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On the hidden temporal dynamics of plant adaptation

Keely E. Brown[1] and Daniel Koenig[1,2]

Abstract

Adaptation to a wide range of environments is a major driver of plant diversity. It is now possible to catalog millions of potential adaptive genomic differences segregating between environments within a plant species in a single experiment. Understanding which of these changes contributes to adaptive phenotypic divergence between plant populations is a major goal of evolutionary biologists and crop breeders. In this review, we briefly highlight the approaches frequently used to understand the genetic basis of adaptive phenotypes in plants, and we discuss some of the limitations of these methods. We propose that direct observation of the process of adaptation using multigenerational studies and whole genome sequencing is a crucial missing component of recent studies of plant adaptation because it complements several shortcomings of sampling-based techniques.

Addresses

[1] Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

[2] Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA

Corresponding author: Brown, Keely E. (keelyb@ucr.edu)

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Introduction

Plant experimental systems are uniquely suited to the study of environmental adaptation. They are immobile, many can be self-fertilized or cloned to produce identical genotypes across common gardens, and can be collected and stored over long periods of time as seed. Because of these and other advantages, experiments using plants provided some of the earliest evidence linking phenotypes to environmental adaptation [1,2]. Even before these classic studies, breeders had recognized that yield was often improved when plants were grown in the same location as they had been grown historically [3]. Until recent years, very little was understood about the genetic basis for the traits being selected across environments [4]. Establishing connections between genotype and locally adaptive phenotypes [5,6] is imperative to mediate the impacts of a changing climate on plant agronomic production and ecological diversity [7,8].

Advances in sequencing technology have recently made it possible to affordably sequence hundreds to thousands of genomes from a single species. The major challenge moving forward is to link the millions of genomic differences identified in these projects to adaptive phenotypic differences. Common approaches combine large panels of sequenced genomes from extant individuals with phenotypic, environmental, or geographical data to produce statistical associations that pinpoint genes underlying adaptation (what we refer to throughout as "sampling" approaches, see below for further description). These approaches have been hugely successful in uncovering the mechanism of plant adaptation at the genetic level [9].

However, the study of plants collected from a single time point provides an incomplete picture of the dynamic process of evolution. A necessary complement is to directly track changes in the genetic composition of a population throughout the entire genome across generations [10–12]. In this review, we term these studies multigenerational, where samples are taken at different time points to compare earlier and later generations. Adaptive changes in the genome can be pinpointed by comparing observed allele frequency changes to neutral evolution [13], or by associating genotypic change with an increase in fitness over time [14]. Such studies have already opened a window into the process of evolution across many systems revealing the genetic architecture underlying the evolution of a novel trait [15], the predictability of evolution in response to environmental change [16], and the number of genes that underlie the response to selection [17,18].

Multigenerational studies have long been a staple of plant evolutionary biology, and many of the oldest experimental evolution studies have been conducted in

plants. Hallauer's Tuśon [19], the Illinois long term selection experiment [20], the Buxton Climate Change Impacts Lab [21], the Saclay Divergent Selection Experiments in maize [22], and the Composite Crosses in barley [23] all demonstrated dramatic phenotypic change in response to new environments or artificial selection over decades. Shorter-term studies in plants spanning fewer than ten generations have also shown substantial evolutionary shifts in genotype and phenotype [24,25]. We propose that these classic multigenerational approaches, when combined with modern genome sequencing techniques, will make substantial contributions to our understanding of the link between genotype and adaptive changes in phenotype in plants.

Sampling the genomic differences underlying plant adaptation

Before discussing multigenerational approaches, we will first briefly outline the common "bottom−up" and "top−down" sampling strategies used to link genomic variation to phenotype in plants [9]:

Bottom−up approaches leverage the availability of large numbers of genome sequences within a species to search for the footprint of selection at specific genes (Figure 1, bottom right). They largely rely on the well-supported assumption that individuals or populations are adapted to their current environment [26]. One bottom−up method searches for regions of the genome that show differentiation in allele frequencies larger than predicted by neutral processes between populations collected from different environments. Genes in these regions are candidates for targets of selection [27]. Another bottom−up approach, environmental genome-wide association [28], combines whole genome sequence data with environmental information about the collection site of each of the sequenced samples. By treating each environmental variable as a phenotype in a genome wide association study, genetic changes associated with differences in specific environmental variables are identified. Environmental GWAS have, for example, rediscovered cold tolerance genes [29], and identified climate-associated variants that change mRNA structure [30], both in *Arabidopsis thaliana*.

