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DISCLAIMER

The Dothideomycetes class of fungi includes many pathogens that infect a broad range of plant hosts. Here, we compare genome features of 18 different members of this class, including 6 necrotrophs, 9 (hemi)biotrophs and 3 saprotrophs, and discuss genome structure, evolution, and the diverse strategies of pathogenesis. The 18 genome sequences show dramatic variation in size due to variation in transposon expansions, but less variation in core gene content. During evolution, gene order in these genomes is changed mostly within boundaries of chromosomes by a series of inversions often surrounded by simple repeats. This is in contrast to major interchromosomal rearrangements observed in other groups of genomes. Several Dothideomycetes contain gene-poor and TE-rich putatively dispensable chromosomes of unknown function. In the current set of organisms, biotrophs and hemibiotrophs are mostly phylogenetically separated from necrotrophs and saprobes, which is also reflected in differences between gene sets represented in each group. The 18 Dothideomycetes offer a rich catalogue of genes involved in cellulose degradation, proteolysis, Cys-rich small secreted proteins and secondary metabolism, many of which are enriched in proximity of transposable elements, suggesting faster evolution because of both TE mobility and RIP effects.

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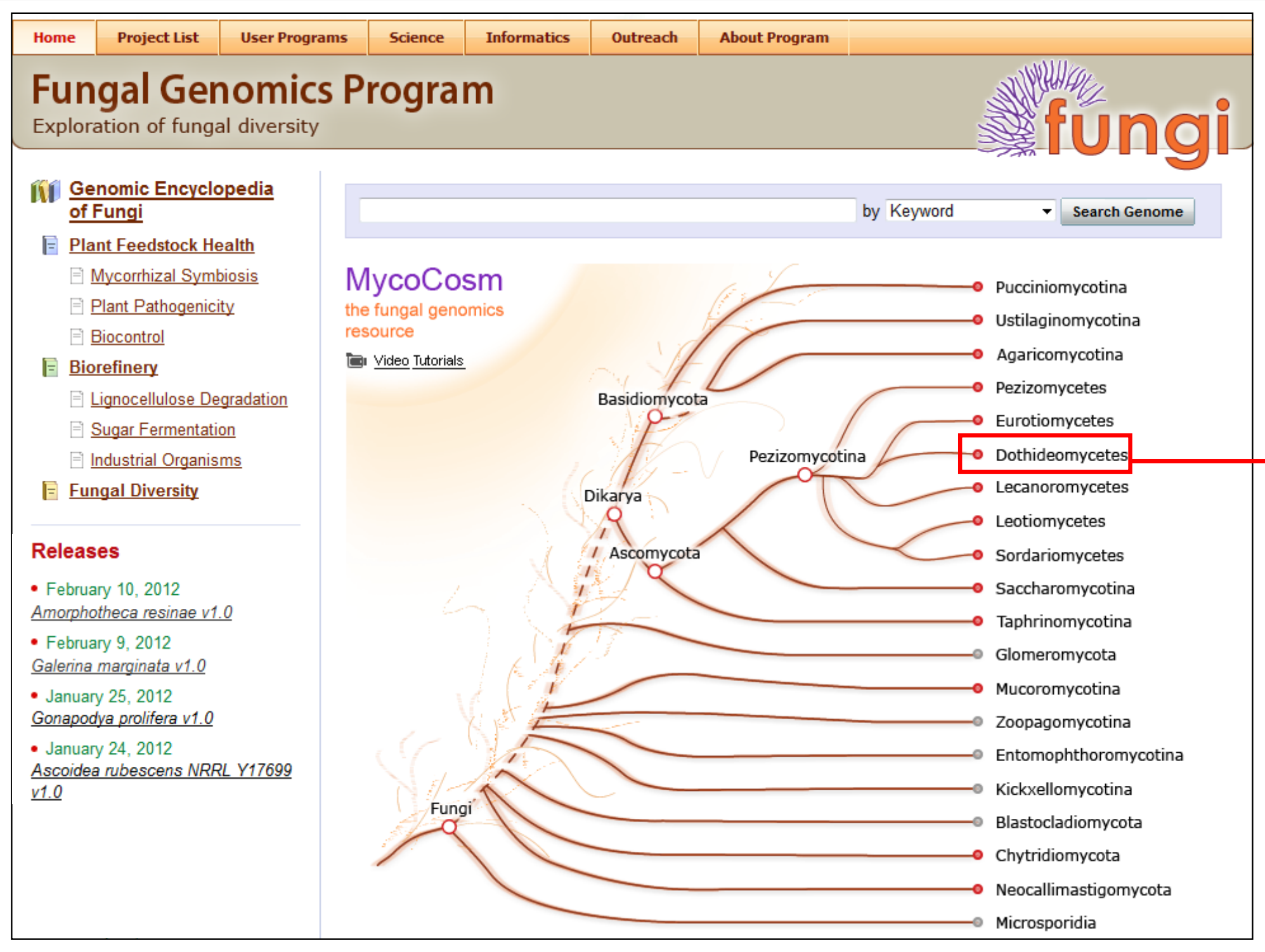
Introduction

The class of *Dothideomycetes* is one of the largest and most diverse groups of fungi. Many are plant pathogens and pose a serious threat to agricultural crops grown for biofuel, food or feed. Most *Dothideomycetes* have only a single host and related species can have very diverse host plants. Eighteen genomes of *Dothideomycetes* have currently been sequenced by the Joint Genome Institute and other sequencing centers. Here we describe the results of comparative analyses of the fungi in this group.



