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# Developing maps of fitness consequences for plant genomes

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Predicting the fitness consequences of mutations, and their concomitant impacts on molecular and cellular function as well as organismal phenotypes, is an important challenge in biology that has new relevance in an era when genomic data is readily available. The ability to construct genomewide maps of fitness consequences in plant genomes is a recent development that has profound implications for our ability to predict the fitness effects of mutations and discover functional elements. Here we highlight approaches to building fitness consequence maps to infer regions under selection. We emphasize computational methods applied primarily to the study of human disease that translate physical maps of within-species genome variation into maps of fitness effects of individual natural mutations. Maps of fitness consequences in plants, combined with traditional genetic approaches, could accelerate discovery of functional elements such as regulatory sequences in non-coding DNA and genetic polymorphisms associated with key traits, including agronomically-important traits such as yield and environmental stress responses.

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One of the great challenges in biology is to determine the fitness consequences of individual polymorphisms across the genome. Over the last few years, high-throughput functional genomics and whole genome resequencing have enabled discovery of functional elements in non-coding DNA and comprehensive descriptions of single nucleotide polymorphisms (SNP) and other genetic variants in plant genomes. For example, genome-wide SNPs have been catalogued for many species including *Cicer* (chickpea) [1], *Zea* (maize) [2], *Oryza* (rice) [3], date palms [4], and Chlamydomonas [5], and the 3000 Rice Genomes Project recently reported more than 30 million polymorphisms in 3024 rice varieties [6••]. In the next few years such 'SNP atlases' will become available for many other crops and their wild relatives.

With whole genome sequences now widely available, evolutionary biologists are revisiting the long-standing challenge [7,8] of predicting the fitness effects of mutations. In principle, expanding these predictions to a genomewide scale would allow us to construct maps of fitness variation that describe the probability that a mutation will impact fitness and predict both the magnitude and sign (i.e. beneficial or deleterious) of their effect. In practice, estimating the fitness effect of mutations remains one of the great objectives in molecular evolution [8], but recent advances in diverse evolutionary and experimental approaches have improved the prospects of constructing maps of fitness consequences in plant genomes.

Maps of fitness consequences have potentially widespread applications. From an evolutionary perspective, they provide a basis for predicting whether a mutation is beneficial and improves a fitness-related trait or is deleterious and negatively impacts traits such as crop yield or resistance to disease that are targeted for improvement. From the perspective of molecular biology, maps of fitness consequences provide clues as to which positions in the genome impact a cellular function (Box 1). Since mutations that impact fitness must also affect function, identification of sites that affect fitness may assist with quantifying the fraction of the genome that is functional — a subject of recent controversy in human genetics [9] — and identifying polymorphisms that modify traits of interest [10,11].

Here we review recent advances in methods to construct genomewide maps of fitness variation. Although many of these approaches have primarily been applied to the study of human diseases, we focus on methods that are applicable to plants and highlight how they may profoundly improve efforts to discover functional elements in plant genomes.

## Genome-wide maps of fitness consequences

Regions of the genome that are conserved in evolution represent a special class of sites, where purifying selection

**Box 1 The link between fitness and function.**

Mutations that impact fitness must also impact a cellular function. This observation is the basis for applying principles of molecular evolution to assess the impact of a mutation's function indirectly by predicting its fitness effect. Such fitness effect predictions are made through methods based on population genetics theory that quantify the proportion of sites under selection or the strength of selection acting on collections of sites in the genome (Box 2).

What is the relationship between fitness and function? The fitness-function relationship is the extent to which a change in allele function will lead to a change in fitness. This relationship is often assumed to be linear. However limited experimental data indicate non-linear relationships that vary from locus to locus and depend on genetic background and the environment [65]. Rest *et al.* [66•] quantified the effect of changes in expression in *LCB2* in yeast on fitness and reported an 'S'-shaped, or sigmoidal, relationship. Hartl *et al.* [65] quantified this relationship for activity at β-*galactosidase* and fitness in *E. coli* and found it approximates a saturation curve as it does in other metabolic contexts. The exact nature of the relationship between fitness and function is sure to be complex and will remain unknown except for exceptional study systems.

