Global Change Biology

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RESEARCH ARTICLE OPEN ACCESS

Local Adaptation Drives Leaf Thermoregulation in Tropical Rainforest Trees

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Received: 15 November 2024 | **Revised:** 29 April 2025 | **Accepted:** 15 May 2025

Funding: This work was supported for this research was provided by funding to K.B.M. from the Skyrail Rainforest Foundation and Holsworth Wildlife Endowment, and from the Australian Research Council (ARC, LP190100484 and DP210103186).

Keywords: functional traits | intraspecific trait variation | leaf energy balance | leaf temperature | leaf thermoregulation | limited homeothermy | local adaptation | population genomics | thermal tolerance | tropical rainforest

ABSTRACT

Tropical forests play a critical role in biodiversity, carbon sequestration, and climate regulation, but are increasingly affected by heatwaves and droughts. Vulnerability to warming may vary within and between species because of phenotypic divergence. Leaf trait variation can affect leaf operating temperatures—a phenomenon termed 'limited homeothermy' when it helps avoid heat damage in warmer conditions. However, evidence for this capacity and the relative roles of acclimation or adaptation remain limited. We measured photosynthetic heat tolerance and leaf thermal traits of three co-occurring rainforest tree species across a thermal gradient in the Australian Wet Tropics. Using a leaf energy balance model parameterised with field-measured traits, we predicted variation in leaf-to-air temperature differences (ΔT_{trait}) and resulting thermal safety margins. We combined this with individual-based genome-wide data to detect signals of adaptive divergence and validated findings in a glasshouse trial with provenances grown under contrasting temperature and humidity conditions. Intraspecific trait variation reduced ΔT_{trait} and increased heat tolerance in warmer sites for *Darlingia darlingiana* and *Elaeocarpus grandis,* but not *Cardwellia sublimis*. As a result, thermal safety margins declined less steeply with increasing growth temperature in species capable of increased heat tolerance and avoidance, indicating these strategies can effectively buffer warming. All species showed genomic signals of selection, with associations to temperature and moisture variables. In *E. grandis*, glasshouse results confirmed a negative cline in ΔT_{trait} with temperature of origin. Although contrasting growth temperature and humidity lead to acclimation of individual traits, their coordination maintained ΔT_{trait} across the conditions imposed. Our findings provide evidence of limited homeothermy and suggest climate gradients have selected for trait combinations that reduce leaf temperatures at warmer sites in some but not all species. Given the rapid pace of climate change, those species with limited capacity to adjust their thermal safety margins through acclimation or adaptation may be at greater risk of local extinction.

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1 | Introduction

Global warming is rapidly increasing temperatures, raising concerns about the ability of species to persist (IPCC 2022). Tropical forest trees, vital for biodiversity, carbon sequestration and water cycling (Mitchard 2018), may be particularly vulnerable because of their evolution under relatively stable thermal environments (Trew and Maclean 2021). However, populations across climate gradients may respond differently to environmental change because of plasticity and/or local adaptation (Barton et al. 2020; Halbritter et al. 2018; Leites and Garzón 2023). Although local adaptation can enhance fitness under stable conditions, it may inhibit future persistence if climate change creates a mismatch between the historic conditions a population is adapted to and the new environment (Jordan et al. 2024). Therefore, understanding how tropical trees have adapted to temperature is crucial for evaluating their resilience to global warming and informing effective management strategies.

Plants have evolved various mechanisms to cope with heat stress and maintain physiological function, such as altering leaf-level biochemistry to increase their photosynthetic heat tolerance (Geange et al. 2021; Middleby, Cheesman, and Cernusak 2024). Such adjustments help prevent leaf damage and maintain carbon uptake during heatwaves (Drake et al. 2018). However, recent studies indicate that this may be insufficient to preserve thermal safety margins—the difference between a leaf's thermal tolerance threshold and the temperatures it experiences—in warmer environments (Kitudom et al. 2022; Perez and Feeley 2020). As such, species or populations near their thermal limits, such as those in lowland tropical forests, may be particularly vulnerable to even small temperature increases in the coming decades (Araujo et al. 2021; Doughty et al. 2023; Pau et al. 2018).

Plants can also cope with thermal stress by avoiding high temperatures through morphological and physiological trait variations that influence leaf energy balance (Michaletz et al. 2015). Traits like leaf width, absorptance, leaf angle and stomatal conductance to water vapour (g_s) interact with the canopy microclimate to influence the fluxes of sensible and latent heat and the interception of radiation (Campbell and Norman 1998; Jones 2013). This leads to significant differences between leaf and air temperatures ($\Delta T = T_{leaf} - T_{air}$), because of differences in water use (Fauset et al. 2018), leaf morphology (Leigh et al. 2017) and canopy architecture (Leuzinger and Korner 2007; Woods et al. 2018). Given the importance of maintaining T_{leaf} within safe operating limits, it is hypothesized that traits influencing ΔT may undergo selection (Mahan and Upchurch 1988; Michaletz et al. 2015). Although such thermoregulation has been noted across different ecosystems (Guo et al. 2023; Kitudom et al. 2022), studies within species are limited (Kullberg et al. 2023; Kullberg and Feeley 2022), and few have explored the relative roles of phenotypic plasticity and ecotypic variation (Middleby, Cheesman, and Cernusak 2024; Posch et al. 2024).

Understanding whether intraspecific variation in leaf traits affecting ΔT results from plasticity or genetic differentiation is essential for predicting future vulnerability and for guiding revegetation efforts to match provenance with future site conditions (Breed et al. 2013; Jordan et al. 2024). Although common garden and reciprocal transplant experiments are ideal for studying local adaptation, they are resource-intensive (Sork et al. 2013), especially in remote areas. Population and ecological genomic methods, such as genotype–environment (GEA) and genotype–phenotype associations (GPA), offer complementary insights into adaptive potential without extensive field trials (Arab et al. 2020; Breed et al. 2019; Shryock et al. 2021).

To assess whether tropical trees have the capacity to cope with future climate change, we examined the patterns and drivers of intraspecific variation in leaf traits affecting thermoregulation. We define leaf thermoregulation as coordinated trait variation that alters the difference between leaf and air temperature (ΔT_{trait}) across a thermal gradient. Since leaves are typically warmer than air during the day because of solar radiation, ΔT values are generally positive. We use ΔT_{trait} to represent the component of ΔT attributable to trait variation and interpret limited homeothermy as a negative slope of ΔT_{trait} versus maximum temperature of the warmest month (MTWM) or MTWM of origin—indicating that leaves heat up less in warmer environments because of trait adjustments. A flat slope would indicate no thermoregulatory adjustment, whereas a positive slope would suggest increased leaf warming at warmer sites. By isolating the effects of trait variation and focusing on the slope of ΔT_{trait}, this approach avoids confounding by environmental influences on T_{leaf} and circumvents the flawed assumption that limited homeothermy requires T_{leaf} to fall below T_{air}.

We hypothesized that traits associated with leaf cooling are under selection, specifically:

1. Within species, individuals from warmer climates of origin show coordination of leaf thermal traits that enhance leaf cooling relative to individuals from cooler climates.
2. Adaptive genetic variation associated with leaf traits is positively correlated with temperature gradients across species distributions.
3. Both plasticity and ecotypic differentiation will affect the observed patterns of leaf thermoregulation in *E. grandis*, grown under controlled thermal environments.

To test these predictions, we measured photosynthetic heat tolerance and leaf traits in populations of three tree species across a thermal gradient and predicted leaf temperatures with a leaf energy balance model. We also examined genome-wide single nucleotide polymorphisms (SNP), using GEA and GPA analyses to explore genome-wide signals of selection and identify the environmental drivers of adaptive variation in thermoregulation using generalized dissimilarity modeling. Finally, this approach was validated in *E. grandis* using a climate-controlled glasshouse trial with seedlings collected across a thermal gradient.

2 | Methods

2.1 | Study System: Rainforest Tree Species in the Wet Tropics of Queensland, Australia

The Wet Tropics of Queensland World Heritage Area comprises 8,945 km^2 of mostly rainforest vegetation in northeastern Queensland, Australia. Its mountainous terrain creates geographic and spatial variation in climatic conditions across elevation over small distances. This presents an ideal opportunity for exploring patterns of intraspecific trait variation and local adaptation. The Wet Tropics of Queensland experiences warm temperatures year-round, with high but seasonal rainfall and periodic cyclone disturbance (UNESCO World Heritage Centre 1988). We selected three tropical rainforest species—*Elaeocarpus grandis* F. Muell. (Elaeocarpaceae), *Cardwellia sublimis* F. Muell. (Proteaceae) and *Darlingia darlingiana* F. Muell. L.A.S. Johnson (Proteaceae)—that are relatively abundant, upper canopy species occurring across a wide elevational range (0–1300 m). Both *C. sublimis* and *D. darlingiana* are endemic to the Wet Tropics of Queensland and have wind-dispersed seeds, whereas *E. grandis* has a wider distribution extending into Southeast Asia and the Australian subtropics and has large fleshy fruits dispersed primarily by birds.

2.2 | Field Sampling

During October 2021 to May 2022, we sampled trees from 16 mature remnant forest sites from across the Wet Tropics of Queensland, spanning an elevation range of 1299 m a.s.l (5 to 1304 m a.s.l), a mean annual temperature range of 7.1°C (18.6°C to 25.8°C) and a mean annual precipitation range of 2940 mm (1355 to 4295 mm). At each site, 3–10 individuals per population (median 6), spaced > 100 m apart, were selected, with the intention to avoid sampling closely related individuals. Sun-exposed branches from the upper canopy were sampled using a pole pruner or big shot, placed in large opaque, water-sprayed bags, and kept shaded during transport to the field lab (up to 4 h). At the lab, branches were recut underwater and rehydrated in the dark for trait measurements (*c.* 3 h for functional traits and 12 h for chlorophyll fluorescence). Leaf samples for genomic analysis were dried on silica beads. In total, we measured leaf traits on 105 *C. sublimis*, 96 *D. darlingiana*, and 104 *E. grandis* individuals, and generated genomic data for 98 *C. sublimis*, 96 *D. darlingiana*, and 89 *E. grandis* individuals (Table 1). Additionally, seeds were collected from 11 *E. grandis* individuals from six sites for the glasshouse experiment (Figures 1 and S1, Table 1).

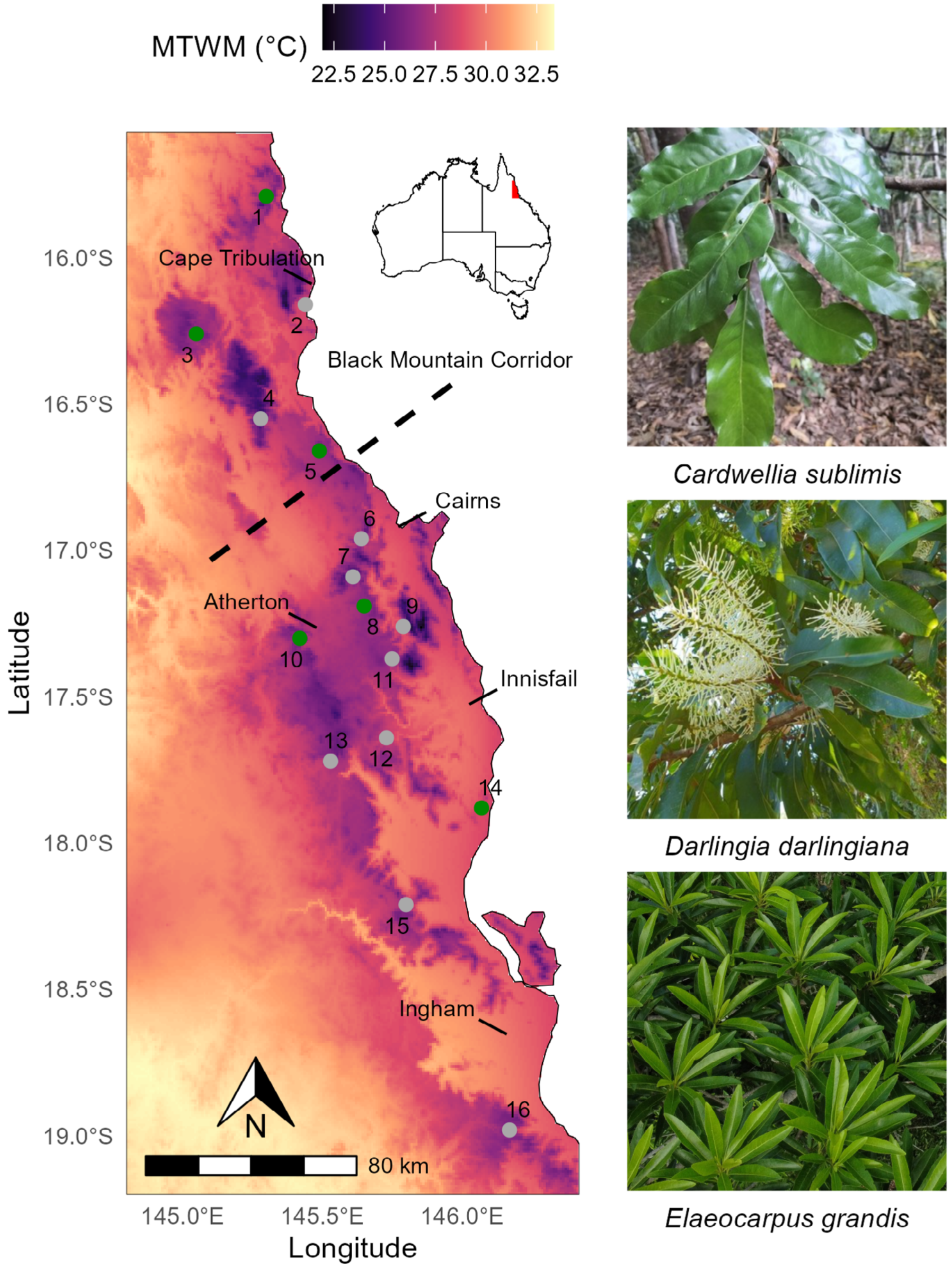
2.3 | Leaf Traits

We measured leaf traits known to reflect trade-offs between resource acquisition and conservation strategies (Wright et al. 2004) and those impacting leaf energy balance. These included leaf mass per area (LMA, $\mathrm{g\,m^{-2}}$), leaf dry matter content (LDMC, $\mathrm{mg\ g^{-1}}$), leaf thickness (μm), effective leaf width (cm), leaf reflectance spectra including absorptance (Abs, %) and reflectance (Ref, %) to shortwave radiation, leaf carbon isotope composition (δ^{13}C), leaf nitrogen (N_{mass}, %), and the mass ratio of carbon to nitrogen. We also measured stomatal density (stoma $\mathrm{mm^{-2}}$) and size ($\mu\mathrm{m}^2$) to calculate theoretical maximum conductance (g_{max}, $\mathrm{mol\ m^{-2}\,s^{-1}}$). Leaf traits were measured according to standard techniques on fully expanded sun-exposed

TABLE 1 | Site coordinates and means for environmental variables. Sites are ordered by decreasing latitude, and numbers correspond to those in Figure 1. Sample size per population is given for trait measurements, and, where this differs, for the genetic dataset in brackets.

