

# Climate Constrains Photosynthetic Strategies in Darwin's Daisies: A Test of the Climatic Variability and Jack-of-All-Trades Hypotheses

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**ABSTRACT:** Empirical evidence for the climate variability and performance trade-off hypotheses is limited to animals, and it is unclear whether climate constrains the photosynthetic strategies of plants. The plant genus *Scalesia* Arn. ex Lindl (family Asteraceae), endemic to the Galápagos archipelago, provides an ideal study system to test these hypotheses because of its species with markedly different leaf morphologies that occupy distinct climatic zones. In this study we tested the classic hypotheses that (1) climate constrains leaf size, (2) high climatic temperature variability selects for thermal generalists (i.e., the climate variability hypothesis), and (3) there is a trade-off between the breadth and rate of photosynthetic performance (i.e., jack-of-all-trades but master of none hypothesis). To do this we measured the leaf morphologies and photosynthetic temperature response curves of 11 *Scalesia* species. In support of a priori predictions, we found that small-leaved *Scalesia* species were more likely to occupy hotter and drier climates than large-leaved species, there was a positive relationship between climatic temperature variability and the breadth of photosynthetic performance, and photosynthetic performance was negatively correlated with photosynthetic breadth. Our study is among the first to provide evidence for the performance-breadth trade-off hypothesis in photosynthesis, suggesting that climate change may select for photosynthetic thermal generalists.

**Keywords:** jack-of-all-trades, climate change, Galápagos, leaf temperature, macroecology, climate variability hypothesis.

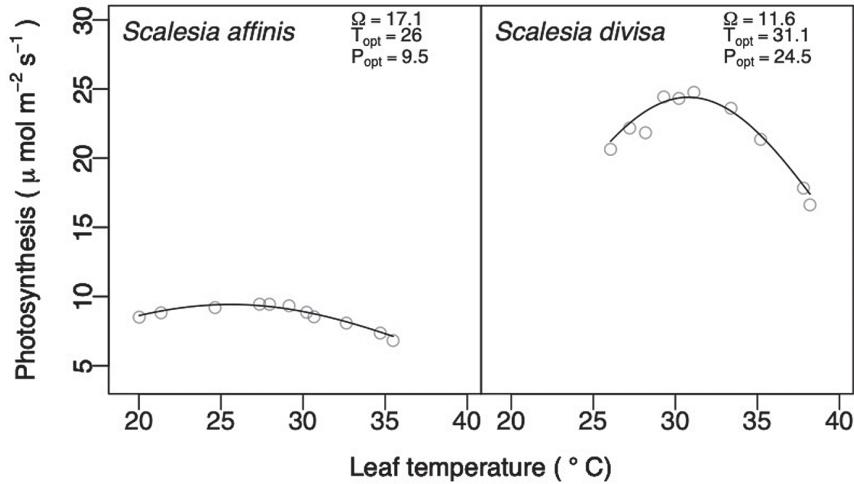
## Introduction

The climate variability hypothesis, formalized by Janzen (1967), proposes that variable climates select for species

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with large breadths of physiological function (Ghalambor et al. 2006; Perez et al. 2016). The jack-of-all-trades but master of none hypothesis proposes that there are trade-offs between species' breadth of performance and their maximum performance (Huey and Slatkin 1976). Assuming a trade-off between species' breadth of function and their maximum performance, climatic variability should also influence species' performance. However, empirical evidence for both the climatic variability hypothesis and the jack-of-all-trades but master of none hypothesis is limited to ectothermic animals (Sunday et al. 2019), and it is unclear whether climate constrains the photosynthetic strategies of plants.

Since photosynthesis is highly temperature sensitive, it follows that climate variability, and especially temperature variability, should select for plants capable of photosynthesizing over a broad range of temperatures in accordance with the climatic variability hypothesis. The breadth of photosynthetic function can be characterized by the width of the unimodal temperature–carbon assimilation curve for photosynthesis ( $\Omega$  [ $^{\circ}$ C]; June et al. 2004; Perez et al. 2021a). Large values of  $\Omega$  indicate an ability to photosynthesize over a broad range of temperatures, while smaller  $\Omega$  values are found in species with narrow photosynthetic thermal tolerances (fig. 1). In other words, high temperature variation is expected to select for photosynthetic thermal generalist species characterized by large values of  $\Omega$ , while small climatic temperature variation is expected to favor thermal specialist species with low values of  $\Omega$ .



**Figure 1:** Carbon assimilation as a function of temperature using equation (1) for two different species of *Scalesia*. Here, *Scalesia affinis* exhibits a broad photosynthetic temperature response curve ( $\Omega$ ) and a low maximum rate of photosynthesis ( $P_{\text{opt}}$ ), which is characteristic of thermal generalists, while *Scalesia divisa* exhibits a narrow  $\Omega$  and a high  $P_{\text{opt}}$ , which is expected for thermal specialists.

If climatic variability selects for thermal generalist strategies of carbon assimilation, then it may also influence species' optimum rates of photosynthesis. The performance trade-off hypothesis posits that thermal generalists are jacks-of-all-trades but masters of none. In other words, they should be characterized by broad breadths of performance (e.g., fig. 1; *Scalesia affinis*; Huey and Hertz 1984; Huey and Kingsolver 1989), but at optimal temperatures they should be outperformed by thermal specialists that have greater maximum performance over a narrower breadth of temperatures (e.g., fig. 1; *Scalesia divisa*). Therefore, if high climate variability selects for large  $\Omega$ , it may also correspond to a low maximum rate of photosynthesis ( $P_{\text{opt}}$ ), resulting in a negative relationship between  $P_{\text{opt}}$  and  $\Omega$ .

However, leaf temperatures can be decoupled from climatic temperatures because of leaf thermoregulatory traits, such as leaf size (Perez and Feeley 2020). Leaf size and shape are generally considered adaptations that help leaves maintain temperatures within optimal ranges for photosynthesis ( $T_{\text{opt}}$  [°C]; Michaletz et al. 2015; Leigh et al. 2017). Leaf morphology (specifically width and dissection) can influence leaf temperature because of its effect on leaf boundary layer conductance. The leaf boundary layer defines the transition between slow-flowing air adjacent to the leaf surface and fast-flowing air away from a leaf (Jones 2014). As the distance that air travels across a leaf surface increases, measured as leaf width, so too does the thickness of this boundary layer (Campbell and Norman 1998). Since slow-flowing air reduces heat dissipation via lowered boundary layer conductance, increases in leaf width or decreases in leaf dissection (i.e., reducing the thickness of the boundary layer) can cause increases in leaf temperature

when other leaf thermoregulatory traits remain constant (Nobel 1999). Increases in leaf area would not necessarily cause an increase in leaf temperature, since leaves can be highly divided with very small leaf widths.

