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Genomic Studies of Local Adaptation in Natural Plant Populations

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Victoria L. Sork is a professor in the Department of Ecology and Evolutionary Biology at UCLA. She earned a B.S. from University of California Irvine and a Ph.D. from the University of Michigan. Her research program integrates field and genomic studies to identify the genetic and epigenetic basis of local adaption in tree populations, with a conservation goal of understanding how to manage tree populations experiencing rapid climate change. To aid this and other oak evolutionary and conservation research programs, she and collaborators have produced a high quality annotated genome of Quercus lobata. Over the last few years, she has also served as the Dean of Life Sciences at UCLA with the aspiration to promote inclusive excellence in research and education.

Abstract

Local adaptation arises as a result of selection by the local environment favoring phenotypes that enhance fitness. Geographic patterns of phenotypic variation are in part due to this selective process. Classically, the genetic basis of those phenotypes has been studied in plant populations using a quantitative genetic approach in which plants from different source populations are grown in common environments, in reciprocal transplant experiments, or in studies across a wide geographic and environmentally heterogeneous area. Limitations of these approaches to understanding the genetic basis of phenotypic variation can now be addressed with next generation sequencing, gene expression profiles, and epigenetic analysis. In this paper, I summarize contemporary genomic research on local adaptation by comparing findings from the *Arabidopsis* annual plant model system with long-lived tree species in four kinds of local adaptation studies: 1) genomic studies of transplant experiments; 2) landscape genomic studies; 3) gene expression studies; 4) epigenetic studies of local adaptation. Although the basic study designs of common garden, reciprocal transplants, and geographic variation have remained constant, the inclusion of contemporary genomic approaches has provided substantive advances in our understanding of the genetic underpinnings of local adaptation, including the impact of climate, the identification of candidate genes involved in genotype-by-environment interactions, and evidence for the potential

role of epigenetic modification. Despite these advances, new questions are arising and key areas for future research include more exploration of gene networks in response to biotic and abiotic stressors and improved statistical tools for traits with polygenic inheritance.

Introduction

Long before Darwin and Wallace, naturalists recognized geographic patterns of phenotypic variation of organisms and questioned the causes of this variation. The ways in which genetic variation may shape these patterns of phenotypic variability lies at the core of the field of evolutionary ecology. Although some spatial variation is due to the history of genetic drift and gene flow, the local environment plays a large role in shaping phenotypic differences across a species range—both in its proximate effect on traits through phenotypic plasticity and its ultimate impact on the evolution of local adaptation. An early source of evidence for local adaptation came from the reciprocal transplants of Clausen, Keck, and Heiseys, which highlighted the fact that plants often grow best where they occur naturally. This ecological approach has also illustrated the role of natural selection in the diversification of populations and in speciation (Hendry et al. 2007). Meanwhile the field of forestry has long used common gardens located in difference environments to compare growth and other traits of trees from different provenances (i.e., sets of trees from the same locality), making it possible to assess the genetic basis of population differences (Matyas 1996; Savolainen et al. 2007; Sork et al. 2013; de Villemereuil et al. 2016). The foresters' motivation was to improve management of forest populations by identifying proper seed sources for traits and growth forms that would maximize the growth and economic value of plantation trees (Langlet 1971; Grattapaglia et al. 2009). Thus, common garden and reciprocal transplant experiments have been crucial for decades to understanding how natural selection shapes geographic phenotypic variation.

The emergence of genomic tools has led to a new wave of studies that can map spatial patterns of adaptive genetic variation and also identify genes underlying locally adaptive traits (Stapley et al. 2010; Anderson et al. 2011; Franks and Hoffmann 2012; Savolainen et al. 2013). Interest in the genomic approach is illustrated by the plethora of recent reviews on this topic (see a subset in Table 1, A). These reviews promote the idea that genomic information can add value to, rather than replace, studies that use reciprocal transplants, common gardens, or treatment experiments to examine the genetic basis of phenotypes. Genomic information adds value to these studies by providing novel types of data that enhances our understanding of the evolution of local adaptation, its role in shaping responses to climate change or types of global change, and the ways in which conservation strategies can most effectively manage threatened or at-risk populations or species. Moreover, by sampling individuals in natural populations along an environmental gradient, new genomic tools create the opportunity to study landscape patterns of DNA sequences and create spatial maps of the genomic signatures of natural selection with a new level of clarity and resolution. These two basic study designs—experimental gardens and natural population sampling—can be combined with DNA sequence data, gene expression levels, and/or epigenetic analysis to explore the evolution of local adaptation in plant populations. Moreover, in cases where experimental gardens are not available or feasible, genomic tools provide viable alternatives.

Beyond developing new evolutionary insight, the availability of genomic tools creates useful applications for climate change and conservation biology studies (see articles in Table 1, B and C). With recent increases in the rate of climate change, many biologists are concerned that locally adapted populations may not survive and thrive under new climatic conditions, may not be able to adapt in time to new conditions, or will not have the capacity to migrate to new locations to keep up with the pace of a changing climate (Etterson and Shaw 2001; Davis, Shaw and Etterson 2005; Aitken et al. 2008; Merila and Hendry 2014; Colautti, Ågren and Anderson 2017). Genomic tools may help us assess these concerns and develop mitigation strategies, when and where needed, to preserve populations, species, interactions among species, and ecosystems.

In this paper, I will present the lessons we are learning from new genomic sequencing tools by comparing findings from *Arabidopsis* and tree species. *Arabidopsis* has become a valuable evolutionary model system due to its short generation time, which is conducive for transgenerational experiments and its increasingly available genomic and germplasm resources. Trees provide an alternative opportunity to examine the genetic basis of local adaptation in nature because their high outcrossing and gene flow rates reduce genetic structure that confounds the search for gradients of adaptive genetic variation, and their high levels of genetic and phenotypic variation are valuable for association mapping (Gonzalez-Martinez, Krutovsky and Neale 2006; Savolainen et al. 2007). Although both types of studies also have their limitations, it is useful to examine the extent to which we can leverage information from *Arabidopsis* to make inference about trees, and vice versa. In the last ten years, many studies across a range of species and life history traits have emerged. Here, I will *not* attempt to review all of those empirical studies, nor will I provide an overall review of the potential and promise of genomic tools for the study of local adaptation or population responses to rapid climate change, as this topic has been extensively treated (see Table 1). Instead, I will illustrate new insights about local adaptation and its genetic/epigenetic basis by presenting four pairs of studies with different genomic approaches, and within each pair, I will compare one study from the *Arabidopsis* literature and one from the tree literature.

