# Differences in stomatal conductance between leaf shape genotypes of *Ipomoea hederacea* suggest divergent ecophysiological strategies

Yash Kumar Singhal[1§], Julia Anne Boyle[1,2], John R. Stinchcombe[1,3§]

1. Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada
2. Department of Ecology and Evolutionary Biology, University of Michigan–Ann Arbor, Ann Arbor, Michigan, United States
3. Koffler Scientific Reserve, University of Toronto, Toronto, Ontario, Canada

§ To whom correspondence should be addressed: yashkumarsinghal2003@gmail.com; john.stinchcombe@utoronto.ca

## Abstract

Intraspecific variation in leaf shape affects many physiological processes but its impact on plant distribution is underexplored. Using a common garden, we studied daytime thermoregulation of lobed and entire leaf genotypes of *Ipomoea hederacea*, which displays a latitudinal leaf shape cline. Both leaf shapes maintained similar temperatures but entire leaf genotypes had significantly increased stomatal conductance in warmer/sunnier weather. With less potential water loss, lobed genotypes may have advantages in drier conditions. Lobed genotypes are more common in the north of the species' range, which receives less summer precipitation, suggesting water availability as a potential clinally varying selective agent.

**Keywords: Intraspecific Variation, Thermoregulation, Ecophysiology, Latitudinal Cline, Morning Glory**

## Description

A fundamental goal of ecology and evolution is to understand the distribution of intraspecific variation, as it affects responses to selection within and across environments (DeMarche et al., 2019; Song and Li 2023). Climate change is expected to greatly change selective landscapes (Parmesan and Hanley 2015; Chiang et al., 2021), and plant species are predicted to undergo latitudinal range-shifts to track optimum environmental conditions (Chen et al., 2011; Lenoir and Svenning 2015; Tomiolo and Ward 2018). Species persistence will rely, in part, on existing genetic variation as well as phenotypic and physiological plasticity (Anderson et al., 2012; Bellard et al., 2012). Here, we examine how genetic variation in leaf morphology in *Ipomoea hederacea* (Ivy-leaf morning glory) leads to differences in ecophysiology, and whether these differences are consistent with existing phenotypic clines.

There is an incredible diversity of leaf shapes between and within species, and leaves are involved in crucial processes such as photosynthesis, thermoregulation, and defence (Nicotra et al., 2011). A single Mendelian locus controls leaf shape in *Ipomoea hederacea,* resulting in a homozygous lobed genotype, a homozygous entire genotype, and an intermediately lobed heterozygote (Figure 1a; Elmore 1986; Bright 1998). The species' range extends from Mexico to southern Pennsylvania with a latitudinal cline in leaf shape, where lobed plants dominate northern populations while southern populations are either entire or

polymorphic (Bright 1998; Campitelli and Stinchcombe 2013a). The agents of selection maintaining this cline remain unclear (Campitelli and Stinchcombe 2013b).

