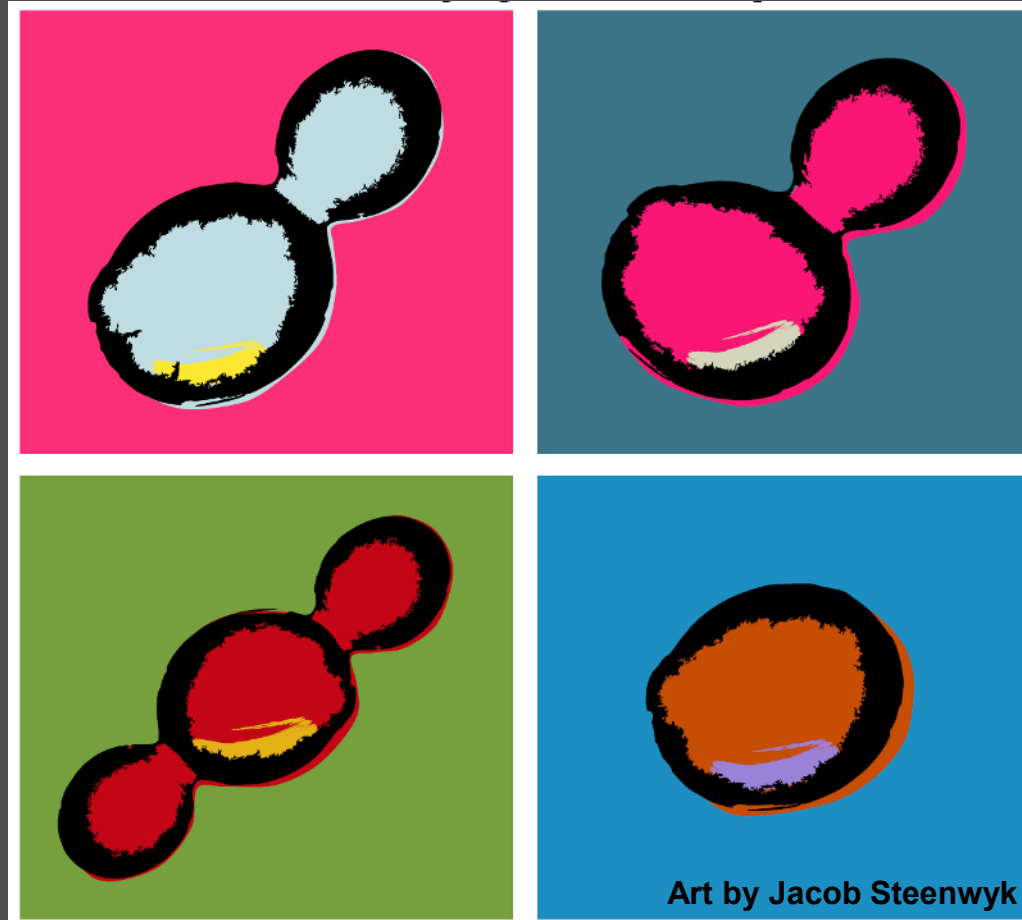


Fantastic Budding Yeasts and How They Evolved to Be



Antonis Rokas

Department of Biological Sciences, Vanderbilt University

<http://www.rokaslab.org/>

@RokasLab

The Evolution of Budding Yeast Biodiversity

- ❖ **Sequence the genomes of all ~1,100+ known budding yeast species**
- ❖ **Construct their definitive phylogeny and timetree & use it to revise their taxonomy**
- ❖ **Examine how genomic variation transforms into metabolic variation across an entire subphylum**



The Evolution of Budding Yeast Biodiversity



**Hittinger lab
genetics**



**C. Kurtzman
taxonomy**



**M. Groenewald
taxonomy**



**Rokas lab
evolution**

The Team

**Dana
Opulente**



**Jacek
Kominek**



**Xing-Xing
Shen**



**Xiaofan
Zhou**



**Drew
Doering**



**Yuanning
Li**



**Jacob
Steenwyk**



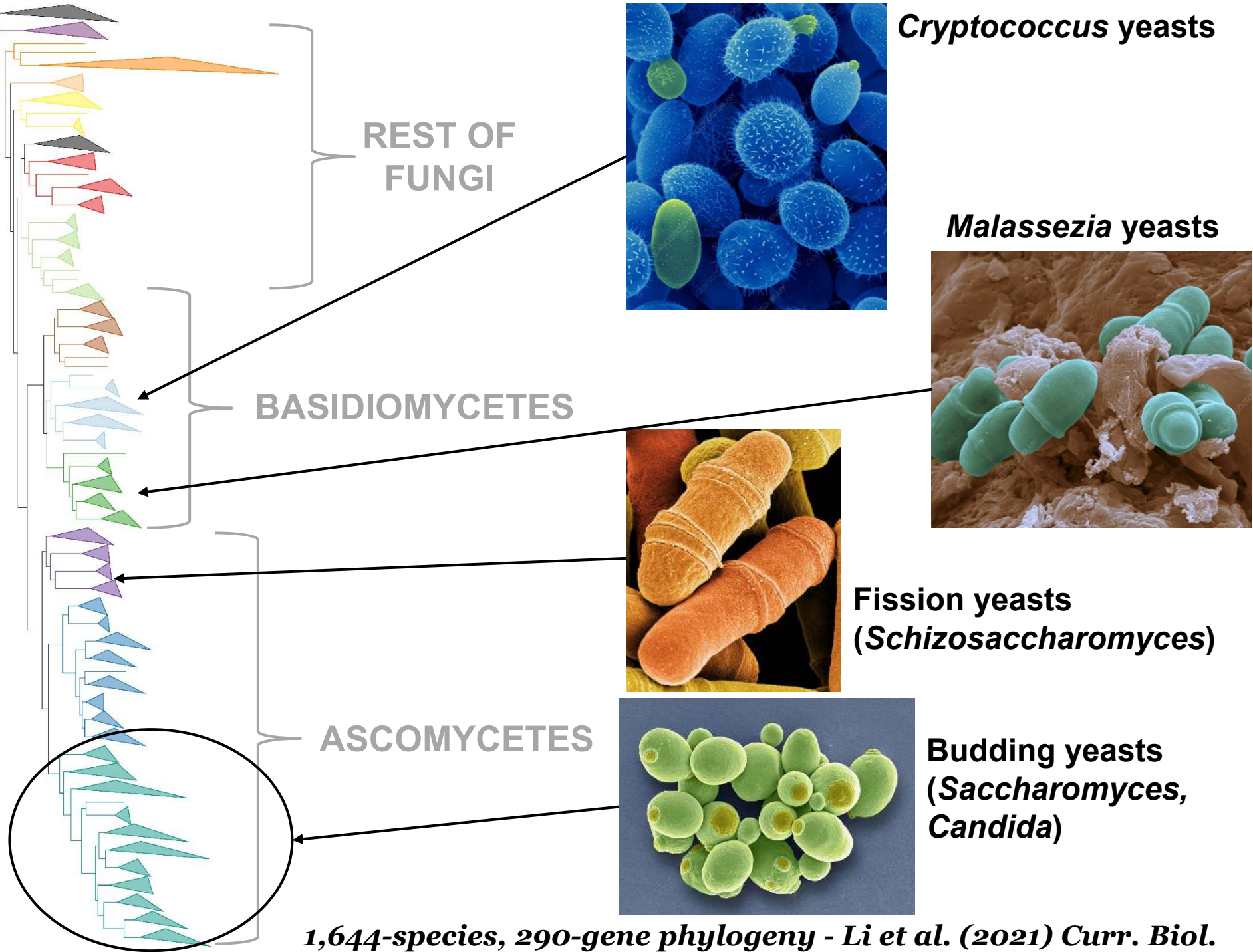
**Abbe
LaBella**

why yeasts?

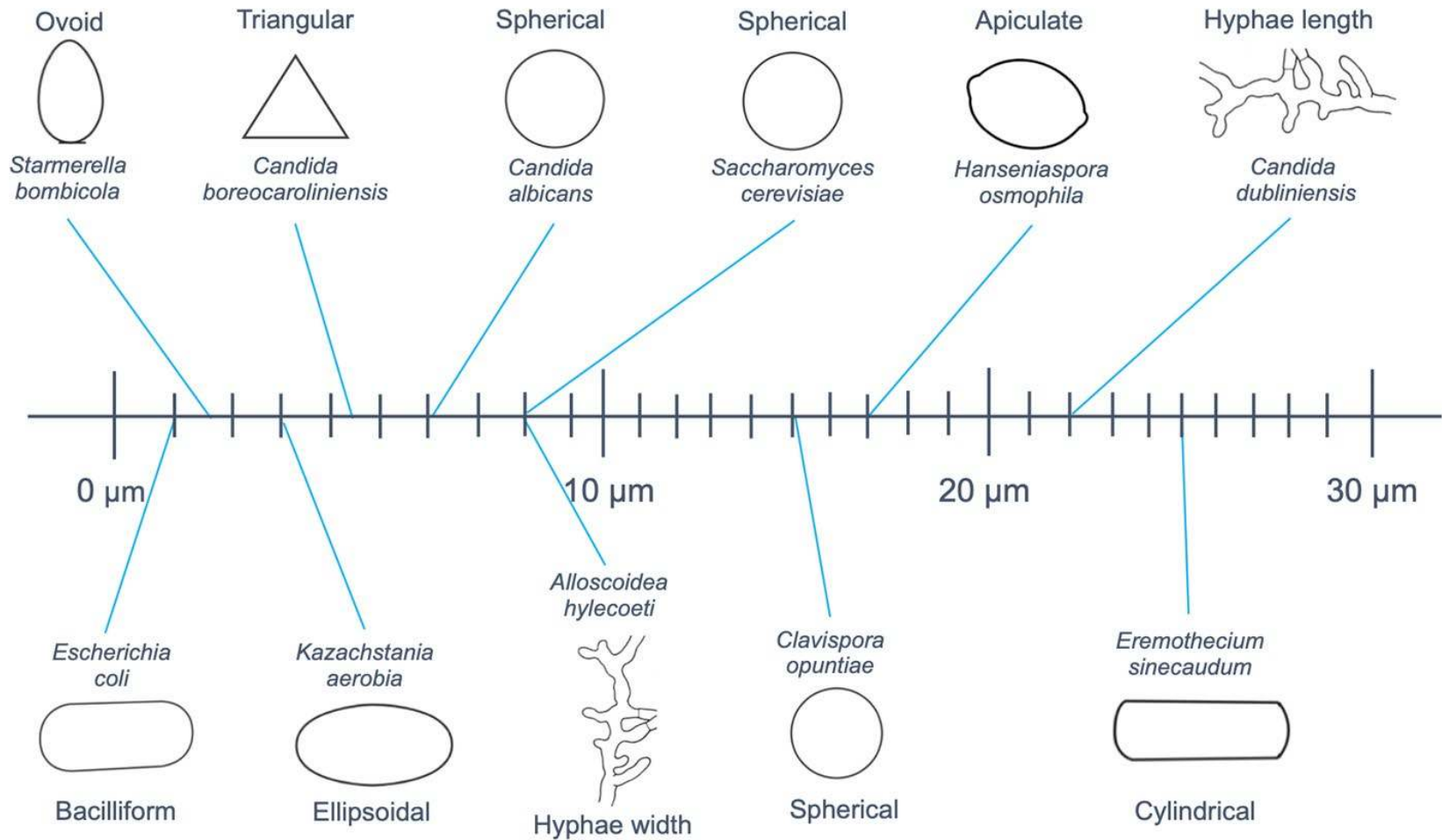
What is a Yeast?

Yeasts: fungi that asexually reproduce by budding or fission, which results in growth that is comprised mainly of single cells

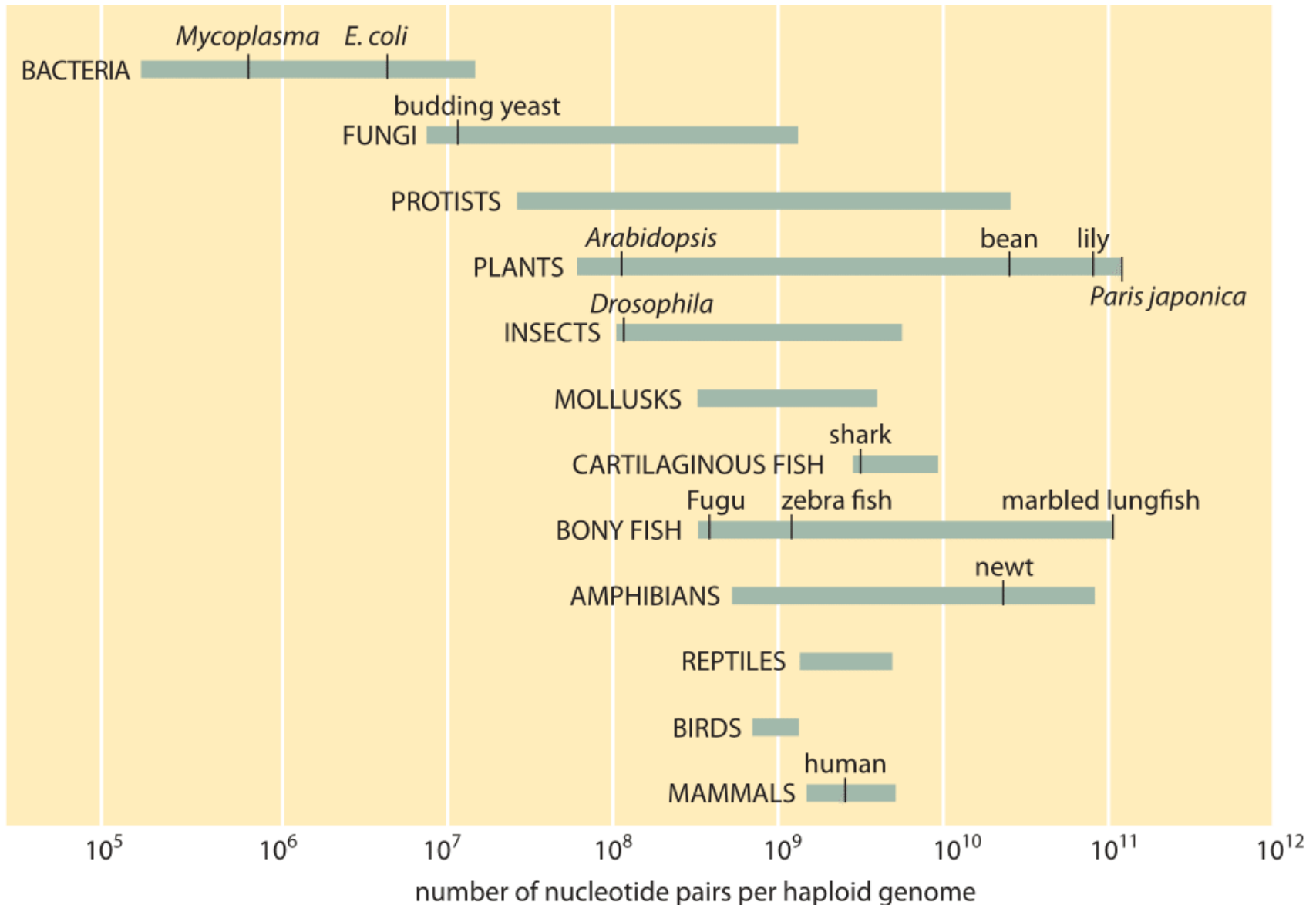




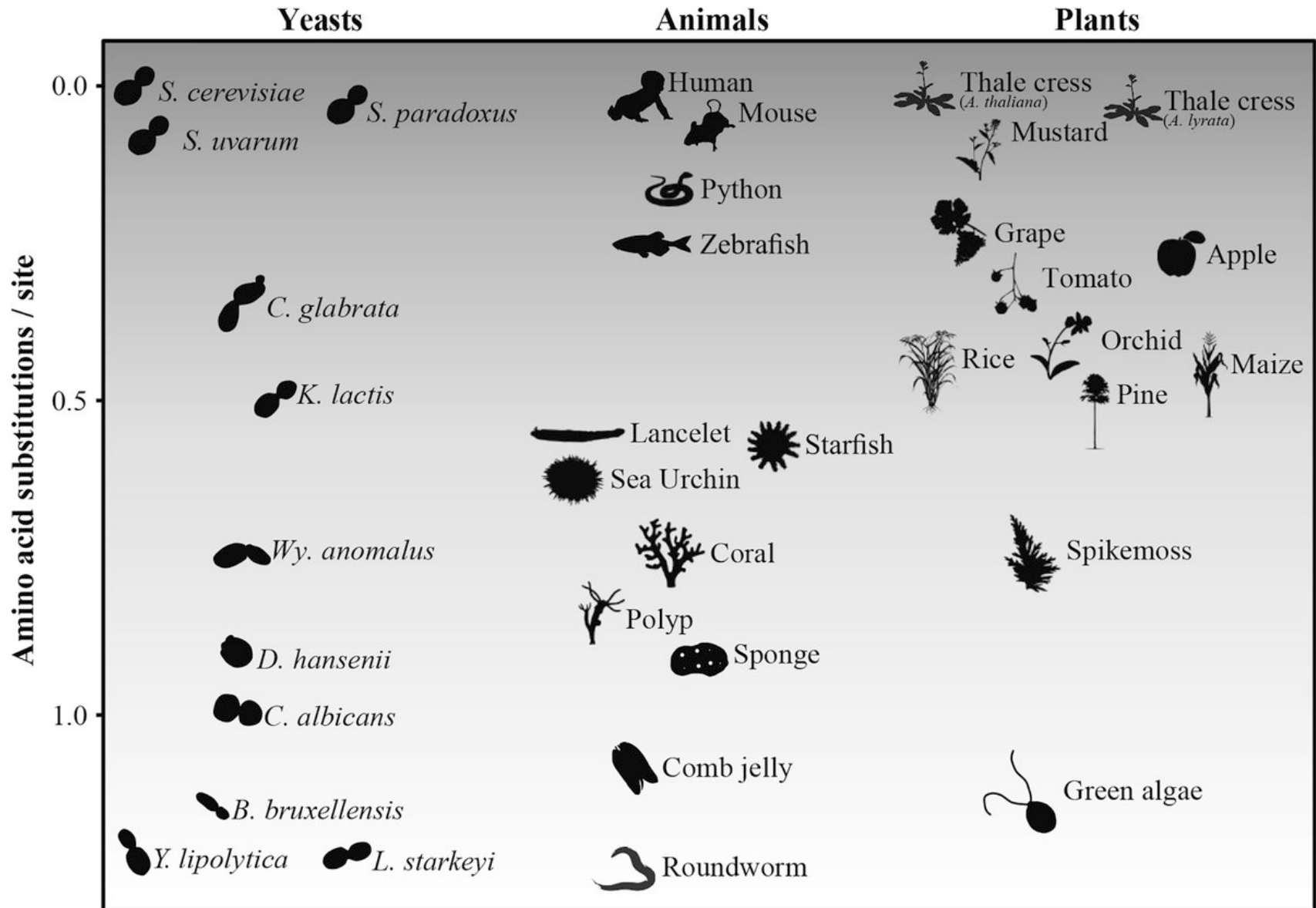
Not All *Saccharomycotina* Species are Yeasts