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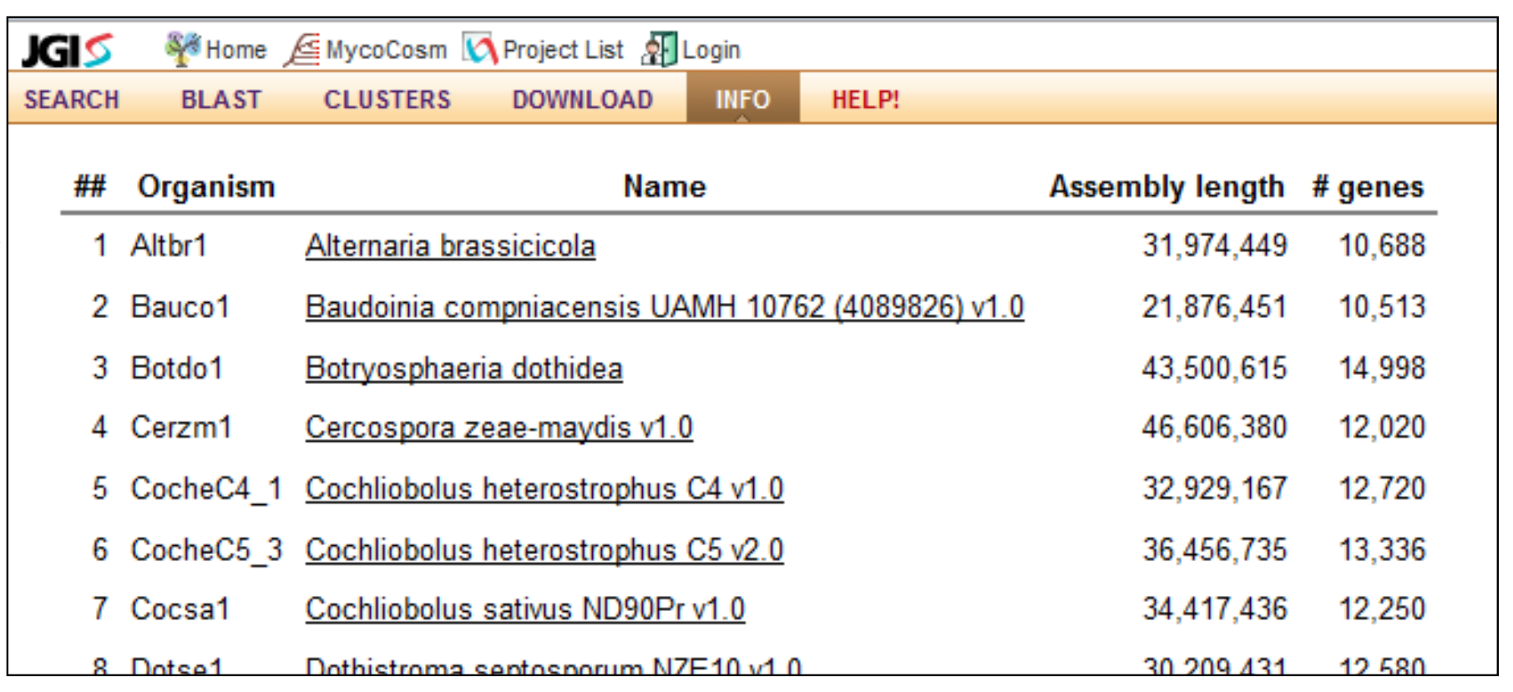
Mycocosm. The web portal Mycocosm contains the genomes and annotations of all 18 sequenced *Dothideomycetes*, as well as over 100 other fungal genomes, sequenced by the JGI and other sequencing centers. Organism-specific and comparative tools are available to the user on http://jgi.doe.gov/fungi