Transplant Experiments and Genomic Studies of Local Adaptation

A good source of evidence of local adaptation comes from transplant experiments—either common garden or reciprocal transplant experiments—because they allow one to test the genetic basis of population differences within and among environments. Through common garden experiments, it is feasible to compare the growth of populations from different regions growing across a set of different environments (Kawecki and Ebert 2004; Leimu and Fischer 2008). Through reciprocal transplant experiments, it is possible to demonstrate local adaptation if plants survive and thrive best in their home environment and if they outperform plants from other parts of the species range that evolved in different environments (Kawecki and Ebert 2004). Reciprocal transplant experiments have the advantage

Table 1 Selected recent reviews discussing promise and potential of genomic tools for studies of (A) local adaptation, (B) climate change adaptation, and (C) conservation biology
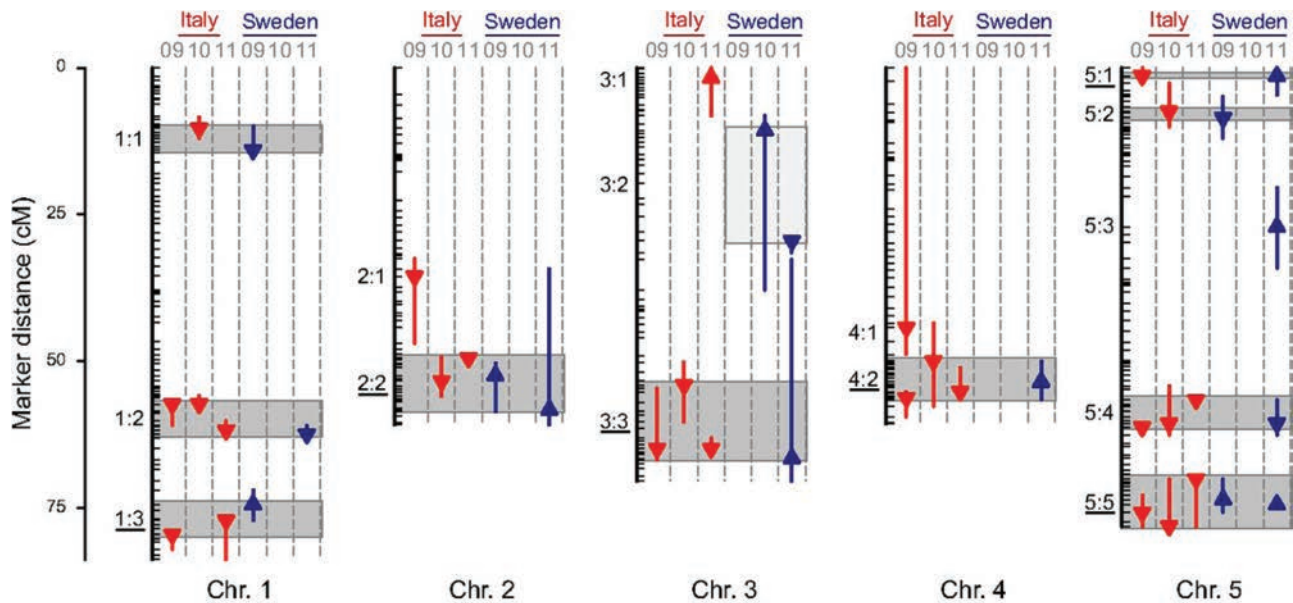
Citation	Description
A. Local Adaptation	
Barrett and Hoekstra (2011)	This review article cautions that identification of adaptive genetic variation requests that the fitness benefits of phenotypes under selection must also be demonstrated. They provide ways to do so.
Blanquart et al. (2013)	Demonstration showing that the adaptive divergence of populations requires good statistical design.
De Mita et al. (2013)	Useful analysis of the methods used to detect adaptive genetic variation; environmental association may be more robust than genetic differentiation tests.
de Villemereuil et al. (2016)	Advocates combining population genomics and genome-wide association studies with the settings of a common garden to generate an integrative demonstration of local adaptation
Des Marais et al. (2013)	They review genomic studies of quantitative trait loci mapping and gene expression studies to understand the molecular basis of genotype-by-environment interactions, which are commonly found and exhibit complex underlying genetic factors.
Hoban et al. (2016)	The article presents a good summary of the use of genomic scans to implement F_{ST} outlier methods and environmental association analysis of genetic gradients to find evidence of local adaptation
Leimu and Fischer (2008)	Analyzed published reciprocal transplant studies and found local adaptation is less common than assumed and more prevalent with species with large effective population size.
Rua et al. (2016)	After conducting a meta-analysis on 1170 arbuscular mycorrhizal studies, they observe potential to detect local adaptation for mycorrhizal relationships.
Savolainen et al. (2013)	Excellent review of full range of genomic approaches that can be used to identify adaptive genetic variation.
Siol, Wright and Barrett (2010)	Like other reviews, they point out that demographic history confounds the study of selection in plant populations. However, they argue that population and landscape genomic studies should examine the interaction between population history and selection when testing for selection, rather than simply controlling for history.
Sork et al. (2013)	Defines landscape genomics with an emphasis on the opportunities and challenges of studying tree species. They summarize landscape, ecological, and evolutionary genomic approaches for insight about evolution of local adaptation and the conservation and resource management applications.
Stapley et al. (2010)	Outlines some of the key ways that next generation sequencing can help to identify the genes underpinning adaptation.
Tiffin and Ross-Ibarra (2014)	They summarize the ways that population genetics can enhance our understanding of local adaption. The article presents an excellent synthesis about the statistical issues of detecting adaptive genetic variation.
B. Climate change adaptation	
Aitken and Whitlock (2013)	Describes how assisted gene flow can be a powerful tool for managing species with large populations and broad ranges to create adaptation to local climatic conditions.
Aitken et al. (2008)	Considers how locally adapted tree populations will respond to climate change and which kinds of populations will be most at risk.
Alberto et al. (2013)	Discusses the need for more common gardens and genomic information to understand how tree populations will respond to climate change.
Anderson, Panetta and Mitchell-Olds (2012)	They propose hypotheses and present experimental approaches to test how plants are to respond to climate change.
Christmas, Breed and Lowe (2016)	Present most appropriate methods for understanding adaptive responses and capacity to adapt to future climate, especially in trees and alternative conservation approaches.
Davis et al. (2005)	Discusses models that show that adaptive divergence of populations to differing environmental conditions can occur within decades for herbaceous plant and within centuries or millennia for longer-lived trees, implying populations in the past have been able to respond on an evolutionary time scale comparable to that historic climate change.
DeBiasse and Kelly (2016)	This review of recent studies describes how comparative transcriptomics can provide plastic and evolutionary responses to changing environments. The discuss how transcriptomics can help understand local adaptation and plant response to multiple stressors.
Franks and Hoffmann (2012)	The authors discuss how climate change provides the opportunity to study the genetic basis of climate change adaptation using techniques such as association analysis, genome scans, transcriptome profiling, and epigenetic effects.
Savolainen (2011)	The short introduction to a special issue makes the point that the best genomic resources for studying genetic basis of climate adaptation come from model species such as *Arabidopsis thaliana* whereas informative fitness studies are derived from natural populations spread over multiple species with less developed genomic resources.
C. Conservation Biology	
Allendorf, Hohenlohe and Luikart (2010)	The availability of complete genome sequences from thousands of species and populations within species will transform the impact of conservation science. The article suggests best strategies for using these new tools.
Frankham (2010)	This paper describes the value of using genetic information to clarify the definition of species for conservation purposes and the management of captive and threatened animal species.
Harrisson et al. (2014)	The discuss benefits and limitations of using genomic tools for incorporating knowledge of evolutionary potential of populations into conservation management practices.

of testing for fitness advantages of local genotypes (e.g., Ågren and Schemske 2012; Ågren et al. 2013), whereas common gardens can utilize a quantitative genetic design to identify the genetic basis of specific phenotypes underlying local adaptation (Savolainen et al. 2013), which has been nicely demonstrated in many forest provenance studies (Matyas 1996; De Kort et al. 2014; McKown et al. 2014a). An additional advantage of well-designed common gardens is that they allow a search for genes underlying these phenotypes by utilizing genomic information to conduct Genome-Wide Association Studies (GWAS), in which genetic variants are associated with phenotypes (Lepais and Bacles 2014; McKown et al. 2014b; Steane et al. 2014; de Villemereuil et al. 2016).