Top−down studies can measure a number of traits and estimate plant fitness in a diverse set of sequenced samples grown together in one or more common gardens [26]. Loci that contribute to local adaptation are expected to differ in frequency among individuals with high or low fitness in contrasting environments (Figure 1, bottom left). Associations between genotype and proxies for fitness can be tested for every one of the potentially millions of genomic differences that segregate amongst the studied lines. Unlike the phenotype-free bottom−up approaches, these experiments can directly link adaptive phenotypes to fitness estimates. Using this approach, specifically climate associated loci have been identified in many plant species [31−34].

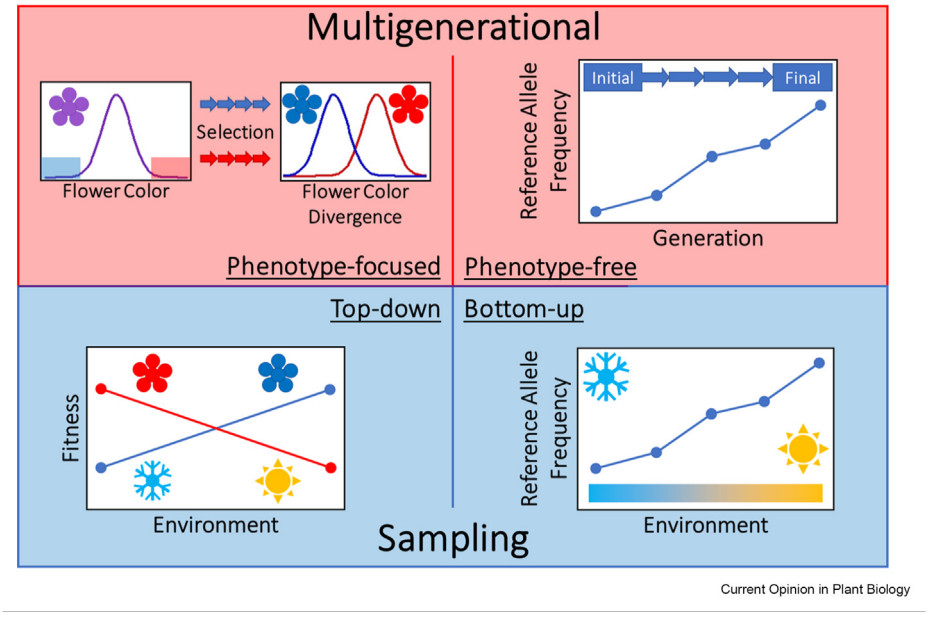
Both top−down and bottom−up approaches reveal candidate loci that may be involved in adaptation. However, both approaches observe the outcome rather than the process of evolution, which limits our understanding of the genetic variation that contributes to adaptation. Multigenerational experiments complement sampling strategies by directly recording the process of evolution across time. While large, well-designed multigenerational experiments have the potential to provide a great deal of information about the process of adaptation, it is important to note that they suffer from their own set of challenges (Box 1). Below, we discuss two major categories of multigenerational studies: those which directly impose selection on a single or small number of phenotypes (phenotype-focused) and those which use the environment to generate a selective landscape without experimenter-imposed selection (phenotype-free). We argue that by watching evolution in action we can reduce the effort required to assay large numbers of recombinants, mitigate the non-randomness of sampling natural variation, study more complex allelic interactions, explore forces maintaining variation, and uncover the cumulative contribution of small-effect loci.

Genetic change accompanying constant selection on phenotypes of interest

Phenotype-focused multigenerational studies attempt to understand the genetic response to selection on a single trait or suite of traits of interest (Figure 1, top left). In the simplest form, strong divergent selection is imposed by the researcher on a genetically and phenotypically diverse founding population [19,20,35]. The resulting change in genetic composition of the population can then be monitored by calculating allele frequencies in pooled or individual sequenced genomes [36]. Alleles favored in replicate populations are then inferred to be targeted by selection. In the absence of a reference genome, differential gene expression can also be used to identify candidate genes, such as for herbicide resistance [37]. Selection can also be applied in two directions simultaneously. The resulting divergent populations then serve as parents in crosses to generate segregating populations that can be genotyped and used in QTL mapping to provide further evidence of a link between phenotypic change and candidate genomic regions [38−40]. Importantly, because phenotype-focused studies often involve applying selection at the same time as a population is exposed to a naive environment (a laboratory or greenhouse for example), appropriate controls are necessary to account for inadvertent selection for "lab adaptation" [41].

