MINIREVIEW

Horizontal gene transfer in fungi

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Abstract

Horizontal gene transfer (HGT) is frequently observed in prokaryotes and until recently was assumed to be of limited importance to eukaryotes. However, there is an increasing body of evidence to suggest that HGT is an important mechanism in eukaryotic genome evolution, particularly in unicellular organisms. The transfer of individual genes, gene clusters or entire chromosomes can have significant impacts on niche specification, disease emergence or shift in metabolic capabilities. In terms of genomic sequencing, the fungal kingdom is one of the most densely sampled eukaryotic lineages and is at the forefront of eukaryote comparative genomics and enables us to use fungi to study eukaryotic evolutionary mechanisms including HGT. This review describes the bioinformatics-based methodologies commonly used to locate HGT in fungal genomes and investigates the possible mechanisms involved in transferring genetic material laterally into fungal species. I will highlight a number of fungal HGT events and discuss the impact they have played on fungal evolution and discuss the implications HGT may have on the fungal tree of life.

Introduction

Horizontal (or lateral) gene transfer is defined as the exchange and stable integration of genetic material between different strains or species (Doolittle, 1999). Horizontal gene transfer (HGT) differs from vertical gene transfer, which is the normal transmission of genetic material from parent to offspring. Whole-genome sequencing projects have shown that HGT is a major evolutionary force in prokaryotic evolution (Eisen, 2000).

Until recently, the impact of HGT on eukaryotic evolution was thought to be limited (Kurland *et al.*, 2003). The reasons for this viewpoint included limited eukaryotic genomic data, perceived problems associated with overcoming germ and soma separation in multicellular organisms and the apparent inhibition of large-scale searches for HGT following high-profile erroneous reports of prokaryotic genes in the human genome (Lander *et al.*, 2001; Stanhope *et al.*, 2001).

The rapid increase in publicly available eukaryotic genomic data has changed our views on the frequency and subsequent important roles HGT may play in eukaryotic evolution (especially unicellular organisms). For example, the transfer of a number of prokaryotic genes into the amoeba *Entamoeba histolytica* has altered its metabolic capabilities increasing its range of substrates to include tryptophanase and aspartase (Loftus *et al.*, 2005). Similarly, prokaryote genes transferred into the social amoebae *Dictyostelium discoideum* give it the ability to degrade bacterial cell walls (dipeptidase), resist the toxic effects of tellurite (terD) and scavenge iron (siderophore; Eichinger *et al.*, 2005). The presence of bacterial genes in phagotrophic eukaryotes was initially explained by the 'you are what you eat hypothesis' (Doolittle, 1998). However, the presence of bacterial genes in nonphagotrophic organisms (including members of the fungal kingdom) has shown that mechanisms other than phagocytosis are responsible.

Because of their roles as human/crop pathogens, relative small genome size and importance in the field of biotechnology, over 100 fungal species have been fully sequenced to date. This abundance of fungal data permits us to investigate the frequency and possible consequences HGT has played in fungal evolution.

This review sets out to describe the methodology commonly used to locate HGT, the consequences it has

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played in fungal evolution and possible concerns for reconstructing the fungal tree of life (FTOL).

In silico detection of HGT

Several approaches can be taken to detect incidences of HGT. These include patchy phyletic distribution of a gene (Fitzpatrick *et al.*, 2008; Fig. 1a), locating shared introns in the genes of unrelated species indicating monophyly (Kondrashov *et al.*, 2006), alternatively locating intronless genes in a species that is generally intron rich could indicate an acquisition from a bacterial source (Garcia-Vallve *et al.*, 2000; Schmitt & Lumbsch, 2009), also finding similar genes shared amongst unrelated species that share a specific niche/geographical location (Kunin *et al.*, 2005) or locating genes with conserved synteny blocks that are present in two or more species but absent from close relatives (Fitzpatrick *et al.*, 2008; Rolland *et al.*, 2009; Fig. 1b).

