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SI GENOME TO PHENOME

Evolutionary and ecological functional genomics, from lab to the wild

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SUMMARY

Plant phenotypes are the result of both genetic and environmental forces that act to modulate trait expression. Over the last few years, numerous approaches in functional genomics and systems biology have led to a greater understanding of plant phenotypic variation and plant responses to the environment. These approaches, and the questions that they can address, have been loosely termed evolutionary and ecological functional genomics (EEFG), and have been providing key insights on how plants adapt and evolve. In particular, by bringing these studies from the laboratory to the field, EEFG studies allow us to gain greater knowledge of how plants function in their natural contexts.

Keywords: quantitative trait loci mapping, genome-wide association study mapping, ecological transcriptome, lab–field gap, environment.

INTRODUCTION

A key goal of biology has been to understand the genetic basis of complex traits, including the genetic architecture of quantitative phenotypes that dominate variation in plant populations (Tanksley, 1993; Mitchell-Olds *et al.*, 2007), and the role of the interaction between environment and genetics in trait expression (Des Marais *et al.*, 2013). Plants have varied life histories and can grow in a wide range of environments, and whether plants are able to adapt to perturbations in their environment depends on the extent to which plants can render appropriate phenotypic responses. Over the last few years, a growing area of research – broadly termed as evolutionary and ecological functional genomics (EEFG) – has developed with a focus on studying the genetic architecture of quantitative traits (Feder and Mitchell-Olds, 2003), understanding how genes and genomes function in natural environments (Ungerer *et al.*, 2008), and how genetic features affect ecological success and evolutionary fitness (Mitchell-Olds *et al.*, 2007). In general, EEFG involves the examination of evolutionary routes of genetic change that can be fully understood only by considering environmental influences on the phenotype throughout organismal development.

Questions that are being addressed in this new area are varied. How much are traits affected by genotype-by-environment (G × E) interactions, i.e. what is their level of canalization? What are the roles of regulation at the epigenetic, transcriptional and post-transcriptional levels on plastic responses in the wild? How do organisms integrate multiple, dynamic and constantly fluctuating environmental signals in their life cycles? How are signaling networks fine-tuned to generate adaptive plastic responses within and among species? Why are genetic differences maintained in populations? When and how can genetic architecture constrain phenotypic plasticity?

As the drive to understand how organisms adapt in the wild has advanced over the last 10–15 years, researchers have explored numerous avenues of investigation. Finding answers to EEFG-type questions requires combinations of approaches in controlled laboratory and natural environments. With recent advances in genomics, quantitative and population genetics, and molecular and systems biology it has been possible to shift from a reductionist to a systems-level approach, and to bring functional genomics to non-model organisms as well. Here we focus on three major approaches for unearthing the genetic/genomic and environmental basis for plant phenotypic variation, which are increasingly being applied for large-scale analysis in the field. These approaches are genetic mapping, epigenomic analysis and high-throughput gene expression (transcriptome) studies; all three have provided new insights into the nature of plant traits in the laboratory and in the field. These have enriched how G × E interactions, normally not detectable in a particular genotype if not for a shift in the conditions of their environment, are currently being addressed.

FINDING ASSOCIATIONS BETWEEN GENOTYPE AND PHENOTYPE: LINKAGE MAPPING

The first key approach in evolutionary and ecological functional genomics consists of mapping quantitative trait loci (QTL; Figure 1), and finding the underlying genes or genetic variants associated with complex traits. Before the boom in next-generation sequencing approaches, QTL mapping studies were often limited in the number of markers along the chromosomes that could be used. Popular markers were microsatellite length polymorphisms (short, tandem, or simple sequence repeats: STRs/SSRs), single-nucleotide polymorphisms (SNPs) or derivatives thereof. For example, SNPs could form the basis of the presence/absence of polymorphisms in restriction sites (e.g. restriction fragment length polymorphisms). SNP markers can now be generated with much higher throughput through microarray analysis, whole-genome sequencing or genotyping-by-sequencing methods, such as RAD-seq (Baird *et al.*, 2008), Pool-seq (Futschik and Schlötterer, 2010) and QTL-seq (Takagi *et al.*, 2013). Traditional QTL mapping efforts have worked with artificial populations developed specifically for this purpose, including bi-parental mapping populations such as advanced backcrosses, heterogeneous inbred families, isogenic/near-isogenic lines, and recombinant inbred lines (Crow, 2007; Kooke *et al.*, 2012). More recently, advanced mapping populations such as multi-parent advanced generation inter-cross (MAGIC) populations (Kover *et al.*, 2009) have been developed to increase the number of QTLs that segregate in a cross. The large number of parental accessions used to create populations provides higher allelic and phenotypic diversity.

Mapping populations have been generated for a wide array of plant species, and have remained important resources for uncovering the genetic architecture of traits of interest. Examples of these species are the model plant *Arabidopsis thaliana* (Ehrenreich *et al.*, 2007), crops such as maize (Buckler *et al.*, 2009), rice (Zhou *et al.*, 2016) and soybeans (Diers *et al.*, 2018), and ecologically and evolutionarily interesting species such as Mimulus spp. (Monnahan and Kelly, 2017) and Helianthus spp. (Anderson *et al.*, 2011). Mapping studies on these species have demonstrated that many quantitative traits appear to be controlled by some QTLs of large effect, and have facilitated the isolation of specific genes important for these traits. One classic example has been the fine-mapping of the QTL responsible for the branched architecture in maize associated with its domestication from teosinte, which was initially mapped as a domestication QTL and subsequently fine-mapped as the *tb1* gene (Doebley *et al.*, 1995). In *A. thaliana*, an early flowering time QTL was shown to be a large-effect allele of the *CRY2* gene (El-Din El-Assal *et al.*, 2001). These and myriad other studies have advanced our understanding of the genetic basis for complex traits in plants, and have been a major advance in dissecting key plant traits (Mauricio, 2001).