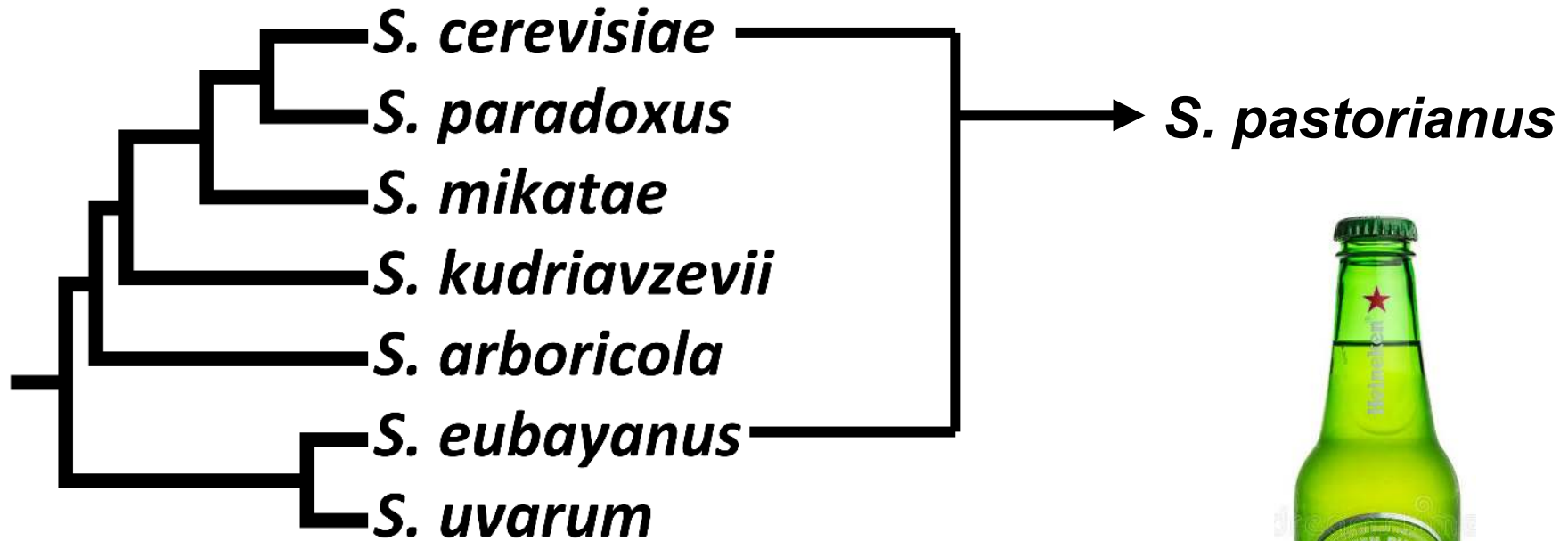
Budding Yeasts Have Very Small Genomes



Budding Yeasts Have Amazing Genomic Diversity



Budding Yeasts Have Genomic “Flexibility”



S. cerevisiae – *S. eubayanus*
divergence $\approx$ human – chicken
divergence



Budding Yeasts Vary Widely in their Diets

Nectar yeasts

(*Wickerhamiella* / *Starmerella* clade)

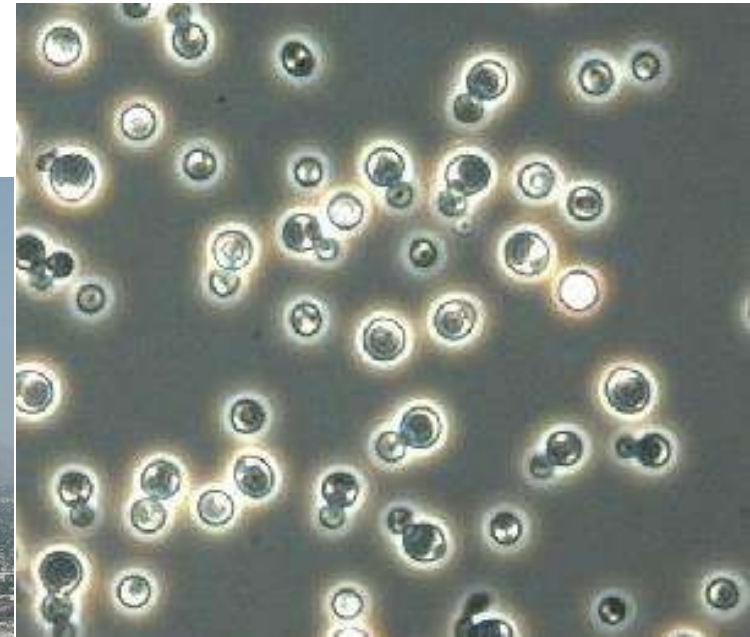


Cactophilic yeasts



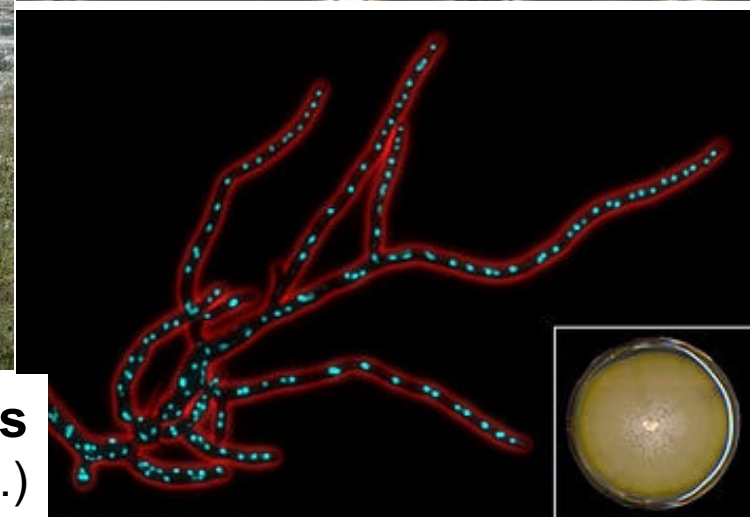
Oil producers

(*Lipomyces*, *Yarrowia*)



Human pathogenic yeasts
(*Candida*)

Plant pathogens
(*Eremothecium* sp.)



Strains & Metabolite Growth Data for All 1,100+ Species



**Cletus Kurtzman
(1939-2017)**

Fermentation

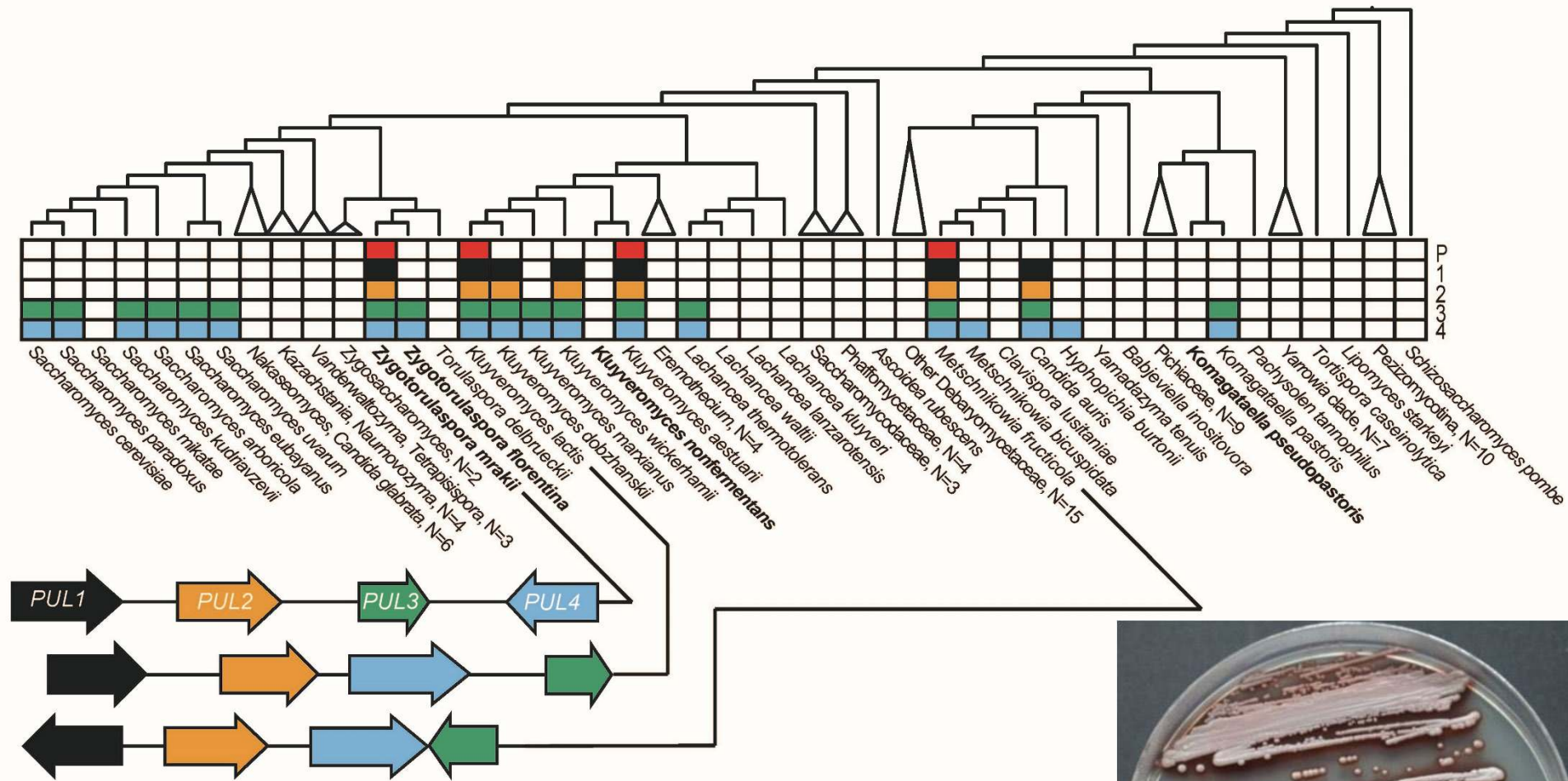
Glucose	+	Lactose	—
Galactose	—	Raffinose	—
Sucrose	—	Trehalose	—
Maltose	—		

Growth (in Liquid Media)

Glucose	+	D-Ribose	—
Inulin	—	Methanol	—
Sucrose	—	Ethanol	—
Raffinose	—	Glycerol	—
Melibiose	—	Erythritol	—
Galactose	—	Ribitol	—
Lactose	—	Galactitol	—
Trehalose	—	D-Mannitol	—
Maltose	—	D-Glucitol	—
Melezitose	—	myo-Inositol	—
Methyl- α -D-glucoside	—	DL-Lactate	—
Soluble starch	—	Succinate	—
Cellobiose	+	Citrate	—
Salicin	+	D-Gluconate	+
L-Sorbose	—	D-Glucosamine	—
L-Rhamnose	—	N-Acetyl-D-glucosamine	n
D-Xylose	—	Hexadecane	n
L-Arabinose	—	Nitrate	—
D-Arabinose	—	Vitamin-free	—

Budding yeasts are a fantastic system for studying the evolution of the genotype – phenotype map and for inferring principles of genome evolution in eukaryotes

Pathway Discovery using Phylogenomic Footprinting



Pathway Discovery using Machine Learning

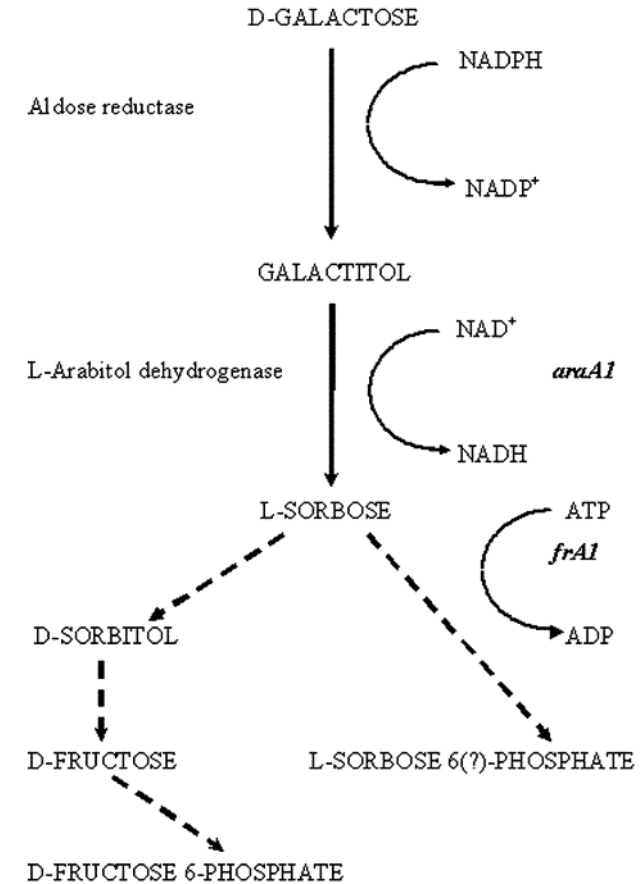
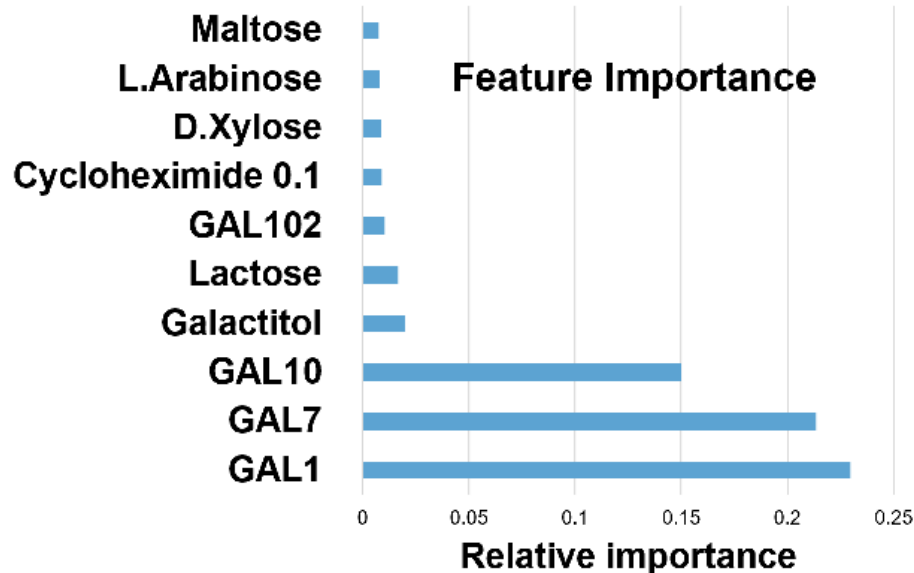
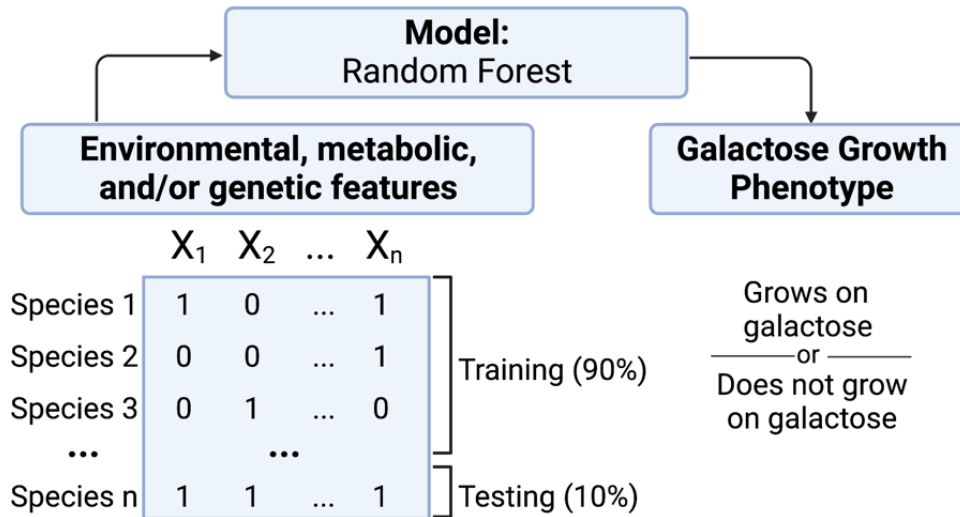