Hallauer's Tusón featured selection for maize early female flowering time in a temperate environment from

Figure 1



Examples of each discussed method for associating genotype with adaptation. Common to all depicted methods is the generation of groups between which allele frequency differences can be compared. Top left: Phenotype-focused multigenerational experiments use researcher-imposed selection on a phenotype to generate groups of individuals with divergent phenotypes. Top right: Phenotype-free multigenerational experiments track allele frequency differences between subsequent generations of a population exposed to a (sometimes naïve) environment. Bottom left: Top−down sampling methods use differences in fitness between groups in a set of common environments to associate phenotypes with adaptation to different conditions. Bottom right: Bottom−up sampling methods use allele frequency differences between populations found in varying environments to find putatively adaptive gradients in frequency.

a starting population composed of tropical-adapted landrace accessions (first citation). In 2019, Wisser et al. found that phenotypic change in this population occurred in two stages; early generations revealed changes in few loci of large effect, while change in later generations accompanied changes in allele frequencies at many initially rare loci [36]. This kind of non-constant path to phenotypic change in a novel environment, coupled with the retention of the vast majority of starting genetic diversity, would be difficult to study in the absence of direct comparison of subsequent generations.

Because multigenerational studies directly compare across generations or selective regimes, they can, given sufficient population sizes, exclude drift and demographic history as major drivers of change, as least to a degree sufficient for inferences of selection. This does not mean that drift does not occur in these populations, but rather that the expected rate of change due to drift can be estimated from simulations using known founding genetic diversity and population size. Substantial phenotypic change in the direction of imposed selection can occur rapidly in fewer generations than expected by drift alone [22], suggesting that selection is strong enough to swamp the effects of drift. Additionally, the rate of phenotypic change is often remarkably constant regardless of genetic diversity of starting material, only reaching phenotypic plateaus where further change in phenotype is physiologically constrained [42], such as by approaching a lower limit of detectability on oil content in maize kernels [20]. For decades, artificial selection experiments have demonstrated the efficacy of strong selection in changing a wide range of traits across organisms spanning the tree of life [18,43−45]. This ability for researcher imposed selection to consistently change phenotypes suggests another utility of phenotype-based multigenerational studies, the ability to detect the cumulative effects of rare or small effect loci genome-wide.

However, phenotype-focused studies typically apply artificially strong selection under controlled conditions. If study design is not carefully considered, these characteristics can limit the ability to pinpoint causal variation and inform on the process of adaptation. Strong selection acting on standing variation can lead to substantial hitchhiking [46], which can affect power to map the genes that underlie a trait of interest [47] (also see Box 1). Simulating phenotype-based multigenerational studies should be leveraged to inform experimental design to maximize detection of the genetic basis of adaptive changes [48−50]. Particularly important parameters are the effective population size, effective

Box 1. : Caveats.

Identification of causal variation: Using a multigenerational approach to link genotype to phenotype does not inherently decrease the risk of overestimating the contribution of identified loci to phenotypic variation when sample sizes are small (also known as the Beavis effect, [75]). Nor are multigenerational studies inherently better for capturing the contribution of rare alleles, which is a major challenge in association mapping [76]. But, multigenerational studies are amenable to a level of true replication that is hardly possible using sampling methods. Sampling can, of course, tap the potential of naturally-generated replication across environmental clines [77], but a multigenerational study can track parallel changes across replicated populations adapting to the same environment or responding to the same selection from the same initial genetic diversity. Replicated populations increase the probability of capturing a causal rare variant, and can be used to estimate the bias in effect size (when sample sizes are appropriate) [78]. It should be noted that increased replication means more space, time, and money for genotyping, which still does present a challenge for long-lived or large plants, or plants with large genomes. It is true that this could bias the types of species for which replicated multigenerational studies are possible, but those challenges have always limited the types of plants that become model organisms.

Drift: Random chance can cause large changes in allele frequencies independent of selection, in both natural and experimental populations [79,80]. Throughout this review, we have argued that, given strong enough imposed selection and large enough experiments, drift is unlikely to be the primary driver of genetic change in multigenerational studies. However, for many plants, replicated multigenerational studies with thousands of individuals might not be feasible, especially for outcrossing plants where non-random mating might reduce effective population sizes [81]. Despite these challenges, multigenerational studies still have several features that make them amenable to creative methods for distinguishing drift from selection. For one, the expected change under scenarios with drift alone can be simulated in multigenerational studies where population size and genetic diversity are known. We advocate for performing simulations prior to the initiation of a new multigenerational study if the founding source of genetic diversity is known, to estimate the population size necessary to overcome detectable allele frequency changes by drift alone. Additionally, recent modeling frameworks developed by Buffalo and Coop [82] utilize an advantage of multigenerational studies: selection generates autocorrelation between generations in allele frequency changes, while drift does not. Though this framework was developed to account for linked selection in multigenerational studies, it could be employed to assess whether drift is likely obscuring associations.

