 temperate species thermal tolerance breadth.

423 **Heat and cold thermal limits vary among biomes**

424 Plants exposed to drought and/or freezing share a similar physiological response: cell dehydration
425 (Anisko and Lindstrom, 1996, Blake and Hill, 1996, Lintunen et al., 2013). The physiological
426 response to drought conditions may partially explain our apparently counterintuitive result that

desert species were more freezing tolerant (more extreme $T_{crit\text{-}cold}$ and $T_{nucleation}$) than either alpine or temperate species. Plants can use osmotic adjustments to offset water loss from cells along the water potential gradient caused by both drying and freezing (Larcher, 2003, Siminovitch and Cloutier, 1983). Thus, the ability of desert species to cope with dehydration induced by long periods of drought might also confer the ability to cope with freezing, even though these desert species are unlikely to ever be exposed to such extreme freezing temperatures. In addition, leaf adaptations to aridity such as small leaves, thick cuticles, smaller, more densely packed cells, thicker cell walls with small capillary structures and small xylem vessel diameter (Dörken et al., 2020) also can prevent ice formation, which increases supercooling capacity and freezing tolerance (Lintunen et al., 2013). Such mechanisms may reflect that species living in environments where freezing temperatures are rare use supercooling as a mechanism to avoid freezing damage, whereas in alpine environments, species tolerate ice formation. Indeed, the desert species studied here generally had nucleation temperatures much lower than their $T_{crit\text{-}cold}$, from which we can infer a high supercooling capacity that effectively serves to delay freezing. Indeed, Andean alpine species' low $T_{nucleation}$ at the end of the growing season are attributed to low soil moisture and thus potential water stress, rather than low temperatures (Sierra-Almeida et al., 2016). Drought also improves both heat and cold tolerance in an Australian alpine grass (Sumner et al., 2022). Likewise, drought exposure can increase heat tolerance through raising intracellular sugar contents, which stabilise thylakoid membranes (Hüve et al., 2006, Brestic et al., 2012). The extreme freezing, cold and heat tolerances found in our desert species might reflect a secondary outcome of plant adaptation to aridity.

The relatively high $T_{crit\text{-}hot}$ of some of our alpine species may seem surprising; however, these heat tolerance ranges are similar to recent measures of heat tolerance in other alpine species (43-55°C, Sumner and Venn, 2022). Alpine plants can be exposed to very high temperatures, especially those

with prostrate growth forms (Körner, 2003). Leaf temperatures of European alpine species can reach up to 50 °C in summer, especially on calm sunny days, and leaf heat tolerances can be higher than 50 °C (Buchner and Neuner, 2003). Maximum leaf temperature has been measured at nearly 40 °C in early summer in Australian alpine species (Bird et al., 2023, unpublished data) and may yet be much higher in hot and still conditions in late summer. Therefore, thermal tolerance thresholds could be breached in alpine species if the combination of heat with other environmental stress, such as high light, caused heat tolerance to decrease, as found for some European alpine species (Buchner et al., 2015, Buchner and Neuner, 2003).

Heat and cold thermal limits relate to different climatic variables

Predicting plant responses to changing temperature regimes is a significant global challenge and requires a mechanistic understanding of how temperature affects species physiology and distributions. Although the physiological mechanisms underpinning freezing and cold tolerance (Guy, 2003) and heat tolerance (Wahid et al., 2007) have been established in model plants, responses of species exposed to natural conditions remain understudied. While the thermal limits of plants have recently been shown to broadly vary with latitude (O'Sullivan et al., 2017, Lancaster and Humphreys, 2020), comparisons employing standard techniques across multiple biomes remain rare, particularly for both heat and cold tolerance concomitantly (Geange et al., 2021). Importantly, the capacity to predict thermal tolerance from key bioclimatic parameters such as MAT and MAP remains complex (Perez and Feeley, 2021). For our cross-biome dataset, we used a PCA approach due to the strong correlations among local climate variables, which means that we cannot state explicitly that MAT or MAP or T_{range} are strong explanatory variables individually *per se*. Rather, these biomes are defined clearly by climate variables in combination: PC1 from cool and wet to extreme hot and dry; and PC2 from thermally stable and benign minimum temperatures to thermally variable and cold minimum temperatures. The combination of mean

