Unknown

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1 Notes

In preamble: - short codes for commonly used symbols - ?

Terminology to standardize:

- the native light habitat or environment: the SPLASH paper refers to what they calculated as 'habitat' PPFD, so I am going to use that terminology

- growth condition versus measurement conditions

- ?

2 Authors

definite: Wei Shen Lim, Dachuan Wang, Chris Muir question: Sam McKlin, Tom Buckley

3 Main

[introduction] Stomata are important

Stomatal ratio is important unsolved problem

Amphi leaves are more common in sunny habitats and confer a great benefit. (this pattern is also true in Solanum? could we analyze herbarium data here?)

[maybe make distinction between amphi leaves and var in SR here since all tomato species are amphi]

Leaves with greater stomatal density ratio are more in open, sunny habitats because they deliver the greatest benefit in those circumstances. An amphistomatous leaf increases photosynthetic carbon gain compared to an otherwise identical hypostomatous leaf by increasing conductance through the leaf intercellular airspaces and boudary layers; the additional water loss through a second boundary layer is typically small [cites]. We quantify this benefit as the amphistomy advantage (AA = log(Aamphi/Ahypo). Why would AA be greater in sun than shade? There are three nonmutually exclusive hypotheses that we classify as 'acclimatory', 'plastic', and 'constitutive'.

Acclimatory hypothesis: Leaves acclimated to high light intensity typically increase total leaf stomatal conductance (increased CO$_2$ supply) and upregulate Rubisco activation (increased CO2 demand). A one-dimensional circuit model using the Farquhar-von Caemmerer-Berry biochemical model

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of C$_3$ photosynthesis shows that both increased stomatal conductance and Rubisco activity should increase AA, all else being equal (Supporting Information). If the acclimatory hypothesis is correct, we predict that AAhigh > AAlow for all species regardless of native habitat or growth environment. Plants adapted to sunny, open habitats will evolve greater stomatal density ratio to take advantage of regular exposure to high light intensity.

Plastic hypothesis: Individuals of the same genotype often develop dramatically different leaves in sun and shade conditions (cite). Plastic responses are likely adaptations to optimize photosynthesis at different light intensities (cites), but changes in leaf anatomy and biochemistry could modulate AA as a byproduct. Thicker or less porous leaves, both of which are associated with high leaf mass per area (LMA), will have lower g_ias; leaves with increased total stomatal density and Rubisco concentration have greater potential CO2 supply and demand. Under the plastic hypothesis, we predict that AAsun > AAshade for all species and light intensities. AAsun and gsmax,sun should be positively associated with native light habitat. [transition] We assume that genotypes adapted to sunny, open habitats will express a phenotype best adapted to that environment when leaves develop under high light intensity; genotypes adapted to shaded closed habitats may be plastic, but suboptimal for light intensities they do not regularly experience in nature.

[conceptual figure could also show differential benefit of amphi leaves in sun/shade]

to plants growing there. in those It has been hypothesized that amphistomy increases photosynthesis more in sunny places. Three nonmutually exclusive adaptive explanations for why amphi leaves:

Conceptual figure explaining each hypothesis

- acclimation: greater demand, higher gs increase AA
- developmental plasticity: light-induced changes in leaf anatomy modulate AA
- constitutive: genetic differences in leaves adapted to different light habitats

We distinguished among these hypotheses by comparing AA among wild tomato species from different native light habitats, grown under simulated sun and shade light treatments, and measured under contrasting light intensity (Figure of hypotheses and predictions). We measured AA on 600 individual plants from 30 accessions (average of 10 replicates per light treatment) using a recently developed method (*1*). With this method, we directly compare the photosynthetic rate of an untreated amphistomatous leaf to that of the same leaf with gas exchange blocked through the adaxial (upper) surface by transparent plastic, which we refer to as 'pseudohypostomy'. To compare amphi- and pseudohypostomatous leaves at identical whole-leaf g_{sc}, we measure A over a range of g_{sc}, inducing stomatal opening and closure by modulating humidity (see Materials and Methods for further details).

Table of directional predictions (with table summarizing results? part of conceptual figure? in supplement?)

caption: Directional predictions associated with each hypothesis explaining why amphistomy advantage (AA) might be greater for leaves in sunny, open habitats. For each hypothesis, we make predictions for how native light habitat, light treatment, and light intensity would affect AA.

Table 2: Solanum accessions

hypothesis	native light habitat	light treatment	light intensity
acclimatory	cor(PAR, AA) = 0	AAsun = AAshade	AA2000 > AA150
plastic	cor(PAR, AA) = 0	AAsun > AAshade	AA2000 = AA150
constitutive	cor(PAR, AA) > 0	AAsun = AAshade	AA2000 = AA150

[add rows for when multiple hypotheses are supported simultaneously? or put that in SI?]