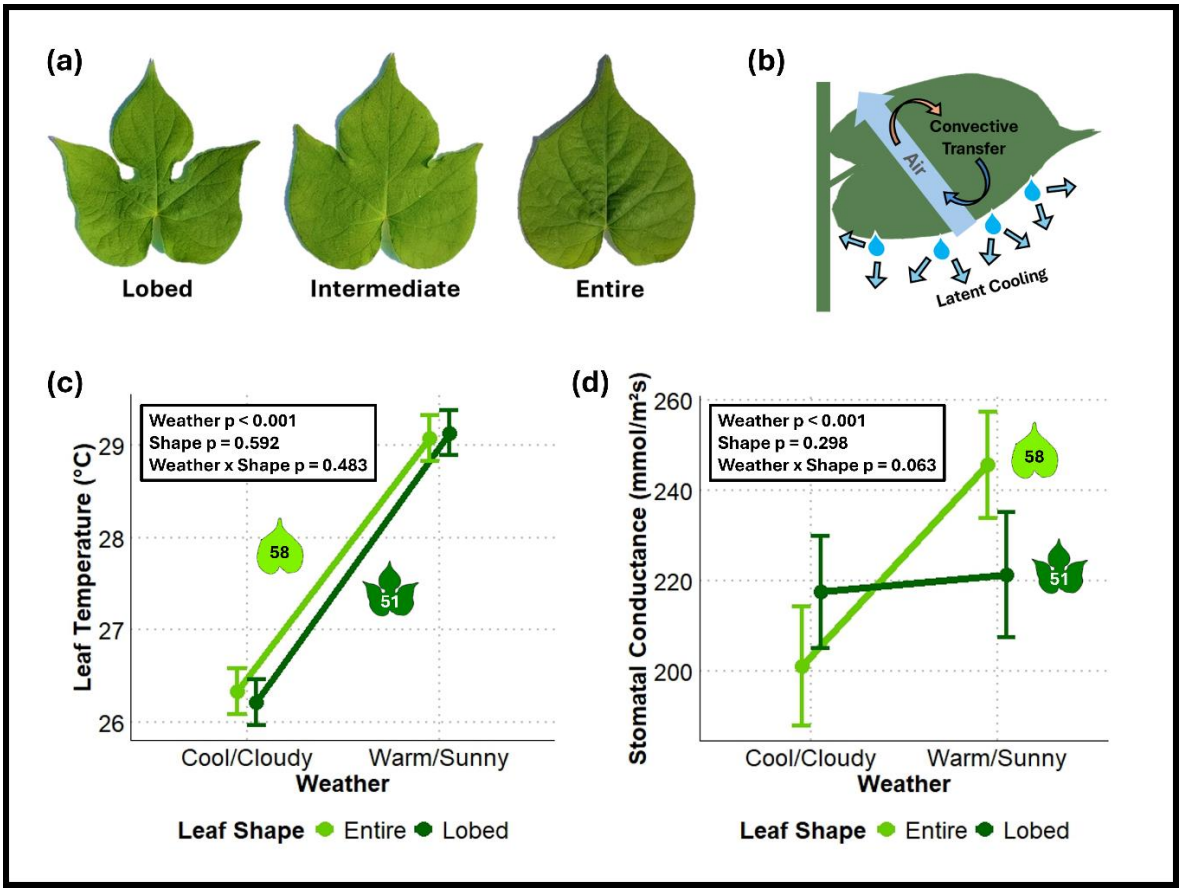


**Figure 1. Differing ecophysiology between leaf shape genotypes of *Ipomoea hederacea*:** (a) Leaf shape phenotypes of *Ipomoea hederacea*. (b) Schematic showing two major modes of leaf thermoregulation, convective transfer and latent cooling. (c) Daytime leaf temperature varies with abiotic environment but not leaf shape. (d) Stomatal conductance varies with abiotic environment for entire leaf plants but not lobed genotypes. Overall linear model statistics are displayed on the graphs in (c) and (d). Points represent marginal means ± standard error.

Plants thermoregulate through three main mechanisms (Figure 1b): radiative heat transfer (heat exchange in the form of radiation), convective heat transfer (exchange of heat with surrounding air), and latent cooling (loss of heat through transpiration; Nobel 2020). Ecophysiology theory predicts that leaf shape influences thermoregulation as it affects air flow around leaves and thus convective heat transfer between leaf surfaces and surrounding air (Vogel 1970; Schuepp 1993). Lobed leaves are predicted to have smaller effective boundary layers and thus more efficient convective cooling, which keeps them more closely matched to air temperature than entire leaves (Vogel 1970; Gurevitch and Schuepp 1990, Schuepp 1993; Nobel 2020).

We grew *I. hederacea* of all leaf shape genotypes in a long-term common garden at a northern latitude, and measured leaf temperature and abaxial stomatal conductance to characterize thermoregulation. We repeated these measurements on two days differing in temperature and light (Table 1): a cool/cloudy day and a warm/sunny day 48 hours apart, to study the same plants under different environmental conditions.

**Table 1:** Average weather metrics calculated from 181 measurements, taken every minute between 12:00 and 15:00 hrs (on July 24th and July 26th, 2024) at the Koffler Scientific Reserve Weather Station, near the common garden. All weather metrics except precipitation were significantly different between the two days ($p < 0.001$).

| Day | Average Air Temperature (℃) | Average PAR (µE) | Average Pressure (mbar) | Average Wind Speed (m/s) | Average Gust Speed (m/s) | Total Rain (mm) |
|---|---|---|---|---|---|---|
| **July 24, 2024 (cool/cloudy)** | 23.8 ± 0.067 | 678 ± 26.1 | 977 ± 0.033 | 1.12 ± 0.048 | 2.76 ± 0.071 | 0.00 ± 0.00 |
| **July 26, 2024 (warm/sunny)** | 24.9 ± 0.045 | 1730 ± 2.52 | 985 ± 0.020 | 0.897± 0.049 | 2.17 ± 0.067 | 0.00 ± 0.00 |