annual temperature, precipitation and maximum temperatures explained considerable variation in $T_{crit\text{-}cold}$ and $T_{nucleation}$. However, because desert species had much lower $T_{nucleation}$ than $T_{crit\text{-}cold}$, the patterns with the climate axes differed slightly. These contrasting alpine and desert cold threshold responses suggest that $T_{nucleation}$ is more strongly associated with aridity and minimum temperatures than $T_{crit\text{-}cold}$, supporting the idea of shared mechanisms of tolerance to freezing and dehydration under drought.

Although plants growing in extreme hot and dry environments have higher heat tolerance, due to the high correlation of MAP with both MAT and MaxT, the role of temperature *vs* precipitation cannot be completely separated. For a range of common-grown Australian *Acacia* species from broad climatic and geographic origin, precipitation does not strongly drive variation in $T_{crit\text{-}hot}$ (Andrew et al., 2023). Yet, drought or low water availability might drastically reduce stomatal conductance and therefore cooling capacity during exposure to heat, thus increasing both leaf temperature and heat tolerance (Cook et al., 2021), demonstrating that water availability and temperature are intrinsically linked. Local climate, and particularly temperature, seems to play a significant role in driving variation in $T_{crit\text{-}hot}$ in our field study. Our findings contrast with another common garden study of 123 taxonomically diverse species, for which climate variables generally had very weak predictive power (<5%) for heat tolerance (Perez and Feeley, 2021).

Despite some clear macroclimatic patterns in our study, a large proportion of the variation in cold and, to a lesser extent, heat tolerance was attributable to among-species differences, unrelated to phylogeny (cold: 27.7 - 46.5%, heat: 24.7%). The contribution of species differences could reflect their different leaf traits, particularly those contributing to variation in leaf temperature, the signal of which may be amplified when sampled *in situ*. Mahan and Upchurch (1988) proposed that the lower limit of temperature for a plant is controlled by its environment, while the upper limit can be controlled by the plant, suggesting that plants are ‘limited homeotherms’. Recent studies have

499 shown that what drives variation in heat tolerance is leaf temperature, rather than air temperature
500 (Cook et al., 2021, Perez and Feeley, 2020). Leaves can avoid heat stress through different
501 mechanisms that decouple leaf from air temperature, such as anatomical traits (Leigh et al., 2017,
502 Leigh et al., 2012, Tserej and Feeley, 2021), patterns of display such as leaf inclination (Ball et al.,
503 1988) and transpirational cooling through stomatal conductance (Deva et al., 2020, Drake et al.,
504 2018), all of which can vary among co-existing species (Urban et al., 2017, Marchin et al., 2022).
505 Leaf-to-air temperature decoupling (Blonder et al., 2020) might also explain why relationships
506 between heat tolerance and latitude are smaller than expected for the air temperature span
507 considered (O'Sullivan et al., 2017, Curtis et al., 2016, Lancaster and Humphreys, 2020).

508 Thermal tolerance breadth captures both lower and upper limits. Unlike for animals, global
509 patterns of variation in the TTB of plants have received little attention. Despite the existence of
510 large thermal tolerance datasets, the lack of paired, concurrently measured low and high thermal
511 tolerance thresholds has hampered the ability to adequately assess thermal tolerance breadth in
512 plants. In our study, TTB increased non-linearly (hyper-allometrically) with increasing PC1:
513 species exposed to the high mean temperatures, coupled with aridity and heat extremes in our
514 desert biome had much wider thermal tolerance breadth than temperate or alpine plants (Fig. 6B).
515 However, although we found that species played a role in explaining variation in TTB, the capacity
516 for different species to respond to increasingly extreme and variable temperatures by changing
517 their TTB is only recently emerging. Thermal tolerance breadth of temperate and tropical alpine
518 species appears to differ only at the end of the temperate growing season, due to an increase in
519 cold acclimation (Sklenář et al., 2023). For common-grown seedlings from the same biomes that
520 were tested here, TTB became narrower when exposed to a combination of heat and freezing
521 temperatures in controlled climate chambers (Harris et al., *in press*). However, the same authors
522 found that the effect of biome is minimal, which contrasts the clear biome-based differences in