The effective integration of reciprocal transplant design with genomic data, including a well-annotated genome, is nicely illustrated by the work of Ågren, Potsma, and Schemske and their colleagues who have conducted a series of reciprocal transplant studies using populations of *Arabidopsis thaliana* from Italy and Sweden (e.g., Ågren and Schemske 2012; Ågren et al. 2013; Postma and Ågren 2016). Their next step was to explore the genetic basis and adaptive significance of freezing tolerance as a potential mechanism for those fitness differences by identifying quantitative trait loci (QTL) associated with freezing tolerance to genetic and fitness trade-offs in reciprocally planted genotypes. Their analysis identified QTLs across five chromosomes that are associated with increased fitness of Swedish genotypes when planted locally or away (see Figure 1). Using the genomic resources available for *Arabidopsis*, they were able to implicate a major regulator of freezing tolerance, CBF2, as a candidate gene for one of the freezing tolerance QTL (Ågren et al. 2013; Oakley et al. 2014). Further studies used recombinant inbred lines derived from a cross between populations of *A. thaliana* in Italy and Sweden at the parental sites, mapped QTL associated with early seedling establishment to demonstrate that these QTL contributed to local adaptation and genetic
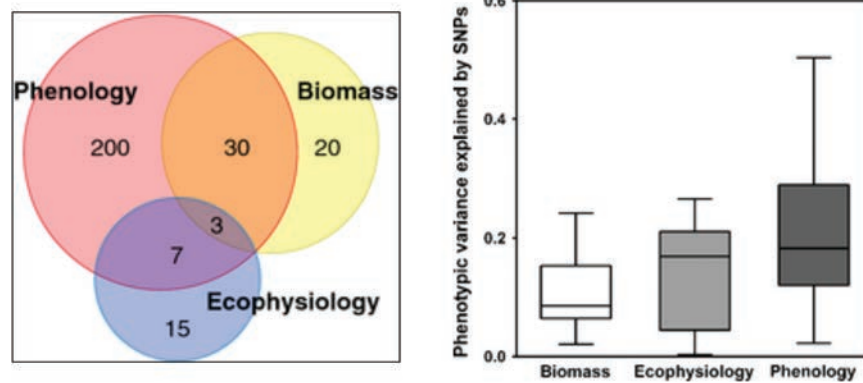
fitness trade-offs (Postma and Ågren 2016). This set of studies and the extensive body of work using the *Arabidopsis* model-system points to benefits of integrating field studies, controlled experiments, and extensive genomic resources to understand the genetic basis of local adaptation.

The availability of provenance studies in forestry has opened other doors to our understanding of local divergence in tree populations that may reflect local adaptation. Transplanting genotypes from throughout the species range into two or more common gardens has enabled a quantitative genetic analysis of population differentiation of traits that may be associated with local adaptation and the heritability of phenotypes. Poplars (*Populus spp.*) provide an excellent opportunity to integrate genomic information into provenance studies to identify genes underlying phenotypes. Because they are clonal, it is feasible to estimate the heritability of traits measured in common gardens, and additionally, their high commercial value has motivated the establishment of common gardens. A recent study by McKown et al. (2014a) utilized over 400 accessions of *Populus tricharpoca* collected several years ago from throughout the species range and grown in a single garden. These accessions were phenotyped for 40 traits associated with phenology, biomass, and ecophysiology, and genotyped using a 34 000-single nucleotide polymorphism (SNP) array. Using GWAS, they detected significant associations with about 300 genes, most of them associated with phenology traits (Figure 2A). They estimated that the significantly associated SNPs can explain 10–30 % of the variation in the phenotypic variation depending on the category of the trait (see Figure 2B). This study nicely demonstrates the benefits of combining common garden and genomic studies. For long-lived outcrossing species, the QTL approach requiring recombinant inbred lines is not always feasible, but the use of common gardens that capture extensive phenotypic and genotypic variation across a species range allows us to benefit from the integration of genomic techniques into these studies.

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Figure 1. Quantitative Trait Loci (QTL) of five chromosomes of *Arabidopsis thaliana* from populations that are correlated with fitness in field experiments located in Italy and Sweden in 2009, 2010, and 2011. This figure shows 15 quantitative trait loci (QTL) associated with fitness. Their analysis discovered six QTLs (underlined) with trade-offs in fitness, that is loci that are locally favored (arrows pointing up) but reduce fitness elsewhere (arrows pointing down). Shaded boxes indicate the range of point estimates associated with the detected QTLs. [Figure and details are taken from Ågren et al. (2013).]

Figure 2. (**A**) Genome-wide association study (GWAS) identified 275 unique genes distributed across trait categories associated with biomass, ecophysiology, phenology with number of genes indicated and circle sizes representing relative proportion of significant genes. The majority of genes are associated with phenological traits. (**B**) The amount of phenotypic variance for each category explained by SNPs. Quantile distributions are indicated by lines and boxes. [Details and figures are taken from McKown et al. (2014a).]
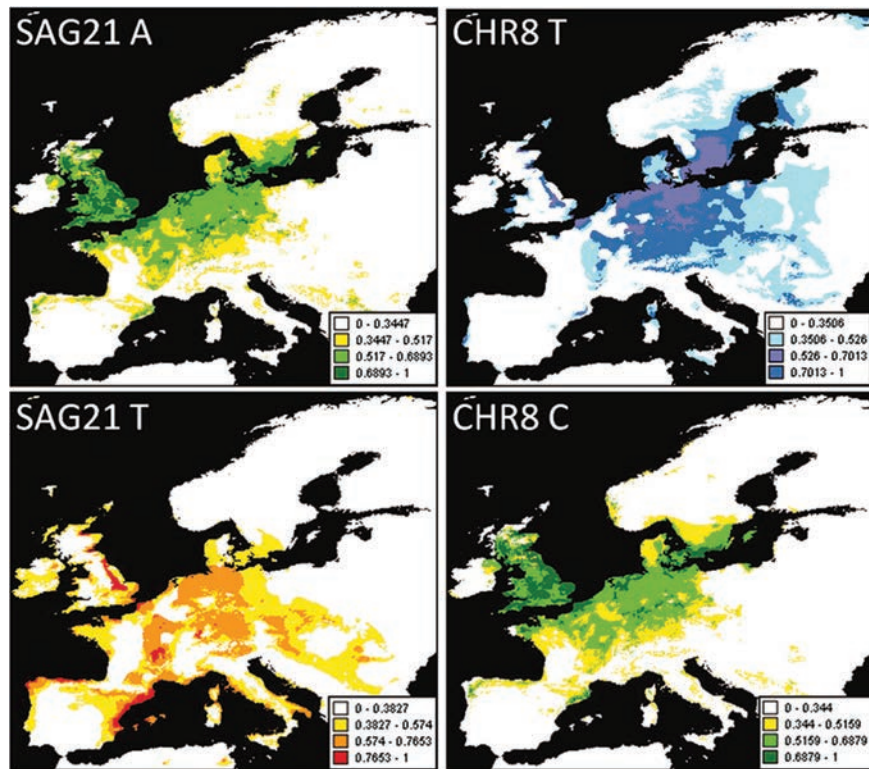
Population and Landscape Genomic Studies of Local Adaptation