has preserved a sequence over long periods of evolutionary history. Constraint-based, or conservation-based methods aim to identify these slow evolving sequences and the functional elements they encode using multiple sequence alignments from phylogenetically diverse species. In practice, such 'phylogenetic footprinting' methods assign scores to positions in the genome indicating the degree of conservation across species [12–14] (Box 2) thereby enabling the discovery of elements that, when mutated, are expected to impact fitness. Application of these approaches in plants benefits from 70 published genomes [15], which enable the localization of sequences that have been conserved over different evolutionary time scales and discovery of functional elements restricted to closely-related species (Figure 1).

**Box 2 Evolutionary genetic approaches to mapping fitness effects to the genome**

The development of maps of fitness consequences benefit from a number of complementary approaches.

- Constraint-based methods: These methods primarily use phylogenetic and homology-based inference to identify sites with low rates of substitution across a phylogeny. Sites are assigned scores that are typically interpreted in the context of fitness (e.g. neutral vs. deleterious) without the need to pre-classify sites into groups [12–14].
- SFS methods: A class of methods that use a histogram of allele frequencies, or SFS, to estimate the magnitude of fitness effects in a pre-defined class of sites relative to a neutral class based on allele frequency distributions. In principle, classes of sites subject to selection can be distinguished from those evolving neutrally and estimates of the distribution of fitness effects can be obtained [8].
- Comparative population genomic methods: This class of methods uses intra-specific diversity and between-species divergence data in pre-defined classes of sites to estimate the proportion of sites subject to selection [24••] or the magnitude and sign of the fitness effect [27]. Methods in this class require a neutral class of sites to be contrasted with the site class of interest.
- Effect class methods: These methods predict the impact of mutation on fitness or function by considering properties unique to each effect class. Most methods return a score indicating the likelihood that an individual mutation will impact function [67].

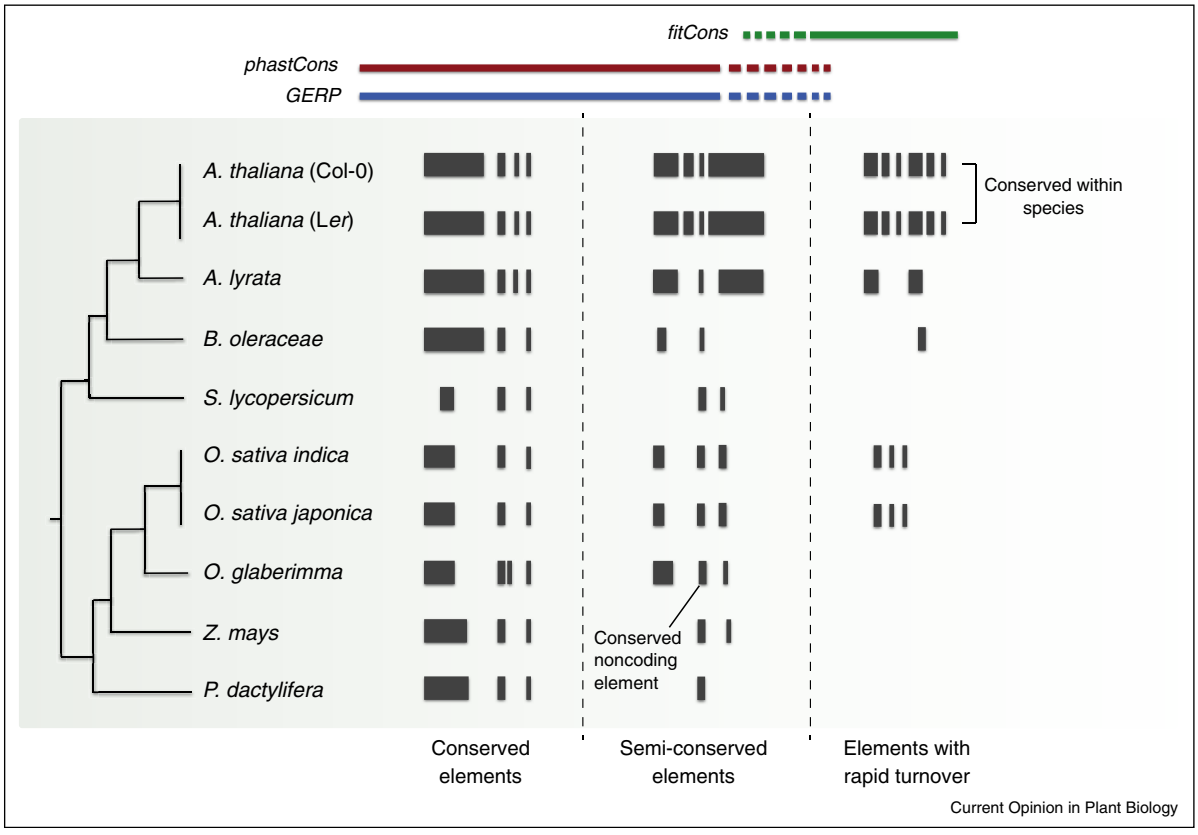
The power of such comparatives approaches is illustrated by a genome-wide, high-resolution atlas of >90 000 conserved noncoding sequences (CNSs) in the Brassicaceae family [16••]. In this study, whole genome sequences from nine closely-related crucifer species and intra-specific diversity data from two species were used to establish that CNSs sequences identified in multi-species alignments are under purifying selection in *Arabidopsis thaliana* and *Capsella grandiflora* populations [16••].