	Lat.	Long.	Elev	MTWM	PDM	Sample size per population		
Population	**(°)**	**(°)**	**(m)**	**(°C)**	**(mm)**	**CS**	**DD**	**EG**
1. Cedar Bay*	−15.79	145.3	172	28.0	35	6	6	6
2. Daintree	−16.16	145.44	43	28.1	69	6 (5)	6	6 (5)
3. Mt. Windsor*	−16.26	145.05	1098	26.1	17	6	6	6 (5)
4. Mt. Lewis	−16.55	145.28	1028	25.1	28	9 (8)	6	6
5. Kuranda*	−16.66	145.49	450	26.8	31	6	6	6
6. Dinden	−16.96	145.64	493	27.4	31	6	4	6
7. Mt. Edith	−17.09	145.62	977	25.7	39	6	6	3
8. School for field studies*	−17.19	145.65	770	26.5	61	10 (8)	9	10 (6)
9. Goldsborough	−17.26	145.79	149	28.8	87	0	6	6
10. Mt. Baldy*	−17.30	145.42	1162	25.8	30	7	6	5 (4)
11. Topaz	−17.37	145.75	743	26.4	111	6	0	6
12. South Johnstone	−17.64	145.73	540	27.9	109	8 (7)	8	8 (6)
13. Koombaloomba	−17.72	145.53	870	26.9	59	6	6	6
14. Mission Beach*	−17.88	146.07	28	28.7	90	10 (7)	7	10 (6)
15. Kirrama	−18.21	145.8	677	27.1	51	6	6	6
16. Paluma Range	−18.98	146.17	832	26.4	34	8	8	8 (6)

Note: Lat = latitude, Long = longitude, Elev = elevation (m a.s.l), MTWM = max temperature of the warmest month (Bio5, °C), PDM = precipitation of the driest month (Bio14, mm). CS = *Cardwellia sublimis*, DD = *Darlingia darlingiana*, and EG = *Elaeocarpus grandis*. Sites with an * had *E. grandis* seedings included in the glasshouse trial.



Cardwellia sublimis

Darlingia darlingiana

Elaeocarpus grandis

FIGURE 1 | Map of study region. Map color shows the max temperature of the warmest month from 1981 to 2010 retrieved from the CHELSA database at 1 km resolution (Karger et al. 2017). The dashed black line indicates position of the Black Mountain Corridor, a putative geographic barrier during glacial maxima (Schneider et al. 1998). Grey points labeled with numbers indicate the populations where genetic and trait samples were collected for each of the three species shown to the right (see Table 1 for further information on populations). Green points highlight populations where *E. grandis* seedlings were collected from in the subsequent glasshouse experiment. Map lines delineate study areas and do not necessarily depict accepted national boundaries. Photos of *D. darlingiana* were provided by David Tng, *C. sublimis* by Mathew Borella, and *E. grandis* by Alexander Cheesman.

leaves from each tree (Perez-Harguindeguy et al. 2013), with 10 leaf replicates for leaf functional traits and three for absorptance and stomatal traits (for detailed measurement protocols, see Supporting Information, Methods S1).

2.4 | Leaf Temperature (T_{leaf}) Modelling

We tested how covariation of leaf traits affects predicted leaf-to-air temperature differences (ΔT) across a thermal gradient and whether this helps maintain T_{leaf} within safe margins. T_{leaf} was predicted using a steady-state leaf energy balance model based on the Penman-Monteith equation (Jones 2013; Monteith and Unsworth 2013), requiring plant trait-based inputs (leaf width, absorptance to shortwave radiation, stomatal ratio, and conductance), and microclimate inputs (air temperature, vapour pressure deficit, radiation, and wind speed).

Microclimate inputs were parameterized using the *micro_global* function in 'NicheMapr' v.3.2.0 (Kearney and

Porter 2020) which uses the New et al. (2002) global monthly climate database at a 10 × 10 km grid centered on 1960 to 1990. Hourly estimates of T_{air}, VPD, and solar radiation (converted to photosynthetic photon flux density, PPFD) were obtained for the 15th of each month, using tree coordinates and elevation, with solar radiation adjusted for terrain using the 'microclima' setting (Maclean et al. 2019). From this typical monthly, diel data, daytime means (09:00 to 15:00) were calculated for each individual tree as well as an overall mean across all individuals and species.

To separate changes in ΔT due to microclimate from those due to leaf trait covariation, we used two microclimate parameterisations when running the leaf energy balance model. For ΔT based solely on leaf traits (hereafter $\Delta T_{\mathrm{trait}}$), microclimate inputs were set to the overall daytime means described above, with $T_{\mathrm{air}} = 24.8°\mathrm{C}$, $\mathrm{VPD} = 1.4\,\mathrm{kPa}$, $\mathrm{PPFD} = 1403\,\mu\mathrm{mol\,m^2\,s^{-1}}$, and assuming calm conditions with a wind speed of $0.5\,\mathrm{m\,s^{-1}}$. For ΔT based on both traits and climate (ΔT_{clim}), we used the tree-level daytime mean modelled microclimate described above.

We implemented the leaf energy balance model using the *PhotosynEB* function in 'plantecophys' v.1.4–6 (Duursma 2015), which couples an energy balance model to the Farquhar, von Caemmerer, and Berry photosynthesis model (Farquhar et al. 1980) and the optimality-based unified g_s (USO) model (Medlyn et al. 2011), with T_{leaf} solved iteratively. The leaf traits that were ultimately involved in ΔT parameterization include effective leaf width, absorptance to shortwave radiation, N_{mass}, and $\delta^{13}\mathrm{C}$. For methodological comparison, we also calculated $g_{\max}$ from stomatal anatomy (Supporting Information, Methods S1), this was not used for downstream genetic analysis. To convert estimated T_{leaf} to ΔT, we used the same T_{air} values used as inputs for each respective model (i.e., a common $T_{\mathrm{air}} = 24.8°\mathrm{C}$ for $\Delta T_{\mathrm{trait}}$ and a tree-level T_{air} for ΔT_{clim}).

2.5 | Stomatal Conductance Modelling

An assumption of the USO model used to predict g_s is that plants maximize carbon gain while minimizing water loss. To obtain g_s, traits that impact gas exchange are required, such as the maximum rate of carboxylation (V_{cmax25}) and light-saturated rate of electron transport (J_{max25}), as well as the slope parameter (g_1) that describes the relationship between photosynthesis and stomatal conductance and responses to changes in VPD (Medlyn et al. 2011). Since characterizing V_{cmax25}, J_{max25} and g_1 in the field for each tree was not feasible, we varied these according to observed linear relationships with leaf N_{mass} (V_{cmax25}) determined during the glasshouse experiment (Figure S9), and as a ratio of V_{cmax25} to J_{max25} and used thermal sensitivity of V_{cmax} and $J_{\max}$ parameters determined in Australian tropical trees (Kelly 2014). The parameter g_1 was calculated using the following equation (Medlyn et al. 2011):

$$g_1 = \frac{\left(\frac{C_i}{C_a}\sqrt{\mathrm{VPD}}\right)}{\left(1 - \frac{C_i}{C_a}\right)}$$

where VPD is the tree-level mean daytime vapour pressure deficit (kPa), and C_i/C_a is the ratio of intercellular to ambient CO_2 concentrations, estimated from leaf $\delta^{13}\mathrm{C}$ using the 'isocalcr' package in R (Mathias and Hudiburg 2022).

This model effectively captures dynamic gas exchange and produces realistic leaf temperature estimations under non-stressful conditions (Guo et al. 2022; Kearney and Leigh 2024). However, evidence suggests that at higher temperatures, g_s and photosynthesis decouple, with g_s maintained or even increasing (De Kauwe et al. 2019; Diao et al. 2024; Drake et al. 2018; Marchin et al. 2023; Urban et al. 2017). This decoupling may reflect a strategy to enhance transpirational cooling rather than optimizing carbon gain, especially under heat stress. Relying solely on the USO model could thus overlook plant responses aimed at maintaining T_{leaf} within a viable range. In addition, our parameterisation relies on a within-species correlation between V_{cmax25} and leaf N_{mass} that was only determined in *E. grandis*. To address these assumptions, we also calculated $\Delta T_{\mathrm{trait}}$ and ΔT_{clim} using theoretical $g_{\max}$ determined from stomatal anatomy via the *findTleaf* function, which estimates T_{leaf} independently of photosynthesis. These are referred to as anatomical $\Delta T_{\mathrm{trait}}$ and anatomical ΔT_{clim} throughout the manuscript. By incorporating $g_{\max}$, we aim to account for the upper limits of stomatal conductance that plants may employ in response to heat stress.

2.6 | Photosynthetic Heat Tolerance

Photosynthetic heat tolerance was assessed by examining the decline in maximum photosynthetic efficiency of photosystem II with increasing incubation temperature. To do so, the ratio of variable fluorescence (F_v) to maximum fluorescence (F_m) was measured following an established protocol (Leon-Garcia and Lasso 2019) modified from Krause et al. (2013). For each tree, 15 to 20 rehydrated leaves were sampled, and six 9 mm leaf discs per leaf were pooled and randomly assigned to heat treatments. Discs were incubated for 30 min in water baths at 36°C, 40°C, 42°C, 44°C, 46°C, 48°C, 50°C, 52°C, 54°C, and 58°C, with an untreated control at ~24°C, then placed in petri dishes at 24°C for 24 h. Chlorophyll fluorescence was then measured on leaves that had been dark adapted for 30 min using a chlorophyll fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Five sites (and six for *E. grandis*) at varying elevations were measured during October–November 2021. Individuals showing photoinhibition (control $F_v/F_m < 0.6$) were excluded from analysis. A 4-parameter log-logistic curve was fitted using 'drc' v.3.0–1 (Ritz et al. 2015), with the lower asymptote set to 0. The thermal tolerance metric T_{50} (T_{leaf} at 50% decline in F_v/F_m) was obtained from the curves.

2.7 | Thermal Safety Margins

To determine how intraspecific trait variation may influence thermal safety margins (TSM) across species distributions, TSMs were calculated as the difference between thermal tolerance (T_{50}) and modeled T_{leaf} ($\Delta T + T_{\mathrm{air}}$) as described above. We calculated TSMs using both $\Delta T_{\mathrm{trait}}$ and ΔT_{clim}, where $\Delta T_{\mathrm{trait}}$

assesses the contribution of trait variation to TSMs, and ΔT_{clim} assesses whether this variation offsets differences in local climate conditions across the species' range. Additionally, we calculated anatomical TSMs using anatomical ΔT calculated from stomatal anatomical traits.

We were interested in assessing relative changes in TSM across a species distribution, as such our TSM estimates were calculated using daytime average (09:00 to 15:00) conditions and are likely higher than studies assessing instantaneous point measurements of T_{leaf} or those that use T_{crit} (the temperature at which photosystem II function first begins to decline) as the upper threshold instead of T_{50}. Canopy leaves of tropical forests already surpass T_{crit} (Doughty et al. 2023), and although some photosynthetic function can recover after a single exposure, recovery is less likely after T_{50} exposure (Cook et al. 2024; Javad et al. 2025; Tarvainen et al. 2022) but see Javad et al. (2025). It is also important to note that heat tolerance itself can be dynamic and influenced by recent thermal exposure (Drake et al. 2018), which may introduce variability into TSM estimates.

2.8 | Climate and Environmental Variables

To explore environmental drivers of trait and genomic variation, we retrieved gridded maps of bioclimatic variables, as well as wind speed and relative humidity for the Wet Tropics of Queensland from the CHELSA database at 1 km resolution, averaged for 1981–2010 (Brun et al. 2022; Karger et al. 2017). Gridded soil data products at 5–15 cm depth, 30 m resolution, for the period 1950–2013 were obtained from the Soil and Landscape Grid of Australia and resampled to 1 km resolution using 'raster' v.3.6–11 (Hijmans 2022) to match the bioclimatic data. To avoid multicollinearity and ensure interpretability, we selected uncorrelated variables ($r < 0.7$, calculated using values for the sampled sites) relevant to tropical tree functioning: maximum temperature of the warmest month (Bio5, °C), precipitation of the driest month (Bio14, mm), minimum relative humidity (RH_{min}, %), mean wind speed ($\text{Wind}_{\text{mean}}$, m s^{-1}), soil pH (pH), and total soil phosphorus (P).