A second source of evidence of local adaptation stems from the field of landscape genomics, wherein genetic variation in natural populations sampled throughout a region or species range using sequence data from genome-wide scans, candidate genes, exome capture, transcriptomes, and other genomic methods. Genomic signatures of selection in natural populations can be revealed through landscape genomic statistical tests that look for spatially varying selection between populations or associations between genetic and environmental gradients (Sork et al. 2013; Tiffin and Ross-Ibarra 2014). Several landscape genomic methods are available to test for evidence of selection (Table 1, and for a review of multiple methods, see Hoban et al. 2016).

A major contrast between approaches to detect selection is whether the focus is on spatially divergent selection on outlier loci with significantly high genetic differentiation (Price et al. 2006; Excoffier, Hofer and Foll 2009), or selection correlated with environmental gradients (De Mita et al. 2013; Rellstab et al. 2015; Rellstab et al. 2017). In the former, sample design is critical to this approach (Lotterhos and Whitlock 2014), and some evidence suggests that F_{ST} outlier analysis is less robust to detecting loci than environmental association analysis (De Mita et al. 2013; Rellstab et al. 2015; Nadeau et al. 2016). In the latter framework, outlier SNPs that show a significant association with environmental gradients such as a climate factor provide good candidate genes under selection (De Mita et al. 2013; Sork et al. 2013; Rellstab et al. 2015). Many statistical applications now exist. Some that identify individual loci (e.g., Coop et al. 2010; Kang et al. 2010; Hancock et al. 2011; Frichot et al. 2013), some that take into account the fact that many traits have a polygenic basis (e.g., Berg and Coop 2014), and some that examine the multivariate relationship between genetic and environmental gradients together (e.g., Oksanen et al. 2007; Frichot et al. 2013). These methods can use genomic data that has been generated in multiple ways, such as reduced representation libraries, genome-wide SNP arrays, candidate genes, or whole genome sequencing (all are reviewed by articles in Table 1). Regardless of the choice of genomic data or statistical model, the commonality of all the landscape genomic methods is the incorporation of spatial data to detect local adaptation, whereas controlling for background genetic structure created by demographic processes that affect the entire genome.

An excellent example of a landscape genomic approach to the study of local adaptation in the *Arabidopsis thaliana* model system concerns a study of geographic and climatic associations of fitness-associated loci (Fournier-Level et al. 2011). To find those loci, Fournier-Level et al. (2011) planted genotypes from accessions throughout the species range into four common gardens with a range of climate conditions. Using GWAS, they found SNPs significantly associated with fitness traits, whereas controlling for geography, to identify candidate genes for local adaptation and demonstrate their additional association with climate. They also discovered that the alleles associated with higher fitness were more abundant in the planting sites closer to their source of origin than randomly sampled genomic controls (see Figure 1 in Fournier-Level et al. (2011)). Using species distribution modeling of specific loci, they predict the distribution of climate associated alleles on the landscape (see Figure 3). By using a landscape approach, they illustrate that selection across environments, and not restricted gene flow and genetic drift, contribute to spatial variation in genotypes. This kind of information also has obvious utility for species that require management or conservation, such as the forest tree species discussed above.

The use of predictive models of spatial genetic structure that integrate multivariate environmental predictors with single loci patterns is currently in early stages of development, but it is likely to be increasingly common in upcoming years, especially for studies trying to predict response to climate change. In an elegant landscape genomic study of balsam poplar, *Populus balsamifera*, Fitzpatrick and Keller (2015) applied nonlinear spatial models of genetic and climate gradients to model contemporary geographic distribution of genetic variation that incorporate climate variation. Their study focused on candidate genes in the flowering time pathway, which includes multiple SNPs in the GIGANTEA-5 gene, which is represented by multiple SNPs. They also controlled for genetic structure by generating a randomly selected set of SNPs to provide the genome-wide genetic background. The two landscape models, General Dissimilarity Modeling (Ferrier et al. 2007) and Gradient Forest (Ellis, Smith and Pitcher 2012), made similar predictions. In the GF model, the reference SNP-based dataset, selected to represent background structure due to demographic history, showed different weightings across climate variables than the one based on the GI-5 gene (Figure 4A and B). Their study illustrates how spatial models can identify regional sources of seeds for resource management practices, keeping in mind that the multiple SNP dataset will capture the influence of selection

Figure 3. Geographic distribution of the probability of survival-associated alleles, based on MaxEnt models, within two candidate genes that are associated with local adaptation in *Arabidopsis thaliana.* The *SAG21* gene (**left**), which may be involved in water stress tolerance, has an A allele that is associated with high survival in Finland. Note that the alternative T allele is modeled to be more southern in its distribution. The *CHR8* gene (**right**), which may be related to DNA repair after viral infection, has a T allele with high survival in Germany, whereas an alternative allele, C, is modeled to have a distribution towards the west and England. [Figure is from Fournier-Level et al. (2011); see publication for details.]
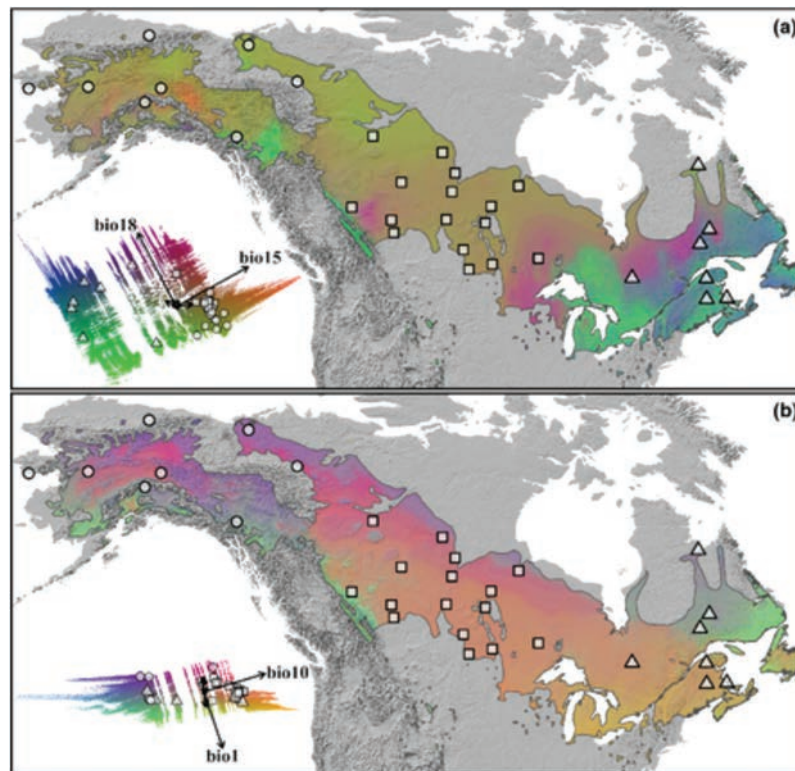
across multiple genes whereas a single gene dataset will represent the climate gradients most associated with that gene only. This investigation, like Fournier-Level et al.'s (2011) study, provides evidence of the spatial patterns of local adaptation and illustrate how a landscape genomic approach can place adaptive genetic variation on a map, which is critical for understanding how to manage and conserve populations facing changing environmental conditions.