[results] Amphistomy increases A in all accessions, in both sun and shade leaves, and light intensities. We infer this from the fact that blocking gas exchange in pseudohypostomatous leaves reduced A by X-X% depending on the accession, light treatment, and light intensity (Table/figure). The AA is equivalent to an X-X% change in total g_{sc} (see SI section gs equivalency). But whereas increasing g_{sc} would increase water loss as a necessary by-product, amphistomy can increase A without any appreciable affect on transpiration.

Sun leaves from high light habitats [not sure if htis result is true yet] benefit the most from amphistomy because of a both developmental plasticity and constitutive differences among accessions. [quantify difference in AA and contribution of different affects]. Surprisingly, light intensity had a little effect… (should this be in this paragraph, or it's own?).

[discussion] - sun/shade has long been appreciated and this shows new trait that should be considered and that CO2 diffusion becomes major limitation

4 Materials and Methods

[this will be moved to SI eventually]

4.1 Accessions

We compared AA among 30 ecologically diverse accessions of wild tomato, including representatives of all described species of *Solanum* sect. *Lycopersicon* and sect. *Lycopersicoides* (*2*) and the cultivated tomato *S. lycopersicum* var. *lycopersicum* **??**. Due to constraints on growth space and time, we spread out measurements over 80 weeks from May 1, 2022 to October 31, 2023. Replicates within accession were evenly spread out over this period to prevent confounding of temporal variation in growth conditions with accession. [anything else to say here? maybe explain accession selection and phylogeny?]

4.2 Plant growth conditions

In all growth spaces, we recorded PPFD using full spectrum quantum sensors (SQ-500-SS, Apogee Instruments, Logan, Utah, USA); we recorded temperature, RH, and $[CO_2]$ using an EE894 sensor (E+E Elektronik, Engerwitzdorf, Austria) protected by a radiation shield. All environmental measurements were taken every 10 minutes from the middle of plants racks at approximately the same height as the leaves we measured. We measured leaf temperature of focal leaves prior to measurement using an infrared radiometer (SI-111-SS, Apogee Instruments, Logan, Utah, USA).

4.2.1 Germination and seedling stage

Seeds provided by the Tomato Genetics Resource Center germinated on moist paper in plastic boxes after soaking for 30-60 minutes in a 50% (volume per volume) solution of household bleach and water, followed by a thorough rinse. We transferred seedlings to cell-pack flats containing Pro-Mix BX potting mix (Premier Tech, Rivière-du-Loup, Quebec, Canada) once cotyledons fully emerged, typically within 1-2 weeks of sowing. We grew seeds and seedlings for both sun and shade treatments under the same environmental conditions (12:12 h, 25:20 °C, 40:60 RH day:night cycle). LED light provided PPFD $=$ $200\,\mu mol\,m^{-2}\,s^{-1}$ (Fluence RAZRx, Austin, Texas, USA).

4.2.2 Light treatments

Seedlings were randomly assigned in alternating order within accession to the sun or shade treatment during transplanting. After seedlings established in cell-pack flats for $\approx$ 2 weeks, we transplanted them to 3.78 L plastic pots containing 60% Pro-Mix BX potting mix, 20% coral sand (Pro-Pak, Honolulu, Hawai'i, USA), and 20% cinders (Niu Nursery, Honolulu, Hawai'i, USA). Percentage composition is on a volume basis. The soil mixture contained slow release NPK fertilizer following manufacturer instructions (Osmocote Smart-Release Plant Food Flower & Vegetable, The Scotts Company, Marysville, Ohio, USA). We determined pot field capacity one week after transplanting using a scale (Ohaus V12P15 Valor 1000, Parsippany, New Jersey, USA) and watered to field capacity three times per week to prevent drought stress.

We assigned sun and shade treatment to lower and upper racks of a $1.22\,m \times 2.44\,m$ shelving unit in a climate-controlled growth room. We assigned the sun treatment to the lower rack to limit diffuse light from reaching the shade treatment. The average daytime PPFD was $800\,\mu mol\,m^{-2}\,s^{-1}$ and $125\,\mu mol\,m^{-2}\,s^{-1}$ for sun and shade treatments, respectively. To isolate the effect of light intensity from quality, we used the same LED model with the the same spectrum (Fluence SPYDR 2i, Austin, Texas, USAS), but dimmed the lights in the shade treatment. To maintain homogeneous environmental conditions other than light, we mixed air within the growth room using an air circulator (Vornado 693DC, Andover, Kansas, USA) and within racks using a miniature oscillating air circulator (Vornado Atom 1, Andover, Kansas, USA). Despite these efforts, the air in the sun treatment was on average 1 °C warmer and the average RH was consequently 5 lower. However, because of evaporative cooling, the leaves in the sun treatment were only 1 °C on average ($n = 600$ leaves).

