Ecological Genomics of Adaptation and Speciation in Fungi

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Abstract

Fungi play a central role in both ecosystems and human societies. This is in part because they have adopted a large diversity of life history traits to conquer a wide variety of ecological niches. Here, I review recent fungal genomics studies that explored the molecular origins and the adaptive significance of this diversity. First, macro-ecological genomics studies revealed that fungal genomes were highly remodelled during their evolution. This remodelling, in terms of genome organization and size, occurred through the proliferation of non-coding elements, gene compaction, gene loss and the expansion of large families of adaptive genes. These features vary greatly among fungal clades, and are correlated with different life history traits such as multicellularity, pathogenicity, symbiosis, and sexual reproduction. Second, micro-ecological genomics studies, based on population genomics, experimental evolution and quantitative trait loci approaches, have allowed a deeper exploration of early evolutionary steps of the above adaptations. Fungi, and especially budding yeasts, were used intensively to characterize early mutations and chromosomal rearrangements that underlie the acquisition of new adaptive traits allowing them to conquer new ecological niches and potentially leading to speciation. By uncovering the ecological factors and genomic modifications that underline adaptation, these studies showed that Fungi are powerful models for ecological genomics (eco-genomics), and that this approach, so far mainly developed in a few model species, should be expanded to the whole kingdom.

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4.1 Introduction: The Importance of Fungi in Ecology

Fungi are probably the most underexplored kingdom of Eukaryotic life. Paradoxically, they also contain one of the most studied organisms: the budding yeast *Saccharomyces cerevisiae*. This astonishing contrast stems from two specificities of Fungi. First, unlike most plants and animals, but like many bacteria, the majority of Fungi are either microscopic, non free-living, or dependant on highly specific biotic and abiotic conditions to live. Hence, a substantial part of fungal diversity cannot be observed *in natura*, isolated, or described and studied with the standard methods used in other eukaryotes. Second, fungal diversity has been mischaracterized for a long time, mostly because of the high diversity of sexual and growth forms, whereas these two features have been highly conserved during plant and animal evolution. This has often resulted in several species described for the same organism. For all these reasons, as Hibbet and Taylor (2013) recently pointed out, experimental genetics and evolutionary research on the fungal kingdom often focused on few organisms that, like *S. cerevisiae*, had small genomes and could be easily isolated, described and studied, but that represented a biased view of the actual fungal diversity.

Given their ability to absorb complex carbon sources and minerals from many environments, their resistance to unfavourable conditions and high dispersal abilities of their *spores*, Fungi have adopted various life history traits, and colonized most ecological niches (James et al. 2006; Stajich et al. 2009). Their simple organization – a mass of mostly undifferentiated cells, the thallus – allows them to form complex structures and associations with other organisms. Hence, even in purely mineral habitats, they are able to associate with algae or cyanobacteria to form lichen, enabling them to draw carbon from the air (Nash 2008). In the soil, Fungi form vast networks of rhizomes that recycle organic material, and they can make billions of connections with plant roots – *mycorrhizae* – providing plants with essential minerals in exchange for complex carbon compounds (Read 1991). They are present in the stomach of herbivorous animals where they help degrade fibres in return for a favourable reproductive environment (Nicholson et al. 2010). Many Fungi are also efficient pathogens and parasites of many plants and animals, able to perform part or all of their metabolism and life cycle at the expense of their hosts (San-Blas and Calderone 2008). Finally, Fungi play a central role in human societies with respect to health, industry, agronomy and research (Kendrick 2011).

Most Fungi can reproduce both asexually and sexually. However, the features of sexual reproduction vary greatly among and within species, and all modes of sexual reproduction can be found, from isogamy to anisogamy, from *homothallism* to *heterothallism*, with sometimes thousands of different mating *idiomorphs* (Billiard et al. 2011).

The high diversity and divergence in life history traits and reproductive modes among species has left a profound imprint on the evolution of fungal genomes. Here I review recent progresses in fungal eco-genomics and their contribution to the understanding of the following issues: (1) what are the main genomic features of adaptation to contrasting life history traits and to various modes of sexual reproduction? (2) How does adaptation to contrasting environments

shape fungal genome evolution? And (3) what are the mechanisms underlying speciation at the genomic level?

4.2 Macro-ecological Genomics in Fungi

Fungi are the result of about 1 billion years of evolution, during which genomes of different clades underwent profound molecular changes. Recent advances in molecular biology and genomics have allowed partial reconstruction of the evolutionary history of the kingdom, and improved the taxonomic classification (Fig. 4.1). Here I present recent contributions of fungal genomics to the understanding of how the main clades of Fungi emerged and how their adaptation to life histories shaped their genome organization.

4.2.1 Main Evolutionary Features of Ascomycota and Basidiomycota Genomes

Comparative genomics has been extensively used to infer the evolutionary history of Ascomycota genomes (Fig. 4.2). Evolutionary reconstruction from divergent clades suggests that the ancestral Ascomycota genome was likely small (about 12 Mb) and remained highly conserved in size during the evolution of Ascomycota yeasts, while the diversification of higher Ascomycota (Pezizomycotina) was characterized by a global increase in genome size that stabilized at about 40 Mb (Kelkar and Ochman 2012). Although the phylogenetic organization of Basidiomycota is less well defined, the same observation holds true for this clade, with a generally small genome size (around 15 Mb) in Pucciniomycotina, located at the root of the evolutionary tree, and a

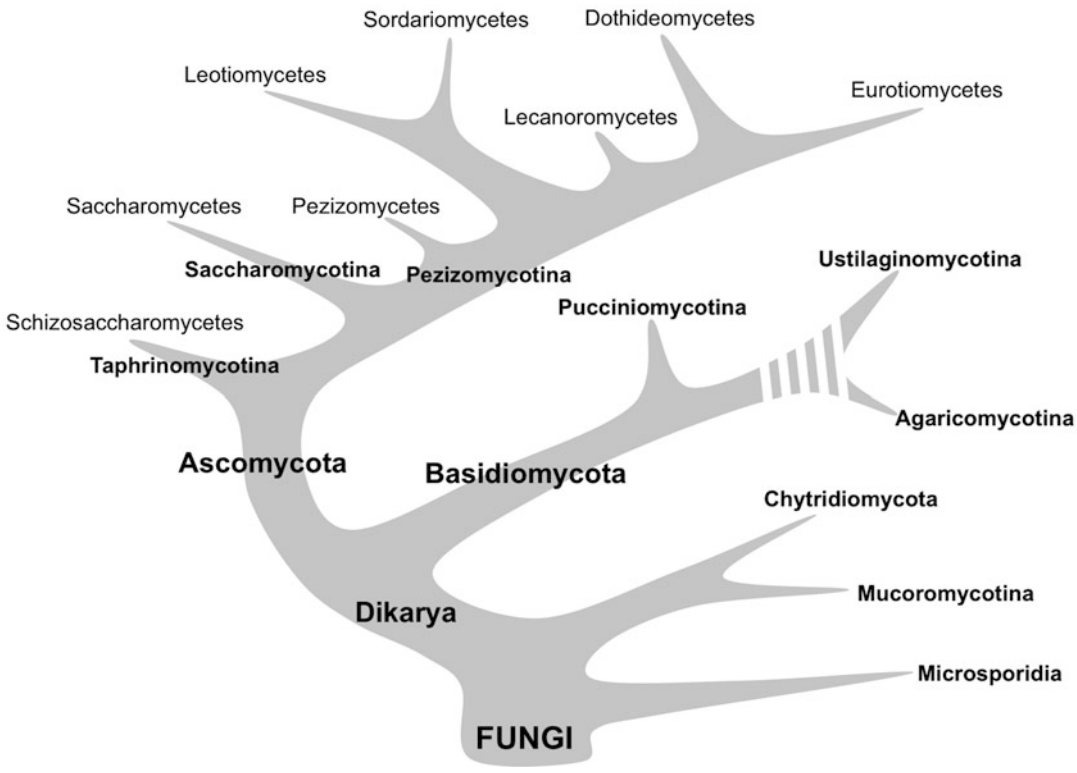

Fig. 4.1 Evolutionary tree of fungi based on whole-genome sequencing. Only main taxonomic groups are represented. Uncertain phylogenetic positions are hatched (Adapted from Kelkar and Ochman (2012); Wang et al. (2009); branch width is unrelated to the number of species in the taxonomic groups)