However, the most convincing method to detect HGT uses phylogenetic inference (Ragan, 2001; Fig. 1c). Highly supported topological disagreement (incongruence) between a strongly supported gene tree and the known species phylogeny can often be parsimoniously explained

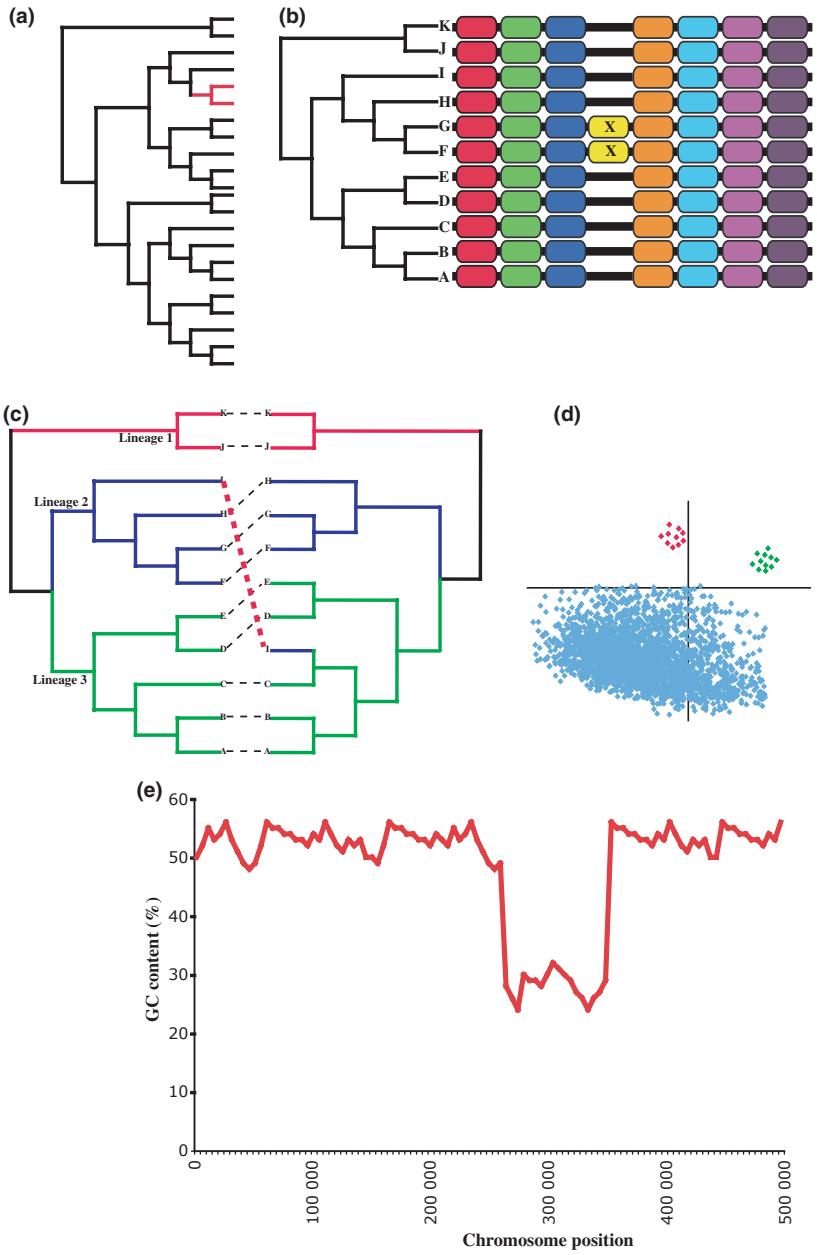

Fig. 1. Detecting incidences of HGT. (a) Patchy phyletic distribution, gene of interest is not found in closely related relatives, and orthologs can only be located in distantly related species. (b) Gene of interest located in conserved synteny block and absent from closely related species. May also indicate a gene loss but similarity-based searches can help validate if it is a potential HGT event or loss. (c) Phylogenetic inference, species gene tree on the right differs from gene tree on the left. Phylogenetic incongruence can be used to detect HGT and also determine the donor species. (d) Codon usage variation, native genes have a preferential codon usage pattern (blue dots); recently transferred genes have yet to ameliorate to their new hosts genome and still display the codon usage pattern of their cognate genome. (e) Variation in GC composition along a chromosome may indicate that alien genetic material has recently been acquired. In this case, the transferred DNA has a GC content lower than the recipient genome.

only by invoking HGT (Andersson, 2005; Keeling & Palmer, 2008). Phylogenetic reconstruction methods remain the only way to reliably infer historical events from gene sequences as they are the only methods that are based on a large body of work (Eisen, 2000). For example, phylogenetic methods are designed to accommodate variation in evolutionary rates and patterns within and between taxa (Ragan *et al.*, 2006). However, it is not easy to extend phylogenetic methods to all genes, for example some gene families evolve so rapidly that orthologs cannot be confidently identified (Ragan, 2001). Other problems that may arise are the computational difficulties in inferring trees and assessing confidence intervals for large data sets.