523 thermal thresholds observed here in plants *in situ*. The distinct responses between these contexts
524 implies that life stage and current growing conditions are likely to be important modifiers of
525 thermal tolerance. A recent study in an alpine species found that growth conditions far outweighed
526 other environmental and genetic sources of variation in trait changes (Arnold et al., 2024).
527 Research into what influences the capacity for species to shift their TTB in response to
528 environment is ongoing. Current research generally suggests that the evolution of plant niches is
529 constrained by heat tolerance (Araujo et al., 2013), supported by evidence of reduced variation in
530 heat tolerance across landscapes (Lancaster and Humphreys, 2020). However, we found the
531 opposite, with heat tolerance having greater among-species variability (~30 °C) than cold tolerance
532 (~20 °C) across our diverse biomes. This disparity points to the need for examination into whether
533 wider TTB or more extreme thermal thresholds contribute to greater plasticity to warming and
534 future climate extremes.

535 Narrow physiological thermal limits have been identified as a feature of species vulnerability to
536 climate change (Pacifici et al., 2015, Williams et al., 2008). Nevertheless, the use of PSII thermal
537 tolerance metrics determined at the leaf-level, requires careful interpretation. When leaves reach
538 their critical thermal limits, damage to PSII can occur, leading to decreased electron transport rate
539 and photosynthetic failure, thus compromising leaf survival. The likelihood that such damage
540 compromises whole plant survival, however, is less clear. PerezSocha et al. (2021) found heat
541 tolerance thresholds to correlate with carbon assimilation limits and therefore are linked to plant
542 productivity. To further unpick these patterns, we need to better understand the relationship
543 between physiological thermal limits of modular components of the whole plant with plant survival
544 and fitness across the plant life cycle. Further, to explain additional variation in these thermal
545 tolerance thresholds, explorations of the plasticity in both cold and heat tolerance across space and
546 time are also warranted.

Concluding summary

Thermal tolerance breadth (TTB), a parameter broadly used in animal thermal biology literature, has rarely been measured using a consistent method in such a diverse range of plant species and environments. To explore the climate variability hypothesis, we concomitantly measured the lower ($T_{crit\text{-}cold}$ and $T_{nucleation}$) and upper ($T_{crit\text{-}hot}$) photosystem thermal thresholds and TTB of 69 plant species across three biomes: two climatically extreme (alpine and desert) and one more stable (temperate). Considering climate from a discreet biome perspective, our results provide only partial support for the CVH, with plants from the climatically variable desert biome having the widest TTB. Across local climate variables, we found limited support for the CVH, with some evidence that ice nucleation temperature was driven by thermal range and minimum temperature, but heat tolerance was not, and substantial variation was explained by species. TTB of leaves was extremely wide; much wider than the air temperature extremes to which plants are exposed in these biomes. It is important to remember that sustained stressful temperatures within critical limits could still have far-reaching effects, depending on species life history, morphological traits and plasticity, as well as microclimatic and temporal heterogeneity. While local climate may partially explain variability in plant thermal tolerance and breadth, incorporating species traits, incorporating leaf temperature and microhabitat information is an important next step to better understand the vulnerability of different vegetation communities to climate extremes.

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Supporting Information

Table S1. Selected species in each biome, family, growth form and mean thermal tolerance thresholds.