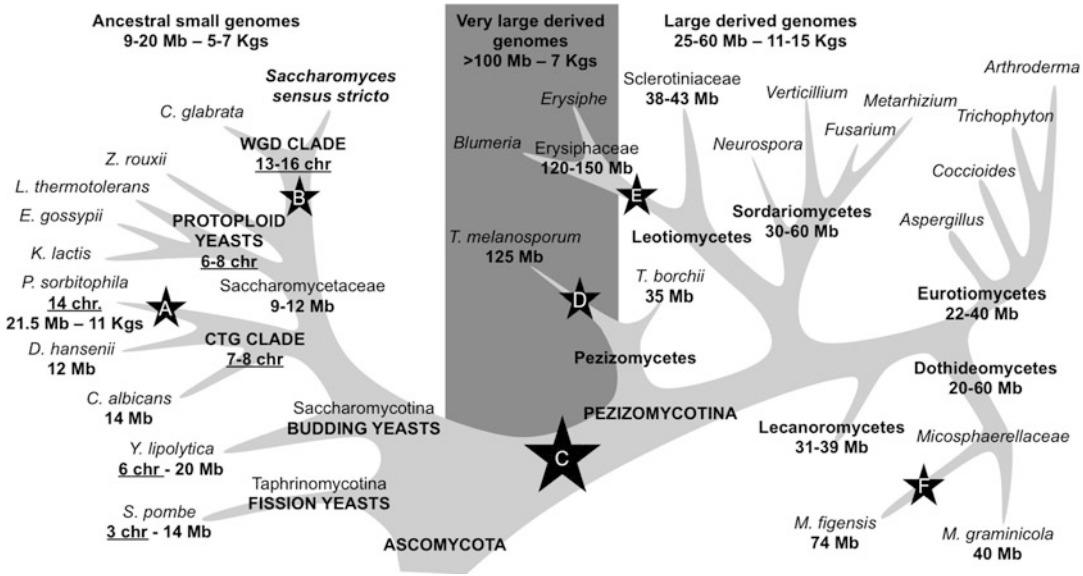

Fig. 4.2 Genome size evolution in Ascomycota based on whole-genome sequencing (Burmester et al. 2011; Dujon 2005; Gao et al. 2011; Kelkar and Ochman 2012; Souciet et al. 2009; Wang et al. 2009). Only representative taxa are shown. Number of chromosomes (chr), genome size (Mb) and number of genes in thousands (kg) are given in intervals when available for several species (Grigoriev et al. 2012; Kelkar and Ochman 2012; Souciet et al. 2009). The ancestral Ascomycota genome was presumably small (9–20 Mb and 5–7 kg) and remained relatively stable during the evolution of budding yeasts. Stars indicate examples of important genome expansions and remodelling. (**a**) *Pichia sorbitophila* resulted from a recent hybridization by polyploidization, as attested by the doubling of genome size and number of genes (Louis et al. 2012). (**b**) Unlike the ancestral and paraphyletic group of protoploid yeasts, yeasts of the WGD clade (including *S. cerevisiae*) resulted from a whole-genome duplication, as attested by the doubling of chromosome number followed by gene losses (Kellis et al. 2004; Souciet et al. 2009). (**c**) Genome expansion and increase in the number of genes during early evolution of Pezizomycotina, followed by (**d**–**f**) several independent genome expansions after proliferation of transposable elements in several symbiotic (**d**) or pathogenic species (**e**–**f**), and (**d**–**e**) massive gene loss (Kelkar and Ochman 2012; Martin et al. 2010; Santana et al. 2012). Branch width is unrelated to the number of species in taxonomic groups

global increase of up to 40 Mb or more in some Agaromycotina (Fig. 4.3). In both cases, this increase in genome size resulted from an increase in gene number from 5,000–7,000 to 10,000–20,000 genes and, at least in Pezizomycotina, from an increase in number of introns and mobile elements (Grigoriev et al. 2012; Kelkar and Ochman 2012). Many other structural features were revealed by comparative genomics. For instance, important increases in genome size (75–150 Mb) occurred despite massive gene loss (about 7,000 remaining genes), because of the proliferation of mobile elements in independent clades of Pezizomycotina (Kelkar and Ochman 2012; Martin et al. 2010; Santana et al. 2012; Fig. 4.2). The evolution of Ascomycota yeasts was marked by a strong conservation in genome size, but a whole-genome duplication (WGD) occurred in Saccharomycetaceae, as attested by the presence of numerous duplicated genes and the doubling of chromosome number. This was likely followed by a massive gene loss in this clade, since the number of genes is equivalent between post-WGD and protoploid yeasts (Dujon et al. 2004; Kellis et al. 2004; Souciet et al. 2009). This evolutionary event gave rise to the so-called "WGD clade", containing the model yeast species *S. cerevisiae* (Fig. 4.2). Another interesting feature of the evolution of Ascomycota yeasts is a slight modification in the standard genetic code, which is unique among eukaryotes and gave rise to the so-called "CTG clade", which includes the model *Candida albicans*. In these yeasts, 97 % of CUG codons are translated into serine and 3 % into leucine, whereas 100 % are translated into leucine in most eukaryotes. The comparison of proteins

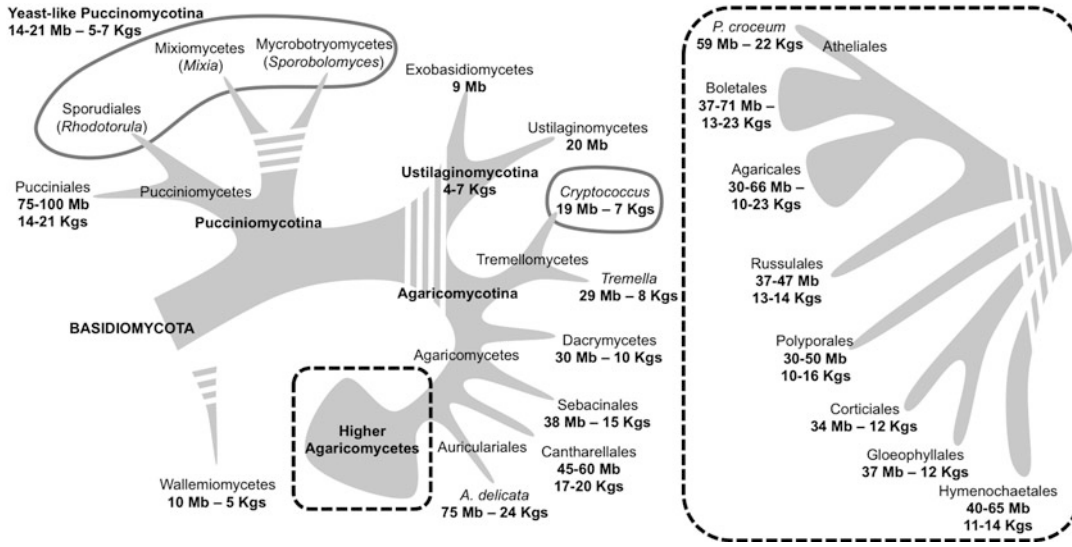

Fig. 4.3 Genome size evolution in Basidiomycota (Aime et al. 2006; Hibbett 2006; Wang et al. 2009). Only main taxonomic groups are represented. Uncertain phylogenetic positions are hatched. Genome size (Mb) and number of thousands of genes (kgs) are given in rank when available for several species (Grigoriev et al. 2012). Yeast-like species, characterized by small genome sizes, are circled in *grey*. The detail of phylogeny in higher Agaricomycetes is represented in the *dotted frame* on the *right*. Branch width is unrelated to the number of species in taxonomic groups

from CTG with their orthologs from non-CTG yeasts showed that the replacement of leucine by serine in the CTG ancestor was not random, but preferentially occurred in regions where it had no effect on protein function (Rocha et al. 2011). Inversely, when serine were experimentally replaced by leucine in *C. albicans*, it had only slight effects on its fitness, like changes in colony morphology (Gomes et al. 2007). Moreover, Gomes et al. (2007) showed that the proportion of CUG codons translated into leucine in *C. albicans* significantly increased in stressful conditions, as temperature increase, pH decrease or oxidative stress. These two studies suggest that flexibilities in the standard genetic code could enhance adaptability of CTG yeasts to changing environments.

4.2.2 Evolution of Life History Traits Modulates Fungal Genomes

Comparative genomics provides insights in the evolutionary processes that remodelled fungal genomes, and is thus tightly linked to the study of life history traits. The large diversity of life forms, reproductive modes and metabolism of Fungi makes them perfect models to investigate the role of adaptation in modelling genome architecture.

4.2.3 Genome Size Variation in Relation to Lifestyle

The most obvious feature of adaptation to lifestyle in Fungi is the clear correlation between genome size and multicellularity (see Fig. 4.4). Indeed, smaller genomes are usually observed in unicellular or "yeast-like" Fungi, (9–20 Mb), whereas most Fungi producing multicellular thalli have larger genomes (20–125 Mb) and a higher number of genes. This evolutionary trend is independently observed in Ascomycota and Basidiomycota (Fig. 4.4), and broadly observed in multicellular organisms in other branches of life (Koonin 2011). However, many Fungi deviate from this universal pattern because of diverse forms of life, such as parasitism or mycorrhization (Fig. 4.4).

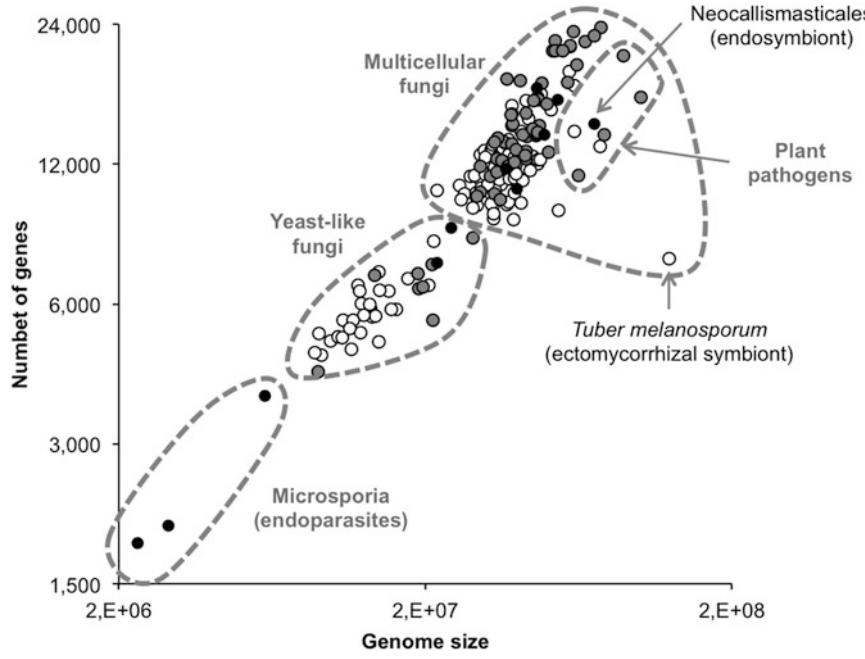

Fig. 4.4 Correlation between genome size and the number of genes reveals footprints of genome evolution on some life history traits of Fungi. *Grey circles* represent Basidiomycota; *white circles* represent Ascomycota; *black circles* represent other sub-phyla of Fungi. All available genomic data from Fungi were compiled here (Grigoriev et al. 2012; Peyretaillade et al. 2011; Souciet et al. 2009)