Climate is known to influence leaf size, and these effects of climate are predictable at global scales (Moles et al. 2014; Wright et al. 2017). For example, large (read “wide”) leaves can be advantageous for raising leaf temperatures to optimal levels for photosynthesis in cool climates (Meinzer and Goldstein 1985). Large leaves are also advantageous in warm climates where there is adequate water supply to sustain stomatal conductance, which reduces leaf temperature and increases photosynthesis as a result of greater stomatal opening (Smith 1978; Wright et al. 2017). Conversely, hot, dry environments are likely to favor species that maximize boundary layer conductance and minimize their water requirements, which is accomplished with small (read “narrow”) or highly dissected leaves (Nicotra et al. 2011). Therefore, photosynthetic strategies may not conform to the predictions of the climate variability hypothesis if the climatic constraints on leaf morphology buffer leaf temperatures from climatic temperatures.

To understand how climate may constrain leaf morphology, photosynthetic strategy, and performance, we investigated the plant genus *Scalesia* Arn. ex Lindl. (family Asteraceae). *Scalesia* species are also referred to as Darwin's daisies, which include morphologically diverse woody species distributed in different habitats throughout the Galápagos's many large and small islands (Fernández-Mazuecos et al. 2020). Molecular evidence suggests that a common ancestor of the extant *Scalesia* species originally colonized the Galápagos's hot, dry lowlands within the past 1 million

years (Fernández-Mazuecos et al. 2020). Today, there are 15 known extant *Scalesia* species that occupy a diversity of habitats and climate zones, including the hot, dry lowlands; the cool, moist highlands; and transitional midelevations. The *Scalesia* radiation's extant species may be the product of a rapid diversification process since the Middle Pleistocene (0.13–0.7 million years ago; Fernández-Mazuecos et al. 2020). Despite recent advances in understanding the diversification process in *Scalesia*, the adaptations that have facilitated their occupancy of the Galápagos's various environments are unclear. However, *Scalesia* species are readily distinguished by their distinct leaf sizes and shapes—traits that may indicate how climate has constrained leaf morphology, selected for carbon assimilation strategies, and contributed to radiation into diverse habitats.

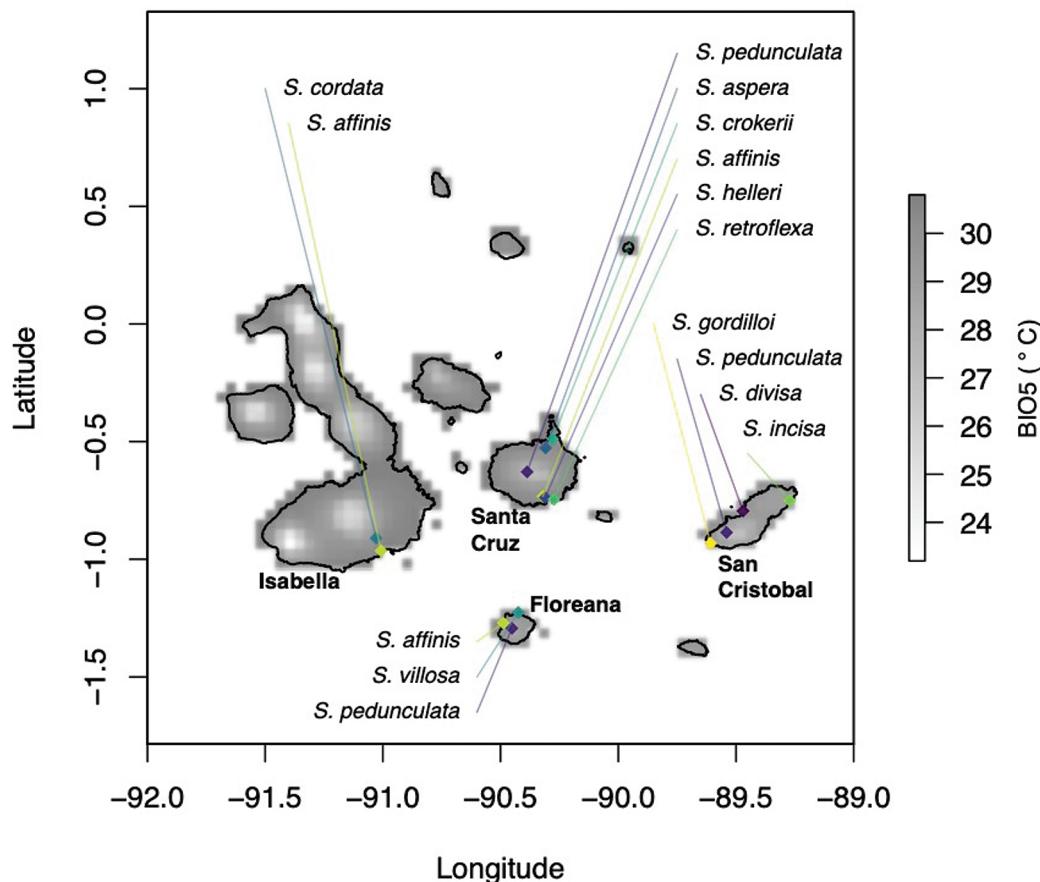
Building off the proposed relationships between climate, leaf morphology, and photosynthetic performance, we used 11 species within the *Scalesia* genus to test three hypotheses. First, we tested whether climate constrains *Scalesia* leaf morphology by testing the hypothesis ( $H_1$ ) that small-

leaved *Scalesia* species correspond to hot, dry climates and that large-leaved species correspond to cool, wet climates. We then tested the hypothesis ( $H_2$ ) that  $\Omega$  is positively related to climatic temperature variability. Finally, we tested the hypothesis ( $H_3$ ) that  $\Omega$  and  $P_{\text{opt}}$  are negatively correlated across *Scalesia* species.

## Methods

### Study Site and Species Selection

Our study was conducted on the Galápagos Islands of Santa Cruz, San Cristóbal, Isabela, and Floreana using new field data collected in July 2019. Collectively, these islands provided access to 11 of 15 different *Scalesia* species that occupy low- to high-elevation environments. We visited one population each of *S. affinis*, *S. aspera*, *S. cordata*, *S. crokerii*, *S. divisa*, *S. gordilloi*, *S. helleri*, *S. incisa*, *S. retroflexa*, *S. pedunculata*, and *S. villosa*. We also visited three disjunct populations each of *S. affinis* and *S. pedunculata* that occurred on different islands (fig. 2). These species and populations were selected



**Figure 2:** Galápagos archipelago and the locations of the *Scalesia* spp. populations sampled in this study along with the maximum temperature of the warmest month (BIO5).

because they are among the most accessible (access and logistics are extremely complicated and expensive in the Galápagos Islands outside of the few main tourist centers) but still represent considerable variation in leaf morphology. The locations of the study populations are illustrated in figure 2. We measured leaf morphological and photosynthetic traits for at least three individuals within each population of each species. We did not include *S. stewartii*, *S. atractyloides*, *S. microcephala*, or *S. baurii* in our study because of prohibitive costs and logistics.

