Genome Evolution in Plant Pathogenic and Symbiotic Fungi

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Advances in Botanical Research, Vol. 49
Incorporating Advances in Plant Pathology

0065-2296/09 $35.00
DOI: 10.1016/S0065-2296(08)00603-4

ABSTRACT

Approximately 100,000 species of fungi have been described so far, of which, a high percentage obtain nutrients by living in close association with other organisms (mainly plants), being pathogens or symbionts (i.e. commensalists or mutualists). At the genomic level, an association between broad-scale genetic changes and the emergences of the parasitic and symbiotic life style in fungi has been proposed. Although comparative genomic studies in fungi are still in the early stages, they have enormous potential to improve our understanding of how such life styles evolve. In this chapter, we review the main characteristics of genome evolution in fungi, particularly in species that are pathogenic or symbiotic with plants, by focusing on the mechanisms involved in host interactions. We address the following topics in relation to the pathogenic and symbiotic lifestyles in fungi: the evolution of genome organization, chromosomal rearrangements, evolution of gene families and clusters, suppression of recombination around mating type loci, rapidly evolving genes, horizontal transfer, hybridization, transposable elements, telomeres, introns, mitochondrial genomes, and we finally compare genome evolution between pathogenic bacteria and fungi.

I. INTRODUCTION

Approximately 100,000 species of fungi have been described so far, of which, a high percentage obtain nutrients by living in close association with other organisms, mainly plants. Many fungi are pathogenic and can lead to severe economic losses due to infected crops. Other associations are commensal or particularly mutualistic symbioses, which are beneficial to the host organism, including the mycorrhizal fungi that colonize the roots of many important crops and forest trees. These mutualistic fungi improve the growth of the host plants by facilitating the uptake of nutrients.

Parasitic and symbiotic species are found interspersed with saprophytes in fungal phylogenies (Berbee, 2001), suggesting that transitions between these life history strategies have occurred repeatedly within the fungal kingdom. Moreover, there are numerous cases where the relative harm and benefit to the host plant is largely context dependent (e.g. Clay *et al.*, 1993). To be a pathogen or symbiont, a fungus has to overcome the numerous physical, cellular, and molecular barriers presented by the host. To persist, they must enter another organism, then grow and replicate using nutrients from host tissues, avoid host defenses, and eventually produce propagules that lead to the infection of more than one additional new host. At the genomic level, three genetic mechanisms have been proposed to be associated with the emergences of the parasitic and symbiotic life style in fungi. First, parasitism and symbiosis are associated with the evolution of novel genes. Such genes often have specific roles during host infection and arise by horizontal gene transfer or gene duplication followed by functional divergence. Second, parasitism

and symbiosis are often associated with gene loss and deletions. These losses can involve genes required for free-living saprophytism and those that allow the escape from detection by host defenses. Third, adaptations to the parasitic and symbiotic habits often result from differences in the regulation of gene expression. Further, living in such close association with a host provides the opportunity for coevolutionary dynamics and for the invasion of novel environments through the process of host shifts. These phenomena require rapid adaptations and a more continual evolutionary progression than in response to strictly abiotic factors, which may leave footprints in the genome such as positive selection. Gene gain, gene loss, and rapid adaptation can be investigated using comparative studies. In fungi, such genomic studies are still in the early stages, being limited to a few species, but there is enormous potential to improve our understanding of the molecular mechanisms involved in host–pathogen interactions as many more genomes sequences are under construction.

In this chapter, we will review the main characteristics of genome evolution in fungi, particularly plant pathogenic and symbiotic species, focusing on the mechanisms involved in adaptations to host interactions. We will address the following topics in relation to the pathogenic and symbiotic lifestyles in fungi: the evolution of genome organization, chromosomal rearrangements, evolution of gene families and clusters, suppression of recombination around mating type loci, rapidly evolving genes, horizontal transfer, hybridization, transposable elements, telomeres, introns, mitochondrial genomes, and we will finally compare genome evolution between pathogenic bacteria and fungi. General information on the species cited in this review are given in Table 1.

II. THE EVOLUTION OF GENOME SIZE, CONTENT AND ORGANIZATION

Compared to animals and plants, fungi exhibit streamlined and gene-dense genomes, with an average estimated size of ~37 Mb, and ranging between 6.5 Mb for *Pneumocystis carinii* to 795 Mb for *Scutellospora castanea* (Gregory *et al.*, 2007; see the Fungal Genome Size database: http://www.zbi.ee/fungal-genomesize/N = 762). Gene densities averaged across the genome vary significantly, between 37 and 61 genes per 100 kb (Galagan *et al.*, 2005b). For comparison, average gene density is about 10 genes per 100 kb in *Drosophila* and about 1 gene per 100 kb in humans and other mammals (see http://www.ornl.gov/sci/techresources/Human_Genome/faq/compgen.shtml). Chromosome numbers also vary considerably in fungi, with the smallest number at 3, in the ascomycete *Schizosaccharomyces pombe*, and the largest

TABLE 1

General Information, When Available, about Each Fungal Species Cited in the Review: Genome Statistics, Life Style, and Taxonomy

Genome	Size (Mb)	Approx. gene number	Chromosome number (*n*)	Sequencing status[a]	Life style/habitat	Phylum
Ascosphaera apis	24	~8,092		Incomplete	Bee pathogen	Ascomycete
Ashbya gossypii	9.2	1,443	7	Complete	Plant pathogen	Ascomycete
Aspergillus flavus	36.8	13,071	12	Complete	Weak pathogen	Ascomycete
Aspergillus fumigatus	29.4	9,900	8	Complete	Opportunistic/ pathogen	Ascomycete
Aspergillus niger	33.9	11,200	14	Complete	Weak pathogen	Ascomycete
Aspergillus oryzae	36.7	12,074	12	Complete	Domesticated	Ascomycete
Blastocladiella emersonii				EST data: 16,985	Aquatic	Chytridiomycete
Botrytis cinerea	30	16,448		Complete	Plant pathogen	Ascomycete
Candida albicans	14.9	12,015	8	Complete	Human pathogen	Ascomycete
Candida glabrata	12.3	5,300	13	Complete	Human pathogen	Ascomycete
Cryptococcus neoformans	20	6,500	14	Complete	Human pathogen	Basidiomycete
Debaryomyces hanseii	12.2	6,900	7	Complete	Halotolerant methylotrophic	Ascomycete
Giberella/Fusarium graminearum	36	12,000	4	Complete	Plant pathogen/ necrotroph	Ascomycete
Kluyveromyces lactis	10.6	5,300	6	Complete	Nonpathogenic	Ascomycete
Kluyveromyces waltii	10.7	5,700	8	Complete	Nonpathogenic	Ascomycete
Laccaria bicolor	65	16,100	10, at least	Complete	Symbiont (mycorrhizal)	Basidiomycete
Magnaporthe grisea	38	11,000	7	Complete	Necrotroph	Ascomycete
Malassezia globosa	9	4,285		Complete	Human pathogen (dandruff)	Basidiomycete
Neurospora crassa	39	10,000	7	Complete	Non pathogenic, saprofitic and pathogenic	Ascomycete

Penicillium marneffei	26		3–8	Nearly complete	Pathogenic	Ascomycete
Phycomyces blakesleeanus	40	14,792		Complete	Non pathogenic	Zygomycete
Pichia stipitis	15.4	5,800		Complete	Xylose fermenting	Ascomycete
Pisolithus microcarpus				Incomplete	Symbiont (ectomycorrhizal)	Basidiomycete
Puccinia graminis	88.64	20,567		Complete	Plant pathogen	Basidiomycete
Rhizopus oryzae	45.3	17,467		Draft sequence	Human pathogen	Zygomycota
Saccharomyces cerevisiae	12.1	6,300	16	Complete	Non pathogenic	Ascomycete
Sclerotinia sclerotiorum	38.33	14,522		Complete	Plant pathogen	Ascomycete
Ustilago maydis	20.5	6,900	23	Complete	Facultative biotroph	Basidiomycete
Yarrowia lipolytica	20.5	6,700	6	Complete	Alcane utilizer	Ascomycete
Agaricus bitorquis	34.2	1,100	13	Incomplete	Symbiont (ectomycorrhizal)	Basidiomycete
Agrocybe aegerita				Only the mitochondrial genome is available	Wood decomposer	Basidiomycete
Alternaria alternata	33.6		9–11	Incomplete	Plant pathogen	Ascomycete
Batrachochytrium dendrobatidis	23.72	8,794	20	Complete	Animal pathogen	Chytridiomycete
Botrytis allii				Incomplete	Plant pathogen	Ascomycete
Cochliobolus carbonum				Incomplete	Saprophyte or plant pathogen	Ascomycete
Candida dubliniensis	16			Nearly complete	Human oral pathogen	Ascomycete
Coccidioides immitis	29	10,600	6, at least	Draft assembly	Human pathogen	Ascomycete
Cochliobolus victoriae				Incomplete	Plant pathogen	Ascomycete
Colletotrichum lindemuthianum			9–12	Incomplete	Plant pathogen	Ascomycete
Coniophora puteana				Incomplete	Decomposer	Basidiomycete

