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Regulatory networks of glucosinolates shape *Arabidopsis thaliana* fitness

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Systems biology approaches address higher levels of complex, but dynamic metabolic regulatory networks utilizing single accessions of a species. This contrasts with the likelihood that plants utilize genetic diversity of both individual genes and regulatory networks as a solution to surviving in a complex environment. This would require systems biology to begin a more inclusive search for 'all' networks within a species. In this review, we will highlight how natural genetic diversity within particularly aliphatic glucosinolates in *Arabidopsis thaliana* and related species has resulted in highly complex, dynamic regulatory networks enabling the plant to adapt to a highly changing environment. We will discuss how this diversity is essential for the fitness performance of *A. thaliana*.

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Introduction

The emergence of systems biology approaches has created an awareness of the existence of complex, but dynamic metabolic regulatory networks. However, these models are typically generated using a single accession of a species with the explicit notion of identifying 'the' network. The concept of a single network contrasts with plants being constantly exposed to fluctuating and contrasting environments (drought, flooding, insects, pathogens, UV-B, etc.) that require different solutions to optimize the plant's fitness within that environment. While a plant handles some of this environmental perturbation through the use of complex and intricate regulatory networks, increasing evidence emerges that plants also cope with fluctuating environments through genetic diversity in a bet-hedging strategy [1,2]. This bet-hedging strategy suggests that a species may actually have multiple networks, each specific to a subset of accessions (i.e. specific combinations of alleles at GLS-related loci resulting in a specific GLS profile), each coping with the same stress.

The weedy *Arabidopsis thaliana* species has long been identified as a model genetic system with regards to its genomic and genetic resources. The many natural accessions of *A. thaliana* survive in diverse environmental niches (ranging from the Cape Verde Islands to Siberia), and have been specifically successful in adapting to highly changing environments including the prevalence of plants, pathogens, and insects. Particularly the diverse aliphatic glucosinolates have been recognized for their ability of *A. thaliana* to adapt to fluctuating biotic pest environments, and changes in their profile alter the fitness of *A. thaliana* in the field in response to plant/insect interactions [3,4]. Accordingly, *A. thaliana* glucosinolates are being used as a model system to understand how this genetic diversity can be interconnected with modern systems biology to understand how genetic diversity benefits a species.

Structural and quantitative variations in glucosinolate accumulation among *A. thaliana* accessions are largely under genetic control and have been intensely studied by QTL analyses. This effort has identified a number of genes that produce a modular genetic system controlling the structure of the final bioactive compound. There are three key nodes to the modular system with the first node controlling the side-chain length of the aliphatic glucosinolates and regulated by the *GSL-ELONG* locus in most species (Table 1). Next, genetically diverse side-chain modifications are controlled by the naturally polymorphic enzymes of the *GSL-AOP*, *GSL-OX*, and *GSL-OH* loci (Table 1). Finally, the intact glucosinolate is hydrolyzed to different activation products mainly depending upon specific genes with nitrile formation being driven by the functional allele at the *ESP* locus and isothiocyanates being pushed by the functional *ESM1* locus. Together, these loci combine to make a modular genetic system that has the capacity to create diverse chemical structures with supposedly different biological activities. Recent research is beginning to show that this genetic modularity is essential for the ability of glucosinolate-containing plant species to successfully reproduce in a fluctuating environment. These observations raise the question of how such diverse biosynthetic machineries can be maintained and if the underlying regulatory networks are under similar natural selection such that each glucosinolate profile has a distinct regulatory network.