Recently, there has been a renewed interest in using bulked segregant analysis in QTL mapping. In concert with high-density genotyping via whole-genome sequencing. A method termed as extreme QTL (X-QTL) mapping was developed in yeast that promised a higher resolution and rapid mapping (Ehrenreich *et al.*, 2010). This has been used in mapping seed size (Guo *et al.*, 2015), germination features (Yuan *et al.*, 2016a) salt tolerance (Guo *et al.*, 2015; Yuan *et al.*, 2016b; Figure 2) in *A. thaliana*. X-QTL mapping continues to hold some promise, although it is likely to be more important for species with high recombination rates.

Conventional QTL mapping is still used extensively, especially for fine-mapping efforts, but is now complemented by genome-wide association studies (GWAS). Unlike conventional QTL mapping, which relies on a pedigreed population, GWAS use linkage disequilibrium to link specific SNPs with traits of interest in populations of natural accessions or varieties (Figure 2). This allows for the exploration of more genetic diversity in GWAS than in conventional QTL mapping, where the genetic diversity is limited to the selected parents. Loci identified through GWAS in such populations may therefore have broader relevance for a species as a whole, under the assumption that common genetic variation explains common phenotypic variation (Nordborg and Weigel, 2008).

Pioneered in human studies in the early 2000s (Ozaki *et al.*, 2002), GWAS are currently used to identify the genomic basis of phenotypes in both model and non-model plant species. A seminal plant GWAS by Atwell *et al.* (2010) showcased the method's power for mapping natural genetic variants for more than 100 traits in *A. thaliana*. To date, GWAS have also identified the genetic basis of numerous agronomic traits, speeding up breeding programs of crops such as maize (Jiao *et al.*, 2012; Zila *et al.*, 2013; Wallace *et al.*, 2014; Hu *et al.*, 2017; Xu *et al.*, 2018) and rice (Huang *et al.*, 2010, 2011; McCouch *et al.*, 2016; Wang *et al.*, 2017a; Wang *et al.*, 2018a), as well as crops with less well-developed molecular resources such as wheat (Liu *et al.*, 2017; Kristensen *et al.*, 2018), soybean

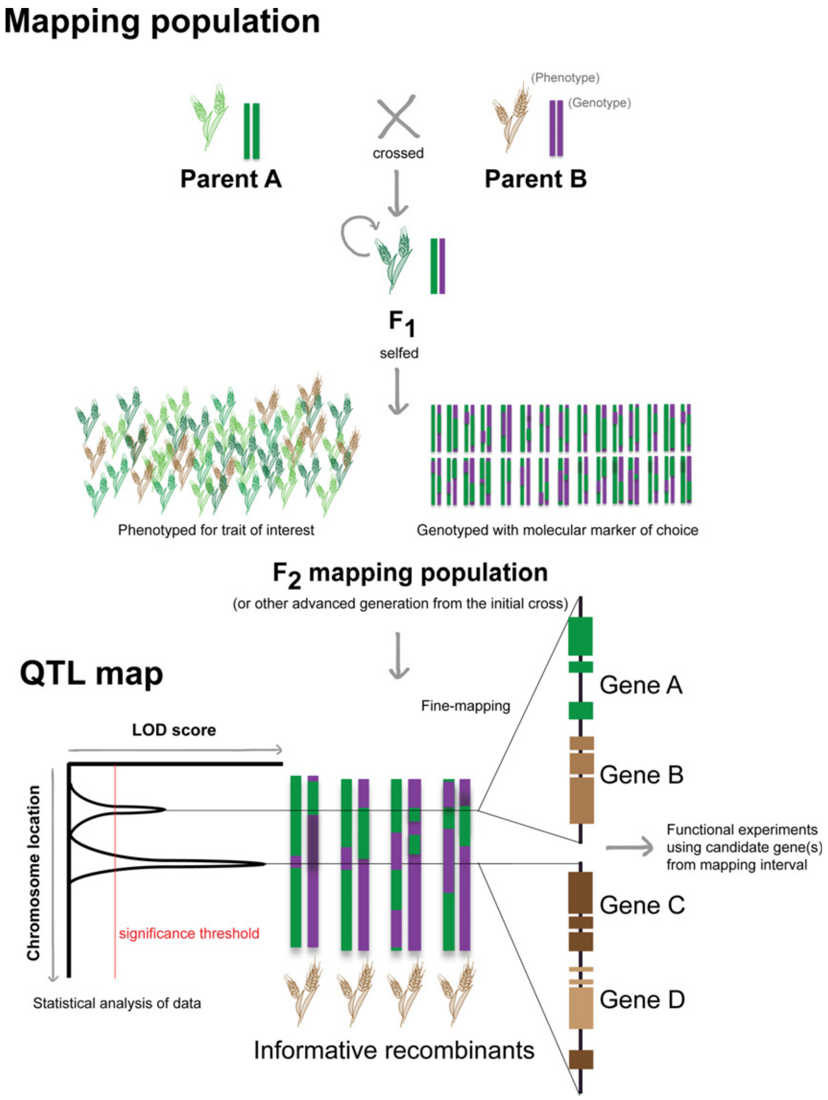

Figure 1. Schematic representation of conventional quantitative trait loci (QTL) mapping steps and analysis to identify genotype–phenotype associations.

(Fang *et al.*, 2017a), barley (Sharma *et al.*, 2018), cotton (Fang *et al.*, 2017b; Du *et al.*, 2018a), cassava (Kayondo *et al.*, 2018), foxtail millet (Jia *et al.*, 2013), spinach (Ma *et al.*, 2016) and cauliflower (Thorwarth *et al.*, 2017). GWAS are a particularly relevant tool for perennial species such as apple (Urrestarazu *et al.*, 2017), citrus (Minamikawa *et al.*, 2017), eucalyptus (Resende *et al.*, 2017) and poplar (Liu *et al.*, 2018a). It is difficult to make QTL mapping populations for such tree species due to their high genetic loads, high levels of heterozygosity and long generation cycles coupled with large sizes.

