

Unknown

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
- ?

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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 inter-cellular airspaces and boundary layers; the additional water loss through a second boundary layer is typically small [cites]. We quantify this benefit as the amphistomy advantage ($AA = \log(A_{\text{amphi}}/A_{\text{hypo}})$).

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 CO₂ supply) and upregulate Rubisco activation (increased CO₂ demand). A one-dimensional circuit model using the Farquhar-von Caemmerer-Berry biochemical model of C₃ 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 AA_{high} > AA_{low} 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 AA_{sun} > AA_{shade} for all species and light intensities. AA_{sun} and g_{smax,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. 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.

| hypothesis | native light habitat | light treatment | light intensity |
|--------------|---|---|--------------------------------------|
| acclimatory | $\text{cor}(\text{PAR}, \text{AA}) = 0$ | $\text{AA}_{\text{sun}} = \text{AA}_{\text{shade}}$ | $\text{AA}_{2000} > \text{AA}_{150}$ |
| plastic | $\text{cor}(\text{PAR}, \text{AA}) = 0$ | $\text{AA}_{\text{sun}} > \text{AA}_{\text{shade}}$ | $\text{AA}_{2000} = \text{AA}_{150}$ |
| constitutive | $\text{cor}(\text{PAR}, \text{AA}) > 0$ | $\text{AA}_{\text{sun}} = \text{AA}_{\text{shade}}$ | $\text{AA}_{2000} = \text{AA}_{150}$ |

[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 g_{sc} 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

Materials and Methods

[this will be moved to SI eventually]

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?]

Table 2: Solanum accessions

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 [CO₂] 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).

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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fluence RAZRx, Austin, Texas, USA).

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 ≈ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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).

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 chlorophyll 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.

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} :

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

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 ($C_a = 415 \mu\text{mol mol}^{-1}$) and $T_{\text{leaf}} = 25.0$ °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 PPFD for 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 97.7:2.3 red:blue) and low light-intensity (PPFD = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 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 (*I*), 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and RH = 70% before inducing stomatal closure with low RH and logging data as described above.

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 $\mu\text{mol m}^{-2} \text{s}^{-1}$), ambient CO_2 ($C_a = 415 \mu\text{mol mol}^{-1}$), RH = 50%, and $T_{\text{leaf}} = 25^\circ\text{C}$. After A and g_{sw} stabilized, we measured A at 20 light-intensity levels between 0 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in descending order.

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 is 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 mm^2) to estimate stomatal density.

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

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 |

images using the FIJI implementation of ImageJ2 version 2.3.0 (7). We dried leaves for 72 hours at 74 °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). 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.

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](#).

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 $410 < C_a < 420 \mu\text{mol mol}^{-1}$ and $A > 0$.

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.

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.

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 $0.05 \log(\text{mol m}^{-2} \text{ s}^{-1})$, retaining the point nearest the midpoint of the interval.

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.

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 provisionally 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

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 |

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.

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](#).

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 (**marshall_model_1980?**) to each $A-Q$ curve using nonlinear regression with the `nlsLM()` function from the *R* package **minpack.lm** version 1.2.4 (**Elzhov_minpack.lm_2023?**). We sequentially removed data points with an absolute external studentized residual > 3 until none remained.

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 $r^2 < 0.99$.

Data analysis

General points (not sure where this goes yet)

- models fit in Stan/brms
- we chose the number of chains, warmup and sampling iterations, and maximum treedepth so that parameter estimates converged ($\hat{R} < 1.01$ (8)) and the effective sample size (ESS) for each parameter was $> 10^3$.

We fit Bayesian models using the *R* package **brms** version 2.20.4 (9) with MCMC sampling in *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.

Parameter estimates (this is a provisional section)

Base model (need to add indices):

$$\log(A) = \beta_0 + \beta_1 \log(g_{sw}) + \beta_2 \log(g_{sw})^2 + \epsilon$$

Estimate AA by integrating over range of g_{sw} overlap (need to add indices for accession, replicate, light intensity):

$$AA = \int_{\min(\log(g_{sw}))}^{\max(\log(g_{sw}))} \log\left(\frac{A_{\text{amphi}}(x; \theta_{\text{amphi}})}{A_{\text{hypo}}(x; \theta_{\text{hypo}})}\right) dx$$

where $\min(\log(g_{sw}))$ and $\max(\log(g_{sw}))$ are the minimum and maximum values of $\log(g_{sw})$ where amphi and pseudohypo curves overlap. A_{amphi} is calculated from parameter estimates $\theta_{\text{amphi}} \in \{\beta_{0,\text{amphi}}, \beta_{1,\text{amphi}}, \beta_{2,\text{amphi}}\}$; A_{hypo} is calculated from parameter estimates $\theta_{\text{hypo}} \in \{\beta_{0,\text{hypo}}, \beta_{1,\text{hypo}}, \beta_{2,\text{hypo}}\}$ for each curve.

