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
- ?

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 CO$*2$* demand). A one-dimensional circuit model using the Farquhar-von Caemmerer-Berry biochemical model 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 [@triplett_amphistomy_2024]. 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	cor(PAR, AA) = 0	AAsun = AAshade	AA2000 > AA150
plastic	cor(PAR, AAsun) > 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

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* [@peralta_taxonomy_2008] and the cultivated tomato *S. lycopersicum* var. *lycopersicum* (tab:accessions). 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 $= 200\ \mu\mathrm{mol\,m^{-2}\,s^{-1}}$.

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).

outline of tab:accessions LA number,Species name, lat, lon, elev, SPLASH light value