Microsporidia are the most primitive fungal organisms (Fig. 4.1) and so far have the smallest known eukaryotic genomes. For instance, *Encephalitozoon intestinalis*, a unicellular and obligate intracellular animal parasite, has 1,833 genes contained in a 2.3 Mb genome (Fig. 4.4). Peyretaillade et al. (2011) found that such small genomes are likely derived from a larger genome, and have been probably highly compacted after the reduction of non-coding sequences and the length reduction of protein-coding sequences. The authors suggested that this gene compaction could result from loss of protein domains involved in many protein-protein interactions, since their related functions became non-essential for the parasite. Large genome expansions frequently occurred in phytopathogenic Ascomycota, in spite of a low gene density (Fig. 4.4). These expansion could result from dispensable elements such as small chromosomes in *Mycosphaerella* (Goodwin et al. 2011) or from the proliferation of non-coding sequences, such as introns and mobile elements in *Mycosphaerella* (Santana et al. 2012), *Erysiphe* and *Blumeria* (Kelkar and Ochman 2012). Genome expansion in relation with pathogenicity was also observed in more basal Fungi like Mucoromycotina, whose genome is composed of about 20 % of transposable elements (Ma et al. 2009). In most cases, this accumulation of repeated and mobile elements is found around genes encoding virulence factors such as proteases, suggesting that these elements may be involved in adaptation to pathogenicity (Kelkar and Ochman 2012).

4.2.4 Expansion of Gene Families in Relation to Ecological Specialization

The adaptation of organisms to new ecological niches is the result of the loss and acquisition of specialized functions. These functions are often regulated by families of genes, i.e. genes with similar functions, resulting from repeated

duplications of an ancestral gene, followed by functional divergence (Demuth et al. 2006). The largest fungal genomes so far are found in truffles (Ascomycota), in particular *Tuber melanosporum* (125 Mb), despite its small gene number (7,500). Some of the typical Ascomycota gene families absent in the *T. melanosporum* genome are involved in secondary metabolism. Those became less essential in this ectomycorrhizal species, since the host plant provides secondary metabolites. Comparative genomics in *T. melanosporum* also provided functional evidence for the effect of *symbiosis* on the genome composition, for instance the rise of gene families encoding enzymes involved in degradation of host cellular walls (Martin et al. 2010). Interestingly, genes with identical functions also exist in other symbiotic Fungi like *Laccaria bicolor* (Basidiomycota, Agaricales; Martin et al. 2008), but they arose from independent evolutionary events (Martin et al. 2010). Wood decay Fungi are characterized by efficient lignin depolymerisation, and many functional evidence of this ability were found in fungal genomes. In Agarycomycetes (Basidiomycota), Floudas et al. (2012) used comparative genomics to highlight the early expansion of gene families involved in lignin depolymerisation, such as genes coding for peroxidases. They suggested that wood decay was the ancestral state in this taxon, but that this ability is highly variable among independent lineages where these genes were lost. For instance, in *Phanerochaete chrysosporium* (Basidiomycota, Polyporales), extra-cellular degradation of lignocellulose is permitted by a complex set of multiple gene families, such as cellulases and pyranoseoxidases. However, in the case of *Postia placenta*, a close relative of *P. chrysosporium*, many of these genes were lost, thus making this organism unable to efficiently depolymerize lignin (Martinez et al. 2009). Similarly, in another relative, *Ceriporiopsis subvermispora*, Fernandez-Fueyo et al. (2012) linked decrease in cellulose depolymerisation efficiency with variation in gene composition and expression.

Comparative genomics of pathogenic Fungi provided evidence for the high diversity in genes and molecular pathways underlying pathogenicity, most of which have evolved independently and result from a long-term arms race between hosts and pathogens. For instance, studies carried out in distinct clades revealed a large expansion of gene families encoding proteins involved in pathogenicity, such as secreted proteases, toxins or cell wall degradation enzymes. These increases in copy numbers occurred independently in dermatophytic Fungi such as *Arthroderma* and *Trichophyton* (Ascomycota; Burmester et al. 2011), in insect pathogens such as *Metarhizium* (Ascomycota; Gao et al. 2011), in yeasts of the CTG clade (Butler et al. 2009), in amphibian pathogens such as the Chytrid Fungi *Bratrachochytrium dendrobatidis* (Joneson et al. 2011) and in phytopathogenic species such as those from the *Fusarium* genus (Rep and Kistler 2010). In the human pathogen *Rhizopusoryzae* (Mucoromycotina; Fig. 4.1), the expansion of gene families involved in virulence factors and drug resistance arose after whole genome duplication (Ma et al. 2009). Some exceptions were found, such as in the other phytopathogenic species *Mycosphaerella graminicola*, which showed a surprisingly small number of genes involved in cell wall degradation as compared to other phytopathogens (Fig. 4.5). This feature suggests that *M. graminicola* evolved from an *endophyte* ancestor, and developed a protease activity rather than host cell wall degradation (Goodwin et al. 2011). Recently, Ohm et al. (2012) compiled available genomic data for three clades of Dothideomycetes Fungi (Ascomycota): Pleosporales, containing only phytopathogens, Hysteriales, including only *saprotrophs* (degrading humus) and Carpnodiales, containing *M. graminicola,* but also many other phytopatogens and a saprotrophic species. Based on genomic sequences, they reconstructed and identified major features of the evolutionary history of pathogenicity within this clade (Fig. 4.5). They observed that, despite a great variation in genome size among these Fungi (20–75 Mb), the number of genes was highly conserved (10,000–14,000), confirming that variation in genome size mainly resulted from proliferation of mobile (Kelkar and Ochman 2012; Martin et al. 2010; Santana et al. 2012) and repetitive elements, altogether

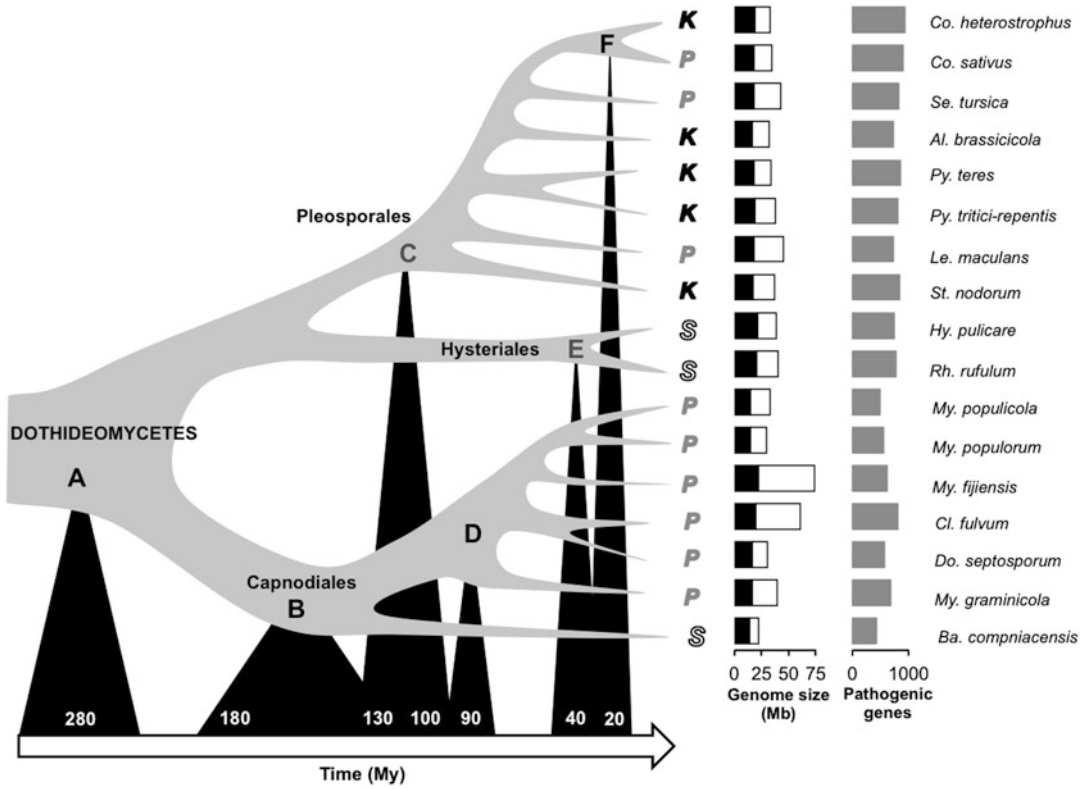

Fig. 4.5 Comparative genomics of 17 genomes allows reconstructing the evolution of pathogenicity in Dothideomycetes Fungi (adapted from Ohm et al. (2012)). The evolutionary tree, based on genomic data, reveals the chronology of the main steps (**a**–**f**) of Dothideomycetes evolution (*black triangles* on the time line indicate the range for age estimation of each node in million years). The following information is indicated for each species to the *right* of the tree: life style symbolized by a bold letter (*K* killing pathogen, *P* pathogen, *S* saprotroph); coding and non-coding genome size in megabases (*black and white horizontal bars*, respectively); number of pathogenic genes (*grey horizontal bars*); and species name (genus abbreviations: *Co.*: *Cochliobolus*; *Se.*: *Setosphaeria*; *Al.*: *Alternaria*; *Py.*: *Pyrenophora*; *Le.*: *Leptosphaeria; St.*: *Stagonospora*; *Hy.* : *Hysterium*; *Rh.* : *Rhydidhysteron*; *My.*: *Mycosphaerella*; *Cl.*: *Cladosporium*; *Do*: *Dothistroma*; *Ba.*: *Baudoinia*)