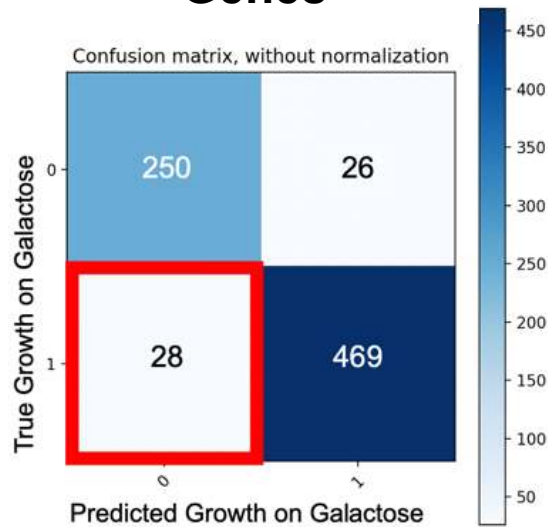
Fig. 8 The reductive D-galactose degrading pathway. *Broken lines* Alternative catabolic pathways

Fekete et al. (2004) Arch. Microbiol.



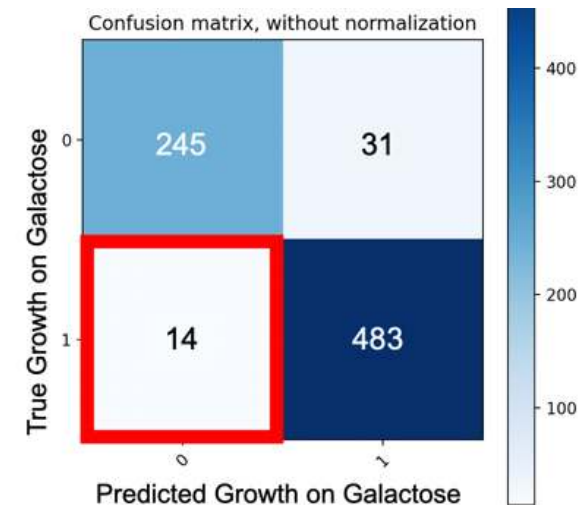
Pathway Discovery using Machine Learning

Genes

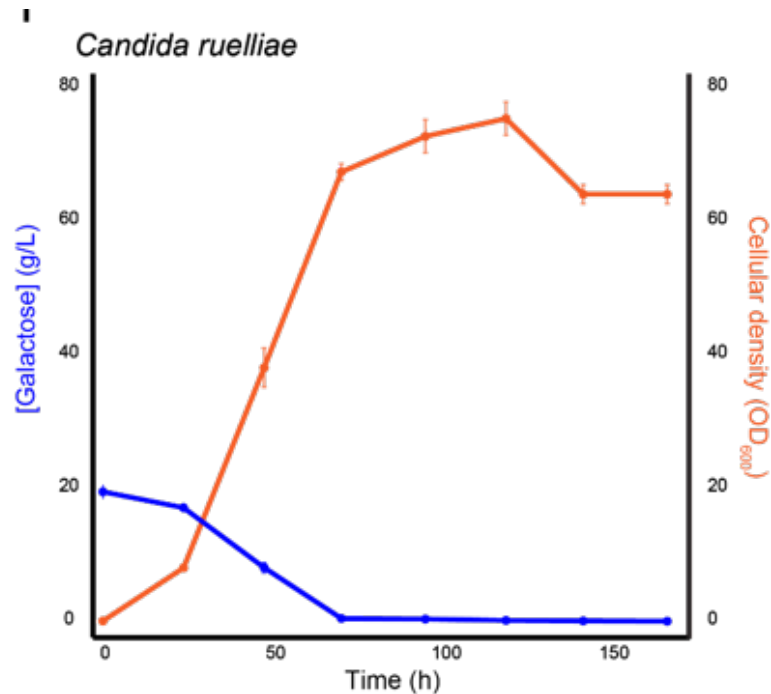


Most of these 14 species grow on both galactose and galactitol

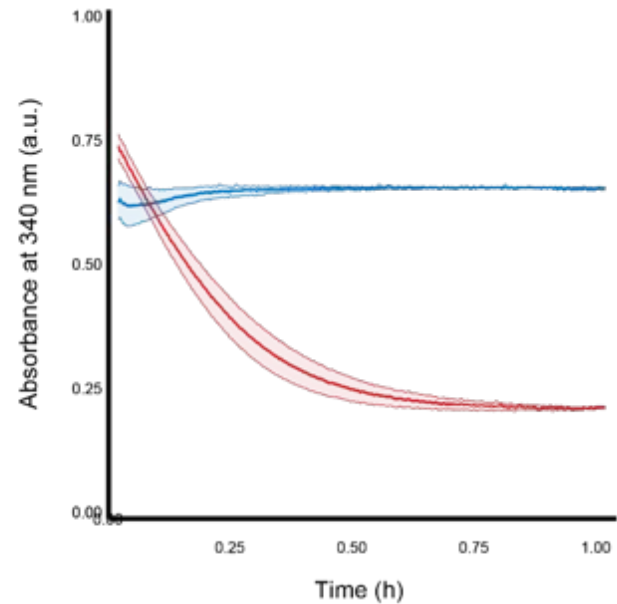
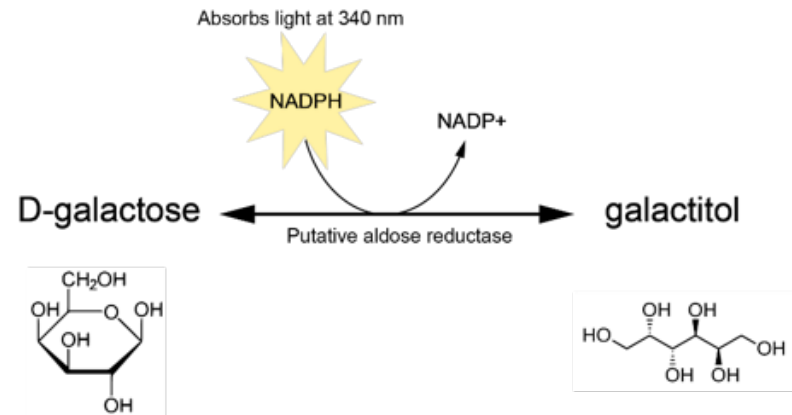
Genes + Galactitol



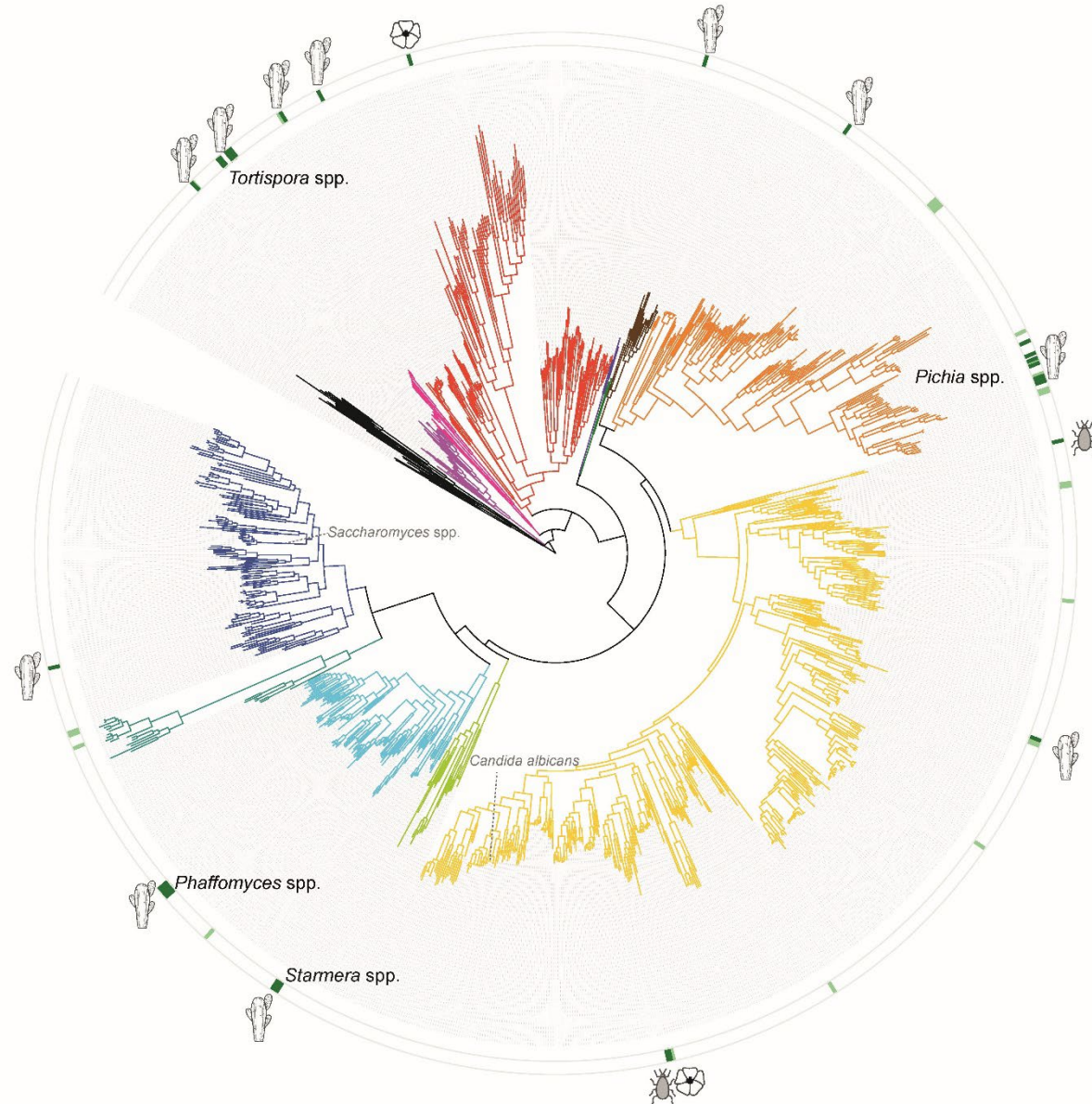
Galactose Consumption and Enzymatic Activity