(*continues*)

TABLE 1 (*continued*)

Genome	Size (Mb)	Approx. gene number	Chromosome number (*n*)	Sequencing status[a]	Life style/habitat	Phylum
Cordyceps aka Beauveria bassiana				Incomplete	Animal pathogen	Ascomycete
Crinipellis perniciosa	39		8–10	Incomplete	Plant pathogen	Basidiomycete
Cryphonectria parasitica				Incomplete	Plant pathogen	Ascomycete
Fusarium oxysporum	33–50	17,735		Complete	Plant pathogen	Ascomycete
Gibberella fujikuroi	40–50		12	Incomplete	Plant pathogen	Ascomycete
Heterobasidion annosum				Incomplete	Plant pathogen	Basidiomycete
Leptosphaeria maculans	34	12,000	15–16	Incomplete	Plant pathogen	Ascomycete
Melampsora medusae				Incomplete	Plant pathogen	Basidiomycete
Melampsora occidentalis				Incomplete	Plant pathogen	Ascomycete
Microbotryum violaceum	25		11–13	EST data: 40,000	Plant pathogen	Basidiomycete
Mycosphaerella graminicola aka Septoria tritici	41	11,395	18	Complete	Plant pathogen	Ascomycete
Nectria haematococca	40	15,707	17	Complete	Saprophytes, rhizosphere colonizers or pathogens	Ascomycete
Neosartorya fischeri	32.6	10,407	8	Complete	Human pathogen	Ascomycete
Neotyphodium = Epichloë	29–57		42	Incomplete	Plant pathogen or mutualist	Ascomycete
Neurospora intermedia				Incomplete	Plant pathogen	Ascomycete
Neurospora tetrasperma				Incomplete	Plant pathogen	Ascomycete
Ophiostoma novo-ulmi		8,000–10,000		Incomplete	Plant pathogen	Ascomycete
Ophiostoma ulmi				Incomplete	Plant pathogen	Ascomycete
Phanerochaete chrysosporium	30	10,048	10	Complete	Wood degrader	Basidiomycete
Phytophthora infestans	237		8–10	Incomplete	Plant pathogen	Oomycete

Pleurotus ostreatus				Incomplete	Saprophyte (Ectomyccorhizal)	Basidiomycete
Pneumocystis carinii	7.7			Incomplete	Animal pathogen	Ascomycete
Podospora anserina	36	10,545	7	Complete	Saprophyte	Ascomycete
Puccinia recondita				Incomplete	Saprophyte	Basidiomycete
Pyrenophora trici-repentis	37.8	12,171	11	Complete	Plant pathogen	Ascomycete
Schizosaccharomyces pombe	12	4,800	3	Complete	Non pathogenic	Ascomycete
Scutellospora castanea	50			Incomplete	Arbuscular mycorrhizal	Zygomycete
Stachybotrys chartarum				Incomplete	Plant pathogen	Ascomycete
Stagonospora nodorum = *Phaeosphaeria nodorum*	37.24	16,597		Incomplete	Plant pathogen	Ascomycota
Ustilago hordei				Incomplete	Plant pathogen	Basidiomycete

[a]Incomplete sequencing status may be either because there is an ongoing sequencing project that is not finished yet, or because there is no sequencing project yet started.

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number at 20, in the basidiomycete *Ustilago hordei* and the chytrid *Batrachochytrium dendrobatidis* (Gregory *et al.*, 2007). Summary statistics, including gene numbers and densities, are highly dependent on the quality of genome annotation, which is often rather poor, and therefore should be considered with caution and frequently updated.

Despite common themes in fungal evolution, fungi are strikingly diverse at the genome level, showing many instances of lineage-specific evolution. Not only can the DNA sequences of genes be highly divergent but there are also important changes in the order and localization of homologous genes among genomes. For example, the comparison of the related ascomycetes *Neurospora crassa* and *Magnaporthe grisea*, which diverged around 200 MYA, reveals that their genomes have only 74% identity at the amino acid level (a distance comparable to that between mammals and fish) and virtually no synteny (conserved gene colinearity between genomes) (Dean *et al.*, 2005). This is perhaps not so surprising, as the comparison of similarly distant species also reveals very few traces of synteny. Another instance of synteny loss between relatively closely related species involves members of the *Aspergillus* genus, whose average amino acid identity is 68% (Galagan *et al.*, 2005a). Not surprisingly, when more distant genomes are compared, it becomes evident that there is a rapid breakdown of conserved synteny over relatively short periods of time, due mostly to genome rearrangements (translocations, inversions), which frequently occur in subtelomeric regions and are associated with repetitive sequence elements (Keely *et al.* 2005). Other mechanisms responsible for the pervasive loss of synteny include spontaneous segmental gene duplications (Koszul *et al.*, 2004), occasional horizontal gene transfers (Andersson, 2005; Rosewich and Kistler, 2000b), as well as extensive differential gene loss following duplications. A dramatic example is found among yeasts where whole-genome duplication and the subsequent lineage-specific sequence losses may have contributed to speciation (Giraud *et al.*, 2008a; Kellis *et al.*, 2004; Scannell *et al.*, 2006). However, regions of microsynteny can be found between closely related species, such as those within the *Fusarium graminearum* species complex (Malz *et al.*, 2005), and even between more distant genomes (e.g. *Magnaporthe grisea* and *Neurospora crassa)*, suggesting cases for a functional role of the conserved gene order (Hamer *et al.*, 2001).

Among fungi showing the signatures of adaptations to the pathogenic lifestyle there has been a tendency for reduced genome size (Yuen *et al.*, 2003), for instance, by losing genes or whole metabolic pathways that are no longer necessary: e.g. Hemiascomycetes have lost the genes needed to survive on the carbon source galactose that was irrelevant in the within-host environment (Hittinger *et al.*, 2004). Adaptations to the life style can also be seen in the

existence of lineage-specific genes. The comparison between the genomes of a human pathogen, *Aspergillus fumigatus,* and two closely related but rarely pathogenic species, *Neosartorya fischeri* and *Aspergillus clavatus*, for instance, revealed that 8–14% of the genes were species-specific, and that many of those were involved in carbohydrate and chitin catabolism, transport, detoxification, secondary metabolism and other functions that may facilitate the adaptation to their specific life styles, i.e. soil or mammalian host (Fedorova *et al.*, 2008). Interestingly, these species-specific genes were preferentially located in subtelomeric regions, and in ''genomic islands'' enriched in pseudogenes and transposable elements (Fedorova *et al.*, 2008).

Most pathogenic fungi have also experienced the expansion of specific gene families related to functions that facilitate the infection of the host. Typically, these genes include proteases, secreted proteins, secondary metabolites, cell wall degrading enzymes, major facilitator transporters, amino acid transporters, G-protein-coupled receptors, and enzymes for detoxification of antimicrobial agents. An example of how the expansion of specific gene families provides pathogenic potential to an organism is given by the genome of *Penicillium marneffei*, the only known pathogenic fungus of the *Penicillium* genus. Compared with its innocuous relatives, *P. marneffei* has experienced reductive genome evolution (17 Mb compared to ~30 Mb in other *Penicillium* species), and its genome is rich in secondary metabolite genes and thioester-mediated non-ribosomal protein synthesis (Yuen *et al.*, 2003). Another fungus showing peculiar genomic features related to its pathogenic lifestyle is *Ustilago maydis,* a biotroph basidiomycete that parasitizes maize and depends on living tissue for proliferation and development. Not surprisingly, it lacks the pathogenicity genes present in more aggressive necrotrophic fungal pathogens. However, it possesses clustered secreted protein effectors favoring the invasion of living tissue while minimizing host damages (Kamper *et al.*, 2006).

The genomic organization of a symbiotic fungal species can now be studied with the recent availability of the genome of the basidiomycete *Laccaria bicolor* (Martin *et al.*, 2008). This genome of 65 million base pairs and 20,000 predicted genes is large relative to other fungi. Only 70% of predicted genes have homologs in other fungi, and its size can be partly accounted for by a large number of transposons and repeated sequences, as well as by the presence of large lineage-specific multi-gene families. In particular, there is evidence for the expansion of numerous protein gene families related to the functions that make possible the symbiotic relationship between *Laccaria bicolor* and its tree host *Populus trichocarpa*. In contrast, the genome of *Laccaria bicolor* shows a marked reduction in the gene families coding for plant cell wall degradation enzymes, while these families are well represented in the genomes of many fungal pathogens (Martin *et al.*, 2008).