It is not surprising therefore that there is considerable interest in developing methods that can rapidly identify HGT without the need of phylogenetic trees. These heuristic methods have been referred to as surrogate methods (Ragan, 2001). An example of a surrogate method includes the examination of the patterns of best matches to different species using similarity search techniques to determine the best match for each gene in a genome. This approach has the advantage of speed and automation but does not have a high degree of accuracy. Some notable failures of this approach include the unsupported claim that 223 genes have been transferred from bacterial pathogens to humans (Lander *et al.*, 2001). These findings were based on top hits from a BLAST database search; however, rigorous phylogenetic analyses showed these initial claims to be unsupportable (Stanhope *et al.*, 2001). Similarly, another study based on BLAST database searches also reported that *Mycobacterium tuberculosis* has 19 genes that originate from various eukaryotes (Gamieldien *et al.*, 2002); again using phylogenetic analyses, this hypothesis was shown to be unsupportable (Kinsella & McInerney, 2003). Reasons for low levels of accuracy with these similarity searches include hidden paralogy, distant slowly evolving genes being detected as best matches or two closely related genes not matching well if they have evolved rapidly (Eisen, 1998). Other surrogate methods identify the regions within genomes that have atypical genomic characteristics (Fig. 1d,e). In theory when a gene is introduced into a recipient genome, it takes time for it to ameliorate to the recipients' base composition. Therefore, foreign genes in a genome can be detected by identifying genes with unusual phenotypes such as atypical nucleotide composition or codon usage patterns (Lawrence & Ochman, 1998; Fig. 1d). This approach is attractive as it only requires one genome but does suffer from some obvious flaws. For example, atypical composition may be the result of selection or mutation bias. Furthermore, this approach cannot detect the transfers between species with similar base compositions. Surrogate methods have proven to be successful in detecting HGT events in prokaryotes (Lawrence & Ochman, 1998); however, eukaryote genomes are larger and more complex because of the presence of isochores, large noncoding regions and fragmented genes, making surrogate methods unsuitable for eukaryotic analysis (Mallet *et al.*, 2010). However, some fungal genomes exhibit characteristics (such as compact genomes, few introns and short intergenic regions) similar to prokaryotic genome, thus permitting the use of surrogate methods in genomewide searches of incidences of HGT (Mallet *et al.*, 2010). While surrogate methods do present a heuristic approach for detecting putative HGT events, comparative analyses have shown that surrogate methods fail to identify a common set of genes involved in HGT (Ragan, 2001). Therefore, it is my opinion that when investigating putative HGT, surrogate methods should never be used in isolation; furthermore, positive results should be carefully scrutinized and validated by more robust methodologies such as phylogenetic inference. A typical *in silico* bioinformatics pipeline for detecting HGT in genomic sequences is shown in Fig. 2.

As all HGT detection methods have limitations, it is recommended that a total evidence approach is undertaken where several independent methods are used and cross-corroborated before inferring that a HGT event has occurred (Fitzpatrick, 2009).

Mechanisms that facilitate fungal HGT

HGT requires foreign genetic material to enter the recipient cell, be incorporated into the host genome and successfully express a functional protein. To avoid pseudogenization, the protein should provide a selective advantage to the recipient species.

