# The sleeping giant needs coffee: overlooked areas for the integration of plant ecophysiology and evolutionary biology

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## ABSTRACT

Interpretations of evolutionary outcomes are limited without incorporation of physiological ecology; and ecophysiological interpretations would benefit from incorporating evolutionary perspectives. Although there has been a rise of studies in the last 20 years between these fields, evolutionary studies that incorporate plant physiology have largely focused on the same traits (i.e., flowering time, specific leaf area, etc.), neglecting to incorporate cellular and developmental traits. This is largely due to the high throughput demands in evolutionary studies and the lack of technological advancements in ecophysiology. However, this bias in measured traits has resulted in limiting our understanding of plant form and function evolution. On the other hand, most detailed studies on plant physiological and anatomical responses to the environment are either in applied sciences, focused on economically important plants, or examine model organisms rather than wild populations. These detailed ecophysiological studies generally do not incorporate evolutionary discourse, even though they often study adaptation. The aim of this paper is to offer a comprehensive resource, building upon previous works, for researchers to bridge the gap between ecophysiology and evolutionary ecology.

Keywords: functional traits, plant traits, evolutionary ecology, anatomy, physiology, rapid evolution, populations, population biology, mechanistic traits, development

## INTRODUCTION

To understand plant adaptation and responses to climate change, it is essential that the sometimes-disparate fields of ecophysiology and evolutionary biology integrate. These two fields have a long history of working in parallel with little overlap (Ackerly and Monson, 2003; Caruso et al., 2020a), resulting in divergent methods for study and little crosstalk. Ecophysiologists have sought questions related to present-day environments and physiological responses at the organismal-level (e.g., anatomy, metabolism, biomechanics, development) often implying adaptation, but not necessarily drawing evolutionary conclusions (Olson, 2019). On the other hand, population-level studies on evolution rarely seek to describe the organismal changes that are abundant in ecophysiological studies and instead, generally focus on macro-morphological traits (e.g., phenology, growth rates, biomass; Geber and Griffen, 2003). Although both fields are interested in examining responses to climatic events, inferences about global change would benefit from including both methodologies into single studies.

In 2003, Ackerley and Monson referred to this separation as “the sleeping giant”, urging ecophysiologists to employ a foundation of evolutionary theory; particularly relating traits with fitness. In the same year, Geber and Griffen (2003) published a meta-analysis on functional trait evolution, urging evolutionary biologists to study organismal-level traits. This showcases the desire from both sides of the aisle to integrate. More recently, a special issue of the *International Journal of Plant Sciences* highlighted the current state of functional trait evolution (Caruso et al., 2020a), providing evidence that “the giant is awake”. In contrast, Olson (2019) highlights areas that are still lacking in necessary overlap, emphasizing that “the giant [is] dozing on some issues”, with which Caruso (2025) later agrees. To this end, there has been great discussion about the role of functional traits on fitness, the need to integrate physiology and genomics, phenotypic plasticity effects on evolution and vice versa, and trait macroevolution (Caruso et al., 2020a; Olson 2019; IPSC, 2023). Therefore, instead of reiterating these works, this paper presents areas limiting the integration of ecophysiology and evolutionary biology that have not been thoroughly discussed. I argue that to obtain true integration, evolutionary biologists need to expand the types of traits they explore and ecophysiologists should incorporate evolutionary discourse (Fig 1).

## IN AN ERA OF BIG DATA, WHAT ARE WE MISSING?

In 2003, Geber and Griffen specifically stated that trait evolutionary studies should broaden the types of traits examined, specifically highlighting the need to study physiology and development. While this call has been made recurrently (Garnier and Navas, 2012; Olson, 2019; Caruso et al., 2020a; Olson, 2020; IPSC, 2023; Cocciardi et al., 2024; Olson, 2024; Caruso, 2025) and publication rate on such questions has been exponential since 1989 (Caruso et al., 2020a), a bias in what we measure remains. Indeed, out of 90 studies included in a meta-analysis on the selection strength of functional traits, 45% measured phenology, 23% physiology, 16% plant size, 14% leaf morphology, and 4% leaf chemistry (Fig. 2A; Caruso et al., 2020b). While this highlights the increase in bridging ecophysiology and evolutionary biology, it also emphasizes the underrepresentation of different phenotypic categories.