At present, GWAS are collectively being mobilized to provide population-based validation for the genotype–phenotype associations of previously identified genes via candidate gene approaches (Todesco *et al.*, 2010), and for newly discovered, relevant genes (Nemri *et al.*, 2010; Slavov *et al.*, 2014; Diepenbrock *et al.*, 2017; Hazzouri *et al.*, 2018; Rajarammohan *et al.*, 2018). Most importantly, population re-sequencing coupled with QTL or GWAS on traits of interest is being actively adopted in studies on trait plasticity and adaptation in natural environments (Brachi *et al.*, 2010; Dell'Acqua *et al.*, 2014; van Heerwaarden *et al.*, 2015; Lasky *et al.*, 2015; Anderson *et al.*, 2016; Kerdaffrec *et al.*, 2016; Meyer *et al.*, 2016; Brunazzi *et al.*, 2018; Du *et al.*, 2018b). GWAS approaches are currently being developed to assess the genetic basis for plant interactions with other members of the species community as well. GWAS that considered both sides of plant–microbe and plant–insect interactions showed the importance of genetic variation in plant and plant colonizer in determining the outcome of an interaction (Nallu *et al.*, 2018; Wang *et al.*, 2018b).

A fusion of linkage analysis (as epitomized by QTL mapping) and GWAS has been undertaken with the development of nested association mapping (NAM; Yu *et al.*, 2008; McMullen *et al.*, 2009; Figure 2). NAM allows for rapid

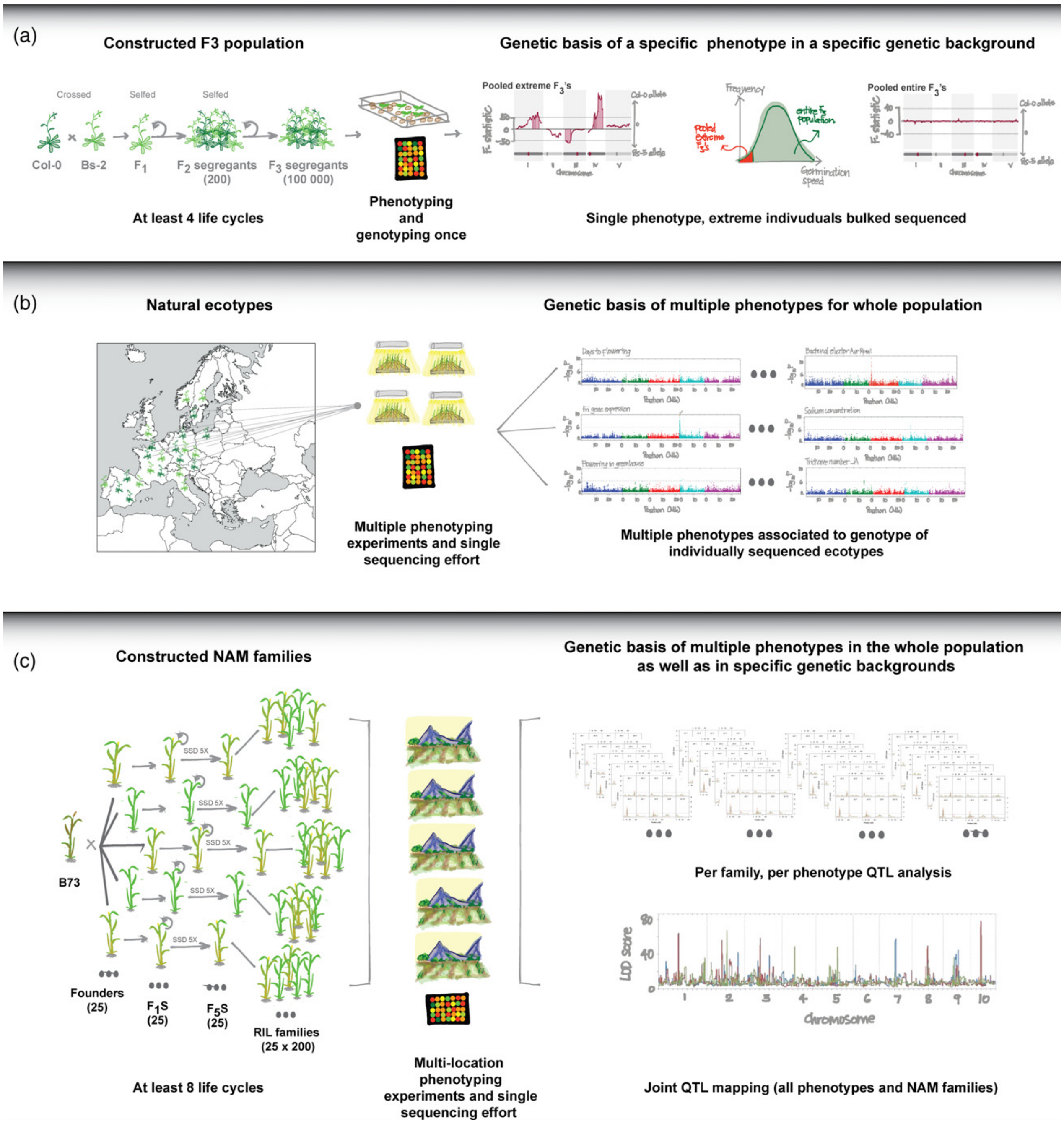

Figure 2. An illustrated comparison of selected linkage mapping approaches for finding associations between genotype, phenotype and environment. Stylized and re-drawn workflow methods for (a) extreme quantitative trait loci (X-QTL) mapping (Yuan *et al.*, 2016a,b), (b) genome-wide association study (GWAS; Atwell *et al.*, 2010) and (c) nested association mapping (NAM; Buckler *et al.*, 2009), which exemplify strengths and weaknesses of these specific linkage mapping approaches.

high-resolution mapping of genes underlying quantitative traits, and employs specially developed NAM populations to great success. In maize, where this technique was developed, it was first used to map genes associated with flowering time (Buckler *et al.*, 2009; Figure 2). Since then it has been extended for use in rice (Fragoso *et al.*, 2017), wheat (Bajgain *et al.*, 2016) and barley (Maurer *et al.*, 2015).

These and other mapping advances continue to provide greater speed and resolution in mapping genes associated with complex traits, both in the laboratory and the field. Depending on the scope of the question, time constraints and required mapping resolution, either QTL or GWAS may be appropriate; a review by Korte and Farlow (2013) provides an account of the strengths and weaknesses of GWAS, with some comparisons to QTL mapping.