#### Temperature-Assimilation Curves

To estimate the carbon assimilation parameters used in this study (i.e.,  $\Omega$ ,  $P_{\text{opt}}$ , and  $T_{\text{opt}}$ ), we performed temperature-assimilation curves for each individual of each study species using a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE). All of our study species occur in open habitats or experience full sun exposure as mature individuals. Temperature-assimilation curves were performed using one healthy, sun-exposed, fully expanded mature leaf from each mature individual. The LI-6800 system's ability to cool leaves in hot environments or heat leaves in cold environments is limited to approximately  $\pm 5^{\circ}\text{C}$  ambient temperature. Consequently, carbon assimilation was not necessarily measured at identical temperatures for each individual, and the range of temperatures that each leaf was exposed to varied with the environment. We subjected leaves to a gradient of temperatures from  $20^{\circ}\text{C}$  to  $27^{\circ}\text{C}$  and from  $27^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  in the coolest and hottest environments, respectively.

During all of the measurements, the LI-6800 leaf chamber was maintained at high light levels of  $1,000 \mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ . The  $\text{CO}_2$  concentration was maintained at 410 ppm (mean = 409.97, SD = 0.06) in the reference chamber. The sample chamber's relative humidity was set to 50% during sampling, but it was automatically varied as needed to prevent moisture condensation within the LI-6800 system. To avoid measurement error, the LI-6800 reference and sample chamber's infrared gas analyzers were matched any time the sample chamber's leaf temperature was changed by  $\leq 5^{\circ}\text{C}$  or  $\geq 5^{\circ}\text{C}$  since the previous match. We visually assessed stabilization of leaf temperatures, assimilation rates, and stomatal conductance before recording carbon assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). We measured carbon assimilation at 5–11 different temperatures per leaf.

Assimilation was modeled as a function of temperature following the model presented in June et al. (2004) and Perez et al. (2021b):

$$P(T) = P_{\text{opt}} \times e^{-(T_{\text{leaf}} - T_{\text{opt}})/\Omega^2}, \quad (1)$$

where  $T_{\text{leaf}}$  is leaf temperature ( $^{\circ}\text{C}$ ), and  $\Omega$  ( $^{\circ}\text{C}$ ) is defined as the difference between the temperatures above and below

$T_{\text{opt}}$  at which assimilation ( $P$ ;  $\mu\text{mol } [\text{CO}_2] \text{ m}^{-2} \text{ s}^{-1}$ ) is reduced by  $\sim 37\%$  from  $P_{\text{opt}}$  (fig. 1).

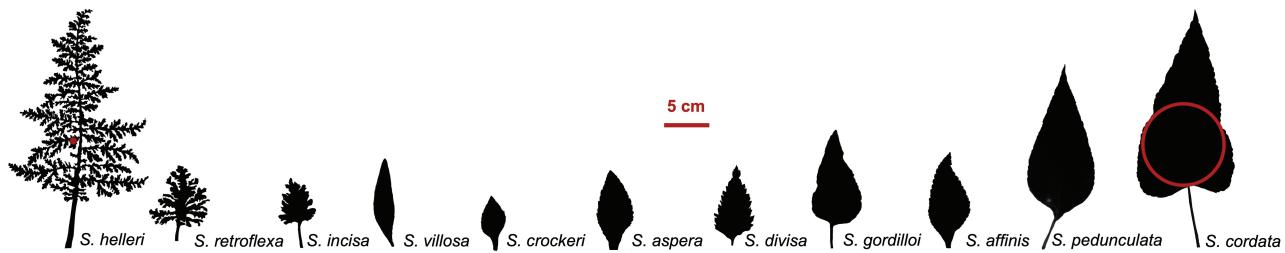
The  $P_{\text{opt}}$ ,  $T_{\text{opt}}$ , and  $\Omega$  parameters of equation (1) were estimated according to the fits of nonlinear least squares (nls) functions in R's base stats package (R Core Team 2021). We bootstrapped the parameter estimates for each species by randomly resampling our leaf temperature and assimilation data set 1,000 times with replacement. The bootstrapped means for  $P_{\text{opt}}$ ,  $T_{\text{opt}}$ , and  $\Omega$  from equation (1) were calculated for each individual. The mean  $P_{\text{opt}}$ ,  $T_{\text{opt}}$ , and  $\Omega$  were then calculated for each species for use in subsequent analyses.

#### Leaf Size and Effective Leaf Width

Following gas exchange measurements, we photographed leaves with a Canon EOS Rebel T5i against a homemade plexiglass scanner bed while leaves remained attached to the plant (destructive sampling of our leaves was prohibited). Leaf images were used to adjust the leaf area used in estimating gas exchange rates and to characterize the mean leaf size of each individual. Leaf size was quantified using the effective leaf width (cm), which is the diameter of the largest circle capable of fitting within a leaf margin. We used the effective leaf width, also referred to as the characteristic dimension, to characterize leaf size because it is used to estimate leaf boundary layer conductance, and leaf boundary layer conductance influences leaf temperature (Campbell and Norman 1998; Jones 2014). The effective leaf width is commonly used to understand leaf thermoregulation and is a more physiologically relevant metric of leaf size than leaf area given the context of our study (Parkhurst and Louks 1972; Leigh et al 2017). We measured the effective leaf width of each scanned leaf using ImageJ software (Rueden et al. 2017) and calculated the mean effective leaf width for each species. Leaf silhouettes that are representative for each species are displayed in figure 3 and include an illustration depicting the species with the largest and smallest effective leaf widths in our study.