III. CHROMOSOMAL REARRANGEMENTS

One of the main mechanisms responsible for the evolution of genome organization is chromosomal rearrangement. The relatively small genomes of fungi contain chromosomes that are very difficult to observe by conventional microscopy. However, the chromosomes are in fact small enough to separate by pulsed-field gel electrophoresis, providing a powerful approach to quantify any number or size differences across related taxa. Intra-specific polymorphisms in electrophoretic karyotypes are extremely common in both the ascomycete and basidiomycete phyla and have their origins in mutations associated with chromosome breakage or fusion and recombination between non-homologous loci during meiosis or parasexual cycles (Zolan, 1995).

Chiasma formation between copies of repetitive DNA, such as transposable elements, has been suggested as a primary mechanism for generating chromosome length variation (Fraser *et al.*, 2005; Zolan, 1995). In several fungal pathogens, genetic variation created by chromosomal rearrangements has been reported to favor adaptation to novel hosts or nutritional environments (Larriba, 2004). For example, in the pathogenic yeast *Candida albicans* phenotypic mutants derived *in vitro* often exhibit altered karyotypes and at mutation frequencies varying between 10^{-5} and 10^{-2}, depending upon the strain (Rustchenko, 2007). Specific alterations to chromosome structure of *Candida albicans* have been shown to be selected for in the animal host and to result in increased virulence (Chauhan *et al.*, 2005). Also, extensive chromosome rearrangements in *Candida dubliniensis* relative to its pathogenic sister species *Candida albicans*, may be at the origin of the diminished pathogenicity in the former species compared to the latter, possibly due to karyotypic instability (Magee *et al.*, 2008), although there is no direct evidence for this. The biased distribution of both avirulence genes and transposable element sequences into subtelomeric regions, as suggested by genome analysis of *Magnaporthe grisea* (Dioh *et al.*, 2000), may indicate a functional relationship where frequent rearrangements enhance adaptive abilities (O'Sullivan *et al.*, 1998). However, in other pathogenic fungi, such as *Cryptococcus neoformans*, extensive chromosomal rearrangements have been associated with no obvious beneficial change in phenotype (Boekhout and van Belkum, 1997).

The presence of variation in chromosome structure within species or even within populations (Zolan, 1995) could be expected to cause sterility of certain crosses through the failure of meiosis. It has been proposed that karyotype evolution in sexual species of fungi is constrained by this effect, whereas asexual species may frequently be more polymorphic, i.e. the 'meiotic maintenance hypothesis' for karyotype stability (Kistler and Miao, 1992). Karyotypic polymorphism is for instance frequent in the asexual species *Fusarium*

oxysporum and *Nectria haematoccoca* (Kistler and Miao, 1992). However, a number of studies have since provided evidence to the contrary, of sexual fungi generating and maintaining structurally variable chromosomes through meiotic generations, including species of *Stagonospora*, *Crinipellis* and *C. neoformans* (Boekhout and van Belkum, 1997; Caten and Newton, 2000; Rincones *et al.*, 2006). In some fungi, highly controlled forms of selfing (e.g. automixis with central fusion) can preserve balanced structural heterozygosity in chromosomes, such as in *Microbotryum violaceum* and *Saccharomycodes ludwigii* (Hood and Antonovics, 2004; Yamazaki and Oshima, 1996). Support for the meiotic maintenance hypothesis may come from *Colletotrichum lindemuthianum*, where the presence of high variable minichromosomes appears greater in sterile lineages (O'Sullivan *et al.*, 1998).

Chromosomal rearrangements may give rise to large regions of repeated DNA sequence either by duplicative translocations or aneuploid segregation following hybridization events (Fraser *et al.*, 2005; Greig *et al.*, 2002). The presence of redundant copies allows for contrasting evolutionary fates for paralogous genes sequences. In the case of subtilisins in fungal pathogens of insects, ancient duplications for these essential pathogenicity genes have been retained and diversified for more than 220 million years (Bagga *et al.*, 2004). However, the genomic loss of one paralog is a more common fate of duplicated genes, and in the case of pathogenic fungi, the genes lost may be more likely to belong to common orthologous groups involved in response to environmental stresses (Wapinski *et al.*, 2007).

IV. GENE CLUSTERS

Some genes in the genomes are kept tightly linked despite pervasive chromosome rearrangements. Growing evidence indeed shows that in fungal genomes genes that interact in the same metabolic pathway tend to be clustered together (Keller and Hohn, 1997). Different hypotheses about how such clusters evolved have been proposed. One idea involves the action of horizontal gene transfer (HGT) (Walton, 2000), where whole clusters of genes are passed between organisms, as it is likely to have occurred in fungi possessing the penicillin pathway, thought to be transferred from prokaryotes (Penalva *et al.*, 1990). Another possibility suggests that selective pressure maintains the genes together, their clustering facilitating co-expression and co-regulation. Examples include the regulation of secondary metabolites, such as the tricothecene (Proctor *et al.*, 1995), the aflatoxin/sterigmatocystin clusters (Payne *et al.*, 1993; Woloshuk *et al.*, 1994; Yu *et al.*, 1996) and the epipolythiodioxopiperazine (ETP) gene clusters in filamentous ascomycetes (Patron *et al.*, 2007b).

In fact, several studies show that in pathogenic fungi, the families involved in pathogenicity are frequently clustered, including genes encoding host-specific toxins and secondary metabolites, as well as the enzymes that synthesize them (Keller *et al.*, 2005; Sidhu, 2002). In general, genes associated with a pathogenic lifestyle can be grouped into different types, according to their nature and/or function (for a review see Soanes *et al.* 2007): cell surface receptors like the G-protein-coupled receptors (GPCRs) that bind exogenous ligands and participate in signalling cascades (Cuomo *et al.*, 2007; Dean *et al.*, 2005); secreted proteins, which constitute a diverse group of small peptides such as toxins, proteinaceous effectors, hydrolitic and degrading enzymes (Hane *et al.*, 2007; Machida *et al.*, 2005; Xu *et al.*, 2007); protein effectors that suppress plant defenses and alter cellular metabolism (Hane *et al.*, 2007; Kamper *et al.*, 2006); and secondary metabolites such as non-specific and host-specific toxins, among which key families involved in the biosynthesis of toxins include polyketide synthases (PKS), non-ribosomal peptide synthesis (NRPS), hybrid PKS-NRPSs and cytochrome P450. Recently, PKS-NRPS hybrids were discovered and a phylogenetic analysis suggests a single origin within ascomycetes by the fusion of a PKS and a NRPS (Bohnert *et al.*, 2004; Kroken *et al.*, 2003). Interestingly, hybrid PKS-NRPS genes have been found to encode the avirulence gene ACE1 in *M. grisea,* which unlike most avirulence genes is not secreted (Collemare *et al.*, 2008); another hybrid PKS-NRPSs has been identified in *F. graminearum* (Gaffoor *et al.*, 2005). All secondary metabolites (including antibiotics, potent toxins and ergot alkaloids) are produced by a few common biosynthetic pathways. The most abundant are polyketides (e.g. aflatoxin, a potent toxin with immuno suppressing effects), which are synthesized by PKS genes: many potent toxins are polyketides used by pathogenic species such as *Aspergillus*, *Penicillium*, *Fusarium*, or *Stachybotrys*. NRPS is another important source of bioactive secondary metabolites in fungi (Reiber *et al.*, 2005), also related to the pathogenic fungal life-style. This key mechanism is produced by NRP synthetases, which are large multi-functional enzymes that contain domains for adenylation, thiolation (or peptydil carrier protein PCP) and condensation. Genes encoding NRP synthetases are clustered and are typically co-expressed, as in *Aspergillus* spp., where a linked regulatory gene called LeaA, encoding a methyltransferase, is involved in secondary metabolite gene cluster regulation (Stack *et al.*, 2007). Interestingly, some pathogenicity clusters producing host-specific secondary metabolites are located in conditionally dispensable or supernumerary chromosomes, as in *Nectria haematococca* (Han *et al.*, 2001; Temporini and Van Etten, 2002) or *Alternaria alternata* (Hatta *et al.*, 2002). Note that biotroph fungi, like

Ustilago maydis, possess biotroph-specific gene clusters: about 70% of the predicted genes in that genome encode secreted proteins, most of which are specific to *Ustilago*, and one fifth of which are contained within only twelve gene clusters (Howlett, 2006; Kamper *et al.*, 2006).