4.3 Leaf trait measurements

We selected a fully expanded, unshaded leaf at least six leaves above the cotyledons during early vegetative growth. This typically meant that plants had grown in light treatments for ≈ 4 weeks, ensuring they had time to sense and respond developmentally to the light intensity of the treatment rather than the seedling conditions (*3*). Shade plants grew slower than sun plants, hence leaves at the same developmental stage were measured on chronologically older plants in the shade treatment. In some sun plants, we had to use leaves higher on the stem because short internodes made lower leaves inaccessible with the gas exchange equipment. We measured terminal leaflets in 70.0% of cases, but used the lateral leaflet closest to the terminal leaflet when it was damaged or difficult to clamp into the gas exchange chamber. When a leaflet was damaged during gas exchange measurements, we collected anatomical data from the nearest leaflet on the same leaf (10.0)%. We measured chlorophyll concentration index (CCI) using a chrolophyll concentration meter (MC-100, Apogee Instruments, Logan, Utah, USA) on the lamina of focal leaflets before gas exchange measurements at the same time we measured leaf temperature.

4.3.1 Amphistomy advantage

We estimated 'amphistomy advantage' (AA) *sensu* (*4*), but with modifications previously described in (*1*). AA is calculated as the log-response ratio of A compared at the same total g_{sw}:

$$\text{AA} = \log(A_{\text{amphi}}/A_{\text{hypo}})$$

We measured the photosynthetic rate of an untreated amphistomatous leaf (A_{amphi}) over a range of g_{sw} values. We refer to this as an A–g_{sw} curve. We compared the A–g_{sw} curve of the untreated leaf to the photosynthetic rate of pseudohypostomatous leaf (A_{hypo}), which is the same leaf but with gas exchange through the upper surface blocked by a neutral density plastic (propafilm).

We measured A–g_{sw} curves using a portable infrared gas analyzer (LI-6800PF, LI-COR Biosciences, Lincoln, Nebraska, USA). Light-acclimated plants were placed under LEDs dimmed to match their light treatment during gas exchange measurements. We estimated the photosynthetic rate (A) and stomatal conductance to CO_2 (g_{sw}) at ambient CO_2 ($C_a = 415\,\mu\text{mol}\,\text{mol}^{-1}$) and $T_{\text{leaf}} = 25.0\,°\text{C}$. The irradiance of the light source in the pseudohypo leaf was higher because the propafilm reduces transmission. To compensate for reduced transmission, we increased incident PPFDfor pseudohypo leaves by a factor 1/0.91, the inverse of the measured transmissivity of the propafilm. We also set the stomatal conductance ratio, for purposes of calculating boundary layer conductance, to 0 for pseudohypo leaves following manufacturer directions.

We collected four A–g_{sw} curves per leaf, an amphi (untreated) curve and a pseudohypo (treated) curve at high light-intensity (PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$; 97.7:2.3 red:blue) and low light-intensity (PPFD $= 150\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$; 87.0:13.0 red:blue). We always measured high light-intensity curves first because photosynthetic downregulation is faster than upregulation in these species. To control for order effects,

we alternated between starting with amphi or pseudohypo leaf measurements. Unlike (*1*), preliminary experiments with *Solanum* indicated a strong order effect in that A declined in the second curve. Therefore, we made measurements over two days. On the first day, we measured high and low light-intensity curves for either amphi or pseudohypo leaves; on the second day, we measured high and low light-intensity curves on the other leaf type.

In all cases, we acclimated the focal leaf to high light (PPFD $= 2000 \, \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1}$) and high relative humidity (RH $= 70\%$) until A and g_{sw} reach their maximum. After that, we decreased RH to $\approx 10\%$ to induce rapid stomatal closure without biochemical downregulation. Hence, A_{amphi} and A_{hypo} were both measured at low chamber humidity after the leaf had acclimated to high humidity. All other environmental conditions in the leaf chamber remained the same. We logged data until g_{sw} reached its nadir. We then acclimated the leaf to low light (PPFD $= 150 \, \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1}$) and RH $= 70\%$ before inducing stomatal closure with low RHand logging data as described above.

4.3.2 Light-response (A–Q) curves

In 90.0% of plants, we measured light-response (A–Q) curves on the same leaflets as A–g_{sw} curves. However, when a leaflet was damaged during A–g_{sw} curves, we used the next closest leaflet for A–Q curves. Leaves acclimated to high light-intensity (PPFD $= 2000 \, \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1}$), ambient CO_2 ($C_{\text{a}} = 415 \, \mu\text{mol} \, \text{mol}^{-1}$), RH $= 50\%$, and $T_{\text{leaf}} = 25 \, °\text{C}$. After A and g_{sw} stabilized, we measured A at 20 light-intensity levels between 0 and $2000 \, \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1}$ in descending order.

4.3.3 Stomatal anatomy

We estimated the stomatal density and size on ab- and adaxial leaf surfaces from all leaves, using guard cell length as a proxy for stomatal size since it proportional to maximum conductance (*5*). We made surface impressions of leaf lamina from the same area used for gas exchange measurements using a-silicone impression material (Zhermack elite HD+, light body, fast set, Rovigo, Italy). We applied clear nail polish to make positive replicas of the impression. After nail polish dried, we mounted replicas on a microscope slide using transparent tape (*6*). We digitized a portion of each leaf surface replica using a brightfield microscope (Leica DM2000, Wetzlar, Germany). We counted and measured guard cell length on all stomata using the FIJI implementation of ImageJ2 version 2.3.0 (*7*), then divided the count by the visible leaf area ($0.890 \, \text{mm}^2$) to estimate stomatal density.