#### Climatic Distributions

We estimated the climate distributions of each study species according to their known geographic occurrences. Species' occurrences were obtained from the Global Biodiversity and Information Facility (<http://www.gbif.org/>) using the spocc R package (Chamberlain 2020). These records were supplemented with in situ occurrences collected by an ongoing project seeking to map and monitor *Scalesia* populations with drones and inventory plots. We screened records with obvious georeferencing errors (e.g., coordinates within oceans) and excluded them from our data set. Our data set did contain some identical coordinates for occurrences from the plots because of the close proximity of some



**Figure 3:** Representative leaf silhouettes of the *Scalesia* species used in this study arranged by the smallest (left) to largest (right) effective leaf width. Circles illustrate the diameter of the largest circle capable of being inscribed within leaf margins used to estimate effective leaf width.

individuals. Since these were distinct individuals, duplicate coordinates from plots were included in our data set. We extracted the BIO5 (mean maximum temperature of the warmest month), BIO3 (isothermality), and BIO12 (total annual precipitation) variables from the WorldClim database for each occurrence (2.5 arc minute resolution; Hijmans et al. 2005). Isothermality is the ratio of the mean diurnal temperature range (BIO2) to the annual temperature seasonality (BIO4).

We selected the BIO5 and BIO12 climatic variables to test  $H_1$  because hot temperatures and precipitation are likely to act as constraints on leaf size in this system. We selected BIO3 to test  $H_2$  because it incorporates information about temperature variation over both short and longer time-scales that may explain patterns in species distributions (Chan et al. 2016). The mean of each climatic distribution was then calculated for each species. We opted to use observed geolocations rather than species distribution models to avoid overestimating *Scalesia* climatic distributions.

#### Data Analysis

We evaluated  $H_1$  by testing whether small-leaved and large-leaved *Scalesia* species were negatively and positively correlated with BIO5 and BIO12, respectively. We used effective leaf width as our proxy for leaf size because of its influence on leaf thermodynamics. To test this hypothesis, we computed a phylogenetic variance-covariance (VCV) matrix. The phylogeny we used was obtained from genotyping-by-sequencing techniques performed by Fernández-Mazuecos et al. (2020) on 15 different *Scalesia* species, and we assume this phylogeny is the best-supported evolutionary history reconstruction for this group. The four branches representing species not included in our study were dropped before analysis. The VCV matrix was created with the phyl.vcv function in the phytools R package (Revell 2012). The product of this inverse VCV and the observed trait values was divided by the sum of the inverse VCV matrix to calculate the ancestral trait value at the root of our phylogeny

(Blomberg et al. 2003; Swenson 2014). Root trait values were used to calculate a phylogenetically corrected covariance matrix among traits that was rescaled to compute Pearson's  $r$  using the cov2cor function in R's base stats package (R Core Team 2021). The  $t$  statistic and  $\alpha = .05$  were used to test for a significant correlation between effective leaf width and BIO5 and BIO12.

This approach assumes that traits followed a Brownian model of evolution and that trait variance was proportional to the branch lengths between two species and their most recent common ancestor. We decided to use the Brownian motion evolutionary model to test the null hypothesis that evolutionary change in traits associated with climate in *Scalesia* is neutral and can be attributed to genetic drift. This does not assume that a trait following Brownian motion is not under selection but that a trait can evolve following this model as long as selection acts in such a way to maintain the properties of Brownian motion.

To test  $H_2$ , we constructed a maximum likelihood phylogenetic generalized least squares model using the gls function in R's nlme package (Pinheiro et al. 2021) and the same phylogenetic trees as above. The correlation structure of our data used our phylogenetic tree and was defined using the corBrownian function with a  $\gamma$  value of 1 in the ape R package (Paradis and Schliep 2019). We evaluated our hypothesis testing whether the BIO3 climatic variable was a significant predictor of variation in the  $\Omega$  parameter at the  $\alpha = .05$  level.

Finally, to test  $H_3$ , we computed a phylogenetic variance-covariance (VCV) matrix following the steps described to test  $H_1$ . The  $t$  statistic and  $\alpha = .05$  were used to test for a significant correlation between  $P_{opt}$  and  $\Omega$ . Given the potential relationship between  $\Omega$  and  $P_{opt}$ , we also explored how isothermality may influence  $P_{opt}$ . To do this, we used a phylogenetic generalized least squares model to determine whether isothermality predicted  $P_{opt}$ . To account for missing data and data augmentation and to incorporate random effects to test nonindependence structure of the residuals, we performed Markov chain Monte Carlo

(MCMC) generalized linear mixed models (GLMMs) in the package MCMCglmm (Hadfield 2010; see the supplemental PDF). All analyses were performed using the R statistical programming language (ver. 4.1.0; R Core Team 2021).

## Results

Our final data set consisted of a total of 48 temperature-assimilation curves. The mean  $\Omega$ ,  $P_{\text{opt}}$ , and  $T_{\text{opt}}$  across all species were  $14.49^{\circ}\text{C}$  (2.83 SD),  $18.69 \mu\text{mol} [\text{CO}_2] \text{ m}^{-2} \text{ s}^{-1}$  (4.43 SD), and  $26.25^{\circ}\text{C}$  (2.70 SD), respectively. We found no significant correlation between  $T_{\text{opt}}$  and the other photosynthetic traits or climatic variables. Leaf area was not correlated to any of the variables that we report except for the effective leaf width (see the supplemental PDF).

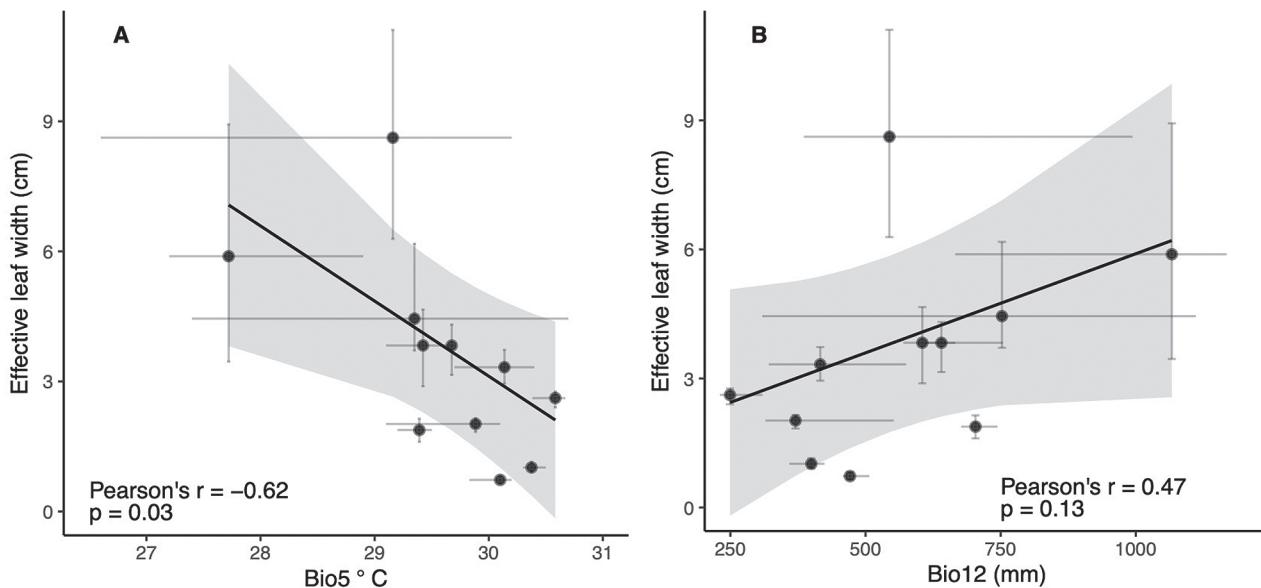
The results from the test of  $H_1$  revealed a significant ( $p = .03$ ) negative correlation ( $r = -0.62$ ) between BIO5 and effective leaf width (fig. 4A). We observed an insignificant correlation ( $r = 0.47, p = .13$ ) between BIO12 and effective leaf width (fig. 4B). When we did not correct for phylogenetic nonindependence, we observed negative correlation ( $r = -0.64, p = .03$ ) between BIO5 and effective leaf width and insignificant correlation ( $r = 0.45, p = .16$ ) between BIO12 and effective leaf width.