2.9 | Genotyping

Dried leaf tissue samples were sent to Diversity Arrays Technology Australia Pty Ltd., Canberra, Australia for DNA extraction and genotyped using DArTseq, a reduced representation sequencing method (Sansaloni et al. 2010). Data quality and SNP filtering were performed using 'RRtools' v.0.1 (Bragg et al. 2020; Rossetto et al. 2019). SNPs with a reproducibility score below 0.96, over 20% missing data, or redundant loci were excluded. Individuals with 80% missing loci were also removed, and a minor allele frequency threshold of 0.05 was applied. The cleaned dataset comprised 8884 SNPs across 98 individuals from 15 populations for *C. sublimis* (6.48% missing data), 8009 SNPs across 96 individuals from 15 populations for *D. darlingiana* (8.15% missing data), and 4935 SNPs across 89 individuals from 16 populations for *E. grandis* (6.65% missing data).

2.10 | Population Genetic Diversity and Structure

We assessed population genetic diversity by estimating allelic richness, expected heterozygosity (H_e), observed heterozygosity (H_o) and the inbreeding coefficient (F_{IS}) using 'diveRsity' v1.9.90 (Keenan et al. 2013), with 1000 bootstrap replicates for confidence intervals. Genetic similarity was evaluated through Principal Component Analysis (PCA) using 'adegenet' v2.1.8 (Jombart 2008). Overall F_{ST}, a measure of genetic differentiation based on allele frequency variation at neutral loci, was estimated for each species using analysis of molecular variance on all SNPs with the 'poppr' package and converted into a χ^2 distribution. Ancestry coefficients were estimated via discriminant analysis of principal components (DAPC) and sparse non-negative matrix factorization (sNMF). For both methods, we tested1 to 16 genetic clusters (K). DAPC was performed with K-means clustering on PCA-transformed genotypes, selecting the optimal K via the Bayesian information criterion and retaining 10 principal component axes using cross-validation. For sNMF, 'LEA' v.3.8.0 (Frichot and François 2015) was used, selecting the optimal K based on the stabilization of cross-entropy values and choosing the best replicate with the lowest cross-entropy.

2.11 | Signals of Selection

To detect potentially adaptive loci, we used F_{ST} outlier analysis, GEA, and GPA. Association analyses were performed using latent factor mixed modeling (LFMM) and partial redundancy analysis (pRDA). This approach combined univariate and multivariate methods to detect both single-locus and multilocus signatures of selection with high power and low false-positive rates (Forester et al. 2018). For GPA, we tested two trait sets: (1) ΔT_{trait} as an integrative trait, and (2) a suite of leaf traits (LMA, thickness, width, Absorptance, N_{mass}, and g_1) selected for their relevance to leaf energy balance, low intercorrelation ($r < 0.7$), and evidence of population differentiation. For *C. sublimis*, g_1 was included despite population effects being only marginally significant ($p = 0.0731$).

To ensure complete SNP datasets, missing values were imputed with the most common allele across individuals. Given multiple potential K values identified in prior population structure analyses, we conducted analyses for $K = 1$–3, treating all significant SNPs as putatively adaptive. F_{ST} outlier analysis was done using 'pcadapt' v.4.3.3 (Luu et al. 2017) using default parameters, with SNPs considered putatively adaptive if the $q$value was < 0.05. LFMM was run for both GPA and GEA analyses with a lasso penalty using 'lfmm' v.1.1 (Caye et al. 2019) testing multiple latent factors. Z scores were calibrated for genomic inflation before being converted into p-values. Candidate SNPs were identified using a false discovery threshold of $q < 0.1$. The pRDA analyses in both GPA and GEA were conducted using 'vegan' v.2.6–4 (Oksanen et al. 2022), with two principal components for genetic structure (see previous section) as conditional variables. For GPA, elevation was also included as a conditional variable to account for non-genetic variation in leaf traits. SNPs associated with phenotypes (GPA), or environments (GEA) were identified by extreme loadings, defined as 3 standard deviations along pRDA axes.

2.12 | Generalized Dissimilarity Modelling

We employed generalized dissimilarity modeling (GDM) to further investigate nonlinear GEA and GPA and identify potential environmental drivers of variation. In contrast to LFMM and pRDA, this approach assesses multivariate, nonlinear patterns of genomic or trait turnover along environmental gradients, while accounting for isolation by distance (Ferrier et al. 2007; Mokany et al. 2022). GDM is useful for evaluating the relative importance of environmental predictors (via the sum of I-spline coefficients) and for identifying where the steepest gradients of change occur (by observing the change in slope).

For the genomic data, we ran GDMs on three SNP datasets. First, we used the putatively adaptive SNPs identified through trait associations (GPA analysis by both LFMM and pRDA). The second dataset included SNPs identified as genomic outliers (F_{ST} outlier analysis) and through association with the environment (GEA analysis). We explored both GEA and GPA datasets because GPA was not measured in a common garden; thus, GEA helps determine if the environmental variables linked to trait variation also drive adaptive variation. Although the environmental drivers of genomic variation are already inherently assessed within the GEA analyses, we performed GDM on this SNP set to allow direct comparison of driver relative importance across the different genomic GDMs. The third dataset investigated whole genome variation across environmental space, using the original cleaned SNP set that includes both adaptive and neutral SNPs. For all three analyses, we used a population-level pairwise F_{ST} distance matrix generated with ‘SNPRelate’ v.1.30.1 (Zheng et al. 2012) using the relative beta estimator in Weir and Hill (2002).

Additionally, we conducted two trait GDMs to assess the extent to which temperature or other environmental factors explained variation in ΔT_{trait} and leaf trait variation. We calculated population-level means for ΔT_{trait} and other leaf traits (the uncorrelated suite described above). For the leaf trait GDM, we reduced variables to two principal component axes using the *princomp* function. Euclidean distance matrices were computed for each dataset using the *dist* function.

All five GDMs were fit using ‘gdm’ v.1.5.9–9.1 (Ferrier et al. 2007; Mokany et al. 2022). Predictor significance was tested with matrix permutation ($n = 50$ permutations) using the *gdm.varImp* function, with predictor significance defined by increases in explained deviance. Variables with the highest sum of I-Spline Coefficients were considered most important. We estimated model sensitivity with bootstrapping ($n = 1000$ iterations), retaining 90% of populations (Shryock et al. 2015).

2.13 | Glasshouse Experiment

Our goal in the glasshouse experiment was to assess how the climate of origin and growth environment influences intraspecific variation in leaf thermoregulation. Specifically, we tested whether plants from warmer climates, or grown under warmer conditions, exhibited lower predicted ΔT_{trait} than those from cooler climates or grown under cooler conditions. To do this, we grew *E. grandis* seedlings collected from multiple mother trees across a temperature gradient under controlled cool-humid, warm-humid, and warm-dry chamber treatments.

The glasshouse experiment included 59 naturally germinated *E. grandis* seedlings collected from the base of 11 likely mother trees across six sites, and grown in pots at James Cook University's Environmental Research Complex in Cairns (Figures 1, 2, and S1). The number of mother trees per site ranged from one to three. Seedlings originated from sites spanning a mean annual temperature range of 18.8°C to 24.9°C.

To test the effects of climate of origin and growth environment on leaf traits, we allocated seedlings to one of three climate-controlled chambers: a cool-humid chamber (26°C, 1 kPa VPD), a warm-humid chamber (32°C, 1 kPa VPD), and a warm-dry chamber (32°C, 2 kPa VPD). These treatments simulated typical summer conditions in the uplands (cool-humid) and lowlands (warm-dry), with the warm-humid chamber used to isolate the effects of VPD from temperature (Table S1).

In August 2022, one to two seedlings per mother tree were randomly assigned across the chambers. Two seedlings in the cool-humid treatment died, leaving 57 surviving seedlings while maintaining representation across all mother trees. After 2 months, leaf-level gas exchange and functional traits were measured on new, fully expanded sun leaves. These trait data were used to calculate ΔT_{trait} following the same approach as for field measured traits. Detailed measurement protocols are described in Supporting Information, Methods S2.

2.14 | Analysis

Our goal was to assess if coordinated variation in leaf traits across tropical tree distributions reflects adaptation to temperature through increased heat avoidance or tolerance at warmer sites.

We first quantified overall intraspecific variation, population differentiation, and the association of trait variation with thermal gradients. Traits included leaf functional traits (e.g., LMA, LDMC, leaf thickness, and $\delta^{13}C$) or thermal parameters (e.g., T_{50}, ΔT_{trait}, and TSM). Intraspecific variation was assessed using the quartile coefficient of variation to account for issues with standard CV (Botta-Dukát 2023). Population differentiation in leaf traits was measured using P_{ST}, the phenotypic analogue of F_{ST}, which quantifies the proportion of total trait variance attributable to differences among populations. If the 95% confidence intervals for P_{ST} exceed F_{ST}, this can indicate trait divergence beyond neutral expectations. We also used R^2 from a linear model with population identity as the predictor to estimate trait differentiation, analogous to P_{ST} but without assuming trait heritability. Finally, linear regression was used to test for associations between leaf traits and the max temperature of the warmest month (MTWM).

To examine if trait variation across sites was an adaptation to thermal gradients across species distributions, we identified signals of selection using association analysis and compared the relative importance of environmental predictors in genomic versus trait generalized dissimilarity modeling. To complement

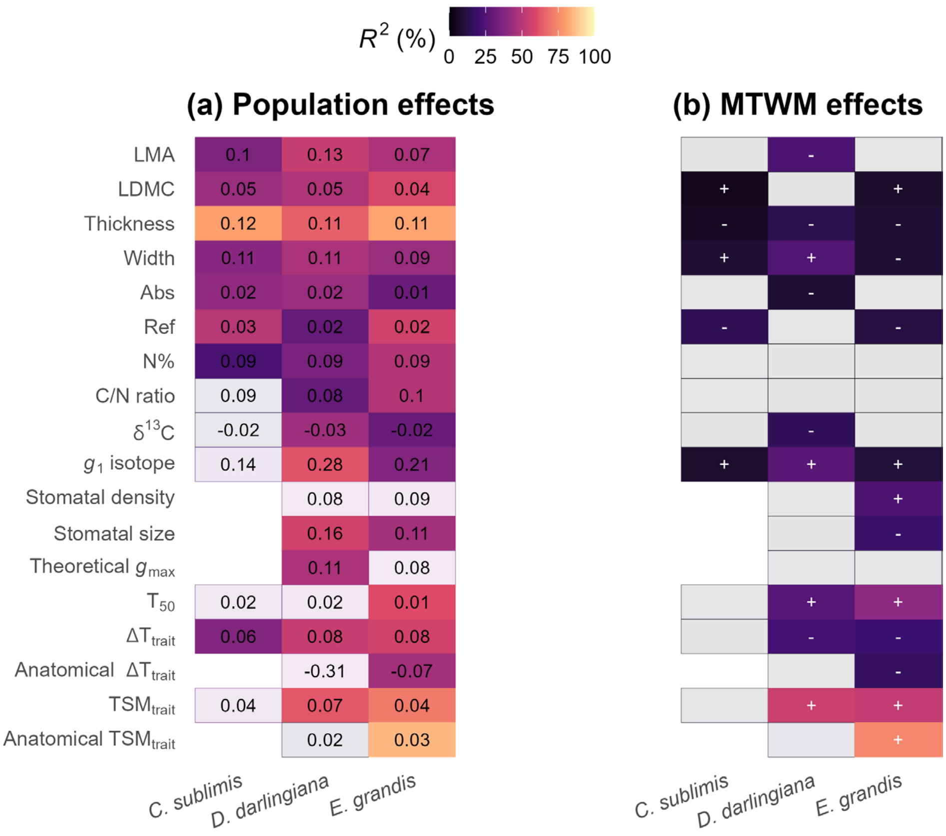


FIGURE 2 | Comparison of regression results for the effect of population or max temperature of the warmest month (MTWM) on trait variation for each species. Tiles are colored by R^2, with transparent tiles indicating non-significant ($p > 0.05$) results. Values within tiles for panel a) show the quartile coefficient of variation, and symbols in panel b) show the sign of the slope estimate for the linear regression. For more information on model results, see Tables S2 and S3.

association analyses and explicitly test the contribution of genotype and environment to intraspecific variation in leaf traits and ΔT_{trait} for *E. grandis* saplings grown in the glasshouse, we used mixed effects models to test the influence of mean annual temperature of origin (continuous) and treatment (cool-humid, warm-humid, warm-dry) on leaf traits and ΔT_{trait}, including ‘mother tree’ as a random effect nested within ‘site’. We then removed non-significant variables and presented estimated marginal means for the final models.

3 | Results

3.1 | Extent of Intraspecific Leaf Trait Variation in Natural Populations

In most cases, intraspecific trait variation (defined as the quartile coefficient of variation, CV) for each trait was similar across the three species (Figure 2a). Traits with the lowest quartile CV (<0.05) included LDMC, Absorptance, Reflectance, δ^{13}C, and T_{50}. The trait g_1, which is calculated from δ^{13}C (but accounts for some influence of the environment), had the highest variation and showed the greatest differences in CV across species, with CV = 0.14, 0.28, and 0.21 in *C. sublimis*, *D. darlingiana*, and *E. grandis*, respectively. For most traits, population effects on trait variation were significant ($p < 0.05$), except for leaf C/N ratio, δ^{13}C, g_1, and T_{50} in *C. sublimis*; stomatal density and T_{50} in *D. darlingiana*; and stomatal density and theoretical g_{max} in *E. grandis* (Figure 2a, Table S2). Where significant population effects on trait variation were observed, population explained between 23% and 85% of trait variation across all species and traits, with the strongest effects observed for leaf thickness (Figure 2a, Table S2). Population differentiation for most traits was stronger than expected because of genetic drift ($P_{ST} > F_{ST}$), except for leaf elemental concentrations or T_{50} in *C. sublimis* (Figure S2).

