THE EVOLUTION OF FUNCTIONAL TRAITS IN PLANTS: IS THE GIANT STILL SLEEPING?

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In the field of plant ecophysiology, the term "functional trait" has multiple overlapping definitions (table 1). However, functional traits are generally considered aspects of plant phenotypes that influence growth, survival, and reproduction by mediating interactions with the biotic and abiotic environment; examples include leaf mass per unit area (LMA), which estimates the construction cost for light interception, and photosynthetic capacity (A_{max}), which estimates the maximum rate of carbon fixation when light is not limiting. As such, functional traits are expected to evolve in response to natural selection, and intra- and interspecific variation in functional traits is often interpreted as being adaptive.

One milestone in the effort to understand the evolution of functional traits in plants was the publication in 2003 of a special issue of the *International Journal of Plant Sciences*. This special issue included papers that have been repeatedly cited in subsequent studies of the evolution of functional traits: examples include Reich et al.'s (2003) review of the mechanisms that affect the evolution of functional trait spectra and Geber and Griffen's (2003) quantitative meta-analysis of estimates of natural selection on and heritability of functional traits. But in the introduction to the 2003 special issue, the guest editors had a "tough love" message for plant functional biologists (Ackerly and Monson 2003, p. S1):

> As students of evolutionary and ecological physiology, we often accept the adaptive nature of morphological and functional traits despite little or no evidentiary connection between function and fitness, or we accept hypotheses of homology or convergence between functional traits without strong phylogenetic hypotheses of ancestry and trait evolution.

Ackerly and Monson (2003) went on to identify four barriers to the integration of plant ecophysiology with evolutionary biology: (1) the difficulty of measuring functional traits, (2) a lack of emphasis on the process by which functional traits evolve, (3) the difficulty of connecting variation in functional traits to variation in fitness, and (4) a paucity of researchers trained in both ecophysiology and evolutionary biology. Despite these barriers, Ackerly and Monson (2003) were optimistic about the prospect of integrating plant ecophysiology with evolutionary biology, referring to it as "waking the sleeping giant."

Now that 17 years have passed since the *IJPS* special issue on the evolution of plant functional traits was published, is the giant awake or still sleeping? To answer that question, we have organized a new special issue on the evolution of functional traits in plants. In this introduction, we first describe the progress that has been made over the past 17 years in overcoming Ackerly and Monson's (2003) four barriers to the integration of plant ecophysiology with evolutionary biology. We then consider the current state of the field of plant evolutionary ecophysiology by summarizing the papers in this special issue, including how they relate to Ackerly and Monson's (2003) four "major traditions" in the study of plant functional trait evolution. Finally, we describe priorities for future studies of the evolution of functional traits in plants.

Progress in Overcoming Barriers to the Integration of Ecophysiology with Evolutionary Biology

The Difficulty of Measuring Functional Traits

One challenge for studies of functional trait evolution is that measuring plant function can be time-consuming. Because physiology follows circadian rhythms (reviewed in McClung 2006) and is sensitive to microenvironmental conditions (e.g., Anderson and Gezon 2015), physiological states are highly dynamic over space and time, even within clones of the same genotype (e.g., Smith et al. 2011). This dynamism means that either standardizing measurement conditions or making multiple measurements over a variety of conditions is necessary to robustly characterize physiological states (Murchie et al. 2018). In addition, considerable time must be spent equilibrating plants to environmental conditions prior to measuring physiology (Hanson et al. 2016), as well as training operators to distinguish valid physiological measurements from problematic ones (Pérez-Harguindeguy et al. 2013). Consequently, it has historically been difficult to accurately measure plant function on a sufficient number of individuals to study the evolution of functional traits.