For  $H_2$ , our phylogenetic least squares model revealed a marginally significant positive relationship between isothermality and  $\Omega$  (table 1; fig. 5). We also found that isothermality and  $\Omega$  were significantly correlated before (Pearson's  $r = 0.63, p = .05$ ) and after (Pearson's  $r = 0.57,$

$p = .05$ ) controlling for phylogenetic nonindependence. We found that  $\Omega$  was significantly correlated with diurnal temperature range (BIO2; Pearson's  $r = 0.61, p = .05$ ) and the annual temperature seasonality (BIO4; Pearson's  $r = -0.61, p = .05$ ) only when not controlling for phylogenetic nonindependence.

For  $H_3$ , our analyses indicated that  $\Omega$  and  $P_{\text{opt}}$  were negatively correlated before (Pearson's  $r = 0.85, p < .01$ ) and after (fig. 6; Pearson's  $r = -0.83, p < .01$ ) controlling for phylogenetic independence. Our phylogenetic least squares model indicated that there was a significant positive relationship between isothermality and  $P_{\text{opt}}$  (table 2; fig. 6). We found that  $P_{\text{opt}}$  was significantly correlated with diurnal temperature range (BIO2; Pearson's  $r = -0.78, p < .01$ ) and annual temperature seasonality (BIO4; Pearson's  $r = -0.80, p < .01$ ) after controlling for phylogenetic nonindependence.

Given the relationship between isothermality and  $P_{\text{opt}}$ , we also explored how the effect of isothermality on  $P_{\text{opt}}$  might be altered depending on leaf morphology. We found that there was a marginally significant interaction between the effects of isothermality and effective leaf width on  $P_{\text{opt}}$  (fig. 7; table 2). To better understand this relationship, we used the emtrends function in the emmeans R package (Lenth 2021) to assess how  $P_{\text{opt}}$  may vary across an isothermality gradient for the *Scalesia* species with the largest and smallest leaves. We found that  $P_{\text{opt}}$  was likely to decrease at a marginally and significantly higher rate ( $p = .07$ ) for large-leaved species (slope:  $-72.7 - 8.91$ ) than for small-leaved species (slope:  $-15.1 - 8.04$ ). The results of the



**Figure 4:** Relationship between effective leaf width and maximum temperature of the warmest month (BIO5; A) and mean annual precipitation (Bio12; B). Error bars represent 95% confidence intervals of the species average.

**Table 1:** Phylogenetic generalized least squares coefficients for the photosynthetic temperature response curve ( $\Omega$ ) and isothermality (BIO3)

Predictor	Estimate	95% CI	p
Intercept	-19.63	-51.52 to 12.27	.259
Isothermality	6.10	.42 to 11.78	.064
Observations		11	
R <sup>2</sup>		.401	
AIC		56.463	

Note: AIC = Akaike information criterion; CI = confidence interval.

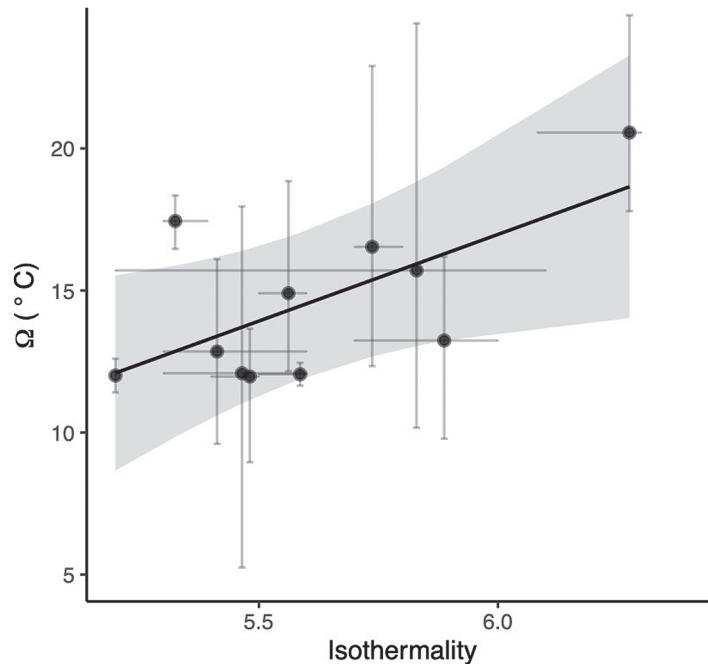
MCMC GLMMs confirm a strong negative relationship between  $\Omega$  and  $P_{\text{opt}}$  irrespective of any phylogenetic dependency (credible intervals with lower bound of -0.8 and upper bound of 0.26; table S1), and we also confirmed that isothermality significantly contributes to variation in  $P_{\text{opt}}$  among *Scalesia* species. Our results confirm that a significant proportion of the variance in  $P_{\text{opt}}$  is better explained by the individual contributions of isothermality and  $\Omega$  instead of the interaction of these two parameters (table S1).

We repeated the analyses above using the median values for our climatic data. The results of these data are presented in the supplemental PDF. The use of median climate data did not drastically alter our interpretation of our results

or our conclusions. However, median climate data did not predict a significant amount of variation in  $\Omega$ .

