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**Mapping the genetic basis of ecologically and evolutionarily relevant traits in
*Arabidopsis thaliana***

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ABSTRACT

There has been a long standing interest in the relationship between genetic and phenotypic variation in natural populations, in order to understand the genetic basis of adaptation and to discover natural alleles to improve crops. Here we review recent developments in mapping approaches that have significantly improved our ability to identify causal polymorphism explaining natural variation in ecological and evolutionarily relevant traits. However, challenges in interpreting these discoveries remain. In particular, we need more detailed transcriptomic, epigenomic, and gene network data to help understand the mechanisms behind identified associations. Also, more studies need to be performed under field conditions or using experimental evolution to determine whether polymorphisms identified in the lab are relevant for adaptation and improvement under natural conditions.

INTRODUCTION

Plants, perhaps due to their limited mobility, show an impressive array of intraspecific and interspecific variation for traits such as drought, disease resistance, tolerance to salinity, etc. [1-3]. Much of the phenotypic variance seem to reflect specific adaptations to climate and seasonality and are thought to have been shaped by natural selection. Underlying all this phenotypic variation is, of course, extensive genomic, transcriptomic, and epigenomic variation. There has been a long standing interest in tapping onto this variation to improve crops and to dissect the genetic basis of adaptation.

Recent technological developments have significantly improved our ability to catalogue all this genomic diversity [e.g. 4-8]. The hope is that a complete catalogue will lead to the unravelling of the relationship between natural genetic and phenotypic variation, what is also known as the “genotype to phenotype map” [9] (Fig 1). Such a map would facilitate the identification of useful candidate genes to improve crops, allowing the identification of gene transfer from model species into crops or the identification of useful orthologs [10-11]. It will also allow a better understanding of the value of different kinds of genetic diversity for the maintenance of evolutionary potential and therefore for species’ persistence [12-13]. These are particularly pressing issues given the challenges plants (and therefore, us) may encounter in the near future when significant changes in the environment are expected [14]. Here, we will review the current approaches to identify the genetic basis of ecological and evolutionary traits and discuss whether we are getting closer to understanding them.

Mapping using experimental populations

Most traits of evolutionary and economical relevance (e.g. germination rate, competitiveness, glucosinolate content, fitness, etc) are complex, that is, they are determined by multiple loci, which may interact with each other, and they are often affected by environmental and parental effects. Thus, identifying the genetic basis of these traits has been quite challenging. Currently, the main approach to identify loci underlying natural variation in complex traits is to associate genotypic and phenotypic variation using either recombinant inbred lines (RILs) or a collection of natural accessions.

Until very recently, RILs were mainly produced by crossing two inbred accessions (genomes independently collected from natural populations) to obtain a segregating F₂ population (Fig. 2a). The recombined genotypes are then inbred for 5 or more generations, to produce homozygous genotypes (RILs) that only need to be genotyped once, and later phenotyped for multiple traits under different environments [e.g. 15-16]. Marker association between mapped genotyped variants and phenotype indicate the locations of genetic factors that significantly affect the trait. However, these lines typically do not offer very good accuracy, with QTL being located to chromosomal regions varying between 5 and 50 cM (which is equivalent to 1.2 to 12 Mb in *A. thaliana*, or 9 to 90 Mb in Maize) [17]. To reduce the interval containing the QTL, more recombinations are needed. In Advanced Recombinant inbred lines (AILs, Fig 2b), two or more generations of recombinations are performed before the inbreeding starts, reducing the confidence interval for the QTL localization [18-19].

Recently, multiparent RILs have been developed: The Arabidopsis Multiparent RILs (AMPRIL) [20], and the Multiparent Advanced Genetic InterCross (MAGIC) lines [21]. The MAGIC lines were derived from intercrossing 19 natural accessions of *A. thaliana* for multiple generations (Fig. 2c). Thus, in addition to extra recombination steps, there are also a larger number of alleles segregating in the lines; as a result QTL can be mapped to significantly smaller regions (< 1 Mb). This resource has been further enhanced by the sequencing of the parental genomes [5], which revealed 3.3 million variable sites that are segregating in the MAGIC lines. The level of nucleotide diversity present among the MAGIC lines is similar to the diversity observed among 96 accessions previously genotyped [5, 8]. Also, they contain 35% of all the non-private SNPs uncovered so far by the 1001 genome project (which includes at the moment 452 accessions), and 68% of the SNPs with frequency higher than 0.05 (Cao, pers. comm.). This suggests that although only 19 accessions were used to make the MAGIC RILs, they do capture a significant proportion of the common molecular variation present in the species. While MAGIC and AMPRIL lines

allow higher precision than a traditional RIL, they may detect fewer QTL [20]. However, more data is needed to determine if this is indeed the case since only a couple of studies have yet been published, and what would be the cause of such reduction.