Constraint-based approaches rely on comparisons across multiple species to detect functional elements maintained over million-year timescales [12–14] and can be limited by a number of factors [17•] including low sensitivity to recent changes in constraints associated with either losses or gains in function in protein-coding genes or non-coding DNA. For example, characterizing recently evolved elements is limited by the fact that closely related species sequences are conserved owing to recent common ancestry and distinguishing between conservation owing to recent ancestry or evolutionary constraint is problematic [17•]. In plant genomes, CNSs, such as transcription factor binding sites (TFBSs), experience a more rapid evolutionary turnover compared with animal TFBSs [18••]. Thus, while constraint-based methods are a powerful means to detect elements associated with ancient conserved functions, they are limited in their ability to characterize elements conserved over shorter timescales, like plant TFBSs.

The site frequency spectrum (SFS) is a histogram of allele frequencies, which can be used to infer population demography, identify genomic regions subject to selection, and estimate the distribution of fitness effects of mutations [19]. The shape of the SFS is sensitive to the strength of selection acting on a class (e.g. nonsynonymous) of mutations; purifying selection, for example, shifts the site frequency spectrum towards lower frequencies relative to neutral mutations. SFS methods can thus estimate the distribution of fitness effects (i.e. the fraction of sites subject to different magnitudes of selection, Box 2).

In the context of the study of fitness consequences of genetic polymorphisms, SFS methods have been used to estimate the proportion of new mutations that are neutral, weakly or strongly deleterious from population data [20,21]. In practice, these methods incorporate the observed shape of the SFS (Box 2) for both a neutral and a selected class of mutation and apply numerical methods to estimate the proportion of mutations that are under selection by assuming a distribution of fitness effects and incorporating population parameters such as the mutation

**Figure 1**




Comparison of constraint-based approaches and fitCons to uncover functional elements conserved over different evolutionary time scales in plant genomes. The phylogeny shows representatives of diverged monocot and eudicot species as well as ecotypes for the species *O. sativa* and *A. thaliana*. The grey blocks represent regions of the genome with the following characteristics: (left) conserved syntenic blocks across multiple genomes, (middle) blocks showing stronger synteny between closely related species (*A. thaliana* and *A. lyrata*) but also having smaller elements (e.g. conserved non-coding sequences) that are conserved across more diverged species (semi-conserved elements), and (right) blocks that show intraspecies rather than interspecies conservation (rapid turnover). While constraint-based approaches (e.g. phastCons and GERP) are designed to uncover conserved and semi-conserved elements, fitCons integrates both divergence between relatively closely related species and population genomic data to enable the discovery of semi-conserved elements but also those exhibiting rapid turnover.

rate and effective population size [21]. Although these methods are limited by a strong dependence on prior site class definitions and the relative scarcity of intra-specific polymorphism in strongly selected site classes, they should enable characterization of deleterious mutations in plant genomes [22,23•] and may assist in quantifying the proportion of selected mutations.