Fortunately, many of these difficulties in accurately measuring plant function can be overcome. The dynamism of physiological states can be directly characterized by measuring physiology as a function of a continuous variable such as temperature or ontogeny (i.e., a function-valued trait; reviewed in Gomulkiewicz et al. 2018). Although classic methods for measuring function-valued traits are time-consuming, new rapid measurement techniques have been developed (e.g., Stinziano et al. 2017 for A–C_i curves; Zhang et al. 2018 for xylem embolism vulnerability curves). Alternatively, the difficulty of measuring dynamic physiological traits

Table 1

Examples of Definitions of the Term "Functional Trait"

Definition	Reference
"Any attribute that has potentially significant influence on establishment, survival, and fitness."	Reich et al. 2003, p. S143
"Any trait which impacts fitness indirectly via its effects on growth, reproduction and survival."	Violle et al. 2007, table 3
"Functional traits are morphological, biochemical, physiological, structural, phenological, or behavioral characteristics that are expressed in phenotypes of individual organisms and are considered relevant to the response of such organisms to the environment and/or their effects on ecosystem properties."	Díaz et al. 2013, box 1

can be avoided by using less dynamic proxies for physiological function. Examples of such proxies include anatomical or morphological traits (e.g., xylem anatomy rather than hydraulic conductivity; Hacke and Sperry 2001) and spectral data (Yendrek et al. 2017).

A Lack of Emphasis on the Process by Which Functional Traits Evolve

Since 2003, more studies of plant functional traits have focused on the process of evolution by natural selection: approaches such as artificial selection (e.g., Delph et al. 2005), resurrection ecology (reviewed by Franks et al. 2018), and experimental evolution (e.g., Collins et al. 2006) have increasingly been used to trace the evolution of plant functional traits across generations. These approaches have been used to test hypotheses about the mechanisms of selection on plant functional traits. For example, Emms et al. (2018) used artificial selection to test (and ultimately reject) the hypothesis that drought escape physiology in *Clarkia unguiculata* evolves as an indirect consequence of selection for obligate self-pollination. These approaches have also been used to test for limits on the evolution of plant functional traits. For example, Sheth and Angert (2016) used artificial selection to determine that a lack of genetic variation for flowering time could limit the adaptation of *Mimulus cardinalis* at its northern range limit but not at its southern range limit. Although these kinds of studies are not yet common enough to draw broader conclusions about the "rules" by which plant functional traits evolve, they have provided unique insights into the evolution of functional traits in specific study systems.

The Difficulty of Connecting Variation in Functional Traits to Variation in Fitness

Since 2003, many more studies have explicitly estimated the relationship between variation in plant functional traits and variation in fitness among individuals within a population (i.e., phenotypic selection analysis; see Conner and Hartl 2004 for an overview of this approach). This increase is evident from com-

paring the meta-analysis of phenotypic selection estimates from the 2003 *IJPS* special issue (Geber and Griffen 2003) to the updated meta-analysis included in this special issue (Caruso et al. 2020): for example, only 10 published estimates of selection on gas exchange and water-use efficiency traits were included in Geber and Griffen (2003), whereas >200 estimates of selection on these types of traits were included in Caruso et al. (2020). Phenotypic selection analysis has been used to test hypotheses about the evolution of correlated suites of functional traits. For example, Kimball et al. (2013) detected selection for a combination of traits (high water-use efficiency and high growth rate) that was never observed in natural populations, suggesting that the evolution of these traits was genetically constrained. In addition, estimates of phenotypic selection have been used to test whether interspecific variation in functional traits evolved in response to natural selection. For example, Dudley et al. (2012) found that selection for increased photosynthesis was stronger in *Clarkia exilis* than in *C. unguiculata*, suggesting that differences in photosynthetic rate between these species evolved in response to natural selection.