3.2 | Relationship Between Leaf Traits and Maximum Temperature of the Warmest Month

We observed differences in the relationship between traits and max temperature of the warmest month (MTWM) across the three target species, although the explained variance was relatively low for many of these traits (Figures 2b and S3, Table S3). Across all species, g_1 increased with MTWM, suggesting a decline in intrinsic water use efficiency potentially driven by higher stomatal conductance at warmer growth temperatures. Leaf thickness also consistently decreased with increasing MTWM across all species, indicating the development of thinner leaves in warmer climates. In *C. sublimis*, LDMC and leaf width increased with MTWM, and reflectance decreased (Figures 2b and S3, Table S3). For *D. darlingiana*, as MTWM increased, LMA, Absorptance, and δ^{13}C all decreased, whereas leaf width increased (Figures 2b and S3, Table S3). In *E. grandis*, as MTWM increased, width, reflectance, and stomatal size decreased, whereas LDMC and stomatal

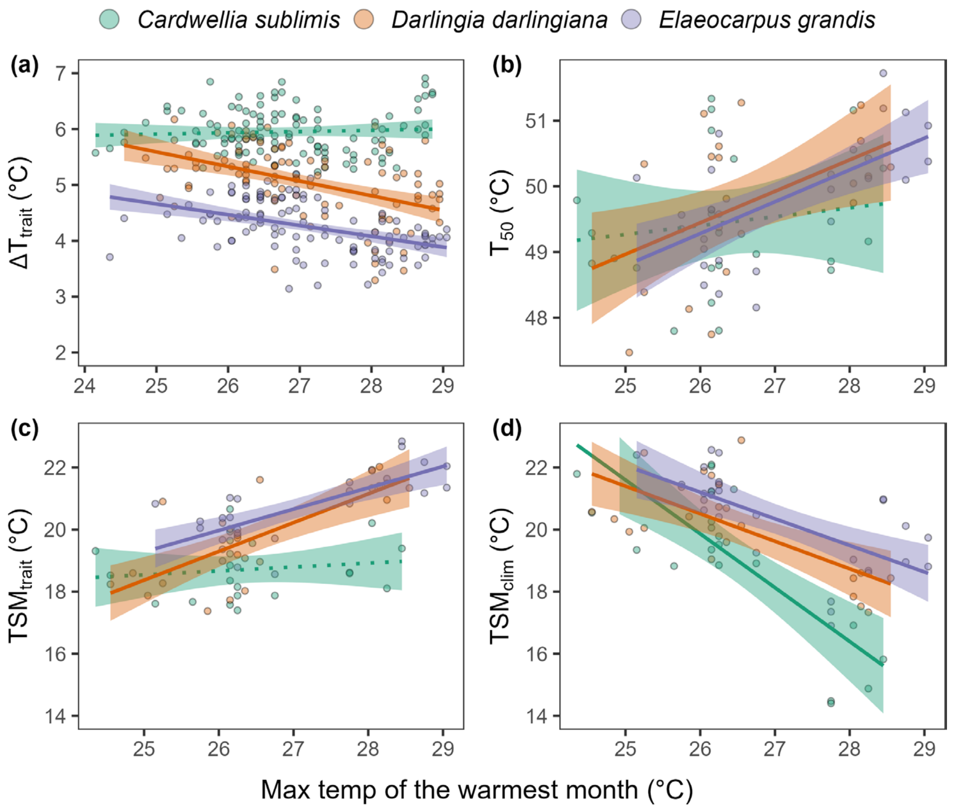


FIGURE 3 | Variation in daytime mean leaf-to-air temperature differences (ΔT_{trait}, a), thermal tolerance metrics (T_{50}, b), and thermal safety margins (TSM; c, d) with max temperature of the warmest month across the distribution of three tropical tree species. Note in a and c ΔT is only based on leaf trait variation, with the same microclimate inputs for all trees, whereas in d, both traits and microclimate inputs vary with each individual tree (TSM_{clim}). Microclimate inputs are derived from NicheMapR and not from max temp of the warmest month. Each point represents an individual-tree level average. Significant correlations with max temperature of the warmest month are denoted by solid regression lines, whereas non-significant correlations are dotted. The shaded region represents standard errors.

density increased (Figures 2b and S3, Table S3). For *D. darlingiana* and *E. grandis*, the covariation between stomatal density and size resulted in no significant change in theoretical g_{max} with MTWM (Figures 2b and S3, Table S3).

3.3 | Implications of Intraspecific Trait Variation on Leaf Thermoregulation

The consequences of intraspecific trait trade-offs for leaf thermoregulation were investigated through the prediction of trait-based leaf-to-air temperature differences (ΔT_{trait}) and how these vary with MTWM. Note that ΔT_{trait} is calculated using a single set of microclimate parameters and thus represents the influence of traits only. Mean ΔT_{trait} was 6.0°C, 5.1°C, and 4.2°C in *C. sublimis*, *D. darlingiana*, and *E. grandis*, respectively (Table S2). Intraspecific variation in ΔT_{trait} was similar across the three species, with quartile CV = 0.06 to 0.08 (Figure 2a). Population was a significant predictor of ΔT_{trait} for all species, although the proportion explained was slightly higher for *D. darlingiana* and *E. grandis* than for *C. sublimis* (Figure 2a and Table S2). We found evidence of leaf thermoregulation for two of the three species, with ΔT_{trait} decreasing with increasing MTWM for *D. darlingiana* ($R^2 = 0.22$, $p < 0.001$) and *E. grandis* ($R^2 = 0.2$, $p < 0.001$) but not for *C. sublimis* (Figures 2b and 3, Table S3). This represented a 0.26°C (95% CI: 0.16-0.36) and a 0.19°C (95% CI: 0.12-0.27) decrease in ΔT_{trait} per 1°C increase in MTWM for *D. darlingiana* and *E. grandis,* respectively (Table S3). In other words, in these two species, leaf traits varied with climate in such a way that modeled leaf temperatures were closer to air temperatures under common conditions, and populations from warmer sites were predicted to experience less leaf heating relative to air temperature than those from cooler sites.

Unsurprisingly, using theoretical g_{max} from stomatal anatomy to calculate trait-based leaf-to-air temperature differences (anatomical ΔT_{trait}) led to substantially lower values of ΔT_{trait} (Figure S4). Mean anatomical ΔT_{trait} was equal to −0.6°C for *D. darlingiana* and −1.8°C for *E. grandis* (with negative values indicating T_{leaf} cooler than T_{air}). Levels of intraspecific variation for anatomical ΔT_{trait} in *D. darlingiana* and *E. grandis* were different, with a quartile CV = −0.31 for *D. darlingiana* and −0.07 for *E. grandis* (Figure 2a). Population was only a significant predictor of anatomical ΔT_{trait} for *E. grandis*, with $R^2 = 0.45$, $p = 0.014$ (Figure 2a and Table S2). This time we found evidence of thermoregulation only for *E. grandis* ($R^2 = 0.17$, $p = 0.00495$) where anatomical ΔT_{trait} decreased 0.09°C (95% CI: 0.03-0.15) per 1°C increase in MTWM (Figures 2b and S4, Table S3).

3.4 | Intraspecific Variation in Thermal Tolerance

Thermal tolerance metrics were similar for all three species, with species-mean T_{50} ranging from 49.5°C to 49.8°C (Table S2).

Intraspecific variation was low relative to other traits, and population differences were only significant in *E. grandis* (Figure 2a and Table S2). We observed significant positive relationships between T_{50} and MTWM for both *D. darlingiana* and *E. grandis*, but not *C. sublimis* (Figures 2b and 3, Table S3). T_{50} increased by 0.48°C per 1°C rise in MTWM for both *D. darlingiana* ($R^2 = 0.26$, $p = 0.044$) and *E. grandis* ($R^2 = 0.40$, $p < 0.001$, Figure 3).

3.5 | Isolating Trait-Mediated Effects on Thermal Safety Margins

The combined variation in T_{50} and ΔT_{trait} was assessed through calculation of trait-based thermal safety margins ($\text{TSM}_{\text{trait}} = T_{50} - T_{\text{leaf}}$ °C), using common microclimate inputs across all individuals. Because environmental conditions are held constant, all variation in $\text{TSM}_{\text{trait}}$ reflects the effects of either T_{50} or trait-mediated cooling. We observed a significant increase in $\text{TSM}_{\text{trait}}$ with MTWM for *D. darlingiana* and *E. grandis*, with $\text{TSM}_{\text{trait}}$ increasing by 0.93°C per 1°C increase in MTWM for *D. darlingiana* (95% CI: 0.53–1.32), and by 0.69°C for *E. grandis* (95% CI: 0.42–0.95). No significant relationship was found for *C. sublimis* (Figures 2b and 3, Table S3). This pattern reflects coordinated variation in both thermal tolerance and trait-mediated leaf cooling, with thermal tolerance contributing approximately twice as much to the increase in thermal safety margins across the temperature gradient. When calculating trait-based thermal safety margins using stomatal anatomical traits (anatomical $\text{TSM}_{\text{trait}}$), we only observed a relationship with MTWM in *E. grandis* ($R^2 = 0.74$, $p < 0.0001$), representing an increase in $\text{TSM}_{\text{trait}}$ of 0.83°C (95% CI: 0.55-1.11) per 1°C increase in MTWM (Figures 2b and S4, Table S3).

3.6 | Variation in Operational Thermal Safety Margins

To demonstrate the effect of this intraspecific variation on operational TSMs, we plot TSM calculated with individual tree-level variation in both traits and modeled microclimate (daytime mean radiation, humidity, wind speed, and air temperature) (Figure 3). T_{leaf} is still higher in warmer climates, but for *E. grandis* and *D. darlingiana*, this is to a lesser extent than would have been if they exhibited no trait variation across the thermal gradient. As a result, TSM_{clim} declined significantly more slowly with MTWM in *D. darlingiana* and *E. grandis* (slopes = −0.89 and −0.86, respectively) than in *C. sublimis* (slope = −1.74), indicating a stronger buffering capacity in species that exhibit trait-based thermoregulation and adjustments in heat tolerance.

3.7 | Genetic Diversity and Population Structure

Genetic diversity was relatively low in our study species. Expected heterozygosity across all populations was 0.19 (±0.012 SD) in *C. sublimis*, 0.19 (±0.011 SD) in *D. darlingiana*, and 0.23 (±0.013 SD) in *E. grandis* (Table S4). Observed heterozygosity was less than expected heterozygosity for all populations of all species and was 0.15 (±0.014 SD) in *C. sublimis*, 0.12 (±0.010 SD) in *D. darlingiana*, and 0.18 (±0.016 SD) in *E. grandis* (Table S4). All three species showed strong isolation by distance, with mantel tests showing R^2 of 0.86 ($p = 0.001$) in *C. sublimis* (Figure S5), R^2 of 0.77 ($p = 0.001$) in *D. darlingiana* (Figure S6), and R^2 of 0.51 ($p = 0.011$) in *E. grandis* (Figure S7). Ancestry analysis indicated the presence of 1, 2, or 3 genetic clusters across the sampled range, supported by PCA analysis. The main break for all species corresponds to the Black Mountain Corridor, a biogeographic barrier for multiple taxa coinciding with a break in the Great Dividing Range (Figures S4–S6) (Schneider et al. 1998).

3.8 | Genomic Signals of Adaptation

We identified genomic signals of adaptation in all three species using multiple complementary methods (Figure 4). These included F_{ST} outlier detection, GPA, and GEA, applied using both univariate (LFMM) and multivariate (pRDA) approaches.

LFMM identified relatively few candidate SNP overall (Table S5), reflecting its conservative detection of strong single-locus signals. *D. darlingiana* exhibited the clearest LFMM signal, particularly with N_{mass} (25 SNPs) and g_1 (11 SNPs)—traits tied to photosynthesis and stomatal behavior. In contrast, few or no SNPs were detected in *E. grandis* and *C. sublimis* for ΔT_{trait} or leaf traits. *D. darlingiana* also showed broader environmental associations, particularly with RH_{min}, MTWM, and soil phosphorus.

Multivariate pRDA identified more candidate SNPs than LFMM across all species (Table S6), highlighting its greater sensitivity to polygenic patterns and small-effect loci that may be missed by univariate approaches. Trait-based pRDAs yielded 28 to 69 SNPs per species, and environment-based pRDAs identified between 31 and 74 SNPs. In trait-based pRDA, RDA loadings were most strongly influenced by thickness and LMA (*C. sublimis*), absorptance and g_1 (*D. darlingiana*), and thickness and N_{mass} (*E. grandis*) (Figure S8). In environmental pRDAs, Bio14, RH_{min}, and MTWM consistently explained the most variation (Figure S8), aligning with LFMM results. The explanatory power of these pRDAs was low (Adjusted R^2 values all < 0.01) but within the expected range for genome scans, where most genetic variation is neutral. Explanatory power was higher for the environment pRDAs (0.0063–0.0177) than for trait pRDAs (0.0019–0.0031), suggesting that some key traits under selection may not have been captured.

PCAdapt identified the largest number of candidate SNPs, especially in *C. sublimis* (243 SNPs), which also exhibited the strongest isolation by distance. Although this may indicate adaptation, it could also reflect underlying neutral structure, as PCAdapt is sensitive to demographic effects.

