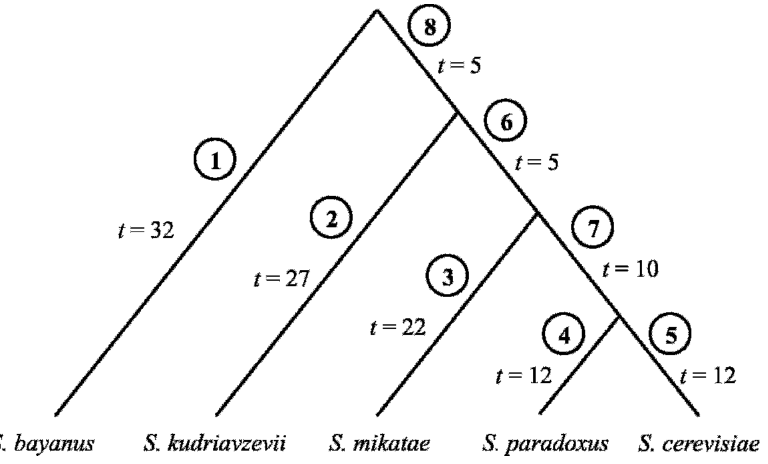
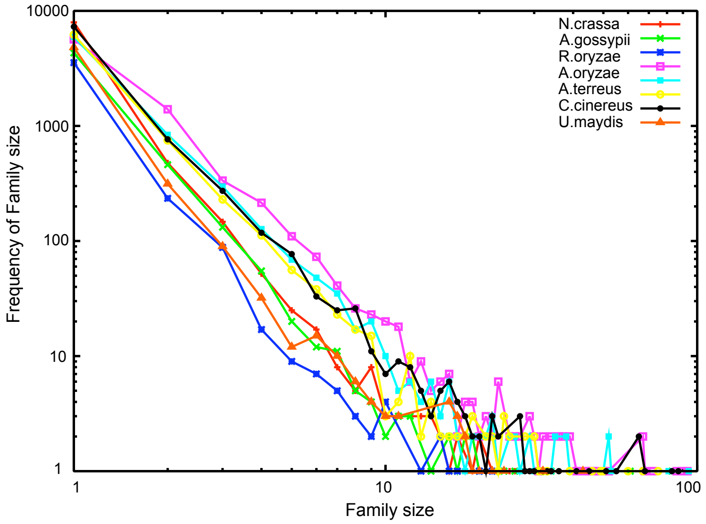
Evolution of gene family size change in fungi

Jason Stajich
University of California, Berkeley





Outline

- Gene family size change - a model
- Cornucopia of fungal genomes
- Methodology for comparing family size
- Lineage specific expansions

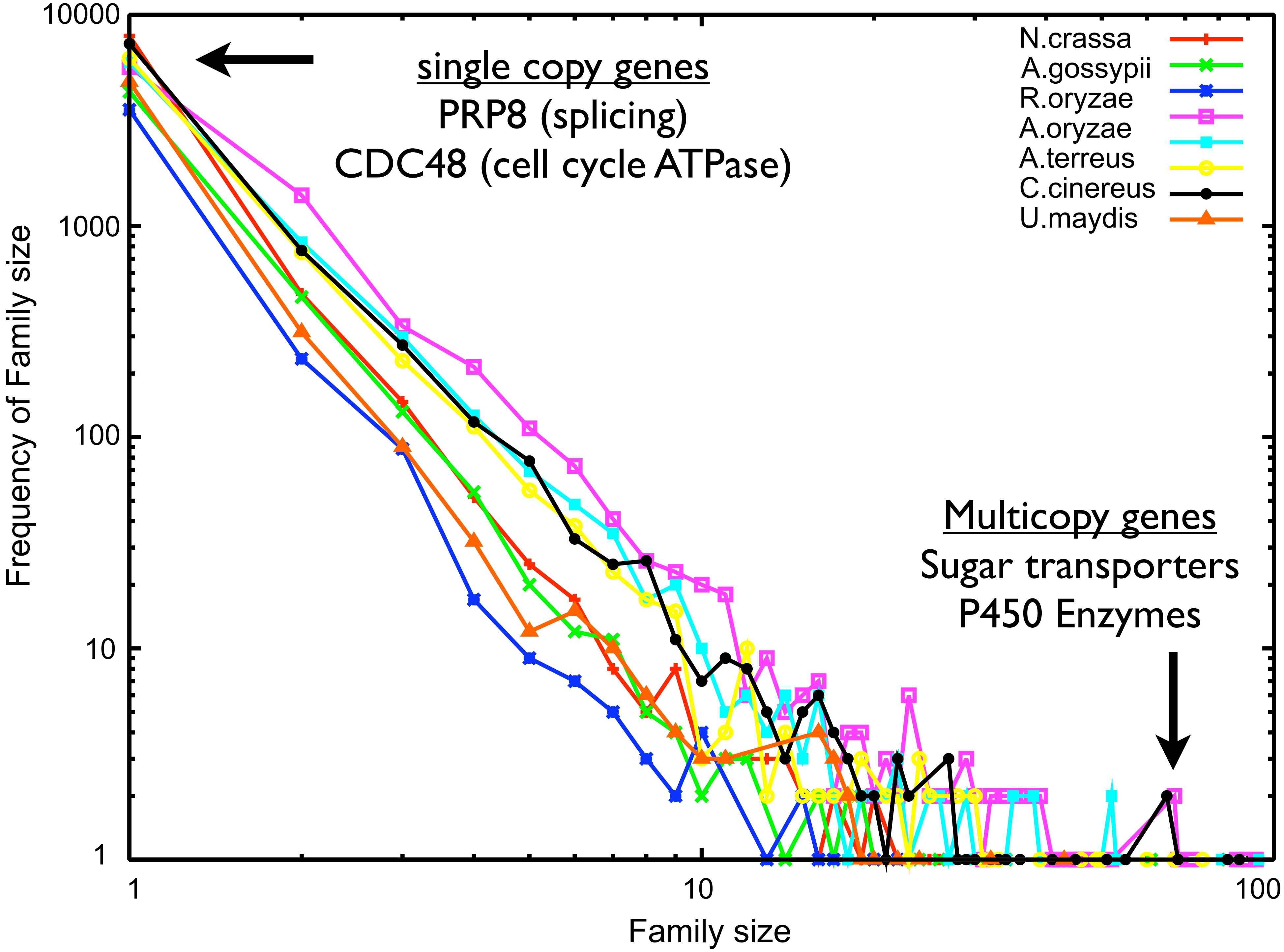
Gene family evolution

- Gene duplications are the crucible of new genes and thus new functions
- Many comparative approaches focus only on identifiable one-to-one orthologs.
  - Signature of adaptive evolution can be confounded in multi-gene families
- How important is lineage-specific expansion in adaptive changes?

Identifying family expansions

- Previous work only considered pairwise
- *Ad hoc* comparison of gene family sizes
  - *C.elegans-C.briggsae* - GPCR family expansions (Stein et al, *PLOS Biology* 2004)
  - *A. gambiae-D. melanogaster* - Mosquito specific family expansions related to symbiotic bacteria (Holt et el, *Science* 2002).
- Need a null model

Gene family sizes follow power law distribution



single copy genes

PRP8 (splicing)

CDC48 (cell cycle ATPase)

Multicopy genes

Sugar transporters

P450 Enzymes

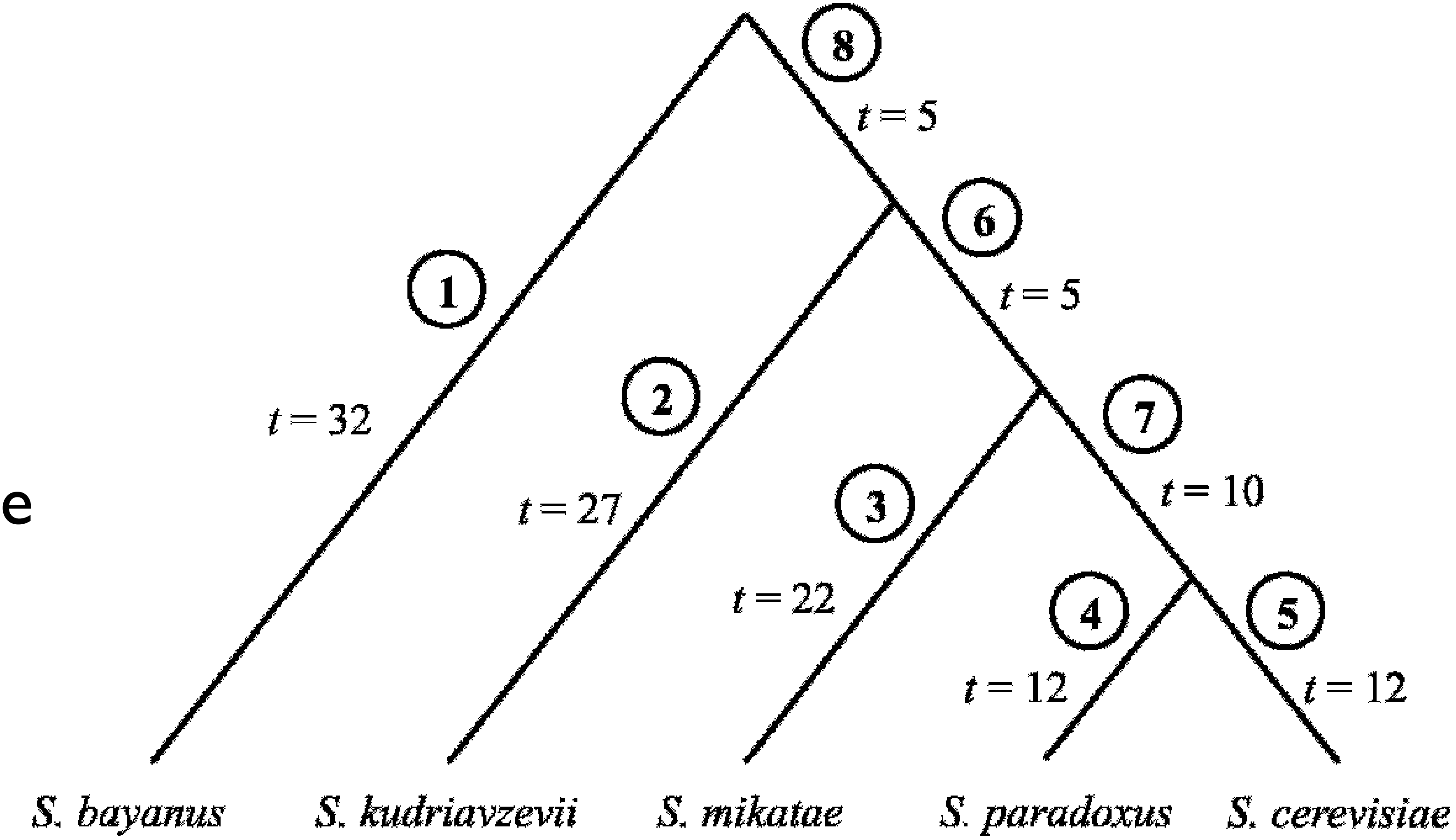
Phylogenetic evaluation of gene family size change