In addition to individual-level studies, there have also been many studies published since 2003 that estimated the relationship between plant functional traits and fitness at the species level (e.g., Poorter et al. 2008; Martinez-Vilalta et al. 2010; Visser et al. 2016). Many of these cross-species studies have been guided by the concept that variation in resource availability and life history result in the repeated evolution of combinations of functional traits representing different ecological strategies (i.e., trait spectra). Trait spectra, by explicitly linking ecological interactions to patterns of plant resource allocation, make predictions about the relationship between functional traits and fitness (e.g., Donovan et al. 2011). However, cross-species studies of the predicted relationship between plant functional traits and fitness have found mixed results (reviewed by Swenson et al. 2020); some studies have found that variation in functional traits predicts variation in mortality or growth rate, but others have not.

A Lack of Researchers Trained in Both Ecophysiology and Evolutionary Biology

Two lines of evidence suggest that there has been an increase since 2003 in the number of researchers trained in both ecophysiology and evolutionary biology. First, the number of papers on the evolution of functional traits in plants has exploded (fig. 1). Since 2003, >1300 papers have been published on the evolution of plant functional traits, and of those papers, more than half have been published since 2015. This accelerating rate of publication suggests that the next few decades hold great promise for our understanding of the evolution of plant functional traits. Second, early-career researchers no longer perceive the divide between ecophysiology and evolutionary biology described by Ackerly and Monson (2003): when we organized a Botany 2019 colloquium to accompany this special issue, we found that the graduate students and postdoctoral researchers currently studying the evolution of functional traits were not aware that there was a recent period when the study of plant ecophysiology was not explicitly informed by questions and methods from evolutionary biology. Given that it has been fewer than 20 years since the publication of Ackerly and Monson's (2003) special issue, this is perhaps among the most compelling evidence that
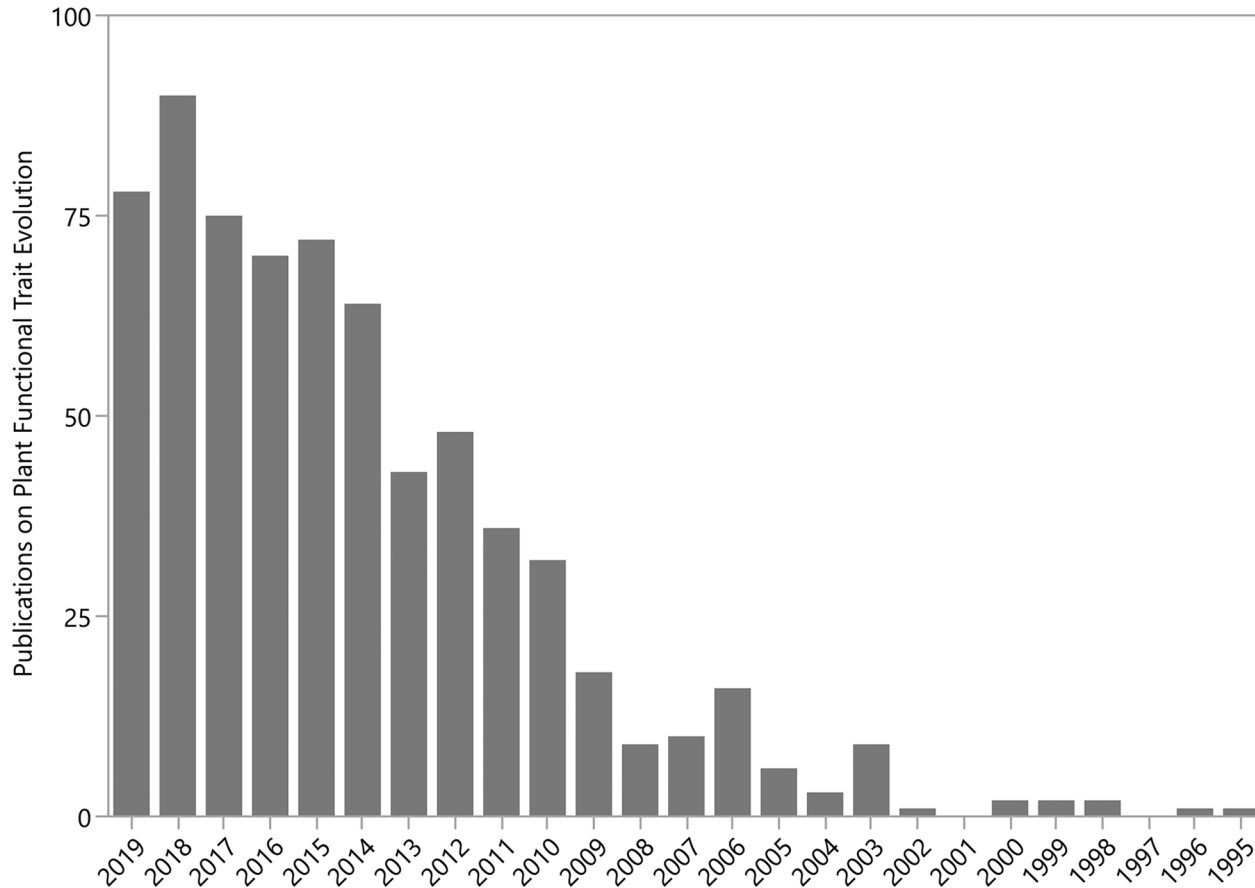
Fig. 1 Number of publications on plant functional trait evolution over the past 25 years. New publications per year were estimated from the Web of Science Core Collection (ver. 5.33; Clarivate Analytics, Philadelphia) as of October 20, 2019, using the following Boolean language: TS = (plant*) AND TS = (functional NEAR/5 trait*) AND TS = (evolution). Note the rapid increase in publications since 2015 and the 2003 publication of the original *IJPS* special issue on plant functional trait evolution.