Species	Family	Growth form	$T_{crit\text{-}cold}$ (°C)	$T_{nucleation}$ (°C)	$T_{crit\text{-}hot}$ (°C)	TTB (°C)
Alpine						
Aciphylla glacialis	Apiaceae	Herb	-7.3±0.4	-7.2±0.3	39.5±1.2	46.8±1.4
Astelia alpina	Asteliaceae	Herb	-13.9±0.7	-9.8±0.5	46.4±1.0	60.4±0.8
Carex breviculmis	Cyperaceae	Sedge	-12.0±1.2	-15.3±1.7	47.0±0.8	59.4±0.7
Celmisia costiniana	Asteraceae	Herb	-9.6±0.7	-7.6±0.7	44.9±0.4	54.4±0.9
Epacris paludosa	Ericaceae	Shrub	-18.2±1.2	-19.4±1.1	45.2±1.7	63.0±2.5
Eucalyptus pauciflora (ssp niphophila)	Myrtaceae	Tree	-8.7±0.3	-9.1±0.4	39.0±0.7	47.7±0.8
Ewartia nubigena	Asteraceae	Mat	-13.1±1.8	-11.9±1.9	43.0±0.9	56.1±2.2
Grevillea australis	Proteaceae	Shrub	-11.3±1.1	-10.8±1.8	44.1±1.4	55.4±1.9
Hovea montana	Fabaceae	Shrub	-18.5±0.3	-8.0±2.5	44.6±1.3	63.1±1.3
Luzula acutifolia	Juncaceae	Rush	-8.6±0.4	-10.3±1.3	44.2±2.3	52.9±2.3
Nematolepis ovatifolia	Rutaceae	Shrub	-7.5±0.4	-8.1±0.3	45.5±3.4	53.0±3.7
Olearia brevipedunculata	Asteraceae	Shrub	-14.3±1.3	-11.9±1.7	43.6±0.6	57.9±1.4
Orites lancifolius	Proteaceae	Shrub	-8.6±0.4	-8.5±0.5	45.2±1.7	53.8±1.7
Oxylobium ellipticum	Fabaceae	Shrub	-16.2±1.6	-19.4±1.3	42.3±1.2	58.5±2.4
Pimelea ligustrina	Thymelaeaceae	Shrub	-9.6±0.6	-8.6±0.6	37.1±0.4	46.6±0.6
Plantago muelleri	Plantaginaceae	Herb	-6.0±0.4	-5.5±0.4	39.9±2.9	45.9±3.1
Podocarpus lawrencei	Podocarpaceae	Shrub	-10.3±0.6	-13.7±1.7	45.8±1.2	56.0±1.2
Prostanthera cuneata	Lamiaceae	Shrub	-12.5±1.5	-15.9±1.7	39.1±2.0	51.7±1.5
Psychrophila introloba	Ranuculaceae	Herb	-7.3±0.4	-6.8±0.5	41.6±0.5	48.9±0.8
Richea continentis	Ericaceae	Shrub	-13.0±1.4	-14.4±2.3	43.6±0.4	56.7±1.3
Tasmannia xerophila	Winteraceae	Shrub	-8.4±0.5	-8.69±0.4	44.1±0.7	52.4±1.1
Viola betonicifolia	Violaceae	Herb	-6.6±1.0	-7.7±0.7	43.5±0.4	50.1±1.3
Temperate						
Acacia binervata	Fabaceae	Tree	-12.0±0.7	-11.8±0.9	39.8±1.5	51.8±1.0
Acacia longifolia	Fabaceae	Tree	-10.2±0.7	-10.3±0.6	43.7±1.7	53.8±2.2
Acmena smithii	Myrtaceae	Tree	-11.5±0.8	-6.3±1.6	48.0±0.7	59.4±1.0
Backhousia myrtifolia	Myrtaceae	Tree	-8.5±0.8	-6.8±1.0	48.0±0.4	56.5±1.1
Banksia integrifolia	Proteaceae	Tree	-6.7±0.5	-5.1±0.3	38.1±1.8	44.8±2.2
Brachychiton acerifolius	Malvaceae	Tree	-6.6±0.8	-7.7±0.5	48.6±0.4	55.2±0.7
Carex appressa	Cyperaceae	Sedge	-9.3±0.3	-5.0±0.7	50.1±1.2	59.4±1.1
Cayratia clematidea	Vitaceae	Climber	-8.0±0.8	-8.0±0.5	46.5±0.2	54.6±0.9
Ceratopetalum apetalum	Cunoniaceae	Tree	-10.8±1.0	-8.5±1.8	47.3±2.0	58.1±3.0
Cryptocarya microneura	Lauraceae	Tree	-8.5±1.1	-11.9±0.3	45.4±1.2	53.9±1.7
Doryphora sassafras	Atherospermataceae	Tree	-8.4±0.7	-5.1±0.7	47.6±0.8	55.9±1.1
Ficus coronata	Moraceae	tree	-8.7±0.6	-5.8±0.7	46.5±0.5	55.2±0.8
Guioa semiglauca	Sapindaceae	Tree	-7.7±0.3	-5.3±0.9	50.8±0.4	58.5±0.5
Lomandra longifolia	Asparagaceae	Sedge	-10.9±1.1	-6.7±1.2	49.5±1.6	60.4±2.3
Luzuriaga cymosum	Luzuriagaceae	Climber	-10.1±1.7	-10.9±1.3	48.8±0.2	58.9±1.7
Myoporum acuminatum	Scrophulariaceae	Tree	-9.4±0.6	-7.3±1.7	49.0±0.9	58.4±0.9
Oplismenus hirtellus	Poaceae	Grass	-4.0±0.7	-6.2±1.2	43.8±1.0	47.8±1.0