comprising about 40 % of the *Mycosphaerella fijiensis* genome (Fig. 4.5). Moreover, these repetitive elements likely resulted from many chromosomal rearrangements occurring during the evolution of this clade, some of them linked to genes involved in pathogenic traits, such as the ability to degrade host cellulose or to lyse host proteins. Interestingly, genomic rearrangements observed in these clades likely enhanced the expansion of pathogenic gene families. These expansions were more important in Pleosporales (700–1,000 pathogenic genes) than in Capnodiales (500–800), in agreement with the more serious pathogenicity of Pleosporales on its plant host, often resulting in cell destruction, compared to Capnodiales, which often leaves the host cell alive until pathogen reproduction (Fig. 4.5). Expansions of pathogenic gene families also occurred in Hysteriales, which have a more recent common ancestor with Pleosporales, suggesting that these *saprotroph* species possibly derived from a phytopathogen ancestor and took advantage of these genes to efficiently degrade fresh plant debris. Conversely, the *saprotroph Baudoinia compniacensis* (Capnodiales) has likely adopted

a distinct strategy to degrade decayed debris, since its small, compact genome (10,000 genes for 20 Mb) only harbours 435 genes related to pathogenicity.

4.2.5 Effect of Sexual Reproduction on Fungal Genomes

Fungi are known to form large asexual colonies, allowing them to efficiently exploit their habitat. However, when ecological conditions are changing, sexual recombination could be necessary to allow the rise of new allelic combinations, which may be advantageous in the new environment. The most obvious evidence of this evolutionary constrain is that stressful conditions are usually used in Fungi to induce sexual reproduction in laboratory conditions. Even apparent asexual species could occasionally perform sex to favour the maintenance of genetic diversity and to colonize new ecological niches (Billiard et al. 2011; Sun and Heitman 2011; Tsui et al. 2013). Hence, sexual reproduction has a great impact on fungal genome evolution and could alter the synteny between genomes of closely related species. For instance, in Dothideomycetes (Ascomycota), genomes of distinct species have similar chromosomes with the same gene composition, but their organization was shuffled by frequent recombination, possibly during meiosis or horizontal transfers between closely related species (Hane et al. 2011).

Sexual recognition in Fungi is governed by one or several mating-type loci (*MAT*), each containing genes coding for complementary sexual *idiomorphs*. When this system is functional, reproduction can only occur between two individuals expressing complementary *MAT idiomorphs* (*heterothallism*). This system of reproduction is widespread and presumably ancestral in most Fungi (Billiard et al. 2011). However, many fungal taxa hijacked or modified this system, resulting in independent and contrasting evolutionary modifications, such as the augmentation of the number of *MAT idiomorphs*, the evolution from *heterothallism* to *homothallism* or the possible loss of sex (Billiard et al. 2011; Lee et al. 2010; Sun and Heitman 2011). With the emergence of genomic data, genomic regions of *MAT* loci have been intensively studied in many Fungi and revealed the high diversity and complexity of function, gene composition and organization underlying the evolution of reproductive systems (Butler et al. 2009; Fraser et al. 2007; Lee et al. 2010; Metin et al. 2010; Tsui et al. 2013). For instance, Butler et al. established that the observed co-occurrence of homothallic and heterothallic species in the CTG clade resulted from multiple losses and reorganizations of *MAT* genes (Butler et al. 2009). In *Neurospora* species (Ascomycota), Wik et al. (2008) found genomic evidence that multiple transitions from *heterothallism* to *homothallism* resulted from independent disruptions of different *MAT* genes in different species. In this particular clade, the genomic region subjected to the effect of *MAT* locus evolution is so large that an entire chromosome was affected. For instance, in the *heterokaryotic* and pseudohomothallic species *N. tetrasperma*, the maintenance of self-fertility is allowed by the co-transmission of two nuclei of opposed *MAT idiomorphs* (Fig. 4.6). Menkis et al. (2008) and Ellison et al. (2011b) showed that this enforced co-transmission is likely to have occurred after inversions of large genomic regions surrounding the *MAT* locus, therefore preventing recombination events between homologous chromosomes containing *MAT idiomorphs*. The non-recombinant region encompasses one fifth of the entire genome (Fig. 4.6). Evidence for similar mechanisms of recombination suppression by complex genomic rearrangements of the *MAT* locus has also been observed in the *Mycrobotrium* (Basidiomycota; Votintseva and Filatov 2009) and *Cryptococcus* (Basidiomycota; Metin et al. 2010) genus. In these species, like in *N. tetrasperma* (Whittle and Johannesson 2011), the absence of homologous recombination between *MAT* chromosomes allows mutations to accumulate without being purged, but also enhances deterioration in preferred codon usage, suggesting a decrease of translation efficiency for genes located in the non-recombinant region

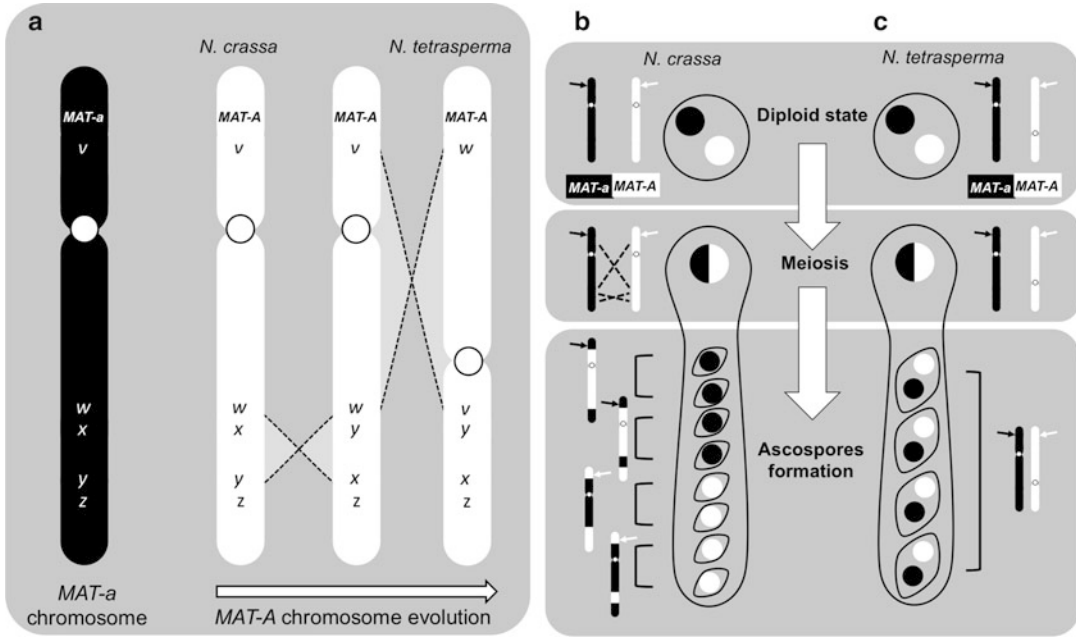

Fig. 4.6 Main genomic features of evolution from self-sterility (*heterothallism*) to self-fertility (pseudo-*homothallism*) in two *Neurospora* species, adapted from Menkis et al. (2008) and Ellison et al. (2011b). (**a**) Evolution of chromosomes including the gene coding for two opposite mating-type determinants (*MAT-a* and *MAT-A*) in *N. tetrasperma*. The *MAT-a* chromosome (*left*) is similar in *N. crassa* and *N. tetrasperma*, and is collinear with the homologous *N. crassa MAT-A* chromosome. In *N. tetrasperma*, the *MAT-A* chromosome underwent two main inversions (*dotted lines*), so that it is not collinear with the *MAT-a* chromosome. Positions of *MAT* locus and five hypothetical loci *v*, *w, x*, *y* and *z* are indicated; *circles* indicate the centromere position. (**b**) Segregation of *MAT-a* and *MAT-A idiomorphs* during meiosis and ascospore formation in *N. crassa* (*heterothallism*). A cell with two nuclei is represented on *top*, with the respective karyotype of each nucleus including either the *MAT-a* (*left*; *black*) or the *MAT-A* (*right*; *white*) chromosome. *Arrows* indicate the *MAT* locus position. During the fusion of nuclei and meiosis (*middle*), all chromosomes, including *MAT* chromosomes, undergo crossing over (*dotted lines*). The resulting ascus contains eight *ascospores*, each having a single *MAT* determinant. (**c**) The same steps are indicated for *N. tetrasperma* (pseudo-*homothallism*). Mismatch occurs during meiosis between *MAT-a* and *MAT-A* chromosomes, because they are not collinear and chromosomes are co-transmitted in all of four *ascospores*, all then co-expressing both *MAT idiomorphs*

(Whittle et al. 2011). Such accumulations could possibly lead to chromosome degeneration, which was likely an early step of sexual chromosome evolution in animals (Fraser et al. 2004). Another interesting case of *MAT* chromosome degeneration could be found in Saccharomycetaceae, where *heterothallism* is ancestral but evolved in *homothallism* in higher taxa such as for instance in *C. glabrata* and *S. cerevisiae* (Fig. 4.2). In these species, *homothallism* consists in recombination between homologous regions of active copies of genes coding for *idiomorphs* (either *MAT-a* or *MAT-α*) and silent copies of these genes located somewhere else in the chromosome, allowing recurrent *idiomorph* switching in haploid cells. As a result, a *MAT-a* cell could switch to a *MAT-α* cell and vice versa, allowing mating between any cells. By comparing *MAT* chromosomes of different Saccharomycetaceae species, Gordon et al. (2011) showed that recurrent DNA damages and mis-repairs occurring during *idiomorph* switching progressively resulted in the erosion of the *MAT* chromosome through the loss and transposition of genes flanking the recombination hotspot.