the barriers between plant ecophysiology and evolutionary biology are dissolving.

Studies of the Evolution of Plant Functional Traits: Where Are We Now?

Ackerly and Monson (2003) recognized four major traditions in the study of plant functional trait evolution: (1) the adaptive value of functional traits, (2) the genetic and developmental basis of functional trait variation, (3) the evolution and evolutionary consequences of phenotypic plasticity, and (4) comparative and paleobotanical studies of functional trait evolution. Below we place the 11 papers included in this special issue in the context of these four major traditions.

The Adaptive Value of Functional Traits

Ackerly and Monson (2003, p. S1) noted that one key limitation of studies of the evolution of plant functional traits was the lack of an "evidentiary connection between function and fitness." This limitation was addressed by two of the studies in this special issue (Maherali 2020; Petipas et al. 2020), both of which explicitly estimated the relationship between plant functional traits and fitness. Maherali (2020) estimated the contribution of fitness components (survival, reproduction, individual growth) to population growth rate (i.e., elasticities) by comparing species that differ in their association with arbuscular mycorrhizal (AM) fungi. He found that the contribution of individual growth rate to variation in population growth rate was significantly larger in species that either facultatively associated with AM fungi or were nonmycorrhizal than in species that obligately associated with AM fungi. Given this link between mycorrhizal state and fitness, Maherali (2020) argues that plant responses to mycorrhizal fungi should be considered functional traits that can evolve in response to selection imposed by the biotic and abiotic environment. Petipas et al. (2020) estimated natural selection on root functional traits in the model plant *Panicum virgatum*. Petipas et al. (2020) found that the strength of selection varied depending on the biotic and abiotic environment: the effect of the soil microbial community (a biotic factor) on selection on root tissue density depended on soil nitrogen (an abiotic factor). This is one of the few studies to date to estimate selection on belowground functional traits,

and it illustrates how biotic and abiotic factors can interact to shape selection on plant functional traits.