Corollary to these initial genetic mapping strategies that identify regions of the genome associated with the trait of interest, fine-mapping and successive confirmatory experiments are still often necessary to pinpoint specific trait-causal gene/s. In some cases, trait associations are located in regions of the genome that contain the genes previously shown to be causal for the trait, i.e. (Yang *et al.*, 2014; Hu *et al.*, 2017; Ogawa *et al.*, 2018). However, for uncovering mechanisms involving novel genes, additional experiments including fine-mapping and necessity-sufficiency experiments still provide the needed confirmation and are still the norm for most studies to date.

PHENOTYPIC VARIATION AND THE EPIGENOME

A second major approach in the study of plant phenotypic variation was to focus on characterizing the plant epigenome and its role in the regulation of phenotypes. Epigenetics form a particular focus of attention as these underlie phenotypic variation and also could influence the process of adaptation to the environment (Eichten *et al.*, 2014). Epigenetic modifications encompass a wide range of biochemical changes to the chromatin state that stem from DNA methylation, alterations in the type or placement of histones (phosphorylation, acetylation, ubiquitylation and sumoylation), or even the replacement of auxiliary RNAs or proteins (Dowen *et al.*, 2012; Figure 3). Correspondingly, a host of techniques have been developed to assess different aspects of the epigenome. Common high-throughput methods to catalog DNA methylation marks and nucleosome positioning are bisulfite sequencing (Shiraishi and Hayatsu, 2004; Lister *et al.*, 2008; Lewsey *et al.*, 2016) and MNase-seq (Zaret *et al.*, 2005; Liu *et al.*, 2015a; Zhang *et al.*, 2015), respectively. On the other hand, DNase-seq (Zhang *et al.*, 2012a,b; Qiu *et al.*, 2016), Chip-seq (Kaufmann *et al.*, 2010) and ATAC-seq (Buenrostro *et al.*, 2015; Lu *et al.*, 2017; Maher *et al.*, 2018; Sijacic *et al.*, 2018) help to determine chromatin accessibility and to demarcate possible regulatory regions of the genome.

Although these techniques often need to be used in tandem with other techniques to ascertain the signals obtained, they have revolutionized the way we look at the genetic basis of phenotypes. The first whole-genome, single-nucleotide-resolution, bisulfite sequencing study for any organism was on the *A. thaliana* epigenome (Lister *et al.*, 2008). This study analyzed various mutants, and studied the distribution, context and periodicity of genome methylation including at rDNA genes and telomeres, which were inaccessible to previous methods. Although single-base-pair resolution epigenomes have been obtained for plant species as diverse as melon (Martin *et al.*, 2009), rice (Li *et al.*, 2012), tomato (Zhong *et al.*, 2013), wheat (Jiao *et al.*, 2018), spruce (Ausin *et al.*, 2016) and apple (Daccord *et al.*, 2017), most work on dissecting epigenomic mechanisms has been done in the model plant *A. thaliana* (Liu, 2013; Seymour and Becker, 2017; Springer and Schmitz, 2017).

One exceptional study has been the comprehensive analysis of DNA methylomes of 34 diverse angiosperm species at single-base resolution (Niederhuth *et al.*, 2016). Their comparative analysis uncovered widespread natural variation of DNA methylation within angiosperms, and found that methylation patterns broadly reflect the evolutionary and life histories of plant species. Systematic comparisons of published epigenomic data have provided further insights into the regulation (Zhang *et al.*, 2018a), and evolution (Choi and Purugganan, 2018; Wang *et al.*, 2018c) of epigenetic mechanisms. Furthermore, studies on the epigenomic landscape have revealed the functional portions of the genome in detail. In both plants and animals, nucleosomes are generally enriched in GC-rich portions of the genome, and different nucleosome densities can be observed across different tissues (Zhang *et al.*, 2015). Different cell types also have different chromatin accessibility patterns (Sijacic *et al.*, 2018). In maize, open chromatin has been shown to be about less than 1% of the genome, although despite being a small portion of the genome these regions explain an immense amount (~40%) of heritable phenotypic variation in different complex traits (Rodgers-Melnick *et al.*, 2016). Pushing on from the point of merely understanding patterns of epigenetic changes, there have been considerable studies that show how these changes play important roles in plant developmental processes, and responses to the environment (Ko *et al.*, 2010; Dowen *et al.*, 2012; Daccord *et al.*, 2017; Friedrich *et al.*, 2018; Kumar *et al.*, 2018; Zhang *et al.*, 2018b).

In terms of inheritance, a study of epimutations in the F_1 generation and the corresponding parents showed that, although less stable than genetic mutations, epimutation clusters are strongly associated with regions where the production of siRNA differs between parents (Chodavarapu *et al.*, 2012). Genomic regions that harbor these epimutations, explaining as much as 90% of the variation observed in some mapping populations, have been shown to control complex traits such as flowering time and root length (in Arabidopsis; Cortijo *et al.*, 2014). Heritable epimutations have also been shown to be involved in local adaptation in several plant species (Lämke and Bäurle, 2017; Zheng *et al.*, 2017; Groot *et al.*, 2018; Herrera *et al.*, 2018; Weinhold, 2018), and are especially relevant for studies in the EEFG framework.