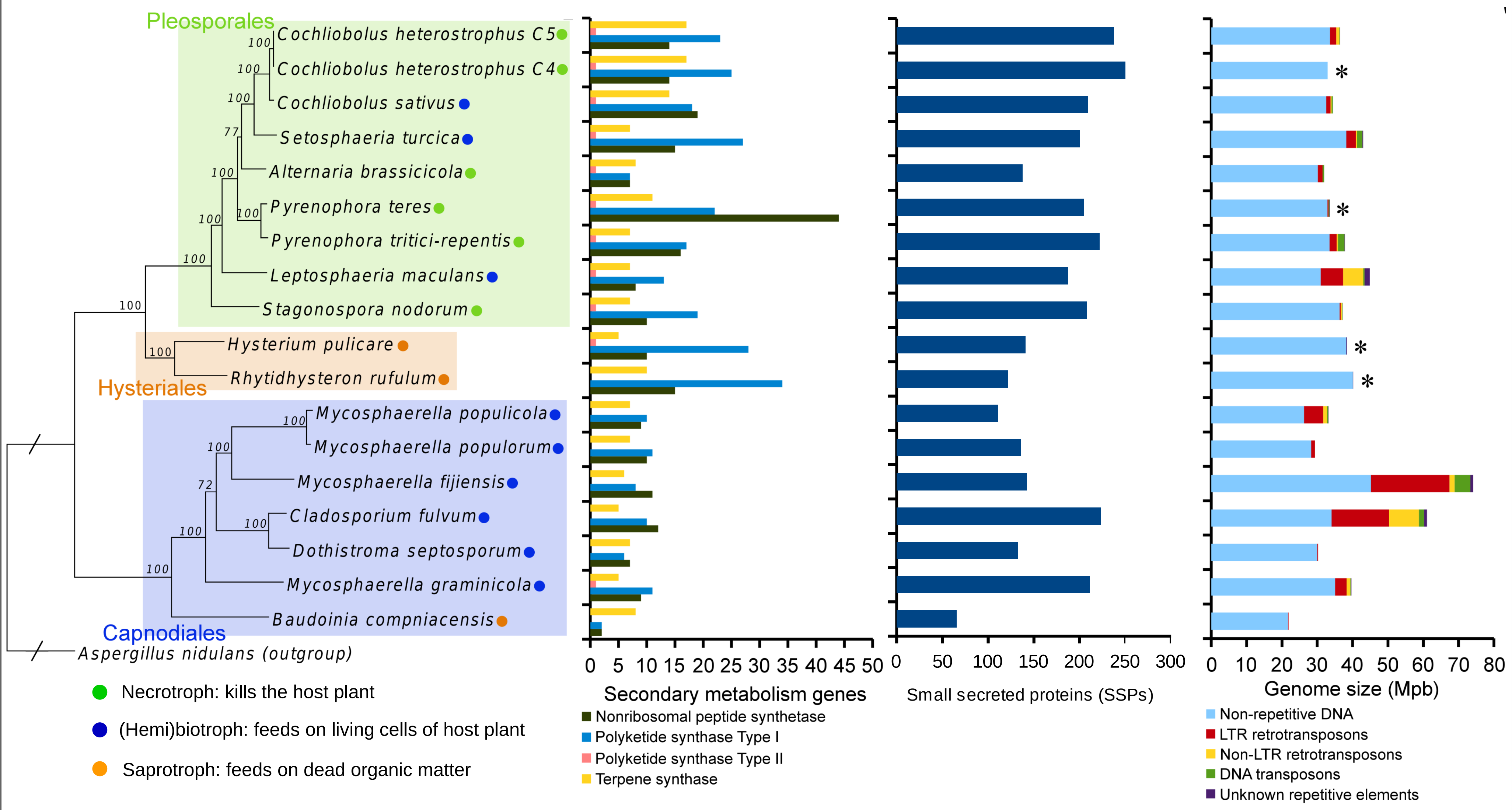


##	Organism	Name	Assembly length	# genes
1	Altbr1	Alternaria brassicicola	31,974,449	10,688
2	Bauco1	Baudoinia compniacensis UAMH 10762 (4089826) v1.0	21,876,451	10,513
3	Botdo1	Botryosphaeria dothidea	43,500,615	14,998
4	Cerzm1	Cercospora zeae-maydis v1.0	46,606,380	12,020
5	CocheC4_1	Cochliobolus heterostrophus C4 v1.0	32,929,167	12,720
6	CocheC5_3	Cochliobolus heterostrophus C5 v2.0	36,456,735	13,336
7	Cocsa1	Cochliobolus sativus ND90Pr v1.0	34,417,436	12,250
8	Dotse1	Dothistroma septosporum NZE10 v1.0	30,209,431	12,580