In addition to Maherali (2020) and Petipas et al. (2020), two other studies in this special issue (Caruso et al. 2020; Swenson et al. 2020) assess the evidence for a relationship between plant functional traits and fitness. Swenson et al. (2020) tackled an apparent contradiction in trait-based ecology studies: plant functional traits are typically hypothesized to affect survival, growth, and reproduction, yet empirical studies often find that the relationship between traits and demographic performance is weak. Swenson et al. (2020) argue that because most trait-based ecology studies analyze species mean values, trait-demographic performance relationships are obscured by variation in biotic and abiotic environmental factors. They suggest that trait-based ecology studies should use the tools and frameworks of evolutionary ecology, which has well-established approaches for studying the relationship between traits and fitness among individuals within populations. One of these approaches, phenotypic selection analysis, is the focus of Caruso et al.'s (2020) meta-analysis of published estimates of natural selection on plant functional traits. Caruso et al. (2020) found that selection on plant functional traits was four to eight times stronger than selection on all trait classes of both plants and animals (Kingsolver et al. 2012), suggesting that the connection between plant functional traits and fitness is particularly strong. In addition, they found that experimentally manipulating abiotic factors had a larger effect on selection on functional traits than manipulating biotic factors, which supports the hypothesis that variation in plant functional traits primarily reflects adaptation to the abiotic environment.

The Genetic and Developmental Basis of Functional Trait Variation

For functional traits to evolve in response to natural selection, there must be genetic variation for those traits. Guilherme Pereira and Des Marais (2020) describe the advances made over the past two decades in our understanding of the genetic basis of leaf functional traits. Using leaf structural, nutrient, and stomatal traits as charismatic examples, they find that much of what we know about the genetic basis of functional traits still comes from traditional model systems such as *Arabidopsis* and cereal crops. However, Guilherme Pereira and Des Marais (2020) also describe the substantial progress that has been made in developing techniques for identifying the genetic basis of functional traits in emerging model systems, including woody species such as *Populus*.

While Guilherme Pereira and Des Marais (2020) focused on progress that has been made in determining the genetic basis of plant functional traits, Roddy et al. (2020) focused on the effect of genome size on the evolution of these traits. Roddy et al. (2020) found that genome size predicts variation in cell size, cell packing density, and the maximum rate of photosynthesis across vascular plant species. They conclude that genome size therefore acts as a "first-order constraint" on carbon acquisition and should limit allocation to plant growth, reproduction, and defense.

The Evolution and Evolutionary Consequences of Phenotypic Plasticity

Plant functional traits can vary across ontogeny (i.e., heteroblasty; reviewed in Zotz et al. 2011) and/or with changing en-

vironmental conditions (i.e., phenotypic plasticity; reviewed in Nicotra et al. 2010). However, variation in these traits is generally ignored in phylogenetic comparative studies of functional trait evolution, which rely on trait measurements at a single developmental point or in a single environment. To determine the effect of ignoring trait variation on phylogenetic comparative studies of functional trait evolution, Mason et al. (2020) analyzed repeated measurements of leaf traits across a growing season in the genus *Cornus*. They found that analyses of repeated measurements reveal evolutionary correlations between leaf traits and environmental factors that are not apparent from analyses of measurements at single time points; for example, seasonal plasticity in LMA is correlated with precipitation, but measurements of LMA at a single time point are not. This study demonstrates how explicitly analyzing trait variation caused by heteroblasty and/ or plasticity is essential for understanding the evolution of plant functional traits.

Comparative and Paleobotanical Studies of Functional Trait Evolution

Ackerly and Monson (2003, p. S1) noted that comparative studies of plant functional trait evolution were limited by the lack of "strong phylogenetic hypotheses of ancestry and trait evolution." This limitation was addressed by two of the studies in this special issue (Medeiros et al. 2020; Veromann-Jürgenson et al. 2020*b*), both of which analyzed variation in plant functional traits within a phylogenetic context. Working in the genus *Rhododendron*, Medeiros et al. (2020) used formal phylogenetic comparative methods to test whether the relationship between leaf economics and stem xylem hydraulics traits depends on leaf phenology and plant architecture. Medeiros et al. (2020) found that leaf-stem coordination varies among clades that differ in phenology (e.g., deciduous vs. evergreen) and architecture (e.g., maximum height). This work highlights how the evolution of cross-organ integration can be limited by correlations with other functional traits. Working with a sample of species from all extant gymnosperm clades, Veromann-Jürgenson et al. (2020*b*) tested whether net assimilation rate is limited by mesophyll conductance to CO_2. They found that mesophyll conductance is highly variable within the gymnosperms and can be the primary limit of net assimilation in some clades. Veromann-Jürgenson et al.'s (2020*b*) results suggest that mesophyll conductance should be included in models to better predict species' responses to climate change.