Consistent with previous results (Campitelli et al., 2013; Campitelli and Stinchcombe 2013b), there was no significant effect of leaf shape on daytime leaf temperature on either day (Tukey's post hoc test, $p = 0.595$ on the cloudy day and 0.775 on the sunny day); leaves on all plants were on average 2.83℃ warmer in warm/sunny conditions (Tukey's post hoc test, $p < 0.001$ for both shapes; Figure 1c). Increased leaf temperature was coupled with a significant 44.45 mmol/m$^2$s increase in average stomatal conductance of entire shaped leaves in warm/sunny conditions (Tukey's post hoc test, $p = 0.027$; Figure 1d), but not lobed leaves (Tukey's post hoc test, $p = 0.856$; Figure 1d). Higher entire leaf stomatal conductance suggests increased investment into latent cooling, as plants were losing more water through their stomata when it was hotter. As lobed leaves did not increase latent cooling in hotter temperatures, yet all plants maintained similar leaf temperatures, plants with lobed leaves may thermoregulate via another strategy, potentially through greater convective cooling facilitated by their shape.

Stomatal conductance affects water loss and gas exchange (Cowan 1982) which is strongly correlated with photosynthesis (Lawson et al., 2018). Thermoregulation and photosynthetic activity are thus likely both altered between genotypes. We suggest that reduced dependency on latent cooling may allow lobed genotypes to keep their stomata closed, reduce evaporative water loss, and conserve water on hot and sunny days, which would be beneficial in areas with low water availability. In contrast, greater stomatal conductance in entire genotypes on sunny days implies more open stomata, facilitating greater latent cooling, water loss, and gas exchange. Such a strategy would be advantageous in areas with greater water availability. Our hypothesis is consistent with the leaf shape cline in *I.*

*hederacea,* where lobed genotypes are more frequent in northern latitudes which receive less summer precipitation (e.g., Martin-Benito and Pederson 2015), and entire genotypes are more common in southern sites with greater summer precipitation. These findings suggest water availability as a potential selective agent contributing to the leaf shape cline.

Future work could test the hypothesis of divergent strategies by measuring biomass accumulation, photosynthetic efficiency, and fitness of the genotypes under different water availability conditions in the field. Experiments that factorially manipulate temperature and photosynthetically active radiation (PAR) could also test whether thermoregulatory strategy and photosynthetic activity explain the trends in stomatal conductance.

Intraspecific variation in thermoregulation and other physiological traits is likely a factor underlying species distributions (Westerband et al., 2021) and may affect species resilience to ecological challenges (Niu et al., 2020). One implication of our results is that species and genotypes that rely strongly on latent cooling or have other water-intensive physiological traits may face novel ecological challenges with changing precipitation patterns.

**Methods**

We used an experimental population of *I. hederacea* grown north of their natural range at the Koffler Scientific Reserve (KSR, www.ksr.utoronto.ca) in Ontario, Canada (44°01'48”N, 79°32'01”W). We started by collecting both genotypes from North Carolina, USA, and selfing them for seven generations to create the parents (P1). We crossed homozygous entire and lobed P1 individuals; an F1 heterozygote was selfed to produce an F2 population (Campitelli and Stinchcombe 2013b; Boyle et al., 2024). We allowed heterozygous F2 individuals to self-fertilize, producing F3 seeds planted into the common garden in 2019. We let plants naturally dehisce in 2019 and each subsequent year, ploughing the field in early summer each year to maintain the population. We sampled the 5th generation in July 2024, measuring 58 entire and 51 lobed individuals (heterozygotes and lobed grouped). We grouped heterozygous and homozygous lobed individuals because our ecophysiological hypotheses were based on differences between lobed and entire shaped leaves; distinguishing between heterozygous and homozygous lobed individuals in the field is also challenging. Because heterozygotes are intermediately lobed, considering them with homozygous lobed individuals should make our results conservative.

*Measurements*

We measured leaf temperature (°C) and abaxial stomatal conductance (mmol/m$^2$s) using a hand-held leaf porometer (Decagon Devices, SC-1 Model). We calibrated and used the device without a desiccant. We surveyed the field systematically using quadrats of equal size, and measured the first fully open leaf from the top of each plant; measured leaves were of similar size. We took measurements of the same plants on a cool/cloudy day (July 24th, 2024) and a warm/sunny day (July 26th, 2024) to study thermoregulation under

different environmental conditions (Table 1). All measurements were taken between 12:00 and 15:00 hrs, based on a preliminary time-series.