- Previous methods only used *ad hoc* statistics
- Explicit model for gene family size change according to a Birth-Death models
- Apply BD to family size along phylogeny using probabilistic graph models
- CAFE - Computational Analysis of gene Family Evolution

Hahn et al, *Genome Res* 2005
De Bie, et al *Bioinformatics* 2006
Demuth et al, *submitted*

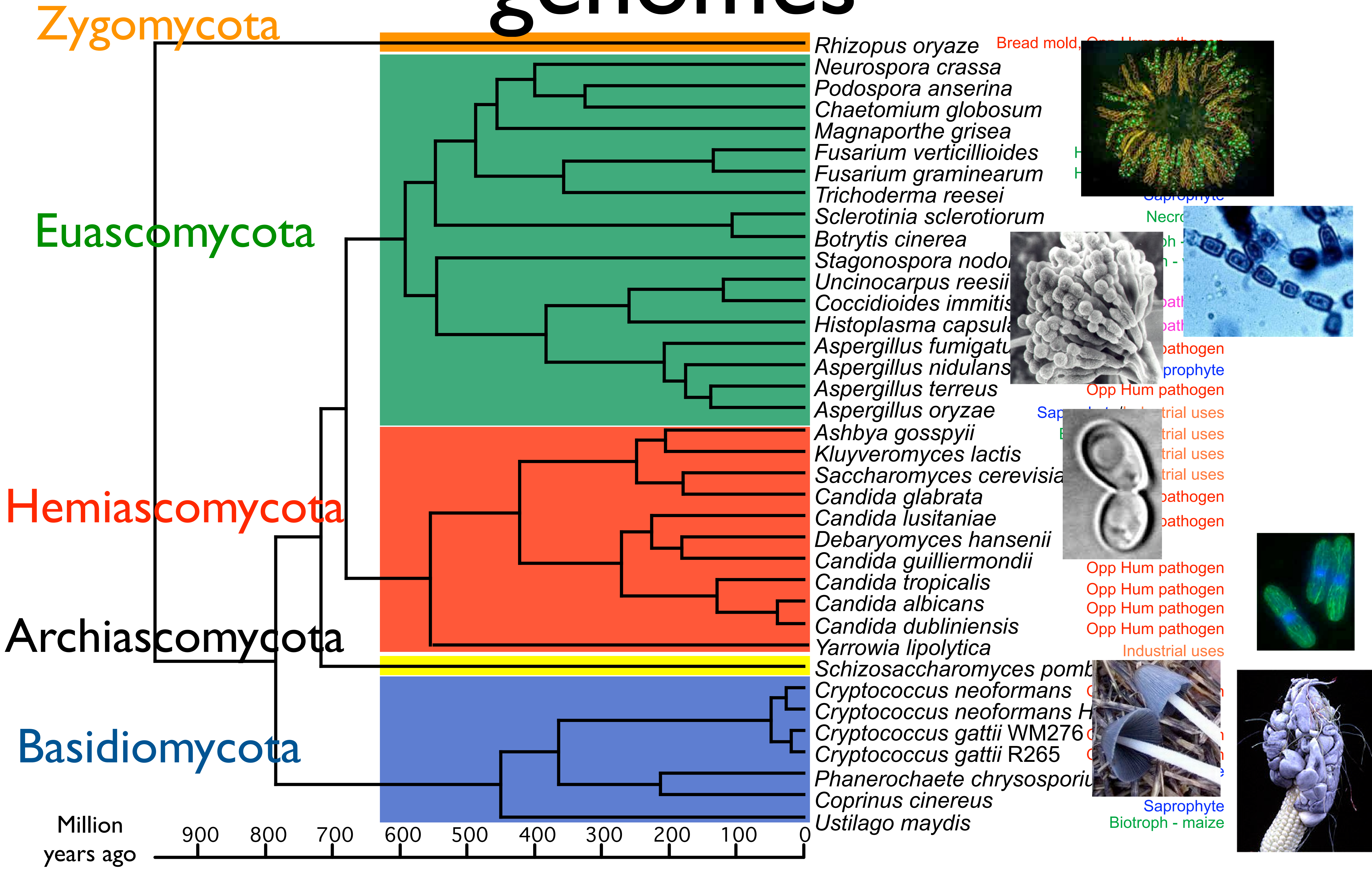
CAFE

- Use a Probabilistic Graph Model for:
- Ancestral states
- Birth and Death rate (lamda)
- Per branch changes
- P-values



Hahn et al, *Genome Res* 2005

37 Fully sequenced fungal genomes



Zygomycota

Euascomycota

Hemiascomycota

Archiascomycota

Basidiomycota

Rhizopus oryaze Bread mold, Opp Hum pathogen
Neurospora crassa
Podospora anserina
Chaetomium globosum
Magnaporthe grisea
Fusarium verticillioides H
Fusarium graminearum H
Trichoderma reesei Saprophyte
Sclerotinia sclerotiorum Necr
Botrytis cinerea ph -
Stagonospora nodo h -
Uncinocarpus reesii
Coccidioides immitis path
Histoplasma capsula path
Aspergillus fumigatu pathogen
Aspergillus nidulans prophyte
Aspergillus terreus Opp Hum pathogen
Aspergillus oryzae Sap trial uses
Ashbya gosspyii E trial uses
Kluyveromyces lactis trial uses
Saccharomyces cerevisia trial uses
Candida glabrata pathogen
Candida lusitaniae pathogen
Debaryomyces hansenii
Candida guilliermondii Opp Hum pathogen
Candida tropicalis Opp Hum pathogen
Candida albicans Opp Hum pathogen
Candida dubliniensis Opp Hum pathogen
Yarrowia lipolytica Industrial uses
Schizosaccharomyces pomb
Cryptococcus neoformans C n
Cryptococcus neoformans H
Cryptococcus gattii WM276 C n
Cryptococcus gattii R265 C n
Phanerochaete chrysosporiu e
Coprinus cinereus Saprophyte
Ustilago maydis Biotroph - maize

50+ More funded and
in progress world-wide

Sequencing In-Progress*

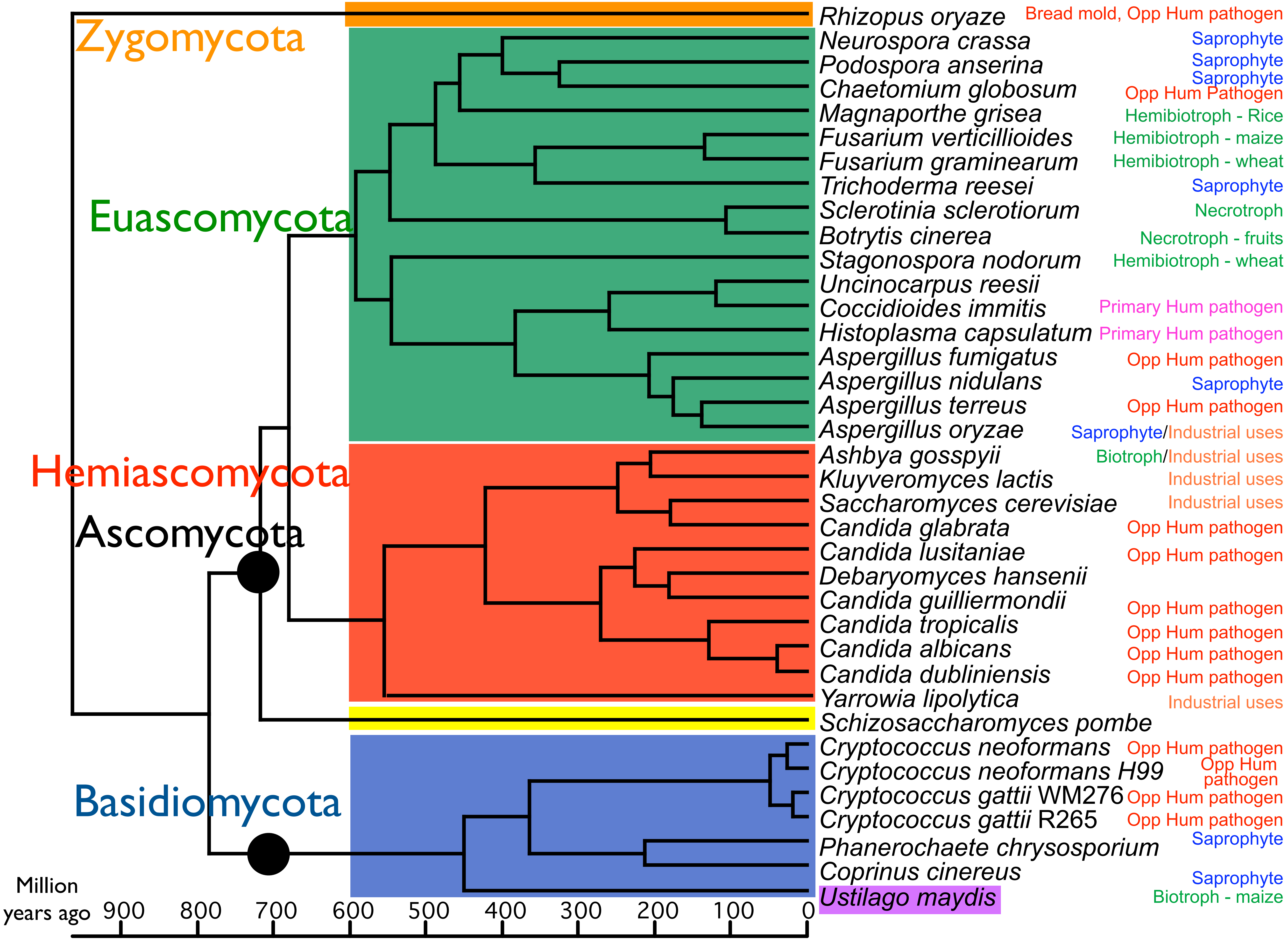
Species	**Clade**	**Sequencing center**	
Schizosaccharomyces japonicus	Archaeascomycta	Broad-FGI	
Schizosaccharomyces octosporus	Archaeascomycta	Broad-FGI	
Pneumocystis carinii	Archaeascomycta	Sanger, UC, Broad-FGI	
Pneumocystis carinii hominis	Archaeascomycta	UC, Broad-FGI, UC	
Amanita bisporigera	Basidiomycota: Homobasidiomycota	MSU	
Crinipellis perniciosa	Basidiomycota: Homobasidiomycota	Univ Campinas	
Ganoderma lucidum	Basidiomycota: Homobasidiomycota	Yang-Ming Univ	
Hebeloma cylindrosporum	Basidiomycota: Homobasidiomycota	INRA	
Laccaria bicolor	Basidiomycota: Homobasidiomycota	JGI-DOE	R
Phakopsora pachyrhizi	Basidiomycota: Homobasidiomycota	JGI-DOE	
Postia placenta	Basidiomycota: Homobasidiomycota	JGI-DOE	
Schizophyllum commune	Basidiomycota: Homobasidiomycota	JGI-DOE	
Sporobolomyces roseus	Basidiomycota: Urediniomycota	JGI-DOE	
Phakopsora meibomiae	Basidiomycota: Urediniomycota	JGI-DOE	
Batrachochytrium dendrobatidis	Chytridiomycota	Broad-FGI & JGI-DOE	
Piromyces sp.	Chytridiomycota	JGI-DOE	
Glomus intraradices	Glomeromycota	JGI-DOE	
Phycomyces blakesleeanus	Zygomycota	JGI-DOE	R
Brachiola algerae	Microsporidia	Genoscope	
Nosema (Antonospora) locustae	Microsporidia	MBL	R
Enterocytozoon bieneusi	Microsporidia	Tufts Univ	

