




# How and why? Synthesizing evolution and physiology

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Organisms are functionally constrained by physical and chemical laws, yet natural selection has produced seemingly endless forms within these fundamental limits. I combine empirical and theoretical approaches to tackle basic evolutionary and ecological questions grounded in physiological mechanisms. Physiologists have discovered numerous tradeoffs that organisms must confront in order to survive and reproduce, and such fitness tradeoffs are the theoretical foundation for most evolutionary and ecological models. Despite this seemingly natural integration between disciplines, an *evolutionary physiology synthesis* has largely eluded us. The result is that we know little about the role of physiological tradeoffs in adaptive evolution, even though such tradeoffs are widely assumed to be a major source of natural selection.

There are two major conceptual barriers to synthesis between disciplines that I aim to overcome. The first barrier is that evolutionary biologists are often not familiar with significant physiological details that impact the evolutionary inference. My work on stomatal and leaf evolution illustrates why the details matter. The second conceptual barrier is that physiologists generally focus on optimization but neglect the variety of evolutionary processes that maintain variation within and between species. I study the processes that maintain physiological variation from macro- to microevolutionary scales using multiple empirical systems suited to address different questions:

 Micro: within species	 Meso: within genus	 Macro: flowering plants
Monkeyflowers ( <i>Mimulus</i> )	Wild tomatoes ( <i>Solanum</i> )	Stomatal evolution

**Quantitative approaches – mathematical models, statistics, and computation – are integral to my research:** Mathematical models force us to explicitly state assumptions and rigorously derive logical conclusions. Biology may seem too complex to reduce to equations, but in my experience nonmathematical models are simpler, founded on unstated assumptions, and derived with faulty logic. I analyze mathematical models using integral calculus, ordinary differential equations, stochastic differential equations, and simulation models in R and Mathematica (see e.g. [Muir 2013](#), [2015](#), [Muir & Hahn 2014](#)).

Biological predictions are often complex and the data are unavoidably messy. Advanced statistical methods are needed to derive insight and not be misled by spurious patterns. I strongly believe that the statistics should be molded to the data and the biological question, not the other way around. I most often use generalized linear mixed effects models ([Muir et al. 2017](#)), nonlinear regression ([Muir & Thomas-Huebner 2015](#)), finite mixture models ([Muir 2015](#)), and phylogenetic comparative methods ([Muir 2015](#), [2017](#) (in press)). Moving forward, I will increasingly use Bayesian tools I've learned in academia (Bontrager et al. in review) and industry ([link](#))

Science should be open and reproducible. I use computational tools for programmatic data collection, wrangling, analysis, and reporting to generate reproducible manuscripts with transparent methods. Examples of reproducible manuscripts can be found on my public [GitHub](#) page. Moving forward, I aim to make my science more open by publicly preregistering predictions and publishing open lab notebooks in real time.

## I. Macroevolutionary physiology: variation and constraint on stomatal evolution

The major unresolved question in macroevolution is how fitness optima vary across large spans of time and space. Stomata are a fascinating trait for studying macroevolutionary processes because of their important but potentially conflicting roles in both abiotic and biotic interactions. Stomata are microscopic pores in a leaf that play an outsized role in plant ecology by allowing plants to tightly regulate water loss and CO<sub>2</sub> gain. Interestingly, stomata are also a major route of infection by foliar pathogens such as fungi and bacteria. I have been particularly interested in why most stomata are only on the lower surface of the leaf (hypostomy). Biophysical models actually predict that all plants should be amphistomatous (stomata on upper and lower leaf surfaces) to maximize photosynthesis, yet ~90% of plant species are hypostomatous. Across flowering plants, the relationship between stomatal ratio and growth indicates remarkably strong selection on efficient CO<sub>2</sub> supply in species with fast life histories ([Muir 2015](#)) from high light habitats

([Muir 2017](#) in press). Annual forbs from open habitats are nearly always amphistomatous; trees and shade tolerant herbs are nearly always hypostomatous. However, there must be a cost to amphistomy, otherwise it would be ubiquitous. Stomata on the upper surface might increase susceptibility to foliar pathogens that infect through stomata. Thus, stomata could mediate a tradeoff between photosynthesis and pathogen defense, but this is poorly studied.