While lateral transfer has been observed for a number of selfish genetic elements including mycoviruses (van Diepeningen *et al.*, 1998), plasmids (Kempken, 1995), group I introns (Gonzalez *et al.*, 1998) and transposons (Belbahri *et al.*, 2008), the mechanisms of HGT in fungi are not fully understood. A number of possible mechanisms have been reported, however. For example, bacterium to *Saccharomyces cerevisiae* conjugation followed by DNA exchange via bacterial conjugative plasmids has been observed (Heinemann & Sprague, 1989). Similarly, no dedicated DNA uptake mechanisms have ever been reported in *S. cerevisiae,* yet transformations have been observed under specific artificial laboratory conditions (Nevoigt *et al.*, 2000). *Saccharomyces cerevisiae* was also one of the first fungal species to be amenable to *Agrobacterium tumefaciens*-mediated transformation (ATMT; Bundock *et al.*, 1995). A number of fungal species have since been shown to undergo ATMT under specific laboratory conditions including the presence of acetosyringone (de Groot *et al.*, 1998; Chen *et al.*, 2000), a phenolic

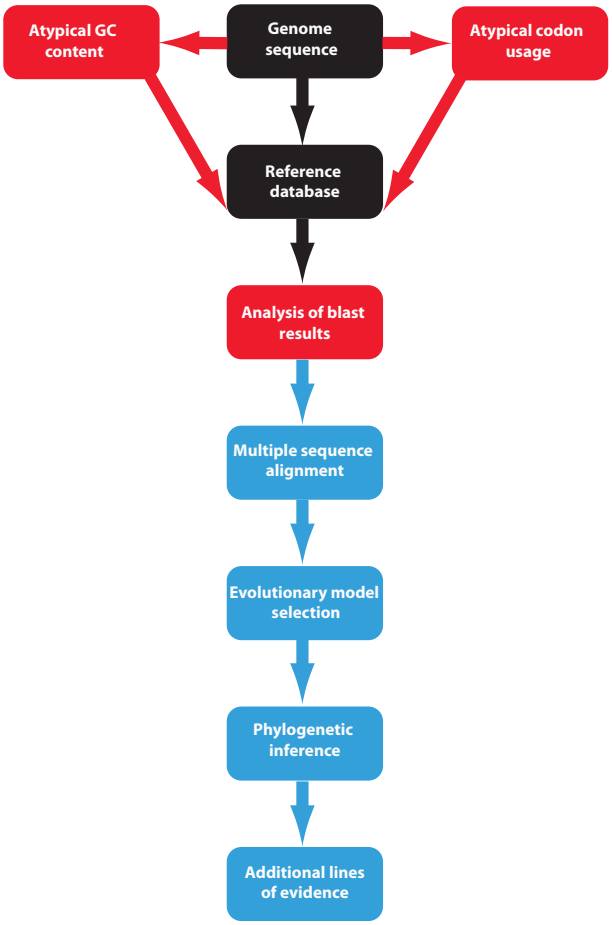

Fig. 2. Typical bioinformatics HGT pipeline. Surrogate methods (red boxes) such as detecting genes with atypical GC content, atypical codon usage patterns or top database hits to nonrelated organisms may be used as an initial step in detecting incidences of HGT. Robust HGT analyses should always verify putative cases of gene transfers via phylogenetic analysis (blue boxes), and this process requires all homologues to be retrieved from the reference database and aligned (and manually edited if required). The optimum model of sequence evolution is usually located, and phylogenies are inferred using reliable phylogenetic reconstruction methods implemented in a maximum-likelihood or Bayesian framework. Gene trees that are strongly supported and deviate significantly from the species tree are indicative of HGT. Additional lines of evidence such as synteny or patchy phyletic distribution may also be investigated to provide further evidence for that a gene transfer event has occurred.

plant wound hormone that is involved in plant-pathogen recognition that induces the expression of virulence genes in *A. tumefaciens*. Transfer of genetic material between *Candida glabrata* and *Saccharomyces cereviaiae* has also been observed, although the mechanisms are not fully understood, plasmid transfer due to cell lysis or cytoduction are possibilities (Mentel *et al.*, 2006).

Other studies have shown that ecological proximity may be linked to HGT. For example, a yeast wine strain (*S. cerevisiae* EC118) has gained 65 KB of genetic material from *Zygosaccharomyces bailii* (a major contaminant of wine fermentations; Novo *et al.*, 2009). The genome of *Mycosphaerella graminicola* also displays evidence of whole chromosomal transfer (Goodwin *et al.*, 2011). *M. graminicola* contains 21 chromosomes; eight of these are dispensable and originated from an unknown fungal source, which is most likely the result of a somatic fusion with another species that had eight or more chromosomes (Goodwin *et al.*, 2011).