Sequencing In-Progress*

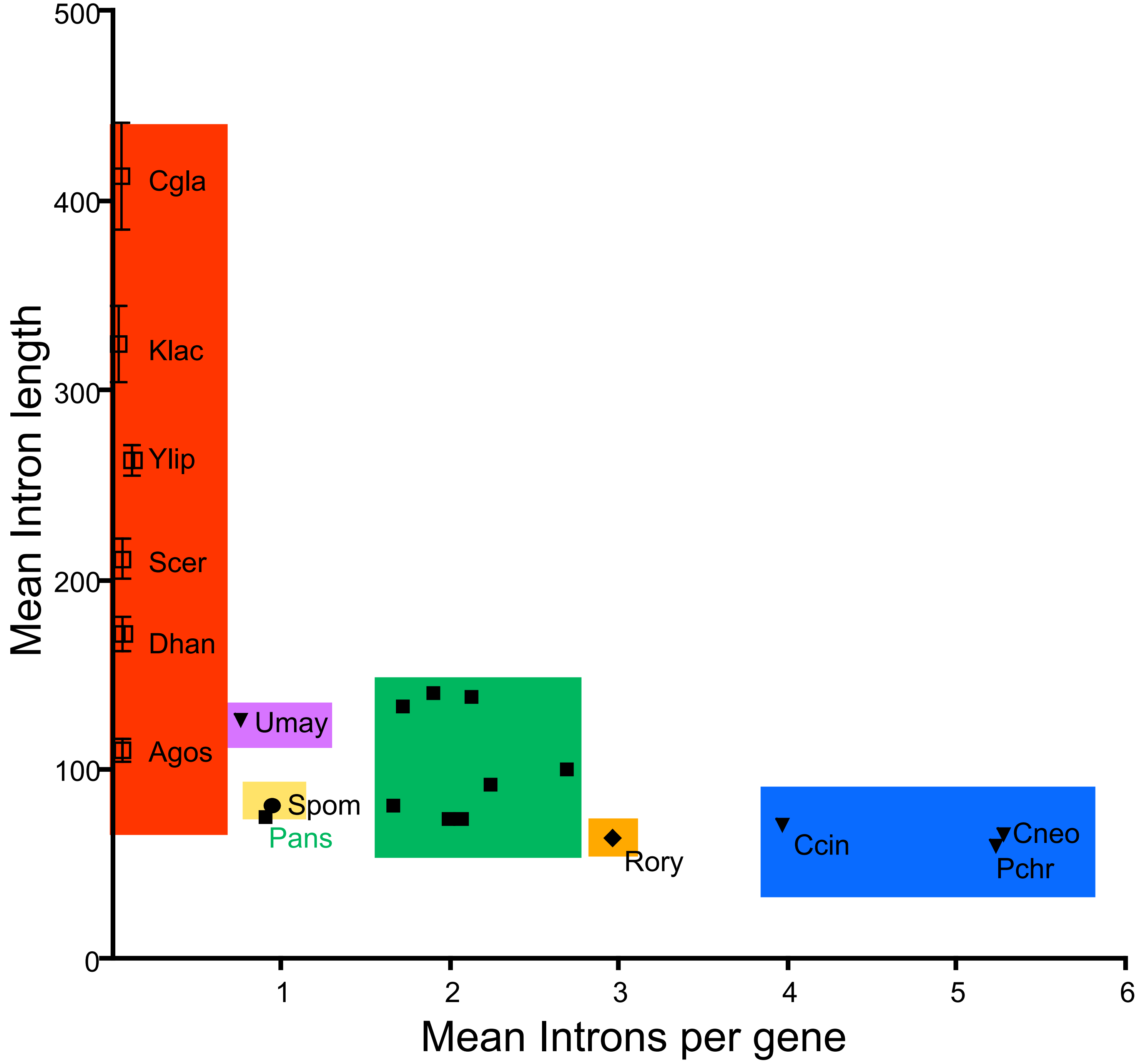
Species	**Clade**	**Sequencing center**
Aspergillus niger	Euascomycota: Eurotiomycota	DOE-JGI R
Aspergillus flavus	Euascomycota: Eurotiomycota	NCSU
Aspergillus clavatus	Euascomycota: Eurotiomycota	OU
Neosartorya fischeri	Euascomycota: Eurotiomycetes	TIGR
Histoplasma capsulatum WU24	Euascomycota: Eurotiomycota	Broad-FGI R
Histoplasma capsulatum 186R,217B	Euascomycota: Eurotiomycota	WUSTL
Coccidioides posadasii	Euascomycota: Eurotiomycota	TIGR
Coccidioides immitis 10 strains	Euascomycota: Eurotiomycota	Broad-FGI & TIGR
Paracoccidioides brasiliensis	Euascomycota: Eurotiomycota	Univ of Brazil
Ascosphaera apis	Euascomycota: Eurotiomycota	BCM
Epichloe festucae	Euascomycota: Sordariomycetes	UK
Podospora anserina	Euascomycota: Sordariomycetes	Broad-FGI
Trichoderma atroviride	Euascomycota: Sordariomycetes	DOE-JGI
Trichoderma virens	Euascomycota: Sordariomycetes	DOE-JGI
Leptosphaeria maculans	Euascomycota: Dothideomycetes	Genoscope
Alternaria brassicicola	Euascomycota: Dothideomycetes	VPI & WUSTL R
Xanthoria parietina (lichen)	Euascomycota: Lecanoromycetes	DOE-JGI
Candida albicans WO-1	Hemiascomycota	Broad-FGI
Lodderomyces elongisporus	Hemiascomycota	Broad-FGI R
Pichia stipitis	Hemiascomycota	JGI-DOE
Saccharomces bayanus	Hemiascomycota	(49, 167)
Saccharomces castellii	Hemiascomycota	(49)
Saccharomces cerevevisiae RM11-1A	Hemiascomycota	Broad-FGI
Saccharomces cerevevisiae YJM789	Hemiascomycota	(113) +++
Saccharomyces kluyeri	Hemiascomycota	WUSTL (finishing)
Saccharomces kudriavzevii	Hemiascomycota	(49)
Saccharomces mikatae	Hemiascomycota	(49, 167)
Saccharomces paradoxus	Hemiascomycota	(167)
Saccharomyces pastorianus	Hemiascomycota	Kitasato Univ
Zygosaccharomyces rouxii	Hemiascomycota	CNRS-Genoscope

Genome annotation

- Many of the fungal genomes were only assembled genomic sequence.
- Automated annotation pipeline was built to generate to get systematic gene prediction.
- Several gene prediction programs were trained and results were combined with GLEAN (Liu, Mackey, Roo, et al unpublished) to produce composite gene calls.