Lastly, work has been started in modeling epigenetic trajectories for understanding of the evolutionary forces driving epigenome evolution at different time-scales and conditions (Gallusci *et al.*, 2017; Huang and Ecker, 2018). Modeling efforts are either statistics-based or processed-based, and have been shown to furnish a mechanistic

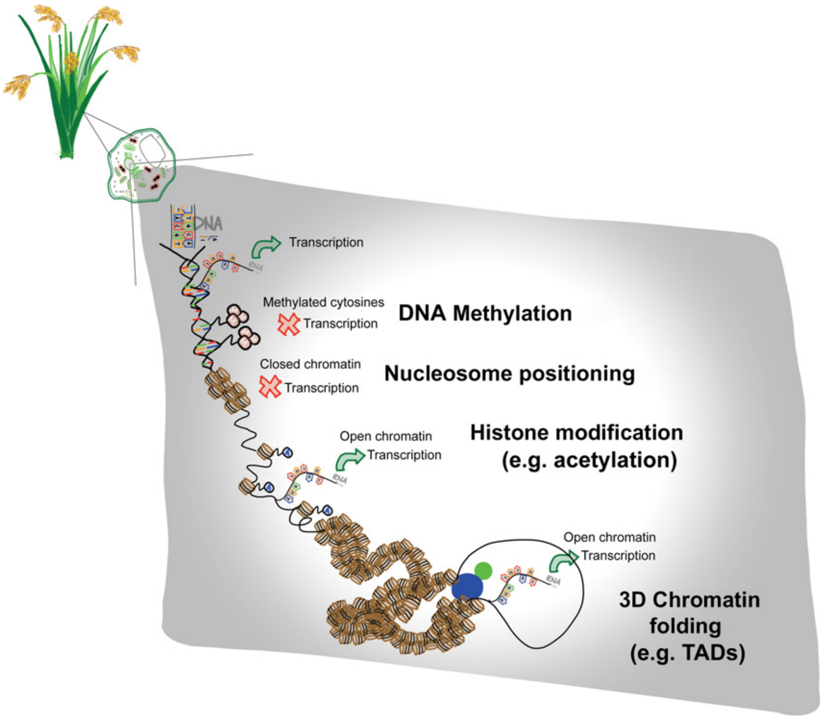

Figure 3. An illustration of several epigenetic factors known to affect phenotypic variation.

understanding of how the pieces fit together to form a well-matched whole and quantify their impact on plant performances (Gallusci *et al.*, 2017). Some modeling work on classic epigenetic-mediated examples includes description of the Polycomb-based switching mechanism during the vernalization process involving Arabidopsis *FLC* (Richards *et al.*, 2012a), and using DNA methylation as the predictor of variance in plant height (Hu *et al.*, 2015).

THE TRANSCRIPTOME SHAPES TRAIT VARIATION

The third approach in the study of plant EEFG focuses on gene expression patterns in plants with the aim of understanding how such patterns contribute to trait plasticity and adaptation. By examining the mRNA transcripts and small RNAs, it is possible to find out which loci are important in a particular environment, developmental stage and even a specific cell type.

Just as high-throughput next-generation DNA sequencing has allowed researchers to readily scale up plant genotyping, microarray and RNA sequencing (RNA-seq) technologies have scaled up our capacity of measuring transcript levels at the genome scale. Early microarray work showed that it was possible to gain a systems-level understanding of transcript heritability and transcriptome regulation (Schadt *et al.*, 2003; Keurentjes *et al.*, 2007; West *et al.*, 2007). Three RNA-seq papers (Lister *et al.*, 2008; Mortazavi *et al.*, 2008; Nagalakshmi *et al.*, 2008) on Arabidopsis, yeast and mice, respectively, mark the start of this tool's use for functional genomic studies. Contemporarily, RNA-seq is used at ever-larger scales for functional characterization of developmental, environmental response and economically important phenotypes (Becker *et al.*, 2017; Feng *et al.*, 2017; Giacomello *et al.*, 2017; Leydon *et al.*, 2017; Liu *et al.*, 2018b), and is often used to validate epigenomic measurements (Wang *et al.*, 2017b). There are numerous studies that employ whole-genome gene expression analysis not just for model plant species and crops, but also for lesser-known plant species including Japanese lawn grass (Xie *et al.*, 2015), *Cunninghamia lanceolata* (Cao *et al.*, 2016), mangrove fern (Zhang *et al.*, 2016), wild oil-tea camelia (Chen *et al.*, 2017), curry tree (Meena *et al.*, 2017) and Banksia (He *et al.*, 2018). At the same time, computational tools like eRD-GWAS (expression read depth GWAS mapping) used to uncover expression variation (Lin *et al.*, 2017) can now be harnessed much more efficiently to connect genomic variation with phenotypes.

The importance of examining how genes are expressed in the field and their role in adaptation is currently given more relevance. Works from numerous groups, including Shimizu *et al.* (2011), Nagano *et al.* (2012), Richards *et al.* (2012a,b), Kobayashi *et al.* (2013), D'Agui *et al.* (2016), Holmes *et al.* (2016), Kudoh (2016) and Mähler *et al.* (2017), highlight some key findings in this area and will be discussed more extensively in the succeeding sections. We specifically chose these studies because they feature how RNA-seq has assumed an important role for characterizing the temporal, spatial, regulatory and evolutionary gene expression landscapes.

The scope of studies utilizing linkage, epigenetics and transcriptome analyses in the purview of the EEFG framework will be discussed in greater detail below.

THE EEFG RESEARCH PROGRAM: EXPLOITING VARIATION

Technological advances in molecular biology have stimulated the rapid generation of large-scale sequencing data in model (Mir, 2009; Faino and Thomma, 2014) and non-model organisms (Ekblom and Galindo, 2011; Nawy, 2011; Unamba *et al*., 2015; da Fonseca *et al*., 2016) at a reasonable cost. Furthermore, there is an expanding realization that the genome or transcriptome of a single individual (i.e. a reference individual) is inadequate to represent the diversity within and among closely related species (Borevitz and Nordborg, 2003; Gasch *et al*., 2016). Conclusions based on work done solely on a chosen genotype in one study may not be readily extrapolated to the rest of the species (Gan *et al*., 2011; Ristova *et al*., 2018).

Fortunately, it is now possible to capture the diversity of accessions, varieties, ecotypes, related species and plant interactors in genetic studies, thereby making EEFG comparative approaches and association studies to identify genetic components of traits and adaptations possible (Juenger *et al*., 2006; Lai *et al*., 2008; Fournier-Level *et al*., 2011; Brachi *et al*., 2015; Gloss *et al*., 2017; Mähler *et al*., 2017; Exposito-Alonso *et al*., 2018; Nallu *et al*., 2018; Wang *et al*., 2018a,b,c; von Wettberg *et al*., 2018). Work on the model plant Arabidopsis (and other Brassicaceae species) in natural habitats, for example, has provided valuable insights into the role of natural selection on disease-resistance genes, and on the metabolic fluxes in auxin and glucosinolate metabolism (Tian *et al*., 2003; Prasad *et al*., 2012; Olson-Manning *et al*., 2013, 2015; Horton *et al*., 2014; MacQueen and Bergelson, 2016). Studies such as these could have direct applications in the management of disease-resistance alleles in crop varieties.