## Discussion

The evolutionary advantage of generalized versus specialized phenotypes has long been hypothesized to constitute a trade-off between a species' maximum performance and its performance breadth. To our knowledge few empirical works have explicitly tested this trade-off hypothesis regarding the relationship between plant functional traits and climate. In this study, we investigated how climate constrains leaf morphology (i.e., effective leaf width) and influences the ecological strategies of carbon assimilation ( $P_{\text{opt}}$ ,



**Figure 5:** Marginally significant relationship between isothermality (BIO3) and the breadth of the photosynthetic temperature response curve ( $\Omega$ ) for 11 *Scalesia* species. Phylogenetic generalized least squares model parameters are presented in table 1. Error bars represent 95% confidence intervals of the species average.

**Table 2:** Phylogenetic generalized least squares coefficients for maximum rate of photosynthesis ( $P_{\text{opt}}$ ), isothermality (BIO3), and effective leaf width

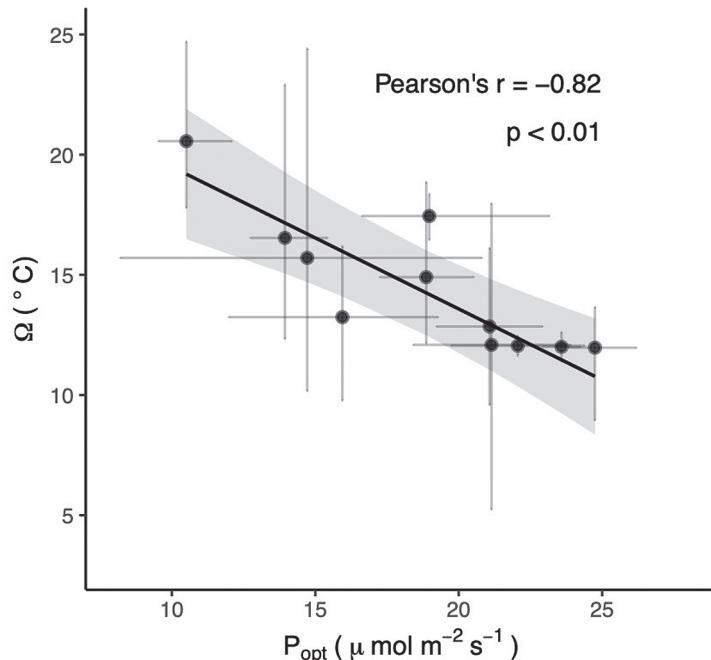
Dependent variable, predictor	Estimate	95% CI	p
$P_{\text{opt}}$ :			
Intercept	86.93	55.79 to 118.07	<.001
BIO3	-1.22	-1.78 to -.67	.002
Observations		11	
$R^2$		.719	
AIC		55.94	
$P_{\text{opt}}$ :			
Intercept	20.40	-47.03 to 87.82	.572
BIO3	-.02	-1.25 to 1.21	.974
Effective leaf width	26.54	2.35 to 50.73	.069
BIO3 × effective leaf width	-.47	-.90 to -.04	.070
Observations		11	
$R^2$		.876	
AIC		54.242	

Note: AIC = Akaike information criterion; CI = confidence interval.

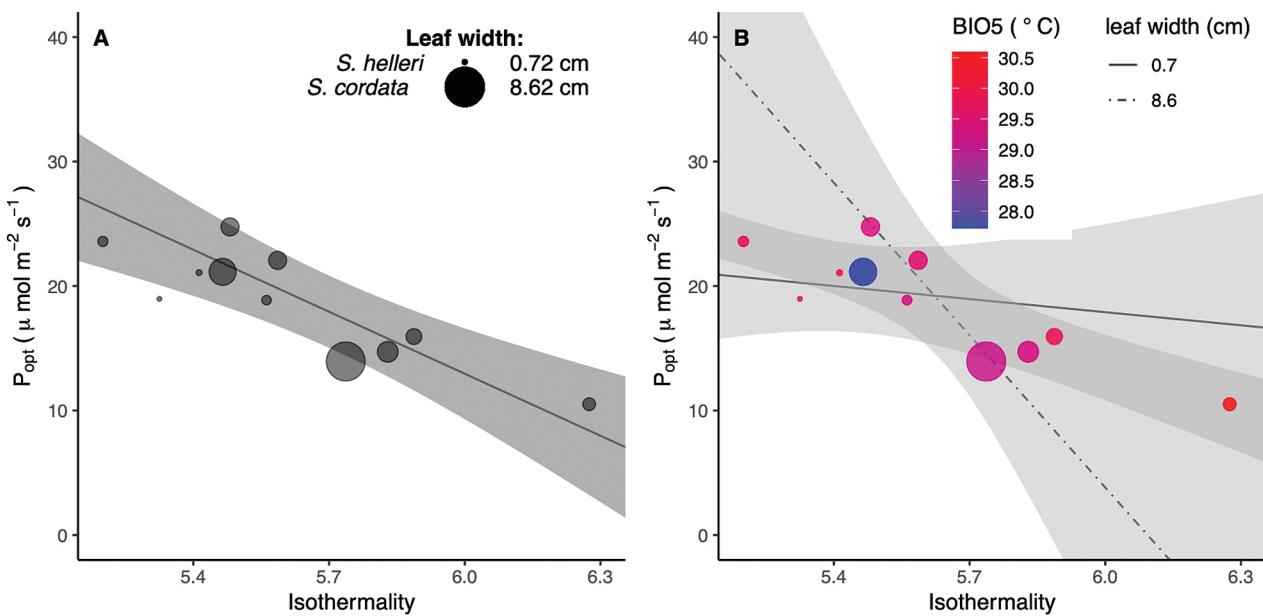
$T_{\text{opt}}$ , and  $\Omega$ ) for 11 species within the *Scalesia* genus that has radiated to occupy various habitats throughout the Galápagos archipelago.

We found mixed support for our first hypothesis ( $H_1$ ) that high temperatures and limited water availability are likely to constrain leaf size and morphology in this emblematic genus. Our results indicated that *Scalesia* species with large effective leaf widths are more likely to occur in cli-

mates with low mean maximum monthly temperatures. Our results also suggest that annual precipitation has a weaker influence on effective leaf width relative to mean maximum monthly temperatures. Although not significant, the positive relationship we observed between mean annual precipitation and effective leaf width is consistent with the hypothesis that water availability in hot climates is likely to facilitate larger leaf sizes. Indeed, populations of the tree



**Figure 6:** Significant correlation between the maximum rate of photosynthesis ( $P_{\text{opt}}$ ) and the breadth of the photosynthetic temperature response curve ( $\Omega$ ). Error bars represent 95% confidence intervals of the species average.