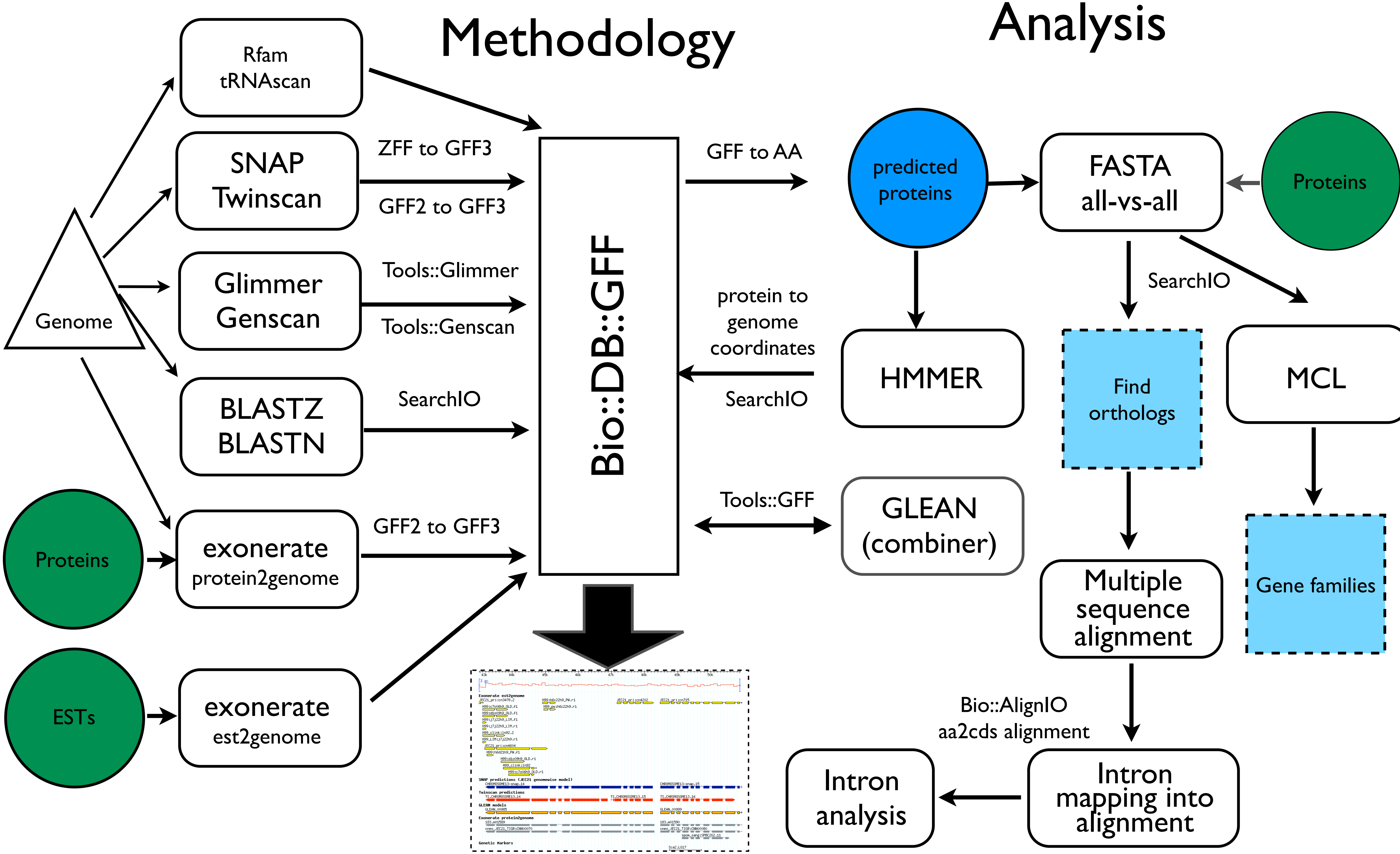

Zygomycota
Euascomycota
Hemiascomycota
Ascomycota
Basidiomycota
Rhizopus oryaze
Bread mold, Opp Hum pathogen
Neurospora crassa
Saprophyte
Podospora anserina
Saprophyte
Chaetomium globosum
Saprophyte
Opp Hum Pathogen
Magnaporthe grisea
Hemibiotroph - Rice
Fusarium verticillioides
Hemibiotroph - maize
Fusarium graminearum
Hemibiotroph - wheat
Trichoderma reesei
Saprophyte
Sclerotinia sclerotiorum
Necrotroph
Botrytis cinerea
Necrotroph - fruits
Stagonospora nodorum
Hemibiotroph - wheat
Uncinocarpus reesii
Coccidioides immitis
Primary Hum pathogen
Histoplasma capsulatum
Primary Hum pathogen
Aspergillus fumigatus
Opp Hum pathogen
Aspergillus nidulans
Saprophyte
Aspergillus terreus
Opp Hum pathogen
Aspergillus oryzae
Saprophyte/Industrial uses
Ashbya gosspyii
Biotroph/Industrial uses
Kluyveromyces lactis
Industrial uses
Saccharomyces cerevisiae
Industrial uses
Candida glabrata
Opp Hum pathogen
Candida lusitaniae
Opp Hum pathogen
Debaryomyces hansenii
Candida guilliermondii
Opp Hum pathogen
Candida tropicalis
Opp Hum pathogen
Candida albicans
Opp Hum pathogen
Candida dubliniensis
Opp Hum pathogen
Yarrowia lipolytica
Industrial uses
Schizosaccharomyces pombe
Cryptococcus neoformans
Opp Hum pathogen
Cryptococcus neoformans H99
Opp Hum pathogen
Cryptococcus gattii WM276
Opp Hum pathogen
Cryptococcus gattii R265
Opp Hum pathogen
Phanerochaete chrysosporium
Saprophyte
Coprinus cinereus
Saprophyte
Ustilago maydis
Biotroph - maize
Million years ago
900
800
700
600
500
400
300
200
100
0

Intron frequency varies among the fungi



Methodology

Analysis



Genome

Rfam
tRNAscan

SNAP
Twinscan

ZFF to GFF3

GFF2 to GFF3

Glimmer
Genscan

Tools::Glimmer

Tools::Genscan

BLASTZ
BLASTN

SearchIO

Proteins

exonerate
protein2genome

GFF2 to GFF3

ESTs

exonerate
est2genome

Bio::DB::GFF

GFF to AA

protein to
genome
coordinates

SearchIO

Tools::GFF

predicted
proteins

HMMER

GLEAN
(combiner)

FASTA
all-vs-all

Proteins

SearchIO

Find
orthologs

MCL

Gene families

Multiple
sequence
alignment

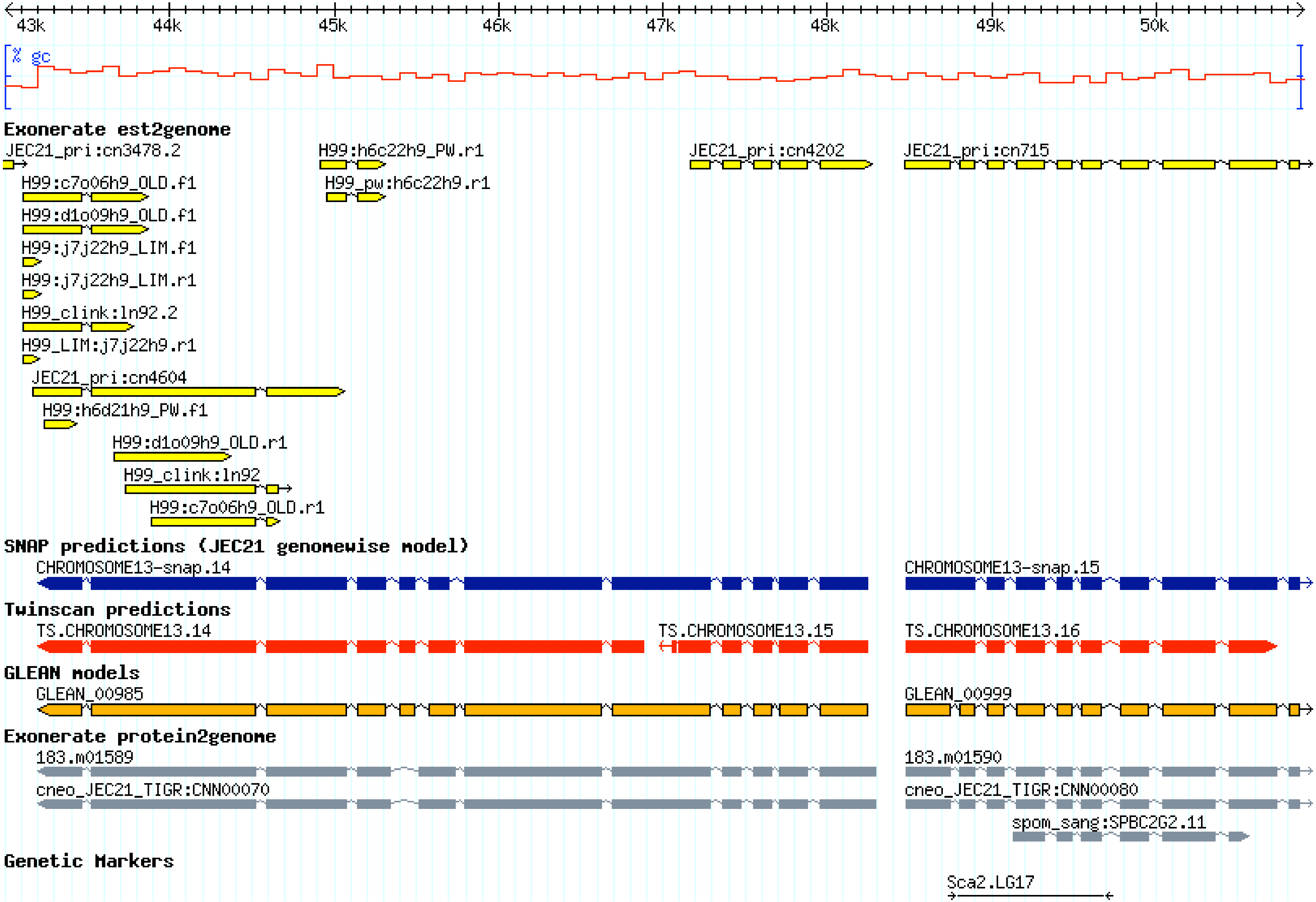
Bio::AlignIO
aa2cds alignment

Intron
mapping into
alignment

Intron
analysis

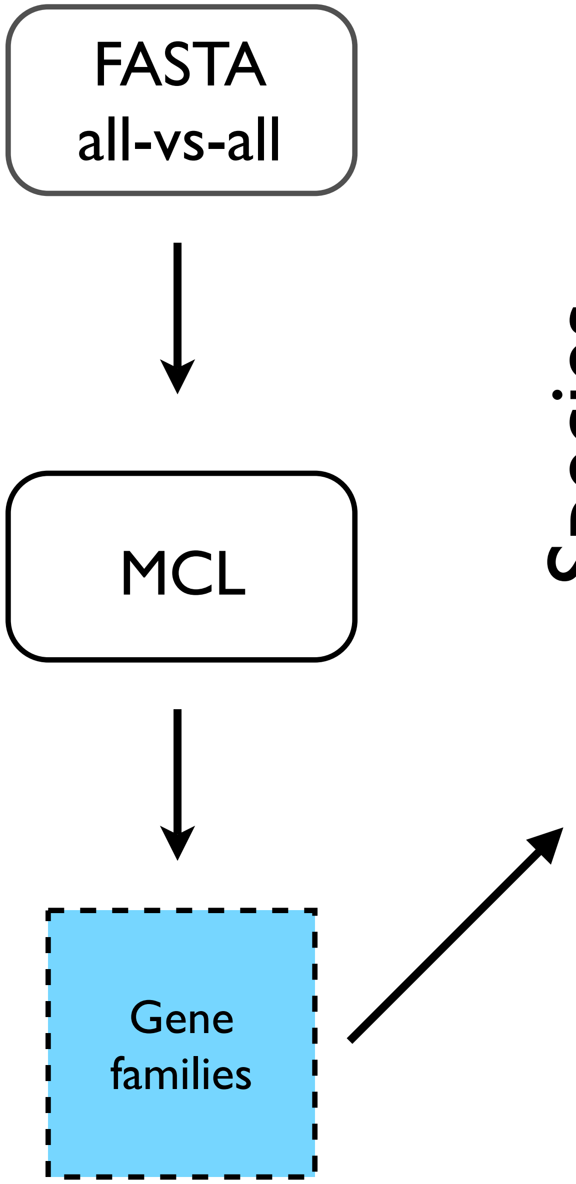
http://fungal.genome.duke.edu

Generic Genome Browser



Methods: gene family identification

- All-vs-All pairwise sequence searches (FASTP)
- Cluster genes by similarity using Markov CLustering (MCL) algorithm
- Identify families with unusually large size changes along phylogeny with CAFE
- Use 37 fungal genomes from 5 major clades