V. SUPPRESSION OF RECOMBINATION AROUND MAT LOCI

Another remarkable example of gene clusters in fungal genomes corresponds to the genes determining mating compatibility, which are clustered at the mating-type (MAT) loci (Herskowitz, 1989). MAT loci reside in genomic regions with properties similar to sex chromosomes in plants and animals. One principle among these shared traits is the suppression of meiotic recombination. In fact, the best studied models for the origin of sexes begin with haploid systems and the linkage of genes for non-self recognition and a second trait, such as control over cytoplasmic inheritance (Hoekstra, 1987; Hurst and Hamilton, 1992). The consequences for local recombination suppression are many, including changes in gene content both for regions linked to mating type and the evolution of the genome as a whole.

Because compatible gametes in fungi must differ at the mating types, which code for haploid cell-to-cell recognition and post-zygotic compatibility, these loci are always heterozygous in the diploid stage of the life cycle. In fungi that require mating prior to infection of the host, the mating type loci themselves are sometimes considered to be pathogenicity factors, such as in *Ustilago hordei* (Bakkeren and Kronstad, 1996). However, protein products of mating type loci additionally may serve functions for mating and virulence through common G-protein-mediated environmental sensing and response pathways (Bolker, 1998). Moreover, the lack of recombination at and around mating type loci can favor the differential fixation of alleles that impact disease interactions. For fungi that cause infections during the haploid stage, the alternate mating types may differ in developmental traits and virulence, such as in the basiodiomycete *Cryptococcus neoformans* (Barchiesi *et al.*, 2006). Among ascomycetes, reports are more limited for recombination suppression near mating type loci. However, examples do include differences in pathogenicity between haploids of the alternative mating types in *Mycosphaerella graminicola* (Zhan *et al.*, 2007) and the contribution of heterozygosity for mating types in *Candida albicans* to virulence (Lockhart *et al.*, 2005), suggesting that additional traits not directly related to sexual development may be linked to mating compatibility. The best evidence of recombination suppression in ascomycetes involves the automictic fungus *Neurospora tetrasperma*,

where the majority of the mating type chromosome exhibits structural rearrangements between the homologous pair that prevent proper alignment or chiasma formation (Jacobson, 2005). This recombination suppression appears to have been built in different steps, forming two "evolutionary strata" (Menkis *et al.*, 2008). Among fungi with automictic reproduction (i.e. intratetrad selfing, as is common among secondary homothallic species), recombination suppression between mating type and the centromere is frequent and can facilitate the preservation of heterozygosity (Zakharov, 2005).

An additional characteristic of fixed heterozygosity is the accumulation of loci with genetic load because a deleterious mutation may be permanently sheltered by the still functional allele on the homologous chromosome (Malefijt and Charlesworth, 1979). Such patterns are readily seen in non-self recognition systems in plants and animals, but also occur in linkage to mating types across diverse fungi. A phenomenon called "mating type bias" is common to pathogens in both the Ustilaginales and Microbotryales, where deleterious recessive mutations are linked to one mating type and prevent its growth during the haploid stage. Theoretical models have attempted to explain the persistence of such load due to an association with beneficial effects in the heterozygous stage, either for the load locus itself or for tightly linked loci (i.e. hitchhiking) (Tellier *et al* 2007; Antonovics *et al*. 1998).

Preservation of heterozygote advantage, continued sheltering of genetic load, or the recruitment of other mating type specific genes may contribute to the expansion of recombination suppression around the mating type locus, giving rise to large regions of compositionally divergent haploid sex chromosomes in fungi such as *Cryptococcus neoformans*, *Ustilago hordei*, and *Microbotryum violaceum* (Hood, 2002; Hood *et al.*, 2004; Fraser and Heitman, 2005; Giraud *et al.*, 2008b). Structural heterozygosities or even overall chromosome size dimorphism may be preserved in linkage to mating types of fungi, in ways reminiscent of sex chromosomes in plants and animals. Although gene composition around the mating types of the basidiomycetes remains to be fully characterized, there is a substantial contribution of repetitive DNA and transposable elements (Bakkeren *et al.*, 2006; Hood, 2005), where it is likely that the rate of chromosomal rearrangements and divergence is enhanced among related fungal taxa.

VI. EVOLUTION OF GENE FAMILIES

In addition to genomic rearrangements, genome evolution can occur via gene family evolution and gene duplication (Force *et al.*, 1999; Ohno, 1999), or even whole genome duplications (Dujon *et al.*, 2004). These mechanisms can

generate redundant genetic material upon which evolution can act to generate new functions. Gene families arise and expand through functional divergence following duplication: examples in fungi include cellular motors called kinesins (Schoch *et al.*, 2003), the ABC transporters and MFS drug efflux systems that help fungi detoxify products from the plants defenses (Howlett, 2006), the multidrug resistance transporter families (Gbelska *et al.*, 2006), major surface glycoproteins, related proteins and proteases (Keely *et al.*, 2005). The genes that make possible a symbiotic or a biotrophic relationship (e.g. hydrophobins and mannoproteins, adhesins, phospholipases, and transporters) between fungi and plants (e.g. endomycorrhizae and ectomycorrhizae) also seem to have evolved more extensively by gene-family expansions. They are mostly part of specific biochemical pathways, including genes that trigger regulatory cascades (Martin *et al.*, 2007, 2008), and are often clustered (e.g. Jargeat *et al.*, 2003).

Examples of lifestyle-associated gene family expansions are numerous in fungi: the Dandruff-associated species *Malassezia globosa*, which lacks a fatty acid synthase gene and is thus lipid dependent, shows the expansion of a number of secreted lipases and phospholipases which help this fungus harvest the host's lipids (Cornell *et al.*, 2007; Xu *et al.*, 2007). This lifestyle-associated expansion also occurred in the distant species *Candida albicans,* which has similarly adapted to the human skin environment, but is not present in the more closely related *Ustilago maydis*, which is a plant pathogen. Also, host-specific toxins have been found in *Stagonospora nodorum* and some of its closely related pathogenic species (Hane *et al.*, 2007). Other gene family expansions are related to particular phenotypic traits: in a recent study, the repertoires of filamentous fungi (Pezizomycotina) and yeasts (Saccharomycotina), which do not form filaments, were compared (Cornell *et al.*, 2007). Typically, the two classes of Ascomycota have different metabolic capabilities and there is a correspondent divergence in their proteome repertoires. The authors found that in the Pezizomycotina gene families that provide greater metabolic flexibility and a broader substrate range (e.g., transport proteins, transcription factors, proteins that allow for the use of different carbon sources) have expanded relative to the Saccharomycotina. Inversely, proteins involved in cell wall structure have expanded in the yeast relative to the filamentous ascomycetes. Interestingly, both ascomycete and basidiomycete filamentous fungi show an expansion of the chitosanase gene family that is involved in hyphal cell walls, an example of convergent evolution, which on the contrary is lacking in yeast cell walls.

Once new genes have arisen, recombination and gene conversion further contribute to the evolution of gene families. The extent to which gene

duplication promotes gene family evolution has been investigated in a recent study (Wapinski *et al.*, 2007), where comparison of the genomes of seventeen ascomycetes showed that genes related to stress-responses have undergone many duplications and losses while growth-related genes were markedly less prone to such evolutionary dynamics. Also, duplicated genes appear to diverge through changes in regulatory sequences more often than through changes in biochemical function (Wapinski *et al.*, 2007).

The generation of repeated sequences can occur at different scales, ranging from whole-genome duplications (WGD) to short segmental or tandem duplications (Dujon *et al.*, 2004). WGD was elegantly demonstrated to have occurred within the Ascomycete phylogeny, specifically within the yeasts (Kellis *et al.*, 2004; Scannell *et al.*, 2006). The WGD event was followed by massive differential gene losses that deleted most of the duplicated regions in lineage-specific patterns across the yeasts group, possibly contributing to speciation. Duplication, particularly WGD, and subsequent gene loss have been identified as powerful forces shaping the genomes across the fungi kingdom, and as lineage-splitting forces since the different fate of duplicates can bring about speciation (Semon and Wolfe, 2007). Indeed, following WGD, duplicated genes can have a different probability of retention: in order to be maintained, copies can either rapidly change function thus avoiding redundancy (neofunctionalization); duplicates can share a function through subfunctionalization; there can be a combination of neo- and subfunctionalization at different stages following duplication; also, redundant copies can serve as buffers or back-up for a given function; copies can be retained due to their slow rate of evolution; they can be maintained in order to preserve the relative stoichiometry of protein complexes or to maintain expression levels or dosage compensation (Semon and Wolfe, 2007). Interestingly, WGD can result in polyploidization (having extra sets of homologous chromosomes). This situation can explain relatively large genome sizes in, for example, mycorrhizal fungi (Glomeromycota), where it has been shown to contribute to the long-time maintenance of high genetic variability within asexual species (Pawlowska and Taylor, 2004).