By examining these studies further, I find that even within categories, certain traits are more represented than others (Fig. 2B). Only one study measured chlorophyll fluorescence (physiological trait), compared to 13 that measured assimilation rate, and only one studied attractance compounds (leaf chemistry), compared to five that examined defense compounds. Critically, only two of the 90 studies examined development (in trichomes and reproduction), for which Geber and Griffen (2003) explicitly expressed the need to improve, and roots were poorly explored, having only been examined in five studies. Flowering time, in contrast, was the most commonly recorded trait overall (44 studies). These results showcase the narrow breadth of traits that are often explored and the need to broaden our plant phenotyping.

The phenotyping of plants, specifically the types of traits measured, has a storied past (Belluau and Shipley, 2018) that could be contributing to the reduced traits that are examined. One key issue is likely the concept of a ‘functional trait’. There are several definitions, which have been continuously discussed in the literature (Violle et al., 2007; Brodribb, 2017; Caruso et al., 2020a; Klimešová et al., 2025), but for evolutionary biology, functional traits are described as affecting a fitness component (reproduction, survival, or growth). However, without studying all traits and their impact on reproductive output over the lifespan of an organism, it is difficult to know what does or does not constitute a functional trait (MacColl, 2011). Others have sought to categorize traits as functional versus mechanistic (Brodribb, 2017) or “soft” versus “hard” (Belluau and Shipley, 2018), while others have claimed that all traits are functional (Violle et al., 2007), and yet we still have major dissonance between these definitions. Furthermore, these delimitations between "types of traits" are not helpful and risk perpetuating the divide between ecology and evolutionary biology, by harboring axiological assumptions on what is more rigorous.

Indeed, historical delimitations of what constitutes rigorous science could be to blame for missed opportunities between the fields of ecophysiology and evolutionary biology. For instance, Olson (2024) explains the significance of the historical belief that the comparative method of studying evolution and adaptation is less rigorous, as compared to controlled experimental methods. In contrast, Caruso (2025) argues that historically, experimental studies were often viewed as non-representative of natural conditions and therefore population comparative approaches were preferred. Interestingly, these perspective pieces were both written within a year of each other with differing viewpoints. Both criticize such divides and support increased overlap between the fields. This showcases that these arbitrary divides have often been associated with legacies of different fields of study.

Therefore, I propose that it is better to think about traits on a continuum from isotopic to phenological, not in a binary of "hard" vs. "soft" or "functional" vs. "mechanistic". Furthermore, I suggest treating traits as they are, rather than as accessible proxies of other traits, such as measuring stomatal conductance instead of using leaf mass per area (LMA, the inverse of specific leaf area (Pérez-Harguindeguy et al., 2013; Belluau and Shipley, 2018; Blonder et al., 2020). This is especially important since across environments and species, these proxy relationships are not always consistent (Blonder et al., 2020); providing rationale for studying more and different traits. As evolutionary biologists, it is our responsibility to expand the types of traits we measure (Fig. 2; for a detailed and exhaustive description of traits and methodologies see Weiher et al., 1999 and Pérez-Harguindeguy et al., 2013). As ecophysiologists, it is our responsibility to broaden our understanding of the long-term consequences of such traits, by incorporating reproductive output into our studies and thereby evolution (whether via selection or not; Fig.1). Doing so will help us to better understand plant adaptation at the within-species scale and the consequences of future climate change.

## WHERE IS THE SLEEPING GIANT?

To fully wake the "sleeping giant", we need to incorporate the developmental, anatomical, and biochemical components of ecophysiology and evolutionary biology. By not integrating these fields, we overlook information about the ways that plants exist, evolve, and respond (Box 1).