Gene Expression Studies of Local Adaptation

The transcriptome, that is the entire RNA sequence of expressed DNA, can be a valuable source of evidence for studying local adaptation. Gene expression data is available for model and non-model species because it is possible to create a *de novo* 'transcriptome' through techniques such as RNA-Seq (Wang et al. 2009), even without a reference genome. Although there are many ways to measure gene expression, here I will focus on the use of RNAseq because it is feasible for evolutionary and ecological studies and the gene expression profiles generated by RNAseq provide an informative indication of how genes vary in their response among treatments or conditions. Such experiments allow insight into the molecular mechanisms underlying local adaptation (Des Marais et al. 2012; Lasky et al. 2014; Akman et al. 2016; Gugger et al. 2016). Gene expression profiles can reveal genotype-by-environment (G×E) interactions in expression that underlie phenotypic plasticity (Des Marais et al. 2013). RNAseq data can also be used to identify networks of genes that are co-regulated in response to an environmental stressor (Langfelder and Horvath 2008; Akman et al. 2016). This technique both reduces the problem of multiple testing, but also groups genes

into meaningful networks that may be involved in the same molecular pathways. Thus, transcriptome analysis presents an extremely valuable tool for finding genes and gene networks involved in plant responses to the environment.

For the *Arabidopsis* case study, Lasky et al. (2014) illustrate how gene-expression profiles generated by previous studies of *Arabidopsis thaliana* with drought and cold treatments (Hannah et al. 2006; Des Marais et al. 2012) can be used to identify locally adapted genes in response to climate. They distinguished genes with consistent responses to environmental stress (expression stress response, eSR) from genes with variable expression response to stress, indicative of a genotype-by-environment interaction (expression gene-by-environment interaction, eGEI). The eSR genes should play a role in stress tolerance across all environments and, therefore, be subject to directional selection whereas eGEI genes will allow plasticity across variable environments enhancing local adaptation. As predicted, Lasky et al. (2014) show that the early flower genotypes for *Arabidopsis* were under-represented for eSR genes when testing for associations with several cold-related climate variables, whereas eGEI genes were enriched for associations with several climate variables (see Figure 5). Similar results were found for drought-related variables. Lasky et al. (2014) also found that eGEI genes that showed associations with drought and cold also had greater polymorphism in promotor regions than did eSR genes. This study demonstrates that some genes enable all individuals within a species to respond to environmental change with plasticity, which allows them to survive different conditions. In contrast, other genes will be expressed differently across populations, exhibiting a G×E interaction that would

Figure 4. (**A**) Predicted spatial distribution for a set of reference SNPs from the genomic background, and (**B**) SNPs in the circadian clock gene GIGANTEA-5 (GI-5), which are associated with adaptive bud phenology traits. Gradients in genetic turnover based on transformed environmental predictors generated by Gradient Forest modeling (Ellis et al. 2012) Colors represent gradients in genetic turnover based on transformed environmental predictors. [Figure excerpted from Figure 5 in Fitzpatrick and Keller (2015), see publication for details of study.]

reflect local adaptation. These G×E expression patterns that improve plant performance or survival can be used to find genes that shape local adaptation.
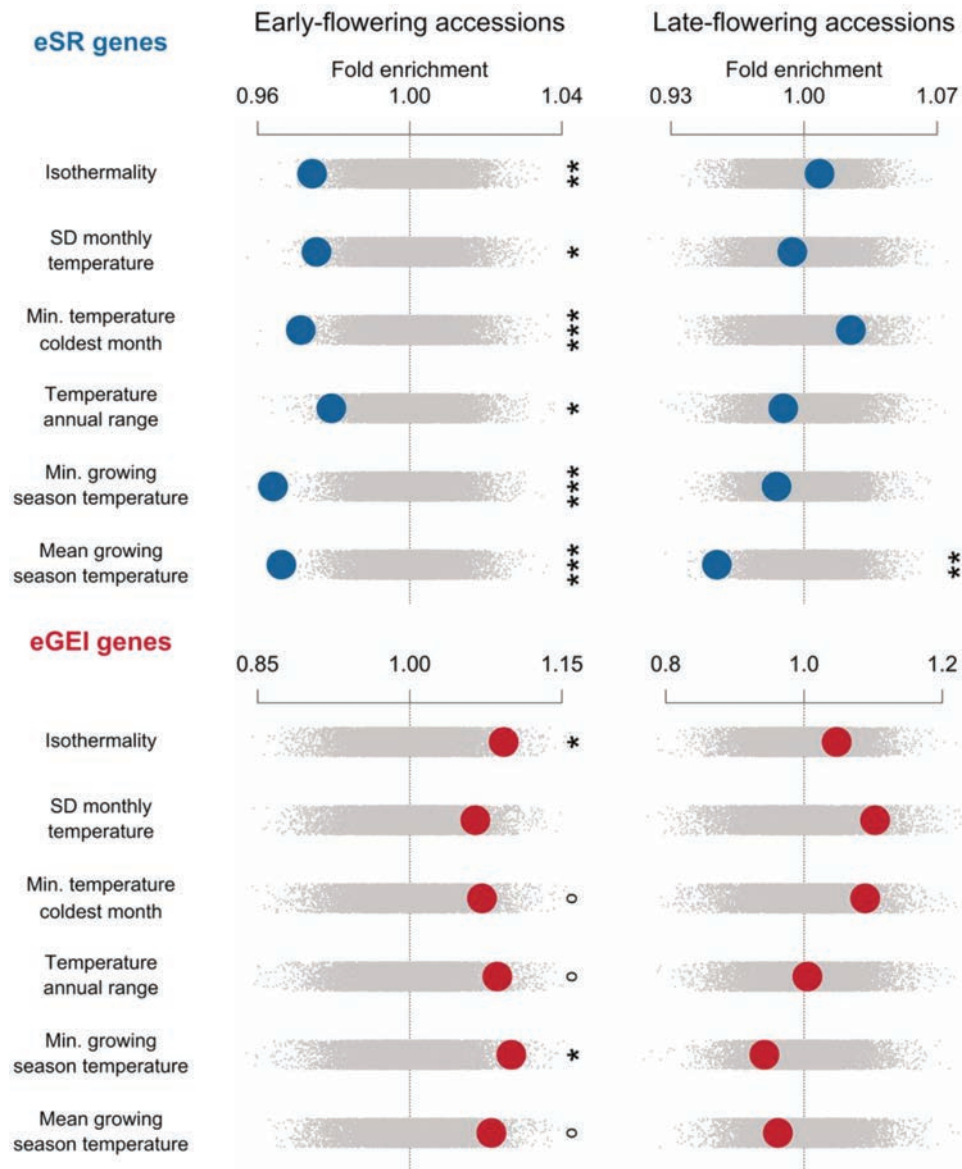
The following tree study provides another good example of how local adaptation can be observed through gene expression patterns. Gugger et al. (2016) examined water stress response of one-year-old seedlings grown in a greenhouse from acorns collected from three populations of valley oak, *Quercus lobata*, distributed in climatically different regions of the species range (Figure 6A). They found an extremely strong transcriptomic response to the water stress treatment: 52% of the ~68 000 contigs were differentially expressed before and after the drought treatment. In addition, they found 56 contigs (i.e., "eGEI genes") that showed a population by treatment interaction (Figure 6B). These contigs showed similar sequences as several stress response genes as well as metabolic and regulatory functional genes, and may be involved in local adaptation, especially given that the patterns of response of the three populations show a gradient in gene expression that follows the precipitation gradient. This work illustrates an approach for long-lived tree species to identify the extent to which tree populations might be genetically differentiated, with the caveat that seedling response may not be indicative of tree response throughout its life span. Nonetheless, such experiments provide an indication of how young trees would respond to climate conditions during establishment, which others have noted is a critical phase for the impact of natural selection (Postma and Ågren 2016).