Spontaneous segmental duplication also contributes significantly to the evolution of gene families and functions (Dujon *et al.*, 2004; Koszul *et al.*, 2004). Furthermore, gene duplication can also be mediated by the reverse transcriptase of Class I transposable elements. These act in a ''copy and paste'' fashion to create retrogenes (or ''processed pseudogenes'') that have been identified in fungi by the lack of intronic sequences (Daboussi, 1997).

VII. RAPIDLY EVOLVING GENES AND GENES EVOLVING UNDER POSITIVE SELECTION

Changes in gene functions, in particular after gene duplication, can arise either due to changes in gene expression, through the action of positive selection or by the relaxation of selective constraints (Ohno, 1999; Ohta, 2002). In the latter cases, the molecular signature of diversifying selection can be detected through the analysis of sequence data, if nonsynonymous substitution rates (amino acid-changing) are significantly higher than synonymous substitution rates (Yang, 1997; Yang and Bielawski, 2000). The evolutionary rate of a gene can be influenced by various factors, including selective constraints (Yang and Bielawski, 2000), expression level (Hastings, 1996; Pal *et al.*, 2001), dispensability or essentiality (Hirsh and Fraser, 2001; Krylov, 2003), and the existence of duplicated genes (Force *et al.*, 1999).

In fungal genomes, positive selection has been found to act in the evolution of functionally important gene families, in particular those that confer an adaptation to a pathogenic life-style. These include genes coding for defense systems or for evading the host resistance mechanisms, toxic protein genes, and other virulence-related genes (Staats *et al.*, 2007). Particular examples of genes under positive selection in fungal genomes include the mycotoxin gene cluster in *Fusarium* (Cuomo *et al.*, 2007; Ward *et al.*, 2002), various phytotoxin genes in *Botrytis* (Staats *et al.*, 2007) and *Phytophthora infestans* (Liu *et al.*, 2005), the aflatoxin gene cluster in *Aspergillus* (Carbone *et al.*, 2007), host specific toxin the wheat pathogen *Phaeosphaeria nodorum* (Stukenbrock and McDonald, 2007), antigens in *Coccidioides* human pathogens (Johannesson *et al.*, 2004) and serine proteases in 10 fungal species (Hu and Leger, 2004). Positive selection in the defense R-genes of the plant is frequently followed by coevolution in the avirulence genes of the fungal parasite (Jones and Jones, 1997; Meyers *et al.*, 1998; Parniske *et al.*, 1997). This gene-for-gene interaction with corresponding responses in both the host and the parasite genomes is referred to as an ''arms-race'' process (Dawkins and Krebs, 1979).

Some regions in the genomes appear to be rapidly evolving. In *Fusarium graminearum* for instance, localized and highly polymorphic genomic regions are significantly enriched with genes favouring plant infection, such as secreted proteins, major facilitator transporters and cytochrome P450s (Cuomo *et al.*, 2007). Another example is found in the *Saccharomyces* strain YJM789, a human pathogen in patients with compromised immunity, where nonrandom regions with high polymorphism may be associated with the strain pathogenicity (Wei *et al.*, 2007). These rapidly evolving regions may have been selected to be hotspots of mutations if they contained many genes

under diversifying selection. Even in the absence of positive selection, a relaxation of selective constraints can be associated with rapidly evolving genomic regions, which provide raw material for selection.

VIII. HORIZONTAL GENE TRANSFER

Besides selection for rapidly changing genes, another means to acquire novelty in genomes is via horizontal gene transfer (HGT), which has been defined as the stable transfer of genetic material between individuals, but not directly attributable to vertical transmission from a parent to a descendent cell or individual (Kidwell, 1993). Fungi are likely candidates for experiencing HGT events since they readily undergo hyphal anastomoses, and heterokaryon incompatibility is rarely completely successful in preventing cytoplasmic or nuclear exchange (Walton, 2000). For many reported cases of HGT, there is prolonged physical contact among organisms due to shared habitat and symbiotic or antagonistic interactions that may favor the eventual genetic transfer (Garcia-Vallve *et al.,* 2000; Rosewich and Kistler, 2000a). Moreover, types of genetic elements that are particularly prone to exchange and introgression in the classic sense of vertical transmission also provide the bulk of evidence for HGT, including transposable elements (Diao *et al.*, 2006; Silva and Kidwell, 2000) or components of the mitochondrial genomes (Bergthorsson *et al.*, 2004). HGT may potentially provide the recipient organisms with new genetic materials that extend or improve capabilities for adaptation (e.g., Gojkovic *et al.*, 2004; Hall *et al.*, 2005), and this may be particularly important for pathogens and symbionts (Oliver and Solomon, 2008). In fact, HGT between pathogens and their hosts has been reported in diverse systems, including parasitic plants (Davis and Wurdack, 2004) and between bacteria and their animal hosts (Kondo *et al.*, 2002).

For most eukaryotes, transfer mechanisms are poorly understood, and HGT has been detected by indirect evidence, usually from incongruences between the phylogeny of the suspected genetic element and the accepted phylogeny of the organism harboring it. There are different types of "character-state discordance" for a given genetic element that lead to the suggestion of HGT: (*i*) high sequence similarity between distantly related organisms, (*ii*) irregular, or "patchy" phylogenetic distribution in a variety of lineages, (*iii*) sequence patterns (GC content, codon usage, introns, etc.) inconsistent with respect to its genomic context (Rosewich and Kistler, 2000b). Different methods, based on the sequence and phylogenetic analyses of discordances, have been proposed to detect the action of HGT (summarized by Bull *et al*. (1993) although none provides unequivocal evidence.

A number of alternative explanations for observed discrepancies include phylogenetic error, use of paralogous sequences, sporadic retention of shared-ancestral characters, introgressive hybridization and rate-variation among lineages (Rosewich and Kistler, 2000b).

Despite difficulties for proving HGT in fungi, several examples have been suggested involving different types of genetic elements. The literature on HGT in fungi shows a bias towards transfer from prokaryotes to fungi (Andersson, 2005; Andersson and Roger, 2002; Hall *et al.*, 2005), but counterexamples have also been suggested (Brown and Doolittle, 1999). Mitochondrial plasmids are widespread in filamentous fungi and are likely to be horizontally transferred, like the plasmid kalilo in *Neurospora intermedia* (Kempken *et al.*, 1992). HGT has also been proposed for transposable elements in fungi (Daboussi and Langin, 1994). There are fewer examples of HGT involving nuclear genes, however, interesting cases have been suggested (Klotz *et al.*, 1997; Li *et al.*, 1997; Liu *et al.*, 1997; Moens *et al.*, 1996), especially related to pathogenic gene clusters [for a comprehensive summary see Temporini and Van Etten (2004), but also Walton (2000); Friesen *et al.* (2006); Richards *et al.* (2006)]. It is known that host-specific toxins contribute to host-range in plant pathogenic fungi. In a remarkable example, Friesen *et al.* (2006) showed how the horizontal transfer of a gene encoding the ToxA protein, from *Stagonospora nodorum* to *Pyrenophora tritici-repentis*, gave rise to a pathogen population with a significantly enhanced virulence, thus providing the opportunity for the latter species to exploit a new niche. Note that introgression could give rise to the same pattern, and current methods to detect HGT do not necessarily distinguish between these two scenarios. HGT has been credited with the victorin toxin produced by *Cochliobolus victoriae* having been potentially transferred to *Cochliobolus carbonum*, thus giving rise to the pathogen that destroyed oats during the blight epidemic of the 1940s (Scheffer, 1991). HGT has also been supported for the transfer of ETP clusters (Patron *et al.*, 2007a), the ACE1 avirulence gene cluster (Khaldi *et al.*, 2008), and in a review exploring the likely association of interspecific HGT, new diseases and host-specific toxins (Oliver and Solomon, 2008).

Interestingly, whole chromosomes seem to have been transferred horizontally in fungi. HGT may also explain the origin of supernumerary chromosomes, which are present in some but not all members of a fungal lineage (patchy distribution), and contain DNA absent in other parts of the genome (discordant sequence patterns), as they may contain repetitive elements found only in the supernumerary chromosome, and conversely, lack repetitive sequences found in the other chromosomes (He *et al.*, 1998; Masel *et al.*, 1996; Poplawski *et al.*, 1997). However, it may be very difficult to distinguish horizontal transfers from introgression as the result of hybridization events.

IX. MIXING DIFFERENT GENOMES: HYBRIDIZATION

A more conventional means of mixing genes from different species is indeed hybridization. There has been growing concern during the last decade over the number of reported hybridizations in fungi, particularly among pathogenic species (see Olson and Stenlid, 2002, for a review). The increasing global transportation of plant and plant products creates new combinations of their associated pathogens. Such evolutionary events require serious attention because they may lead to the emergence of diseases with new epidemiological properties or host specificities (Brasier, 2001). Hybridization events can give rise to a variety of genomic constitutions as well as evolutionary consequences (Mallet, 2007). Here, we outline examples in fungal pathogens of hybridizations resulting in restricted introgression, homoploid hybrid speciation (emergence of a new species heterozygote at almost all loci), and allopolyploid hybrid speciation (emergence of a new species by addition of the two parental genomes, hence with higher ploidy than the parents).