Despite the large number of SNPs identified, overlap across all three methods within each species was limited (Figure 4), with only one SNP in *C. sublimis* shared across GPA, GEA, and outlier analyses. However, all species showed overlap between at least two methods, including between GPA and GEA, providing stronger evidence that phenotypic variation is shaped by environmental selection. This partial concordance reflects the different selection signals captured by each method and suggests a

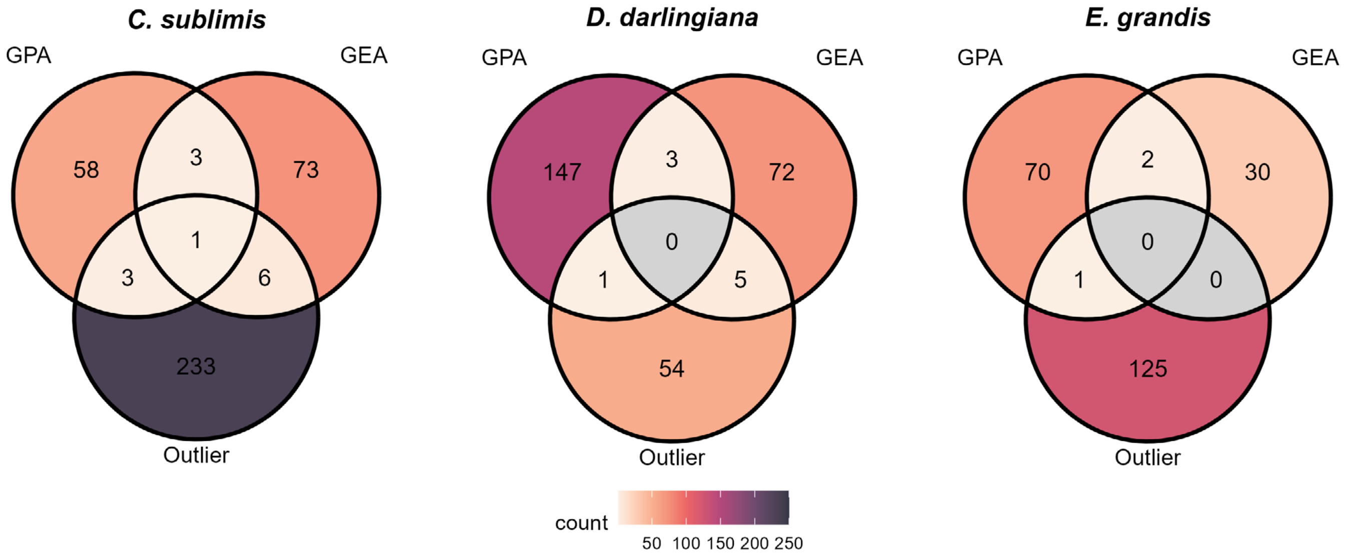


FIGURE 4 | Overlap of candidate SNPs identified using F_{ST} outlier analysis, and genotype phenotype (GPA) and genotype environment (GEA) association analyses for three tropical tree species. Numbers indicate the total number of overlapping SNPs across analyses. Candidate SNPs identified from GPA and GEA include all SNPs identified using either latent factor mixed modeling (LFMM) or partial redundancy analysis (pRDA). For GPA analyses, candidate SNPs were identified using two different sets of response variables: modeled leaf-to-air temperature differences ($\Delta T_{\mathrm{trait}}$), and the principal component axes derived from a suite of leaf traits (Trait PCA). Intersections are colored by the total number of SNPs, with gray panels indicating no shared SNPs detected.

polygenic basis of adaptation involving many small-effect loci. Together, these findings highlight the value of combining multiple approaches to detect complementary signals of selection in natural populations.

3.9 | Genotypic and Trait Turnover Across Environmental Gradients

We used generalized dissimilarity modeling to investigate the relative importance of environmental predictors in explaining deviance in the spatial patterns of genetic turnover based on (1) GPA SNPs, (2) GEA and outlier SNPs, and (3) all SNPs, as well as phenotypic turnover in (4) $\Delta T_{\mathrm{trait}}$, and (5) leaf functional traits-PCA values. Genetic turnover refers to differences in allele frequencies among populations, whereas phenotypic turnover refers to differences in population-mean trait values across the landscape. Across all three species, the generalized dissimilarity models based on SNP datasets had generally high explained deviance (28.8% to 76.8%) and low intercepts (0 to 0.07), indicating the included predictors had good explanatory power. The generalized dissimilarity models based on $\Delta T_{\mathrm{trait}}$ or the leaf trait PCA values explained less variance in the response variables (explained deviance 7.2 to 36.3%, intercepts 0.04 to 0.35) (Table S7).

For SNP-based GDMs, geographic distance, maximum temperature of the warmest month, and precipitation of the driest month (Bio14) consistently emerged among the strongest predictors of allele frequency turnover across all three species GDMs, though their relative importance differs (Figure 5 and Table S7). For the two Proteaceae species, *C. sublimis* and *D. darlingiana*, soil pH was also influential, whereas minimum relative humidity ($\mathrm{RH}_{\mathrm{min}}$) was influential for *E. grandis*.

Trait-based GDM analyses differed somewhat from SNP-based GDMs, with geographic distance no longer a consistent key predictor of trait variation (Figure 5 and Table S7). Instead, environmental factors were primary drivers, although their relative importance varied among species. Notably, environmental predictors identified by trait-based GDMs strongly overlapped with those identified by SNP-based GDMs for *C. sublimis* and *D. darlingiana* but differed slightly for *E. grandis*. Specifically, for *C. sublimis*, both maximum temperature of the warmest month and moisture-related ($\mathrm{RH}_{\mathrm{min}}$, or Bio14) and soil-related (soil pH or soil phosphorus) variables were important predictors. In *D. darlingiana*, the maximum temperature of the warmest month and Bio14 were again the dominant predictors of population variation in $\Delta T_{\mathrm{trait}}$ and trait PCA values. By contrast, in *E. grandis*, the maximum temperature of the warmest month, followed by minimum relative humidity, overwhelmingly explained variation in both $\Delta T_{\mathrm{trait}}$ and trait PCA analyses, with Bio14, important in SNP-based analyses, playing minimal to no role.

3.10 | Effects of Climate of Origin and Growth Environment on Leaf Traits in *E. grandis*

In the climate-controlled glasshouse experiment, *E. grandis* showed trait variation resulting from both plastic responses to treatment conditions and adaptation to mean annual temperature of origin, but not their interaction (Figure 6 and Table S8). Leaf width had a negative correlation with MAT of origin and had significant effects of treatment, exhibiting narrower leaves when grown under warmer conditions. We observed a significant positive correlation between g_1 and MAT of origin, but no effect of treatment. Variation in N_{mass} (a proxy for V_{cmax25}) was not associated with MAT of origin, but was influenced by treatment, with plants grown under cool-humid conditions having

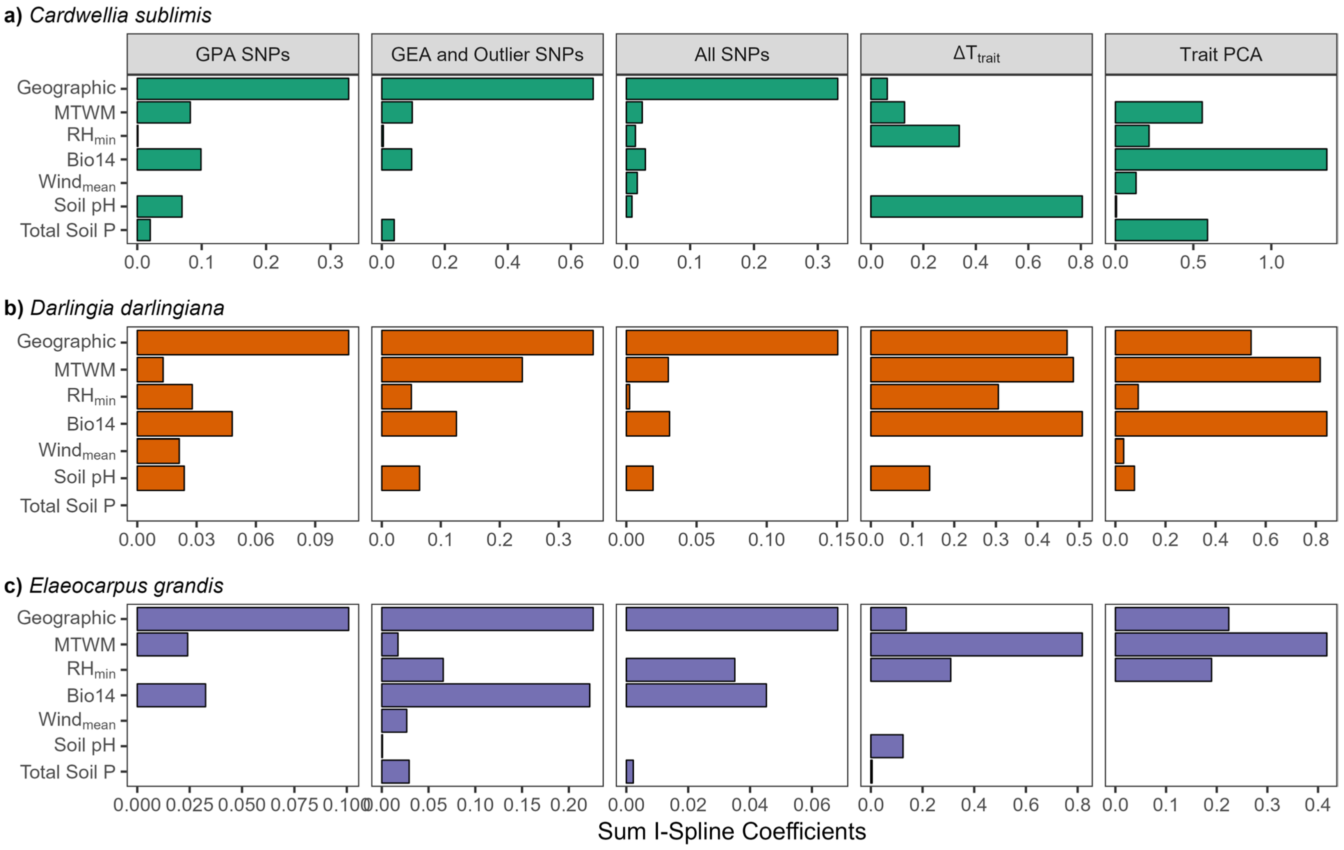


FIGURE 5 | Relative importance of predictors in generalized dissimilarity models (GDM) for three tropical tree species. Relative importance is calculated as the sum of I-Spline coefficients for that predictor in the model. Panels present results for a) Cardwellia sublimis, b) Darlingia darlingiana, and c) Elaeocarpus grandis. For each species, there are five separate analyses presented, with the response variable a population-level pairwise F_{ST} matrix on SNPs identified by GPA analyses (GPA SNPs), the SNPs identified from GEA or F_{ST} outlier analyses (GEA and Outlier SNPs), or the entire suite of neutral and non-neutral SNPs (All SNPs). For the last two columns, the response variable was a Euclidean distance matrix based on either population-mean ΔT_{trait} or principal component axes on a suite of leaf traits (Trait PCA). Model performance metrics can be found in Table S7. For the predictor variables, Geographic = geographic distance, MTWM = max temperature of the warmest month, RH_{min} = minimum relative humidity, Bio14 = precipitation of the driest month, $\text{Wind}_{\text{mean}}$ = mean wind speed.

a higher N_{mass} than plants grown under both warm-humid and warm-dry conditions.

When these traits were input into a leaf energy balance model parameterised with standard microclimate to determine ΔT_{trait}, we found evidence of adaptation to MAT of origin but no apparent effect of treatment (Figure 6 and Table S8). Seedlings from warm-origin provenances exhibited lower ΔT_{trait} than seedlings from cool-origin provenances, meaning that their traits acted to reduce leaf temperatures more effectively under common conditions. This supports the idea that intraspecific variation in leaf traits can offset the effect of higher air temperatures in warmer climates, contributing to enhanced evaporative cooling. These patterns were driven by intraspecific variation in both leaf width and g_1. The decline in ΔT_{trait} with increasing MAT of origin is likely due to the combined effects of narrower leaves and higher g_1 (indicating lower water use efficiency), both of which reduce T_{leaf} under common conditions and thus decrease ΔT_{trait}. The lack of a clear treatment effect on ΔT_{trait} likely arises from opposing responses of individual traits to treatments. Specifically, wider leaves observed in the cool treatment increase the boundary layer resistance, which decreases leaf total conductance compared to narrower leaves in the warm treatment. However, cool treatment leaves also show increased N_{mass}, which the stomatal model translates into higher stomatal conductance (for leaves with common g_1). These trait responses effectively cancel each other out, so whereas trait acclimation did affect ΔT_{trait}, the outcome was a maintenance of ΔT_{trait} across treatments.