Methods that combine population genomics and divergence data represent a powerful means of discovering sites in the genome with fitness consequences. Prominent among these methods is the fitCons method, which estimates the probability of fitness effects of mutations in classes of sites defined by a common function (e.g. a TFBS) by integrating intra-specific polymorphism and between-species divergence data with functional genomic information [24••]. Its foundation is a statistical method called Natural Selection from Interspersed Genomically Coherent Elements (INSIGHT) [25••], which is conceptually similar to population genetics methods that use patterns of polymorphism and divergence to identify departures from neutral expectations [26–29] (Box 2). The contrast between polymorphism and divergence is a powerful approach to inferring recent selection and the INSIGHT approach to pooling dispersed sites enables the discovery of noncoding elements that may have been subject to recent selection [25••]. In this respect, fitCons complements constraint-based methods by identifying functional elements that are recent in origin (Figure 1).

To generate a fitCons map, genomic regions are first partitioned into classes of sites that share similar functional attributes determined across multiple assays (e.g. RNA-seq, DNase-seq, ChIP-seq). To be successful, this will require generating highly informative genomic data types (e.g. non-redundant data sets) with high quality sequencing (e.g. depth, assembly). Sites within a class are assigned a fitCons score that reflects the probability of a

fitness consequence of mutations as inferred by INSIGHT. This approach has the advantage of being annotation-free and facilitates prediction of *cis* regulatory elements and measurement of the global influence of recent natural selection across the genome [24••]. With the increased availability of plant genomic and functional data, we believe that the approach described by Gulko *et al.* [24••] will facilitate assessment of the fitness effects of mutations, estimates of the proportion of plant genomes subject to selection, and discovery of functional elements in plant genomes.

A related approach is the Combined Annotation-Dependent Depletion (CADD) method that integrates information from diverse genome annotations into a single measure (*C* score) to identify putative deleterious, or pathogenic, variants [30•]. The approach relies on mutation-disease association databases such as ClinVar [31] to train a machine learning algorithm to predict fitness consequences. At present, this limits the application of CADD to humans, but highlights the need for development of comparable plant databases [30•,32].

## Characterizing fitness effects by functional effect class

Genomewide approaches to assess fitness consequences are complemented by methods that predict the effects of mutations in specific functional classes. Perhaps most well known in this class of methods are those that evaluate the effect of missense mutations on protein structure with the aim of identifying pathogenic effects (e.g. Sift [33], PolyPhen2 [34], and Provean [35]). Many of these methods make predictions by combining diverse sources of information including sequence conservation across phylogeny, protein structure, gene network topology (e.g. SuSpect [36]), and clinical information on known mutation-disease associations (e.g. GESPA [37]). Such methods may assist in identifying mutations for crop improvement. For example, Shihab *et al.* [38•] implemented the Functional Analysis Through Hidden Markov Models (FATHMM) method to prioritize mutations in starch pathways and storage proteins for improvement in wheat.

Other methods aim to assess fitness effects of mutations in other annotation classes including microRNAs (mrSNP [39]), non-coding RNAs (RNAsnp [40]), and splice sites (SNPlice [41]). Most of these predictive methods can be implemented in plants and it should become possible to pre-compute exhaustively the effects of all possible mutations in each of these effect classes in well-annotated plant genomes [42].

## Mapping the effects of single mutations

Fitness is manifested through specific organismal phenotypes; thus, another way to examine the genetic basis for fitness is via forward genetic approaches whose aim is to associate mutations with traits of interest. Genetic mapping techniques including quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) have been widely applied in plant genomics to map key phenotypes such as fitness-related traits relevant to crop improvement [11].