**Figure 7:** *A*, Significant relationship between isothermality (BIO3) and maximum rate of photosynthesis ( $P_{\text{opt}}$ ). *B*, Significant effect of the interaction between isothermality and effective leaf width on  $P_{\text{opt}}$ . Shaded areas represent the 95% confidence interval of the slope of each relationship. Phylogenetic generalized least squares model parameters are presented in table 2.

species *Scalesia pedunculata* and *Scalesia cordata*, both with unlobed leaves and larger effective leaf widths, mostly inhabit the humid highlands of Isabela, Santa Cruz, and San Cristóbal Islands. The negative relationship between leaf size and temperature was expected according to the physical principles that constrain energy balance. A predominance of larger-leaved species in colder habitats has been observed for other species groups along climatic gradients, including in the Galápagos, and also across the global latitudinal gradient (Hamann 1979; Castillo et al. 2013).

Although our results are generally consistent with global patterns in leaf morphology across climate gradients (Wright et al. 2017), plants in the Galápagos are unlikely to experience extremely cold nighttime temperatures. In cold climates and climates with low nighttime temperatures, species with large leaves are at risk of lethal freezing damage because of their thick boundary layers (Wright et al. 2017). These thick boundary layers prevent water loss from the leaves to offset nighttime radiation that can cause leaf temperatures to plunge (Jones 2014). Consequently, the negative relationship we observed between effective leaf width and maximum temperatures of the warmest month may become obscured in colder climates.

We found support for our second hypothesis ( $H_2$ ) that temperature variability predicts the breadth of  $\Omega$ . We observed that  $\Omega$  was significantly correlated with isothermality, and the phylogenetic generalized least squares model indicated that isothermality was a marginally significant

predictor of  $\Omega$ . These support the climate variability hypothesis formalized by Janzen (1967) and the tendency of the breadth of plant thermal tolerances to increase with latitude (Lancaster and Humphreys 2020). However, it is unclear how the heat tolerances in this study correspond to carbon assimilation (Perez et al. 2021b) or whether this pattern in heat tolerances is biased by sampling procedures. For example, sampling bias may have occurred because we were not able to visit different populations of each species because of logistical difficulties. Furthermore, the relationship between  $\Omega$  and isothermality was sensitive to the use of mean versus median climate data, indicating that *Scalesia* may have skewed climatic distributions and that this result should be interpreted cautiously. We also observed a negative relationship between  $P_{\text{opt}}$  and isothermality, which was expected given the strong correlation between  $P_{\text{opt}}$  and  $\Omega$ .

Our study did not explicitly consider the effects of vapor pressure deficit (VPD), but it is possible that VPD contributed to the relatively weak relationship between  $\Omega$  and isothermality. There is growing evidence that high VPD has a stronger negative effect on photosynthesis than high temperatures alone (Slot and Winter 2017b; Smith et al. 2020; Perez et al. 2021a). Rising leaf temperature increases the leaf-to-air VPD and causes stomatal closure, which in turn curtails carbon assimilation (Slot and Winter 2017a). Indeed, the hottest environments throughout the Galápagos are at low elevations, which are also likely to have the lowest

climatic temperature variability. It is possible that the relatively high temperatures in these environments result in high leaf VPD that causes consistently low rates of stomatal conductance. The high temperatures in these environments could also lead to high heat tolerance of photosystem II (PSII) photochemistry (Perez and Feeley 2020) and broad  $\Omega$  (Perez et al. 2021a) that could obfuscate the expected relationship between  $\Omega$  and isothermality.

According to the Akaike information criterion (AIC), isothermality was a better predictor of  $P_{\text{opt}}$  than  $\Omega$ . Additional analyses revealed that isothermality and effective leaf width interacted to predict variation in  $P_{\text{opt}}$ . An important prediction of this model, assuming that high  $P_{\text{opt}}$ 's are advantageous, is that *Scalesia* species with large effective leaf widths are more likely to occur in environments with low isothermality. Conversely, species with small effective leaf widths will be most successful in environments with high isothermality. However, our data also show that the species with the smallest effective leaf widths actually occur in the locations of the lowest isothermality. The lack of large-leaved *Scalesia* species in environments with extremely low isothermality may be due to a combination of the high temperatures and low water availability that could result in lethally high leaf temperatures (Leigh et al. 2012; Cook et al. 2021). More information is needed to understand the inter- and intraspecific changes in *Scalesia* leaf size across climatic gradients, but the presence of large-leaved species in wet, stable climates and small-leaved species in dry, variable climates are generally consistent with those observed at broader macroecological scales (Wright et al. 2017).

According to the AIC of our phylogenetic generalized least squares model, the interaction between isothermality and effective leaf width was more parsimonious than any model we tested for predicting variation in  $P_{\text{opt}}$  or  $\Omega$  as a function of climate. This interaction model explained 15% more of the variation in  $P_{\text{opt}}$  than isothermality alone. The ability of isothermality to explain a greater portion of variation in  $P_{\text{opt}}$  than  $\Omega$  suggests that climatic temperature variation may have a strong influence on  $P_{\text{opt}}$ , but the mechanism for this relationship is unclear. Alternatively, the strong negative relationship between isothermality and  $P_{\text{opt}}$  could be a statistical artifact of the negative correlation we observed between  $P_{\text{opt}}$  and  $\Omega$  that is strengthened through an interaction with leaf size.

The negative correlation we observed between  $P_{\text{opt}}$  and  $\Omega$  supports our third hypothesis ( $H_3$ ) that there is a trade-off in the maximum performance and the thermal performance breadth of photosynthesis. In other words, our results indicate that *Scalesia* species occupy carbon assimilation strategies on a continuum from thermal generalists that photosynthesize at low rates over a broad range of temperatures to thermal specialists that photosynthesize at high rates over a narrow range of temperatures. To our

knowledge these results are the first to support this performance trade-off hypothesis for carbon assimilation in plants and are contrary to previous studies of  $P_{\text{opt}}$  and  $\Omega$  that have failed to find this relationship. It is possible that this disparity represents a type II error resulting from a lack of adequate control for phylogenetic nonindependence in other studies (e.g., Perez et al. 2021a). Alternatively, our results may represent a type I error if the photosynthetic traits we measured are highly plastic and violate the assumptions of our phylogenetic analyses (Nicotra et al. 2005; Way and Yamori 2014; Kumarathunge et al. 2019). However, even when not controlling for phylogenetic independence, we find that  $P_{\text{opt}}$  and  $\Omega$  remain highly correlated, suggesting that there is in fact a trade-off in these traits within the *Scalesia* genus.