Mapping using natural populations

QTL maps are not the only method available to dissect complex traits; an alternative is to perform GWAS by looking for associations between SNPs and phenotypic variation across a large panel of naturally-occurring inbred accessions [4]. This has been possible due to the reduction in sequencing cost, and the development of new technologies [reviewed in 23] , leading to the genome sequencing of >400 natural accessions of *A. thaliana* ([5,22,24] and <http://1001genomes.org/>). The reduction in price and build up of species being sequenced will probably allow GWAS to also be pursued soon in many other species.

The advantages of using GWAS in *A. thaliana* is that in a large worldwide population, many sequence variants have arisen by mutation and many recombinations have accumulated, resulting in a situation where the linkage disequilibrium between common variants decays on average to background levels within a few kilobases [25]. Thus, a SNP association with a complex trait is likely to be very close to the causal variant. This was clearly shown by the identification of a natural polymorphism in *HKT1* as the cause of natural variation in salt accumulation, and its association with coastal populations [26]. However, there is extensive population structure in *A. thaliana*, and a large number of rare variants (frequency < 5%) [27]. Population structure means that distant variants (even on different chromosomes) are sometimes in disequilibrium with each other, causing false positive genetic associations (estimated to be about 40% in one study [28]). This can be ameliorated by control for population structure, but this can also reduce the power to detect associations [4]. In addition, rare variants affecting a phenotype are hard to detect or map accurately, first because they are unlikely to account for much of the phenotypic variation, and second, if they are of recent origin, they are likely to be in linkage disequilibrium with a larger region of the surrounding genome. GWAS has also been successfully implemented in rice and maize [29-31], illustrating how natural variation is helping identify the genetic basis of complex agronomically valuable traits.

Combining approaches

QTL analysis and GWAS have complementary characteristics: RILs control for allele frequency (the minor allele frequency should never be much under $1/[\# \text{ of parental founders}]$) and population structure is broken up. However, RILs experience less recombination, being limited to those accumulating in meioses during their production. Thus mapping resolution is lower, but there are fewer false positives. It has been suggested that a possible powerful approach is to use RILs and natural accessions to map the same trait simultaneously [28]. The GWAS would provide good resolution for the causal polymorphisms, and the QTL analysis would allow detection of false-positives. It is possible that some of the associations observed with GWAS and not with QTL analysis are true, but there is not sufficient power to detect it on the QTL analysis. This would result in a slightly elevated false negative rate, but that seems less of a problem when the goal is to identify some or the major loci contributing to the variation of a given loci. Another issue is the choice of RILs, since different associations may be due to alleles from different accessions. Synthetic populations derived from multiple accessions, such as the MAGIC or the AMPRIL lines [18] are likely to be particularly useful for this purpose.

Another approach pioneered in maize [32] to combine GWAS and QTL mapping, and particularly useful for species with larger genomes, is to sequence a few accession (25 in the case of maize) and produce a set of RILs between these founders. This approach, also known as nested association mapping (NAM) has led to the successful identification of loci associated with many traits, including disease resistance [31].

Further searching; Transcriptomics and Epigenetics

Despite the impressive depth of knowledge about genomic variation available in *A. thaliana*, recent studies suggest that we have only scratched the surface. Detecting associations between SNPs and phenotype is only the first step in elucidating the mechanism through which genomic variation affects phenotype. More data on quantitative and qualitative variation at the protein, RNA, and chromatin level would provide valuable additional information to unravel the genotype to phenotype map (Fig 1).