In genomes of several Microsporidia species, Lee et al. (2010) found a sex-related locus, similar to that known in heterothallic Mucoromycotina species. This locus was unrelated to the *MAT* locus and was not found in genomes of higher Fungi (namely Chytridiomycota, Basidiomycota and Ascomycota), suggesting that it is specific to basal lineages. Like in the *MAT* locus, the structure and composition in genes of the sex-related locus greatly vary between Microsporidia and Mucoromycotina species. However, the absence of *idiomorphism* and functional evidence for the sex-related locus in Microsporidia suggests that these species are either homothallic or asexual. Even in higher Fungi, as in some *Candida* species where the *MAT* locus is present but partially lost and inactivated, sex could be maintained, suggesting that more complex and unknown genomic features control for cryptic sex in Fungi (Sun and Heitman 2011). In species reproducing asexually, functionality of *MAT* genes can be maintained, either to perform cryptic metabolism, as the regulation of asexual development in response to light in *Neurospora* (Wang et al. 2012b), or to keep the potential to reproduce sexually, providing the opportunity to increase genetic variability and thus to colonize new ecological niches, as it is the case in Ophiostomatales (Sordariomycetes, Ascomycota), an order of pathogenic Fungi (Tsui et al. 2013).

4.3 Micro-ecological Genomics in Fungi

Large-scale evolutionary changes that shaped fungal genomes were tightly associated with main lifestyle characteristics of Fungi: multicellularity, pathogenicity, *symbiosis*, lignin degradation and reproduction. At a lower evolutionary scale, fungal genomes were also marked by slight modifications such as mutations, translocations and regulatory changes that progressively accumulated to confer advantageous adaptations in changing environments. Here, I review experimental and population studies that have examined the roles of local adaptation and speciation on early steps of fungal genomic evolution and have captured evolution in action.

4.3.1 Genomics of Local Adaptation and Recent Evolution in Fungi

Because genomes are shaped by long-term evolution, it is often impossible to predict the phenotypic response of organisms to a particular environmental variation only from the knowledge of gene function, gene composition, or gene orthology based on inter-species comparisons. The increased genomic data available for closely related species or for several individuals from the same species, and the use of experimental evolution, quantitative trait loci (QTL) approaches and population genomics, now allow the investigation of recent imprints of environmental pressures on fungal genomes.

4.3.2 Using Experimental Evolution to Understand the Early Steps of Genomic Adaptation

Experimental evolution coupled with genomics provides a powerful and developing tool to explore the primary mutations and genomic rearrangements underlying adaptation to different types of environments in eukaryotes. Because of their short generation time and their small genomes, yeast-like Fungi are ideal eukaryotic models for this approach. For instance, Araya et al. (2010) compared the genome of a *S. cerevisiae* strain evolved in sulphate-limited conditions with its ancestor genome. They found single-point mutations responsible for adaptation to this limitation, such as a mutation affecting the regulation of *RRN3*, a gene involved in modulating ribosomal gene expression during nutrient-limiting conditions. Anderson et al. (2010) adopted a similar approach to uncover early mutations underlying high salt concentration and low glucose tolerance in *S. cerevisiae* (see also Sect. 4.4.1). In *C. albicans*, a genome-wide survey of gene expression

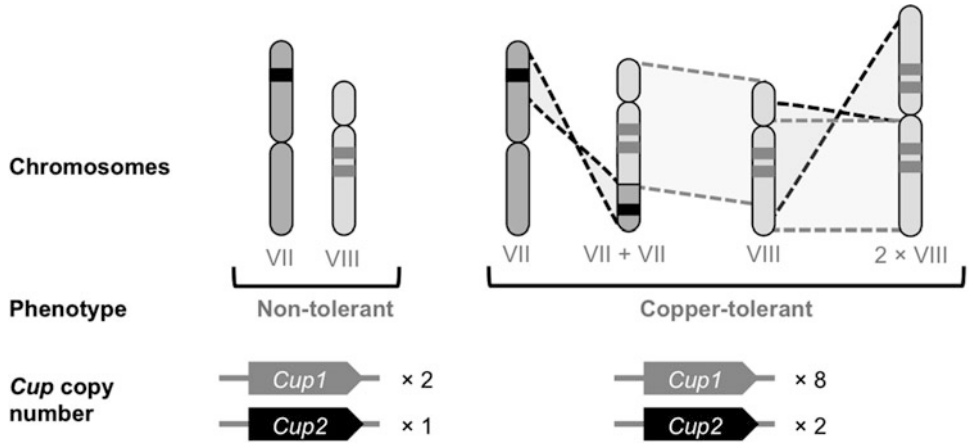

Fig. 4.7 Copper tolerance in natural populations of *Saccharomyces cerevisiae* is allowed by reversible rearrangements (*dotted lines*) of chromosomes VII and VIII fragments. The formation of two additional chimeric chromosomes results in the increase in copy number of *CUP* genes involved in copper regulation in cell (Chang et al. 2013). The position of genes *CUP1* and *CUP2* on chromosomes VIII and VII are indicated by *grey* and *black rectangles*, respectively

conducted in strains adapted to drug resistance suggested that at least two adaptive patterns could occur in response to this stress. These patterns mostly involved nine genes, whose expression levels varied among four independently evolved populations. Hence, one population showed a high expression of a single gene, *CDR2*, controlling drug export, whereas three other populations independently converged on high expression levels of eight other genes, such as *MDR1*, also involved in efflux of drugs, or *YPX98*, *YPR127W*, and *ADH4,* involved in cell protection during oxidative stress (Cowen et al. 2002). Recently, Chang et al. (2013) found that natural strains of *S. cerevisiae* were copper tolerant because of higher expression level of genes *CUP1* and *CUP2* encoding proteins involved in copper regulation. They showed that this increase in expression resulted from an increase in *CUP1* and *CUP2* copy number, allowed by duplications and translocations of large genomic regions encompassing these genes (Fig. 4.7). Interestingly, translocations were reversible when these strains were evolved in less selective environment, suggesting that chromosomal rearrangements could be an important mechanism of rapid adaptation to fluctuating environments.

4.3.3 Population Genomics and Quantitative Trait Loci (QTL) Approaches

An alternative to studying the mutations underlying adaptation is population genomics, which allows detection of natural genomic variations and can associate particular traits to these variations. Because environmental factors are numerous, complex and highly variable within natural populations, the resulting phenotypes can rarely be explained by limited genomic variation, as they can under controlled conditions. The QTL approach allows to experimentally identify genetic variation underlying complex quantitative traits and to measure the influence of selection shaping these traits (Rice and Townsend 2012) by recombining genomes of two phenotypically different individuals and then, by identifying the putative loci involved and their functions. For instance, Liti et al. (2009b) found that telomere length, which is an important factor for buffering DNA loss during replication, varies among *S. paradoxus* populations. Using a QTL approach, they identified two genes likely associated to this variation. Cubillos et al. (2011) crossed individuals from two diverging populations of

S. cerevisiae and grew the progeny in 23 distinct experimental conditions. They found that most of the traits were polygenic, but some, such as copper tolerance or ability to grow on galactose or maltose, were linked with genes whose predicted functions were consistent with the trait, such as genes *CUP1* and *CUP2* involved in copper tolerance (See Sect. 4.3.2), or *GAL3*, involved in galactose metabolism. Using the same approach, Will et al. (2010) showed that the freeze-tolerance of *S. cerevisiae* strains depended on a few mutations in two genes coding for water-transport proteins. Moreover, these genes showed a strong signature of balancing selection. The balanced polymorphism was distributed between two distinct groups of *S. cerevisiae* populations that had contrasting profiles of freeze-tolerance phenotypes.

4.3.4 Population Genomics and Ecological Approaches

When a collection of genomes representing natural variation is available, population genomics can associate the natural environment with natural genomic variations, and then make functional predictions about the genes affected by these variations. By comparing the distribution of synonymous mutations among the genomes of 44 clinical and 44 non-clinical strains of *S. cerevisiae*, Muller et al. (2011) identified a handful of genes likely to be pathogenicity determinants and involved, for instance, in cell wall resistance and cell detoxification, which gives valuable indications about what mechanisms pathogenic strains use to evade the human immune system. Conversely, reverse ecology uses natural genomic variation to predict environmental factors affecting genes, without assumptions about their functions. Ellison et al. (2011a) used a reverse ecology approach to identify factors underlying population structure of *Neurospora crassa* (Ascomycota). Using whole genome sequencing, they found two genetically diverging populations, which also diverged in their ability to grow at low temperature. They looked for genomic distribution of molecular divergence between the two populations, without focusing on particular genes, and found two large genomic regions containing several genes that showed deep divergence between populations. When two of these genes, *MRH4* and *PAC10*, were deleted, strains lost their ability to grow at low temperature, confirming that the divergence between the two genomic regions was responsible for adaptation to cold. When the function of genes and the metabolic pathways underlying a particular trait are known, one can use genome sequences to identify mutations that potentially affect the pathway. This was the case in the yeast *S. kudriavzevii*, where two divergent populations differed in their ability to use galactose as carbon source. Hittinger et al. (2010) identified the underlying mutations affecting genes of the galactose pathway by genome sequencing. They established that, despite frequent gene flow between these two populations, balancing selection maintained inactive copies of these genes in the population unable to metabolise galactose. In this case, the ecological differences between the two *S. kudriavzevii* populations are poorly known. One can only speculate that, because strains were found on different substrates, those provided different sources of carbon: strains able to metabolize galactose were found on barks of trees, whereas strains unable to metabolize it were found in soil.