Oxidoreductive Pathway

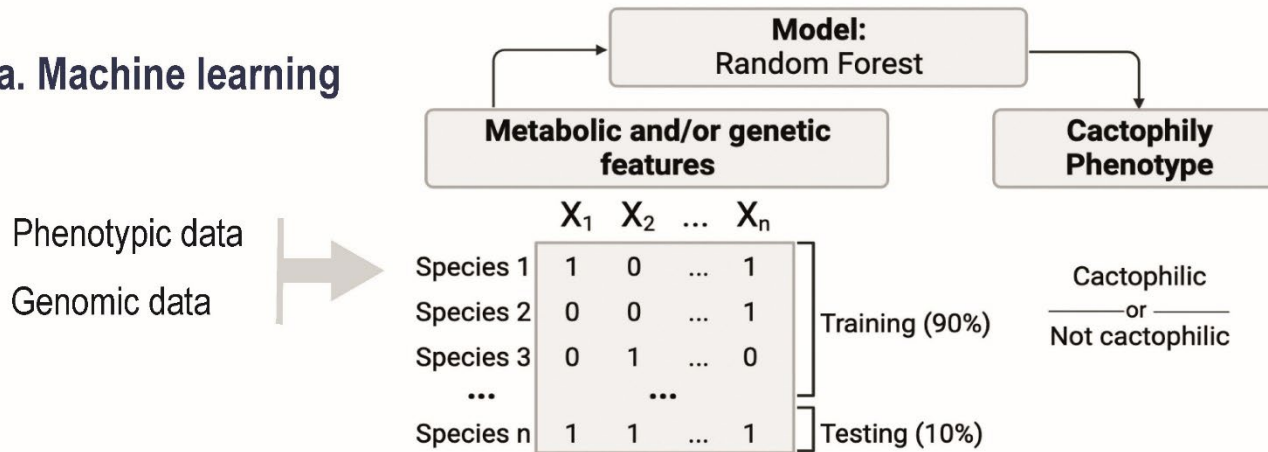


Signatures of Convergent Evolution in Cactophilic Yeasts

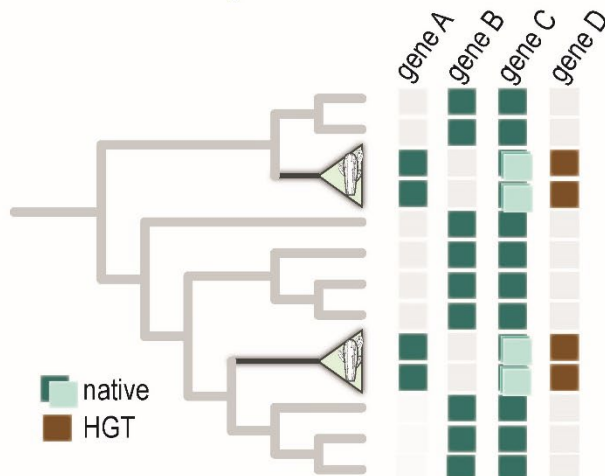


Leveraging AI and Phylogenomics

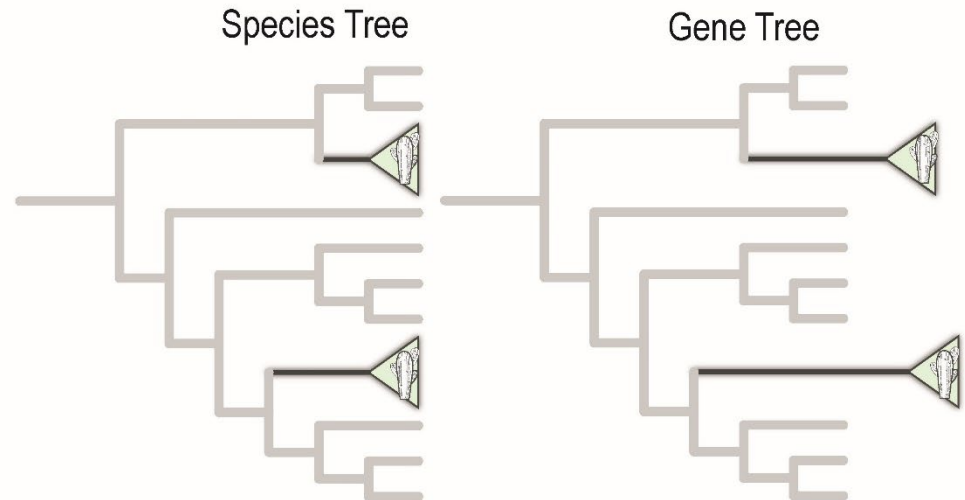
a. Machine learning



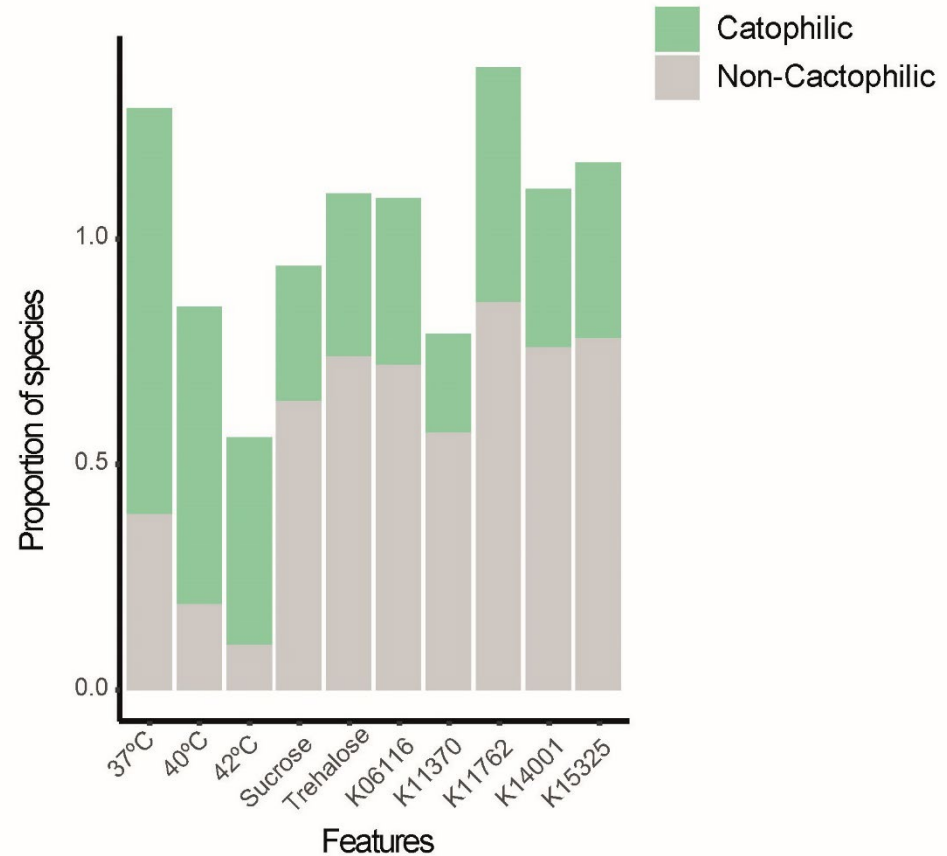
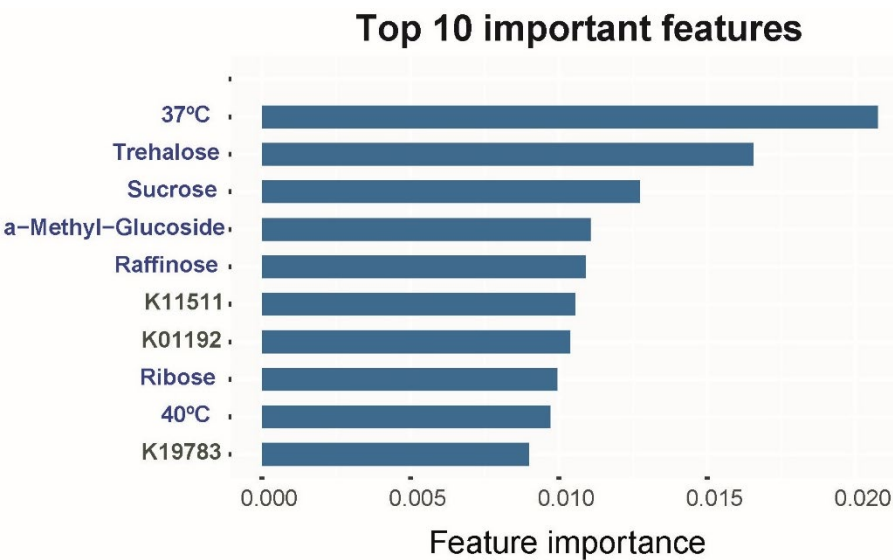
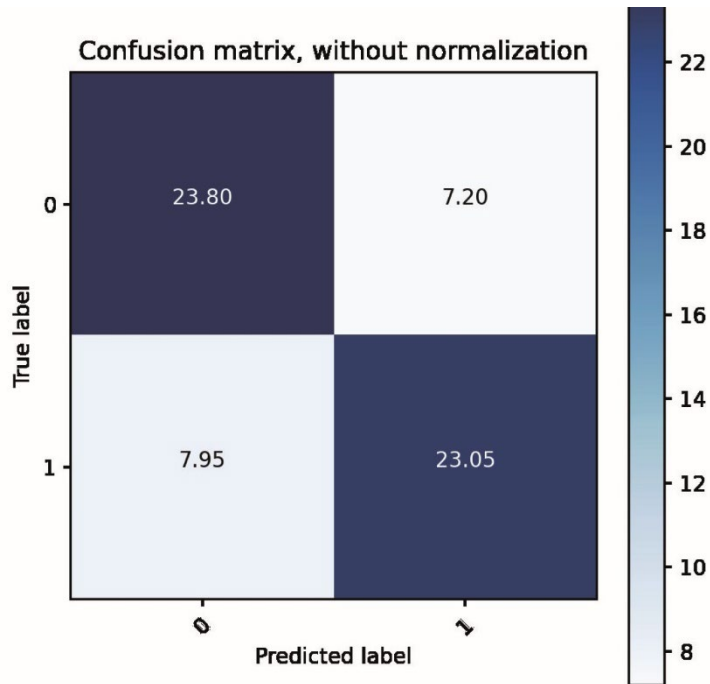
b. Gene family evolution



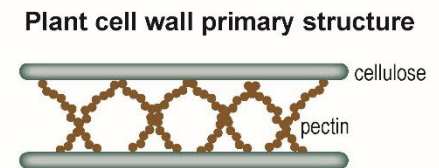
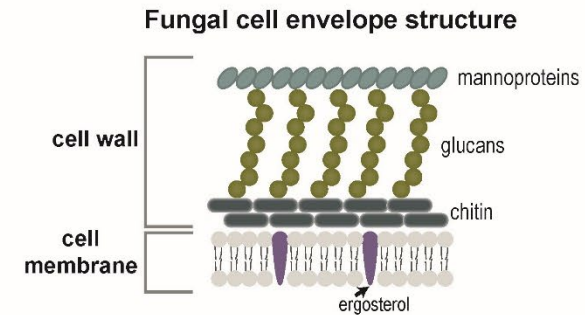
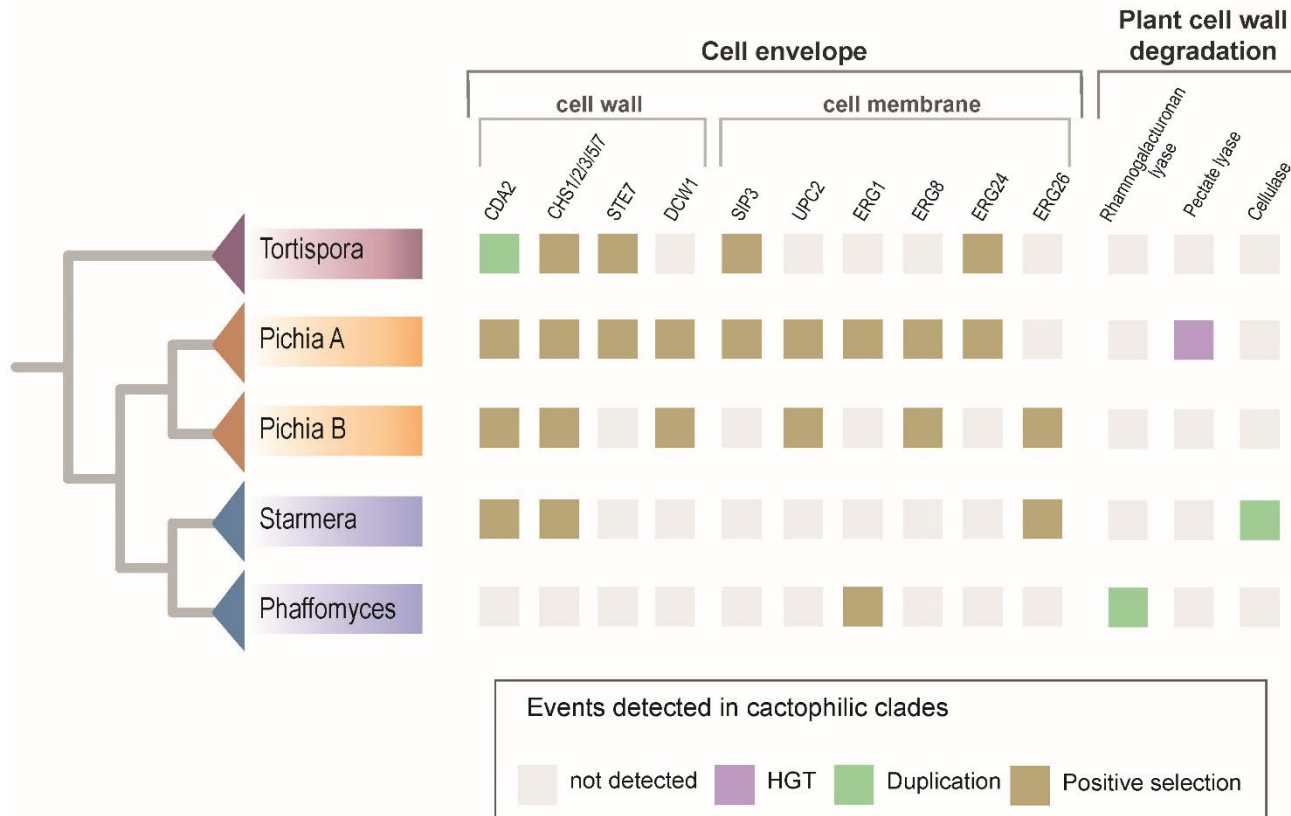
c. (Relative) evolutionary rates