Another process linked to HGT in fungal species is anastomosis. Filamentous fungi frequently fuse conidia and conidial germlings using a specialized hypha known as conidial anastomosis tubes; these allow interconnected germlings to act as a single coordinated individual (regulating water, nutrients, signal molecules, nuclei and organelles; Read *et al.*, 2009) and also allow for genetic exchange (Roca *et al.*, 2004). Although non-self-recognition systems have evolved in fungi (Glass & Kaneko, 2003), there is evidence to suggest that interspecies anastomosis between fungal pathogens may have occurred (Friesen *et al.*, 2006; Xie *et al.*, 2008).

As well as mechanisms that facilitate fungal HGT, there are also potential barriers that may oppose it. For example, fungal nuclei are membrane bound, and also differential intron processing and incompatible gene promoters may need to be overcome (Keeling & Palmer, 2008). Furthermore, fungal genetic material is stored in chromatin; while gene-silencing mechanisms such as repeat induced point mutation and methylation induced premeiotically systems have the potential to pseudogenize foreign genes with repetitive elements. The process of meiotic silencing by unpaired DNA (Shiu *et al.*, 2001) is yet another possible barrier to HGT; indeed, it has been proposed that (meiotic) sex has evolved in eukaryotes as a mechanism to check the identity and limit the impact of foreign DNA (Glansdorff *et al.*, 2009). Another possible barrier to HGT is an alternative genetic code. The human pathogen *Candida albicans* and close relatives translate the codon CTG as serine instead of leucine. Recent analyses of species from the CTG clade (Fitzpatrick *et al.*, 2006) could only locate four incidences of bacterial to fungal HGT since the CTG codon reassignment approximately 170 million years ago (Fitzpatrick *et al.*, 2008; Marcet-Houben & Gabaldon, 2010). Such low incidences of HGT over such a long time period support the hypothesis that genetic code alterations act as barriers to HGT.

Consequences of fungal HGT

Comparative fungal genomic analyses have shown the importance that HGT plays in the evolution of fungi. For example, Hall and Dietrich have shown that *S. cerevisiae*

S288C has acquired 13 prokaryotic genes via HGT because it diverged from its close relative *Ashbya gossypii* (Hall *et al.*, 2005). This number corresponds to a small minority of the *S. cerevisiae* genome (< 1%); however, these genes have contributed to important functional innovations, including the ability to synthesize biotin, the ability to grow under anaerobic conditions and the ability to utilize sulphate from several organic sources (Hall *et al.*, 2005). Similarly, a recent sequencing project of the commercial wine yeast strain EC118 uncovered three genomic regions that have been transferred horizontally from other fungal sources (Novo *et al.*, 2009). The three regions encode 34 genes, which are important in wine fermentation including nitrogen and carbon metabolism, cellular transport and stress responses, that aid yeast wine strains adapt to high sugar, low nitrogen and high ethanol concentrations (Novo *et al.*, 2009).

Other HGT events that have contributed to niche specification include the acquisition of glycosyl hydrolases (GHs) by rumen fungi from prokaryotes (Garcia-Vallve *et al.*, 2000). GHs have permitted rumen fungi to establish a niche in the rumen of herbivorous mammals where cellulose and plant hemicellulose are the main carbon sources (Garcia-Vallve *et al.*, 2000). Similarly, the entomopathogenic fungus *Metarhizium anisopliae* has acquired a phosphoketolase (Mpk1) from an unspecified bacterial source. It has been demonstrated that Mpk1 is necessary for insect virulence and is highly expressed in trehalose-rich insect haemolymph, thus playing an important role in niche adaptation for this fungus in the insect haemocoel.

Slot & Hibbett (2007) have also uncovered an ancient transfer of a nitrate assimilation cluster from the Oomycota to an ancestral Dikarya species and propose that the acquisition of high-affinity nitrate assimilation contributed to the success of Dikarya on land by allowing exploitation of nitrate in aerobic soils. Furthermore, the subsequent transfer of a complete Basidiomycete nitrate assimilation cluster into the ascomycetous mould *Trichoderma reesei* improved fitness and corresponds to a change in nutritional mode (wood decayer), providing further evidence that horizontal transfer can facilitate niche shift in fungi (Slot & Hibbett, 2007).