As both studies illustrate, gene expression studies provide a phenotype that is a direct response to the environment. Many biologists are examining the transcriptomes in natural populations to detect which genes are involved in environmental response through

hypothesis testing and experimental treatments. The transcriptome is also extremely versatile for adaptation studies because it can be used for species with no reference genome, even if the genome is large. For example, Yeaman et al. (2016) compared the transcriptomes of two distant related conifers (> 140M years) to demonstrate that the two species are using the same genes to produce the same phenotypes associated with cold adaptation.

Epigenetic Studies of Local Adaptation

The last pair of case studies address the topic of epigenetic processes as a mechanism resulting in local adaptation (Verhoeven, Vonholdt and Sork 2016). Plants have the potential to respond to the local environment through epigenetic modifications of DNA sequences that alter gene expression and influence plant phenotypes (Law and Jacobsen 2010; Schmitz 2014). In plants, DNA methylation occurs on cytosines in the CG, CHG, and CHH contexts of the DNA sequence (where H is any nucleotide except for C) (Law and Jacobsen 2010) and each has different potential for genetic regulation and transgenerational stability (e.g., Schmitz et al. 2011). Epigenetic variation may result in phenotypic plasticity and in locally adapted phenotypes (Merila and Hendry 2014), and may be especially important in tree populations as a mechanism for faster response to rapid environmental change for species with long generation times (Bräutigam et al. 2013). Epigenetic modifications can occur through histone modification or DNA methylation (Law and Jacobsen 2010), but, so far for ecological and evolutionary studies, DNA methylation is the most commonly studied epigenetic phenomenon (Kilvitis 2014; Verhoeven et al. 2016). Across a range of species, studies have found an association of DNA-methylation levels with environmental
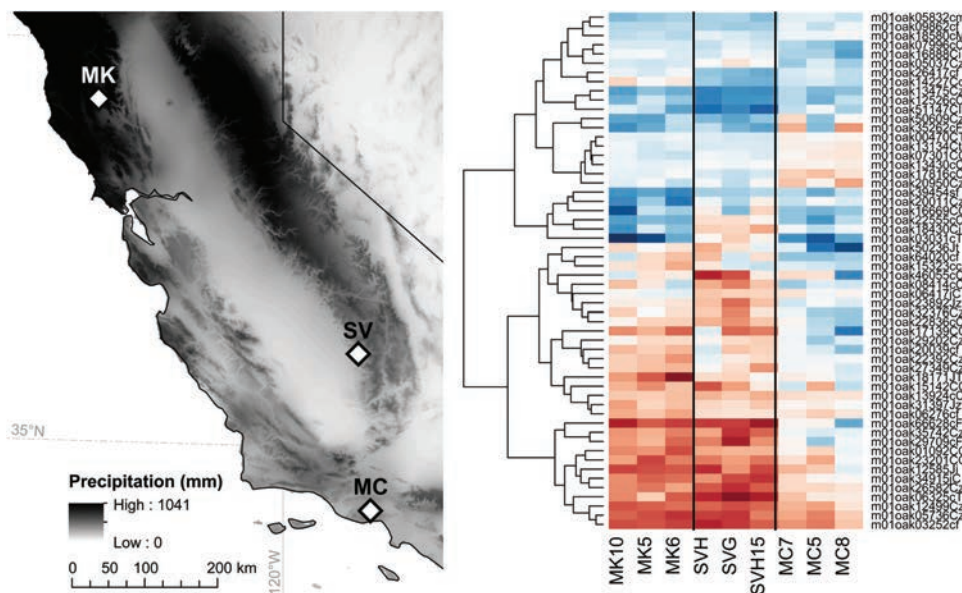
Figure 5. Lasky et al. (2014) found that eSR genes in early accession genotypes of *Arabidopsis thaliana* were significantly under-represented for their association with cold-related climate variables compared to genome-wide expectation based on low z-scores (upper left panel). Significant under-enrichment for late accession genotypes was found for Mean growing temperature seasons only (upper right panel). In contrast, eGEI genes measured in early accession genotypes showed significant enrichment at most climate variables (lower left panel), but not in late accession genotypes (lower right panel). Significance level: °$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$). [This figure is Figure 2 in Lasky et al. (2014); see publication for details.]
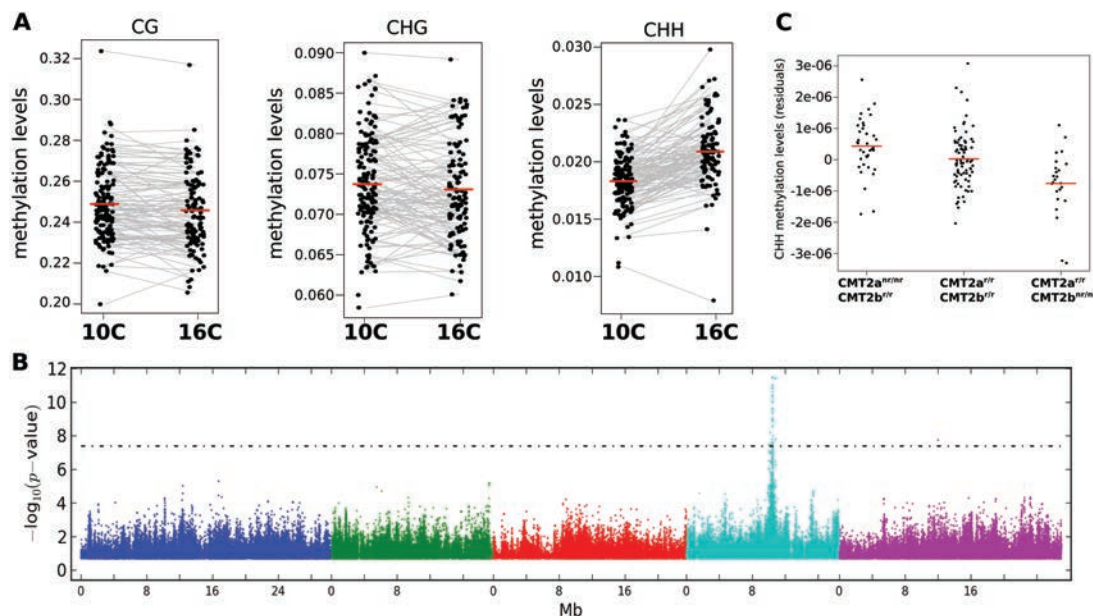
gradients, which suggests a role of natural selection (e.g., Richards, Schrey and Pigliucci 2012; Dubin et al. 2015; Gugger et al. 2016; Keller, Lasky and Yi 2016). DNA methylation has the potential to modify gene expression within the promoter regions and to become heritable, at least for a few generations (Schmitz et al. 2011; Becker and Weigel 2012; McNamara et al. 2016), which may be long enough for spontaneous genetic mutations to arise that maintain adaptive phenotypes (Klironomos et al. 2013). It can also be found within gene bodies, usually in the CG context and probably facilitated through a Cytosine Methyltransferase gene (CMT) (Law and Jacobsen 2010). Methylation may be associated with higher gene expression or silencing transposons (Law and Jacobsen 2010). CHH methylation, which occurs in much lower levels of methylation than CG and CHG methylation, is less stable across generations, but more environmentally sensitive than the other two contexts (e.g., Dubin et al. 2015).