Specific Traits Predict Cactophily



Convergence in Genes Involved in Cell Wall & Envelope



Saccharomyces

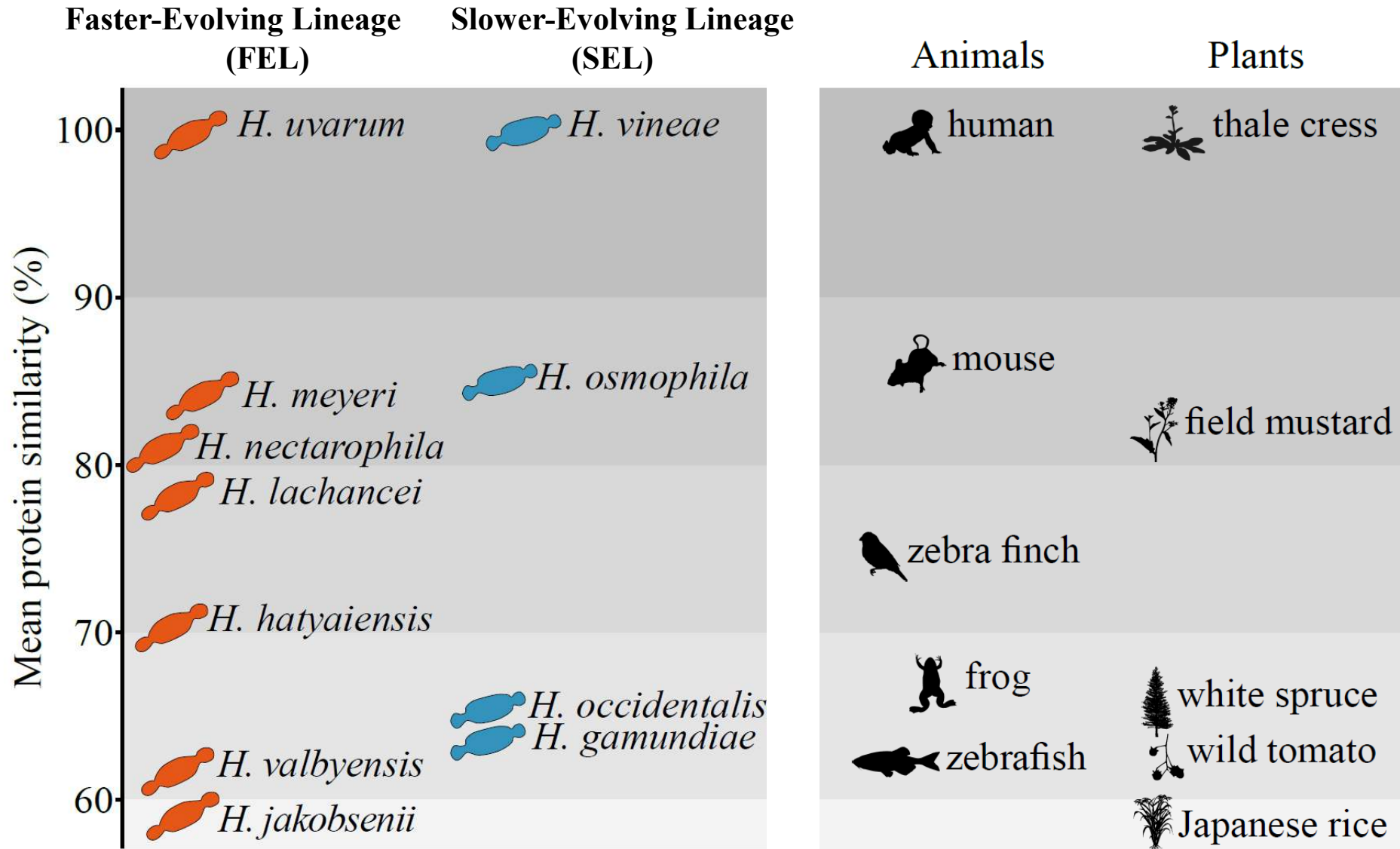
0.1 substitutions/site

C. albicans

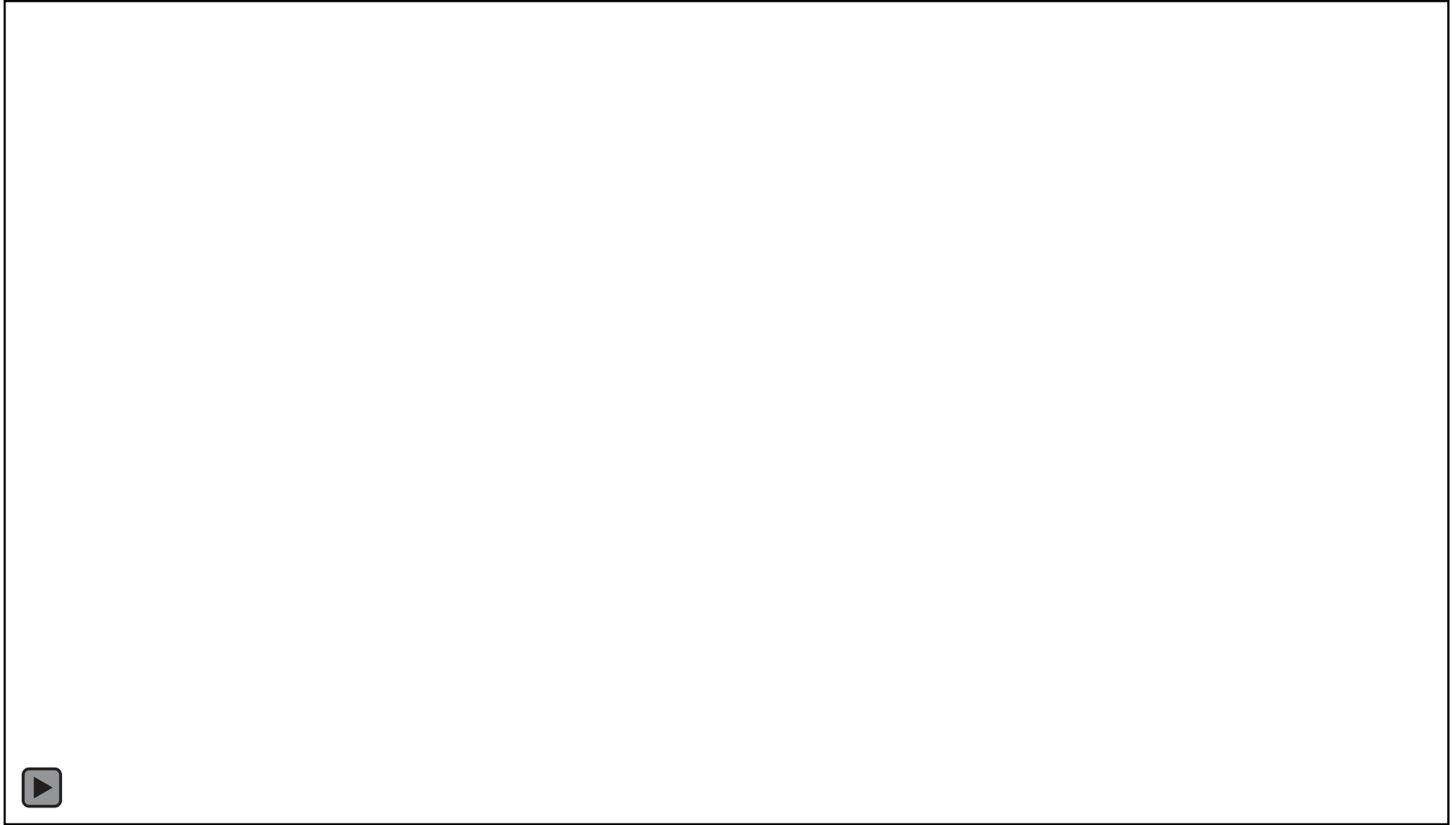
Hanseniaspora



Hanseniaspora Genomic Diversity Exceeds that of Vertebrates



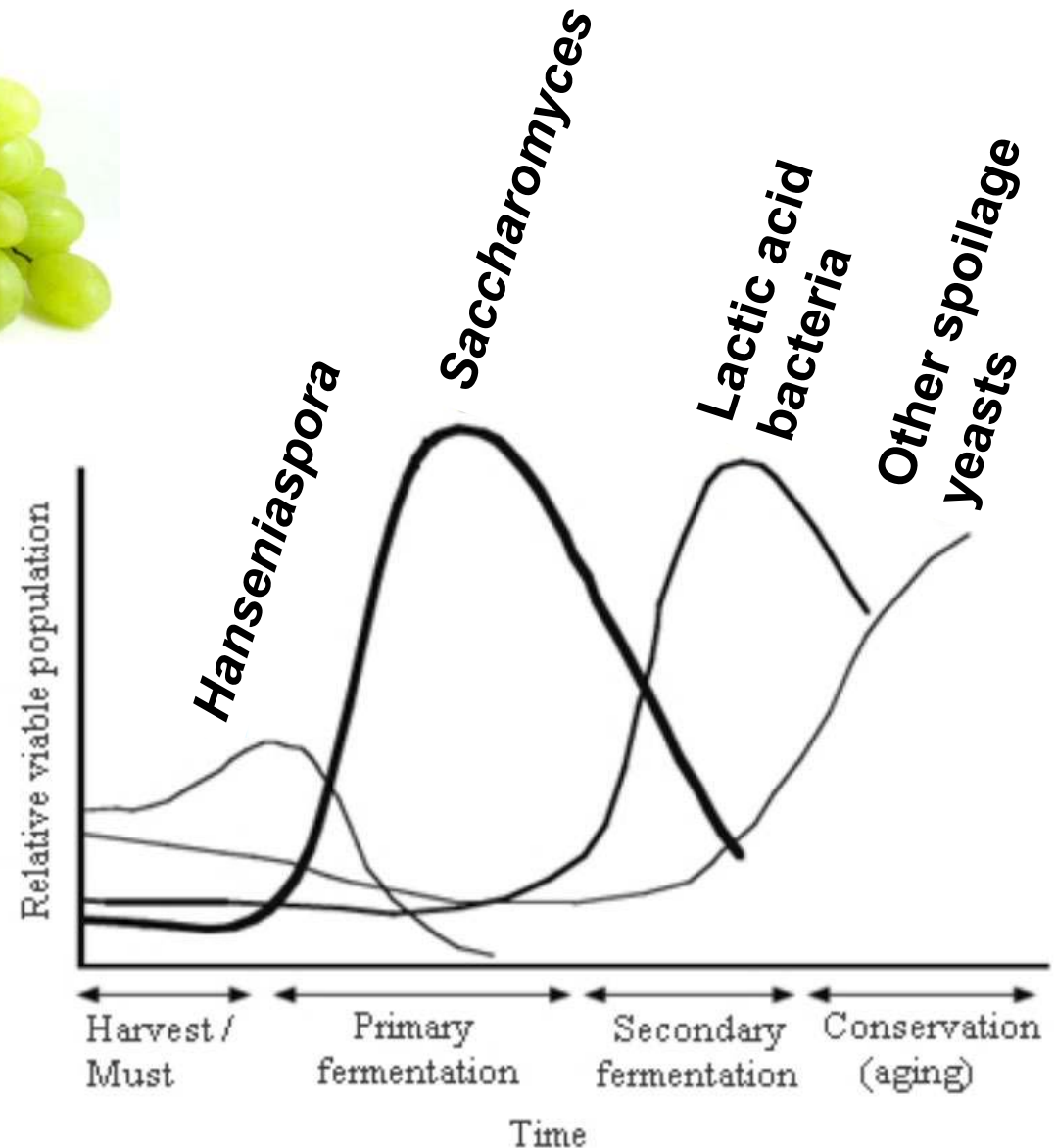
Hanseniaspora *Divide Rapidly, Have Bipolar Budding*



<https://www.youtube.com/watch?v=qFN9oZe5VlM>
Genetik Universität Osnabrück



Hanseniaspora are the Dominant Yeasts on Grapes



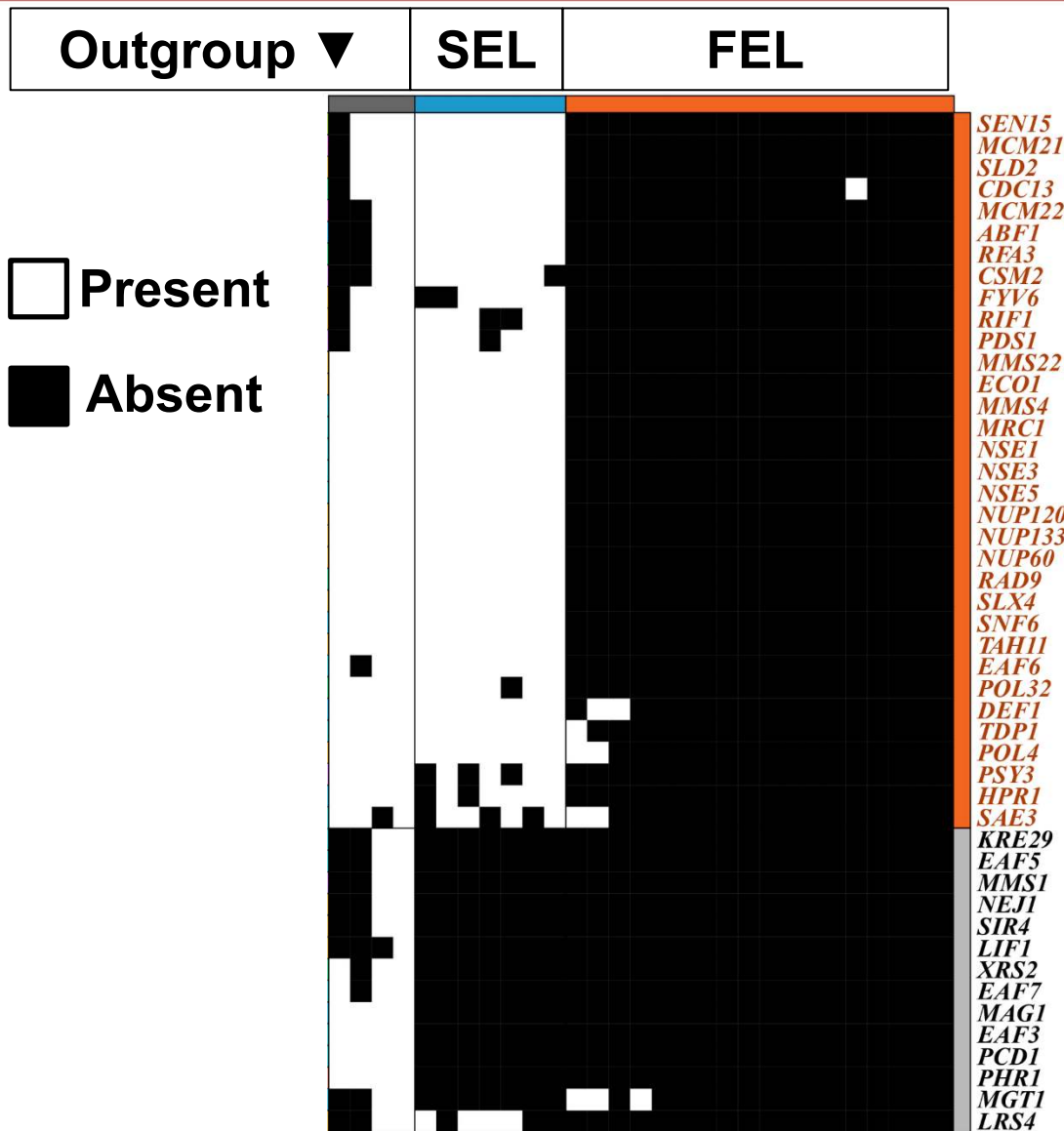
Hanseniaspora *Lifestyle Stems from Extensive Gene Losses*

high evolutionary rate ← loss of DNA repair genes

fast growth ← loss of cell cycle genes

specialized diet ← loss of metabolic genes / pathways

Hanseniaspora Lost Many DNA Repair and Maintenance Genes



TDP1 repairs damage from topoisomerase activity

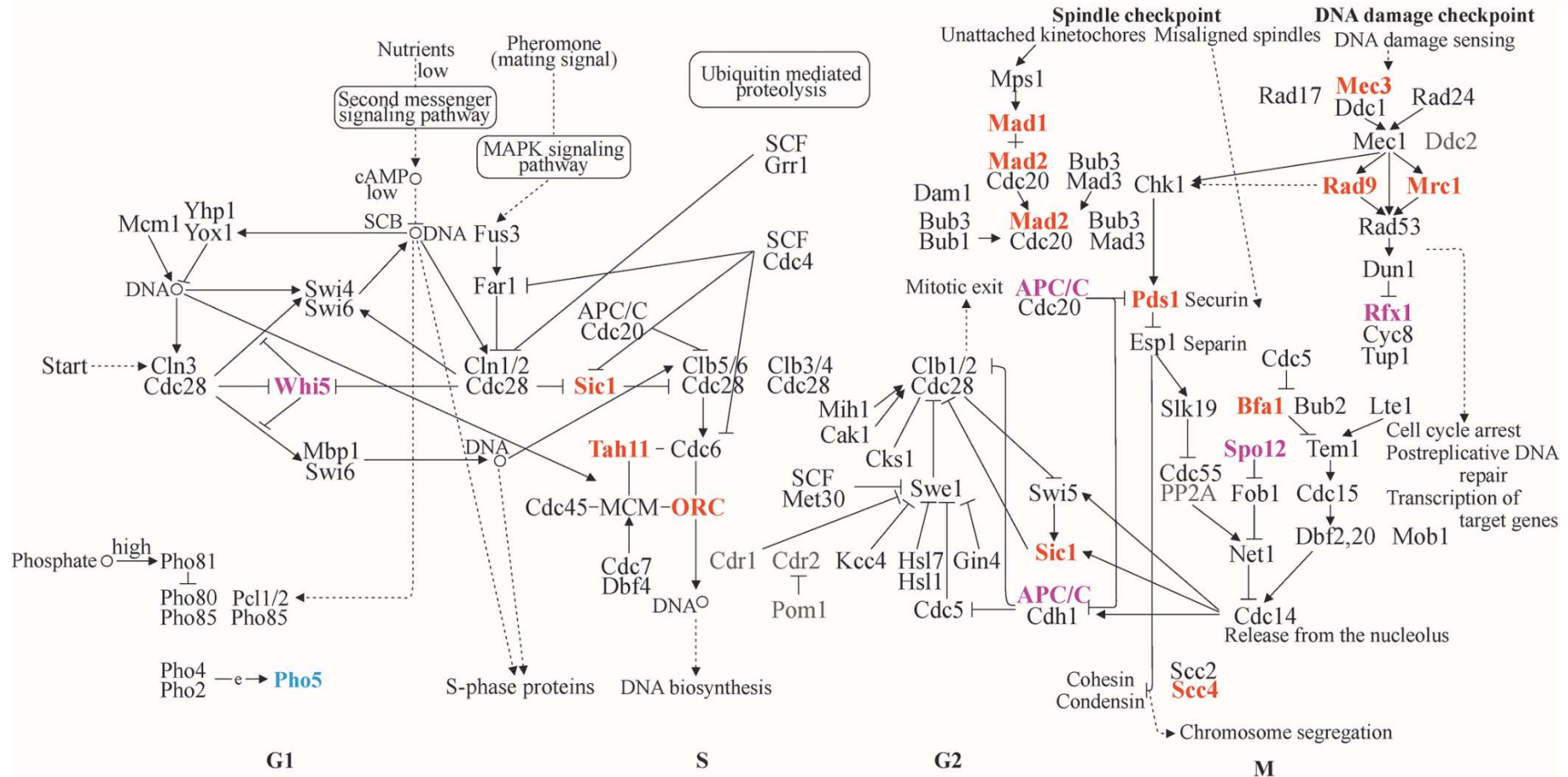
POL32 fills in gaps after excision

PHR1 repairs UV damage

MAG1 excises bases in the base-excision repair pathway



Hanseniaspora Yeasts Lost Many Cell Cycle Genes



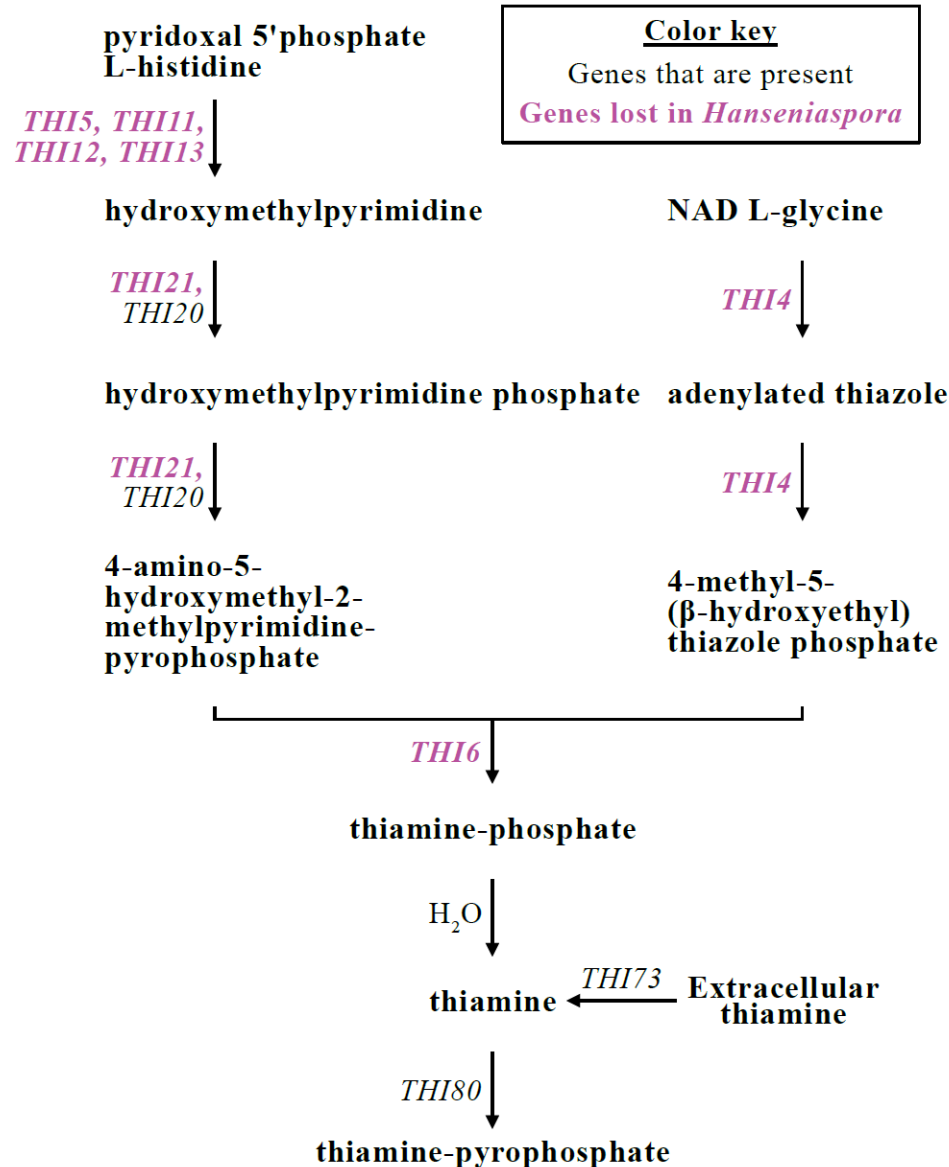
Complexes

ORC (Origin Recognition Complex)		MCM (Mini-Chromosome Maintenance) complex		Anaphase-promoting complex (APC)			Condensin		Cohesin	
Orc1	Orc2	Mem2	Mem3	Cdc27	Apc11	Cdc23	Smc2	Smc4	Smc1	Smc3
Orc3	Orc4	Mem4	Mem5	Apc4	Swm1	Ama1	Ycs4	Bml1	Med1	Irr1
Orc5	Orc6	Mem6	Mem7	Cdc26	Cdh1	Mnd2	Ycg1			
				Cdc20	Doc1	Cdc16				