4 | Discussion

Globally increasing temperatures threaten to push tropical rainforest plants beyond their physiological limits. However, populations adapted to contrasting climates may exhibit phenotypic divergence because of local adaptation in trait means, phenotypic plasticity, or both, that affect their capacity to cope with warming. Intraspecific variation of leaf traits across species distributions can enhance leaf cooling, thereby avoiding lethal temperatures in warmer regions. Yet, evidence of this thermoregulatory ability and the roles of acclimation or adaptation remain limited despite it being key information to assess population resilience. Here we show how leaf energy balance modeling combined with landscape genomics can assess patterns and drivers of variation in thermoregulatory traits in mature tropical rainforest trees. We found

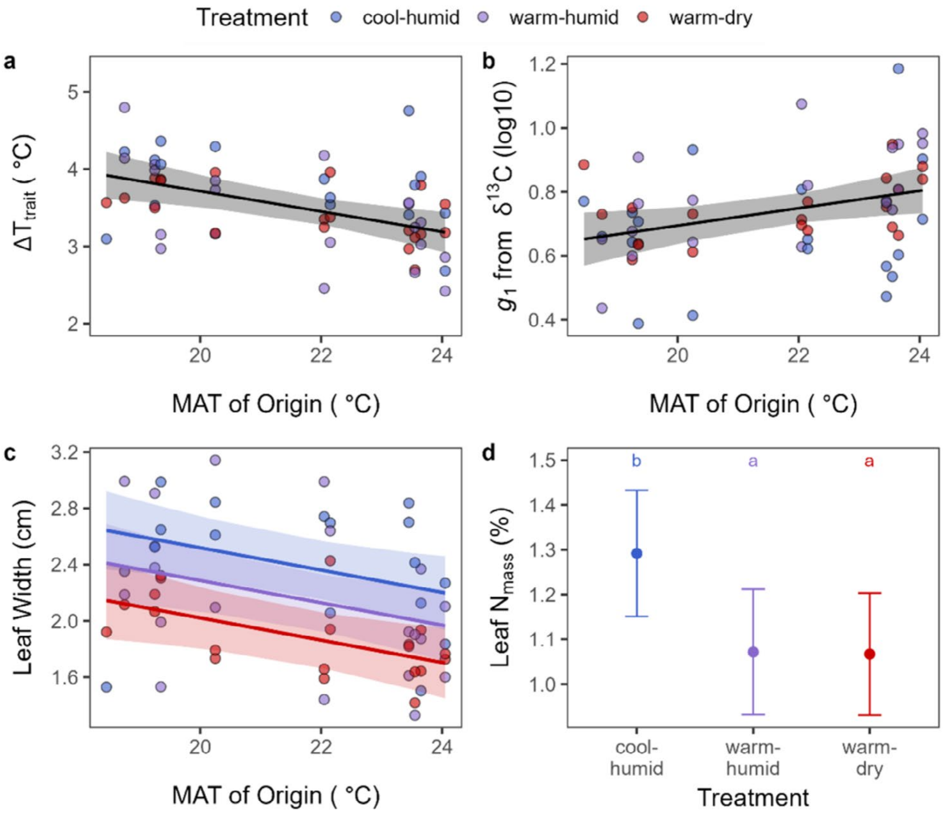


FIGURE 6 | Results from the glasshouse experiment showing significant effects ($p<0.05$) of both treatment conditions and mean annual temperature of origin for mother trees in *E. grandis*. Panels show (a) response of predicted leaf-to-air temperature differences based on leaf traits (ΔT_{trait}), (b) g_1 calculated from leaf δ^{13}C plotted as log10 transformed values, note that g_1 is inversely proportional to intrinsic water use efficiency, (c) effective leaf width, and (d) leaf N_{mass} (a proxy for V_{cmax25}). Points represent observed plant-level averages, and lines and shaded regions show the estimated marginal means and 95% confidence intervals. Letters in panel (d) show significant differences.

intraspecific variation of field-measured leaf traits associated with enhanced leaf cooling and partial maintenance of modeled thermal safety margins in warmer sites for two of the three species, providing partial support for our first hypothesis. Signals of selection were detected in all species; however, contrary to our second hypothesis, adaptive genomic variation associated with predicted ΔT_{trait} was best explained by geographic distance and moisture-related variables rather than temperature alone. Finally, our glasshouse trial of *E. grandis* seedlings supported our third hypothesis: clines in ΔT_{trait} with MAT of origin were a result of both phenotypic plasticity and adaptation.

4.1 | Intraspecific Trait Variation Leads to Enhanced Leaf Cooling in Warmer Climates

Our expectation that leaf trait variation would enhance leaf cooling in plants from warmer sites was observed in two of the three species. This is consistent with the limited homeothermy hypothesis (Mahan and Upchurch 1988; Michaletz et al. 2015), which suggests that trait-based regulation of leaf temperatures may help maintain carbon uptake in suboptimal environments. Although evidence for this phenomenon varies across biomes and species (Blonder et al. 2020; Fauset et al. 2018; Guo et al. 2023; Helliker et al. 2018; Liancourt et al. 2020; Still et al. 2022; Zhou et al. 2023), the theory is debated because of inconsistencies in its definition and testing (Garen et al. 2023; Still et al. 2023). By using a leaf energy balance model parameterized with observed leaf trait variations and common microclimate inputs, we isolate ΔT variations resulting from leaf trait covariation, excluding passive changes because of radiation, humidity, wind speed, and air temperature.

We found that traits varied significantly with MTWM in *E. grandis* and *D. darlingiana*, but their responses differed: leaf width increased with MTWM for *D. darlingiana* and decreased for *E. grandis*. Wider leaves have lower boundary layer conductance, leading to higher leaf temperatures (Leigh et al. 2017; Wright et al. 2017). Despite this, both species showed similar declines in ΔT_{trait} with increasing MAT. This suggests that variables associated with stomatal conductance (g_s) played crucial roles in offsetting the heating effect of wider leaves in *D. darlingiana*. When comparing predicted ΔT_{trait} using the coupled photosynthesis-stomatal conductance model with predictions based on theoretical maximum conductance, the ΔT_{trait} trends aligned for *E. grandis* but not for *D. darlingiana*. Given the impact of g_s modeling on our results, it is crucial to develop approaches that accurately account for how g_s varies with temperature.

We examined whether variation in leaf traits and thermal tolerance helps maintain thermal safety margins across a wide thermal gradient. Thermal tolerance increased with MTWM, whereas predicted ΔT_{trait} decreased for two of the three species. These adjustments worked together to increase both heat tolerance and avoidance in mature trees at warmer sites. When accounting for observed microclimate, this trait variation was not sufficient to perfectly maintain thermal safety margins but did lead to a shallower decrease in thermal safety margin with MTWM than for *C. sublimis*, which showed no variation in either ΔT_{trait} or thermal tolerance. This supports other studies showing that trait variation (both intra- and inter-specific) increases thermal safety margins in plants grown under warm environments compared to cooler environments (Kitudom et al. 2022; Kullberg et al. 2023; Perez and Feeley 2020). Notably, the species that exhibited thermoregulation were also those that showed increased thermal tolerance with MTWM, suggesting that avoidance and tolerance strategies can co-occur. In contrast, the species that showed no evidence of either trait-based cooling or increased thermal tolerance (*C. sublimis*) may be more vulnerable to climate change or rely on alternative mechanisms, such as leaf shedding, to mitigate heat stress. Failure to consider intraspecific trait variation in trait-based thermal safety margins may bias assessment of species vulnerability to climate change.

4.2 | Environmental Drivers of Adaptive Genomic and Phenotypic Divergence

Population differentiation for most traits was stronger than expected because of genetic drift ($P_{\text{ST}} > F_{\text{ST}}$), indicating phenotypic plasticity, divergent selection, or both in natural populations. We detected evidence of selection in all three species, with both GEA and GPA analyses identifying SNPs associated with climate and leaf traits. Moisture-related variables, particularly precipitation of the driest month and relative humidity, were the most consistent selective drivers, though the maximum temperature of the warmest month also contributed. SNPs shared across GEA and GPA approaches in all species suggest that trait variation is shaped by environmental selection. This was further supported by GDM results, which revealed similar environmental predictors of genomic turnover for both traits and climate, highlighting the role of climatic selection in driving adaptive differentiation. Moisture availability and temperature are well-established drivers of mortality (Aleixo et al. 2019; Bauman, Fortunel, Delhaye, et al. 2022), growth (Bauman, Fortunel, Cernusak, et al. 2022), regeneration (Comita and Engelbrecht 2017) and thereby species distributions (Gaviria et al. 2017) in tropical trees. Interestingly, the importance of edaphic variables such as soil pH was also reflected in our GDM for the two Proteaceae species. This is likely related to their formation of proteoid roots (Cheesman et al. 2018), an adaptation that enhances phosphorus availability in nutrient-poor and acidic soils (Griebenow et al. 2022; Lamont 2003).

Geographic distance emerged as a stronger driver of genomic variation than any single environmental variable across all species. This correlation highlights the need for caution in interpreting our results, as the adaptive signal may in part reflect neutral population structure. This aligns with evidence that functional genetic variation might be influenced by neutral processes rather than selection alone (Kardos et al. 2021; Mathur et al. 2023). Alternatively, it could indicate that loci under selection, which contribute to trait clines, may be associated with other environmental variables not explored here that correlate with geographic distance, or they may not form monotonic allele frequency clines in response to environmental gradients (Lotterhos 2023). This does not negate the presence of adaptive signals but highlights the complexity of adaptation, driven by intricate genetic systems. Thus, our findings, while pointing to potential adaptive significance, also reflect the challenges in distinguishing selection and drift in natural populations. To address this, we complemented our field-based analyses with a genotype × environment study using climate-controlled glasshouse chambers.

4.3 | Local Adaptation Drives Leaf Thermoregulation in *E. grandis*

The implications of local adaptation from the genomic analysis were supported by the climate-controlled experiment for *E. grandis* seedlings. We found that local adaptation to the climate of origin was likely responsible for the decline in predicted ΔT_{trait} with temperature across the species distribution. This was despite the acclimation of some traits to temperature or VPD. Essentially, although leaf N_{mass} (a proxy for photosynthetic capacity) and leaf width showed plasticity to growth conditions, the net effect of this trait variation on predicted ΔT_{trait} cancelled out. As a result, the variation in predicted ΔT_{trait} was primarily driven by ecotypic variation in leaf width and water use efficiency. This does not mean that plasticity is unlikely to contribute to patterns of ΔT_{trait} with temperature, as sensitivities to covarying environmental factors may differ across species and lead to different plastic and adaptive responses (Middleby, Cheesman, Hopkinson, et al. 2024). Interestingly, trait-based GDMs indicated that temperature had a greater relative importance for predicting phenotypic variation in ΔT_{trait} than it did for underlying genetic variation, suggesting that acclimation may have contributed to trait differences in the field. However, in the glasshouse experiment, although individual traits showed plastic responses to temperature and humidity, there was no significant treatment effect on ΔT_{trait} itself. This may reflect differences between the acclimation capacity of seedlings and mature trees.

Interestingly, we observed that g_1 increased with MAT of origin in the glasshouse experiment for *E. grandis* and in the field measurements for all three species, indicating a decline in intrinsic water use efficiency and increased evaporative cooling in warmer-origin populations. Although temperature and VPD were positively correlated across our field sites, declining iWUE with increasing VPD would be unexpected under typical drought adaptation scenarios, where reduced stomatal conductance is generally favored to conserve water. However, in the Wet Tropics of Queensland, warmer areas tend to be wetter, as coastal lowlands receive higher annual rainfall than upland sites in the rain shadow. In this context, lower iWUE may reflect an adaptation to warm-wet conditions, where prioritizing transpirational cooling and carbon uptake is increased in the lowlands compared to the cooler but drier uplands. Our results suggest that temperature and/or soil moisture, rather than atmospheric dryness per se, may be the dominant selective force shaping ecotypic differences in stomatal behavior in *E. grandis*.

Moreover, the generalized dissimilarity modeling on outlier SNPs identified using GPA indicated that precipitation of the driest month was a more important explanatory variable than MAT. It is possible then that ecotypic variation observed in the glasshouse was driven by changes in precipitation rather than temperature. However, because precipitation of origin was positively correlated with temperature of origin in our sampled provenances, it is difficult to disentangle their individual effects.

4.4 | Conclusions and Recommendations for Future Research

We found that across some of the species tested, local adaptation to climate significantly influences variation in leaf thermoregulatory traits. However, it remains unclear whether observed ecotypic variation is a response to thermal or moisture gradients, and whether leaf thermoregulation itself is under selection or if this is just a byproduct of adaptive variation to maintain carbon uptake or avoid water stress. Our findings indicate that limited homeothermy is present in some, but not all, tropical tree species, supporting growing evidence that although some species exhibit acclimation and/or adaptation in leaf thermal traits, patterns of intraspecific variation differ across species (Blonder et al. 2020; Kullberg and Feeley 2022; Middleby, Cheesman, and Cernusak 2024; Middleby, Cheesman, Hopkinson, et al. 2024; Tarvainen et al. 2022). As climate change continues to rapidly alter local temperature and moisture regimes, species that can increase their heat tolerance and/or heat avoidance may be less vulnerable to heat stress and better able to maintain carbon uptake and growth. Conversely, species lacking such strategies may face a heightened risk of decline or shifts in distribution (Fortier et al. 2024). This underscores the importance of considering intraspecific variation when assessing forest vulnerability to future climate change.

Future research should focus on expanding studies of plant thermoregulation by leveraging existing trait and genetic datasets to better understand the range of responses of tropical rainforest trees. Incorporating additional traits such as leaf angle and canopy architecture and accounting for dynamic rather than steady-state responses to fluctuating environments will also be important steps forward to more fully capture the processes influencing leaf thermal regulation strategies.

Author Contributions

Kali B. Middleby: conceptualization, formal analysis, funding acquisition, investigation, writing – original draft. **Rebecca Jordan:** methodology, validation, writing – review and editing. **Alexander W. Cheesman:** funding aquisition, investigation, writing – review and editing. **Maurizio Rossetto:** methodology, resources, writing – review and editing. **Martin F. Breed:** funding acquisition, supervision, writing – review and editing. **Darren M. Crayn:** funding acquisition, supervision, writing – review and editing. **Lucas A. Cernusak:** funding acquisition, supervision, writing – review and editing.

Acknowledgements

We would like to sincerely thank volunteers Flossie Brown, Tombo Warra, Michael Bewley Green, Rachael Walshe, Jayden Engert, Nara Vogado, and Sebastian Garavito Ramirez for their assistance during measurement campaigns. We are grateful to James Cook University's Daintree Rainforest Observatory and the School for Field Studies for their logistical support and access to facilities during fieldwork. We also thank Martijn Slot for editorial comments on this manuscript. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets underlying this study have been made publicly available on the Dryad data repository and can be accessed at the doi: 10.5061/dryad.wdbrv162h.

References

Aleixo, I., D. Norris, L. Hemerik, et al. 2019. "Amazonian Rainforest Tree Mortality Driven by Climate and Functional Traits." *Nature Climate Change* 9: 384–388. https://doi.org/10.1038/s41558-019-0458-0.

Arab, M. M., A. Marrano, R. Abdollahi-Arpanahi, et al. 2020. "Combining Phenotype, Genotype, and Environment to Uncover Genetic Components Underlying Water Use Efficiency in Persian Walnut." *Journal of Experimental Botany* 71, no. 3: 1107–1127. https://doi.org/10.1093/jxb/erz467.