4.3.4 Leaf mass per area

Leaf mass per area (LMA) is the dry mass divided by the leaflet area. We scanned fresh leaflets on a flat bed scanner (Epson V600, Los Alamitos, California. USA) and measured leaflet area from digital images using the FIJI implementation of ImageJ2 version 2.3.0 (*7*). We dried leaves for 72 hours at $74 \, °\text{C}$ in a food dehydrator (Cosori CP267-FD, Vesync Co., Anaheim, California, USA) and weighed using a benchtop analytical balance (Ohaus PR64 Analytical Balance, Parsippany, New Jersey, USA).

Table 3: Six sequential steps for cleaning A–g_{sw} curves. The rationale and procedure for each step are described in the text. The rightmost columns summarize the number of curves and mean number of points per curve remaining after each step. For reference, there are four possible A–g_{sw} curves per replicate: all combinations of leaf type (amphi or pseudohypo) and light intensity (high or low).

Step: description	Number of curves	Number of points per curve
1. remove unreliable and unusable data points	2,361	63.0
2. remove hysteretic portion of A–g_{sw} curves at low g_{sw}	2,360	59.2
3. remove outliers within each A–g_{sw} curve	2,360	58.7
4. remove replicates with no overlap between amphi and pseudohypo A–g_{sw} curves	2,268	58.5
5. thin redundant data points within each A–g_{sw} curve	2,268	28.1
6. Trim extreme AA values	2,210	28.0

In 5.0% we measured LMA on the adjacent leaflet because the focal leaflet was damaged or wilted while making surface impressions and we could not reliably estimate area. LMA data are missing from 3.0% of individuals because the area or mass was not recorded at all or recorded incorrectly.

4.4 Cleaning A–g_{sw} curves

The raw data set consisted of 2,370 A–g_{sw} curves with an average of 63.2 points per curve. Manual curation of a data set this size in a principled, consistent manner is not feasible. Therefore, we automated data cleaning using custom *R* scripts. Cleaning is divided into six sequential steps (Table 3).

4.4.1 Remove unreliable and unusable data points

Rationale: Unreliable data points consisted of those where chamber $[CO_2]$ was unstable and therefore measurements are not biologically meaningful. Unusable data points were those where $A < 0$ because the logarithm of a negative number is undefined.

Procedure: We retained data points where $410 < C_a < 420\,\mu\text{mol}\,\text{mol}^{-1}$ and $A > 0$.

4.4.2 Remove hysteretic portion of A–g_{sw} curves at low g_{sw}

Rationale: In most A–g_{sw} curves, we observed a hysteretic response at low g_{sw}. After g_{sw} and A declined simultaneously, A increased slightly as g_{sw} continued to decline or stabilize, indicating some

leaf acclimation to low RH. We removed this portion of the curve to focus curve-fitting on the primary domain where A increases monotonically with g_{sw}.

Procedure: For each curve, we removed data points after g_{sw} had reached its minimum unless there were fewer than 10 data points remaining.

4.4.3 Remove outliers within each A–g_{sw} curve

Rationale: Individual outliers within A–g_{sw} curves, usually caused by transitory changes in chamber conditions, exert undue leverage on parameter estimates and cause bias and/or low precision in parameter estimates.

Procedure: We fit provisional quadratic regressions for each curve using ordinary least squares with the `lm()` function in *R*. We sequentially removed data points with an absolute external studentized residual > 3 until none remained.

4.4.4 Thin redundant data points within each A–g_{sw} curve

Rationale: Data points closely spaced along the A–g_{sw} curve provide redundant information and may be highly correlated (i.e. pseudoreplication). This occurred because data was logged at a constant temporal interval, but the rate at which g_{sw} declined was not constant. Thinning reduces parameter estimation bias toward densely sampled regions of the curve which may not be the most biologically informative.

Procedure: We retained the maxima and minima g_{sw} for each curve and thinned all but one point per thinning interval of $0.05 \ \log(\mathrm{mol\,m^{-2}\,s^{-1}})$, retaining the point nearest the midpoint of the interval.

4.4.5 Remove replicates with no overlap between amphi and pseudohypo A–g_{sw} curves

Rationale: We could not estimate AA for replicates where amphi and pseudohypo A–g_{sw} curves did not overlap.

Procedure: We removed replicates where the range of g_{sw} values for amphi and pseudohypo A–g_{sw} curves did not overlap.

Table 4: Two sequential steps for cleaning A–g_{sw} curves. The rationale and procedure for each step are described in the text. The rightmost columns summarize the number of curves and mean number of points per curve remaining after each step.

