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Integrative genetical genomics in *Arabidopsis*

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An integrative genetical genomics study in *Arabidopsis* reports that six QTL hot spots have system-wide effects on a wide range of molecular and phenotypic traits, providing empirical evidence for phenotypic buffering.

The phenomenon of genetic buffering was initially described by Waddington in 1942 (ref. 1). One of the key examples of genetic buffering is that of Hsp90, a molecular chaperone that secures the proper functioning of many different developmental signaling pathways in *Drosophila* and *Arabidopsis*. In the presence of Hsp90, much of the genetic variation remains hidden, but upon impairment of Hsp90 function, novel and discrete phenotypic variants appear[2]. This evolutionarily conserved buffering system may allow organisms to accumulate mutations without negative impacts on fitness, and increase their chances for evolutionary adaptation in conditions when the genetic variation is expressed[3].

Ritsert Jansen and colleagues[4], on page 166 of this issue, provide the first system-wide evidence for phenotypic buffering in *Arabidopsis*. Using a genetical genomics approach, the authors profiled 162 L*er* × Cvi recombinant inbred lines (RIL) of *Arabidopsis thaliana* for variation in transcript, protein and metabolite abundance, and mapped quantitative trait loci (QTL) for 40,580 of these molecular traits. The data were integrated with QTL for a total of 139 publicly available phenotypic data collected for the same RIL population over many years by various research laboratories (**Table 1**). They found that only six QTL hot spots underlie variation in 16% of the transcript traits, 25% of the protein traits, 55% of the metabolite traits and 77% of the phenotypic traits for which QTL could be mapped. Although the parental lines L*er* and Cvi differ by more than 500,000 SNPs, it is notable to find that a large proportion of the variation in such a wide range of molecular and morphological traits is explained by these six QTLs, and this finding suggests that phenotypic buffering may be a mechanism of robustness to molecular variation in this system.

Table 1 Integrative analysis of *Arabidopsis* QTL

Mapping population	Traits	Method	Traits with one or more of six QTL hot spots (%)	Hierarchy of buffering
Arabidopsis RIL $n = 162$	24,065 transcripts	Microarray	16	
	2,843 proteins	2D-PAGE	25	
	13,672 metabolites	GC-TOF-MS; LC-QTOF-MS; ^{1}H-NMR	55	
	139 phenotypes	Biomass, morphology, etc.	77	

Taking an integrative genomics approach, Fu *et al.*[4] characterize a wide range of molecular (transcript, protein and metabolite abundance) and phenotypic traits using the noted methods. The percentage of traits with QTL that mapped to at least one of the six QTL hot spots is given.

Robustness and pleiotropy

As predicted previously[5], and as recently substantiated with experimental data in yeast[6], robustness to perturbations is an inherent property of biological networks[7]. Biological networks are characterized by a small number of highly connected nodes, called hubs. On a cellular level, a hub represents a transcript, protein or metabolite that either interacts or is correlated with a high number of other transcripts, proteins or metabolites. In the study of Fu *et al.*[4], correlations between transcript, metabolite and protein levels are evident, as they are mainly controlled by the same six QTL hot spots. Whether such QTL hot spots correspond to network hubs at some level needs further investigation, and a possible role of these hot spots in evolutionary adaptation should also be considered.

Fu *et al.*'s observation that QTL for 77% of all phenotypic traits with QTL map to six hot spots has important general implications. It suggests that morphological screens for mutants may be biased toward a limited number of loci with pleiotropic effects. In addition, the Fu *et al.* study[4] demonstrates that apparently unrelated phenotypes may often have some shared genetic basis, a concept that also emerges from human disease studies[8–10].

Furthermore, Fu *et al.*[4] find that these six QTL hot spots influence less of the molecular traits with QTL, which may suggest lower levels of buffering at the molecular level. QTL for 16%, 25%, 55% of all transcript, protein and metabolite traits with a QTL, respectively, map to the same six QTL hot spots, compared to 77% of phenotypic traits (**Table 1**). Consequently, screening for mutants at the molecular level will increase the probability of identifying new causal loci that could not be identified from morphological screens. One such example in *Arabidopsis* was given by a study showing that mutations in the gene encoding phenylalanine ammonia lyase (PAL), the entry point in phenylpropanoid biosynthesis, evoke far-reaching effects at the transcript and metabolite level, yet do not cause morphological abnormalities[11].

Resolving causal variation

Fu *et al.*[4] provide an ideal demonstration of how taking an integrative genetical genomics approach, in which transcriptomic,

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metabolomic and proteomic data are analyzed in a concerted way with morphological data, can bring significant insight into the genetics of complex traits. However, Fu *et al.*'s approach of QTL mapping in RIL segregating populations comes with several limitations. First, the mapping resolution remains limited, and hot spots may contain a number of candidate genes. Association mapping is a complementary approach with higher precision in identifying causal genetic variation underlying QTL hot spots. Second, the amount of segregating genetic variation within a RIL population is limited. Although in Fu *et al.*[4] the two parental lines are rather diverse, they may lack polymorphisms for a number of other hot spots segregating in the natural range of *Arabidopsis thaliana* accessions, resulting in an underestimate of the number of crucial nodes in the molecular networks underlying complex traits. Further studies in larger numbers of accessions would be useful, as would be studies of these accessions in multiple selected environments.

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