Araujo, I., B. S. Marimon, M. C. Scalon, et al. 2021. "Trees at the Amazonia-Cerrado Transition Are Approaching High Temperature Thresholds." *Environmental Research Letters* 16, no. 3: 034047. https://doi.org/10.1088/1748-9326/abe3b9.

Barton, K. E., C. Jones, K. F. Edwards, A. B. Shiels, and T. Knight. 2020. "Local Adaptation Constrains Drought Tolerance in a Tropical Foundation Tree." *Journal of Ecology* 108, no. 4: 1540–1552. https://doi.org/10.1111/1365-2745.13354.

Bauman, D., C. Fortunel, L. A. Cernusak, et al. 2022. "Tropical Tree Growth Sensitivity to Climate Is Driven by Species Intrinsic Growth Rate and Leaf Traits." *Global Change Biology* 28, no. 4: 1414–1432. https://doi.org/10.1111/gcb.15982.

Bauman, D., C. Fortunel, G. Delhaye, et al. 2022. "Tropical Tree Mortality Has Increased With Rising Atmospheric Water Stress." *Nature* 608, no. 7923: 528–533. https://doi.org/10.1038/s41586-022-04737-7.

Blonder, B., S. Escobar, R. E. Kapas, and S. T. Michaletz. 2020. "Low Predictability of Energy Balance Traits and Leaf Temperature Metrics in Desert, Montane and Alpine Plant Communities." *Functional Ecology* 34, no. 9: 1882–1897. https://doi.org/10.1111/1365-2435.13643.

Botta-Dukát, Z. 2023. "Quartile Coefficient of Variation Is More Robust Than CV for Traits Calculated as a Ratio." *Scientific Reports* 13, no. 1: 4671. https://doi.org/10.1038/s41598-023-31711-8.

Bragg, J. G., P. Cuneo, A. Sherieff, and M. Rossetto. 2020. "Optimizing the Genetic Composition of a Translocation Population: Incorporating Constraints and Conflicting Objectives." *Molecular Ecology Resources* 20, no. 1: 54–65. https://doi.org/10.1111/1755-0998.13074.

Breed, M. F., P. A. Harrison, C. Blyth, et al. 2019. "The Potential of Genomics for Restoring Ecosystems and Biodiversity." *Nature Reviews Genetics* 20, no. 10: 615–628. https://doi.org/10.1038/s41576-019-0152-0.

Breed, M. F., M. G. Stead, K. M. Ottewell, M. G. Gardner, and A. J. Lowe. 2013. "Which Provenance and Where? Seed Sourcing Strategies for Revegetation in a Changing Environment." *Conservation Genetics* 14, no. 1: 1–10. https://doi.org/10.1007/s10592-012-0425-z.

Brun, P., N. E. Zimmermann, C. Hari, L. Pellissier, and D. N. Karger. 2022. "Global Climate-Related Predictors at Kilometer Resolution for the Past and Future." *Earth System Science Data* 14, no. 12: 5573–5603. https://doi.org/10.5194/essd-14-5573-2022.

Campbell, G. S., and J. M. Norman. 1998. *Introduction to Environmental Biophysics*. Springer.

Caye, K., B. Jumentier, J. Lepeule, and O. François. 2019. "LFMM 2: Fast and Accurate Inference of Gene-Environment Associations in Genome-Wide Studies." *Molecular Biology and Evolution* 36, no. 4: 852–860. https://doi.org/10.1093/molbev/msz008.

Cheesman, A. W., N. D. Preece, P. van Oosterzee, P. D. Erskine, and L. A. Cernusak. 2018. "The Role of Topography and Plant Functional Traits in Determining Tropical Reforestation Success." *Journal of Applied Ecology* 55, no. 2: 1029–1039. https://doi.org/10.1111/1365-2664.12980.

Comita, L. S., and B. M. J. Engelbrecht. 2017. "Drought as a Driver of Tropical Tree Species Regeneration Dynamics and Distribution Patterns." In *Forests and Global Change*, edited by D. A. Coomes, D. Burslem, and W. D. Simonson, 261–308. Cambridge University Press.

Cook, A. M., E. L. Rezende, K. Petrou, and A. Leigh. 2024. "Beyond a Single Temperature Threshold: Applying a Cumulative Thermal Stress Framework to Plant Heat Tolerance." *Ecology Letters* 27, no. 3: e14416. https://doi.org/10.1111/ele.14416.

De Kauwe, M. G., B. E. Medlyn, A. J. Pitman, et al. 2019. "Examining the Evidence for Decoupling Between Photosynthesis and Transpiration During Heat Extremes." *Biogeosciences* 16, no. 4: 903–916. https://doi.org/10.5194/bg-16-903-2019.

Diao, H., L. A. Cernusak, M. Saurer, A. Gessler, R. T. W. Siegwolf, and M. M. Lehmann. 2024. "Uncoupling of Stomatal Conductance and Photosynthesis at High Temperatures: Mechanistic Insights From Online Stable Isotope Techniques." *New Phytologist* 241, no. 6: 2366–2378. https://doi.org/10.1111/nph.19558.

Doughty, C. E., J. M. Keany, B. C. Wiebe, et al. 2023. "Tropical Forests Are Approaching Critical Temperature Thresholds." *Nature* 621: 105–111. https://doi.org/10.1038/s41586-023-06391-z.

Drake, J. E., M. G. Tjoelker, A. Varhammar, et al. 2018. "Trees Tolerate an Extreme Heatwave via Sustained Transpirational Cooling and Increased Leaf Thermal Tolerance." *Global Change Biology* 24, no. 6: 2390–2402. https://doi.org/10.1111/gcb.14037.

Duursma, R. A. 2015. "Plantecophys—An R Package for Analysing and Modelling Leaf Gas Exchange Data." *PLoS One* 10, no. 11: e0143346. https://doi.org/10.1371/journal.pone.0143346.

Farquhar, G. D., S. V. Caemmerer, and J. A. Berry. 1980. "A Biochemical-Model of Photosynthetic CO_2 Assimilation in Leaves of C-3 Species." *Planta* 149, no. 1: 78–90. https://doi.org/10.1007/bf00386231.

Fauset, S., H. C. Freitas, D. R. Galbraith, et al. 2018. "Differences in Leaf Thermoregulation and Water Use Strategies Between Three Co-Occurring Atlantic Forest Tree Species." *Plant Cell & Environment* 41, no. 7: 1618–1631. https://doi.org/10.1111/pce.13208.

Ferrier, S., G. Manion, J. Elith, and K. Richardson. 2007. "Using Generalized Dissimilarity Modelling to Analyse and Predict Patterns of Beta Diversity in Regional Biodiversity Assessment." *Diversity and Distributions* 13, no. 3: 252–264. https://doi.org/10.1111/j.1472-4642.2007.00341.x.

Forester, B. R., J. R. Lasky, H. H. Wagner, and D. L. Urban. 2018. "Comparing Methods for Detecting Multilocus Adaptation With Multivariate Genotype-Environment Associations." *Molecular Ecology* 27, no. 9: 2215–2233. https://doi.org/10.1111/mec.14584.

Fortier, R. P., A. T. Kullberg, R. D. Soria Ahuanari, L. Coombs, A. Ruzo, and F. K. J. Hotter. 2024. "Hotter Temperatures Reduce the Diversity and Alter the Composition of Woody Plants in an Amazonian Forest." *Global Change Biology* 30: e17555. https://doi.org/10.1111/gcb.17555.

Franks, P. J., and D. J. Beerling. 2009. "Maximum Leaf Conductance Driven by CO2 Effects on Stomatal Size and Density Over Geologic Time." *Proceedings of the National Academy of Sciences* 106, no. 25: 10343–10347. https://doi.org/10.1073/pnas.0904209106.

Frichot, E., and O. François. 2015. "LEA: An R Package for Landscape and Ecological Association Studies." *Methods in Ecology and Evolution* 6, no. 8: 925–929. https://doi.org/10.1111/2041-210x.12382.

Garen, J., L. Aparecido, B. Blonder, M. Cavaleri, M. Slot, and S. Michaletz. 2023. "Canopy-Top Measurements Do Not Accurately Quantify Canopy-Scale Leaf Thermoregulation." *Proceedings of the National Academy of Sciences of the United States of America* 120, no. 15: e2301914120. https://doi.org/10.1073/pnas.2301914120.

Gaviria, J., B. Turner, and B. Engelbrecht. 2017. "Drivers of Tree Species Distribution Across a Tropical Rainfall Gradient." *Ecosphere* 8: e01712. https://doi.org/10.1002/ecs2.1712.

Geange, S. R., P. A. Arnold, A. A. Catling, et al. 2021. "The Thermal Tolerance of Photosynthetic Tissues: A Global Systematic Review and Agenda for Future Research." *New Phytologist* 229: 2497–2513.

Griebenow, S., N. P. Makunga, S. Privett, et al. 2022. "Soil pH Influences the Organic Acid Metabolism and Exudation in Cluster Roots of Protea Species From the Mediterranean-Type Fynbos Ecosystem, Western Cape, South Africa." *Rhizosphere* 21: 100486.

Guo, Z., C. J. Still, C. K. F. Lee, et al. 2023. "Does Plant Ecosystem Thermoregulation Occur? An Extratropical Assessment at Different Spatial and Temporal Scales." *New Phytologist* 238, no. 3: 1004–1018. https://doi.org/10.1111/nph.18632.

Guo, Z. F., Z. B. Yan, B. M. Majcher, et al. 2022. "Dynamic Biotic Controls of Leaf Thermoregulation Across the Diel Timescale." *Agricultural and Forest Meteorology* 315, no. 11: 108827. https://doi.org/10.1016/j.agrformet.2022.108827.

Halbritter, A. H., S. Fior, I. Keller, et al. 2018. "Trait Differentiation and Adaptation of Plants Along Elevation Gradients." *Journal of Evolutionary Biology* 31, no. 6: 784–800. https://doi.org/10.1111/jeb.13262.

Helliker, B. R., X. Song, M. L. Goulden, et al. 2018. "Assessing the Interplay Between Canopy Energy Balance and Photosynthesis With Cellulose Delta O-18: Large-Scale Patterns and Independent Ground-Truthing." *Oecologia* 187, no. 4: 995–1007. https://doi.org/10.1007/s00442-018-4198-z.

Hijmans, R. 2022. "Raster: Geographic Data Analysis and Modeling." R Package version 3.6–11.

IPCC. 2022. *Climate Change 2022: Impacts, Adaptation, and Vulnerability (Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change)*, edited by H.-O. Pörtner, D. C. Roberts, M. Tignor, et al. 3056. Cambridge University Press. https://doi.org/10.1017/9781009325844.

Javad, A., V. Premugh, R. Tiwari, et al. 2025. "Leaf Temperatures in an Indian Tropical Forest Exceed Physiological Limits but Durations of Exposures Are Currently Not Sufficient to Cause Lasting Damage." *Global Change Biology* 31, no. 2: e70069. https://doi.org/10.1111/gcb.70069.

Jombart, T. 2008. "Adegenet: A R Package for the Multivariate Analysis of Genetic Markers." *Bioinformatics* 24, no. 11: 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Jones, H. G. 2013. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. 3rd ed. Cambridge University Press. https://doi.org/10.1017/CBO9780511845727.

Jordan, R., P. Harrison, and M. Breed. 2024. "The Eco-Evolutionary Risks of Not Changing Seed Provenancing Practices in Changing Environments." *Ecology Letters* 27: e14248. https://doi.org/10.22541/au.168570191.19000524/v1.

Kardos, M., E. E. Armstrong, S. W. Fitzpatrick, et al. 2021. "The Crucial Role of Genome-Wide Genetic Variation in Conservation." *Proceedings* *of the National Academy of Sciences of the United States of America* 118, no. 48: e2104642118. https://doi.org/10.1073/pnas.2104642118.

Karger, D. N., O. Conrad, J. Böhner, et al. 2017. “Climatologies at High Resolution for the Earth's Land Surface Areas.” *Scientific Data* 4, no. 1: 170122. https://doi.org/10.1038/sdata.2017.122.

Kearney, M., and A. Leigh. 2024. “Fast, Accurate and Accessible Calculations of Leaf Temperature and Its Physiological Consequences With NicheMapR.” *Methods in Ecology and Evolution* 15, no. 9: 1516–1531. https://doi.org/10.1111/2041-210X.14373.

Kearney, M. R., and W. P. Porter. 2020. “NicheMapR—An R Package for Biophysical Modelling: The Ectotherm and Dynamic Energy Budget Models.” *Ecography* 43, no. 1: 85–96. https://doi.org/10.1111/ecog.04680.

Keenan, K., P. Mcginnity, T. F. Cross, W. W. Crozier, and P. A. Prodöhl. 2013. “diveRsity: An R Package for the Estimation and Exploration of Population Genetics Parameters and Their Associated Errors.” *Methods in Ecology and Evolution* 4, no. 8: 782–788. https://doi.org/10.1111/2041-210x.12067.

Kelly, J. W. 2014. “Productivity and Water Use of Australian Tree Species Under Climate Change.” Thesis, Macquarie University.

Kitudom, N., S. Fauset, Y. Y. Zhou, et al. 2022. “Thermal Safety Margins of Plant Leaves Across Biomes Under a Heatwave.” *Science of the Total Environment* 806: 150416. https://doi.org/10.1016/j.scitotenv.2021.150416.

Krause, G. H., A. W. Cheesman, K. Winter, B. Krause, and A. Virgo. 2013. “Thermal Tolerance, Net CO_2 Exchange and Growth of a Tropical Tree Species, Ficus Insipida, Cultivated at Elevated Daytime and Nighttime Temperatures.” *Journal of Plant Physiology* 170, no. 9: 822–827. https://doi.org/10.1016/j.jplph.2013.01.005.