Step: description	Number of curves	Number of points per curve
1. remove outliers within each A–Q curve	658	19.1
2. remove A–Q curves with poor fit	652	19.1

4.4.6 Trim extreme AA values

Rationale: Extreme AA values were likely due to measurement error or leaf damage. Since amphi and pseudohypo A–g_{sw} curves are measured on consecutive days, a poor calibration or a damaged leaf could cause a large difference in A between days, which would appear as an extreme AA value.

Procedure: We provionsally estimated AA for each replicate by integrating over the range of g_{sw} values where amphi and pseudohypo A–g_{sw} curves overlap. In this procedure, curve parameters were provisionally estimated using ordinary least squares with the `lm()` function in *R*. We then used point estimates of AA for each replicate as the response variable in a linear model with light treatment, light intensity, accession, and all interactions as explanatory variables. This model was also fit using ordinary least squares with the `lm()` function in *R*. We classified extreme AA values as those with an absolute internal studentized residual > 3.5. Because these values likely indicate significant measurement error or leaf damage, we removed A–g_{sw} curves at both light intensities if either was classified as extreme.

4.5 Cleaning A–Q curves

The raw data set consisted of 658 A–Q curves with an average of 19.4. Manual curation of a data set this size in a principled, consistent manner is not feasible. Therefore, we automated data cleaning using custom *R* scripts. Cleaning is divided into two sequential steps (Table 4).

4.5.1 Remove outliers within each A–Q curve

Rationale: Individual outliers within A–g_{sw} curves, usually caused by transitory changes in chamber conditions, exert undue leverage on parameter estimates and cause bias and/or low precision in parameter estimates.

Procedure: We fit provisional nonrectangular hyperbola (*8*) to each A–Q curve using nonlinear regression with the `nlsLM()` function from the *R* package **minpack.lm** version 1.2.4 (*9*). We sequentially removed data points with an absolute external studentized residual > 3 until none remained.

Table 5: Summary of differences among competing models of how AA varies with light intensity, light treatment, and among accessions as a function of native PPFD. The models are numbered from simpler to more complex. All models include fixed effects of light intensity and light treatment; some models include interactions between. All models include a phylogenetic random effect of accession on AA; some models include varying effects of light intensity and light treatment among accessions. The last column indicates the accession-level AA variable we used as a response to native PPFD.

Model	Fixed effects	Phylogenetic random effects	Response to native PPFD
1	light intensity light treatment	varying intercept among accessions	$AA_{0,acc}$
2	light intensity light treatment intensity $\times$ treatment	varying intercept among accessions	$AA_{0,acc}$
3	light intensity light treatment	varying intercept among accessions varying effect of high light intensity among accessions	$AA_{2000,acc}$
4	light intensity light treatment	varying intercept among accessions varying effect of sun treatment among accessions	$AA_{sun, acc}$
5	light intensity light treatment intensity $\times$ treatment	varying intercept among accessions varying effect of high light intensity among accessions varying effect of sun treatment among accessions	$AA_{2000,sun, acc}$

4.5.2 Remove A–Q curves with poor fit

Rationale: A–Q curves with a poor fit to the nonrectangular hyperbola most likely indicate systematic measurement error and/or the leaf was not fully acclimated to the chamber environment.

Procedure: As described above, we fit provisional nonrectangular hyperbola to each A–Q curve and calculated the model r^2. There was a clear break between typical curves and poorly fitting curves where $r^2 < 0.99$. We therefore removed A–Q curves with $r^2 < 0.99$.

4.6 Bayesian data analysis in *Stan*

We fit five models to test predictions of competing hypotheses about why amphistomy advantage (AA) might be greater for leaves in sunny, open habitats. This section provides an overview of differences among models (Table 5). The next sections describe how we fit models in *Stan*, all model parameters and priors, and specific predictions about parameter values for each hypothesis.

4.6.1 Fitting models in *Stan*

We fit Bayesian models with MCMC sampling in the probabilistic programming language *Stan* (*10*). We used CmdStan version 2.33.1 and **cmdstanr** version 0.7.1 (*11*) to interface with *R* version 4.3.2 (*12*). We sampled the posterior distribution from 4 chains with 1000 iterations each after 1000 warmup iterations per chain. We estimated parameters and confidence intervals as the median and 95% quantile intervals of the posterior, respectively. We chose the number of chains, warmup and sampling iterations, and maximum treedepth so that parameter estimates converged ($\hat{R} < 1.01$ (*13*)) and the effective sample size (ESS) for each parameter was $> 10^3$.

4.6.2 Parameter estimation and priors

There were four levels of parameter estimation in our analysis:

1. Estimate A–g_{sw} curve parameters
2. Estimate AA for each light intensity with leaf using A–g_{sw} curve parameters
3. Estimate the effects of light intensity, light treatment, and accession on AA (assimilatory and plasticity hypotheses)
4. Estimate the effects of native light habitat on accession-level AA (constitutive hypothesis)

Although the higher-level parameter estimates depend on the lower-level parameter estimates, we fit all models simultaneously to ensure that the uncertainty in lower-level estimates propagated to higher-level estimates.