FASTA all-vs-all

MCL

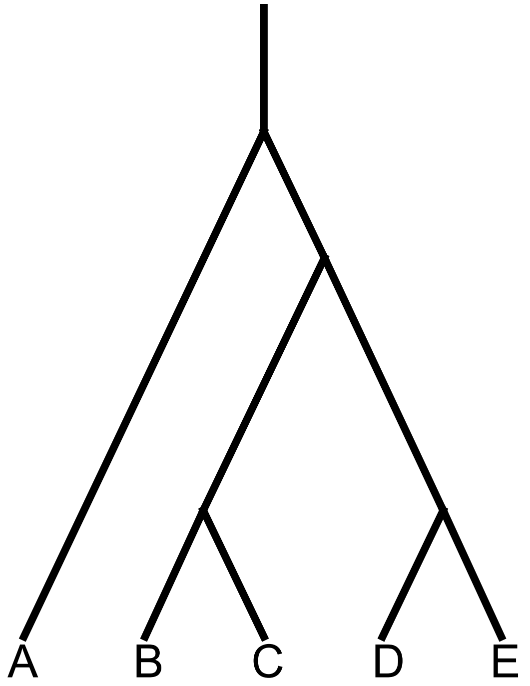
Gene families

Family count

Species

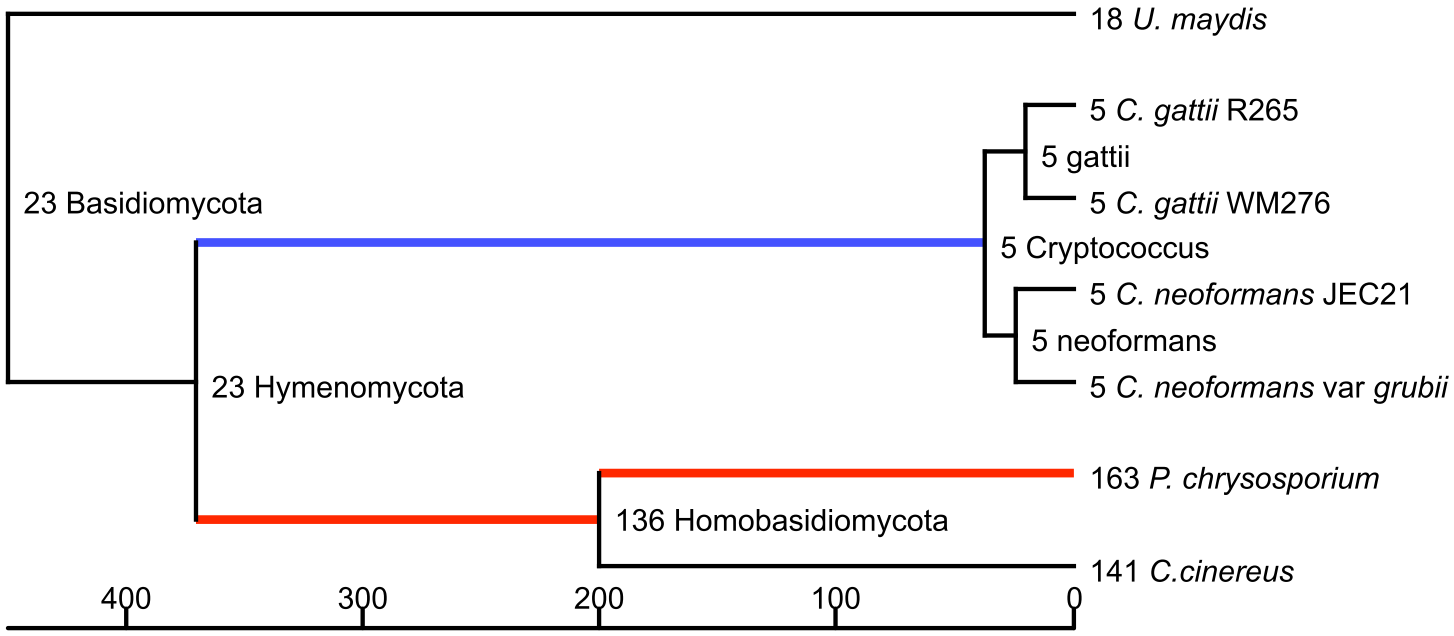
🟧	10	1	2
🟦	14	18	2
🟨	7	1	1
🟩	6	1	12
🟩	6	1	8
🟥	3	1	1

+



CAFE

Family 1	$P < 0.001$	Branch A
Family 2	$P < 0.001$	Branch B
Family 3	$P=0.02$	Branch C,E
Family 4	$P=0.03$	Branch D



Families with significant expansions

49 significant families

Transporters
Kinases
P450
Oxidation

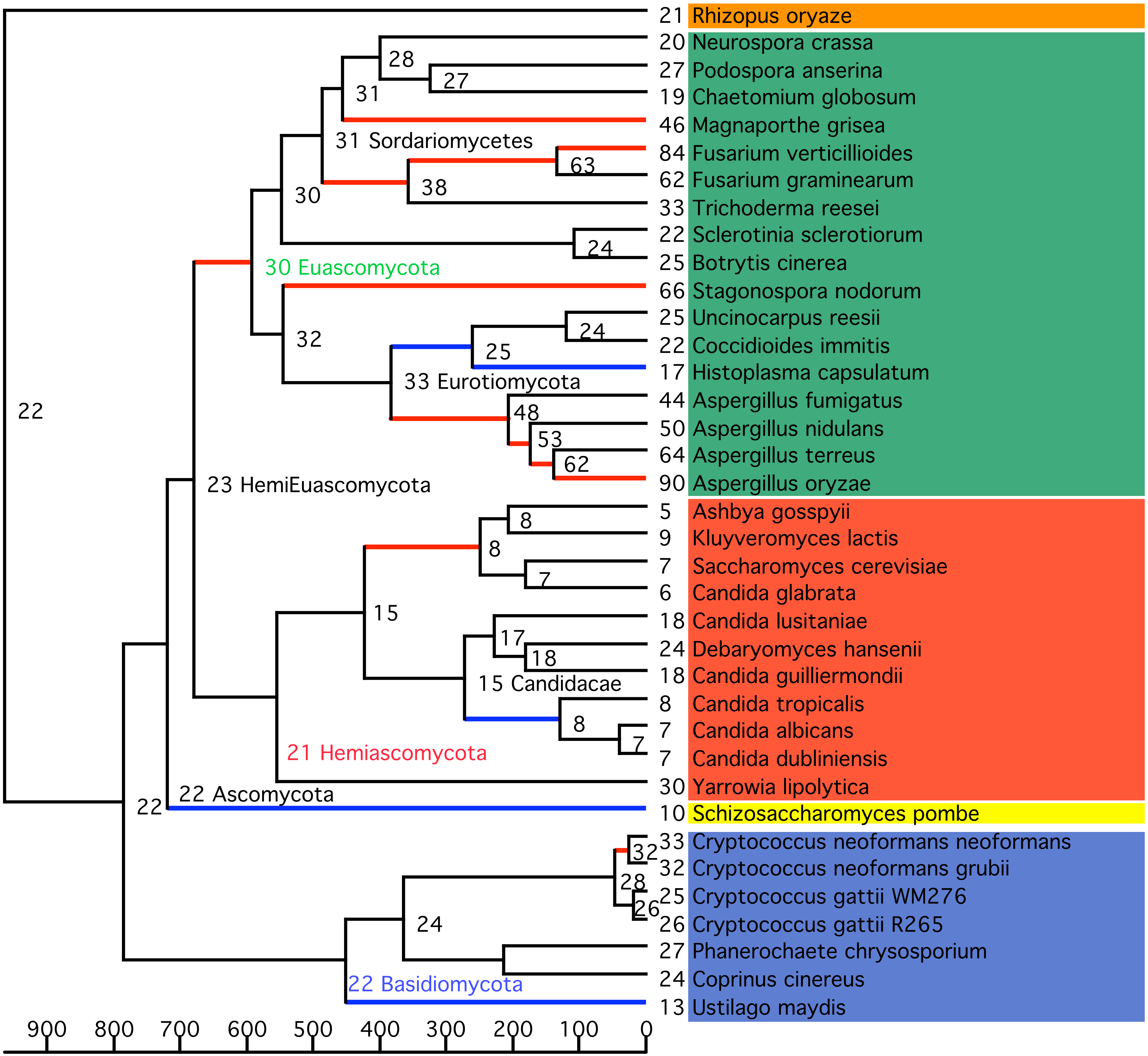
- Vitamin & Cofactor transport
- Lactose & sugar transport
- Amine transport
- Myo-instol, quinate, and glucose transport
- Oligopeptide transport
- ABC transporter
- MFS, drug pump, & sugar transport
- Transport
- Monocarboxylate & sugar transport
- ABC transport
- Amino acid permease

- Methytransferase
- Cytochrome P450: CYP64
- Cytochrome P450: CYP53,57A
- Cytochrome P450
- Kinase
- Subtilase family
- NADH flavin oxidoreductase
- Aldehyde dehydrogenase
- Aldo/kedo reductase
- Multicopper oxidase
- AMP-binding enzyme

Transporters

- Of 45 significant families, 22 were related to transport
- Vitamin and amino acid transport
- Sugar and sugar-like transporters
- Multidrug and efflux pumps
- ABC transporters (ATP Binding Cassette)

