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NEWS AND VIEWS

PERSPECTIVE

A new approach to quantify the adaptive potential of gene expression variation in gymnosperms

SÉBASTIEN RENAUT

Biodiversity Research Centre and Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

Variation in patterns of gene expression contributes to phenotypic diversity and can ultimately predict adaptive responses. However, in many cases, the consequences of regulatory mutations on patterns of gene expression and ultimately phenotypic differences remain elusive. A standard way to study the genetic architecture of expression variation in model systems has been to map gene expression variation to genetic loci (Fig. 1a). At the same time, in many nonmodel species, especially for long-lived organisms, controlled crosses are not feasible. If we are to expand our understanding of the role of regulatory mutations on phenotypes, we need to develop new methodologies to study species under ecologically relevant conditions. In this issue of *Molecular Ecology*, Verta *et al.* (2013) present a new approach to analyse gene expression variation and regulatory networks in gymnosperms (Fig. 1b). They capitalized on the fact that gymnosperm seeds contain an energy storage tissue (the megagametophyte) that is directly derived from a single haploid cell (the megaspore). The authors identified over 800 genes for which expression segregated in this maternally inherited haploid tissue. Based on the observed segregation patterns, these genes (Mendelian Expression Traits) are most probably controlled by biallelic variants at a single locus. Most of these genes also belonged to different regulatory networks, except for one large group of 180 genes under the control of a putative *trans*-acting factor. In addition, the approach developed here may also help to uncover the effect of rare recessive mutations, which usually remain hidden in a heterozygous state in diploid individuals. The appeal of the work by Verta *et al.* (2013) to study gene expression variation is in its simplicity, which circumvents several of the hurdles behind traditional expression quantitative trait locus (eQTL) studies, and could potentially be applied to a large number of species.

Keywords: eQTL, gene duplication, gene network, microarray, *Picea*, RNAseq, transcriptomics

Correspondence: Sébastien Renaut, Fax: 1 604 822 6089;
E-mail: srenaut@interchange.ubc.ca

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Gene expression regulation

Nearly 40 years ago, King & Wilson (1975) wrote a seminal paper in which they argued that mutations affecting patterns of gene expression may be more important than changes at the protein coding level in explaining adaptive phenotypic differences. While the idea that divergent selection acts predominantly on patterns of gene regulation as individuals adapt to new environmental conditions has grown in popularity since these early days, it, nevertheless, remains controversial (Hoekstra & Coyne 2006; Carroll 2008). As such, a major goal of molecular evolution should be to identify the relative fraction of genetic variants responsible for expression differences and ultimately how they affect phenotypes in wild populations. Do large groups of genes tend to be controlled by a single genetic factor with widespread pleiotropic effects (such as transcription factors) or do patterns of gene expression tend to be under the influence of nearby regulatory regions, each independent from one another (*cis* regulation)? What fraction of individual gene expression variation is heritable? Do certain categories of genes tend to show more expression variation? Thanks to the development of genomic technologies such as microarrays and more recently RNA sequencing (Romero *et al.* 2012), researchers have started shedding some light on those matters. However, the inherent complexities of biological systems have meant that they have almost entirely been explored in laboratory model systems (Fay & Wittkopp 2006).

White spruce

Most trees are long-lived species with large and complex genomes that are expensive to sequence and difficult to assemble. White spruce (*Picea glauca*) is no exception: it can live several 100 years and has a highly repetitive and very large genome (~20 GB, Rigault *et al.* 2011). It is also an abundant species of the boreal forest that supports a large timber industry in North America. As the effects of global warming are predicted to disproportionally affect the Northern latitudes (Pachauri & Reisinger 2007), tree species will need to adapt to new environmental conditions or face local extinction. Therefore, there is an urgent need to better characterize the adaptive potential of species in the boreal forest (AdapTree 2012).

Mendelian expression traits

Here, the authors studied how patterns of gene expression segregate in wild spruce trees. In gymnosperms, the seed

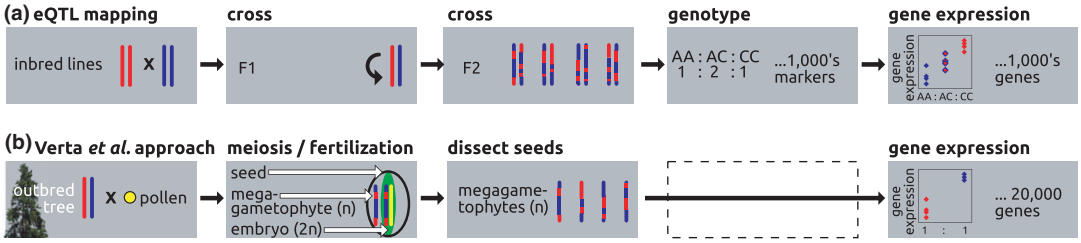

Fig. 1 (a) Simplified overview of the traditional eQTL approach. Two inbred lines are crossed, genotyped and gene expression measured using high-throughput methods (microarray, RNAseq). The novel approach (b) developed by Verta *et al.* (2013) does not require controlled crosses and can be applied directly to field-collected individuals. In addition, it does not involve a time- and resource-consuming genotyping step (the 'dashed box' step in b). Red and blue bars represent homologous chromosomes. Yellow chromosome in (b) comes from pollen cell.

stores energy in a haploid mother tissue, the megagametophyte. By dissecting megagametophytes coming from a single mother tree, we can study how heterozygous alleles controlling patterns of gene expression segregate in the haploid tissue. As such, they quantified gene expression from 18 different haploid megagametophytes in two distinct replicate trees (see simplified schematic of their experimental approach in Fig. 1b).