Incidences of HGT have also been linked to virulence in fungi, and the recent acquisition of a toxin gene (ToxA) by *Pyrenophora tritici-repentis* from *Stagonospora nodorum* has resulted in serious *Pyrenophora* infestations of wheat (Friesen *et al.*, 2006). ToxA exerts its toxic effect via internalization into sensitive wheat mesophyll cells where it localizes to chloroplasts (Manning & Ciuffetti, 2005); however, the mechanisms involved in ToxA-mediated cell death remain to be elucidated.

Interfungal HGT of a pea pathogenicity gene (PEP) cluster from *Fusarium oxysporum* to *Nectria haematococca* has also been linked to disease. The PEP cluster increases pathogenicity by converting a pea phytoalexin (pisatin) into a less toxic compound (Matthews & Van Etten, 1983). Also, a recent comparative genomic study of *Fusarium* species showed that four of *F. oxysporum*'s 15 chromosomes have been acquired through HGT from a fungal source (Ma *et al.*, 2010). One of these chromosomes (chromosome 14) is essential for pathogenicity of tomato plants (Ma *et al.*, 2010). Using a simple co-incubation procedure, the authors demonstrated that chromosome 14 could be transferred between different *F. oxysporum*'s strains converting nonpathogenic strains into a pathogenic strains (Ma *et al.*, 2010).

Direction of transfers

Initially, a large proportion of documented HGT events into fungi involved bacterial donors (Table 1). This phenomenon may be due to the fact that bacterial HGT events are easier to detect than eukaryotic transfers. Furthermore, the majority of systematic fungal genomic HGT searches performed to date have only searched for genes from a bacterial source (Hall *et al.*, 2005; Fitzpatrick *et al.*, 2008; Marcet-Houben & Gabaldon, 2010). Ignoring these experimental biases, there are a number of biological reasons why prokaryote to fungal HGT is more likely than eukaryotic to fungal HGT. First, eukaryotic genes contain introns, and incorrect spicing of these could act as a barrier for eukaryotic to eukaryotic HGT (this may not be an issue between closely related eukaryotes where intron structure and position are highly conserved (Stajich *et al.*, 2007)). Secondly, the number and diversity of bacterial populations is considerably larger than that of eukaryotic populations; therefore, the pool of bacterial genes available in the environment is significantly larger (Keeling & Palmer, 2008). Another factor to be considered is the observation that bacteria contain operons of functionally related genes, meaning that the transfer of a relatively small segment of DNA from bacteria to fungi could result in the gain of a complete metabolic pathway. Whole metabolic pathway transfer from bacteria to fungi has yet to be discovered; however, a recent analysis reported that two of the six genes (*BIO3* and *BIO4*) of the *S. cerevisiae* biotin pathway have been acquired through HGT from a bacterial source (Hall & Dietrich, 2007). Recent analyses have started to locate fungal to fungal interspecies HGTs (Table 1). Interestingly, a number of these studies have uncovered evidence of horizontal transfer of entire metabolic pathways whose genes are clustered within the donor genome (Temporini & VanEtten, 2004; Khaldi *et al.*, 2008; Mallet *et al.*, 2010; Khaldi & Wolfe, 2011; Slot & Rokas, 2011). For example, Slot and Rokas recently showed that a ~57-kb genomic