Color key

Genes that are present
Genes lost in <i>Hanseniaspora</i>
Genes lost in <i>FEL</i> yeast
Genes lost in <i>SEL</i> yeast
Unknown presence or absence

Hanseniaspora Yeasts Lost Many Metabolic Genes

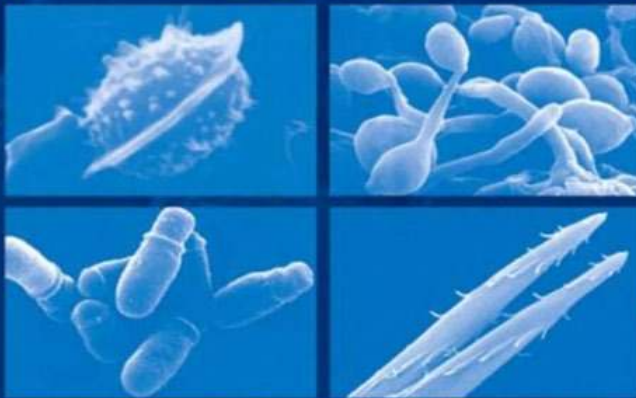


Reconstructing the Evolution of Yeast Metabolism Across the Subphylum



THE YEASTS A TAXONOMIC STUDY

FIFTH EDITION



VOLUME 1

EDITED BY
C.P. KURTZMAN • J.W. FELL • T. BOEKHOUT

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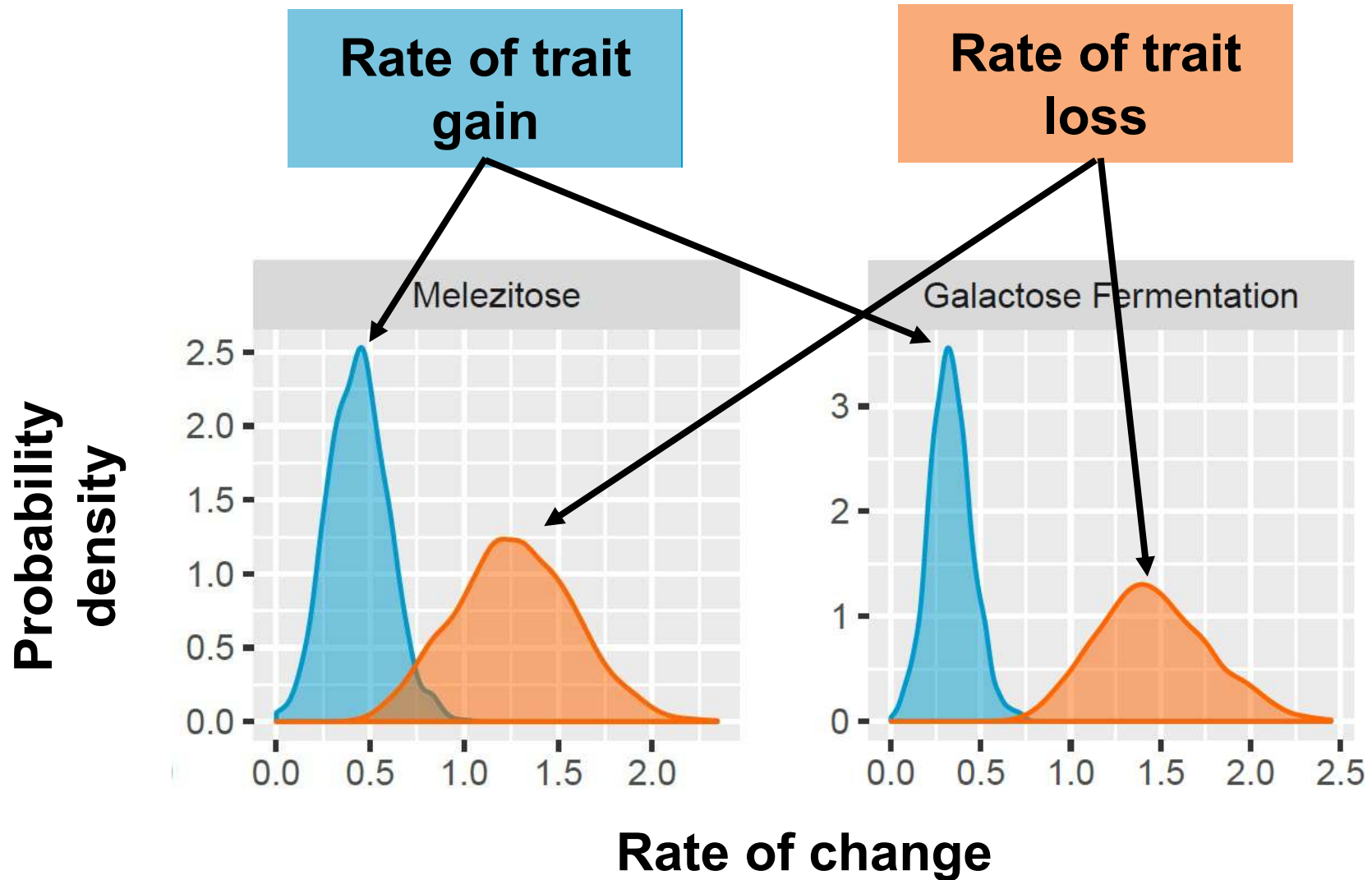
Fermentation

Glucose	+	Lactose	—
Galactose	—	Raffinose	—
Sucrose	—	Trehalose	—
Maltose	—		

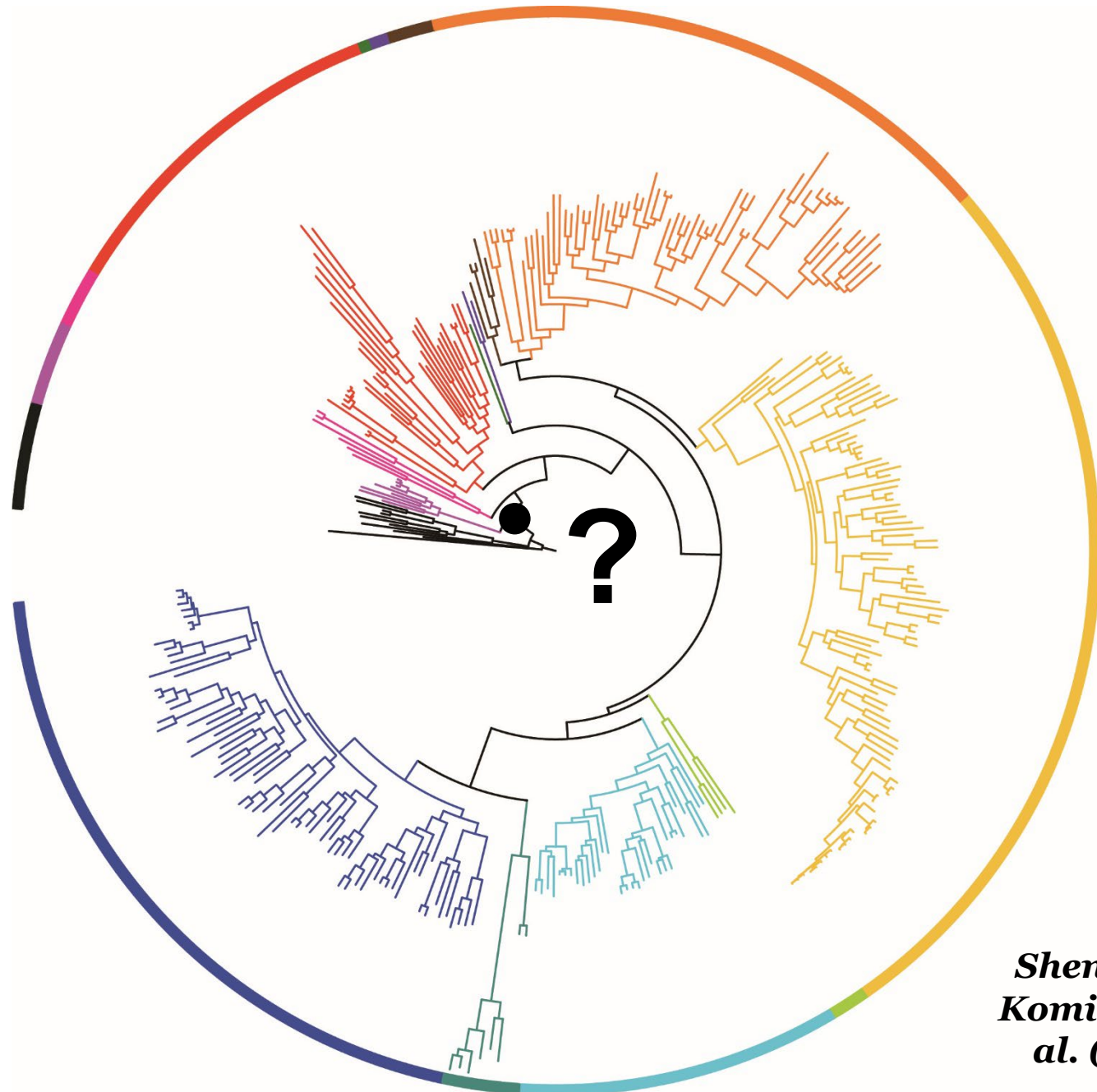
Growth (in Liquid Media)

Glucose	+	D-Ribose	—
Inulin	—	Methanol	—
Sucrose	—	Ethanol	—
Raffinose	—	Glycerol	—
Melibiose	—	Erythritol	—
Galactose	—	Ribitol	—
Lactose	—	Galactitol	—
Trehalose	—	D-Mannitol	—
Maltose	—	D-Glucitol	—
Melezitose	—	myo-Inositol	—
Methyl- α -D-glucoside	—	D,L-Lactate	—
Soluble starch	—	Succinate	—
Cellobiose	+	Citrate	—
Salicin	+	D-Gluconate	+
L-Sorbose	—	D-Glucosamine	—
L-Rhamnose	—	N-Acetyl-D-glucosamine	n
D-Xylose	—	Hexadecane	n
L-Arabinose	—	Nitrate	—
D-Arabinose	—	Vitamin-free	—

Loss Exceeds Gain for 38 / 45 Metabolic Traits

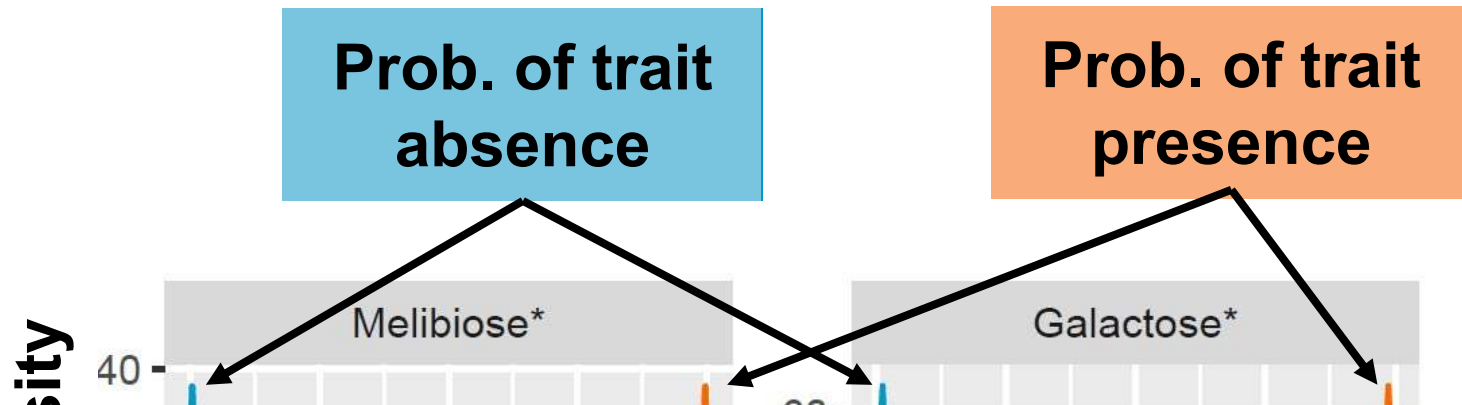


Inferring the Metabolic Capabilities of Yeast Ancestors

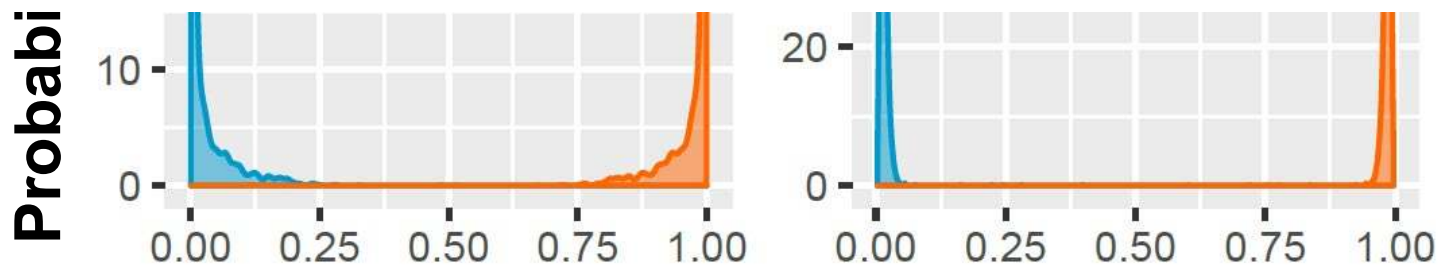


***Shen, Opulente,
Kominek, Zhou et
al. (2018) Cell***

BYCA (Budding Yeast Common Ancestor) was a Generalist



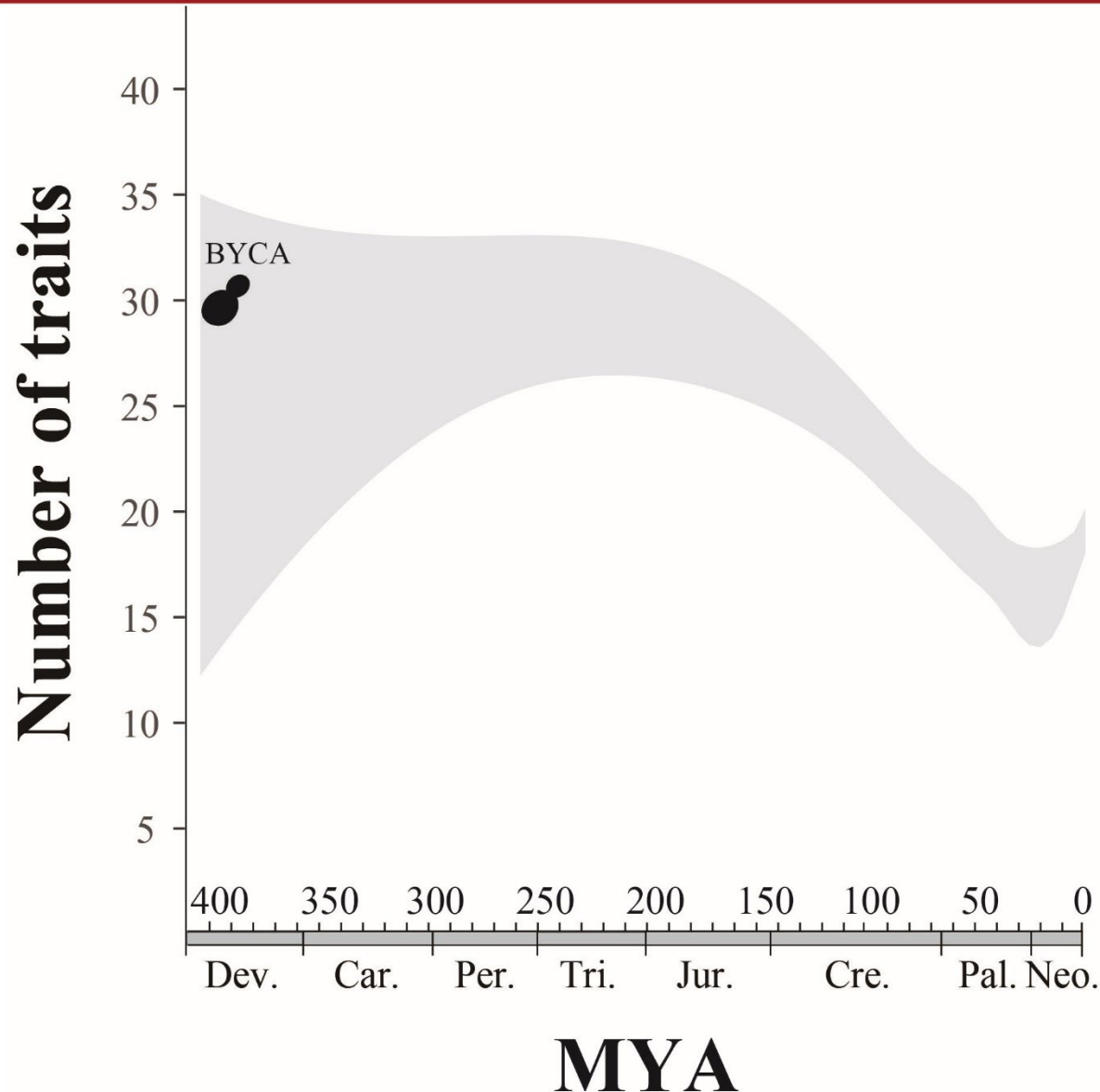
We infer that BYCA could grow on 28 of the 45 substrates