Out of the nearly 20,000 genes they assessed using a spruce-specific microarray, they identified 800 genes segregating in a 1:1 ratio (the *Mendelian Expression Traits* or *METs*). This, in turn, signifies that for each of these MET, gene expression values were divided nearly evenly into two distinct clusters. The authors then argue that this simple segregation pattern implies that it is governed by a single genetic factor with two alleles in the mother tree. This is a reasonable assumption, but invokes the question as to what are the genetic factors controlling those genes exactly. Are they part of a regulatory region in physical proximity to the gene itself (or a tightly linked group of genes, *cis* regulation) or something like a transcription factor acting distally on one or many genes (*trans* regulation)? This question could potentially be answered in the future by genotyping those same individuals using a large panel of genetic markers in a way more akin to the traditional eQTL approach (the 'dashed box' step in Fig. 1b). Although not without its difficulties, one could even combine both the genotyping and gene expression quantification steps through RNA sequencing (see for example: Li *et al.* 2013).

Patterns of co-segregation

The authors then go on to look at patterns of co-segregation. Do METs show similar patterns of expression in the same individuals, thus implying that their expression is governed by one/a few genes? This question has important evolutionary consequences: if expression variation is due to a few genetic variants with wide-ranging effects, there are probably a small number of independent evolutionary paths that can be taken as wild individuals adapt to changing environmental conditions. The authors find a single cluster of 180 genes, which is therefore probably governed by one *trans*-acting factor. On the other hand, most genes form small regulatory clusters, and therefore, expression variation is probably controlled by independent mutations in each of those clusters. Consequently, distinct, yet adaptively similar evolutionary trajectories could be taken during an adaptive event. In future experiments performed using different environmental conditions, tissue types and ploidy levels, one will also be able to ask how robust these regulatory networks are to perturbations (Landry *et al.* 2006, 2007; Kitano 2007).

Finally, the authors also point out that the genes exhibiting segregating expression variation were more likely to have a duplicated (paralogous) gene pair compared with the predicted genome wide number of duplicated genes. In addition, this paralogous copy did not usually show any signs segregating expression variation. Taken together, these intriguing results highlight the role of gene duplication as a prominent source of evolutionary novelty. As one gene copy fills its ancestral function, relaxed selection on the 'extra' copy leads to greater gene expression variance, which can then serve as fuel for natural selection (Adams & Wendel 2005).

The road ahead

The approach developed by Verta *et al.* to study the segregation of gene expression variation is simple, yet elegant. In addition, it could in theory be applicable to a large number of species that have a multicellular haploid life stage such as gymnosperms, but also ferns, bryophytes or even haplodiploid insects (e.g. most bees and wasps). The field of ecological and evolutionary genomics is rapidly changing. While these are exciting times, we must also remain realistic: many of the questions aforementioned are incredibly complex, even in model systems. Nevertheless, we can go beyond simply repeating what has been performed in model systems and develop new methodological approaches, new ecological annotations of genes and better databases to keep track of the wealth of information being generated (Pavey *et al.* 2012). The study by Verta *et al.* is a step in the right direction; it addresses the fundamental aspect of gene expression variation in wild populations and in the future may link this to both genetic and ultimately phenotypic variation.

References

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology*, **8**, 135–141.

AdapTree (2012) *Assessing the Adaptive Portfolio of Reforestation Stocks for Future Climates*. University of British Columbia, Vancouver, Canada. http://adaptree.sites.olt.ubc.ca/

Carroll S (2008) Evo-Devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell*, **134**, 25–36.

Fay J, Wittkopp P (2006) Evaluating the role of natural selection in the evolution of gene regulation. *Heredity*, **100**, 191–199.

Hoekstra H, Coyne J (2006) The locus of evolution: Evo Devo and the genetics of adaptation. *Evolution*, **61**, 995–1016.

King MC, Wilson AC (1975) Evolution at two levels in humans and chimpanzees. *Science*, **188**, 107–116.

Kitano H (2007) Towards a theory of biological robustness. *Molecular Systems Biology*, **3**, 137.

Landry CR, Oh J, Hartl DL, Cavalieri D (2006) Genome-wide scan reveals that genetic variation for transcriptional plasticity in yeast is biased towards multi-copy and dispensable genes. *Gene*, **366**, 343–351.

Landry C, Lemos B, Rifkin S, Dickinson W, Hartl DL (2007) Genetic properties influencing the evolvability of gene expression. *Science*, **317**, 118–121.

Li L, Petsch K, Shimizu R *et al*. (2013) Mendelian and non-mendelian regulation of gene expression in maize. *PLoS Genetics*, **9**, e1003202.

Pachauri RK, Reisinger A (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Intergovernmental Panel on Climate Change*.

Pavey SA, Bernatchez L, Aubin-Horth N, Landry C (2012) What is needed for next-generation ecological and evolutionary genomics? *Trends in Ecology and Evolution*, **27**, 673–678.

Rigault P, Boyle B, Lepage P *et al*. (2011) A white spruce gene catalog for conifer genome analyses. *Plant Physiology*, **157**, 14–28.

Romero IG, Ruvinsky I, Gilad Y (2012) Comparative studies of gene expression and the evolution of gene regulation. *Nature Reviews Genetics*, **13**, 505–516.

Verta JP, Landry CR, Mackay J (2013) Are long-lived trees poised for evolutionary change? Single locus effects in the evolution of gene expression networks in spruce. *Molecular Ecology*, **22**, 2369–2379.

S.R.'s research focuses on ecological adaptation, genetics and speciation.

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