In the EEFG framework, more studies are being undertaken that involve measurements of genome-wide responses to the environment and their evolution (Liu *et al*., 2015a,b; von Wettberg *et al*., 2018). Studies have been able to leverage ‘omics’ technologies in the field to study the basis of local adaptation (Knight *et al*., 2006; Gould *et al*., 2018) and contribute to the understanding of changing environmental conditions and climate change (Fournier-Level *et al*., 2011; Hancock *et al*., 2011; Nagano *et al*., 2012; Plessis *et al*., 2015; D’Agui *et al*., 2016). Experiments that manipulate agents of selection in native field environments using pedigreed populations or GWAS panels also offer unique opportunities for detecting the genetic and environmental mechanisms that generate local adaptation (Wadgymar *et al*., 2017). Combinatorial, multi-location experimental set-ups and reciprocal transplant experiments can unlock fitness-associated loci relative to climate, macro- and microbiota, and local adaptation (Wilczek *et al*., 2009; Fournier-Level *et al*., 2011; Agrawal *et al*., 2012; Prasad *et al*., 2012; Züst *et al*., 2012; Wagner *et al*., 2014; Brachi *et al*., 2015).

A key area that is gradually being integrated into the EEFG framework is phenomics, the practice of high-throughput and high-dimensional phenotyping (Pauli *et al*., 2016). This includes strategies that take into account in-depth environmental characterization and accurate analysis of the environmental variables that can affect plant phenotypes. Phenomics is one more tool to bridge the lab–field gap, which allows accurate high-throughput trait measurements in less controlled environments. The adoption in GWAS to map 13 traditional and two newly defined traits (Yang *et al*., 2014) and QTL mapping for yield components (Tanger *et al*., 2017) in rice is a showcase of the advantage of phenomics for EEFG-type studies. Pushing forward from traditional traits, field imaging platforms capable of measurement of canopy and continuous leaf development have also been shown to dissect more specific traits affecting water use (Vadez *et al*., 2015). Scaling this up even further, unmanned aerial vehicles are currently being developed and streamlined for even higher time and quantity throughput (Shi *et al*., 2016). A comprehensive review on how field phenotyping should be implemented was also published recently by Araus *et al*. (2018).

Importantly, EEFG has ushered direct, real-world applications of ‘omic’ technologies to crops in evolutionary ecological settings. Maize common garden experiments to elucidate genomic, transcriptomic and phenotypic variation uncovered the structure of adaptive diversity in a varied landscape (Liu *et al*., 2015a,b; Kost *et al*., 2017). Some studies have managed to identify specific genomic inversions that harbor loci regulating flowering time and other phenotypes (Fransz *et al*., 2016; Lee *et al*., 2017; Romero Navarro *et al*., 2017). In tomato, researchers were able to decipher the footprints of domestication using gene expression and population genetic analyses (Sauvage *et al*., 2017).

However, while EEFG enables us to answer ecological and evolutionary questions on a scale and precision that was unrealistic only a few years ago, we should point out that the methods that enable systems-level analyses still need more development. Furthermore, if specific genes or mechanisms are uncovered by large-scale analysis, functional tests of necessity or sufficiency are still fundamental.

FROM LAB TO THE FIELD: PLANT GENOMICS AND SYSTEMS BIOLOGY *IN NATURA*

Switching the domain of study from model species towards studies of natural populations comes hand-in-hand with recognizing the significance of shifting from laboratory-based experiments towards (von Wettberg *et al*., 2018) ecological characterization in common gardens, fields and natural habitats. Now, more and more studies are able to distinguish meaningful genetic variation because appropriate environments are being considered (Tian *et al.*, 2003; Franks *et al.*, 2016; Xu *et al.*, 2016; McKown *et al.*, 2018).

Historically, most measurements of the phenotypic consequences of genetic variation have been performed in the confines of the laboratory, thereby reflecting a narrow and sometimes even artificial range of responses to environmental stimuli (Annunziata *et al.*, 2017). When these stimuli are provided by other members of the biota, the behavior of these species may not reflect behavior in field conditions either, potentially leading to unrealistic outcomes; for example, pathogens may only successfully infect leaves when humidity is at sufficient levels (Xin *et al.*, 2016). Undoubtedly, there is abundant evidence for the conclusion that traits observed in laboratory conditions cannot always be used to predict the behavior of those traits in natural conditions, revealing a 'lab–field gap' (Weinig *et al.*, 2002; Malmberg *et al.*, 2005; Casati *et al.*, 2011; Guevara *et al.*, 2012; Mishra *et al.*, 2012; Yamori, 2016; Singh and Roy, 2017). This has led to calls for studying plants, as they were, *in natura* (Shimizu *et al.*, 2011; Richards *et al.*, 2012b; Kudoh, 2016).

Most controlled laboratory experiments have focused on the plant response to a single environmental variable, while plants in a natural environment are responding to multiple, complex, dynamically changing environments. Results from studies on plant responses to combinatorial stresses reinforce the need for caution in comparing results from laboratory and field-grown plants. Such studies have shown that a plant's transcriptional and metabolomic response to combinations of (a)biotic stresses cannot be predicted from the responses to individual stresses (Swindell, 2006; Prasch and Sonnewald, 2013; Rasmussen *et al.*, 2013; Suzuki *et al.*, 2014; Barah *et al.*, 2016; Franks *et al.*, 2016; Gray *et al.*, 2016). In particular, GWAS revealed distinct genetic architectures for single and combined stress responses (Davila Olivas *et al.*, 2017a,b; Thoen *et al.*, 2017). Furthermore, there is phenotypic plasticity for a plant's response to combinations of stresses (Weston *et al.*, 2008; Atkinson *et al.*, 2013; Shaar-Moshe *et al.*, 2018).