***Dothideomycetes* group page.** With tools for comparative analysis

Effector genes involved in pathogenesis

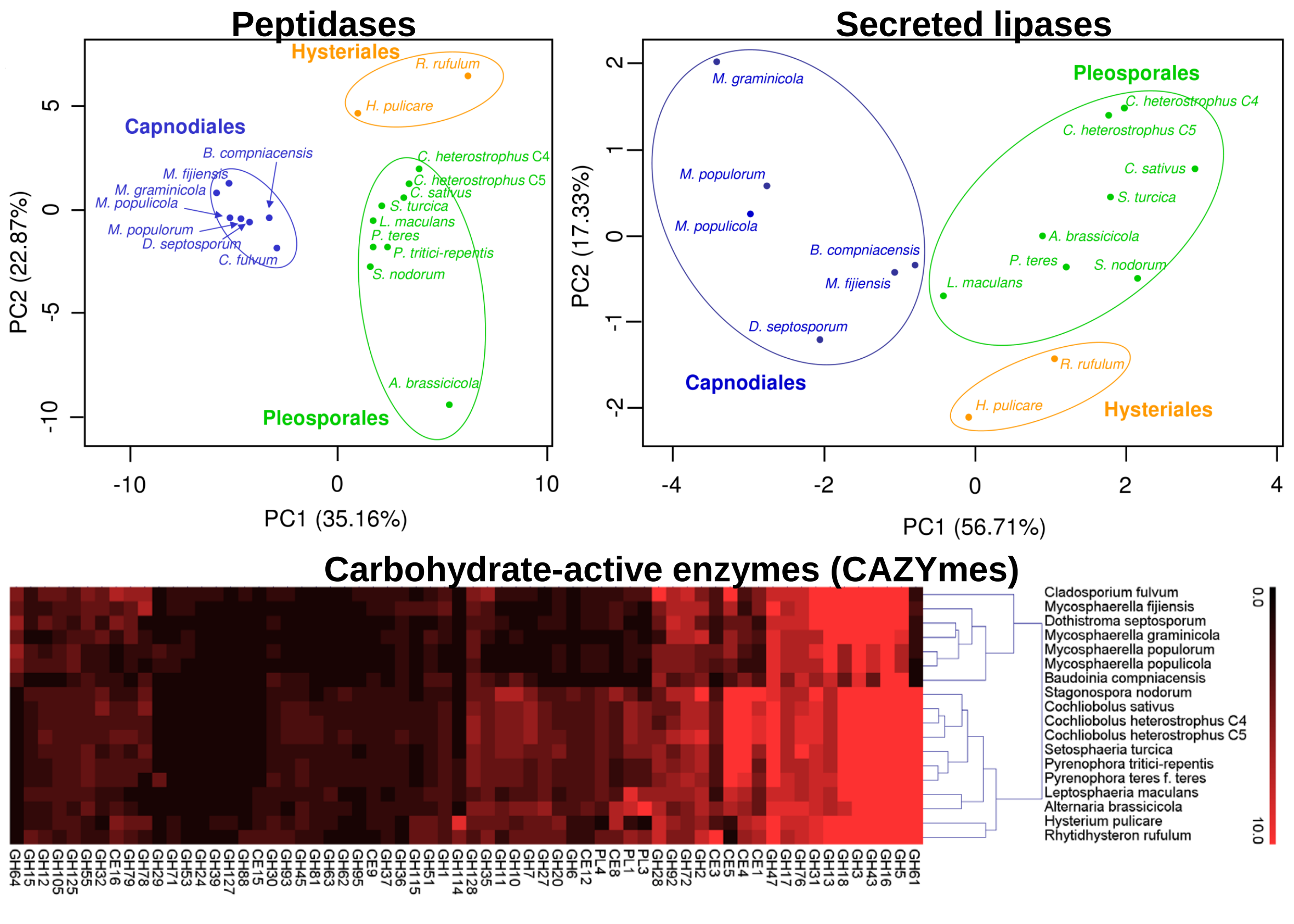
Lifestyles are largely phylogenetically separated. This is also reflected in the gene classes that are involved in plant pathogenesis. Some potential effector gene classes are over-represented near Transposable Elements, speeding up their evolution.



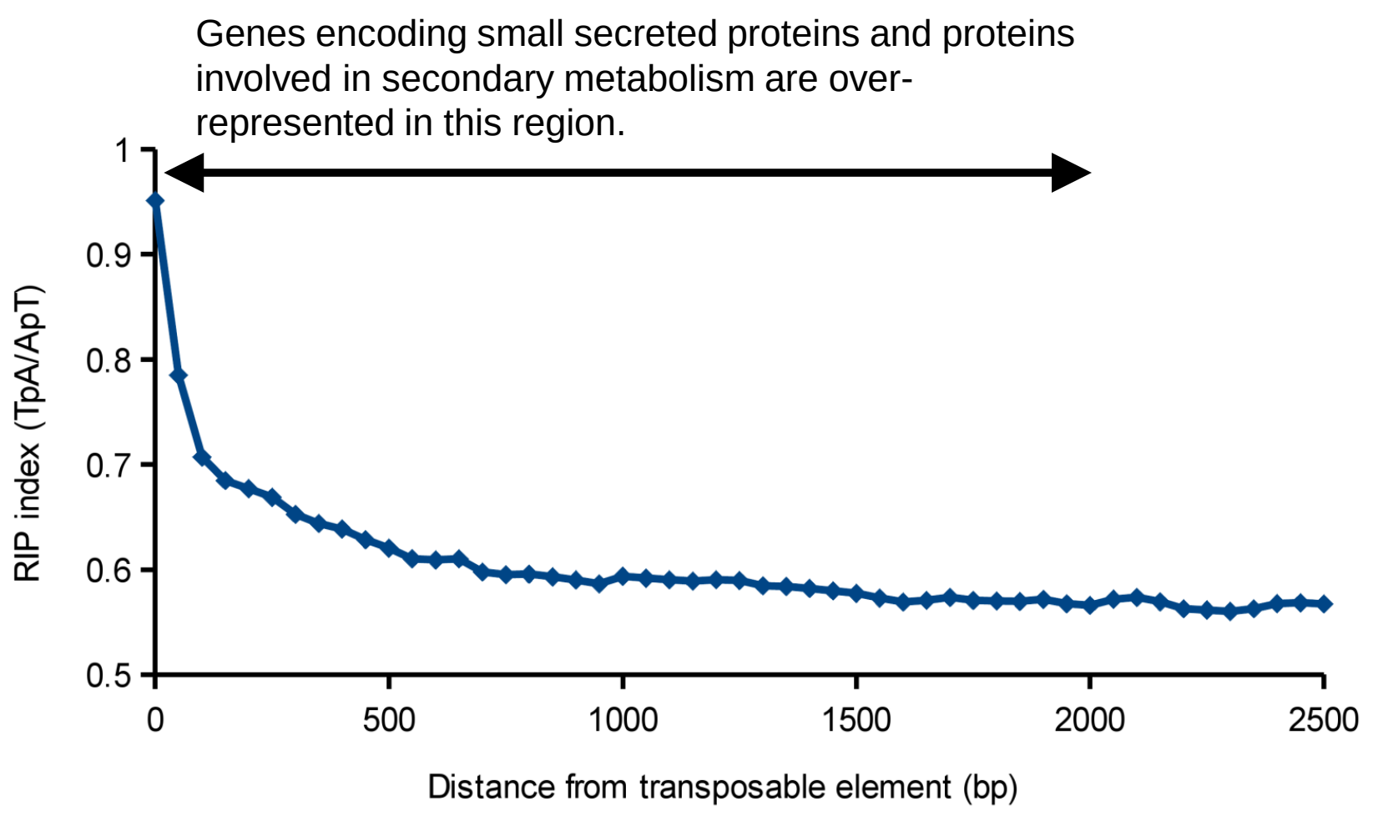
***Dothideomycete* phylogeny.** Different lifestyles are largely phylogenetically separated.