Indeed, much of what we know about plant physiological, cellular, and developmental responses and genetic alterations to their environment stems from the extensive literature on crop species, trees, and *Arabidopsis* (Pereira and Marais, 2020). As an example, the cuticle is important for plant resilience; following drought, cuticle thickness increased across wheat genotypes (Bi et al., 2017) and in *Arabidopsis* (Kosma et al., 2009); increased wax occurred when exposed to a pathogen in *Arabidopsis* (Xia et al., 2010); and changes in wax structure were associated with fungal defense (Arya et al., 2021). However, we know relatively little about the types of waxes produced by and the cuticular structure of wild species (Xue et al., 2017) and whether it is important for climatic responses. Even this simple example highlights several questions that remain to be asked: how does wax differ between populations?; can it evolve rapidly?; does it plastically respond to climate change? Thus, the consequence of not bringing these fields together is a great loss to understanding the natural world.

Furthermore, anatomical development is a staple of botanical sciences, but evolutionary studies that focus on anatomy are predominately related to development across taxa and lineages, rather than present-day evolution within and across populations (Sokoloff et al., 2021). Additionally, most experimental and *in situ* evolution studies are conducted on $C_3$ plants. Out of 90 studies on modern-day trait evolution, there was one $C_4$ plant, only three non-angiosperms, and only orchids represented CAM plants (Caruso et al., 2020b). Although extensive work has been conducted on the evolution of CAM and $C_4$ (Ehleringer et al., 1991) and modern differences (Edwards 2019; Gan and Sage, 2024), few studies have examined present-day rapid evolution of wild species (though *Setaria viridis* is a promising $C_4$ model for such research; Saha et al., 2016; Ethridge et al., 2023). Furthermore, while there are extensive anatomical studies on stress responses in woody species (Dalla-Salda et al., 2011; Guet et al., 2015; Pampuch et al., 2020) and their macro-evolution (48% of species were woody in Whiting et al., 2024 meta-analysis on genetic architecture of local adaptation), much less is known of their modern evolution given their life history. Therefore, much of our knowledge on rapid and modern evolution in plants is understandably from herbaceous or grass species. The lack of anatomical and developmental studies on diverse wild taxa emphasizes the gap in understanding evolutionary responses.

Additionally, although many ecophysiologists combine anatomical, developmental, and phenological traits to study structure-function relations, the connection to adaptation and evolution is not necessarily discussed. The context that these functional traits are often studied is in an adaptationist framework (Garson 2016; Olson, 2019; Olson 2020; Caruso 2025), that a function improves X, yet they do not necessarily measure fitness. Furthermore, this type of analysis does not include other types of evolutionary outcomes (drift, gene flow, mutation). The lack of recognition of this by ecologists, results in studies being overlooked by evolutionary biologists. The likely cause of this oversight is that the majority of ecophysiology studies are in applied settings (e.g., conservation, sustainability, restoration, etc.) or are perceived to be by evolutionary biologists due to differing academic lineages. Therefore, this is an excellent opportunity for evolutionary biologists to have more dialogue with ecophysiologists to bridge their questions together, as many ecophysiologists actually study adaptation (Garson, 2016; Olson, 2019).

## THE HIGH THROUGHPUT PROBLEM

### *Technological advancement disparity*

Across both fields, technological advancements have enabled us to generate data at unprecedented scales (Olson, 2019; Blumstein, 2024), but these advancements have not been equal across fields or across all traits. The era of genomics and remote sensing allows us to ask evolutionary and ecological questions that were once deemed impossible. High throughput genotyping has made it possible for evolutionary biologists to amass ever-larger genomic datasets, conduct genome-wide association studies (GWAS), glean deeper population genomic insights, and quantify plant diversity (single nucleotide polymorphisms and structural changes; Pereira and Des Marais, 2020). In parallel, remote sensing and imaging software has increased vast phenotyping of populations that ecophysiologists use (Blumstein, 2024). Drones equipped with sensing technologies, such as LiDAR can be used to monitor forest growth, health, and carbon absorption (Picos et al., 2020; Qin et al., 2022; Buchelt et al., 2024); *HyperART* images light absorption, reflectance, and transmission of leaves, characterizing chlorophyll content and disease resistance (Bergsträsser et al., 2015). The rate of advancement in these technologies is likely owing to the broader application of molecular techniques in fields such as medicine, and remote sensing in physics, defense, and climate sciences; compared to the lack of broad applicability of many other ecophysiological traits.