The physiological and molecular mechanisms underpinning the trade-off in  $P_{\text{opt}}$  and  $\Omega$  are not well understood and are likely to be the consequence of complex metabolic feedbacks within plant cells. For example,  $P_{\text{opt}}$  and  $\Omega$  are negatively and positively correlated with the heat tolerances of PSII photochemistry, respectively (Perez et al. 2021a). High PSII heat tolerance may promote photosynthesis over a broad range of temperatures at the expense of high  $P_{\text{opt}}$  because PSII tolerance is often achieved via metabolically costly processes like the production of heat shock proteins (Wahid et al. 2007), isoprenoids (Logan and Monson 1999; Taylor et al. 2019), photoprotective pigments (Krause et al. 2015), and membrane-fortifying solutes (Hüve et al. 2006) and the saturation of lipid bilayers (Zhu et al. 2018). The production of heat shock proteins has the potential to facilitate broad  $\Omega$  by preventing denaturation of some enzymes involved in photosynthesis at high temperatures, but it may reduce enzyme efficiency and photosynthetic rates by interfering with binding sites (Al-Whaibi 2011; Chen et al. 2017). Furthermore, the production of metabolites that promote PSII heat tolerance and broad  $\Omega$  may deplete pools of NADPH and ATP that are required for carbon fixation or high  $P_{\text{opt}}$  (Süss and Yordanov 1986; Gershenson 1994; Wahid et al. 2007; Voon and Lim 2019; Perez et al. 2021a).

Since the Galápagos Islands straddle the equator, they are expected to experience low seasonal temperature variation relative to diurnal temperature variation. We found that both diurnal temperature variation (BIO2) and annual temperature seasonality (BIO4) were correlated with  $\Omega$  and  $P_{\text{opt}}$ , but isothermality was the strongest predictor of both photosynthetic traits. This result highlights the importance of understanding the effect of temporal temperature variation on plant thermal ecology. The relative importance of short- versus long-term temperature variability on shaping thermal adaptations is likely to change across latitudes and may influence species' susceptibility to climate change (Chan et al. 2016; Perez et al. 2016). In the Galápagos, temperature has increased 0.06°C per year in the past 20 years, and the

representative concentration pathway (RCP) climate models RCP4.5 and RCP8.5 project an increase in temperature variability by 2050 (Escobar-Camacho et al. 2021). Additionally, future climatic scenarios suggest that precipitation may increase in coastal and highlands areas of the archipelago, which could favor invasive species over thermal specialist species in drier environments (Paltán et al. 2021). Similar global trends could produce scenarios in which thermal generalists with broader geographic ranges are favored over thermal specialists and lead to biotic homogenization (Dornelas et al. 2014).

We found that  $T_{\text{opt}}$  did not vary predictably with any climate variables, potentially indicating that climate selects for leaf sizes that help maintain leaf temperature close to  $T_{\text{opt}}$ . However, the lack of significant correlation between  $T_{\text{opt}}$  and climate variables may have been due to a combination of the relatively small sample sizes within our study and imprecise carbon assimilation measurements resulting from inherent variation in photosynthetic rates within each of the populations that we visited. Plasticity has been observed for  $T_{\text{opt}}$  (Nicotra et al. 2005; Way and Yamori 2014), which has the potential to reduce any selective pressure imposed on leaf size by climate. For example, selection for small leaves may be reduced if individuals are capable of raising their  $T_{\text{opt}}$  in response to increased leaf or air temperature. Common-garden or reciprocal transplant experiments are needed to reveal the adaptive responses of  $T_{\text{opt}}$  and leaf morphology to environmental variation.

Some argue that phylogenetic correction of photosynthetic traits should not be performed except in controlled common-garden settings (Givnish et al. 2004). Common-garden experiments control for environmental conditions that may cause variation in photosynthetic traits and isolate variation caused by genetic differences among individuals. However, reciprocal transplant studies are more feasible and suitable than common-garden experiments for understanding how variable climates have shaped *Scalesia*'s ecological strategies. This is because it is possible that the photosynthetic traits we measured are plastic and the magnitude of plasticity could be selected by climatic variability. Therefore, the *Scalesia* species we studied would need to be grown in a range of environmental conditions experienced by the entire genus (e.g., BIO3, BIO5, BIO12) to determine whether our observed photosynthetic traits were adaptive for a given climate. Although we corrected for phylogeny in our analyses, this correction actually reduced the strength of the relationships we observed when testing hypotheses 1–3, suggesting that our results are robust.

Ultimately, the strong relationships we observed between isothermality and  $\Omega$  and between  $P_{\text{opt}}$  and  $\Omega$  indicate that thermal generalist strategies within the *Scalesia* genus appear to be advantageous for colonizing environments with high climatic temperature variability. Our results also con-

firm that hot climates are likely to select for small leaves. Our findings are notable for being among the first studies to show support for the prediction that greater thermal variability should select for broader physiological tolerance (Janzen 1967; Perez et al. 2016). This is also among the first studies to find support for the performance trade-off hypothesis in photosynthesis. A reciprocal transplant study is needed to determine whether the patterns we observed between photosynthetic traits and climate are the result of plasticity, genetic differences, or a combination of both. Nevertheless, our study provides an important example of how physiological adaptations can explain the environmental distributions of the *Scalesia* radiation and potentially other tropical plants.

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### Statement of Authorship

T.M.P., J.E.G.A., G.R.-T., and K.J.F. conceptualized the study. G.R.-T. and K.J.F. provided funding acquisition. T.M.P., J.E.G.A., G.R.-T., and K.J.F. developed the methods and experimental design. T.M.P. and G.R.-T. collected the data. T.M.P. and J.E.G.A. validated, analyzed, and visualized the data. T.M.P. and J.E.G.A. provided model analysis. G.R.-T. and K.J.F. provided equipment. T.M.P., J.E.G.A., G.R.-T., and K.J.F. wrote, reviewed, and edited all versions of the manuscript.

### Data and Code Availability

The data and code used to produce the results in this study have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.zpc866tb4>; Perez et al. 2022).

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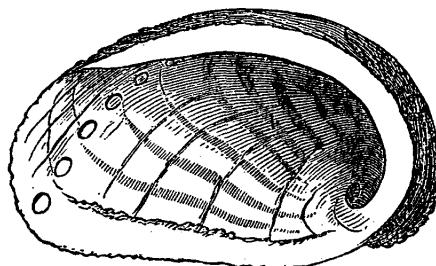
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"There is a family of Mollusca whose beautiful shells are frequently seen ornamenting the parlor mantel or centre table, the admiration of all on account of the brilliant colors and iridescence of their pearly interiors." From "The Haliotis, or Pearly Ear-Shell" by Robert E. C. Stearns (*The American Naturalist*, 1869, 3:250–256).