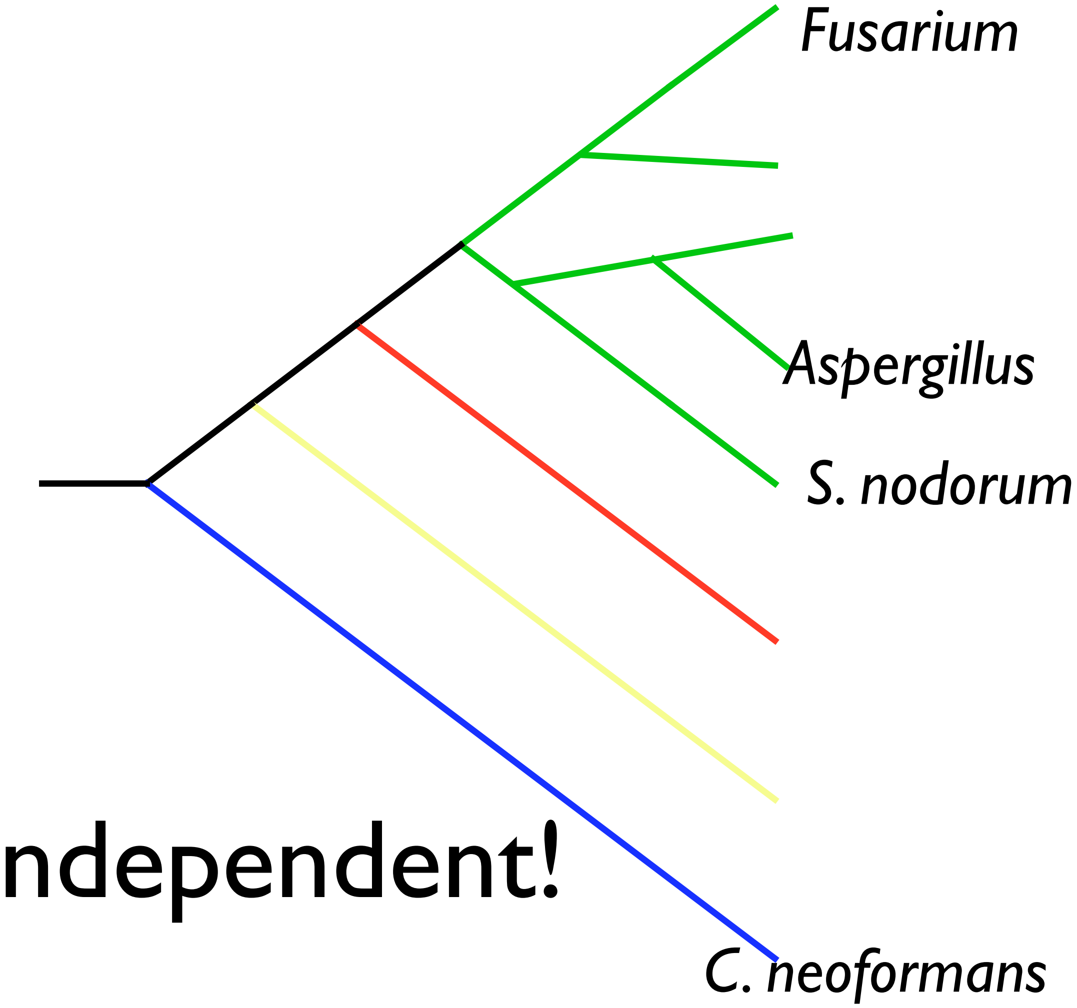
Vitamin & Cofactor Transporters



Marked branches with significant (P<0.05) expansions or contractions

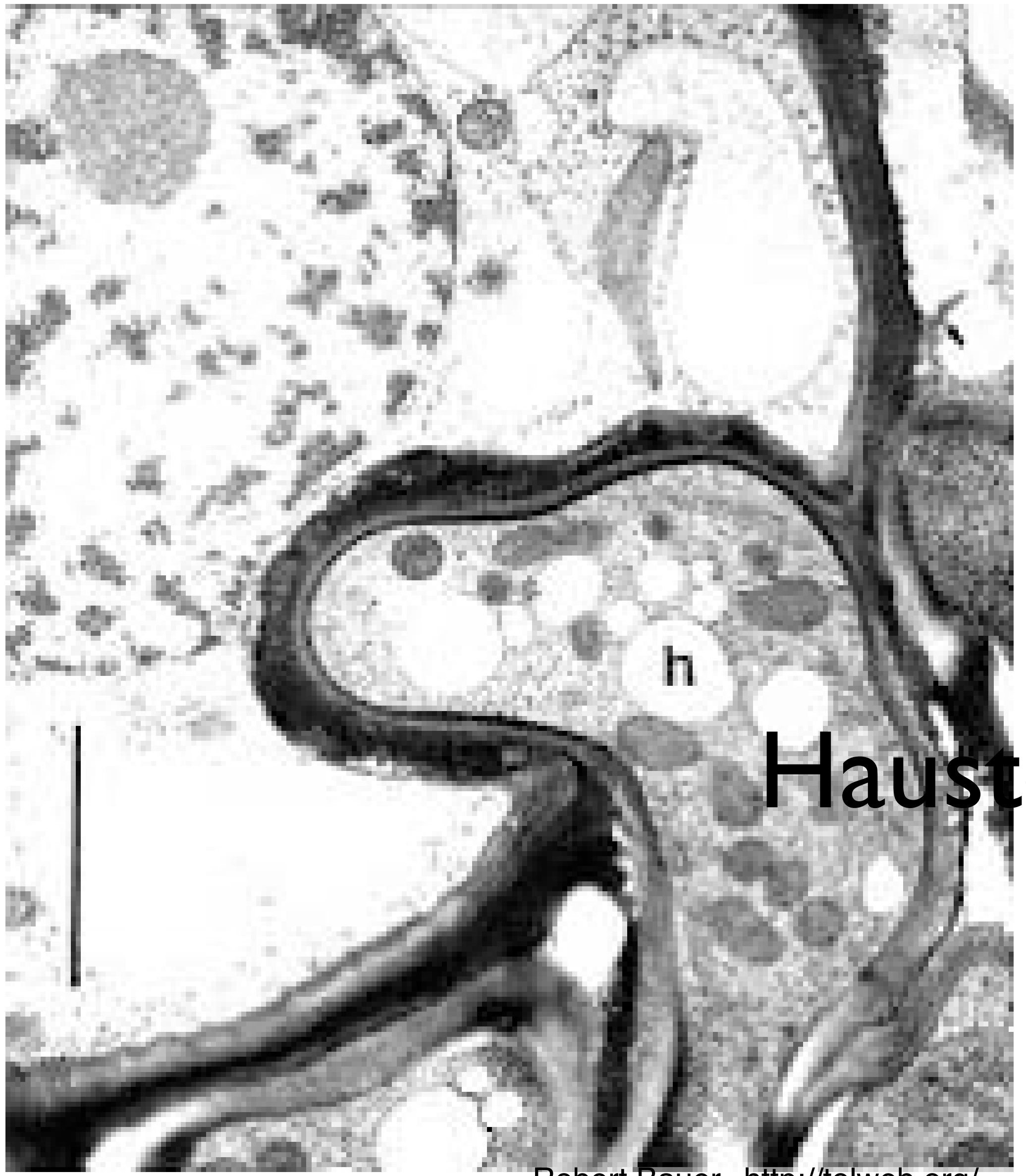
Transporter expansions

- Sugar related, Drug pump, and Major Facilitator Superfamily
  - *Aspergillus spp*, *Fusarium spp*, *S. nodorum*
  - Euascomycota
- Vitamin transport
  - *C. neoformans*, *Fusarium*
  - *A. nidulans* (Biotin)
- Saccharomyces expansions independent!



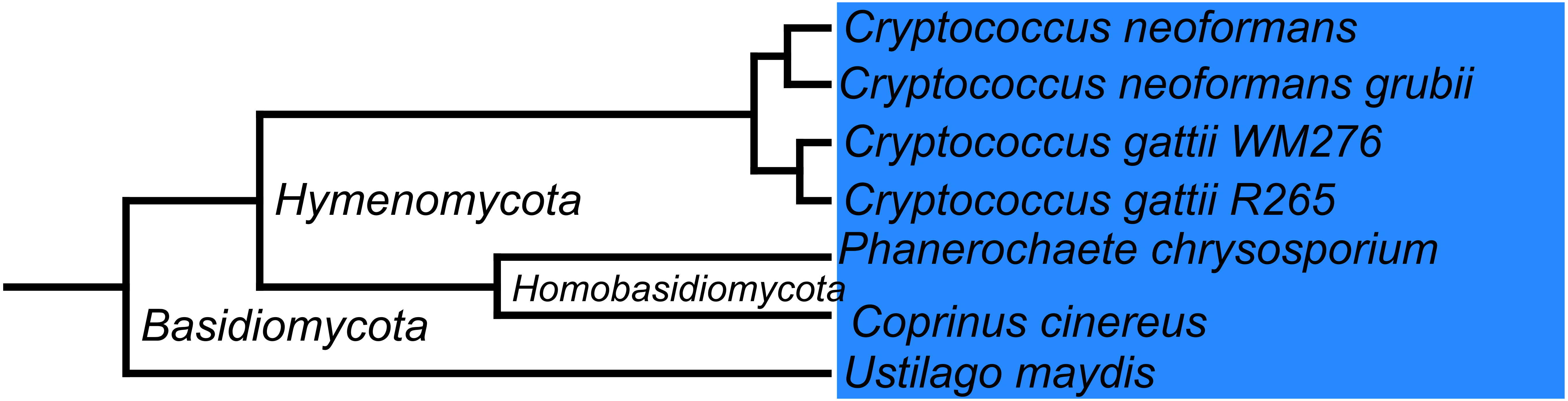
Sugar transporter use in phytopathogens

- Sugar transporters are used to extract nutrients from host
  - Haustorium: specialized structure for plant parasitism
  - Many sugar transporters highly and specifically expressed in haustoria



Robert Bauer http://tolweb.org/

Basidiomycota changes



Cryptococcus neoformans

Cryptococcus neoformans grubii

Cryptococcus gattii WM276

Cryptococcus gattii R265

Phanerochaete chrysosporium

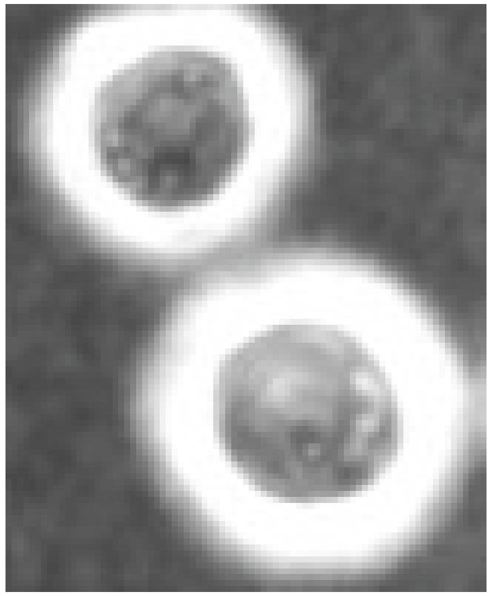
Coprinus cinereus

Ustilago maydis

Hymenomycota

Homobasidiomycota

Basidiomycota



C.neoformans

P.chrysosporium

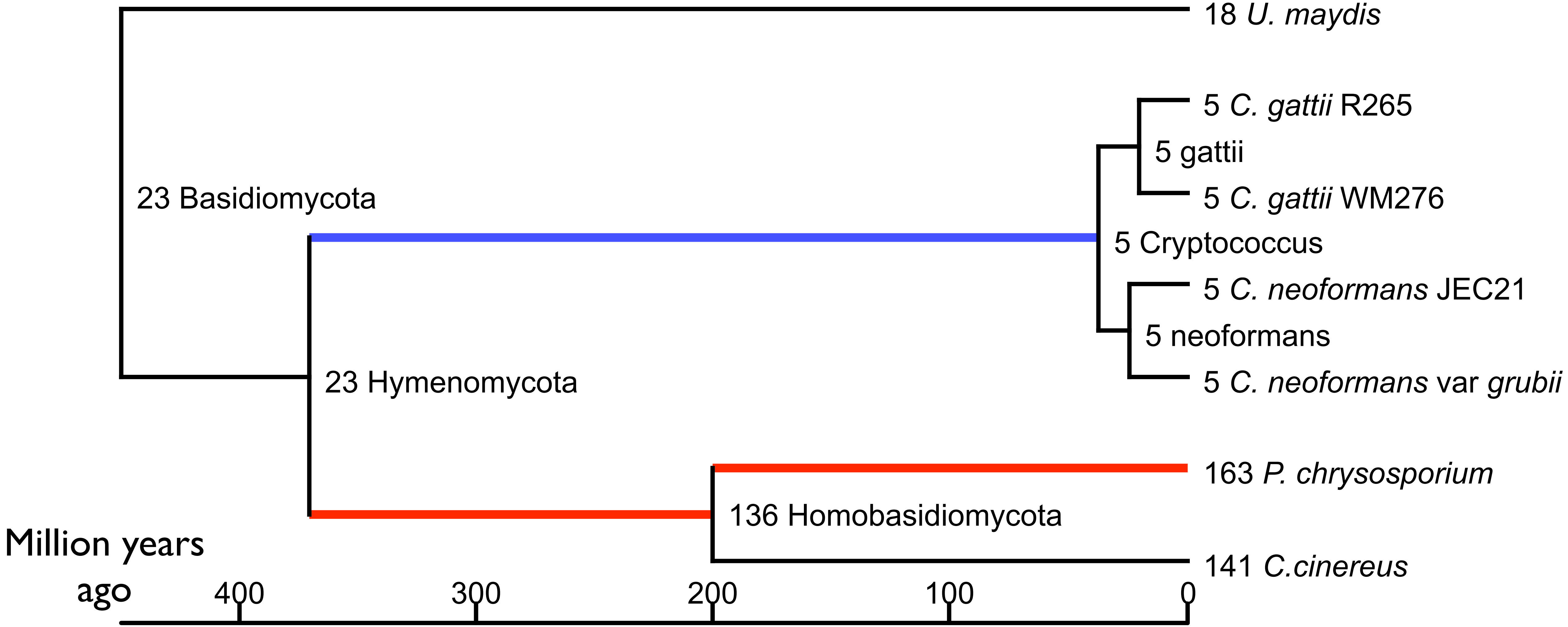
C.cinereus

U.maydis

P450 CYP64

P450 enzymes involved in synthesis and cleavage of chemical bonds. Drug metabolism in animals.

