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Tree immunity: growing old without antibodies

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Perennial plants need to cope with changing environments and pathogens over their lifespan. Infections are compartmentalised by localised physiological responses, and multiple apical meristems enable repair and regrowth, but genes are another crucial component in the perception and response to pathogens. In this opinion article we suggest that the mechanism for dynamic pathogen-specific recognition in long-lived plants could be explained by extending our current understanding of plant defence genes. We propose that, in addition to physiological responses, tree defence uses a three-pronged genomic approach involving: (i) gene numbers, (ii) genomic architecture, and (iii) mutation loads accumulated over long lifespans.

A changing pathogen environment

To survive and be successful all life forms need to defend themselves against invading pathogens. Plants are sessile and need to respond to pathogens *in situ* – which would appear to be a particular problem for long-lived trees. Trees provide food, fibre, and biofuels, and are an essential part of our environment. However, as long-lived perennials, trees are exposed to rapidly evolving pathogens, and a static set of defence genes in a host genome of an individual tree is likely to be overcome by changing pathogen populations. A tree hundreds of years old will have faced very different pathogen genomes at different stages of its life [1–3]. How then do trees maintain their vigour in the face of sustained and changing pathogen attack over time? How does a tree that lives for decades or centuries respond effectively to changing microbe populations?

Tree physiology provides some answers. The capacity to isolate diseased tissue into woody compartments [4], and to dispense with leaves or roots via induced abscission, minimises infection spread [5]. These processes are coupled with multiple meristematic (plant stem cell) zones that allow repair and new growth to compensate for any sections excised due to infection [6]. Systemic acquired resistance (SAR), involving salicylic acid-based priming of plant defences, provides a further level of protection [7]. Defence genes, however, are major contributors to successful long life, and plants retain large numbers of diverse resistance genes (*R*-genes) (Box 1) as well as other defence response genes. Here we propose that tree defence, over a long lifespan, is based on a three-pronged genomic approach to counter pathogen variation over time: (i) gene numbers and diversity, (ii) genomic architecture, and (iii) mutation loads due to lifespan.

Many and varied defence genes

Plant resistance genes (*R*-genes) are important for specific recognition of pathogens and are present in large numbers in plant genomes [8] (Table 1) They are known to undergo diversifying selection, thereby providing the flexibility to respond to rapidly evolving pathogens for the next plant generation [9,10] (Box 2). In fact, diversifying selection has been identified in several plant defence gene families including *R*-genes, *guardees, apoplastic proteases*, and *chitinases* [11–13] (Box 2). For short-lived species and seedling progeny of long-lived plants, diversifying selection provides an opportunity to adapt to variation within and between pathogens. Trees are inheritors of a large and diverse array of defence genes that arise due to genetic recombination and diversifying selection.

Annotated sequences from woody plants provide recent quantitative evidence of defence gene numbers, and indicate that long-lived trees (apple, cocoa, grape, poplar, and rubber) maintain proportionately larger numbers of the nucleotide-binding site leucine-rich repeat (NBS-LRR) class of *R*-genes than do the short-lived plants *Arabidopsis thaliana*, papaya, sorghum, castor oil plant, tomato, common bean, or maize (Table 1) [14–20]. It is suggested that a higher frequency of resistance genes in trees may provide better defence capacity [14,17]. Perennials face ongoing pathogen challenges, perhaps reflected in their accumulation of *R*-gene sequences. There are however exceptions: rice for example, has proportionately larger numbers of *R*-genes than other short-lived plants, potentially as a result of intensive artificial selection [18,21]. Predicted *R*-gene numbers are largely derived from data-mining of newly sequenced plant genomes using homologous sequences. The estimates may therefore not accurately represent true biological gene numbers and should be interpreted with caution. Indeed the numbers of *R*-genes for short-lived and long-lived specimens presented in Table 1, although interesting, are perhaps less important than the fact that all plants maintain such a high frequency of these defence

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Box 1. *R*-genes: an important class of defence genes

Resistance genes (*R*-genes) are a major class of defence genes involved in plant responses to pathogens [8]. Pathogens deliver effector molecules that attenuate host defence responses. R-genes encode proteins, predominantly containing nucleotide binding site and leucine-rich repeat (NBS-LRR) domains, that specifically recognise microbial effector molecules or effector-modified host proteins [50]. Recognition initiates effector-triggered immunity (ETI) that blocks pathogen spread. ETI can involve programmed cell death, antimicrobial accumulation, upregulation of pathogenesis-related proteins and systemic acquired resistance (SAR), whereby a whole plant broad defence-response is elicited through previous localised pathogen exposure [51,52].