Table 1

Genetic loci controlling structural and quantitative variation in leaf glucosinolate profile and structural outcome of glucosinolate activation among *Arabidopsis* accessions (GSL, glucosinolate)

Locus	Genes	AGI code	Gene product function
GSL-ELONG	MAM1 MAM2 MAM3	At5g23010 – At5g23020	Methylthioalkylmalate synthases controlling GSL side-chain length
GSL-AOP	AOP2 AOP3	At4g03060 At4g03050	2-Oxoglutarate-dependent dioxygenases converting methylsulfinylalkyl GSLs to alkenyl GSLs (AOP2) or hydroxyalkyl GSL (AOP3)
GSL-OX	$FMO_{GSL-OX1}$ $FMO_{GSL-OX2}$ $FMO_{GSL-OX3}$ $FMO_{GSL-OX4}$	At1g65860 At1g62540 At1g62560 At1g62570	Flavin-monooxygenases converting methylthioalkyl GSLs to methylsulfinylalkyl GSLs
GSL-OH	GSL-OH1	At2g25450	2-Oxoglutarate-dependent dioxygenase converting 3-butenyl GSL to 2-OH-3-butenyl GSL
MYB28	MYB28	At5g61420	MYB transcription factor, positive regulator of aliphatic glucosinolate biosynthesis
MYB2976	MYB29 MYB76	At5g07690 At5g07700	MYB transcription factors, positive regulators of aliphatic glucosinolate biosynthesis
ESP	ESP	At1g54540	Specifier protein, promotes nitrile formation upon GSL activation
ESM1	ESM1	At3g14210	Reduces nitrile formation upon GSL activation

Glucosinolate diversity shapes a rugged fitness surface

Glucosinolates play important roles in plant/biotic interactions, making them important determinants of plant fitness in the field [3,4,5•,6]. The effect of selection in the field is not linear with respect to either glucosinolate content or structure. Instead, selection is curvilinear on both glucosinolate content and structure because of the presence of both specialist insects (adapted to glucosinolates) and generalist insects (nonadapted but coping with glucosinolates) within the environment [4,5•,7,8•]. In addition, the ability of a given glucosinolate to determine fitness is impacted by its ability to attract or deter specific enemies of the primary foliar pests that are Lepidoptera, aphids, molluscs, or leaf miners [9–12]. Furthermore, curvilinear selection pressures because of the fluctuating presence of interspecific and intraspecific plant competition exists for, at least, glucosinolate content [13,14]. Thus, the optimal glucosinolate content and structure is heavily dependent upon the other plant and insect species present within the environment. Given the disturbed and often fluctuating environmental niches that *A. thaliana* and related species typically occupies, the prevalence of both plant and insect species within the environment will often be highly variable from year to year. As such, balancing selection likely generates a diversity of distinct glucosinolate profiles within *A. thaliana* and relatives optimized for a given environment.

The underlying mechanism behind the ability of *A. thaliana* and relatives to generate distinct and genetically diverse glucosinolate profiles within their foliar tissue is the presence of a complex epistatic interaction network of the main glucosinolate QTL. This epistatic network has a modular architecture such that accessions can quickly shift between glucosinolate structures either via mutation or out-crossing (Table 1 and Figure 1). While all of these genotypic combinations are viable in the laboratory, some combinations are more prevalent than expected while other genotypic combinations are less prevalent than expected in the wild (Figure 2). This is in contrast to the neutral model in which all genotypic combinations would occur at the same frequencies. Thus, glucosinolates and the associated genes within *A. thaliana* identify a fitness surface, which topologically has pronounced peaks and valleys.

Although glucosinolate content can be regulated within each accession, there is no evidence of the ability to regulate large shifts in the predominant glucosinolate structure within *A. thaliana* foliar tissue such that the plant could move from one peak of the fitness surface to another peak within the leaf via regulation (Figure 2). This is likely because each of the genetic polymorphisms controlling side-chain modifications, that is *GSL-ELONG*, *GSL-AOP*, *GSL-OH*, *GSL-ESP*, and *GSL-ESM1*, are exceptional in the sense that they primarily involve the presence of knockout polymorphisms such as nonsense mutations, gene inversions, and promoter deletions, rather than changes in promoter expression. In consequence, this removes the ability to switch on and off these genes in those accessions [15,16,17••,18,19].

Glucosinolate profiles in wild *A. thaliana* accessions do not display intermediate phenotypes, but highly separated bimodal distributions such as either having glucosinolates with side-chain lengths that are all C3 or all C4. In the laboratory, however, it is possible to generate intermediate biochemical phenotypes via transgenic lines or heterozygotes between accessions, but these appear to be