Table 1. Examples of fungal horizontal gene transfer

Recipient	Donor	Chromosome/Gene	References
Candida parapsilosis	Bacterial	Proline racemase and PhzF	Fitzpatrick *et al.* (2008)
Metarhizium anisopliae	Bacterial	*Mpk1*	Duan *et al.* (2009)
60 Fungal species	Bacterial	713 genes	Marcet-Houben & Gabaldon (2010)
Pezizomycotina species	Bacterial	β-glucuronidase	Wenzl *et al.* (2005)
Sordariomycetes and *Saccaromycetes* species	Bacterial	Urea amidolyase	Strope *et al.* (2011)
Saccharomycetaceae species	Bacterial	11 genes	Rolland *et al.* (2009)
Saccharomyces cerevisiae S288c	Bacterial	13 genes	Hall *et al.* (2005), Hall & Dietrich (2007)
Rumen fungi	Bacterial	Glycosyl hydrolases	Garcia-Vallve *et al.* (2000)
Nectria haematococca	Fungal	PEP gene cluster	Temporini & VanEtten (2004)
Podospora anserina	Fungal	Sterigmatocystin cluster	Slot & Rokas (2011)
Aspergillus clavatus	Fungal	*ACE1* cluster	Khaldi *et al.* (2008)
Aspergillus niger	Fungal	Fumonisin cluster	Khaldi & Wolfe (2011)
Saccharomyces cerevisiae EC118	Fungal	34 genes	Novo *et al.* (2009)
Aspergillus oryzae	Fungal	Numerous functions	Khaldi & Wolfe (2008)
Mycosphaerella graminicola	Fungal	Eight chromosomes	Goodwin *et al.* (2011)
Fusarium oxysporum	Fungal	Four chromosomes	Ma *et al.* (2010)
Pyrenophora tritici-repentis	Fungal	*ToxA*	Friesen *et al.* (2006)
Ceratobasidium oryzae-sativae	Fungal	ITS	Xie *et al.* (2008)
Various fungal lineages	Plant	Four genes	Richards *et al.* (2009)

region containing all 23 genes of the sterigmatocystin (toxic secondary metabolite) pathway has been transferred from *Aspergillus nidulans* to *Podospora anserina* (Slot & Rokas, 2011). Very few incidences of eukaryote (nonfungal) to fungal HGT have been located; however, a recent phylogenomic analysis has located four plant to fungi transfers (Richards *et al.*, 2009).

The possible impact of HGT on the FTOL

Resolving the tree of life is a fundamental goal of biology. Fungal evolutionary relationships were historically inferred using cell morphology, physiological/growth tests and sexual states. Today, sequence data are commonly used to infer fungal relationships. The choice of molecular phylogenetic markers for reconstructing robust species trees is difficult and fraught with potential pitfalls (such as hidden paralogy and rapidly evolving genes). Common markers are generally ubiquitous slowly evolving single-copy orthologs. For example, a comprehensive analysis of the early evolution of fungi used six transcription/translation-related genes (18S rRNA, 28S rRNA, 5.8S rRNA, elongation factor 1-α and two RNA polymerase II subunits (RPB1 and RPB2; James *et al.*, 2006). The complexity hypothesis (Jain *et al.*, 1999) assumes that these genes should be immune from HGT, and species phylogenies derived from them should reflect the true evolutionary history of the species being examined. This assumption is being challenged; however, phylogenomic analyses have shown that 24 single-copy genes that are universally distributed throughout the tree of life display evidence of HGT (Creevey *et al.*, 2011). Furthermore, there is a reported case for the transfer of ribosomal genes between two fungal rice pathogens (*Thanatephorus cucumeris* and *Ceratobasidium oryzae-sativae*; Xie *et al.*, 2008). While there is currently no evidence to suggest that any of the six transcription/translation-related genes mentioned above have undergone HGT, the possibility should be considered especially if a phylogenetic inference disagrees significantly with other strongly supported molecular phylogenies or morphological characters. Current evidence suggests that rates of HGT into and between fungi are relatively low; therefore in my opinion, reconstructing the FTOL is a viable endeavour. Furthermore, I don't believe there is evidence yet to suggest that fungal HGT has been so rampant that it undermines a tree of life outlook, replacing it with a web of life hierarchy similar to what we observe in prokaryotes.

Concluding remarks

Currently, the reported rate of fungal HGT is relatively low, but where HGT does occur it can have significant impacts on niche specification, disease emergence or shift in metabolic capabilities. The majority of fungal species that have been sequenced to date belong to the *Ascomycota* phylum; furthermore, there is a significant bias towards species that are pathogens of humans. Reduced costs and recent improvements associated with new sequencing technologies should mean that a wider range of evolutionary, environmentally and biotechnologically interesting fungal organisms will become available in the

coming years. As the diversity of fungal, nonfungal eukaryotes and bacterial genomes expands, I expect the reported incidences of HGT into fungal species to increase. Studies of HGT in the fungal kingdom are still in their infancy, but over the coming years we should gain further insight into the role HGT has played in fungal evolution.

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