Population genomics also provided evidence for very recent evolution of pathogenicity in higher Ascomycota at the species level. For instance, Stukenbrock et al. (2011) looked for evidence of selection in genomes of the wheat pathogen species *Mycosphaerella graminicola* and its related non-pathogenic sister species. They found that the number of genes showing positive selection increased after divergence of *M. graminicola* with its closest relative, whereas it remained low when estimated at a higher evolutionary scale. Interestingly, most of these genes encoded pathogen effectors, suggesting that pathogenicity of *M. graminicola* arose recently, likely with the domestication of wheat. In *Verticillium* (Ascomycota) species, de Jonge et al. (2012) identified *AVE1*, a gene

encoding a virulence effector for multiple plant species, which was found to be part of a large genomic region only present in virulent strains. Interestingly, *AVE1* orthologs were also found in other phytopathogen Fungi and bacteria, usually located in a similar large genomic region also containing many transposable elements, suggesting that at least part of *Verticillium* virulence results from horizontal gene transfers.

Altogether, these studies suggest that not only a few genes allow adaptation to new environments. Mutations occurring elsewhere in the genome could also generate variation in regulation of gene expression and lead to new adaptive responses. Rapid and sometimes reversible genomic rearrangements could also lead to new favourable combinations of genes. In extreme cases, as in some pathogenic Fungi, adaptation could occur after horizontal transfer of advantageous genes between bacteria and Fungi. Finally, the opportunity for Fungi to use distinct and alternative molecular pathways involving different sets of genes to reach the same phenotypic response could also instigate early steps of adaptation in a new environment.

4.3.5 Population Genomics of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*

Population genomics of *Saccharomyces cerevisiae* and *S. paradoxus* has been extensively investigated in the last few years. These sister species have a worldwide distribution and are sympatric – both are found in the wild and are associated with the same tree species (Hyma and Fay 2013; Sniegowski et al. 2002) – but show highly contrasting patterns of population history. On one hand, the population structure of *S. cerevisiae* is highly correlated with its domestication history – association to human pathologies or adaptation to different modes of alcohol fermentation and baking (Hyma and Fay 2013; Liti et al. 2009a; Schacherer et al. 2009) – while almost no geographical signal is observable, except for a few wild and isolated populations in China (Wang et al. 2012a). On the other hand, *S. paradoxus* populations are highly structured according to their geographical locations, with at least three distinct and genetically fixed populations, located in Europe, East Asia and America (Fig. 4.8). This pattern suggests no strong human impact on *S. paradoxus* history and no recent introgression (Hyma and Fay 2013; Liti et al. 2009a), except some evidence of recent hybridization with *S. cerevisiae* in the European *S. paradoxus* lineage (Liti et al. 2006). This structure is emphasized by partial reproductive isolation between the different *S. paradoxus* populations (Kuehne et al. 2007; Liti et al. 2006), although a secondary contact after a recent and likely anthropic immigration event from Europe to America has been reported (Hyma and Fay 2013; Kuehne et al. 2007). Accordingly, Liti et al. (2009a) found that genomic and phenotypic variations were strongly correlated in *S. paradoxus* but not in *S. cerevisiae*. This was in agreement with genomic evidence for frequent introgression events between *S. cerevisiae* lineages, which likely resulted from numerous hybridization during human domestication and acquisition of new and specific genes in industrial strains (Borneman et al. 2011). Additionally, Warringer et al. (2011) found that phenotypic variability was higher in *S. cerevisiae* than in *S. paradoxus*, despite a higher genetic diversity in the latter. Once more, the authors suggested that this paradoxically high phenotypic variation was the result of genetic drift having occurred during multiple and independent domestication events of *S. cerevisiae*. Surprisingly, while selection associated to strong adaptive divergences between species was expected, most of genomic studies failed to detect signal of selection on particular adaptive genes (but see Aa et al. 2006), suggesting either that purifying selection uniformly acted on genomes during *S. cerevisiae* and *S. paradoxus* divergence or that selection was relaxed in *S. cerevisiae* populations after domestication (Liti et al. 2009a). Finally, population genomics studies on these two species quantified how frequently the two species experienced sexual vs. asexual reproduction during their evolution. Based on an estimation of

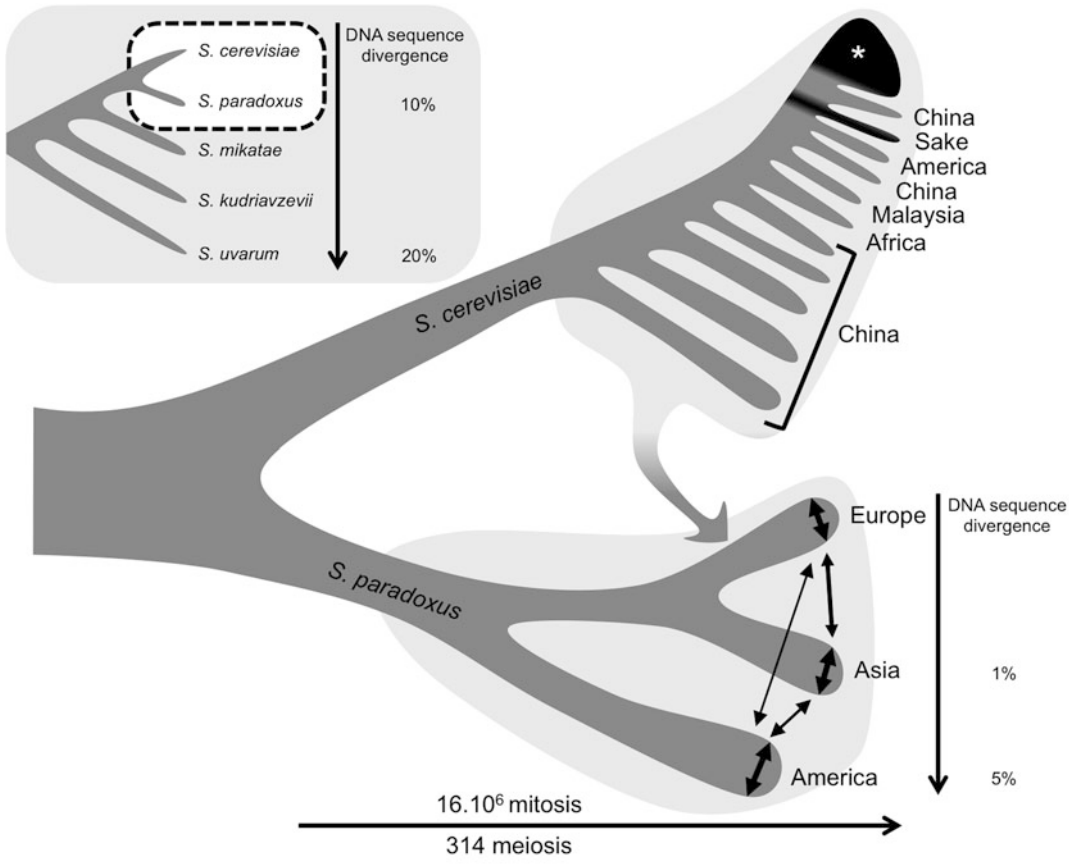

Fig. 4.8 *Saccharomyces cerevisiae* and *S. paradoxus* have contrasting evolutionary histories. *Top left*: evolutionary tree of *Saccharomyces* species indicating the range of genome sequence divergence between *S. cerevisiae* and other species (Dujon 2006). *Dotted frame* indicates the position of *S. cerevisiae* and *S. paradoxus*. Population structure is in agreement with geographical distribution for *S. paradoxus*, whereas *S. cerevisiae*, which underwent human domestication, has no such pattern of structure (Liti et al. 2009a). The *black* area indicates branches in which *S. cerevisiae* domesticated strains are predominant. The *asterisk* indicates the branch containing most of studied strains until recent advances in genomics, and including European, wine, clinical and baking strains. The estimation number of meiosis and mitosis that occurred during species divergence (*arrow on bottom*) was estimated from genomic data (Ruderfer et al. 2006). In *S. paradoxus*, partial reproductive isolation occurred among different lineages. Arrow width is proportional to hybrid progeny survival: 95 % within lineages; 30–70 % among lineages (Kuehne et al. 2007; Liti et al. 2006) and is correlated with sequence divergence among lineages (*vertical axis*) but also with translocations having occurred in some strains (not shown; only results for crosses between collinear genomes are indicated). The *arrow* from *S. cerevisiae* to European *S. paradoxus* indicates evidence for introgression events between the two lineages (Liti et al. 2006)

recombination frequency, Ruderfer et al. (2006) showed that meiosis (i.e. sexual) recombination occurred only once each 50,000 cell divisions in both species, whereas a more recent estimation suggested once per 1,000–3,000 for *S. paradoxus* (Tsai et al. 2008). Both studies support the idea that, despite a fully functional mating system, reproduction in *Saccharomyces* species is mostly clonal (Fig. 4.8). In *S. cerevisiae*, Magwene et al. (2011) proposed an explanation for this phenomenon. They observed that strains with a high proportion of heterozygous sites showed a low capacity to perform meiosis, suggesting that selection favoured asexual reproduction in such strains to maintain advantageous heterozygosity. Similarly, Tsai et al. (2008) estimated that 99 %

of sexual reproduction events that occurred during *S. paradoxus* evolution involved strains originated from the same meiotic events, i.e. from the same parents, thus resulting in highly homozygous individuals.