Pittosporum undulatum	Pittosporaceae	Tree	-10.5±1.2	-8.5±1.4	45.2±1.4	55.7±2.2
Podocarpus elatus	Podocarpaceae	Tree	-8.8±1.1	-6.6±1.1	48.2±0.9	57.1±1.9
Stephania japonica	Menispermaceae	Climber	-7.1±0.5	-5.7±1.1	48.3±0.6	55.4±1.0
Tasmannia insipida	Winteraceae	Shrub	-11.7±1.3	-14.1±0.7	48.0±0.6	59.7±0.8
Trochocarpa laurina	Ericaceae	Shrub	-6.2±0.6	-3.2±0.5	47.9±1.4	54.2±1.4
Viola banksii	Violaceae	Herb	-7.1±1.6	-6.0±0.6	42.2±0.7	49.3±1.6
Desert						
Acacia aneura	Fabaceae	Tree	-13.0±2.2	-16.2±1.2	53.0±4.0	66.0±4.0
Acacia salicina	Fabaceae	Tree	-13.8±0.8	-13.6±1.1	49.6±0.8	63.3±0.9
Acacia victoriae	Fabaceae	Tree	-15.6±1.9	-18.3±0.4	45.1±1.8	60.7±3.3
Alectryon oleifolius	Sapindaceae	Tree	-13.0±0.9	-13.5±1.1	50.3±1.9	63.4±2.1
Atriplex stipitata	Amaranthaceae	Subshrub	-16.7±0.6	-20.5±0.4	47.2±0.5	63.9±1.1
Brachychiton populneus	Malvaceae	Tree	-8.8±1.2	-13.9±1.4	47.4±1.9	56.2±2.1
Capparis mitchellii	Capparaceae	Tree	-13.1±0.6	-14.7±1.8	49.5±1.3	62.6±1.5
Casuarina pauper	Casuarinaceae	Tree	-14.9±0.3	-18.0±0.4	48.7±0.9	63.6±1.3
Dodonaea viscosa	Sapindaceae	Shrub	-12.4±1.4	-14.8±0.7	45.4±0.5	57.8±1.6
Eremophila latrobei	Scrophulariaceae	Shrub	-11.6±0.8	-18.3±1.1	48.5±2.0	60.2±2.1
Eremophila sturtii	Scrophulariaceae	Shrub	-12.7±1.5	-14.3±1.4	51.3±1.8	64.1±2.9
Eucalyptus coolabah	Myrtaceae	Tree	-13.3±2.0	-17.8±0.8	46.3±2.4	59.6±3.7
Eucalyptus largiflorens	Myrtaceae	Tree	-14.3±1.2	-13.9±0.5	45.9±3.1	60.2±2.5
Flindersia maculosa	Rutaceae	Tree	-13.7±0.7	-14.9±1.3	51.7±4.0	65.4±3.6
Geijera parviflora	Rutaceae	Tree	-11.3±1.4	-15.0±1.1	51.4±2.2	62.8±3.2
Grevillea striata	Proteaceae	Shrub	-15.0±1.2	-17.6±0.3	47.6±4.2	62.7±4.3
Myoporum montanum	Scrophulariaceae	Shrub	-13.9±0.4	-16.2±1.1	48.4±0.9	62.2±1.1
Pimelea microcephala	Thymelaeaceae	Shrub	-12.2±2.9	-15.6±0.8	48.7±2.1	60.9±2.1
Ptilotus sessilifolius	Amaranthaceae	Subshrub	-12.7±3.4	-17.2±2.2	51.8±0.5	64.6±3.6
Rhagodia spinescens	Amaranthaceae	Subshrub	-15.4±2.2	-18.1±1.2	54.9±3.4	70.3±4.5
Sclerolaena birchii	Amaranthaceae	Subshrub	-13.9±1.5	-15.4±0.7	48.6±1.6	62.5±2.8
Senna artemisioides	Fabaceae	Shrub	-13.4±2.2	-18.1±0.6	51.9±1.0	65.3±2.7
Sida fibulifera	Malvaceae	Subshrub	-13.1±2.1	-15.2±0.9	50.5±0.9	63.6±2.0
Solanum esuriale	Solanaceae	Herb	-11.9±0.9	-13.5±1.5	49.3±1.1	61.2±0.8