Just as many EEFG approaches have focused on genetic mapping in field studies, the importance of gene expression in the wild is being increasingly appreciated. One example is work on Arabidopsis *FLC* by Aikawa *et al.* (2010), who conducted a 2-year census of the transcript levels of this well-known temperature-dependent flowering time gene to uncover the mechanisms by which environmental factors regulate flowering (Figure 4). This ground-breaking study has since been followed up by others in which *FLC* transcript levels and chromatin states were measured in different localities and field conditions (Nishio *et al.*, 2016; Hepworth *et al.*, 2018), which increasingly provided a clearer account of the complexity and relevance of the environment for *FLC*-mediated responses.

These studies on gene expression in the wild have been expanded to field transcriptome studies. Transcriptome data enrich these classical approaches as well as traditionally employed genomic information for investigating trends in plant ecological and evolutionary responses. Specifically, measurements of genome-wide gene expression variation are helpful in describing the developmental mechanisms that transform genomic information in specific loci to the realized phenotypic responses (Swanson-Wagner *et al.*, 2012; Ren *et al.*, 2018). Furthermore, regulatory variation is clearly abundant within and across populations (Lasky *et al.*, 2014; Lin *et al.*, 2017). And although its evolutionary significance is harder to ascertain, several studies have uncovered heritable gene expression variation among and within species to be the raw material for evolutionary processes (Wang *et al.*, 2005; Konishi *et al.*, 2006; Cong *et al.*, 2008; Jiang and Rausher, 2018).

The potential of transcriptomic analysis in an EEFG framework not only includes identification of functionally relevant portions of the genome in ecological contexts, but also to determine the behavior of phenotypically plastic and adaptive traits in an evolutionary context. Integrating population transcriptome studies in the EEFG framework, we can identify two key insights that can be gained in characterizing the evolutionary, regulatory, spatial and temporal landscapes of plant plasticity and adaptation. First, population-level differences in gene expression can inform us on how distinct environmental gradients shape trait variation and evolution. Second, it can be instrumental to predict species and population level adaptation to environmental (both biotic and abiotic factors) change, and ultimately facilitate the conservation and strategic utilization of specific plant species.

In this regard, broad, high-resolution transcriptomic measurements have been made for several plant species not only in the laboratory but also out in the wild where these plants grow. The levels and patterns of gene expression in the field – which have been referred to as the *ecological transcriptome* (Alvarez *et al.*, 2015) – have the capacity to examine how plants behave when placed in the fluctuating, multi-pronged environments that they face throughout their life cycle. These ecological transcriptome studies allow a connection between genotypic variation into complex trait phenotypes as modulated by the prism of the natural environment. Such studies have now become increasingly popular, and have been undertaken in *A. thaliana* (Richards *et al.*, 2012b; Tyagi *et al.*, 2016) and crops (Plessis *et al.*, 2015; Russell *et al.*, 2016; Zhao *et al.*, 2018), tree species (Philippe *et al.*, 2010; Verta *et al.*, 2013), lesser known plant species (Jia *et al.*, 2017; Yang *et al.*, 2017), and energy crops like switchgrass (Palmer