Saccharomyces species are thus powerful models to investigate the effect of environmental, human and historical factors on population genomics of microbial eukaryotes. Altogether, *S. cerevisiae* and *S. paradoxus* also allow dissecting the genomic imprint of speciation, from early adaptive mutations to complete reproductive isolation.

4.4 Eco-genomics of Speciation and Hybridization

Speciation is a fundamental evolutionary process, but the definition can greatly vary depending on the organisms under consideration. In Fungi, more than in other life kingdoms, the species concept relies on many controversal and conflicting biological, morphological, ecological and phylogenetic criteria (Cai et al. 2011; Giraud et al. 2008; Kohn 2005; Taylor et al. 2000). For instance, depending on the choice of these criteria, one can consider one or three species in *S. paradoxus*, simply because reproductive isolation occurs between different genetic lineages (discussed in Sect. 4.3.5), or four or seven species in *Lentinula* (Basidiomycota), according to morphological or phylogenetic criteria, respectively (Taylor et al. 2006). Similarly in *Neurospora*, most of "genetic" species are reproductively isolated, but exceptions can be found, since some genetically distinct lineages are still able to hybridize (Dettman et al. 2003a, b).

Genomics is the upcoming – but not absolute – criteria to shed light on speciation in Fungi. Here, I review recent studies that investigated a continuum of genomic processes to understand how speciation occurs in Fungi, from the early steps of genetic divergence to the establishment of complete reproductive isolation.

4.4.1 Beginnings of Speciation: Adaptation Drives Early Genetic Incompatibilities

When different strains of the same species occur in distinct ecological niches, they undergo contrasting adaptive constraints. Thus mutations that occur in the genome are differently selected. The accumulation of such advantageous mutations drives toward an optimal fitness for each strain in its own niche, but could also generate incompatibilities if the mutated gene interacts negatively with another one that was selected in a contrasted environment. This phenomenon could be considered as the basis of speciation, since strains from the same species that evolved under contrasted ecological conditions could, in theory, accumulate incompatibilities, leading to progeny with reduced fitness (Gourbiere and Mallet 2010). Anderson et al. (2010) explored the genomes of two experimentally evolved strains of *S. cerevisiae*. They detected early mutations underlying adaptation to high salt and low glucose environments, and found that the progeny of crosses between strains from two experimental populations had a strong fitness decrease in low glucose concentration conditions. They showed that this fitness reduction resulted from strong genetic incompatibility between two mutated alleles, each of them inherited from a distinct parent (Fig. 4.9a). Such within-species incompatibility has also been highlighted in natural populations of *S. cerevisiae* and involved two genes, each present in two allelic states. All combinations between alleles of the two genes could be found in the studied populations, except one. By generating individuals exhibiting the missing allelic combination, Demogines et al. (2008) found that these alleles combined increased the genomic mutation rate, which resulted in long-term accumulation of fitness-defect mutations. These studies suggest that adaptive mutations in a few genes could be sufficient to generate reproductive isolation when selection is strong enough, and eventually lead to speciation. Because yeasts have a

short generation time, one can expect to observe such incompatibilities after a very short evolutionary time. For instance, *S. cerevisiae* and *S. paradoxus* are distinct species that have diverged 0.4–3.5 million years ago. During this time, their genomes have accumulated divergence at 5–10 % of their sites (Fig. 4.8; Liti et al. 2006). This could represent a substantial amount of potential incompatible mutations between species, which however remains to be tested. Large genomic rearrangements, such as chromosomal translocations, could also occur after a very short evolutionary time and generate reproductive isolation. For instance, Liti et al. crossed natural strains of *S. paradoxus* with different chromosome configurations. They found that reproductive isolation, measured as progeny survival, was indeed correlated with the proportion of single nucleotide divergence (Fig. 4.8), but also dramatically decreased when parents had different chromosomal configurations, even if these translocations occurred only a few thousand years ago (Liti et al. 2006).

4.4.2 Genomic Investigation of Inter-species Incompatibilities in Yeasts

When speciation is established after several million years, hybridization is still possible between closely related species, and molecular mechanisms could evolve to maintain and enforce reproductive isolation. For instance, *S. cerevisiae* and *S. paradoxus* produce viable but mostly sterile hybrids, with less than 1 % viability of progeny. Greig et al. (2002) suggested that sterility of hybrids could result from incompatibilities between so-called "speciation genes." To verify this hypothesis, they replaced chromosomes of *S. cerevisiae* by their homologues from *S. paradoxus*. Surprisingly, all tested *S. paradoxus* chromosomes (representing 43 % of the genome) were compatible with the *S. cerevisiae* genome, suggesting that speciation genes were unlikely to play a major role in hybrid sterility (Greig 2007). Two independent genome-wide analyses of progeny from enforced sporulation of interspecies hybrids showed that all expected genomic combinations between *S. paradoxus* and *S. cerevisiae* genomes occurred, even after recombination between parental chromosomes (Kao et al. 2010; Xu and He 2011). Evidence for genetic incompatibilities involving a single pair of speciation genes has been investigated at larger evolutionary scales in *Saccharomyces* species and has so far only been found between the *S. cerevisiae* mitochondrial genome and a nuclear gene of its farthest relative in the group, *S. uvarum* (Lee et al. 2008). Such a lack of evidence for a role of genetic incompatibilities between species is astonishing considering some of the evidence found within *S. cerevisiae* and discussed above, and considering the high genomic sequence divergence (5–20 %) observed between *Saccharomyces* species (Fig. 4.8; Dujon 2006). However, we recently found that high molecular divergence between distant *S. cerevisiae* and *S. kudriavzevii* did not systematically result in functional disorders of essential protein complexes in hybrids, suggesting that vital functions are highly robust to inter-species hybridization (Leducq et al. 2012).

Some other mechanisms that may affect hybrids were also investigated. For instance, genomes of different *Saccharomyces* species are non-collinear, since all of them have undergone independent chromosome translocations. Delneri et al. (2003) demonstrated that these translocations are partly responsible for hybrid sterility between *S. cerevisiae* and *S. mikatae*, since homologous chromosomes could not match perfectly during meiosis, frequently resulting in aneuploid hybrids (Fig. 4.9b). These results confirmed previous findings at the intra-species level (Liti et al. 2006).

All these findings reinforce the hypothesis that no single genetic mechanism is responsible for sterility of inter-species hybrids. Hybrid sterility possibly results from chromosome mismatches during meiosis, combined with multiple complex incompatibilities, which probably involve many genes with individually negligible effects as well as unsuspected underlying molecular processes.