A somewhat less obvious consequence of drift in the context of multigenerational studies is that genetic variation with the potential to impact fitness-related traits might be lost and unavailable for selection (although perhaps not to the degree one might expect, see the study by Desbiez-Piat et al.[22]). Conceptually, this could manifest similarly to Muller's ratchet [83], and only a small subset of the variation present at the experiment's start might end up causing phenotypic change as a result of selection. This issue can, in part, be mitigated using the same replication strategy discussed above. In replicated experiments, each version of a causal allele will have a probability of fixing equal to its starting frequency under drift alone [84,85]. The problem with losing rare variation is compounded when drift is strong, so a balance between population size and level of replication will depend on available resources, the system, the question, and the assumed underlying trait architecture.

Linked selection: Variation that is linked to causal loci under selection in a multigenerational experiment can decrease the resolution of association mapping [86]. It can also facilitate identification of larger regions with multiple, linked loci each of small effect (sometimes manifested as fractionating QTL [87]). This is not unique to multigenerational studies. However, multigenerational studies, especially those employing strong selection on a diverse starting population, might be prone to exacerbating this effect inadvertently by establishing population structure in the early generations, especially in predominantly selfing species [88]. If forming a new population from multiple intercrosses, we suggest choosing a group of founder varieties that are not themselves structured (i.e. all have similar genetic distances). Linkage can also be mitigated by making many independent crosses among founding varieties so that recombination might serve to narrow regions of trait association. As above, simulations might be useful for predicting the severity of linked selection.

recombination rate, and founding genetic diversity. Strong, constant selection on a single trait in absence of other environmental variability may also be unrealistic in natural populations. It is important that phenotype-focused approaches be complemented by studies of adaptation in more realistic conditions if we wish to understand the process of plant adaptation to the environment more generally.

Genetic and phenotypic change during adaptation to a new environment

Plant adaptation to a natural or agricultural environment involves innumerable potential selective forces. To untangle how plants adapt in a complex environment, we must complement phenotype-focused studies with experiments conducted under more realistic selective environmental conditions (phenotype-free studies). Using similar methods to those described for phenotype-focused studies, genetic and phenotypic changes of a population in a specific environment can be tracked through time, agnostic of which phenotypes are expected to be involved in adaptation (Figure 1, top right). Importantly, phenotypes of interest (usually fitness-related) can still be measured during the course of these experiments, though they are not themselves consciously selected by experimenters. Change in the environment often occurs either when a representative sample from an existing population is transplanted [51], or when a newly formed population representing species-wide genetic diversity is established in an unfamiliar location [52]. The new environment could refer to a location with new presumed selective forces [53], a new source of biotic interactions [54,55], or an artificial environment that might mirror a future abiotic climate [56]. Adaptive change can be identified by mapping variation across generations to change in phenotypes, or by genome scans for signatures of selection, like divergence between early and late

generations [57]. Because these differences are measured over many generations, even small allelic effects can accumulate over time to generate substantial phenotypic shifts in the population.

The Buxton Climate Change Impacts Laboratory, an example of an artificial environment, was used by Ravenscroft et al. [56] to quantify genetic divergence after 15 years of exposure to combinations of heat and drought. They found evidence for selection and genetic change that can be further investigated to find candidate genes responsible for climate adaptation. Replicating this type of experiment across many artificial environments representing a variety of climate projections could lead to more robust predictions about plant adaptation, and would not be possible by sampling current environments alone.

In plants, a typical phenotype-free experiment is conducted by comparing stored material, like seed, to descendents some time later after a population has evolved [58]. This resurrection study strategy is appealing when the goal is to predict future species ranges using current local adaptation. Resurrection experiments take advantage of the fact that even in the absence of direct modification, the natural environment is constantly changing [59]. More importantly, it is already changing in the direction that it will continue to change, making conservation or crop improvement applications immediately interpretable [60]. Using common gardens to compare multiple populations subjected to similar changes in climate, resurrection experiments can also detect parallel changes in phenotype [61]. As seen in phenotype focused studies, detectable change can even occur in a relatively short time frame; for example, just 3 generations is enough to develop genetic and phenotypic differentiation in common bean [24]. Given enough generations, further recombination may also occur in the experimental population. Even in predominantly selfing species, new recombinants representing additional combinations of genotypes beyond the initially established population will be generated in sufficiently large studies [62].