Proteins involved in secondary metabolism and small secreted proteins. Generally, genomes of necrotrophs in the *Pleosporales* contain more genes encoding these proteins.

Genome size varies widely. This is caused mainly by differences in repetitive content. Asterisks indicate Illumina-sequenced genomes. In these genomes repeat content is likely an under-estimate.

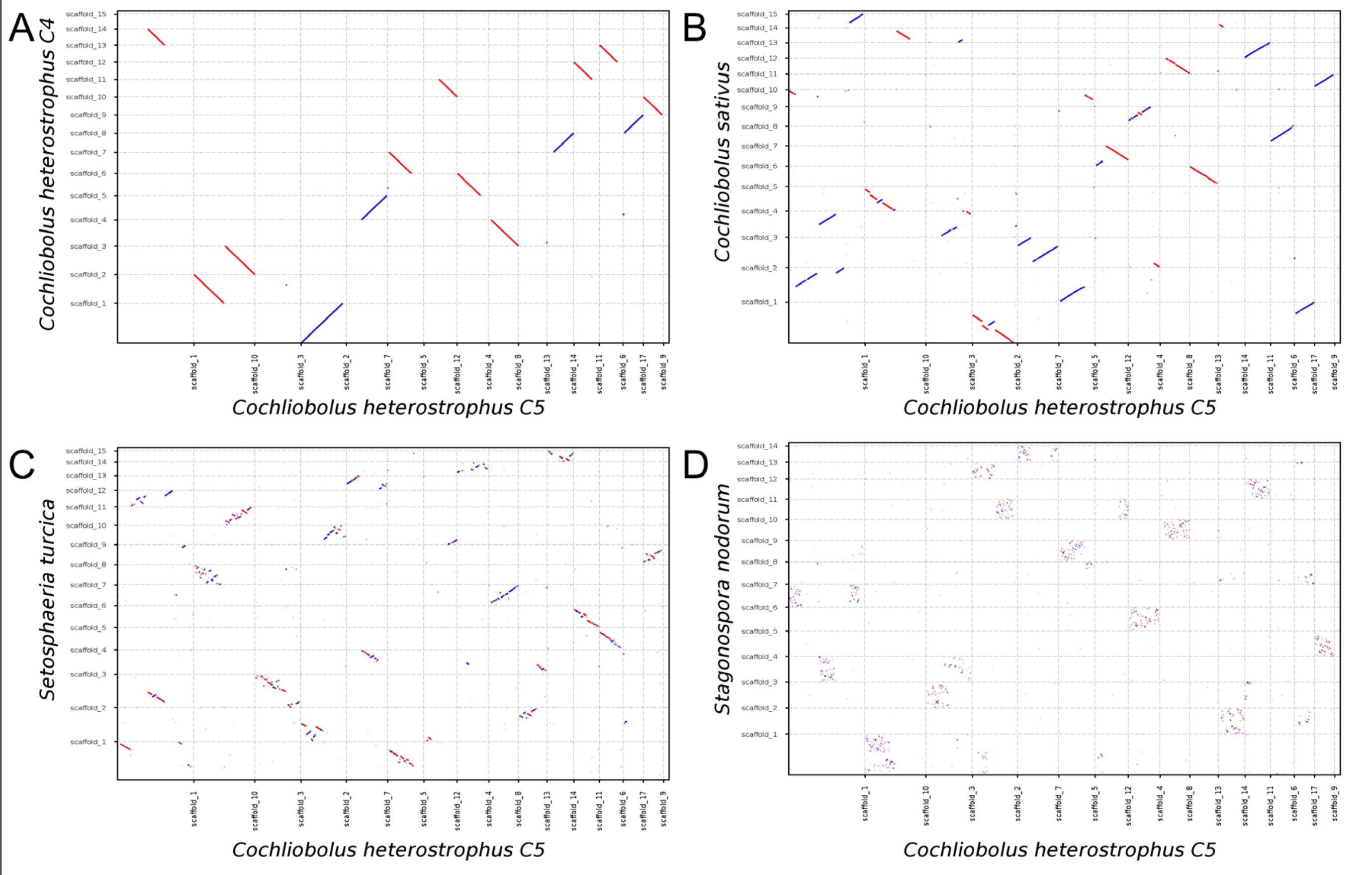


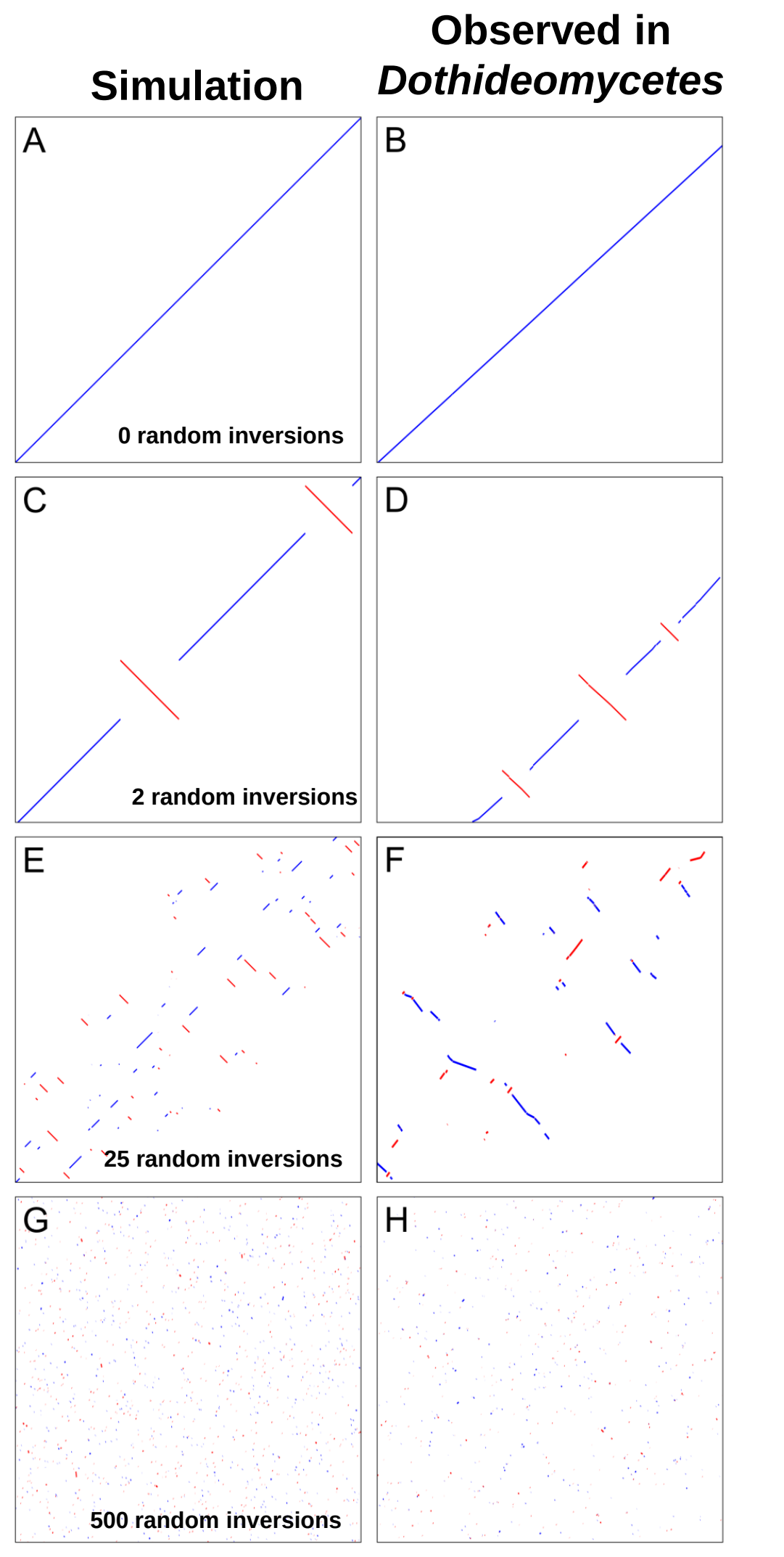
Peptidases, secreted lipases and CAZYmes. In each of these gene classes the organisms belonging to the same order cluster together.



Repeat Induced Point mutations (RIP) occur near Transposable Elements (TEs). Interestingly, some pathogenesis gene classes are over-represented near these TEs. The higher rate of point mutations in this region speeds up their evolution, presumably allowing the pathogens to evade plant defenses.