Kullberg, A. T., L. Coombs, R. D. Soria Ahuanari, R. P. Fortier, and K. J. Feeley. 2023. “Leaf Thermal Safety Margins Decline at Hotter Temperatures in a Natural Warming ‘Experiment’ in the Amazon.” *New Phytologist* 241, no. 4: 1447–1463. https://doi.org/10.1111/nph.19413.

Kullberg, A. T., and K. J. Feeley. 2022. “Limited Acclimation of Leaf Traits and Leaf Temperatures in a Subtropical Urban Heat Island.” *Tree Physiology* 42, no. 11: 2266–2281. https://doi.org/10.1093/treephys/tpac066.

Lamont, B. 2003. “Structure, Ecology and Physiology of Root Clusters - a Review.” *Plant and Soil* 248: 1–19. https://doi.org/10.1023/A:1022314613217.

Leigh, A., S. Sevanto, J. D. Close, and A. B. Nicotra. 2017. “The Influence of Leaf Size and Shape on Leaf Thermal Dynamics: Does Theory Hold Up Under Natural Conditions?” *Plant Cell & Environment* 40, no. 2: 237–248. https://doi.org/10.1111/pce.12857.

Leites, L., and M. B. Garzón. 2023. “Forest Tree Species Adaptation to Climate Across Biomes: Building on the Legacy of Ecological Genetics to Anticipate Responses to Climate Change.” *Global Change Biology* 29, no. 17: 4711–4730. https://doi.org/10.1111/gcb.16711.

Leon-Garcia, I. V., and E. Lasso. 2019. “High Heat Tolerance in Plants From the Andean Highlands: Implications for Paramos in a Warmer World.” *PLoS One* 14, no. 11: e0224218. https://doi.org/10.1371/journal.pone.0224218.

Leuzinger, S., and C. Korner. 2007. “Tree Species Diversity Affects Canopy Leaf Temperatures in a Mature Temperate Forest.” *Agricultural and Forest Meteorology* 146, no. 1–2: 29–37. https://doi.org/10.1016/j.agrformet.2007.05.007.

Liancourt, P., X. Song, M. Macek, J. Santrucek, and J. Dolezal. 2020. “Plant's-Eye View of Temperature Governs Elevational Distributions.” *Global Change Biology* 26, no. 7: 4094–4103. https://doi.org/10.1111/gcb.15129.

Lotterhos, K. 2023. “The Paradox of Adaptive Trait Clines With Nonclinal Patterns in the Underlying Genes.” *Proceedings of the National Academy of Sciences of the United States of America* 120: e2220313120. https://doi.org/10.1073/pnas.2220313120.

Luu, K., E. Bazin, and M. G. B. Blum. 2017. “Pcadapt: An R Package to Perform Genome Scans for Selection Based on Principal Component Analysis.” *Molecular Ecology Resources* 17, no. 1: 67–77. https://doi.org/10.1111/1755-0998.12592.

Maclean, I. M. D., J. R. Mosedale, and J. J. Bennie. 2019. “Microclima: An r Package for Modelling Meso- and Microclimate.” *Methods in Ecology and Evolution* 10, no. 2: 280–290. https://doi.org/10.1111/2041-210x.13093.

Mahan, J. R., and D. R. Upchurch. 1988. “Maintenance of Constant Leaf Temperature by Plants. 1. Hypothesis Limited Homeothermy.” *Environmental and Experimental Botany* 28, no. 4: 351–357. https://doi.org/10.1016/0098-8472(88)90059-7.

Marchin, R. M., B. E. Medlyn, M. G. Tjoelker, and D. S. Ellsworth. 2023. “Decoupling Between Stomatal Conductance and Photosynthesis Occurs Under Extreme Heat in Broadleaf Tree Species Regardless of Water Access.” *Global Change Biology* 29, no. 22: 6319–6335. https://doi.org/10.1111/gcb.16929.

Mathias, J., and T. Hudiburg. 2022. “isocalcR: An R Package to Streamline and Standardize Stable Isotope Calculations in Ecological Research.” *Global Change Biology* 28: 7428–7436. https://doi.org/10.1111/gcb.16407.

Mathur, S., A. Mason, G. Bradburd, and L. Gibbs. 2023. “Functional Genomic Diversity Is Correlated With Neutral Genomic Diversity in Populations of an Endangered Rattlesnake.” *Proceedings of the National Academy of Sciences of the United States of America* 120: e2303043120. https://doi.org/10.1073/pnas.2303043120.

Mcelwain, J. C., C. Yiotis, and T. Lawson. 2016. “Using Modern Plant Trait Relationships Between Observed and Theoretical Maximum Stomatal Conductance and Vein Density to Examine Patterns of Plant Macroevolution.” *New Phytologist* 209, no. 1: 94–103. https://doi.org/10.1111/nph.13579.

Medlyn, B. E., R. A. Duursma, D. Eamus, et al. 2011. “Reconciling the Optimal and Empirical Approaches to Modelling Stomatal Conductance.” *Global Change Biology* 17, no. 6: 2134–2144. https://doi.org/10.1111/j.1365-2486.2010.02375.x.

Michaletz, S. T., M. D. Weiser, J. Z. Zhou, M. Kaspari, B. R. Helliker, and B. J. Enquist. 2015. “Plant Thermoregulation: Energetics, Trait-Environment Interactions, and Carbon Economics.” *Trends in Ecology & Evolution* 30, no. 12: 714–724. https://doi.org/10.1016/j.tree.2015.09.006.

Middleby, K. B., A. W. Cheesman, and L. A. Cernusak. 2024. “Impacts of Elevated Temperature and Vapour Pressure Deficit on Leaf Gas Exchange and Plant Growth Across Six Tropical Rainforest Tree Species.” *New Phytologist* 243: 648–661. https://doi.org/10.1111/nph.19822.

Middleby, K. B., A. W. Cheesman, R. Hopkinson, et al. 2024. “Ecotypic Variation in Leaf Thermoregulation and Heat Tolerance but Not Thermal Safety Margins in Tropical Trees.” *Plant, Cell & Environment* 48: 649–663. https://doi.org/10.1111/pce.15141.

Mitchard, E. T. A. 2018. “The Tropical Forest Carbon Cycle and Climate Change.” *Nature* 559, no. 7715: 527–534. https://doi.org/10.1038/s41586-018-0300-2.

Mokany, K., C. Ware, S. N. C. Woolley, S. Ferrier, and C. M. Fitzpatrick. 2022. “A Working Guide to Harnessing Generalized Dissimilarity Modelling for Biodiversity Analysis and Conservation Assessment.” *Global Ecology and Biogeography* 31, no. 4: 802–821. https://doi.org/10.1111/geb.13459.

Monteith, J., and M. Unsworth. 2013. *Principles of Environmental Physics*. 4th ed. Academic Press. https://doi.org/10.1016/B978-0-12-386910-4.00020-2.

New, M., D. Lister, M. Hulme, and I. Makin. 2002. “A High-Resolution Data Set of Surface Climate Over Global Land Areas.” *Climate Research* 21: 1–25. https://doi.org/10.3354/cr021001.

Oksanen, J., G. Simpson, F. Blanchet, et al. 2022. “Vegan: Community Ecology Package.” R package version 2.6–4.

Pau, S., M. Detto, Y. Kim, and C. J. Still. 2018. “Tropical Forest Temperature Thresholds for Gross Primary Productivity.” *Ecosphere* 9, no. 7: e02311. https://doi.org/10.1002/ecs2.2311.

Perez, T. M., and K. J. Feeley. 2020. “Photosynthetic Heat Tolerances and Extreme Leaf Temperatures.” *Functional Ecology* 34, no. 11: 2236–2245. https://doi.org/10.1111/1365-2435.13658.

Perez-Harguindeguy, N., S. Diaz, E. Garnier, et al. 2013. “New Handbook for Standardised Measurement of Plant Functional Traits Worldwide.” *Australian Journal of Botany* 61, no. 3: 167–234. https://doi.org/10.1071/bt12225.

Posch, B. C., S. E. Bush, D. F. Koepke, et al. 2024. “Intensive Leaf Cooling Promotes Tree Survival During a Record Heatwave.” *Proceedings of the National Academy of Sciences of the United States of America* 121, no. 43: e2408583121. https://doi.org/10.1073/pnas.2408583121.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. “Dose-Response Analysis Using R.” *PLoS One* 10, no. 12: e0146021. https://doi.org/10.1371/journal.pone.0146021.

Rossetto, M., J. Bragg, A. Kilian, H. McPherson, M. van der Merwe, and P. D. Wilson. 2019. “Restore and Renew: A Genomics-Era Framework for Species Provenance Delimitation.” *Restoration Ecology* 27, no. 3: 538–548. https://doi.org/10.1111/rec.12898.

Sack, L., and T. Buckley. 2016. “The Developmental Basis of Stomatal Density and Flux.” *Plant Physiology* 171: 2358–2363. https://doi.org/10.1104/pp.16.00476.

Sansaloni, C. P., C. D. Petroli, J. Carling, et al. 2010. “A High-Density Diversity Arrays Technology (DArT) Microarray for Genome-Wide Genotyping in Eucalyptus.” *Plant Methods* 6: 16. https://doi.org/10.1186/1746-4811-6-16.

Schneider, C. J., M. Cunningham, and C. Moritz. 1998. “Comparative Phylogeography and the History of Endemic Vertebrates in the Wet Tropics Rainforests of Australia.” *Molecular Ecology* 7, no. 4: 487–498. https://doi.org/10.1046/j.1365-294x.1998.00334.x.

Shryock, D. F., C. A. Havrilla, L. A. Defalco, T. C. Esque, N. A. Custer, and T. E. Wood. 2015. “Landscape Genomics of *Sphaeralcea ambigua* in the Mojave Desert: A Multivariate, Spatially-Explicit Approach to Guide Ecological Restoration.” *Conservation Genetics* 16, no. 6: 1303–1317. https://doi.org/10.1007/s10592-015-0741-1.

Shryock, D. F., L. K. Washburn, L. A. DeFalco, and T. C. Esque. 2021. “Harnessing Landscape Genomics to Identify Future Climate Resilient Genotypes in a Desert Annual.” *Molecular Ecology* 30: 698–717. https://doi.org/10.1111/mec.15672.

Sork, V. L., S. N. Aitken, R. J. Dyer, A. J. Eckert, P. Legendre, and D. B. Neale. 2013. “Putting the Landscape Into the Genomics of Trees: Approaches for Understanding Local Adaptation and Population Responses to Changing Climate.” *Tree Genetics & Genomes* 9, no. 4: 901–911. https://doi.org/10.1007/s11295-013-0596-x.

Still, C. J., G. Page, B. Rastogi, et al. 2022. “No Evidence of Canopy-Scale Leaf Thermoregulation to Cool Leaves Below Air Temperature Across a Range of Forest Ecosystems.” *Proceedings of the National Academy of Sciences of the United States of America* 119, no. 38: e2205682119. https://doi.org/10.1073/pnas.2205682119.

Still, C. J., G. F. M. Page, B. Rastogi, et al. 2023. “Reply to Garen et al.: Within-Canopy Temperature Data Also Do Not Support Limited Homeothermy.” *Proceedings of the National Academy of Sciences of the United States of America* 120, no. 15: e2302515120. https://doi.org/10.1073/pnas.2302515120.

Tarvainen, L., M. Wittemann, M. Mujawamariya, et al. 2022. “Handling the Heat – Photosynthetic Thermal Stress in Tropical Trees.” *New Phytologist* 233, no. 1: 236–250. https://doi.org/10.1111/nph.17809.

Trew, B. T., and I. M. D. Maclean. 2021. “Vulnerability of Global Biodiversity Hotspots to Climate Change.” *Global Ecology and Biogeography* 30, no. 4: 768–783. https://doi.org/10.1111/geb.13272.

UNESCO World Heritage Centre. 1988. “Inscription: Wet Tropics of Queensland (Australia).” In *World Heritage List*. UNESCO World Heritage Centre. https://whc.unesco.org/en/decisions/3670/.

Urban, J., M. Ingwers, M. A. McGuire, and R. O. Teskey. 2017. “Stomatal Conductance Increases With Rising Temperature.” *Plant Signaling & Behavior* 12, no. 8: e1356534. https://doi.org/10.1080/15592324.2017.1356534.

Weir, B. S., and W. G. Hill. 2002. “Estimating F-Statistics.” *Annual Review of Genetics* 36: 721–750.

Woods, H. A., M. Saudreau, and S. Pincebourde. 2018. “Structure Is More Important Than Physiology for Estimating Intracanopy Distributions of Leaf Temperatures.” *Ecology and Evolution* 8, no. 10: 5206–5218. https://doi.org/10.1002/ece3.4046.

Wright, I. J., N. Dong, V. Maire, et al. 2017. “Global Climatic Drivers of Leaf Size.” *Science* 357, no. 6354: 917–921. https://doi.org/10.1126/science.aal4760.

Wright, I. J., P. B. Reich, M. Westoby, et al. 2004. “The Worldwide Leaf Economics Spectrum.” *Nature* 428, no. 6985: 821–827. https://doi.org/10.1038/nature02403.

Zheng, X., D. Levine, J. Shen, S. M. Gogarten, C. Laurie, and B. S. Weir. 2012. “A High-Performance Computing Toolset for Relatedness and Principal Component Analysis of SNP Data.” *Bioinformatics* 28, no. 24: 3326–3328. https://doi.org/10.1093/bioinformatics/bts606.

Zhou, Y. Y., N. Kitudom, S. Fauset, et al. 2023. “Leaf Thermal Regulation Strategies of Canopy Species Across Four Vegetation Types Along a Temperature and Precipitation Gradient.” *Agricultural and Forest Meteorology* 343: 109766. https://doi.org/10.1016/j.agrformet.2023.109766.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** gcb70461-sup-0001-Supinfo.docx.