The origin of hybrid genotypes, always in the presence of both parental lineages, presents the opportunity for back-crossing when hybrids are fertile. The result is often the introgression of some genes or genomic regions from one species into the predominant genetic background of the other. Theoretical expectations are that the transferred genes confer a higher fitness or that they are lost by drift. Evidence for introgressive hybridization is increasingly found with the use of multiple loci for phylogenetic reconstruction, where it is evidenced by incongruence among the individual gene trees. Important examples of introgression include *Ophiostoma novo-ulmi*, one of the two ascomycete species causing Dutch elm disease. Several strains show introgression from the less virulent species *Ophiostoma ulmi* (Bates *et al.*, 1993). Interestingly, one of the loci retained from *O. ulmi* belongs to the vegetative incompatibility system (vic #18) that prevents the spread of a virus highly deleterious to the fungus (Paoletti *et al.*, 2006). Other suggested cases of introgression are those of the wooden rotting fungus basidiomycete fungus *Coniophora puteana* (Kauserud *et al.*, 2007), the pathogen of gymnosperms *Heterobasidion annosum* (Gonthier *et al.*, 2007), *Fusarium graminearum,* responsible for the head blight of wheat (O'Donnell *et al.*, 2000), and the species of *Microbotryum* causing anther smut on *Silene acaulis* (Le Gac *et al.*, 2007). In these cases, no clear fitness advantages of hybrids as compared to other natural strains have been detected, but this possibility has not necessarily been investigated.

Hybridization resulting in a new and reproductively independent lineage that maintains a consistent haploid chromosome number is homoploid

speciation, synonymous with allodiploid speciation. Frequently, such hybrids are highly heterozygous and carry alternate alleles derived from each respective parental species (Mallet, 2007). An example of homoploid hybridization event associated with ecological speciation is provided by the combination of the basidiomycetes causing poplar leaf rust: *Melampsora medusae* parasitizing *Populus deltoides*, and *Melampsora occidentalis* parasitizing *Populus trichocarpa*. Although neither of these two fungal species is able to infect the commercial host hybrid *Populus deltoides* x *Populus trichocarpa*, the fungal hybrids between the two rust species can cause disease on the hybrid host (Newcombe *et al.*, 2000, 2001). Ancient homoploid speciation has also been suggested for the ascomycete complex *Gibberella fujikuroi* (O'Donnell and Cigelnik, 1997) and one of the species of *M. violaceum* parasitising *Silene vulgaris* (Devier *et al.*, 2008). In *Puccinia recondita f. sp. tritici*, the leaf rust pathogen of wheat (Park *et al.*, 1999) and *Heterobasidion annosum* (Garbelotto *et al.*, 1996), alleles from two different species have been found within single individuals, but lack of data impedes distinguishing between homoploid speciation and allopolyploid hybridization, described below.

Addition of two different genomes, thereby doubling chromosome number, is called allopolyploid speciation. One of the suggested examples in fungi is the asexual genus *Neotyphodium*, representing symbionts of grasses (Kuldau *et al.*, 1999), which arose from parasitic, sexual *Epichloë* species (Schardl *et al.*, 1997). There is an association between allopolyploid hybridization, asexuality, and mutualism in these endophytes. One of the scenarios to explain this association relies on the meiotic infertility of the hybrids (Selosse and Schardl, 2007). Asexual reproduction of *Neotyphodium* is achieved by seed-borne transmission within the host plant populations, so that the fungi may be selected for favoring mutualism because their reproduction depends entirely on that of their host; i.e. vertical transmission. Further, mutualistic hybrids would benefit from the broader combination of genes for alkaloid production, as these compounds act to protect the host plants from herbivores (Tanaka *et al.*, 2005). Another well-known example of allopolyploid hybridization is *Botrytis allii,* which is also asexual (Nielsen and Yohalem, 2001). Intriguingly, this hybrid exploits the same niche as one of his parents, suggesting that reproductive isolation alone allowed this new lineage to evolve. Polyploid hybrids of *Cryptococcus neoformans*, the human pathogen causing meningoencephalitis, also infect the same host as their parental lineages. Despite sterility, they are highly prevalent and hybrid vigor is invoked to account for their high prevalence (Lin *et al.*, 2007). Allopolyploid hybrids have also been identified among saprophytic *Saccharomyces* species among fermentation selected strains both for wine, beer, and cider production. Their phenotypes suggest that these strains were selected for their ability in combining physical properties like good

alcohol tolerance and fast growth owned by each of the parental species (Gonzalez *et al.*, 2008; Masneuf *et al.*, 1998).

Several hybridization events have thus been identified among parasites and mutualists, among all clades of fungi. Some have been related to an increase in virulence or host range, a shift in host spectrum or a switch towards mutualism (Olson and Stenlid, 2002). The ongoing identification of fungal cryptic species (Arnold and Lutzoni, 2007; Hunter *et al.*, 2006; Redecker and Raab, 2006) may boost the identification of other such events, because hybridization should be most frequent between recently separated species (Kauserud *et al.*, 2007; Le Gac *et al.*, 2007).

X. TRANSPOSABLE ELEMENTS

To understand genome evolution, particular attention should be paid to transposable elements. They are a ubiquitous component of genetic systems and comprise a large proportion of most eukaryotic genomes. The process of inheritance lends itself to the emergence of such cheating elements that gain a transmission advantage by autonomously over-replicating relative to the host genome and inserting copies into new chromosomal locations. In a large number of plant and animal species, transposable elements, or their degraded remnants, make up as much as half of the total nuclear DNA. In fungi, such elements typically comprise less than 20% of the genome, perhaps due to selection against the accumulation of large amounts of DNA sequences that are superfluous to the organism's necessary functions (Wostemeyer, 2002). Still, active proliferation of transposable elements and their dispersed copies across the genome have a major influence upon fungal evolution.

Transposable elements are represented by a diverse array of replication strategies whose origins remain heavily debated (Capy *et al.*, 2000). The mechanisms of replication generally involve either a copy-and-paste strategy typical of Class I retrotransposons, where an RNA compliment is made by the normal transcriptional processes and then reverse-transcribed back to a DNA copy that are inserted often randomly within the genome, or a cut-and-paste strategy typical of Class II transposons, where an element is excised during genome replication from one daughter strand of DNA and inserted ahead of the advancing DNA replication fork. By virtue of the relative efficiency of copy-and-paste, retrotransposons elements usually make a greater contribution to total genome size (Gollotte *et al.*, 2006). However, with the advent of genomic technologies, new types of elements are being discovered that do not fit neatly into existing classifications. For example the

Helitron elements, present in genomes as diverse as vertebrates and the wood rot fungus *Phanerochaete chrysosporium*, are not reverse-transcribed from RNA but instead use a DNA rolling-circle replication (Poulter *et al.*, 2003). Similar in mechanism to bacterial viruses, individual *Helitrons* can produce many descendant copies to proliferate within the genome. *Cryptons* elements, a group of tyrosine-recombinase encoding element discovered in several pathogenic fungi (Goodwin *et al.*, 2003), exhibit a unique combination of sequence characteristics reflecting both DNA transposons and RNA retrotransposons.

Fungi possess the full spectrum of transposable element types, where their influence as powerful forces for genetic change is due to both insertional mutations and to promoting chiasma formation between non-homologous sites (i.e. ectopic recombination). The random nature of integration for most transposable elements can lead to the disruption of coding sequences of genes or the separation of genes from necessary promoter regions. In several pathogenic fungi, such as *Leptosphaeria maculans* and *Magnaporthe grisea*, sequences coding for avirulence genes are found in genomic regions dense with transposable elements (Fudal *et al.*, 2007; Gout *et al.*, 2006; Kang *et al.*, 2001; Rehmeyer *et al.*, 2006), potentially contributing to the extreme variability of avirulence genes that is associated with host–pathogen coevolution. Fungi also have extremely high levels of structural polymorphism for karyotypes, often with chromosome length variation even within populations (Zolan, 1995). The potential contribution of recombination between transposable elements and their enzymes responsible for DNA breakage and repair has been suggested to increase rates of chromosomal rearrangements for a number of species, including *Magnaporthe oryzae* (Thon *et al.*, 2006) and *Fusarium oxysporum* (Daboussi, 1997).