Table 6: Description of parameters estimated in the hierarchical Bayesian model. The `Parameter` column lists the parameter name as it appears in text. The `Description` column provides a brief description of the parameter.

Parameter	Description
A–g_{sw} **curve parameters**	
$\mathbf{B}_{curve}$	$n_{curve} \times 3$ array of random A–g_{sw} curve-level coefficients ($b_{0,j}$, $b_{1,j}$, $b_{2,j}$); $\mathbf{B}_{curve} \sim \mathrm{MVN}(\vec{0}, \boldsymbol{\Sigma}_{curve})$
$\vec{\beta}_{curve}$	vector of mean quadratic coefficients (β_0, β_1, β_2)
$\boldsymbol{\Sigma}_{curve}$	3×3 covariance matrix of curve-level coefficients
$\sigma_{6\,\mathrm{cm}^2,\epsilon}$	minimum residual standard deviation when the measured leaf surface area is 6 cm^2
$\beta_{S,\epsilon}$	slope of the relationship between residual standard deviation and measured leaf surface area (log-link scale)
ρ_ϵ	lag-1 residual autocorrelation
AA **for each light intensity with leaf using agcurve curve parameters**	
$\widehat{\mathrm{AA}}_{klm}$	estimate of AA for the kth leaf at light intensity l in accession m
effects of light intensity, light treatments, and accession on AA	

$\beta_{AA,0}$	intercept of AA at low light intensity in shade treatment
$\beta_{AA,2000}$	effect of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on AA
$\beta_{AA,sun}$	effect of sun treatment on AA
$\beta_{AA,2000,sun}$	effect of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on AA in sun treatment
$\vec{\beta}_{AA,acc}$	vector of n_{acc} phylogenetically structured random accession-level effects on AA; $\vec{\beta}_{AA,acc} \sim \text{MVN}(\vec{0}, \boldsymbol{\Sigma}_{AA,acc})$
$\boldsymbol{\Sigma}_{AA,acc}$	$n_{acc} \times n_{acc}$ covariance matrix of phylogenetically structured random accession-level effects on AA;
$\vec{\beta}_{AA,rep}$	vector of n_{rep} random replicate-level effects on AA; $\vec{\beta}_{AA,rep} \sim \text{Normal}(0, \sigma_{AA,rep})$
$\vec{\beta}_{AA,2000,acc}$	vector of n_{acc} phylogenetically structured random accession-level effects of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on AA; $\vec{\beta}_{AA,2000,acc} \sim \text{MVN}(\vec{0}, \boldsymbol{\Sigma}_{AA,2000,acc})$
$\vec{\beta}_{AA,sun,acc}$	vector of n_{acc} phylogenetically structured random accession-level effects of sun treatment on AA; $\vec{\beta}_{AA,sun,acc} \sim \text{MVN}(\vec{0}, \boldsymbol{\Sigma}_{AA,sun,acc})$
$\sigma_{AA,\epsilon,0}$	intercept of phylogenetically unstructured residual standard deviation of AA
$\beta_{AA,\epsilon,2000}$	effect of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on phylogenetically unstructured residual standard deviation of AA (log-link scale)
$\beta_{AA,\epsilon,sun}$	effect of sun treatment on phylogenetically unstructured residual standard deviation of AA (log-link scale)
$\sigma_{AA,rep}$	standard deviation of random replicate-level effects on AA
$\alpha_{AA,acc}$	decay rate of phylogenetic covariance in random accession-level effects on AA
$\sigma^2_{AA,acc}$	phylogenetic diffusion rate in random accession-level effects on AA
$\alpha_{AA,2000,acc}$	decay rate of phylogenetic covariance in random accession-level effects of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on AA
$\sigma^2_{AA,2000,acc}$	phylogenetic diffusion rate in random accession-level effects of high light intensity at PPFD $= 2000\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ on AA
$\alpha_{AA,sun,acc}$	decay rate of phylogenetic covariance in random accession-level effects of sun treatment on AA
$\sigma^2_{AA,sun,acc}$	phylogenetic diffusion rate in random accession-level effects of sun treatment on AA

effects of native light habitat on accession-level AA

$\beta_{AA,PPFD,0}$	intercept of accession-level AA when native PPFD $= 0\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$
$\beta_{AA,PPFD,1}$	slope of native PPFD on accession-level AA
$\alpha_{AA,PPFD}$	decay rate of phylogenetic covariance in residuals of model testing effect of native PPFD on accession-level AA
$\sigma^2_{AA,PPFD}$	phylogenetic diffusion rate in residuals of model testing effect of native PPFD on accession-level AA