The rate of technological advance for many plant ecophysiological traits has been at a much slower pace, leading to its inability to keep up with genomics. For instance, there are long periods between the development of new and improved key instruments such as the 20 years between the 2016 LI-COR photosynthetic instrument and its previous model (Li-Cor Inc.). Furthermore, advances in reducing measurement time have largely been created by ecophysiologists themselves; for example, rapid carbon accumulation-intercellular carbon concentration (A/Ci) response curves (RACiR; Stinziano et al., 2017), or Fast Assimilation-Temperature Response curves (FAsTeR; Garen and Michaletz, 2024). It is well-known that there is a trade-off between throughput and accuracy and neither field is exempt. RACiR is not as accurate as steady-state A/Ci measurements (Lin et al., 2023), often overestimating carboxylation limitation ($V_{cmax}$) and underestimating RuBP regeneration ($J_{max}$; Taylor and Long, 2019). In genomics, there is a trade-off between greater coverage (the percentage of the genome sequenced) versus the number of samples sequenced (Fountain et al., 2016). However, the dichotomy between the advancement of these two fields is likely a major source of the continued separation and could further their asymmetry.

Furthermore, the high cost of ecophysiology instruments excludes scientists from much of the world, including where most of the world's biodiversity is located (Ciocca and Delgado, 2017). This can result in research groups not investing in new models, which can lead to increased time spent per sample, as well as measurement errors from older models. For instance, the 2016 LI-6800 can conduct environmental assimilation curves significantly faster than older models (up to 75% faster for A/Ci curves; personal observation), and it alleviates much of the thermal measurement error present previously (Garen et al., 2022). Additionally, even when there is access to newer instruments, there is likely only one accessible, making it difficult to process numerous samples. Many necessary tools in ecophysiology are prohibitively expensive and are less likely to be used by other fields, limiting their implementation in population evolutionary studies.

### *Experimental design differences*

The difference in technological advancement and the time-consuming manner of many ecophysiological traits has led to major differences in sampling size and questions that can be answered with statistical confidence. For instance, ecophysiological studies can examine as few as five samples per five individuals within a population (Petruzzellis et al., 2017) or even across a whole species, while population-level evolutionary studies require up to hundreds of individuals depending on the type of study (Hong and Park, 2012). For example, an exhaustive ecophysiology study on xylem embolism in wheat examined 43 plants across three different treatments (a minimum of 20 minutes per scan, personal communication; Harrison-Day et al., 2024). Furthermore, out of 72 studies that examined plant resistance to cavitation ($P_{50}$), only 14 examined more than three separate populations (Anderegg, 2014). In contrast, to study environmental effects on phenotype based on differing levels of additive genetic variation, 3630 individuals of *Brassica rapa* from 62 family lines were studied (So et al., 2022). Indeed, in a meta-analysis of the genetic architecture of local adaptation across 21 studies, the median and average sample sizes were 360 and 523 individuals respectively, and all studies had at least five populations (Whiting et al., 2024). Interestingly, anatomical traits have been critical in the study of paleobotany despite the small sample sizes (e.g., xylem and wood development, seed and floral evolution; Feild et al., 2011; Larter et al., 2017; Mason et al., 2020), as well as macroevolutionary plant biology (e.g., xylem, stomata, leaf architecture of CAM and $C_4$ plant evolution; Sage, 2004; Larter et al., 2017; Olson, 2020; Sokoloff et al., 2021; Muir et al., 2023). However, this same lens has not been pursued widely at the level of modern-day evolution. Of course, these differences in sample size pertain to specific questions, but one cannot ignore the contrast and difficulty it presents in encouraging overlap between population evolution and ecophysiology.

### *Overcoming the high throughput challenge*

Collaboration and population subsets will be at the heart of reducing the high throughput problem and increasing our ability to understand species and population-level differences. Firstly, through a large collaborative effort, Todesco *et al.* (2020) were able to examine ecotype differences of 1400 sunflowers by measuring 16 different traits (albeit many were similar iterations; i.e., nitrogen isotopes, carbon isotopes, C:N ratio, trichome length and densities of different areas, and RGB colouration). This work highlights that the high throughput problem can be overcome when we work collectively. The need for interdisciplinary collaboration between physiologists and plant geneticists to map genotypes to phenotypic responses is widely acknowledged (IPSC, 2023). Furthermore, with collective agreement that physiology and anatomy are important, we can place greater pressure for the necessary advancement in efficient technology development to further increase throughput. The fact that discussions about the need for integration between these fields is long-standing emphasizes that we know the outcomes of such collaboration will be worthwhile.