*Weather*

We gathered weather data from a weather station 530 m from the site (Table 1) using measurements from every minute between 12:00 and 15:00 hrs. The cool/cloudy day of sampling (July 24th) had a lower average air temperature, average PAR, average pressure, average wind-speed, and average gust-speed (Welch Two Sample t-test, all $p < 0.001$) than the warm/sunny day (July 26th). There was no precipitation during the sampling interval on either day.

*Statistical analysis*

We analyzed data using R (v4.4.1; R Core Team 2024) to implement linear mixed effects models in *lme4* (v1.1.35.5; Bates et al., 2015) and *lmerTest* (v3.1.3; Kuznetsova et al., 2017). We used *tidyverse* and *ggplot2* to process data and create plots (v2.0.0; Wickham et al., 2019; v3.5.1; Wickham 2016).

We first modeled leaf temperature as a function of fixed effects of leaf conductance, measurement day (and thus weather, Table 1), leaf shape, and a leaf shape*measurement day interaction; we included sampling quadrant as a random effect. The second model had the same structure except leaf conductance was the response and leaf temperature was a fixed predictor, to account for the relationship between temperature and conductance. In this second model, the random effect of sampling quadrat was estimated to be zero, so we dropped this term and used a linear model.

We used *emmeans* (v1.10.5; Lenth 2024) to obtain marginal mean leaf temperatures and stomatal conductances from each model, grouped by leaf shape and measurement day. We compared extracted means of leaf shape and day using Tukey's Post Hoc tests (Figure 1c and 1d).

Residuals of the model for stomatal conductance measures were normally distributed; residuals from models of temperature were not. We used *bestNormalize* (v1.9.1; Peterson 2021) which identified an Ordered Quantile (ORQ) normalization as the best transformation of temperature. Analysis of ORQ-transformed, log-transformed, and square-root transformed data yielded identical results in direction and statistical significance. We present analyses and means with temperature data in original units for ease of interpretation.

**Acknowledgements:** We want to thank Radana Molnarova, Julio de Almeida, and all other staff at the Koffler Scientific Reserve for accommodating and supporting this project. We are incredibly grateful to the University of Toronto's Centre for Global Change Science for their support and funding (YKS). We thank Amanda Peake for providing feedback on the manuscript and assistance in the field, and NSERC Canada for funding in the form of Discovery Grants (JRS) and CGS M and PGS D graduate fellowships (JAB).

**References**

Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society B: Biological Sciences 279: 3843-3852. DOI: 10.1098/rspb.2012.1051

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67: 1-48 DOI: 10.18637/jss.v067.i01

Bellard Cl, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. 2012. Impacts of climate change on the future of biodiversity. Ecology Letters 15: 365-377. DOI: 10.1111/j.1461-0248.2011.01736.x

Boyle JA, Frederickson ME, Stinchcombe JR. 2024. Genetic architecture of heritable leaf microbes. Microbiology Spectrum 12: e00610-24. DOI: 10.1128/spectrum.00610-24

Bright KL. 1998. Geographic variation and natural selection on a leaf shape polymorphism in the ivyleaf morning glory (*Ipomoea hederacea*) [Dissertation]. Duke University. Campitelli

Campitelli BE, Gorton AJ, Ostevik KL, Stinchcombe JR. 2013. The effect of leaf shape on the thermoregulation and frost tolerance of an annual vine, *Ipomoea hederacea* (Convolvulaceae). American Journal of Botany 100: 2175-2182. DOI: 10.3732/ajb.1300136

Campitelli BE, Stinchcombe JR. 2013a. Natural selection maintains a single-locus leaf shape cline in Ivyleaf morning glory, *Ipomoea hederacea*. Molecular Ecology 22: 552-564. DOI: 10.1111/mec.12057

Campitelli BE, Stinchcombe JR. 2013b. Testing potential selective agents acting on leaf shape in *Ipomoea hederacea*: predictions based on an adaptive leaf shape cline. Ecology and Evolution 3: 2409-2423. DOI: 10.1002/ece3.641

Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD. 2011. Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science 333: 1024-1026. DOI: 10.1126/science.1206432

Chiang F, Mazdiyasni O, AghaKouchak A. 2021. Evidence of anthropogenic impacts on global drought frequency, duration, and intensity. Nature Communications 12: 2754 DOI: 10.1038/s41467-021-22314-w