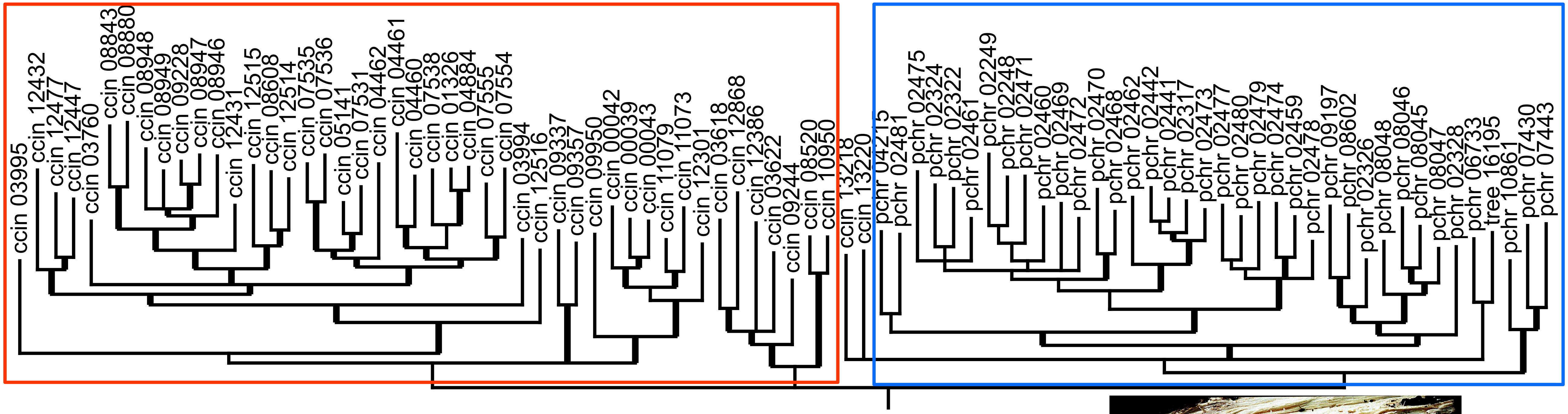
CYP64: Step in *Aspergillus* spp aflatoxin pathway
P. chrysosporium implicated in lignin and hydrocarbon degradation.