The model system of *Arabidopsis* has already provided some insightful explorations of the role of DNA methylation in plant response to the environment. In a very elegant analysis of DNA methylation in Swedish accessions of *Arabidopsis* grown in two temperature treatments, Dubin et al. (2015) compared different contexts of methylation—CG, CHG, CHH—and their genetic basis. They showed that CHH methylation was significantly affected by the temperature where the plants were grown (see Figure 7A). GWAS revealed that CHH methylation was sometimes associated with transposons (Figure 7B), which had a genetic association located on chromosome 4 at the locus of a CHH/CHG methyltransferase. Dubin et al. (2015) also found an association between CG-methylation levels and the climate where the accession came from. This CG methylation is largely due to gene body methylation (GBM), where genes with high GBM tend to be more highly expressed, probably controlled by *trans*-acting polymorphisms.

Figure 6. (**A**) Sampling locations of populations sampled from climatically different regions of *Q. lobata* species distribution (Shading indicates precipitation). (**B**) Heatmap and hierarchical clustering of $\log_2$-fold changes in gene expression in response to drought treatment for 56 eGEI genes. Color scale reflects change in gene expression before and after drought treatment (blue=down-regulated and red= up-regulated. [This figure is based on Figures 1 and 5, respectively from Gugger et al. (2016).]

Figure 7. (**A**) Norms of reaction plots for CG, CHG, and CHH genome-wide methylation levels for *Arabidopsis* accessions grown at 10 °C (125 samples) and 16 °C (116 samples); CHH exhibits significantly higher rates of methylation at 16 °C. (**B**) Manhattan plot of GWAS findings of CHH methylation for accessions grown at 10 °C associated with large transposons as the phenotype. Loci above threshold line are significant based on Bonferroni-corrected *P* value of 0.05. (**C**) Estimates of CHH methylation found on large (over 2 kb) transposons for the samples grown at 10 °C associated with three two-locus genotypes: CMT2a$^{nr/nr}$/CMT2b$^{r/r}$, CMT2a$^{r/r}$/CMT2b$^{r/r}$, CMT2a$^{r/r}$/CMT2b$^{nr/nr}$ demonstrating the association between transposons and CHH methylation. [Figure and details taken from Dubin et al. (2015)]

Similar to these results, (Keller et al. 2016), who used a multivariate environmental association analysis of Eurasian and Swedish accessions of *Arabidopsis thaliana* with climate variables, found single-nucleotide methylation variants (SMVs, also called differentially methylated positions, DMPs) in the CHH context were most strongly associated with climate, and, like the Dubin et al. 2015 study, these methyl sites were often associated with transposons, and possibly RNA-directed methyl transferase genes. Taken as a whole, these two *Arabidopsis* studies suggest that DNA methylation is often genetically based at loci that are under directional selection due to local climate, but temperature-induced methylation cannot be ruled out.
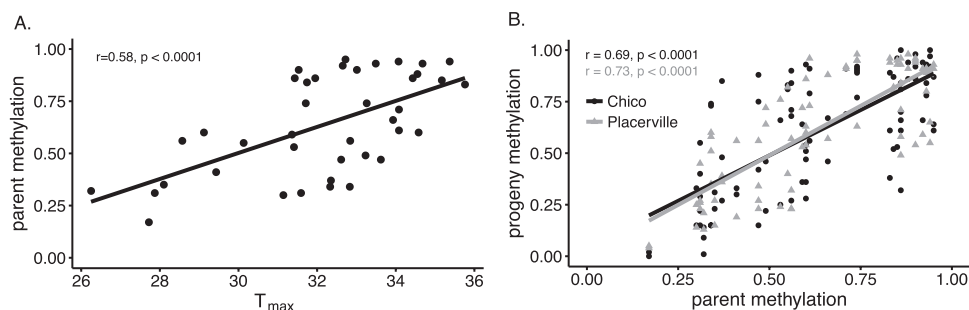
The longevity of trees both provides the opportunity for epigenetic modifications to accumulate over time and a need to utilize a mechanism for rapid response to changing environmental conditions

(Bräutigam et al. 2013). In a study of California oak adults sampled across a broad environmental gradient, Gugger et al. (2016) used reduced representation bisulfite sequencing to identify SMVs (AKA DMPs),which are significant outliers associated with climate. In contrast to the *Arabidopsis* studies, we found that the strongest associations were in the CG context, were most often associated with maximum temperature, and were most frequently found within gene bodies. Using a reference genome (Sork et al. 2016a) and a reference transcriptome (Cokus et al. 2015) of *Quercus lobata*, we searched for genes that might be associated with SMVs and found that the fragment sequence that was most highly correlated with T_{max} (Figure 8A) is associated with a dehydration-responsive element-binding protein. Of the top four most highly correlated outliers, one also included a correlation with a CHH polymorphism, but no gene was located within 1 kb, perhaps suggesting either distant *trans*-acting polymorphism or a distant cis-acting enhancer element (Gugger et al. 2016).

Through current *Q. lobata* studies, we are addressing whether DNA methylation and any context can be environmentally induced and whether it is transgenerational (Sork, Fitz-Gibbon, Burge, Gugger, and Pelligrini, in prep) through a common garden experiment where we planted progeny from the adults sampled in Gugger et al. (2016) into two environments. The challenge to comparing methylation across contexts is that the different levels of methylation bias the detection of outliers towards the more highly expressed context, such as CG methylation. In fact, CHH-methylation levels can be so low (e.g., Dubin et al. 2015 and Gugger et al. 2016). that is difficult to find a significant environmental association. To look for environmental associations, we first re-analyzed the adult oak epigenotypes and analyzed the progeny genotypes with relaxed filters that included SMVs with low methylation levels (Sork, Fitz-Gibbon, Burge, Gugger, and Pelligrini, in prep). Now, we now detect 8 significant associations with climate associated with CHH methylation among adult field-collected samples (Table 2), which is higher than the 2 loci previously reported (Gugger et al. 2016), with similar trends among progeny with their maternal source environment (data not shown). We also find that CHH is under-represented in terms of environmental association (12% of all outlier SMVs) out of the available SMVs (58% of total SMVs; Table 2). In contrast, CG-SMVs are over-represented in our environmental association tests (Table 2). In this same experiment (Sork, et al. in prep), we are also finding many SMPs with evidence of transgenerational inheritance of methylation levels in four-year old progeny by comparing parent-offspring correlations (for example, see Figure 8B), especially for CG-SMVs. When we compare methylation levels between progeny from the same families grown in different gardens, we find that CHH-SMVs are more likely to be influenced by the environment (Sork, et al. in prep). So, in *Q. lobata,* different cytosine contexts show dissimilar tendencies for transgenerational inheritance and sensitivities to environmental factors.