Comparative studies can also be used to develop the structure-function relationships that are necessary for paleobotanical studies of functional trait evolution. In this special issue, Veromann-Jürgenson et al. (2020*a*) tested whether the key functional trait LMA can be estimated from measurements of leaf cuticle thickness in fossil gymnosperms. They found that the relationship between these traits varies depending on leaf structure (i.e., broad-leaved vs. scale-leaved vs. needle-leaved) and is sensitive to growing conditions. They conclude that fossil cuticle thickness can be used to estimate LMA in some gymnosperm clades, assuming that species have experienced similar growth environments. Murray et al. (2020) tested whether the density and geometry of stomata on fossil leaves can be used to infer leaf-level gas exchange. They found that maximum rates of stomatal conductance calculated from stomatal anatomy predict the operational stomatal conductance under field conditions and that this relationship

is consistent across biomes, growth habits, and habitats. Murray et al.'s (2020) results suggest that fossil leaf stomatal traits can be used to infer the ecophysiological function of woody angiosperms contained in the fossil record.

Studies of the Evolution of Plant Functional Traits: Where Should We Go?

The articles in this special issue highlight many future directions for advancing the study of functional trait evolution (table 2). In addition, below we describe three overarching issues for future studies of plant functional trait evolution.

Studying the Mechanisms and Process of Evolution

As we move toward increasing integration of plant ecophysiology and evolutionary biology, it is critical to recognize that our assumptions about the underlying mechanisms and processes of functional trait evolution may be incorrect. This recognition echoes the history of the field of plant ecophysiology itself, where assumed ecological functions of traits have not always stood up to empirical scrutiny: for example, empirical and modeling work shows that desert cactus spines are likely to have evolved for thermoregulation rather than for defense from thirsty mammals, as was traditionally speculated (Lewis and Nobel 1977; Bowers 1988). Today, many hypotheses about the process of plant functional trait evolution come from two types of large-scale studies: first, studies that model functional trait evolution across phylogenies with hundreds to thousands of species (e.g., Sage et al. 2011; Zanne et al. 2014; Moray et al. 2016; Wooliver et al. 2016), and, second, studies that document how functional traits vary across biomes and/or along environmental gradients (e.g., Moles et al. 2014; Atkin et al. 2015; Poorter et al. 2015). Such large-scale studies are excellent sources for hypotheses about the process of evolution (Weber and Agrawal 2012; Shipley et al. 2016), but these hypotheses need to be tested using fine-scale observational or experimental studies (e.g., DeMalach et al. 2019).

Ontology and Conceptual Framework

Along with increased use of evolutionary concepts in the plant functional trait literature, there has also been increased use of evolutionary terminology. However, many of these terms are ill-defined or poorly understood, which can lead to confusion. For example, the terms "acclimation" (Willmer et al. 2000), "phenotypic plasticity" (Pigliucci 2001), and "adaptation" (Stearns 1986) are commonly confused or misused in the plant functional trait literature. But problems concerning terminology are certainly not new or unique to the field of plant functional trait biology. The meaning of conceptual terms such as "constraint" and "trade-off" have long been debated in evolutionary biology (e.g., Stearns 1986; Schwenk 1995; Roff and Fairbairn 2007; Kalisz and Kramer 2008). But a recent focus across biology writ large on ontology development (e.g., Vihinen 2014, 2015) should both improve communication between fields and strengthen the theoretical basis of the field of plant functional trait biology by providing a platform for debate concerning conceptual framing.