I also discovered a wholly unanticipated aspect of stomatal evolution. Stomatal ratio is bimodal and occupies only part of the feasible trait space: plants are completely hypostomatous (all stomata on the lower surface) or have about half of their stomata on each surface, but intermediate trait values are rare. Lack of genetic variation and developmental constraints probably do not explain bimodality ([Muir et al. 2016](#) [in revision]). Instead, mathematical models reveal that bimodality might be a consequence of a tradeoff between pathogen defense and photosynthesis ([Muir 2015](#)). Next, I plan to test this hypothesis using theory and experiments on wild tomatoes as a model system. There is nearly as much variation in stomatal ratio among tomato species as in all flowering plants ([Muir et al. 2014](#)). I have also identified large-effect loci that explain most of the difference between hypo- and amphistomatous species ([Muir et al. 2014](#)). Importantly, genetic tools in tomatoes will allow me to measure the effect of stomatal ratio on photosynthesis and pathogens in isolation from other traits. I will also develop `leafevolvr`, an R package using biophysical models to predict optimal stomatal and other leaf traits across key environmental gradients such as light.



## II. Mesoevolutionary physiology: leaf evolution in wild tomatoes

Leaf size, shape, and internal anatomy are extremely diverse but strongly constrained by functions such as light interception, CO<sub>2</sub> diffusion, and managing scarce resources like water and nitrogen. Two common assumptions are that plants i) cannot build tougher leaves without sacrificing photosynthesis and ii) cannot increase photosynthesis without decreasing water-use efficiency. In collaboration with physiologist Jeroni Galmés (UIB, Spain), I have shown that in contrast to (i) increased leaf toughness (higher leaf mass per area) weakly constrains the evolution of photosynthetic function among closely related species ([Muir et al. 2014, 2017](#)). Instead we find that faster CO<sub>2</sub> diffusion through the mesophyll can simultaneously increase water-use efficiency and photosynthetic rate, contra (ii). This may be important in arid-adapted tomato species that are water-wise yet grow fast during brief periods of water availability ([Conesa et al. 2017](#)).

Harnessing natural variation to improve plant growth without evaporating more water has vast potential for sustainable agriculture. To do this, I will use recently developed high-throughput physiological phenotyping methods, phylogenetics, and quantitative genetics to identify the traits and loci that allow desert tomatoes to break tradeoffs between photosynthesis and water-use efficiency. To that end, I am working with computational biologist Matthew Pennell (UBC) to develop `bayCi`, an R package for studying the evolution of C<sub>3</sub> photosynthesis using Stan, a probabilistic programming language for Bayesian data analysis. My aim is to understand physiological mechanisms while generalizing across many closely related species.



## III. Microevolutionary physiology: local adaptation to climate in monkeyflowers

The theory of evolutionary processes acting within species (microevolution) is more mature than that for macroevolution, but there is surprisingly little evidence of divergent natural selection on physiological traits under natural conditions. To redress this gap, I am collaborating with Amy Angert (UBC) to investigate the physiology of local adaptation to climate in *Mimulus cardinalis*. We want to know what physiological traits are involved in climatic adaptation, what climatic factors shape local adaptation, and how does local adaptation affect heritable variation in fitness? Answering the last question will provide key parameter estimates for predicting whether species can adapt to rapid climate change. We have found latitudinal clines in physiological traits which suggest local adaptation to climate ([Muir & Angert 2017](#)), and are now using reciprocal transplants between Northern and Southern locations in California to measure physiological local adaptation under ecologically realistic field conditions.