4.6.2.1 A–g_{sw} curve parameters

We modeled $\log(A)$ as a quadratic function of $\log(g_{sw})$ for each leaf using the following equation:

$$\log(A_{ij}) = (\beta_0 + b_{0,j}) + (\beta_1 + b_{1,j}) \log g_{sw,i} + (\beta_2 + b_{2,j}) \log g_{sw,i}{}^2 + \epsilon_i$$

where β_0, β_1, and β_2 are the average intercept, linear, and quadratic coefficients, respectively. We used diffuse normal priors with mean 0 and standard deviation 10 on these parameters. We estimated random effects of curve j on the intercept ($b_{0,j}$), linear ($b_{1,j}$), and quadratic ($b_{2,j}$) coefficients. We assumed that the $j \times 3$ array of coefficients was multivariate normal a mean vector of $\vec{0}$ and covariance Σ_{curve}. We used a weakly informative normal prior with mean 0 and standard deviation 1 on the log-transformed standard deviations (i.e. the diagonal of Σ_{curve}). We used a weakly informative LJK(2) prior on the correlation matrix. The off diagonal elements of Σ_{curve} can be calculated from its diagonal elements and the correlation matrix.

The residuals ϵ_i were modeled as a lag-1 autocorrelated time-series. We further assumed that the residual standard deviation of the jth curve ($\sigma_{\epsilon,j}$) was inversely proportional to the leaf surface area (S_j) within the chamber:

$$\log(\sigma_{\epsilon,j}) = \log(\sigma_{6\,\mathrm{cm^2},\epsilon}) + (6 - S_j)\beta_{S,\epsilon}$$

where $\sigma_{6\,\mathrm{cm^2},\epsilon}$ is the minimum residual standard deviation when the $6\,\mathrm{cm}^2$ chamber is completely filled. The residual standard deviation increases on log-linear scale by $\beta_{S,\epsilon}$. We used a weakly informative normal prior with mean -3 and standard deviation 5 on $\log(\sigma_{6\,\mathrm{cm^2},\epsilon})$ and a weakly informative normal prior with mean 0 and standard deviation 1 on $\beta_{S,\epsilon}$.

4.6.2.2 AA for each light intensity with leaf using A–g_{sw} curve parameters

Within the kth leaf, we estimated AA for each light intensity by integrating the difference in $\log(A)$ between the amphi and pseudohypo A–g_{sw} curves over the range of g_{sw} values where the curves overlap (from $\min(\log(g_{sw}))$ to $\max(\log(g_{sw}))$). The estimate of AA for the kth leaf at light intensity l in accession m is:

$$\widehat{\mathrm{AA}}_{klm} = \int_{\min(\log(g_{sw}))}^{\max(\log(g_{sw}))} \log\left(\frac{\widehat{A}_{\mathrm{amphi}}(x; \theta_{klm,\mathrm{amphi}})}{\widehat{A}_{\mathrm{hypo}}(x; \theta_{klm,\mathrm{hypo}})} \right) dx$$

where:

$$\theta_{\mathrm{amphi}} \in \{\widehat{b}_{0,f(\mathrm{amphi},k,l,m)}, \widehat{b}_{1,f(\mathrm{amphi},k,l,m)}, \widehat{b}_{2,f(\mathrm{amphi},k,l,m)}\}, \text{and}$$

$$\theta_{\mathrm{hypo}} \in \{\widehat{b}_{0,f(\mathrm{hypo},k,l,m)}, \widehat{b}_{1,f(\mathrm{hypo},k,l,m)}, \widehat{b}_{2,f(\mathrm{hypo},k,l,m)}\}.$$

The function $f : \Theta_1 \to \Theta_2$ maps the set Θ_1 indexed by leaf type (amphi or pseudohypo), leaf replicate, light intensity, and accession to set Θ_2 indexed by individual A–g_{sw} curve. This mapping is necessary because the random effects structure differs between models of $\log(g_{\text{sw}})$ on $\log(A)$ and that of models predicting AA described in the next section.

4.6.2.3 Effects of light intensity, light treatment, and accession on AA

We tested for effects of light intensity, light treatment, accession, and their interactions on AA. All models included effects of light intensity and light treatment, as well as random effects of accession and replicate within accession. More complex models included interactions between light intensity and light treatment, as well as random effects of accession on the effects of light intensity and light treatment. We used a weakly informative normal prior with mean 0 and standard deviation 10 for fixed effects of light intensity and treatment. We used a weakly informative normal prior with mean -3 and standard deviation 5 for the the random effect standard deviation of replicate within accession. We accounted for the phylogenetic structure among the random effects of accession using an Ornstein-Uhlenbeck (OU) process (*14*). The expected covariance between accessions i and j ($\text{Cov}(i,j)$) is:

$$\text{Cov}(i,j) = \frac{\sigma^2}{2\alpha} \exp(-\alpha D_{ij})$$

where σ^2 is the variance of the random effect, α is the rate of decay of the covariance with phylogenetic distance, D_{ij}, the phylogenetic distance between accessions i and j. We estimated $\sigma^2/(2\alpha)$ and α as a separate parameters and reparameterized them as σ^2 and α. We used a weakly informative normal prior with mean 0 and standard deviation 10 on OU parameters.