Box 2. Diversifying selection

Diversifying selection is tested by looking at rates of non-synonymous (Ka) versus synonymous (Ks) nucleotide substitutions. Non-synonymous substitutions, where an amino acid is substituted, can reduce or remove function, and are therefore presumed to be biologically detrimental. The Ka:Ks ratio is therefore expected to be less than one. A ratio greater than one indicates an evolutionary advantage to non-synonymous mutation and is termed diversifying selection [10]. Several studies have found evidence of diversifying selection in regions of *R*-genes [9,10]. Other defence response genes are also identified as undergoing this mode of selection, including *chitinases* [13,55], *apoplastic proteases* [12], and *guardee molecules* that interact with effectors initiating *R*-gene response [9,11].

genes. Around 1% of all protein coding genes in long-lived woody trees are NBS-LRR *R*-genes.

Studies of relatively long-lived invertebrates, such as sea urchins and snails, indicate that having a large number of diverse pathogen recognition genes provides an effective mechanism for defence [22,23]. Further immune-response diversity in these invertebrates appears to be generated by alternative gene splicing as well as post-translational modifications. Many plant gene transcripts undergo alternative splicing. Sixty-one percent of *A. thaliana* transcripts are known to be alternatively spliced [24]. Little is known of alternative splicing of genes in trees, but analysis of recently sequenced tree genomes (Phytozome; http://www.phytozome.net/) suggest that transcripts from coding genes in *Eucalyptus grandis* (28%), apple (16%), and poplar (77%) undergo alternative splicing. A mechanism for diversity therefore exists at the transcript level, with further potential in post-translational modification of defence gene products, as suggested for the invertebrate sea urchins [22].

Table 1. Predicted NBS-LRR resistance gene numbers in long-lived woody plants and short-lived herbaceous plants

Plants	Predicted NBS-LRR resistance gene numbers	Percentage of predicted protein-coding loci	Refs
Woody plants (long-lived)			
Hevea brasiliensis Rubber tree	618	0.9%	[16]
Malus X domestica Apple	992	1.7%	[15]
Populus trichocarpa Poplar	402	1.0%	[14,17]
Theobroma cacao Cocoa	297	1.0%	[53]
Vitis vinifera Grape	305	1.2%	[19]
Herbaceous plants (short-lived)			
Arabidopsis thaliana Thale cress	178	0.7%	[17]
Carica papaya Papaya	54	0.2%	[16]
Oryza sativa Rice	535	1.3%	[17]
Phaseolus vulgaris Common bean	125	0.5%	[18]
Ricinus communis Castor oil plant	121	0.4%	[20]
Solanum lycopersicum Tomato	266	0.8%	[19]
Sorghum bicolor Sorghum	211	0.6%	[54]
Zea mays Maize	129	0.4%	[15]

In addition, when gene numbers, alternative splicing, and post-translational modification are all combined with diversity afforded through molecular complexing of proteins encoded by different *R*-genes, defence capacity multiplies. Studies of pathogen-challenged rice and tomatoes have identified the pairing of proteins for pathogen blocking [25], as well as modulating of host responses depending on the number and composition of complexed *R*-gene encoded proteins [26].

The Human ENCODE project highlighted the inadequacy of a strict interpretation of Crick's 'one gene–one protein' central dogma of molecular biology [27]. The numbers and variety of transcripts, including alternatively spliced mRNA, other RNA products, and transposable elements (TEs) (Box 3), indicate that the genomic sequence of an organism is simply a starting point for determining its gene product repertoire [27]. It would seem then that the multiplying effect of gene numbers, diversity, splicing variants, and post-translational modifications, in response to pathogens, is likely to contribute to an effective and adaptable defence mechanism during the lifetime of a tree.

Genomic architecture – gene clustering and TEs

The tight clustering of defence-response genes, and *R*-genes in particular, is a frequently observed phenomenon in plant genomes [28]. Tandem duplication of genes, gene conversions, and unequal crossing-over during DNA recombination may account for some of this clustering, but the phenomenon also extends to genes that simply share functional as opposed to sequence attributes [29]. Clustering allows cotranscribed gene expression and is sometimes associated with genes whose products operate within a metabolic pathway [30], as well as in defence [31].

Box 3. Transposable elements (TEs)

TEs are regions of DNA that are mobile within the genome. They have the ability to excise and insert themselves in different genomic regions through direct (cut and paste) or RNA-mediated mechanisms. They were first proposed by Barbara McClintock in the 1940s as an explanation for reversible pigment changes in corn kernels, and were termed 'jumping genes' [56]. Large regions of eukaryotic DNA in plants are made up of TEs that are often methylated to reduce transcription [37].