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Review

Cross-kingdom gene transfer facilitates the evolution of virulence in fungal pathogens

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ABSTRACT

The constant interaction between plants and their pathogens has resulted in the evolution of a diverse array of microbial infection strategies. It is increasingly evident that horizontal acquisition of new virulence functions in fungi is one of the evolutionary processes that maintain pathogens' competitive edge over host plants. Genome analyses of fungi are pointing towards this phenomenon being particularly prevalent in the subphylum Pezizomycota. While the extent of cross-kingdom gene transfer can be determined with existing genomic tools and databases, so far very few horizontally transmitted genes have been functionally characterised, and an understanding of their physiological roles in virulence has been determined for even fewer genes. Understanding the evolutionary selection pressures that drive the retention of acquired genes in particular fungal lineages is important, as it will undoubtedly reveal new insights into both fungal virulence mechanisms and corresponding plant defence processes in the future.

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Abbreviations: NLP, Necrosis and ethylene inducing-like proteins.

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1. Introduction

The interactions between plants and their pathogens are subject to parallel or co-evolution. Pathogens must find innovative strategies to successfully colonise their hosts while plants identify new detection methods and more robust defence mechanisms to ward off pathogen attack. Nevertheless, plants are intrinsically resistant to most pathogens, suggesting that the interaction between plants and successful pathogens are indeed rather specific. Three general classes of pathogenic lifestyles are distinguished within fungal plant pathogens; biotrophic, necrotrophic and hemibiotrophic [1]. Biotrophs feed on living cells and are specialised pathogens that infect only a single or closely related plant species, while necrotrophs that kill host cells to release nutrients have a broader host range and are usually capable of infecting a wider range of plant hosts. Hemibiotrophs are intermediate between these two extremes and typically have an initial biotrophic phase of infection followed by a switch to necrotrohpy.

Virulence in fungi towards a compatible plant host is a quantitative attribute. Many different virulence genes and strategies are used simultaneously by fungi to cause disease [1]. The loss of any one of these genes can cause a quantitative reduction in pathogen virulence. The disease outcome in plant–biotrophic pathogen interactions is often qualitative due to the presence of avirulence genes that encode products recognised by specific host receptors encoded by resistance (R) genes [2]. This phenomenon is also known as effector triggered immunity. However, these avirulence gene products often also contribute to pathogen fitness in a quantitative manner on a host that lacks the corresponding disease R gene(s) [3]. Through co-evolution with their hosts, both biotrophic and necrotrophic fungi have acquired new virulence mechanisms. Increases in pathogen virulence or aggressiveness presumably have evolved in a stepwise manner. Horizontal gene acquisition is one way that a pathogen achieves stepped or quantitative changes in virulence and maintains supremacy over the host plant. Cross-kingdom gene transfer, that is, the acquisition of genes and strategies from outside the fungal kingdom, from for example, bacteria or plants, is one route that has facilitated pathogen's gaining of new virulence functions.

Recent technical advances in genome sequencing and comparative genome analyses have started to reveal unprecedented insights into how cross-kingdom transfers have shaped the evolution of living organisms, and pathogens in particular. In this review, after a brief discussion on the current techniques used in the discovery of such events, we will highlight recent studies that revealed insights into the roles of cross-kingdom horizontal gene acquisitions in the evolution of virulence in plant pathogenic fungi. Horizontal gene transfers that occur within the fungal kingdom will not be reviewed. Readers interested in this topic should refer to other recent reviews [4–6].

2. Detecting evolutionary signatures of cross-kingdom gene transfer events

Detecting the likely presence of cross-kingdom transfer events via comparisons of extant genome databases can be conducted with a number of freely available bioinformatic tools. It is important to note that although these are often referred to as the detection of transfer events, it is in fact the consequence of that historical event or "signature" that is actually detected. While a number of methodologies have been published to identify horizontal gene flow, nearly all use the same or similar principles outlined in Fig. 1. This involves detection of homologies between gene or protein sequences from different organisms, followed by some type of filtering to remove sequences that are not of interest to the biological question being addressed, construction of phylogenetic trees, and analysis for incongruence with expected organismal relationships. Surrogate methods such as patchy distribution and compositional bias (e.g. GC content or codon frequencies) can also be used in conjunction with phylogenetic incongruence to bolster evidence for horizontal transfers [7]. Specific methodologies applied can be found in published analyses and methodological descriptions [5,8–10]. The incongruence with expected organismal relationships can be statistically tested by comparing phylogenetic trees constrained by known relationships and comparing these to unconstrained trees based entirely on gene or protein sequences [e.g. 9, 11]. The direction of gene transfer is usually inferred by identifying the incongruently placed sequence in the phylogenetic tree. For example, if a single fungal gene branches within an otherwise plant-containing clade, then the direction of transfer is presumed to be plant to fungal.

In some cases, alternative scenarios for the presence of a particular gene in a query genome are analysed to determine if a transfer event is the most parsimonious explanation. For example, the number of ancestral gene duplications and subsequent losses required to explain four different events of proposed cross-kingdom gene transfer was analysed in a study [11]. For one gene (the sugar transporter AraJ), either a single transfer or three gene duplications, followed by 22 independent gene losses could explain the organismal distribution. In this case, the former (i.e. gene transfer) is the most parsimonious explanation for the presence of this gene in a number of different oomycetes and fungi. Various studies have applied the basic bioinformatic pipeline shown in Fig. 1 with the filtering stage modified according to the specific biological question being investigated. These examples include identifying the extent of gene transfers from bacteria to fungi [12], comparative analysis to identify genes potentially important for infection of specific or related hosts [13,14], and the detection of fungal to oomycetes (filamentous eukaryotic organisms more closely related to algae than to fungi) transfers [8]. Depending on the chosen approach, this analysis pipeline can be used to identify potential transfers in a single query genome, or in an organism independent manner using approaches that cluster homologous sequences together after an all-versus-all homology search. However, the power of the analysis is highly dependent on the genomes and databases used. For example, if only a few species were present in the database or the species were not taxonomically diverse, the ability to identify incongruence with expected organismal relationships would be severely restricted. In general, more and diverse taxon sampling should provide greater power to identify transfer events. This is evident in analyses that have included the same species but were carried out using different methodologies and databases, which appear to identify gene sets that somewhat vary. For example, the 36 genes identified in *F. graminearum* as being of bacterial origin [12] did not include the amidohydrolase encoding gene FGSG_10599 shown to have extremely limited distribution in fungi and with strong phylogenetic sequence grouping with bacterial sequences [13]. There is currently no single methodology or database that fulfils every need.

3. Cross-kingdom gene transfers have helped shape the genomes of fungi

The potential for transfer of genetic material in microbes across reproductive boundaries and even across kingdoms is well established. Whole genome and metagenome sequencing efforts are now capable of identifying putative events of gene transfer considerably more readily than before, and as more taxa are sampled, the power of these analyses will continue to grow. Incorporating metagenomic analyses in these studies will