Secondly, to better incorporate traits into evolutionary studies, the evolutionary community must be open to adopting smaller sample sizes by using subsets of their populations. Here, a subset of the experimental populations can be examined for anatomical, development, or chemical traits, which can be analyzed alone, alongside other phenotypic data, or extrapolated onto the larger population. For example, Dutch elm disease was studied by inoculating ~1600 trees with *Ophiostoma novo-ulmi* and the timing and percentage of observational wilt across genotypes was examined (Venturas et al., 2014). A subset of twenty-four trees were examined for hydraulic conductivity, wood density, xylem size, and xylem distributions. This experimental design enabled the researchers to disentangle anatomical and physiological characteristics, how they relate to observational indicators of disease, and their heritability in preventing disease spread (Venturas et al., 2014). This is a perfect example of integrating ecophysiological traits into a broader evolutionary context.

## CONCLUSION

Although the merging of ecophysiology and evolutionary biology has been ongoing (Ackerly and Monson, 2003; Caruso et al., 2020a), critical barriers still prevent widespread overlap: the slow rate of ecophysiology technological advancements, large sample sizes in population genomics contrasting with throughput of physiological and anatomical traits, and the cost of ecophysiology tools. Furthermore, contemporary evolutionary studies often neglect physiological, cellular, and developmental traits, therefore limiting our understanding of plant evolution and potentially missing cryptic evolution (Klimešová et al., 2025). In contrast, ecophysiologists often ask evolutionary questions (structure-function as they relate to environments) but do not incorporate this into their discussions or follow-up experiments. By not integrating ecophysiology and evolutionary biology, specifically examining biochemical, anatomical, and developmental traits to complement phenological traits and fitness measurements, we risk losing important drivers of evolutionary change and understanding of plant responses to their environment. Through increased collaboration, inclusion of population subsets, and technological advancements, we will bridge this gap and improve our understanding of plant form, function, and its evolution. As my colleagues have noted before me, the sleeping giant is no longer sleeping, but he is in desperate need of some coffee.

## AUTHOR CONTRIBUTIONS

HAB conceptualized, wrote, and edited.

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Box 1. Here I will use *Mimulus cardinalis* as an example of how the integration of ecophysiology and evolutionary biology improves our understanding of plant morphology and evolutionary change. While each experiment on its own provides information about plants in their environments, together there is a greater depth of understanding.

In experiment 1 (modified from Anstett et al., 2021) evolutionary biologists ask: does climatic history affect population evolution to drought? Ancestor (pre-drought) and descendant (post-drought) seeds from northern and southern regions are grown in a common garden and specific leaf area (SLA) examined. Southern populations evolve rapidly (lower SLA), while northern populations do not. The takeaway being that northern populations are unable to respond fast enough. A clear example of difference in population-level capacity to respond to modern perturbations.

In experiment 2 (modified from Branch et al., 2024), ecophysiologists ask: are there ecotypic differences in plasticity to drought? Leaf architecture and photosynthesis of northern and southern post-drought populations are measured. Both populations exhibit plasticity (increased palisade and stomata in dry treatments). However, northern populations have greater carbon assimilation in wet treatments compared to southern populations that do better in dry conditions. They conclude this is local adaptation to historic water availability.

In experiment 3 evolutionary ecophysiologists ask: do changes in leaf anatomy explain SLA in experiment 1?: The same ancestor and descendant plants from experiment 1 are grown to examine leaf anatomy. Prior to drought, northern plants were not plastic in anatomical traits but following drought they evolved plasticity similar to pre-drought southern plants. Southern plants evolved reduced plasticity, likely corresponding to greater drought specialization. Here the takeaway is that both populations are evolving rapidly, but in different ways. Southern plants are further along the drought tolerance spectrum, while northern plants are catching up, while could have contributed to the difference in SLA observed in experiment 1. This highlights how having narrow breadth of traits that we measure biases our understanding of evolution and how not incorporating evolutionary perspectives reduces our capacity to understand ecological limits.