Whole-genome DNA comparison

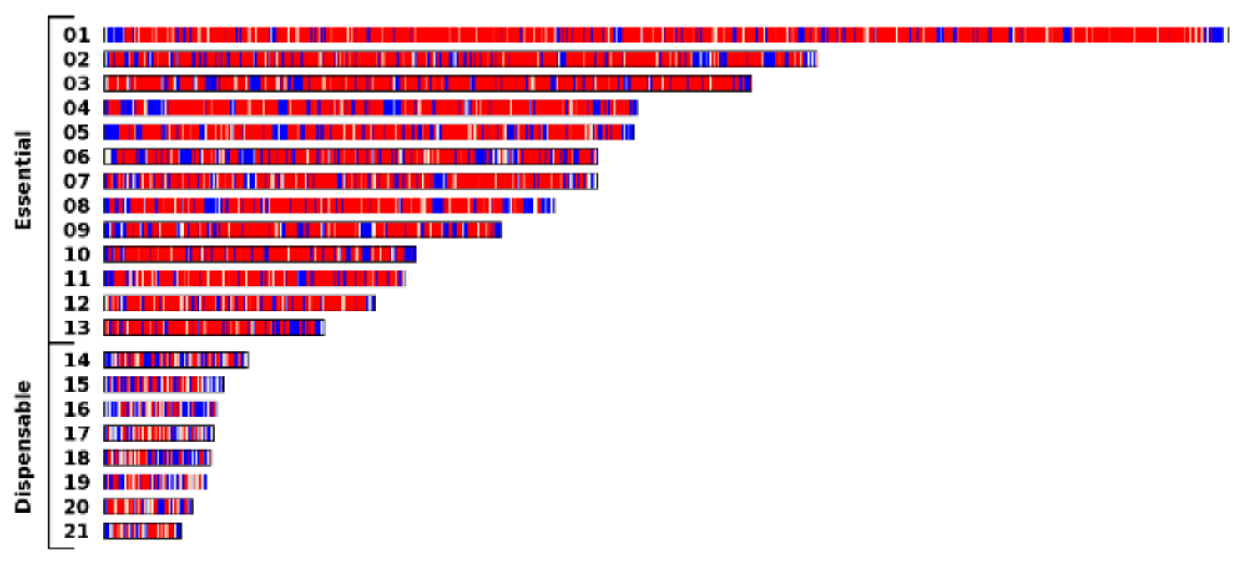




From macrosynteny to mesosynteny. Closely related species show a pattern of nearly perfect macrosynteny (A). This pattern degrades in comparisons with progressively distantly related species (B-D). The syntenic regions become short and spread across scaffold pairs. This phenomenon is most predominant among Dothideomycetes. It has previously been called mesosynteny (Hane et al. 2011). Here we show that mesosynteny appears to be due to intra-chromosomal inversions. Very few inter-chromosomal rearrangements take place. Interestingly, simple repeats (i.e. low-complexity DNA) are over-represented near inversion breakpoints (data not shown).

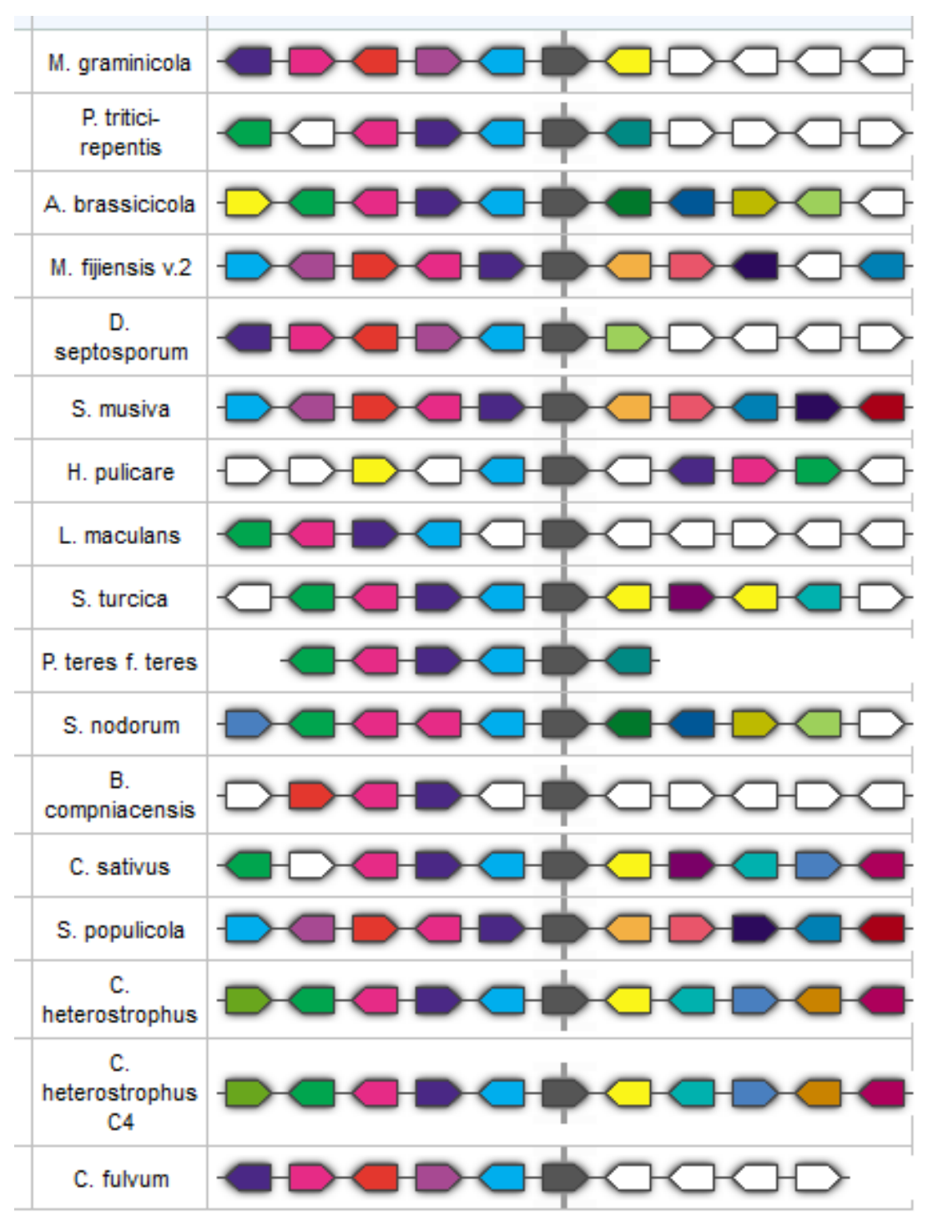
Simulations show that intra-chromosomal inversions lead to a pattern of mesosynteny that is observed among *Dothideomycetes*.