RNA-seq data from seedlings was obtained for the 19 MAGIC parental accessions, and used to re-annotate coding genes in each accession [5]. Surprisingly, it was found that a simple transfer of gene annotations from the reference Col-0 to each accession predicts about one third of the genes to be significantly disrupted in at least one accession (e.g. by a premature stop codon). However, if each accession is annotated *de novo*, backed-up by RNA seq data, it is observed that often the splicing pattern of an affected gene changed in such a way that the new transcript is still functional. Thus, using the default reference gene

annotations to predict the effects of sequence variation is fraught, and the only sure way to correct the annotations is by obtaining transcript data. This study also showed that ~9000 transcripts vary among accessions and may possibly also vary between tissues. Thus, any catalog of genes expressed in one accession of *A. thaliana* represent a biased sample, and better interpretation of associations between SNPs and phenotype will require more RNA seq across accessions and multiple tissues.

Epigenetic studies, which focus on DNA and chromatin modifications, can also provide a useful complement by providing an extra source of heritable variation that may explain some of the phenotypic variation that tend to remain unexplained in many studies of complex traits [33-35]. Intraspecific variation in epigenetic markers has been observed in a number of species [36-38], and it has been shown to affect gene expression in *A. thaliana* [39]. The importance of epigenetic marks on phenotypic expression has been demonstrated by the significant difference in plant heights among *A. thaliana* lines with identical genomes but different methylation patterns [40]. Recent advances in genomic technologies have allowed genome-wide methylation marks to be assayed efficiently [41], opening the doors for the performance of Epigenome-Wide Association Studies (EWAS). EWAS searches for association between DNA methylation marks and phenotype similarly to GWAS. EWAS has been used successfully to identify associations between methylated sites and autism in humans [42], and there are ongoing studies exploring its use in Arabidopsis.

Which genes are being identified?

It is unclear whether most loci identified so far, using the methods discussed above, are relevant to address the genetic basis of adaptation or to improve crops facing significant environmental changes. The first concern comes from the possible effect of environment in determining significant associations [43-44]. A recent study that compared associations found under laboratory and field conditions in *A. thaliana* found a very different set of loci associated flowering time (an important trait to obtain optimal fitness under varying environments) [45]. Of the 25 loci were found to be associated in the field, only 2 were previously identified to be associated with flowering time under lab conditions. A second concern is that selection experiments have suggested that genes previously identified to affect flowering time, do not mediate most of the phenotypic response to selection [21,46-47]. Such discrepancy might be due to differences in environment between QTL and selection studies; but it might reflect the existence of genetic constraints, such that the genes that respond to selection differ from those with detectable associations at a single point in time. Consequently, studies to determine the genetic basis of the response to selection, in which populations that have been allowed to evolve are resequenced, may improve our

understanding of the relationship between existing natural variation and the response to selection.

Conclusions

Statistical developments, sequencing technologies and new mapping methods have significantly improved our ability to detect natural polymorphisms that underlie ecological and evolutionary relevant traits. However, many more studies are still need to understand the adaptive process, and the mechanisms through which causal variants affect phenotype. The biggest challenge ahead is to expand the tools developed in *A. thaliana* to many other non-model species, and to use experimental evolution to understand the relationship between standing variation and evolutionary response. The development of faster and cheaper technologies to assay genomic variation at multiple levels is quickly transforming our knowledge about patterns of natural genetic variation within [48-49] and across related species [50-51]; and it will certainly transform our understanding of the evolutionary process.

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Figure legends

Fig 1. Schematic illustration of the main sources of heritable variation influencing the genotype to phenotype map. Trait 1 illustrates a simple qualitative trait, such as disease resistance in plants where the expression of a receptor protein may be sufficient to elicit defense responses against biotic components of the environment. In contrast, trait 2 and 4 illustrate complex traits mediated by more than one gene. Trait 4 is an example of a composite trait such as fitness, that depends on the combination of multiple traits such as fruit number and number of seeds per fruit, which can have part of its genetic basis in common (Protein 3) and some independent (Protein 4). In reality, we expect much more complex relationships.

Fig. 2. Illustration of the different ways in which Recombinant Inbred Lines (RILs) for mapping complex traits can be produced. Panel A shows a traditional RIL produced from the F₂ of two accessions; panel B shows Advanced Intercross lines, and how the extra generation(s) of recombination reduces the size of the chromosome fragments; and panel C shows a Multiparent Advanced Genetic InterCross (MAGIC) lines, where there are multiple parental accessions and extra generations of recombination.

ABIOTIC ENVIRONMENT
BIOTIC ENVIRONMENT