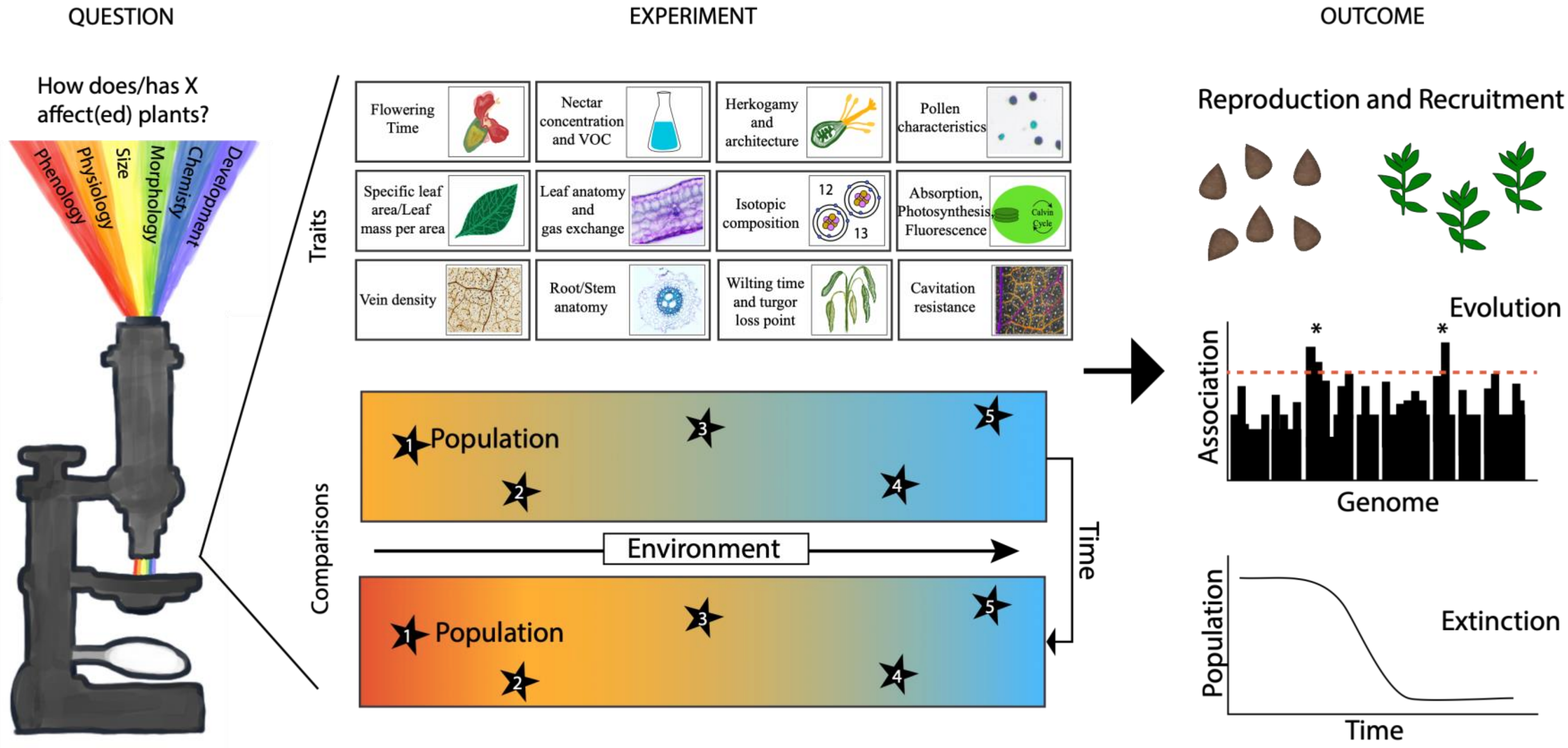

**Figure 1.** Methodological illustration for asking whether X affects plants. Question provides an example of a question that both ecologists and evolutionary biologists ask, with categories of traits often of interest. Experiment shows trait examples and comparisons (populations, environmental, across time). Types of traits are where evolutionary biologists could expand their breadth. Outcome describes lasting effects of whether X affects traits to the point that it is passed on and contributes to future generations or it leads to extinction. Outcomes is where ecophysiologists could expand their work. Images courtesy of Dr. Stefanie Sultmanis (vein density), Dr. Beatrice Harrison Day (root section), and Dr. Vanessa Tonet (leaf cavitation).