Dispensable chromosomes



Eight of the 21 chromosomes of *Mycosphaerella graminicola* are dispensable (i.e. not necessary for survival). These dispensable chromosomes are smaller, less gene-dense and more repeat-rich than the core chromosomes. Proteins encoded by genes on these chromosomes less frequently contain a PFAM domain. Their function is unknown (Goodwin *et al*. 2011).
Scaffolds with similar characteristics are also present in five other *Dothideomycetes*: *Mycosphaerella fijiensis, Cochliobolus heterostrophus C5, Setosphaeria turcica, Leptosphaeria maculans,* and *Stagonospora nodorum*.

Gene order conservation

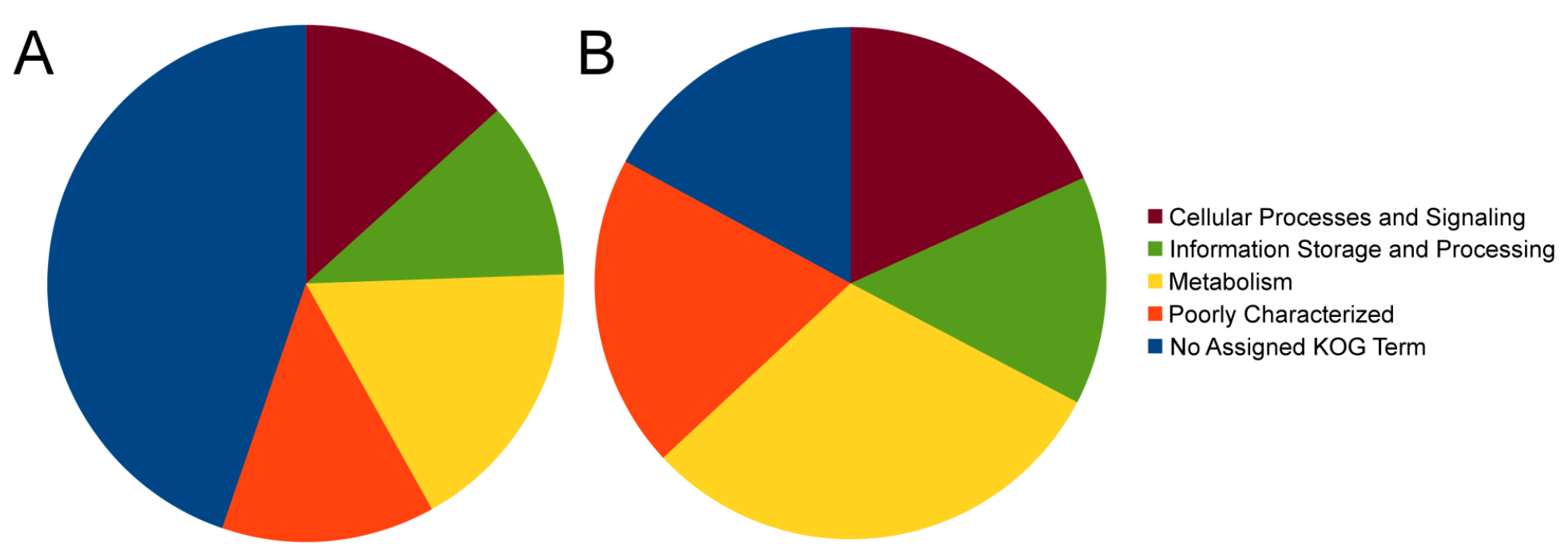


Only two syntenic blocks of genes are conserved in the *Dothideomycetes*. The syntenic block of genes shown here contains mostly oxidoreductases and dehydrogenases. Identical color means the genes are part of the same multi-gene family. Interestingly, in *L. maculans* these 5 genes are up-regulated during pathogenesis.

Conclusions

- The genomes of 18 *Dothideomycetes* (of which 15 are plant pathogens) are currently sequenced and available via MycoCosm.
- Lifestyles are largely phylogenetically separated. Counts of pathogenesis genes follow this pattern.
- Some classes of pathogenesis genes (secretome and secondary metabolism) are over-represented near transposable elements, where they are subject to Repeat Induced Point mutations (RIP). This speeds up their evolution.
- During Dothideomycete evolution many intra-chromosomal, but few inter-chromosomal rearrangements have taken place. This may keep dispensable chromosomes intact.
- Comparative transcriptomics gives insight into conserved fungal responses during pathogenesis, leading to new targets to fight infections.

Core proteome



A. The full proteome of the *Dothideomycetes* contains 215225 proteins and for the majority of these the function according to KOG is unknown or poorly characterized. **B.** The core proteome contains the 66761 proteins from multi-gene families that had at least one member in each *Dothideomycete*. Relative to (A), this set of proteins has more KOG annotations than the full proteome. In particular genes involved in metabolism are over-represented.

Comparative transcriptomics

- Microarray data is available for *M. graminicola* (Keon et al. 2005 and 2007) and *L. maculans* (Rouxel et al. 2011).
- In both cases gene expression was analyzed during early and late stage of infection, allowing comparative analysis.
- 98 orthologous gene-pairs are up-regulated in both organisms during pathogenesis.
  - 24 are oxidoreductases
  - 3 are transcription factors
- Because these 98 genes have a conserved expression responses during infection, they may very well be involved in the pathogenesis process.

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