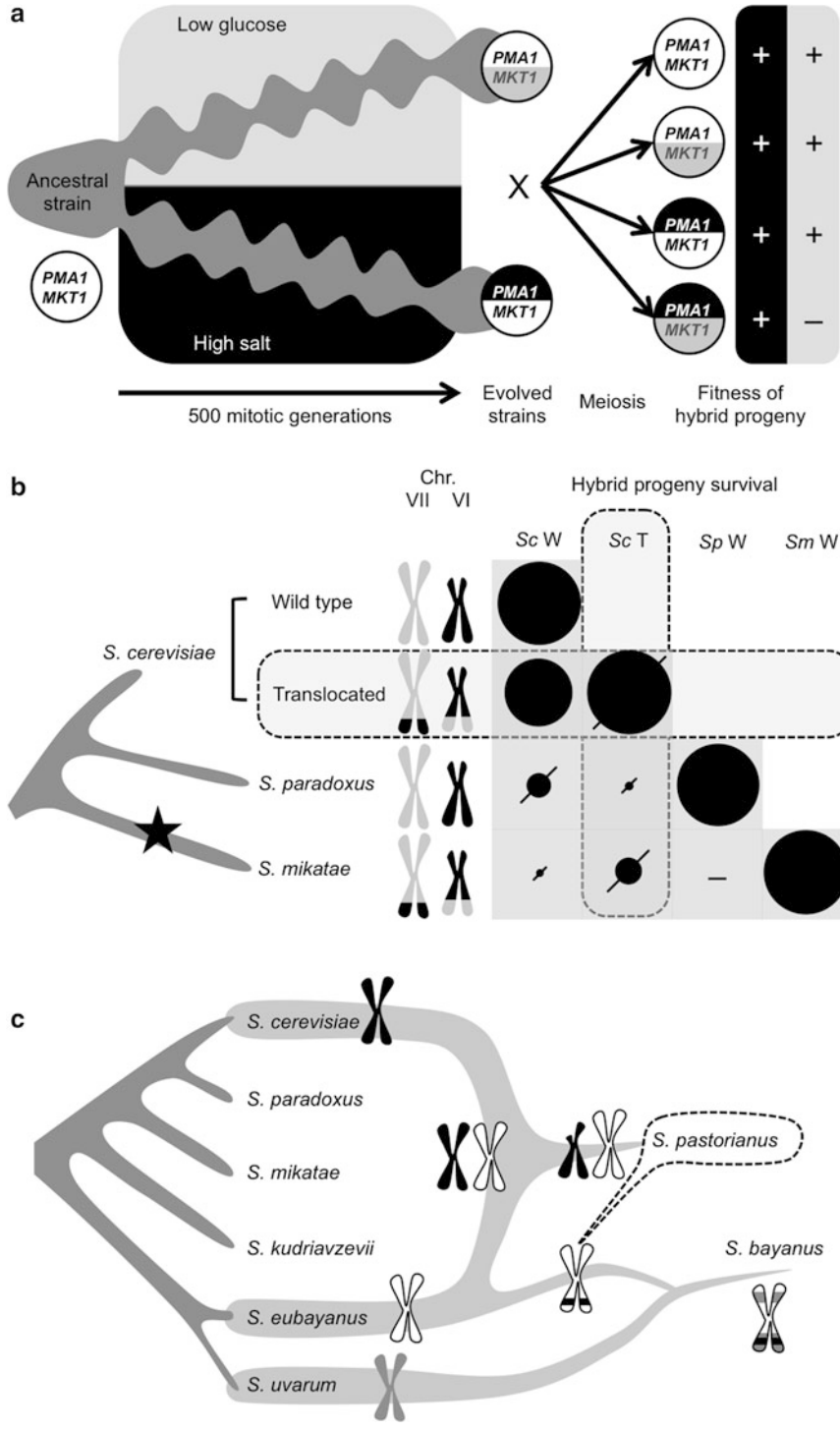

Fig. 4.9 Genomics of speciation and hybridization in *Saccharomyces* yeasts. (**a**) Experimental evolution of *S. cerevisiae* in two contrasting environments (high salt and low glucose concentration, respectively). The analysis of genomes revealed two adaptive mutations affecting genes *PMA1* (high salt; codes for a proton efflux pump) and *MKT1* (low glucose; regulator of mRNA encoding mitochondrial proteins). Hybrid progeny bearing

4.4.3 Genomic Evidence for Speciation by Hybridization

The study of fungal genome evolution provides some evidence that reproductive barriers arising after speciation are not always complete. Indeed, some genomic analyses revealed potential cases of introgression, suggesting that these arose from successful hybridization between two distinct species. Early steps of speciation by hybridization are poorly understood but Dunn et al. (2013) recently showed that rearrangements could experimentally arise among homologous chromosomes of *S. cerevisiae* and *S. uvarum* within their first generation hybrids, when evolved in ammonium-limited conditions. The break points of recombination systematically occurred in the gene *MEP2* coding for an ammonium permease, likely suggesting that chimeric Mep2 proteins confer a higher fitness advantage to the hybrids. However, because interspecific hybrids are often aneuploid and sterile in *Saccharomyces*, this kind of mechanism is likely to lead to an evolutionary dead-end rather than to actual introgression. This is the case of the two sterile brewing yeasts *S. pastorianus* and *S. bayanus*, which resulted from hybridization induced by domestication (Fig. 4.9c). The genome analysis of *S. pastorianus* revealed that it resulted from allotetraploidization between genomes of *S. cerevisiae* and a close relative of *S. uvarum*, *S. eubayanus* (Dunn and Sherlock 2008; Libkind et al. 2011). This hybridization event is likely to have been followed by chromosomal rearrangements resulting in aneuploidy. Similarly, the mosaic genome of *S. bayanus* suggested that it resulted from the fortuitous integration of *S. pastorianus* genomic elements in the *S. eubayanus* genome. In both cases, the genomic hybridization and rearrangements resulted from strong anthropic pressure to select for optimal brewing properties. Similarly, the yeast *Pichia sorbitophila* (CTG clade) is a fortuitous product of industry. Its 14 chromosomes are the results of recent hybridization followed by polyploidization between two unknown but related strains that had only seven chromosomes (Louis et al. 2012) (Fig. 4.2). The evidence of many introgression traces and the absence of entire chromosomes of one of the putative parents was attested by the complete absence of polymorphism between regions of some homologous chromosomes, suggesting that this hybridization was followed by many chromosomal rearrangements and losses. It is interesting to note that the set of genes conferring high osmotic resistance to this species is likely to be the sum of contributions from both parents. For instance, genes enabling metabolism of maltose were inherited from one parent, whereas genes involved in sorbitol metabolism were inherited from both parents.

Finally, in the human pathogenic *Coccioides immitis* (Ascomycota), Neafsey et al. (2010) found evidence for recent genomic introgression from its sister species, especially in geographical areas where both species were sympatric. Introgressed genomic regions contained genes involved in host immune response, once again suggesting that generating new genetic combinations by hybridization could be favoured in selective environments. Hence, fungal genomics provides many examples that it is sometimes advantageous to break reproductive barriers between species in order to generate mosaic

Fig. 4.9 both derived alleles expresses strong fitness decrease (–) in environment with low glucose, suggesting incompatibilities between these derived alleles (Anderson et al. 2010). (**b**) In *S. mikatae* (*Sm* W), a translocation occurred between chromosomes VI and VII (*black star*). Wild-type *S. cerevisiae* (*Sc* W) and *S. paradoxus* (*Sp* W) strains have the ancestral chromosomal configuration. The decrease in *S. cerevisiae* × *S. mikatae* hybrid progeny viability is partly restored when chromosomes of the *Sc*W strains are manipulated so as to be collinear with those of *Sm*W (*Sc* T; *dotted frame*). The *black* disk areas in the cross table are proportional to mean hybrid progeny survival (*bars* indicate standard deviation among replicates; Delneri et al. 2003). (**c**) Multiple anthropic hybridization (*grey*) and *horizontal* transfer (*dotted line*) events between natural *Saccharomyces* species (*dark grey* evolutionary tree) led to the emergence of two brewing species *S. pastorianus* and *S. bayanus*. Evolutionary steps of genome evolution (symbolized by a single duplicated chromosome) were highly simplified. Each color represents the part of the genome from a mother species (Dunn and Sherlock 2008; Libkind et al. 2011)

genomes with new combinations of mutations that evolved independently. Put together, these mutations could bring a fitness advantage in a new ecological niche, but sometimes at the expense of sexual reproduction.

4.5 Conclusion

The advent of genomics in the last 10 years has shed light on the evolution and ecology of Fungi. Budding yeasts, *Neurospora* and other model organisms were yet again the pioneers in exploring the footprint of evolution and ecology on fungal diversity, but unlike classical biological tools, genomics opened this exploration to other diverse forms of Fungi. Using eco-genomics, it is now possible to understand how Fungi conquered such a broad range of ecological niches. First, fungal genomes are the result of 1 billion years of evolution that took place in highly contrasting ecological niches, promoting variable life traits and reproductive modes. During this evolution, genomes were profoundly rearranged through proliferation of mobile elements, whole-genome duplications, gain of adaptive genes, gene compaction and loss of genes linked to metabolisms which became non-essential for Fungi that have developed strong dependency to their host. Second, studies carried out at the lower evolutionary scales investigated early steps of genomic evolution in varying ecological niches. Using experimental evolution, these studies highlighted the role of early mutations in a few key adaptive genes involved in simple traits with strong selection. In natural populations, such adaptive mutations were indeed tightly associated with particular ecological conditions. Adaptive genes could also be gained by horizontal transfers between Fungi and bacteria in some pathogens. In other cases, adaptive traits could be acquired by new, advantageous combinations of genes after chromosomal rearrangements. *Saccharomyces* yeasts are particularly powerful eco-genomics models to investigate all these mechanisms in depth. Finally, local adaptation to contrasting ecological niches could lead to genetic incompatibilities between individuals of the same species, and eventually to reproductive isolation. However, there is little evidence for the occurrence and fixation of such strong incompatibilities in natural populations and for their role in the maintenance of efficient reproductive barriers, even after millions of years of divergence between lineages. Other mechanisms, such as large chromosomal translocations and accumulation of many incompatibilities with smaller effects, are more likely to drive speciation at the genomic level. Moreover, breaking reproductive barriers and reshuffling mutations inherited from different species or divergent strains is sometimes advantageous for organisms to conquer new ecological niches.

Fungi are powerful eco-genomics models to investigate a large range of evolutionary and adaptive mechanisms that drive genome evolution. Of course, the number of studies focusing on a handful of model organisms is still increasing, and these are necessary to understand fundamental mechanisms underlying this evolution. But forthcoming work using eco-genomic approaches will be able to study the remaining underexplored fungal diversity.

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Glossary

Endophyte Organism spending its entire life cycle within a plant.

Heterokaryotic When a cell contains two or more nuclei.

Heterothallism When sexual reproduction can only occur between two phenotypically indistinct individuals from the same species, but expressing different sexual *idiomorphs* (allogamy). Mostly present in algae and Fungi.

Homothallism When sexual reproduction can occur between any individuals from the same species (autogamy), in contrast to heterothallism – **Pseudo-homothallism** derives from heterothallism but the co-transmission of two sexual *idiomorphs* during meiosis allows autogamy.

Idiomorph – or Mating-type Sexual determinants in eukaryotes. Designates compatible sexual partners during reproduction: for instance male and female in plants and animals or *MAT-a* and *MAT-α* in yeasts.

Mycorrhiza Symbiosis between a fungus and roots of a vascular plant. **Ectomycorrhiza** perform the interaction within the host tissues whereas **endomycorrhiza** perform the symbiosis within the host cells.

Quantitative Trait Loci (QTL) Portions of the genome physically co-segregating with an inherited trait, and thus physically linked to at least one gene involved in this trait.

Saprotroph Fungi able to absorb nutrients from dead or decayed organic matter.

Spore In Fungi, a unicellular, resistant reproductive structure formed by meiosis or mitosis, able to produce a new individual after possible dispersal and germination. **Ascospores** are spores produced by Ascomycota.

Symbiosis Close and reciprocally beneficial interaction between two organisms of different species, providing each other with protection, suitable habitat or nutrients.

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