Mini-Review

The fate of gene duplicates in the genomes of fungal pathogens

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Key words: duplication, gene family, expression, evolution, cutinase, gene gain, neofunctionalization, subfunctionalization, redundancy, magnaporthe, pezizomycotina, ascomycota

Understanding how molecular changes underlie phenotypic variation within and between species is one of the main goals of evolutionary biology and comparative genetics. The recent proliferation of sequenced fungal genomes offers a unique opportunity to start elucidating the extreme phenotypic diversity in the Kingdom Fungi.1-4 We attempted to investigate the contribution of gene families to the evolutionary forces shaping the diversity of pathogenic lifestyles among the fungi.5 We studied a family of secreted enzymes which is present and expanded in all genomes of fungal pathogens sequenced to date and absent from the genomes of true yeasts.3,4 This family of cutinases6 predates the division between the two major fungal phyla, Ascomycota and Basidiomycota.5 We discuss our molecular phylogenetic analyses, the number and sequence diversity, and gene gains and losses of cutinase family members between five Ascomycetes: the phytopathogens Magnaporthe oryzae, Fusarium graminearum and Botrytis cinerea; and the model organisms Neurospora crassa and Aspergillus nidulans.5 The functional characterization of three members of the M. oryzae cutinase family,6-10 coupled with the regulatory subfunctionalization and neofunctionalization of most gene pairs5 provide the first justification for the retention of paralogs after duplication and for gene redundancy in the genomes of fungal pathogens.

The fungus M. oryzae (previously known as M. grisea11) causes the destructive rice blast disease and was the first plant pathogenic fungus to have its genome sequenced.12 Its thoroughly studied developmental biology is currently helping to advance our knowledge of fungal-plant interactions (reviewed in ref. 13). The M. oryzae cutinase family comprises 14–17 genes encoding putative serine esterases that breakdown cutin in the cuticle,6,14 the outermost layer coating all aerial parts of plants.15 Three members of this family have been functionally characterized: CUT1 and putative cutinase MGG_02393.5 are dispensable for pathogenicity,7,9,10 whilst CUT2 is required for host surface sensing, normal formation of the infection-structure and complete virulence.6,8 The available functional data and the over-expansion of the cutinase family in the genomes of fungal pathogens render it a suitable multigene family to study the evolution of functional divergence of duplicates in fungal pathogens.

The cutinase family is divided in two ancient subfamilies that predate the split between Ascomycota and Basidiomycota, which occurred nearly 1000 million years ago.16 Furthermore, the cutinase families of all sequenced fungal genomes display extreme coding-sequence divergence, much more so than the significantly larger fungal families of P450 monoxygenases.17 This could reflect the diversity of roles attributed to cutinases, ranging from host-signal perception and transduction to fungal penetration of the host and subsequent carbon acquisition (reviewed in refs. 6, 8 and 15). Such roles could have contributed to fungal adaptation to diverse ecological niches. In fact, a clade of fungal cutinases shows high homology to the acetylxylan esterases, responsible for the degradation of xylan, not the primary substrate of cutinases. Curiously, this presumed functional diversification probably arose before the speciation of B. cinerea, F. graminearum and N. crassa, which occurred at least 230 million years ago.18

Factors Affecting the Gene Family Size and the Rate of Gene Gains and Losses

Our findings support a general trend, whereby the number of family gene-members increases, when the size and repetitive content of the fungal genome increase. In contrast, the numbers of gene family members decrease as repeat-induced point mutation intensifies (RIP is a defense mechanism in Pezizomycotina that removes duplicate genes). Indeed, N. crassa has the lowest genomic ORF redundancy (including cutinases) among Pezizomycotina, due to its highly effective RIP activity, compared with M. oryzae and Aspergillus species.19,20 Our findings are in line with genome-wide studies.1,3 However, the inventory of cutinase genes, also depends on the requirements of each fungal species to successfully complete its lifecycle. Therefore, M. oryzae, B. cinerea and F. graminearum have a great arsenal of cutinase genes due to: their parasitic lifestyle; exposure to cutin from a plethora of host plant species; and their over wintering as saprotrophic mycelia in the soil, degrading different plant debris.12,21,22

The patterns of cutinase gene acquisition compared with gene losses follow a similar trend, with N. crassa having acquired the fewest genes, as seen in the family of P450 genes.17 In direct contrast, M. grisea experienced lineage-specific duplications, coupled with retention of gene duplicates over the millennia, with the implication that...
these ancient duplicates confer a fitness advantage. Such a high gain to loss ratio in *M. oryzae* may be attributed to the positioning of a significant number of cutinase gene family members in close proximity to transposable elements and subtelomeres. The average ratio of gene gain to gene loss in the other three fungal species studied was 2:3. A smaller number of cutinase gene acquisitions than losses in *A. nidulans* could underlie a gradual specificity evolving (over large evolutionary times), until the species adapted to its particular ecological niche. In *Fusarium graminearum* and *Botrytis cinerea*, such a ratio could reflect a slow transition from a strictly saprotrophic to a more parasitic nutritional mode.