Despite various reports for beneficial roles of transposable elements or their "domestication" to perform functions necessary to the organism (Volff, 2006), on average the increased mutations rates caused by insertion and ectopic recombination decrease individual fitness (Arkhipova and Meselson, 2005). Genomes are protected against the proliferation of transposable elements by a variety of mechanisms, and fungi possess perhaps a broader range of defenses than many eukaryotic groups. Moreover, the empirical tractability of fungi has greatly facilitated studies of co-evolution between parasitic DNA and the host genome. A common theme to genomic defense is the detection of repeated or newly inserted DNA sequences. For example, the mechanism of Meiotic Silencing of Unpaired DNA (MSUD) in *Neurospora crassa* detects the hemizygosity of newly inserted elements and employs post-transcriptional gene silencing, related to RNA-interference, to prevent their further replication (Shiu *et al.*, 2001). Also originally found in *N. crassa*, and now known to occur in a broad range of ascomycete and

basidiomycete fungi (Galagan and Selker, 2004; Hood *et al.*, 2005), Repeat-Induced Point Mutation (RIP) is a most effective defense and one which provided the best evidence of having evolved to constrain transposable element populations. RIP is a process that hypermutates repeated DNA sequences of a few hundred base pairs, thus making transposable element copies nonfunctional by inducing non-sense or stop codons and decreasing their sequence similarity between non-homologous sites (i.e. reducing the risk of ectopic recombination). This defense is only known to occur in fungi, and specifically occurs during the dikaryotic stage where the continued intracellular separation of haploid nuclei may facilitate the detection of repeated DNA; whereas in diploid cells all sequences are essentially present in at least two copies. RIP is frequently described as being associated with the sexual stage of the fungal life cycle, which may be interpreted as being involved in meiosis. However, in most ascomycetes, including *N. crassa*, dikaryon formation occurs only briefly prior to meiosis, whereas in basidiomycetes, the dikaryotic is greatly prolonged and the potential impact on the genome defense deserves further study. The paucity of active transposable elements in lineages of *N. crassa* with RIP is evidence of the efficacy of this defense system, but there may be other long-term consequences for fungal evolution. RIP also prevents the duplication of any housekeeping or pathogenicity genes, thus greatly limiting the evolutionary potential of sequence duplications to acquire novel and adaptive functions (Brookfield, 2003).

XI. TELOMERES

Telomeres are genomic regions particularly prone to transposable element accumulation and rapid evolution, thereby playing a role in host adaptation (Sánchez-Alonso and Guzman, 2008). As in other eukaryotes, the ends of fungal chromosomes consist of tandem arrays of simple sequence repeats that are usually GT-rich. The most common telomeric repeat in filamentous fungi is $(TTAGGG)_n$, as is also found in basal metazoans and vertebrates (Traut *et al.*, 2007). The telomere repeats are associated with a number of proteins that protect the chromosome ends from degradation and have roles in the silencing of neighboring genes in the adjacent subtelomeric region. Physical proximity during meiosis between non-homologous chromosome ends is thought to promote ectopic recombination between shared sequences in the subtelomeric regions, leading to the amplification and diversification of telomere-linked genes (Freitas-Junior *et al.*, 2000). The dynamic nature of telomeres may contribute to the variation critical for rapid evolution of

host-parasite interactions (Barry *et al.*, 2003; Freitas-Junior *et al.*, 2000; Schaffzin *et al.*, 1999). In several fungal pathogens of humans, subtelomeres contain gene families encoding immunogenic extracellular proteins. To avoid detection by the host, the pathogens switch gene expression among the different genes of these families, taking advantage of the silencing mechanism resulting from the telomere repeat-associated proteins (De Las Penas *et al.*, 2003; Keely *et al.*, 2005).

Subtelomere regions of plant pathogenic fungi do not harbor families of surface protein genes, but appear to still play an important role in pathogenicity. For instance, a significant proportion avirulence genes is located very near to telomeres both in *Magnaporthe oryzae* (Chen *et al.*, 2007; Rehmeyer *et al.*, 2006) and in *Phytophthora infestans* (van der Lee *et al.*, 2001). For some of these fungal avirulence genes, the importance of the position near telomeres to pathogenicity has been demonstrated, the truncation of chromosome ends allowing loss of the genes and thus the gain of virulence (Orbach *et al.*, 2000). In contrast, silencing near telomeres does not seem to play an important role in pathogenicity in fungi. Rehmeyer *et al.* (2006) proposed that the recruitment of avirulence genes to subtelomeric regions may be adaptive because the subsequent instability favors the emergence of strains that can avoid detection by resistance mechanisms (by truncations of the chromosome parts harbouring the avirulence genes) and that the pathogen can thus colonize a more diverse collection of host genotypes. However, it is important to recognize that recruitment to subtelomeric regions cannot be selected for by an advantage to gene deletion: if the deletion is adaptive (*i.e.,* allows colonizing a new host), then the selected genotype will not have the gene in telomeric position, and the parental genotype with the gene in telomeric position will not be selected for. Yet, telomeric positions can be adaptive by generating allelic variability, and therefore be selected for, and then allow further variability through deletion. Genes near telomeres indeed seem to exhibit higher sequence variability than in other chromosome regions, although it is not clear yet what mechanism is responsible for this variability.

Subtelomeres of pathogenic fungi also contain large families of host adaptation genes, as do other pathogenic microbes. For instance, many fungi contain secondary metabolite gene clusters near telomeres (Rehmeyer *et al.*, 2006), as well as putative secreted proteins that may be involved in plant infection (Rehmeyer *et al.*, 2006). Other gene families are present in subtelomeres in fungi, that may not be directly involved in host-parasite interactions, such as helicase gene families (Gao *et al.*, 2002; Louis, 1995; Sanchez-Alonso and Guzman, 1998).

XII. INTRONS

Introns are also highly dynamic in fungal genomes, and some of them exhibit traits remarkably similar to transposable elements. Fungal genomes are gene dense, with relatively simple gene structure compared to plant and animals. The number of introns per gene ranges from much less than one in *S. cerevisiae* (Goffeau *et al.*, 1996), to between 1 and 2 introns per gene for other recently sequenced ascomycetes (Dean and *et al.*, 2005; Galagan *et al.*, 2003). The basidiomycete *Cryptococcus neoformans* contains an average of seven introns per gene (Loftus *et al.*, 2005). Introns are also typically short in fungi, averaging between 80 and 150 bp in the ascomycetes and averaging 68 bp and down to 35 bp in the basidiomycete *C. neoformans* (Loftus *et al.*, 2005). As in many eukaryotes, intron-poor species of fungi have more introns in 5′ positions. Although fungi appear to use alternative splicing less frequently than metazoans, the most extensive genome-wide survey on alternative splicing in a fungus (in *C. neoformans,* Loftus *et al.*, 2005) revealed evidence of alternative splicing for 4% of the genes (against 40–60% in humans for instance, Modrek and Lee, 2002).

Fungal spliceosomal introns (i.e., the most common insertions found in nuclear pre-mRNA genes) are remarkably dynamic: Nielsen *et al.* (2004) estimated between 150 and 250 gains and between 150 and 350 losses in each of the four Ascomycete lineages they examined (*A. nidulans, F. graminearum, M. grisea* and *N. crassa*), spanning ca. 330 millions years evolution. Fungi also carry retrotransposable elements in the form of Group I and Group II introns in their mitochondria, characterized by a distinct RNA secondary structure and self-splicing pathway (Haugen *et al.*, 2005). In particular, the Group I intron insertion has also been observed in the nuclear genome of parasitic fungi in the genus *Cordyceps* (Nikoh and Fukatsu, 2001). Frequent horizontal transfer between evolutionarily distinct lineages of fungi has been proposed based on phylogenetic distribution of Group I introns (Dujon, 1989; Feau *et al.*, 2007; Hibbett, 1996; Holst-Jensen *et al.*, 1999; Mouhamadou *et al.*, 2006) and this possibility has been demonstrated in experimental settings *in vivo* (Muscarella and Vogt, 1993).

XIII. MITOCHONDRIAL GENOMES

Mitochondrial genomes are another component of genetic information and have their own rules of genomic evolution. Mitochondria are derived from an ancient endosymbiotic α-Proteobacterium and have retained their own genetic system. However, in all eukaryotes, the majority of mitochondrial genes have

been transferred to the nuclear genome and their protein products are imported into mitochondria. The reduction of the mitochondrial genome is probably due to a selection for rapid replication and to rescue conserved genes from the higher rate of mutation in organelles compared to the nuclei (Selosse *et al.*, 2001). Genes whose expression is regulated by reduction/oxidation reactions are preferentially retained in the mitochondrial DNA (mtDNA), perhaps providing more rapid control over their activities (Allen, 2003).