Probability of Trait Presence in Ancestor



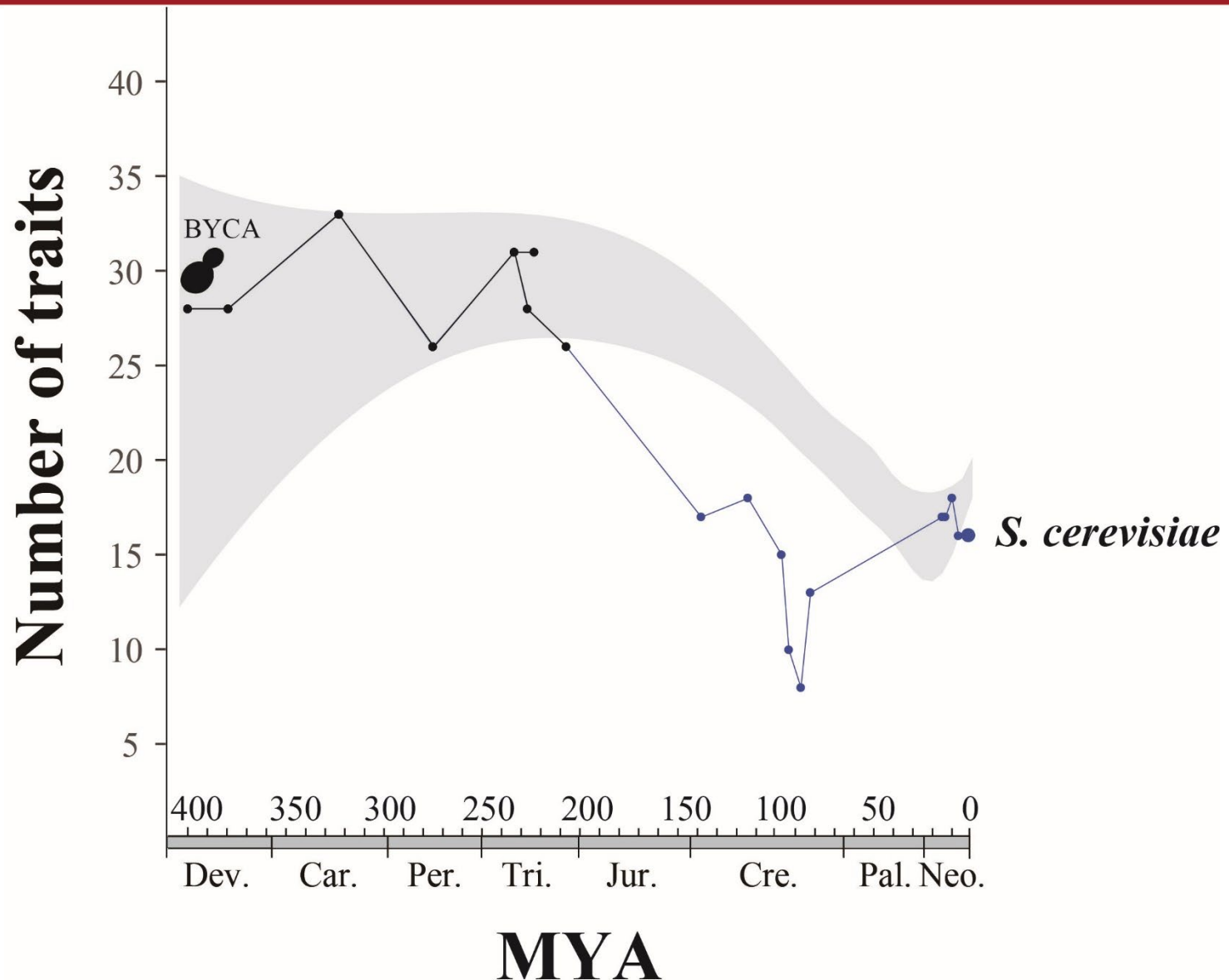
Widespread Loss of Traits



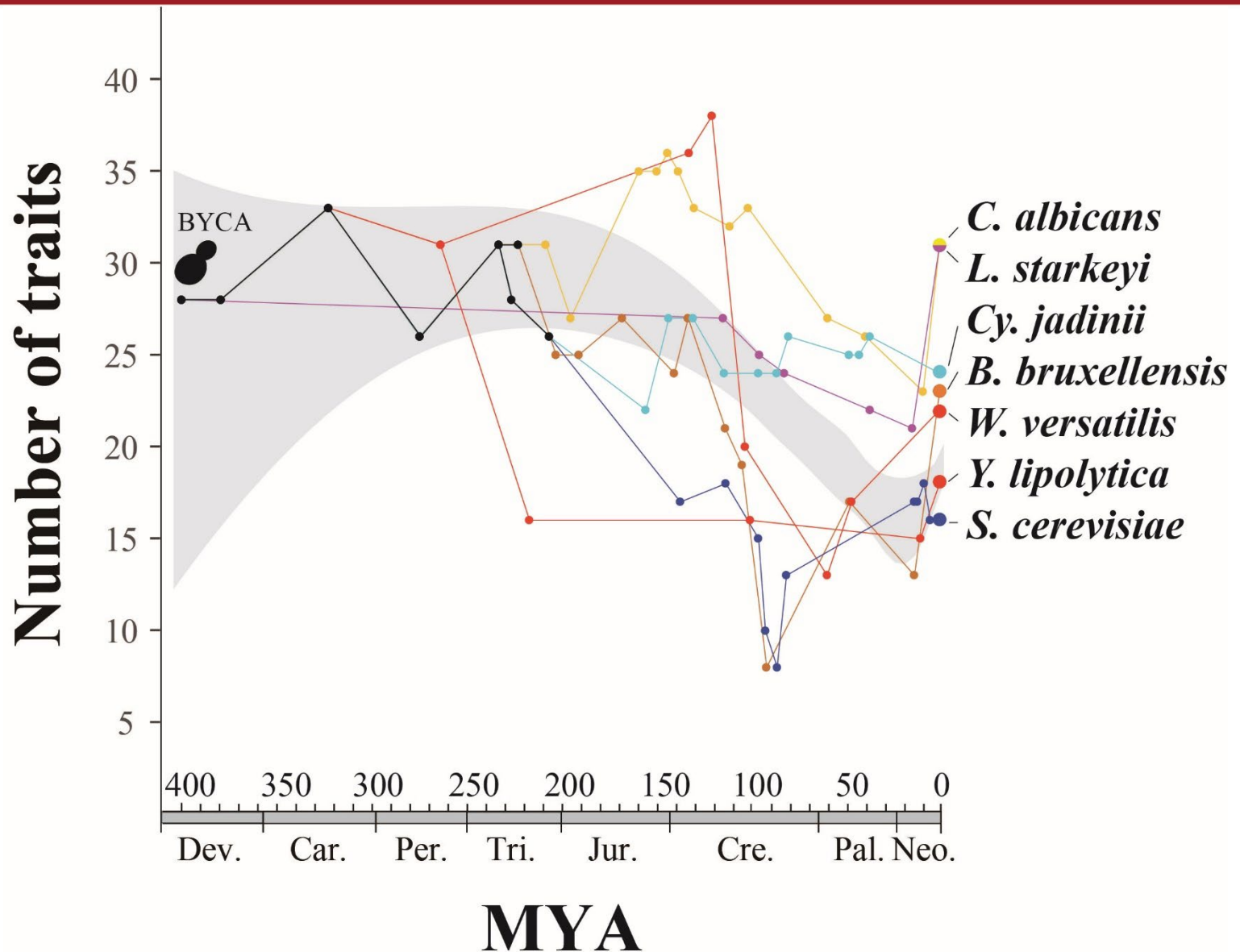
Shen, Opulente, Kominek, Zhou et al. (2018) Cell



Widespread Loss of Traits

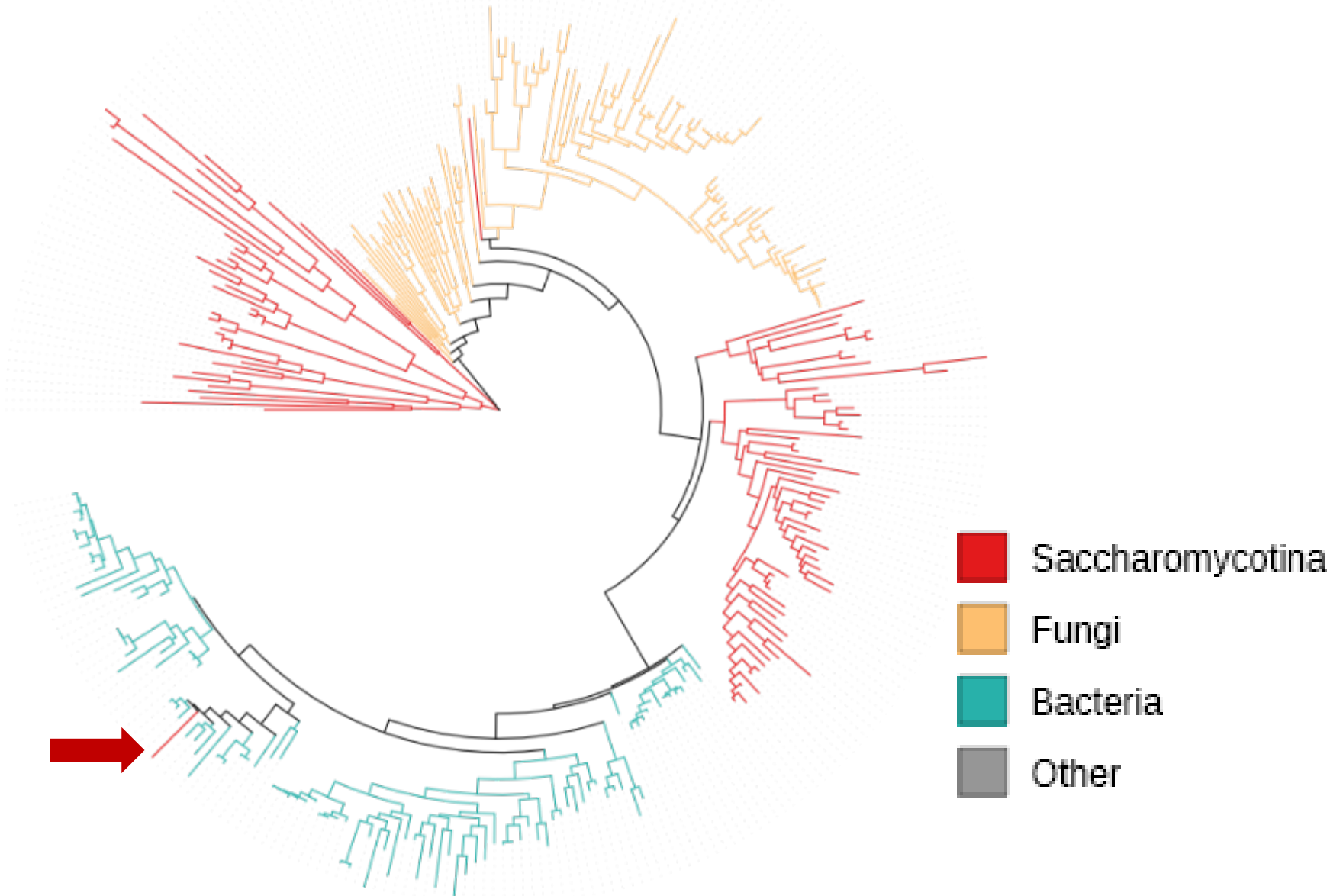


Widespread Loss of Traits

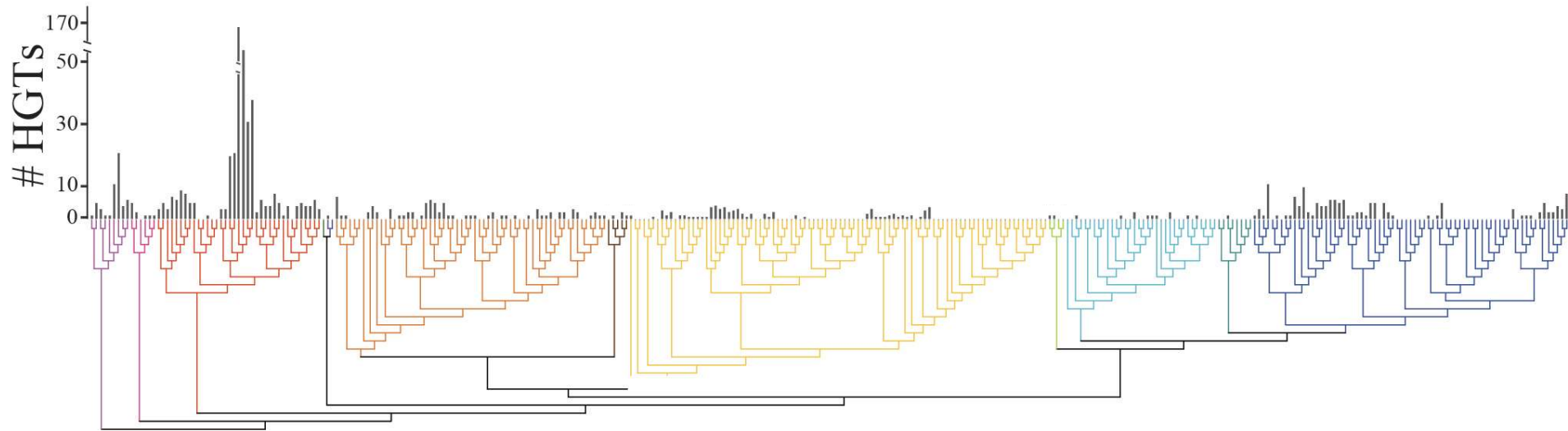


**with so much loss, how
did new metabolic
traits evolve?**

Horizontal Gene Transfer (HGT)



Distribution of 878 Horizontally Acquired Genes



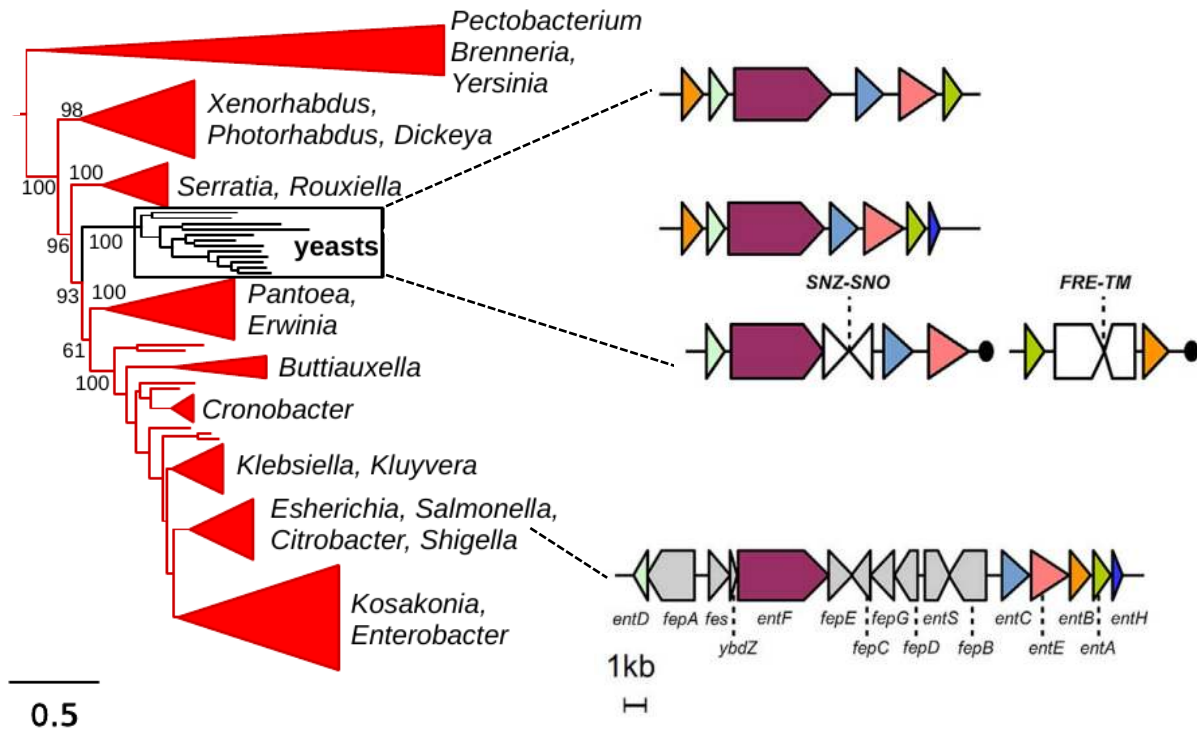
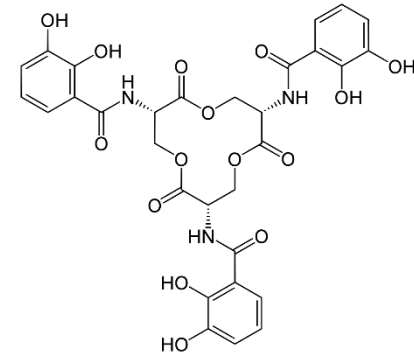
HGT in 226 yeasts with universal code: 0.071%