Cowan IR. 1982. Regulation of Water Use in Relation to Carbon Gain in Higher Plants. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, editors. Physiological Plant Ecology II. Encyclopedia of Plant Physiology, Volume 12/B. Berlin: Springer. p. 589 613. DOI: 10.1007/978-3-642-68150-9_18

DeMarche ML, Doak DF, Morris WF. 2019. Incorporating local adaptation into forecasts of species' distribution and abundance under climate change. Global Change Biology 25: 775-793. DOI: 10.1111/gcb.14562

Elmore CD. 1986. Mode of Reproduction and Inheritance of Leaf Shape in *Ipomoea hederacea*. Weed Science 34: 391-395. DOI: 10.1017/S0043174500067047

Gurevitch J, Schuepp PH. 1990. Boundary layer properties of highly dissected leaves: an investigation using an electrochemical fluid tunnel. Plant, Cell & Environment 13: 783-792. DOI: 10.1111/j.1365-3040.1990.tb01094.x

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software 82: 1-26 DOI: 10.18637/jss.v082.i13

Lawson T, Terashima I, Fujita T, Wang Y. 2018. Coordination Between Photosynthesis and Stomatal Behavior. In: Adams III W, Terashima I, editors. The Leaf: A Platform for Performing Photosynthesis. Advances in Photosynthesis and Respiration. vol 44. Cham: Springer. p. 141-161. DOI: 10.1007/978-3-319-93594-2_6

Lenoir J, Svenning JC. 2015. Climate-related range shifts – a global multidimensional synthesis and new research directions. Ecography 38: 15-28. DOI: 10.1111/ecog.00967

Lenth R. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.5. https://CRAN.R-project.org/package=emmeans.

Martin-Benito D, Pederson N. 2015. Convergence in drought stress, but a divergence of climatic drivers across a latitudinal gradient in a temperate broadleaf forest. Journal of Biogeography 42: 925-937. DOI: 10.1111/jbi.12462

Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H. 2011. The evolution and functional significance of leaf shape in the angiosperms. Functional Plant Biology 38: 535. DOI: 10.1071/FP11057

Niu K, Zhang S, Lechowicz MJ. 2020. Harsh environmental regimes increase the functional significance of intraspecific variation in plant communities. Functional Ecology 34: 1666-1677. DOI: 10.1111/1365-2435.13582

Nobel PS. 2020. Physicochemical and Environmental Plant Physiology. 5th Edition. London, England: Elsevier Academic Press. DOI: 10.1016/C2018-0-04662-9

Parmesan C, Hanley ME. 2015. Plants and climate change: complexities and surprises. Annals of Botany 116: 849-864. DOI: 10.1093/aob/mcv169

Peterson RA. 2021. Finding Optimal Normalizing Transformations via bestNormalize. The R Journal 13: 310-329. DOI: 10.32614/RJ-2021-041

R Core Team. 2024. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Schuepp PH. 1993. Tansley Review No. 59 Leaf boundary layers. New Phytologist 125: 477-507. DOI: 10.1111/j.1469 8137.1993.tb03898.x

Song WH, Li JJ. 2023. The effects of intraspecific variation on forecasts of species range shifts under climate change. Science of The Total Environment 857: 159513. DOI: 10.1016/j.scitotenv.2022.159513

Tomiolo S, Ward D. 2018. Species migrations and range shifts: A synthesis of causes and consequences. Perspectives in Plant Ecology, Evolution and Systematics 33: 62-77. DOI: 10.1016/j.ppees.2018.06.001

Vogel S. 1970. Convective Cooling at Low Airspeeds and the Shapes of Broad Leaves. Journal of Experimental Botany 21: 91-101. DOI: 10.1093/jxb/21.1.91

Westerband AC, Funk JL, Barton KE. 2021. Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. Annals of Botany 127: 397-410. DOI: 10.1093/aob/mcab011

Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al., Yutani. 2019. Welcome to the Tidyverse. Journal of Open Source Software 4: 1686. DOI: 10.21105/joss.01686

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Second Edition. Cham: Springer DOI: 10.1007/978-3-319-24277-4

**Funding:** University of Toronto's Centre for Global Change Science, NSERC.

**Author Contributions:** Yash Kumar Singhal: conceptualization, data curation, formal analysis, investigation, methodology, writing - original draft, writing - review editing, visualization.

Julia Anne Boyle: conceptualization, funding acquisition, supervision, writing - original draft, writing - review editing, formal analysis.

John R. Stinchcombe: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing - review editing, writing - original draft, formal analysis.