Currently, completely sequenced mtDNAs are available from 49 fungal species, representatives of all four fungal phyla (http://megasun.bch.umontreal.ca/ogmp/projects/other/mt_list.html). Fungal mitochondrial DNAs typically encode only 30–40 genes (Bullerwell and Lang, 2005). This is much fewer than the mtDNA of many other eukaryotes, in particular plants and protists, that can carry 50–100 mt genes (Bullerwell and Lang, 2005). This suggests that many of the mitochondrial genes have been lost early in fungal evolution, or even in the opisthokont lineage, since animals seem to have also retained few genes in their mtDNA (Bullerwell and Lang, 2005). Further genome reduction also occurred later in some fungal lineages, some chytridiomycetes having for instance completely lost their mtDNA (Bullerwell and Lang, 2005). A core set of common genes seems to be retained in most fungal mtDNAs, but they exhibit otherwise remarkable polymorphism, mostly due to variation in intron number, gene arrangement, mobile retro-elements, the presence/absence of plasmids, and also gene number (Burger *et al.*, 2003; Gray *et al.*, 1999; Lang *et al.*, 1999).

Fungal mtDNAs seem to be involved in senescence, i.e. growth arrest of the mycelium that appears after some time of laboratory culture in certain filamentous fungi, such as *Podospora anserina*. Senescence has indeed been shown to be associated with rearrangements of the mitochondrial genome, although the causality is not completely clear (Griffiths, 1992). Mutant mtDNAs, called ''suppressive mtDNA'', are however able to induce senescence when transmitted *via* anastomoses between hyphae from different mycelia. Such suppressive mtDNA mutations can thus reduce virulence in some phytopathogenic fungi and be transmitted cytoplasmically. In the chestnut-blight fungus *Cryphonectria parasitica* for instance, the syndrome associated with mutant mtDNAs and their mode of transmission resemble strikingly those of the virus responsible for hypovirulence (Bertrand, 2000). Another example of defective mitochondria is the petite mutation in *Saccharomyces cerevisiae* (Taylor *et al.*, 2002). Mutant mtDNAs can persist even if they lower the fitness of the cells that carry them if they have a sufficient replication advantage compared to wild type mitochondria and if they are under the dynamics of small populations (Taylor *et al.*, 2002): they are then selected for at the cell level and selected against at the organism level.

A means to reduce the frequency of intra-genomic conflicts between different levels of selection is to have mtDNAs variability restricted by uniparental transmission (Aanen *et al.*, 2004; Hoekstra, 1990;). In anisogamous eukaryotes, only the female usually transmits her mitochondria to the zygote (Hurst, 1996). Similarly, only one of the mating types passes on its mitochondria in some bipolar fungi, e.g. in *Cryptococcus neoformans* (Yan and Xu, 2003) and in *Microbotryum violaceum* (Wilch *et al.*, 1992). In some basidiomycetes, unidirectional nuclear migration during mating allows the resulting zygote to carry a single mtDNA type (e.g. *Agaricus bitorquis,* Aanen *et al.*, 2004; Hintz *et al.*, 1988). In other basidiomycetes, mitochondria are sorted out in the young mycelium, a single mtDNA remaining after a few cell divisions, the proximal mechanism being unknown, e.g. in *Agaricus bisporus* (de la Bastide and Horgen, 2003) and in *Agrocybe aegerita* (Barrosso and Labarere, 1997). In some tetrapolar basidiomycetes with multiple mating types, however, the mycelium can remain a mosaic or even heteroplasmic (*e.g.*, *Pleurotus ostreatus,* Matsumoto and Fukumasa-Nakai, 1996), which leaves room for multi-level selection conflicts (Aanen *et al.*, 2004; Hoekstra, 1990). The most frequent occurrence of such conflict is seen in the *S. cerevisiae* yeast, where the unusual cassette system of mating type switching precludes any such control over mitochondrial inheritance and leads to the expectation for higher rates of heteroplasmy.

XIV. COMPARISON WITH THE GENOMICS OF PATHOGENIC BACTERIA

The genomics of prokaryotes has seen a great development in recent years, and has had a major impact on the research of microbial pathogenesis and symbiosis. Comparative genomics of strains and species of bacteria has provided new insights into the evolution of virulence and it may be interesting to compare the genomics of fungal and bacterial pathogens. Although comparative genomics in pathogenic and symbiotic fungi is only at an early stage, it seems that bacteria and fungi have different modes of host adaptation on the genomic level. Analyses of genome sequences in bacteria have demonstrated that many of the genes required for virulence are restricted to pathogenic organisms and that they have been introduced into the genomes by horizontal gene transfer. These HGT events often involve whole cassettes of genes, ranging in size from 5 to 100 kb. Their frequent integration at or near tRNA loci suggests that many were introduced via phage-mediated transfer events (Arnold *et al.*, 2003; Dobrindt *et al.*, 2004; Ochman and Moran, 2001). This process is so pervasive that species-specific chromosomal

regions containing virulence genes are now classified under the general heading of "pathogenicity islands" (Arnold *et al.*, 2003; Dobrindt *et al.*, 2004; Ochman and Moran, 2001). In the animal pathogen *Dichelobacter nodonus* for instance, 20% of the genome is derived from lateral gene transfer and most of these transferred regions seem to be associated with virulence (Myers *et al.*, 2007). Pathogenicity islands have been described in a wide range of both plant and animal bacterial pathogens, and it has become evident that their general features are displayed by a number of DNA regions with functions other than pathogenicity, such as symbiosis and antibiotic resistance, and the general term genomic islands has been adopted (Arnold *et al.*, 2003; Dobrindt *et al.*, 2004). Such lateral gene transfer of genes involved in host adaptation is much less pervasive in fungi, although some examples have been reported (see section on horizontal gene transfers).

A large set of bacterial symbionts and pathogens have undergone massive gene loss, mostly because the host presents a constant environment rich in metabolic intermediates that renders some genes useless under a strictly symbiotic or pathogenic life-style. Such consistent patterns of genome streamlining do not seem to have occurred across the full range of pathogenic and symbiotic fungi, in particular being influenced by whether the fungal species retains some parts of their life cycle as a free-living stage. The ectomycorrhizal and saprophytic fungus *Laccaria bicolor* for instance contains a huge number of genes compared to other fungi (Martin *et al.*, 2007). Some gene losses have been shown to be adaptive in bacteria, such as surface proteins recognized by the hosts (Nakata *et al.*, 1993; Wren, 2000). Also, gene duplication may occur in bacterial pathogens as a mechanisms for generating variation in surface antigenic structure (Wren, 2000). These losses of avirulence genes and gene duplication in families are similar to the processes observed in the genomics of pathogenic fungi.

XV. CONCLUSION

The evolution of fungi is estimated to span at least 500 million years (Taylor and Berbee, 2006), during which time these organisms have explored virtually all available ecosystems and nutritional modes of associations with other living organisms. The adaptations to such a wide range of ecological niches and lifestyles were certainly made possible by the tremendous flexibility of the fungal genomes. This flexibility is due in no small part to pervasive gene duplication at different scales, including that of the whole genome, which has been shown to promote new functions. A complementary mechanism to duplication is the loss of different genes, which can lead to speciation and

specialization, as exemplified in yeasts. Chromosomal rearrangements promote genetic novelty by changing the context of elements already present and introducing new regulatory possibilities. Hybridization contributes significantly to the mixing of fungal genes and horizontal gene transfer, although difficult to prove, is likely to have contributed to the extensive biochemical repertoire that enables fungi to colonize new niches. Genetic elements such as introns, transposable elements, repetitive sequences, supernumerary chromosomes, and the mitochondrial genome further contribute to the enormous genetic variability observed in fungi.

Comparative genomic studies in plant pathogenic and symbiotic fungi, although still in the early stages and limited to a few pathogens, have already brought many insights into the evolution of the pathogenic lifestyle, in particular into the mechanisms of virulence and host adaptations. There is a marked bias in the sequencing efforts towards pathogenic fungi, but current projects are covering the fungal genomes of biotrophs and symbiont species that will hopefully allow us to gain insight into the partnerships between fungi and plants, a critical component of terrestrial and agro-ecosystems. Development of advanced genomics tools and infrastructure is critical for efficient utilization of the vast wealth of available genome sequence information and will form a solid foundation for integrated studies of the biology of plant pathogenic fungi. Future research will also benefit from efforts in the analysis of gene expression evolution through micro-array data and other powerful techniques. Changes in gene expression are indeed difficult to assess from genome sequences, but may play important roles in adaptations (Wapinski *et al.*, 2007). It will also be important to understand the variation of genomes within species, in order to observe fungal evolution in action.

ACKNOWLEDGMENTS

We thank Marc-Henri Lebrun for initiating this review and an anonymous referee for helpful comments. We acknowledge the grants ANR-06-BLAN-0201, ANR 07-BDIV-003, and DEB-0747222.

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