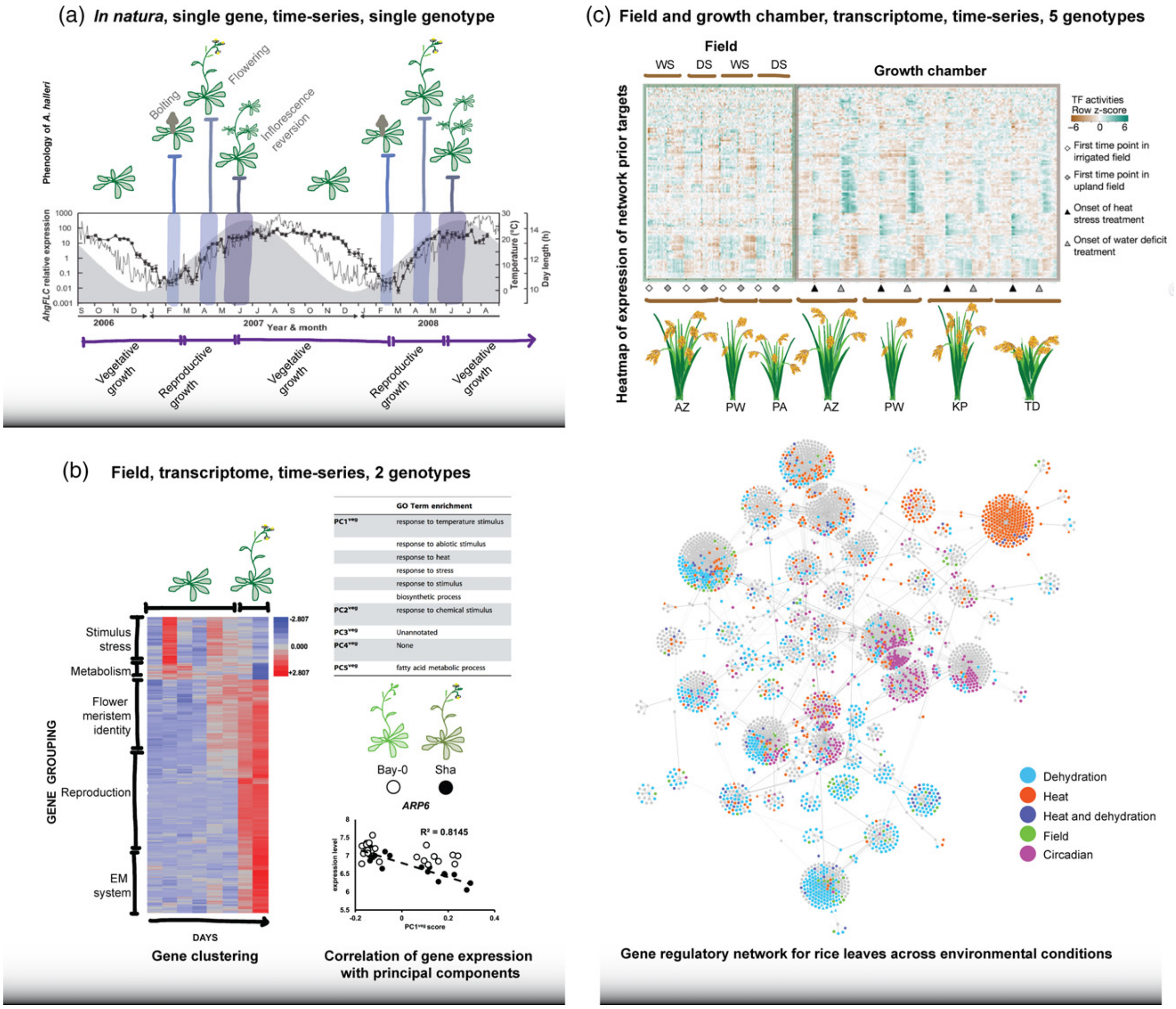

Figure 4. The maturation of *in natura* gene expression studies in an evolutionary and ecological functional genomics (EEFG)-type framework, as illustrated by representative studies.

Stylized and re-drawn key findings for (a) single transcript (*FLC*) expression *in natura* (Aikawa *et al*., 2010), (b) whole transcriptome changes throughout developmental growth of *Arabidopsis thaliana* in the field (Richards *et al*., 2012b), and (c) constructed environmental gene regulatory influence networks from transcriptome and chromatin accessibility data (Wilkins *et al*., 2016). These selected studies highlight the transition to a systems biology approach in an EEFG-type framework.

et al., 2017) and Miscanthus (Song *et al*., 2017; Yan *et al*., 2017; Xing *et al*., 2018).

There has also been more work probing adaptation to harsh and unconventional environments including elevated mountain areas (Yang *et al*., 2015a; Jia *et al*., 2017), inter-tidal mangrove areas (Yang *et al*., 2015b; Guo *et al*., 2017), and even the Antarctic (Cho *et al*., 2018). In the case of biotic interactions, field transcriptomes have been sequenced from microbial plant colonizers and the plant host in tandem in a dual RNA-seq approach (Hubbard *et al*., 2015; Kamitani *et al*., 2016).

In this regard, the initial whole transcriptome study of *A. thaliana* in the field is illustrative (Richards *et al*., 2012b). In this study, two accessions of *A. thaliana* were grown in the field in the Northeastern United States, and the leaf transcriptome was measured throughout the plant's life cycle. The study was able to identify gene clusters that showed differential patterns of gene expression across the life cycle in the field (Figure 4). Moreover, principal components of gene expression could be identified, and these could be correlated with environmental features such as temperature and precipitation. Levels of specific transcripts were associated with each of these principal components, and the function of these transcripts was consistent with their possible environmental response (Figure 4; Richards *et al*., 2012b).

The development of systems biology has also provided the opportunity to use transcriptome data, in conjunction with other data types (Bonneau *et al.*, 2006), to infer key regulatory networks that display the genetic underpinnings of plant responses to the environment. Such regulatory networks include environmental gene regulatory influence networks (EGRINs; Wilkins *et al.*, 2016; Figure 4), as well as other representations of gene regulatory modules reacting to environmental signals (Nagano *et al.*, 2012; Plessis *et al.*, 2015; Fournier-Level *et al.*, 2016; Des Marais *et al.*, 2017; Miao *et al.*, 2017).

While transcriptome studies have become a convention for model species and crop plants in an EEFG context, studies on wild plant species place more emphasis on the basic mechanisms for survival and adaptation. The attention to different environments goes hand-in-hand with increased interest in modeling evolutionary responses in relation to climate change and environmental stress (Nagano *et al.*, 2012; Plessis *et al.*, 2015; Fournier-Level *et al.*, 2016; Watson-Lazowski *et al.*, 2016). Because transcriptional reprogramming was the standard in examining plant defense responses, in the EEFG framework, the transcriptional implications of responses to biotic interactors are more rigorously being considered at the systems level in natural environments (Turner *et al.*, 2013; Liao *et al.*, 2016; Nobori *et al.*, 2018; Young *et al.*, 2018).

Timing in these field transcriptome studies provides a snapshot of the different dimensions of physiological development and even evolution. Molecular mechanisms governing the physiological responses vary across seasons, time-points within a season and even within a given day (Nagano *et al.*, 2012; Plessis *et al.*, 2015; Wilkins *et al.*, 2016). For example, differences in flowering time can reflect on differences in photoperiod sensitivity or vernalization requirements between genotypes (Des Marais *et al.*, 2012; Torres *et al.*, 2013). This would act as a source of G × E interactive effects, and would cause different genotypes to be at different developmental ages, especially later in an experiment. As more field transcriptome studies are undertaken, we can begin to understand plant responses in the field across different seasons and at different time-scales.

OUTLOOK

The developing framework of evolutionary and ecological functional genomics is allowing an integrated examination of the roles of genetics and the environment in shaping plant phenotypes. This can provide important insights into the nature of adaptations and the evolution of plant species, particularly in their natural ecological contexts. Of great interest is the study of EEFG in crop species, particularly as we attempt to meet the challenges of climate-ready crops that can cope with future climate change and feed a burgeoning world population.

This multi-disciplinary approach promises to provide a more holistic approach to the study of plant function. As we consider plant biology in the coming years, EEFG can provide insights into at least two major scientific challenges that we need to tackle. One challenge in this area is to understand how plants respond to complex, dynamic, multi-factorial fluctuating environments that are the norm in nature. While most laboratory studies in plants are focused on examining one or two environmental inputs, in nature plants face multiple changing environmental variables, and how the interplay of these signals with the plant is integrated remains unknown. As we begin to study plants out in nature, we can start to gain traction in learning about plant responses in more realistic contexts.

Another challenge is to enumerate the causal chain of connections from gene to gene network to phenotype and, ultimately, to fitness. This requires studying plants at multiple levels using different techniques – genome sequencing, molecular biology, network inference, plant physiology, phenomics, and evolutionary and ecological analyses. It is a daunting task, but one that must be undertaken if we are to obtain a more complete picture of plant biology.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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