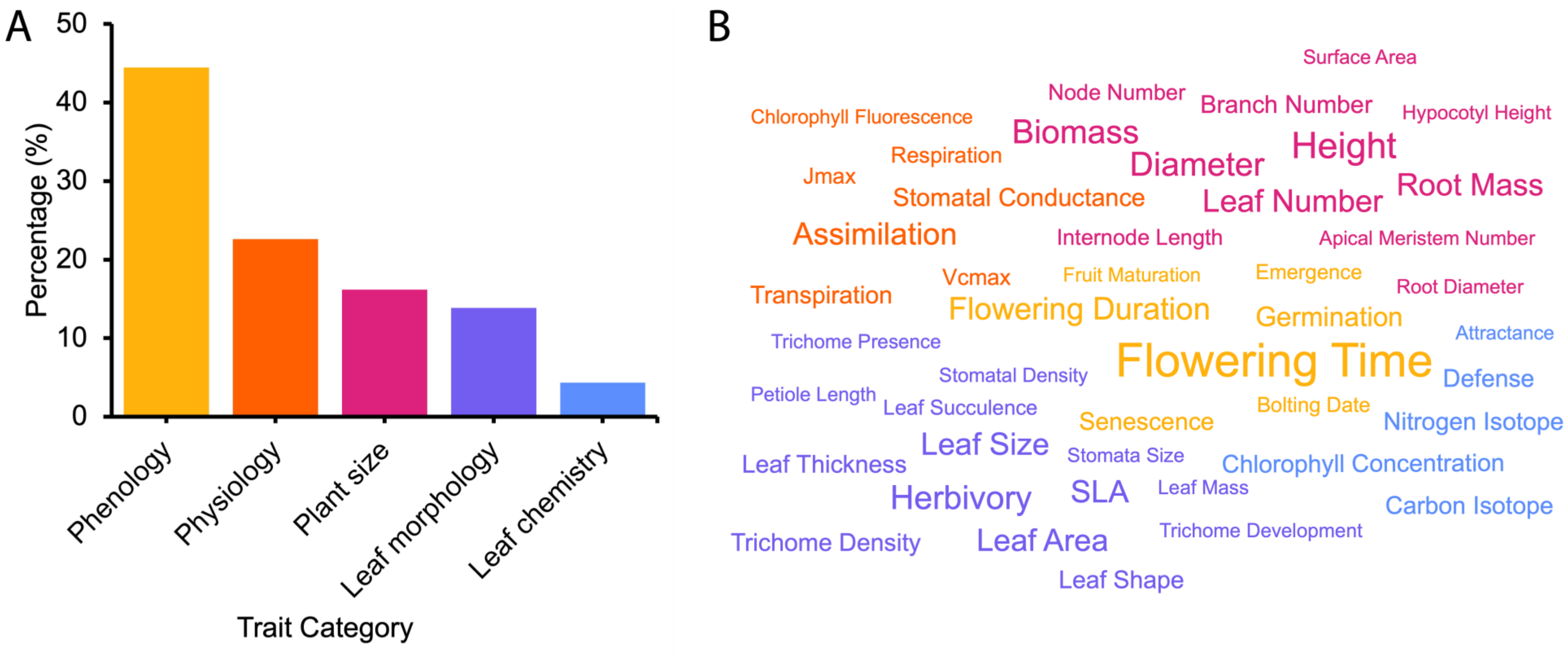

**Figure 2. Visual representations of 90 studies on functional trait selection and the trait categories adapted from Caruso et al. 2020b.** A) Percentage of studies that included the five trait categories used in their meta-analysis: phenology, physiology, plant size, leaf morphology, and leaf chemistry. Categories are in order from greatest number of studies to fewest. B) Wordcloud representation of the underlying traits measured within each of the five categories in panel A. Size of the trait indicates the frequency of use (i.e., flowering time was the most measured trait across all studies). Trait categories are colour-coded to match panel A and are also closer together with in the wordcloud.