Direct observation of evolution in a resurrection design has many experimental advantages over the inference based strategies employed using single time point, extant population samples. The most obvious is that the environmental conditions in an evolution experiment can be measured and directly correlated with phenotypic or genetic change, mitigating the influence of unknown historical environmental factors that may have impacted genetic differences between extant populations. If the population is appropriately large, genetic drift can also be excluded as a driver of population evolution (though, see Box 1 for caveats about this assumption).

By watching evolution happen, either concurrently or in retrospect, phenotype-free multigenerational studies have one huge advantage over sampling large numbers of extant populations: we can study strategies for adaptation that failed in nature [63]. Sampling will always involve a nonrandom set of the possible combinations of genetic diversity. Drift, demographic history, and selection limit the types and combinations of alleles that we can evaluate with extant material. By allowing genotypes to fail, multigenerational studies increase possible genetic variance and the range of resultant possible measured phenotypes. By increasing phenotypic variance, we have more power to connect genotype to phenotype, because we sample more of the total fitness landscape accessible only through combinations of genotypes that would be unrealistic in natural populations, such as cytonuclear incompatibilities (see the study by Pereira et al. [64] for an example in a copepod, but the principal is the same for plants). Intercrosses also generate segregants carrying combinations of alleles that do not exist in natural populations suitable for estimating the contribution of multilocus allelic interactions to local adaptation, although this approach is better suited to selfers since such combinations might take a prohibitively long time to fix given high outcrossing rates.

Linear change over time toward a maximum, as with artificial selection (see above), is only part of understanding adaptation. The same location can vary dramatically through time, for example in severity of pathogen pressure or yearly precipitation. As a result, the fitness advantage of particular alleles may also vary. Maintenance of genetic variation by selection, either through spatial or temporal fluctuations in selective pressure or phenotypic tradeoffs, is termed balancing selection [65]. It is critical that we identify the genetic targets of balancing selection, as previously maintained standing genetic variation might serve as a new mutation-free target of directional selection for adaptation to a changing climate [66,67]. Unfortunately, population genetic signatures of balancing selection, such as skewed allele frequency distributions [68], have multiple interpretations, such as demographic history changes, and are suggestive at best. Even if we assume the footprint of balancing selection is authentic at a locus, the specific form of selection (such as frequency-dependent selection, heterozygote advantage, temporally fluctuating selection, etc.) and the selected phenotype are usually unclear (resistance genes are a notable exception, built on an extensive body of research, for example [69]). With multigenerational studies, one can associate fluctuations in the environment with fluctuations in allele frequencies and phenotype to more directly infer long-term adaptive polymorphism [70]. Breeding or conservation efforts can then make informed decisions about where genetic diversity should be maintained, or whether the effects of

climate change can predict favoring fixation of one allele over another.

Conclusions

Identification of genes that underlie adaptation is one of the grand challenges of ecological and evolutionary genetics. However, as pointed out many years ago [9], in the absence of direct observation of the evolutionary process, the role of alleles in adaptation remains putative. In this review, we advocate for combining multigenerational evolution studies with other strategies for mapping genotype to adaptive phenotype in plants, especially sampling-based approaches (see introduction). In addition to the discussed benefits, it is necessary to use varied methods, because they sometimes provide different answers. Vigouroux et al. [71] found selection for an early flowering allele at the PHYC locus in pearl millet by sampling varieties grown by local subsistence and smallholder farming practices in Niger between 1976 and 2003, during which time flowering time was correlated with rainfall. This provides a smaller target for climate change motivated breeding targeted specifically to improvement of yield in this region than the 22 flowering time QTL identified using a more traditional RIL approach in the same species [72]. Additionally, by combining multigenerational evolution experiments with multiple environment trials, it is possible to associate environmental differences with divergence in complex polygenic traits. We can disentangle divergent selection from change that is consistent across environments [73]. Finally, combining multigenerational studies for which environmental data is available with common gardens is necessary to address genetic changes responsible for adaptation to climate change that decouples previously correlated external cues like temperature and day length [74]. A genome sequencing-assisted return to long-term evolution experiments in plants will supplement current sampling-based approaches for identifying the basis of local adaptation, and better predict evolution driven by environmental change, including both biotic and abiotic factors.

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Declaration of competing interest

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