Both the *Arabidopsis* and valley oak studies suggest that methylation patterns may have an underlying genetic basis. At the same time, some methylation may be environmentally induced, and CHH sites are good candidates for epigenetic effects on local phenotypes. Although evidence exists of correlations between methylation and environmental differences across many species (Becker et al. 2011; Becker and Weigel 2012; Bräutigam et al. 2013; Verhoeven et al. 2016), the genetic versus sole epigenetic basis of those associations

Figure 8. (**A**) Example of one CG-SM where the methylation levels of field collected samples from 40 adults were significantly correlated with maximum temperature of the warmest month (T_{max}), after controlling for background structure. This CG-SNP located within the gene body of a dehydrin-response gene. (Data are a subsample of those used in Gugger et al. (2016)). (**B**) Spearman's correlations between the methylation levels of two progenies per 40 field-sampled adults shown in part (A) versus the methylation levels of those adults. Significant correlations are evidence of broad-sense heritability. Leaf samples were taken from four-year-old progeny at two common gardens located at two US Forest Service sites located at Chico (warmer) and Placerville (cooler), which are both located in northern California. (Figure based on unpublished data of Sork, Fitz-Gibbon, Burge, Gugger, and Pelligrini).

Table 2 Summary of DNA methylation sites found in a sample of 58 *Q. lobata* adults sampled throughout California

Methylation context	Sites (<20% missing data)	Percent of total sites within context	SMVs	Percent SMVs per context out of Total SMVs	Highly significant SMV outliers	Percent outlier SMVs
CG	77179	19%	48984	28%	50	72%
CHG	60602	15%	25665	14%	11	16%
CHH	275393	66%	102903	58%	8	12%
Totals	413174		177552		69	

We found 413174 sites, with the majority (66%) comprised of CHH-methylation sites. Sites containing single methylation variants (SMVs) were enriched for CG (28% CG-SMVs versus 19% CG sites). SMVs that were significant outliers based on environmental association analysis were enriched in the CG and CHG contexts, whereas CHH-SMVs were under-represented (38% outliers versus 58% SMV sites). Data reported in Gugger et al. (2016) and reanalyzed by Sork and Fitz-Gibbon (unpublished data). Similar trends are found among progeny of these same adults (Sork, Fitz-Gibbon, Burge, Gugger, Pelligrini, in prep.).

and the role of methylation in shaping adaptive phenotypes needs much more investigation. Our understanding will require more details on the molecular basis of methylation, including the sequence context and its location within promoter regions, gene bodies, or intergenic sequences. Future studies will need to assess whether the observed methylation is the actual cause of phenotypic change and whether it is transgenerational, both of which are needed for consideration as 'local adaptation' (Verhoeven et al. 2016). Thus, epigenetic processes may be important in the evolution of local adaptation, but we have much to learn about the extent to which these processes are genetic, phenotypically plastic, environmentally induced, and/or transgenerationally stable.

Closing comments

The eight case studies described in this paper were selected to illustrate the lessons we are learning about evolutionary processes in plant populations through the use of rapidly emerging genomic tools. Many more topics could have been explored as demonstrated by numerous review papers (Table 1). Instead, this review focuses on a comparison between findings from *Arabidopsis* and tree species. *Arabidopsis*, a model system with extensive genomic resources, provides a useful study system due to its small size, short life span, ease of cultivation in green houses and growth chambers, and feasibility for experimental manipulations. The fact that *Arabidopsis* populations are highly inbred and can be easily self-pollinated to create recombinant inbred lines (RILs) is an additional advantage for studying the genetic architecture of traits under selection. Collectively, the *Arabidopsis* studies are demonstrating that a large portion of geographic variation in phenotypes has evolved in response to natural selection, despite the fact that their small effective population sizes and opportunity for inbreeding creates extensive differentiation due to genetic drift (Savolainen et al. 2007). Despite these advantages, the same life history traits that make them a suitable model system may limit the generality of the studies to other plant species with more complex life-histories such as trees.

Tree species offer many advantages for evolutionary studies of local adaptation (Gonzalez-Martinez et al. 2006; Nichols et al. 2010; Sork et al. 2013). Their large effective population size due to their highly outcrossing mating systems and extensive gene flow favor the evolution of local adaptation. A long history of provenance studies in forest genetics provides useful data for studying how tree populations have evolved in response to local climate (Savolainen 2011; Sork et al. 2013). These studies have been combined with new genomic tools to provide lessons about tree response to climate across a range of species (Matyas 1996; Alberto et al. 2013). At this point, we need to utilize existing common gardens to ground-truth the findings generated by the genomic approaches for trees in the ways that we are observing for *Arabidopsis* research.

The case studies of *Arabidopsis* and trees species, with so many dissimilarities, point to some important similarities. For example, both have similar scales of adaptive genetic variation shaped more by the environment than distance, both respond to climate gradients through expression of general stress response genes across all populations and through genes that differ across populations in their gene expression response, and both show associations between environmental differences across populations and epigenetic modifications. These parallels would suggest that we can apply genomic tools to the study of local adaptation in trees without always conducting the reciprocal and common garden transplant experiments that can take much space and time before meaningful results are available.

This point has been demonstrated by tree studies that employ genomic tools alone to identify genetic basis of traits underlying locally adapted phenotypes (e.g., Grattapaglia et al. 2009; Eckert et al. 2010; Steane et al. 2014; Fitzpatrick and Keller 2015; Sork et al. 2016b). Encouragingly, the comparisons indicate that both annuals and trees have the ability to adapt to local conditions and to respond to the environment through phenotypic plasticity and epigenetic modifications. We are now equipped with or are currently developing the tools needed to understand in unprecedented detail how plasticity and epigenetic modifications interact with genetic variation and gene expression to mediate adaptation to local conditions.

In closing, I have highlighted some studies here, but many others demonstrate that genomic information has created new, broad horizons for the study of local adaptation and the story will not be simple. Some traits may be characterized with a few key genes or even gene networks, but other traits underlying local adaptation will not only be polygenic but 'omnigenic' (Boyle et al. 2017), which means sample designs and statistical models will need to become increasingly sophisticated. Moreover, as we learn more about the genetic, epigenetic, and transcriptomic processes underlying phenotypes traits and phenotypic plasticity, we will better understand how plants respond to their environment and how to apply this knowledge toward conservation and management strategies for natural populations.

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