When coupled with map-based cloning of causal mutations, these methods complement genomewide maps of fitness consequences by linking specific mutations to traits of interest. For example, the seed protective structure called the awn, varies in structure and number among cereal crop species. Long awns are found in the wild rice *Oryza rufipogon*, while domesticated *Oryza sativa* rice have been selected to have short or no awns to facilitate harvesting and storage [43]. Mapping of phenotypes to specific mutations such as awn traits to naturally-occurring alleles in *Awn-1* (*An-1*) and *LONG AND BARBED AWN1* (*LABA1*) genes in rice [44•,45•] greatly enriches fitness consequence maps by both establishing the trait impacted by specific mutations and suggesting a mechanistic basis for the trait.

Characterization of selective sweeps provides another means of further enriching fitness consequence maps through the identification of regions associated with the fixation of adaptive mutations. Such sweep regions can be identified by various approaches, including local reduction of genetic diversity [46], extended haplotype homozygosity, or local skew in SFS [47]. Regions of positive selection associated with local adaptation can also be inferred by local elevation of sequence divergence or reductions in gene flow using Fst outlier, and other methods [48,49]. These techniques have been widely applied in plant evolutionary genomics [49] and are the basis for important conclusions related to crop domestication [50]. In practice, inferring selective sweeps is challenging due to the confounding effects of population demography as is characterization of the adaptive mutation responsible for a selective sweep which may range in size from $\sim 10^3$ to $10^6$ bps [51]. Nevertheless, when coupled with fine-mapping approaches, these methods provide a means of enriching maps of fitness consequences through the discovery of adaptive mutations.

## Conclusion and outlook

As whole genome resequencing and functional genomic datasets proliferate, the ability to distinguish among neutral, deleterious or adaptive variants in the form of fitness consequence maps will have increasing utility for evolutionary geneticists and plant biologists.

While such maps of fitness consequences will be useful, it should be noted that those generated by approaches such as fitCons are probabilistic in nature and predict fitness consequences for large groups of sites. These maps provide hypotheses for sites subject to selection and verifying the impacts of individual mutations of interest will

require experimental validation [24••,52,53]. Fortunately, new experimental approaches such as CRISPR/Cas and deep mutational scanning [54•], offer the possibility of systematically evaluating the impact of site-specific mutations [55]. The CRISPR/Cas system has been applied in multiple plant species including *Arabidopsis* [56•,57,58], rice [56•], wheat [59], maize [60], sorghum [58], tobacco [57,58], and citrus [61]. Other approaches involve developing and improving high-throughput phenotypic facilities to simultaneously monitor multiple traits [62] or mutations [54•].

Another area that will require attention centers on the need for appropriate repositories to visualize large-scale datasets and fitness maps to facilitate the discovery of functional elements. Phytozome [15] has recently expanded the ability to visualize SNP data, VISTA conservation tracks, and other whole genome plant datasets via JBrowse [63], and CoGE [64] allows users to customize their own instances of JBrowse and conduct comparative analysis that should improve the ability to discover conserved elements. Similar resources that facilitate visualization of information relevant to discovery of mutations with fitness consequences will be important for identifying candidate functional polymorphisms.

We envision that maps of fitness consequences can be seen as part of a tool kit designed to discover gene regions or mutations useful to quantitative geneticists, evolutionary plant biologists and crop breeders. For example, genome-wide maps of fitCons scores coupled with the location of putative selective sweeps or large QTL regions could help in fine mapping genomic regions with important alleles, and provide the basis of subsequent functional analyses. Also, future applications for plant genetics include targeting adaptive mutations (i.e. mutations formed in response to an environment in which the mutations were selected) by comparing multiple fitness consequence maps of a given crop variety under different environmental conditions (e.g. drought, high salinity). Linking adaptive mutations to QTL-mapped traits would also help distinguish mutations that have a deleterious effect from mutations that may be locally adaptive under certain conditions. Deleterious mutations could also be targeted and potentially bred out of a population [23•].

Finally, fitness consequence maps not only have applications in inferring function in plant genomes, but can also help in addressing long-standing problems in evolution including estimation of the proportion of the genome that is subject to selection and predicting the fitness consequence of mutations in regions subject to selection.

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