**Regulatory Subfunctionalization and Neofunctionalization Contributing to the Maintenance of Gene Duplicates**

Gene duplication is a major force in evolution and is thought to represent the main origin of functional novelty. In yeast, functional innovation is not correlated with protein-sequence divergence, but it is associated with regulatory divergence, as is most often the case for Ascomycetes. Therefore, examination of the in vivo regulatory profiles of the *M. oryzae* cutinase gene members informed us of different, and somewhat unexpected aspects of plant pathogenesis that evolved within the same family (e.g., cluster of cutinases upregulated in planta, in the apparent absence of cutin). Whilst there is a possibility for nonfunctionalization of a cutinase gene pair, most cutinase paralogs show asymmetric regulatory profiles. This indicates regulatory neofunctionalization, where one of the genes retained the ancestral function whilst the other copy evolved to a diverged expression pattern, thereby facilitating the acquisition of a novel function. Such role is yet to be experimentally identified for these cutinase gene sets.

On three occasions, however, the regulatory profiles of taxonomically neighboring cutinases are largely conserved. These gene duplicates are thus predicted to undergo regulatory subfunctionalization, or subtle neofunctionalization, as in two cases there is a significant change of expression level between the two paralogs. It is also possible that some of these duplicates have experienced rapid subfunctionalization followed by prolonged neofunctionalization. These gene pairs may have also been retained where increased quantities of protein product have an advantageous effect. There are numerous examples of regulatory subfunctionalization and neofunctionalization in yeast, plants and animals, but our observations are the first in filamentous fungi.

Furthermore, the expression pattern divergence of the cutinase paralogs was not supported by detectable positive selection driving change within their coding sequences. Therefore, regulatory subfunctionalization and/or neofunctionalization account mainly for the stable preservation of such a large number of cutinase paralogs in *M. oryzae*. Functional divergence between duplicates via regulatory sub and neofunctionalization may thus apply to other gene families in this species and in other Ascomycetes.

**Gene Redundancy Guarding Critical Functions**

Our data indicate that certain multigene families members, such as CUT2, have attained a level of functional specificity in the lifestyle of the organism, and may be selected for high dosage. Indeed, highly expressed yeast duplicate genes give stronger fitness effects on deletion than copies with lower expression. Other family members (such as the characterized CUT1 and the putative cutinase MGG_02393.5) may share at least partially overlapping redundant functions. In the case of MGG_02393.5, whole genome microarray studies reveal the gene to be highly transcribed during fungal development on infection-related artificial surfaces; however qRT-PCR from fungal tissues growing on the host reveals the gene to be constitutively expressed. This disparity may explain its dispensability for *M. oryzae* pathogenicity.

In support of the functional redundancy in the *M. oryzae* cutinase and other families, functional complementation from *S. cerevisiae* duplicate genes contributes to the robustness of genetic networks, by reducing the fitness effect of deleterious mutations. Furthermore, in *C. elegans* and Arabidopsis, duplicate pairs with redundant functions have been selected for preservation over extended evolutionary periods, presumably to confer genetic robustness. Lastly, in yeast coding-sequence divergence is negatively correlated with the essentiality of individual genes; this may also apply to the cutinase family. It is therefore plausible that functional compensation through the maintenance of multiple redundant paralogs could occur in *M. oryzae* to ensure vital steps of its lifestyle. In filamentous fungi, further, genome-wide and integrated analyses of coding-sequence and expression divergence, combined with high-throughput functional analyses of gene duplicates will provide a fuller picture of the role of gene families in the origin and evolution of pathogenesis.

**Acknowledgements**

This research was funded by a grant from the BBSRC to Sarah J. Gurr to specifically support Pari Skamnioti. We thank Jesse Alderson (Oxford) for critical reading of the manuscript.

**References**

1. Cornell MJ, Alam I, Soanes DM, Wong HM, Hedeler C, Paton NW, Talbot NJ, Oliver SG. Comparative genome analysis across a kingdom of eukaryotic organisms: Specialization and diversification in the Fungi. Genom Res 2007; 17:1809-22.
2. Wapinski I, Pfeffer A, Friedman N, Regev A. Natural history and evolutionary principles of gene duplication in fungi. Nature 2007; 449:54-61.
3. Skamnioti P, Gurr SJ. Cutinase and hydrophobin Interplay: a herald for pathogenesis? Plant Rev Phytopathol 2007; 45:437-56.
4. Skamnioti P, Lenehan B, Gurr SJ. Pari Skamnioti. We thank Jesse Alderson (Oxford) for critical reading of the manuscript.