We modeled the residual standard deviation of AA, (i.e. phylogenetically unstructured variation unaccounted for by explanatory variables) on a log-link scale with effects of light intensity and light treatment. We used a weakly informative normal prior with mean -3 and standard deviation 5 on the residual standard deviation intercept and a weakly informative normal prior with mean 0 and standard deviation 1 on the effects of light intensity and light treatment on the residual standard deviation.

4.6.2.4 Effects of native light habitat on accession-level AA

We tested a linear effect of native light habitat on accession-level AA. In Models 1 and 2, we used the random intercept of AA as a response variable; in model 3 we used the random intercept plus the random effect of accession at high light intensity; in model 4 we used the random intercept plus the random effect of accession in the sun treatment; in model 5 we used the random intercept plus the random effects of accession at high light intensity and sun treatment. We used a weakly informative normal prior with mean 0 and standard deviation 1 for the slope and intercept. We accounted for the phylogenetic structure among the model residuals using an (OU) process. We used a weakly informative normal prior with mean 0 and standard deviation 10 on OU parameters.

4.6.3 Predictions

The assimilatory, plastic, and constitutive hypotheses make different predictions about the relationship between AA and light intensity, light treatment, and native PPFD among accessions. Since these hypotheses are not mutually exclusive, we describe how we assessed support for one, two, or all three hypotheses simultaneously in Table 7. In general, the assimilatory hypothesis was supported if AA was greater at high light intensity than low light intensity. The plastic hypothesis was supported if AA was greater in sun leaves than shade leaves. The constitutive hypothesis was supported if accession-level AA increased with native PPFD. In interactive models, we only consider positively reinforcing interactions between high light intensity, sun leaves, and native PPFD because these are the only interactions which could explain why amphistomatous leaves are advantageous in high light habitats.

We evaluated predictions using a combination of parameter estimation and model selection. In all cases, we estimated parameters and confidence intervals as described in Section 4.6.1. If the 95% confidence intervals for a parameter did not overlap zero, we considered the parameter to be significantly different from zero. We used the leave-one-out cross-validation information criterion (LOOIC) to compare the fit of models (Table 5) using the *R* package **loo** version 2.7.0 (*15*) to calculate LOOIC values. Note that LOOIC was calculated only from pointwise likelihood values of the submodel estimating effects of light intensity, light treatments, and accession on AA (see Section 4.6.2). We considered models with two standard errors of the mean lower LOOIC value to be a better fit to the data; we considered models with LOOIC values within two standard errors of the mean to be have similar support.

Table 7: Predictions of competing hypotheses about the relationship between AA and light intensity, light treatment, and native PPFD among accessions. The middle column lists specific directional predictions about parameter values and model fit according to leave-one-out cross-validation information criterion (LOOIC), where LOOIC_i is the LOOIC value for model i. The rightmost column describes the predictions in words and explains how accession-level AA is calculated in the relevant model.

Hypothesis	Prediction(s)	Description
Assimilatory	$\beta_{\text{AA,2000}} > 0$	Average AA at high light intensity is greater than that at low light intensity
Plastic	$\beta_{\text{AA,sun}} > 0$	Average AA in sun leaves is greater than that in shade leaves
Constitutive	$\beta_{\text{PPFD,AA}} > 0$	Accession-level AA (AA_{acc}) increases with native PPFD $\text{AA}_{\text{acc}} = \beta_{\text{AA,0}} + \vec{\beta}_{\text{AA,acc}}$
Assimilatory × Plastic	$\beta_{\text{AA,2000,sun}} > 0$ $\text{LOOIC}_1 > \text{LOOIC}_2$	Average AA is highest at high light intensity in sun leaves

Assimilatory × Constitutive	$\beta_{AA,2000} > 0$	Average AA at high light intensity is greater than that at low light intensity
	$\beta_{PPFD,AA} > 0$	Accession-level AA at high light intensity ($AA_{acc,2000}$) increases with native PPFD
	$LOOIC_1 > LOOIC_3$	$AA_{acc,2000} = \beta_{AA,0} + \vec{\beta}_{AA,acc} + \vec{\beta}_{AA,2000,acc}$
Plastic × Constitutive	$\beta_{AA,sun} > 0$	Average AA in sun leaves is greater than that in shade leaves
	$\beta_{PPFD,AA} > 0$	Accession-level AA in sun leaves ($AA_{acc,sun}$) increases with native PPFD
	$LOOIC_1 > LOOIC_4$	$AA_{acc,sun} = \beta_{AA,0} + \vec{\beta}_{AA,acc} + \vec{\beta}_{AA,sun,acc}$
Assimilatory × Plastic × Constitutive	$\beta_{AA,2000,sun} > 0$	Average AA is highest at high light intensity in sun leaves
	$\beta_{PPFD,AA} > 0$	Accession-level AA at high light intensity in sun leaves ($AA_{acc,2000,sun}$) increases with native PPFD
	$LOOIC_3 > LOOIC_5$	$AA_{acc,2000,sun} = \beta_{AA,0} + \vec{\beta}_{AA,acc} + \vec{\beta}_{AA,2000,acc} + \vec{\beta}_{AA,sun,acc}$

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