CYP64 was from independent duplication

C. cinereus expansion

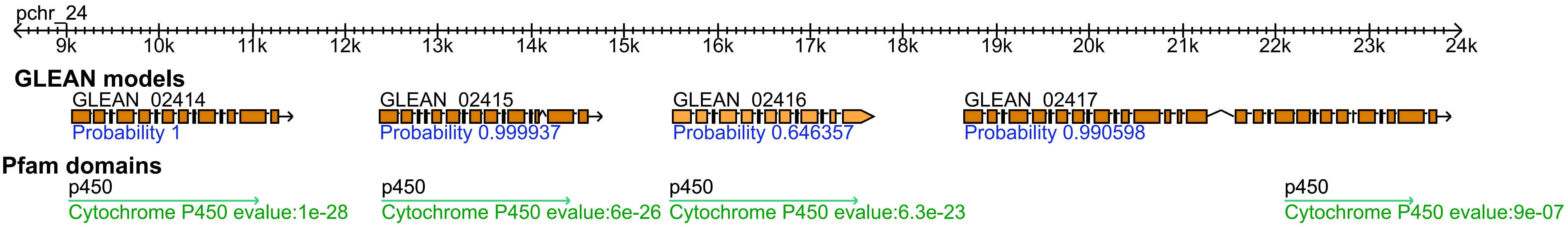
P. chrysosporium expansion



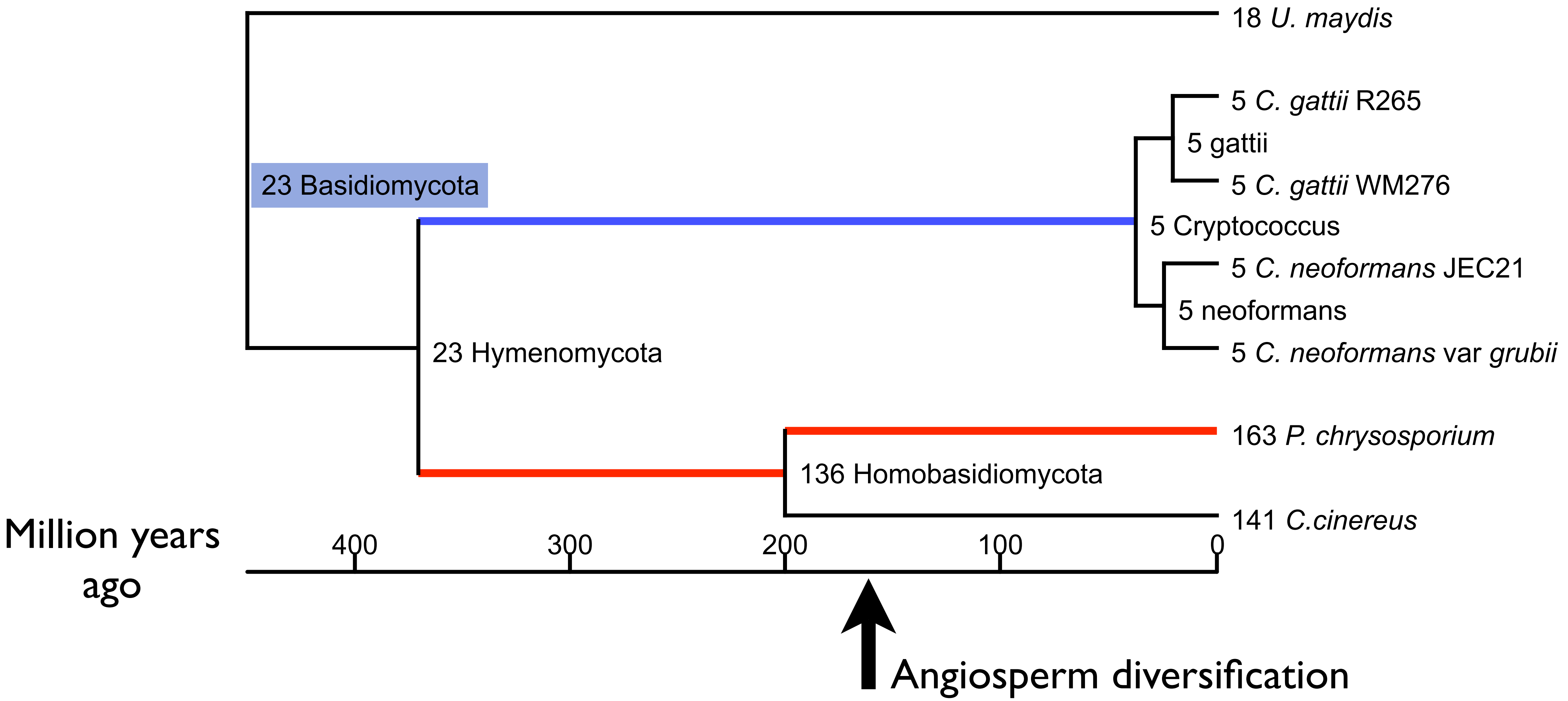
Mario Cervini

Tom Volk

Local duplications created CYP64 expansion



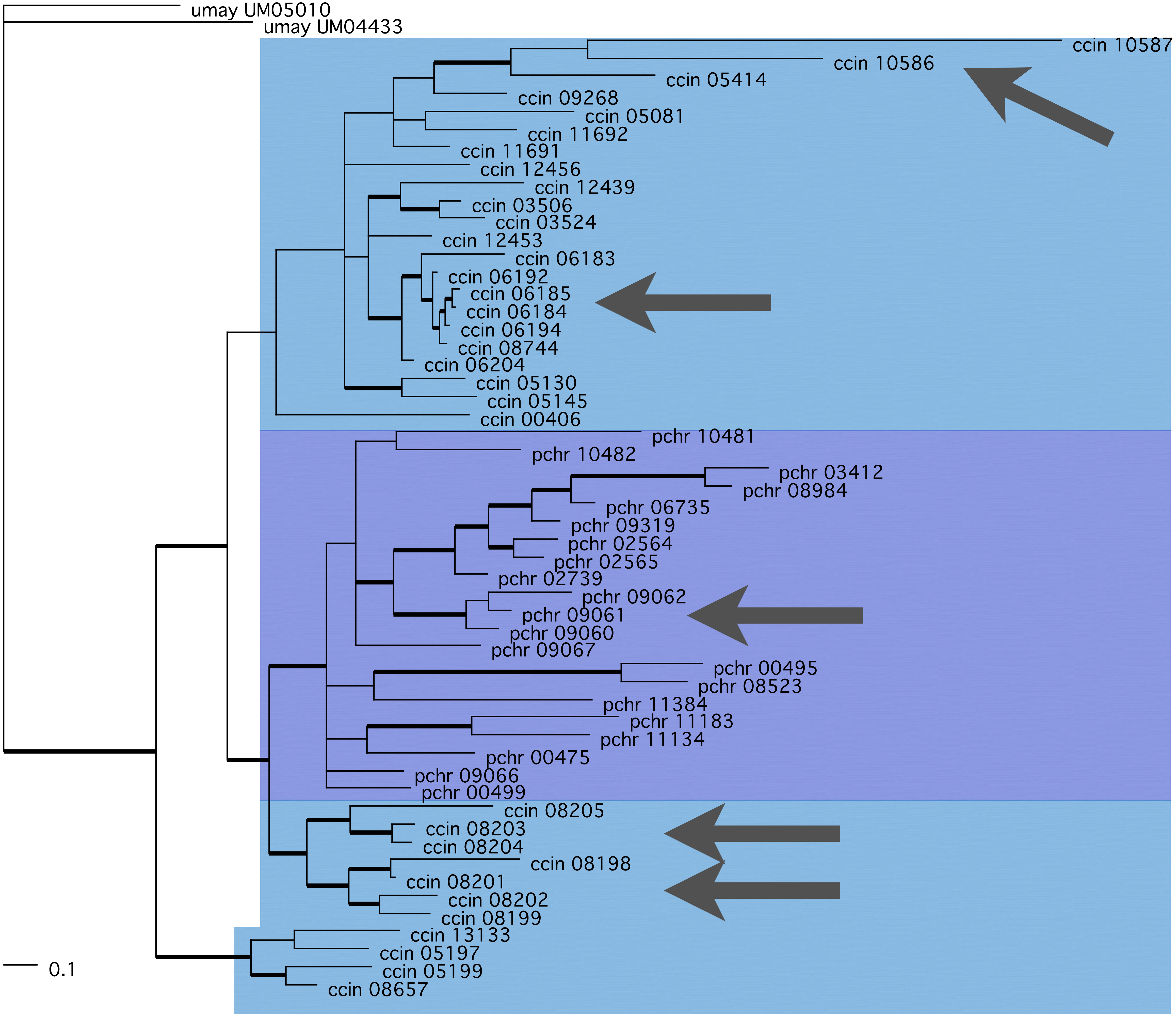
Interpretation of CYP64 expansion



Hydrophobin Family

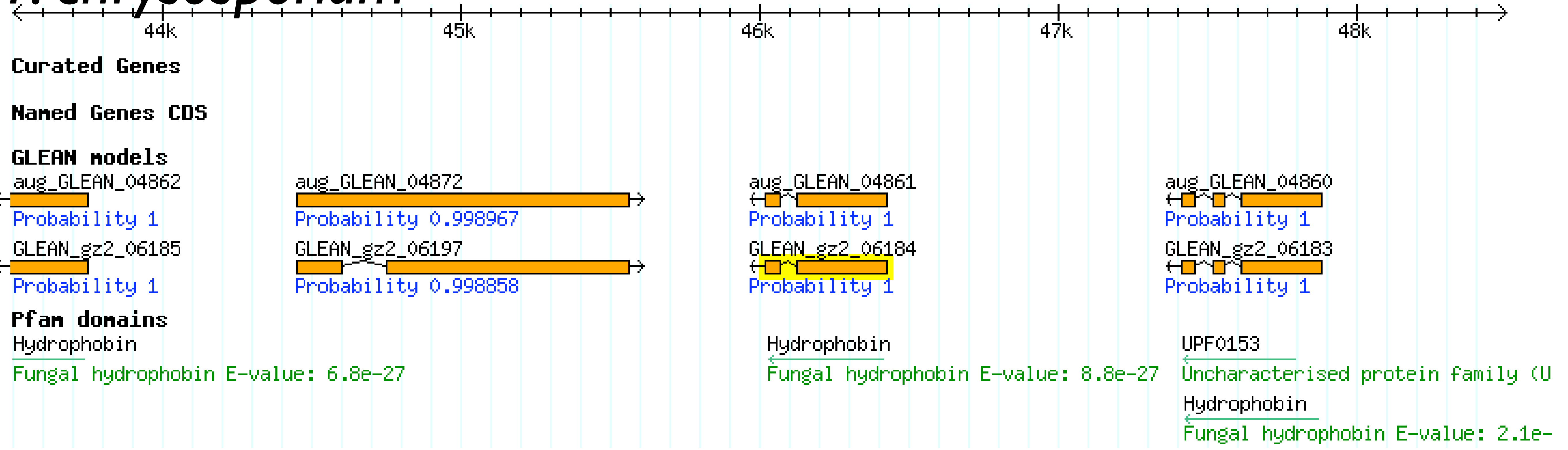
P.chr	*C.cin*	*C.neo*	*U.may*
21	33	0	2

- Self assembling proteins involved in fungal cell wall
- Part of what makes a mushroom
- 8 Cysteine residues critical to function
- Help spores stay airborne resisting water

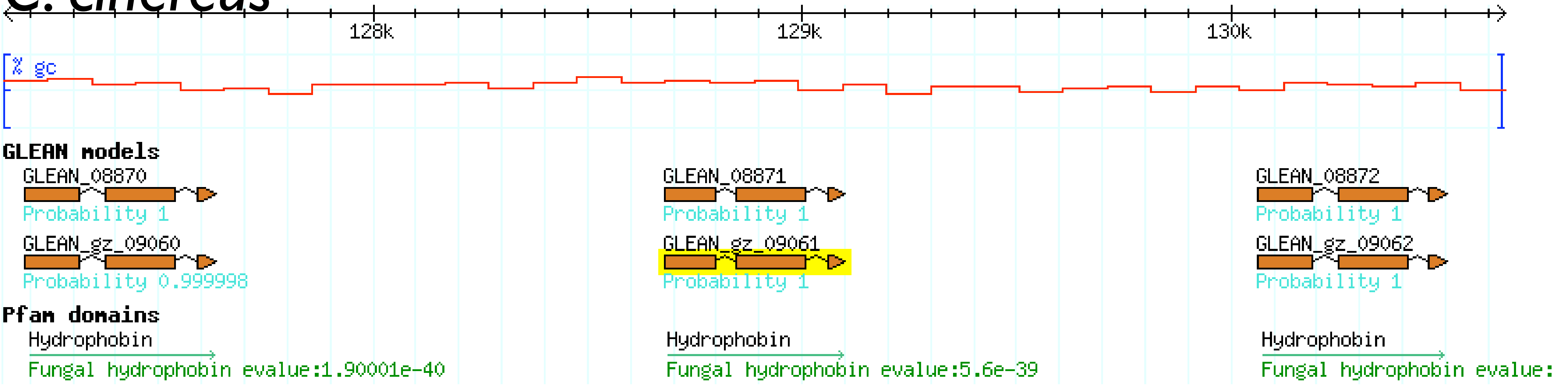

umay UM05010
umay UM04433
ccin 10587
ccin 10586
ccin 05414
ccin 09268
ccin 05081
ccin 11692
ccin 11691
ccin 12456
ccin 12439
ccin 03506
ccin 03524
ccin 12453
ccin 06183
ccin 06192
ccin 06185
ccin 06184
ccin 06194
ccin 08744
ccin 06204
ccin 05130
ccin 05145
ccin 00406
pchr 10481
pchr 10482
pchr 03412
pchr 08984
pchr 06735
pchr 09319
pchr 02564
pchr 02565
pchr 02739
pchr 09062
pchr 09061
pchr 09060
pchr 09067
pchr 00495
pchr 08523
pchr 11384
pchr 11183
pchr 11134
pchr 00475
pchr 09066
pchr 00499
ccin 08205
ccin 08203
ccin 08204
ccin 08198
ccin 08201
ccin 08202
ccin 08199
ccin 13133
ccin 05197
ccin 05199
ccin 08657
0.1

Local Duplications

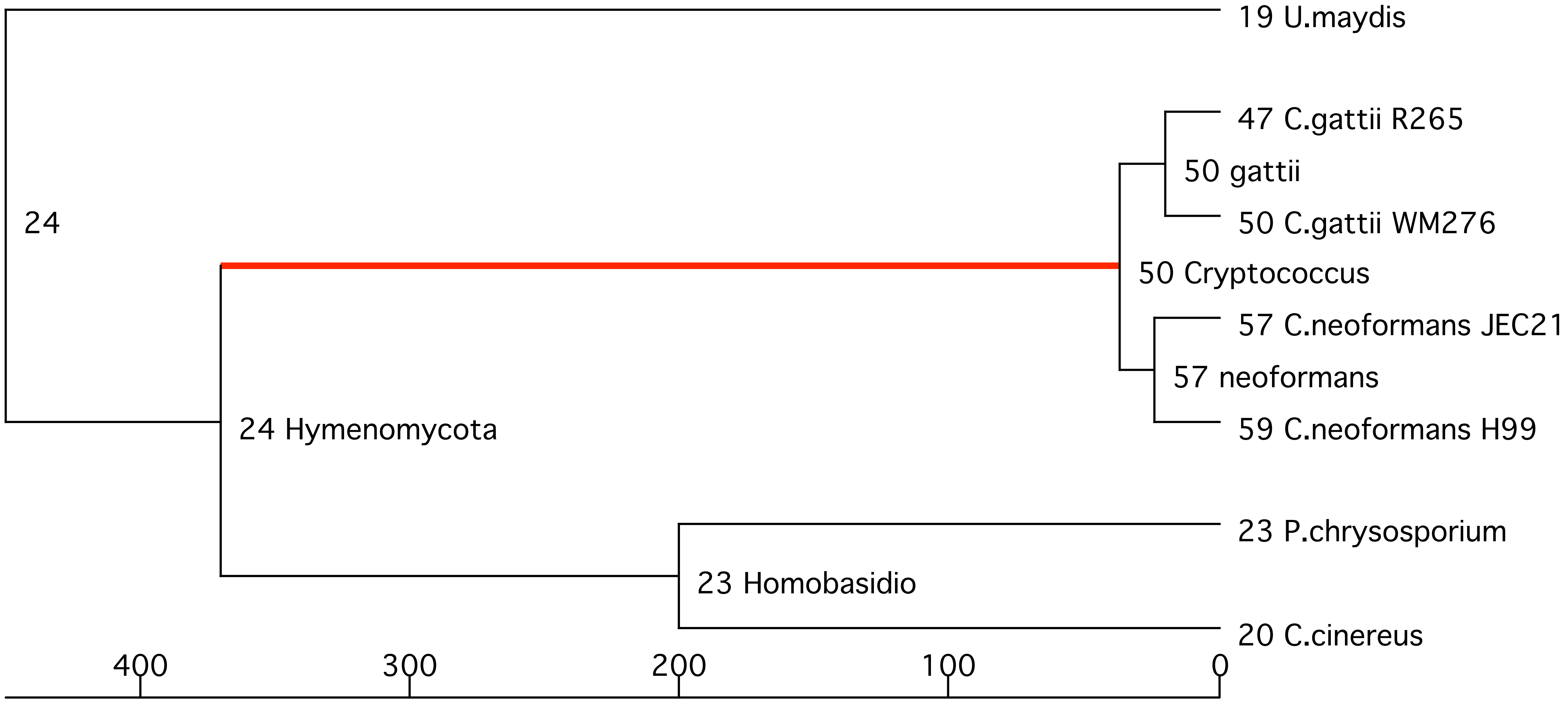
P. chrysosporium



C. cinereus



Cryptococcus sugar transporters expansion



Cryptococcus sugar transporters

- 3x as many sugar transporters in *C. neoformans* (~50) than other basidiomycetes
- "sugar coated killer"
- Capsule is a mixture of glucose, xylose, and mannose.
- Transporters could be important in capsule synthesis

Zerpa et al, 1996

Conclusions

- Transporters are highly expanded in independent lineages
  - Saprophytic and phytopathogenic lifestyles
- Adaptive Homobasidiomycete (mushroom) expansions
  - Lignin degradation - saprophytic lifestyles
  - Hydrophobins - cell wall structures

Acknowledgments

Matthew Hahn (Indiana)
Jeff Demuth
Sang-Gook Han

Tijl De Bie
Nello Cristianini

Aaron Mackey
Ian Korf
Mario Stanke

Fred Dietrich (Duke)

DUKE
UPGG
UNIVERSITY PROGRAM IN GENETICS AND GENOMICS

NSF

CSEM

Sequencing centers
Broad Institute
Joint Genome Institute
Génolevures
Stanford University
TIGR
Welcome Trust Sanger Centre
(NIH and NSF)

BERKELEY MYCOLOGY
1875