Table S2. Climate data extracted for species locations across all biomes used in initial models (see associated spreadsheet).

Table S3. Loadings and variance explained by the first and second Principal Components (PC1 and PC2) that explain 99.6% of the variance among local climate variables. Bold indicates dominant variables.

	Loadings	
Local climate variable	**PC1**	**PC2**
MAP	**-0.498**	-0.158
MinT	0.382	**-0.624**
MaxT	**0.506**	0.098
Trange	0.334	**0.713**
MAT	**0.489**	-0.261
Variance explained	76.5%	23.1%
Cumulative variance	76.5%	99.6%

Table S4. Model AIC and weights for each physiological thermal tolerance threshold and TTB.

Trait	**Model fixed effect structure**	***npar***	**AIC**	**Weight**
$T_{crit\text{-}cold}$	PC1 + PC2	7	1928.7	0.065
	PC1 + PC1^2 + PC2	8	1924.8	**0.458**
	PC1 + PC2 + PC2^2	8	1926.1	0.236
	PC1 + PC1^2 + PC2 + PC2^2	9	1926.1	0.241
$T_{nucleation}$	PC1 + PC2	7	1806.6	0.080
	PC1 + PC1^2 + PC2	8	1805.9	0.113
	PC1 + PC2 + PC2^2	8	1802.6	**0.586**
	PC1 + PC1^2 + PC2 + PC2^2	9	1804.6	0.221
$T_{crit\text{-}hot}$	PC1 + PC2	7	2126.6	**0.525**
	PC1 + PC1^2 + PC2	8	2128.5	0.203
	PC1 + PC2 + PC2^2	8	2128.6	0.194
	PC1 + PC1^2 + PC2 + PC2^2	9	2130.4	0.078
TTB	PC1 + PC2	7	2307.7	0.203
	PC1 + PC1^2 + PC2	8	2306.0	**0.463**
	PC1 + PC2 + PC2^2	8	2308.1	0.164
	PC1 + PC1^2 + PC2 + PC2^2	9	2308.0	0.170

Table S5. Phylogenetic signal in physiological thermal tolerance thresholds.

Trait	C_{mean}	***p*-value**	***I***	***p*-value**	***K***	***p*-value**	**λ**	***p*-value**
$T_{crit\text{-}cold}$	0.112	0.075	0.007	0.235	0.111	0.454	0.560	0.316
$T_{nucleation}$	0.052	0.222	-0.013	0.473	0.095	0.595	0.253	1.000
$T_{crit\text{-}hot}$	-0.181	0.987	-0.063	0.950	0.048	0.957	0.000	1.000
TTB	-0.103	0.854	-0.005	0.378	0.126	0.295	0.000	1.000