The field of plant functional trait biology would also benefit from clarifying the conceptual distinction between states and traits. For example, plant water potential is the energy state of water within the plant, while the traits controlling water potential include leaf anatomical structure, stomatal response to vapor pressure deficit, and cell solute uptake (Nobel 2009). This distinction between states and traits applies to virtually all flux-related ecophysiological traits, including water, carbon dioxide, light, and nutrient fluxes. The problem of confounding states with traits

Table 2

Future Directions for the Study of the Evolution of Plant Functional Traits Identified in This Special Issue

Future directions from this issue	Articles to see for further discussion
Untangling multitrait and cross-organ phenotypic integration among species, especially challenging assumptions about which functional traits limit key aspects of plant performance, and embracing the diversity of alternative functional solutions	Medeiros et al. 2020; Swenson et al. 2020; Veromann-Jürgenson et al. 2020b
Understanding the genetic architecture of functional traits beyond the few well-studied traits using modern mapping approaches, as well as the influence of overall genome size on traits, and how both architecture and genome size may constrain trait evolution or permit rapid adaptation in response to selection	Guilherme Pereira and Des Marais 2020; Roddy et al. 2020
Uncovering whether there are true generalities in how selection acts on functional traits and understanding the relative role of abiotic versus biotic selective pressures on the evolution of plant functional traits	Caruso et al. 2020; Petipas et al. 2020
Better linking functional traits with the demographic processes and vital rates that define fitness, through improved focus on individuals in a given environmental context	Swenson et al. 2020
Digging into the complexities of understudied belowground traits, including the role of the root microbiome as an extended phenotype and mutualisms in driving functional trait variation	Petipas et al. 2020; Maherali 2020
Better understanding plasticity in functional traits, including the evolution of the capacity for plasticity, the role of plasticity under strong trait-trait relationships, how trait plasticity cascades into abiotic and biotic interactions, and developing better methods for incorporating plasticity into phylogenetic comparative approaches	Petipas et al. 2020; Mason et al. 2020
Integrating mechanisms of trait-trait relationships across scales from cell and tissue levels, to within-individual variation over space and time, through intraspecific variation within and among populations, to interspecific variation among species	Roddy et al. 2020; Mason et al. 2020; Medeiros et al. 2020
Determining how far we can extend modern trait patterns into paleo-proxies to understand deep-time evolutionary history of functional traits and gauging their utility for predicting vegetation responses to climate change	Murray et al. 2020; Veromann-Jürgenson et al. 2020a

has been discussed extensively in other fields (clinical psychology [e.g., Fajkowska and Kreitler 2018] and human physiology [Hellhammer et al. 2007]) and is particularly important to consider when modeling dynamic plant functional traits that exhibit strong phenotypic plasticity (Wang et al. 2014).

Systems Biology

One particularly promising approach to studying the evolution of plant functional traits is systems biology, where research is integrated across levels of biological organization from cell to ecosystem (Keurentjes et al. 2011). To date, the systems biology approach to studying plant functional traits has largely been restricted to well-developed model species such as *Arabidopsis thaliana* (e.g., Wilczek et al. 2010). But there are limits to what studies in model species can tell us about the evolution of plant functional traits. For example, *A. thaliana*, as a C_3 annual herbaceous plant, cannot be used to answer questions about the evolution of plant functional diversity (Chang et al. 2016) or the evolution of woody plants in response to climate change (Bradshaw et al. 2000). Consequently, as progress is made in applying a systems biology approach to nonmodel species (reviewed in Richards et al. 2009; Zaidem et al. 2019), there should be many new opportunities to study the evolution of plant functional traits.

Conclusions

Overall, this special issue sends a clear message about the state of plant evolutionary ecophysiology: the giant is awake. We hope that this special issue, like Ackerly and Monson's (2003) *IJPS* special issue, will inspire more researchers at all career stages to study the evolutionary basis of plant function.

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