HGT in 103 yeasts with non-universal code: 0.025%

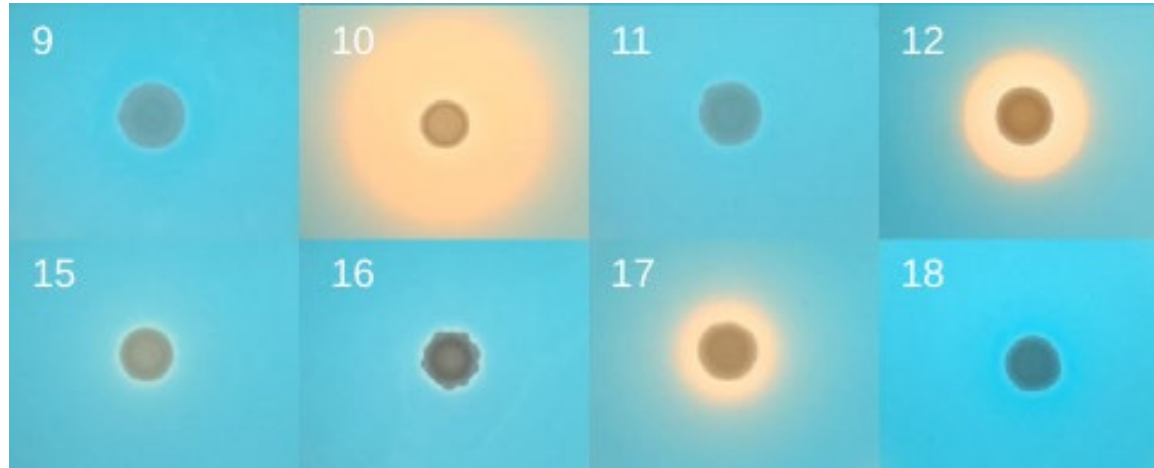


HGT of a Bacterial Siderophore Operon in W/S clade Yeasts

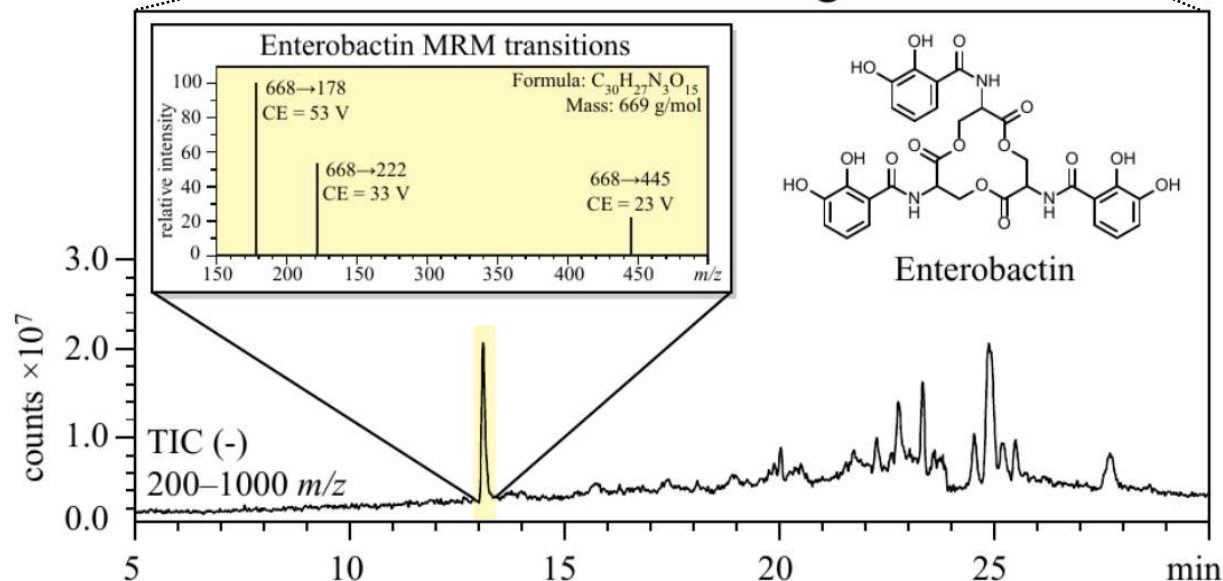
W/S clade yeasts acquired the enterobactin operon from **Enterobacteria** – organisms from both lineages co-occur in insect guts, where iron is a growth-limiting factor

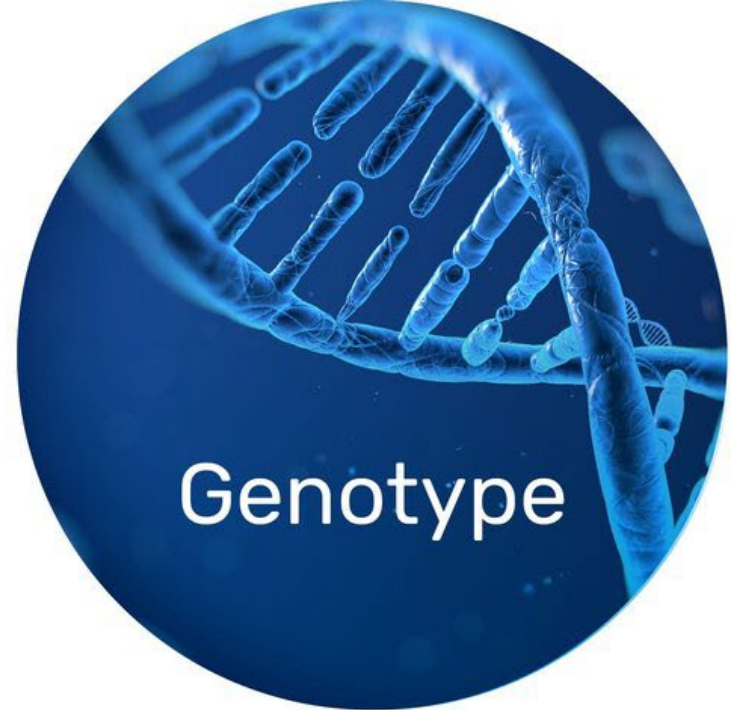


W/S Yeasts with Functional Operon Produce Enterobactin



HPLC-MS chromatogram





“The PIs assume “built it and they will come”. While the yeast community has a long track record of capitalizing on new resources, the PIs are cautioned against arguing for innovation using “field of dreams” reasoning”

Anonymous grant reviewer (2012)

Multiple HGTs, including of bacterial operons, in the W/S clade

Evidence for loss and reacquisition of alcoholic fermentation in a fructophilic yeast lineage

Carla Gonçalves¹, Jennifer H Wisecaver^{2,3}, Jacek Kominek^{4,5,6,7}, Madalena Salema Oom^{1,8}, Maria José Leandro^{9,10}, Xing-Xing Shen², Dana A Opulente^{4,5,6,7}, Xiaofan Zhou^{11,12}, David Peris^{4,5,6,7,13}, Cletus P Kurtzman^{14†}, Chris Todd Hittinger^{4,5,6,7}, Antonis Rokas², Paula Gonçalves^{1*}

Multilayered horizontal operon transfers from bacteria reconstruct a thiamine salvage pathway in yeasts



Carla Gonçalves^a and Paula Gonçalves^{a,1}

Multiple reinventions of mating-type switching

Multiple Reinventions of Mating-type Switching during Budding Yeast Evolution

Tadeusz Krassowski^{1,2,3}, Jacek Kominek^{2,3}, Xing-Xing Shen⁴, Dana A. Opulente^{2,3}, Xiaofan Zhou⁴, Antonis Rokas⁴, Chris Todd Hittinger^{2,3} and Kenneth H. Wolfe^{1,5,*}

Current Biology

Multiple codon reassignments of “universal” genetic code

Evolutionary instability of CUG-Leu in the genetic code of budding yeasts

Tadeusz Krassowski¹, Aisling Y. Coughlan¹, Xing-Xing Shen², Xiaofan Zhou^{2,3}, Jacek Kominek^{4,5}, Dana A. Opulente⁴, Robert Riley^{6,7}, Igor V. Grigoriev⁶, Nikunj Maheshwari¹, Denis C. Shields¹, Cletus P. Kurtzman⁸, Chris Todd Hittinger^{4,5}, Antonis Rokas² & Kenneth H. Wolfe¹

Variation and selection on codon usage bias across an entire subphylum

Abigail L. LaBella¹, Dana A. Opulente², Jacob L. Steenwyk¹, Chris Todd Hittinger²,
Antonis Rokas^{1*}



Signatures of optimal codon usage in metabolic genes inform budding yeast ecology

Abigail Leavitt LaBella¹, Dana A. Opulente², Jacob L. Steenwyk¹, Chris
Todd Hittinger², Antonis Rokas^{1*}

PLOS BIOLOGY

The evolution of the GALactose utilization pathway in budding yeasts

Marie-Claire Harrison,¹ Abigail L. LaBella,¹ Chris Todd Hittinger^{2,*} and Antonis Rokas^{1,*}

Trends in
Genetics

Repeated horizontal gene transfer of GALactose metabolism genes violates Dollo's law of irreversible loss

Max A. B. Haase,^{1,2,†} Jacek Kominek,^{1,†} Dana A. Opulente,¹ Xing-Xing Shen,^{3,4} Abigail L. LaBella,³ Xiaofan Zhou,^{3,5}
Jeremy DeVirgilio,⁶ Amanda Beth Hulfachor,¹ Cletus P. Kurtzman,^{6,†} Antonis Rokas,^{3,*} and Chris Todd Hittinger^{1,*}

SCIENCE ADVANCES | RESEARCH ARTICLE

EVOLUTIONARY BIOLOGY

Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota

Xing-Xing Shen^{1*}, Jacob L. Steenwyk², Abigail L. LaBella², Dana A. Opulente³, Xiaofan Zhou⁴,
Jacek Kominek³, Yuanning Li², Marizeth Groenewald⁵, Chris T. Hittinger³, Antonis Rokas^{2*}

SCIENCE ADVANCES | RESEARCH ARTICLE

EVOLUTIONARY BIOLOGY

An orthologous gene coevolution network provides insight into eukaryotic cellular and genomic structure and function

Jacob L. Steenwyk¹, Megan A. Phillips^{1,†}, Feng Yang^{2,3}, Swapneeta S. Date¹, Todd R. Graham¹,
Judith Berman^{2,†}, Chris Todd Hittinger⁴, Antonis Rokas^{1*}

A genome-scale phylogeny of the kingdom Fungi

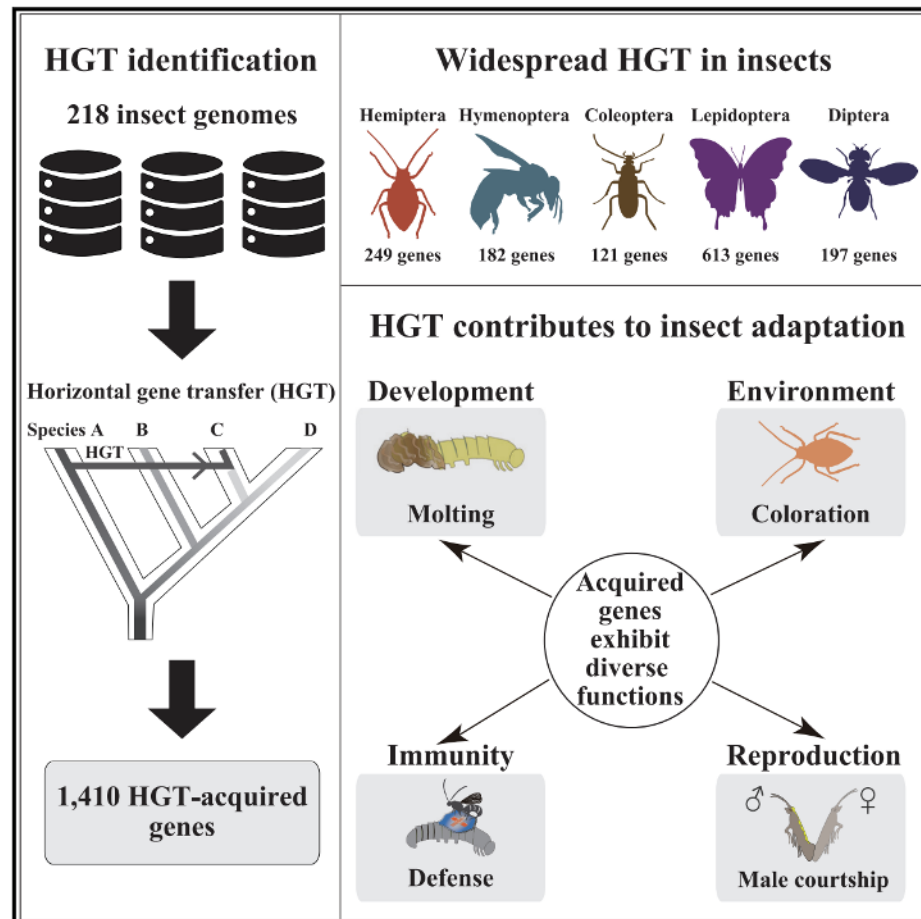
Yuanning Li,¹ Jacob L. Steenwyk,¹ Ying Chang,² Yan Wang,^{3,4} Timothy Y. James,⁵ Jason E. Stajich,³
Joseph W. Spatafora,² Marizeth Groenewald,⁵ Casey W. Dunn,⁷ Chris Todd Hittinger,⁸ Xing-Xing Shen,^{9,*}
and Antonis Rokas^{1,10,*}

GENETICS

Current Biology

HGT is widespread in insects and contributes to male courtship in lepidopterans

Graphical abstract



Authors

Yang Li, Zhiguo Liu, Chao Liu, ...,
Antonis Rokas, Jianhua Huang,
Xing-Xing Shen

Correspondence

antonis.rokas@vanderbilt.edu (A.R.),
jhhuang@zju.edu.cn (J.H.),
xingxingshen@zju.edu.cn (X.-X.S.)

In brief

A comprehensive resource of horizontal gene transfer (HGT) events in 218 insects acquired from non-metazoan sources provides insight into the adaptation of HGTs in insect genomes with the discovery of a functional role for the gene *LOC105383139* in male courtship behavior in lepidopterans.

Acknowledgments

Hittinger lab – UW

**Dana Opulente
Jacek Kominek
Emily Ubbelohde
& many others**

C. Kurtzman – USDA

**M. Groenewald –
Westerdijk**

Rokas lab – Vandy

**Xing-Xing Shen
Xiaofan Zhou
Jacob Steenwyk
Abbe LaBella
Carla Goncalves
Kyle David
& many others**



Budding Yeast Community

**Moriya Ohkuma,
Rikiya Endoh, Masako
Takashima, Ri-ichiroh
Manabe, Neža Čadež,
Diego Libkind, Carlos
A. Rosa and many
others**

<http://y100plus.org>



<http://www.rokaslab.org/>

@RokasLab

Questions?

Yeasts are powerful for studying how genetic variation transforms to phenotypic variation at macroevolutionary scales

Gene conservation is not (never?) universal

Trait & gene loss are “creative” evolutionary forces

Few, but highly impactful, HGT events