Predictions

- Assimilatory:
 - average AA2000 - AA150
 - among accession variation in AA2000 - AA150
- Plastic
 - average AAsun - AAshade
 - among accession variation in AAsun - AAshade

- Constitutive
 - $\text{cor}(\text{PAR}, \text{AA})$ at accession level

Reinforcing interactions (we only consider these because these are ones which could explain why amphileaves occur in high light habitats)

- Assimilatory * Plastic (reinforcing interaction)
 - AAsun - AAshade greater at 2000 than 150
 - AA2000 - AA150 greater in sun leaves than shade leaves
 - there may be var among accessions, but not correlated with PAR
- Assimilatory * Constitutive (reinforcing interaction)
 - $\text{cor}(\text{AA2000}, \text{PAR}) > 0$; $\text{cor}(\text{AA150}, \text{PAR}) = 0$
 - AA2000 - AA150 varies among accessions, correlated with PAR
- Plastic * Constitutive (reinforcing interaction)
 - $\text{cor}(\text{AAsun}, \text{PAR}) > 0$; $\text{cor}(\text{AAshade}, \text{PAR}) = 0$
 - AAsun - AAshade varies among accessions, correlated with PAR
- Assimilatory * Plastic * Constitutive (reinforcing interaction)
 - AAsun - AAshade greater at 2000 than 150
 - AA2000 - AA150 greater in sun leaves than shade leaves
 - AAsun - AAshade varies among accession, correlated with PAR
 - AA2000 - AA150 varies among accession, correlated with PAR
 - $\text{cor}(\text{AAsun2000}, \text{PAR}) > \text{cor}(\text{AAsun150}, \text{PAR})$ and $> \text{cor}(\text{AAshade2000}, \text{PAR})$ and $> \text{cor}(\text{AAshade150}, \text{PAR})$ in nonadditive way

We tested competing predictions through a combination of model selection and parameter estimation from the posterior of selected models. We started with a base model that includes fixed effects of light intensity and light treatment on AA, a random effect of accession on AA, and a linear regression of PAR on accession-level AA. We next compared the fit of the base model to a more complex with an interaction between light intensity and light treatment using LOOIC with the *R* package **loo** version 2.7.0 (**vehtari_practical_2017?**). These comparisons allowed us to test the main predictions of each hypothesis and for reinforcing interactions between assimilatory and plastic hypotheses (tab:predictions). However, these models cannot test predictions when there is an interaction with the constitutive hypothesis, because this hypothesis posits accession-levels variation in responses to light intensity and/or light treatment. Therefore, we tested for significant variation among accessions in light intensity and light treatment by comparing LOOIC values of models with and without random interactions between these factors and accession.

| Hypothesis | Prediction | Model |
|-----------------------------|------------|---|
| Assimilatory | | base model |
| Plastic | | base model |
| Constitutive | | base model |
| Assimilatory * Plastic | | + intensity \times treatment |
| Assimilatory * Constitutive | | + accession \times intensity (random) |
| Plastic * Constitutive | | + accession \times treatment (random) |
| Assimilatory * Plastic * | | + accession \times intensity \times treatment |
| Constitutive | | (random) |

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References

1. G. Triplett, T. N. Buckley, C. D. Muir, [Amphistomy increases leaf photosynthesis more in coastal than montane plants of Hawaiian ‘ilima \(*Sida fallax* \)](#). *American Journal of Botany* **111**, e16284 (2024).
2. I. E. Peralta, D. M. Spooner, S. Knapp, Taxonomy of wild tomatoes and their relatives (*solanum* sect. *Lycopersicoides*, sect. *Juglandifolia*, sect. *Lycopersicon*; Solanaceae). **84** (2008).
3. P.-G. Schoch, C. Zinsou, M. Sibi, [Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L.: 1. Effect of light intensity](#). *Journal of Experimental Botany* **31**, 1211–1216 (1980).
4. D. F. Parkhurst, [The adaptive significance of stomatal occurrence on one or both surfaces of leaves](#). *The Journal of Ecology* **66**, 367–383 (1978).
5. L. Sack, T. N. Buckley, [The developmental basis of stomatal density and flux](#). *Plant Physiology* **171**, 2358–2363 (2016).
6. K. A. Mott, O. Michaelson, Amphistomy as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae). *American Journal of Botany* **78**, 76–79 (1991).
7. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, [Fiji: An open-source platform for biological-image analysis](#). *Nature Methods* **9**, 676–682 (2012).

8. A. Gelman, D. B. Rubin, Inference from iterative simulation using multiple sequences. *Statistical Science* **7**, 457–472 (1992).
9. P.-C. Bürkner, **Brms** : An *r* Package for Bayesian Multilevel Models Using *stan*. *Journal of Statistical Software* **80** (2017).
10. Stan Development Team, *Stan Modeling Language Users Guide and Reference Manual* (2023; <https://mc-stan.org>).
11. J. Gabry, R. Češnovar, *Cmdstanr: R Interface to 'CmdStan'* (2023; <https://mc-stan.org/cmdstanr>, <https://discourse.mc-stan.org>).
12. R Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2023; <http://www.R-project.org/>).