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Minireview

Natural trait variation across *Saccharomycotina* species

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Abstract

Among molecular biologists, the group of fungi called *Saccharomycotina* is famous for its yeasts. These yeasts in turn are famous for what they have in common—genetic, biochemical, and cell-biological characteristics that serve as models for plants and animals. But behind the apparent homogeneity of *Saccharomycotina* species lie a wealth of differences. In this review, we discuss traits that vary across the *Saccharomycotina* subphylum. We describe cases of bright pigmentation; a zoo of cell shapes; metabolic specialties; and species with unique rules of gene regulation. We discuss the genetics of this diversity and why it matters, including insights into basic evolutionary principles with relevance across Eukarya.

Keywords: Saccharomycotina; yeasts; phenotypic variationyeasts

Introduction

The fungal kingdom comprises tens of thousands of species relevant for industry, agriculture, ecology, and biomedicine, with many more likely remaining to be identified (Blackwell 2011, Li et al. 2021). In the context of the fungal tree of life, the subphylum *Saccharomycotina* has achieved some measure of fame for phenotypes that it lacks altogether. Relatives of this group—the rest of the fungal phylum *Ascomycota*—can digest plant cell walls, develop as hyphae and other differentiated cell types, and make complex secondary metabolites. *Saccharomycotina* species often do not have these characteristics. Members of the subphylum have small genomes, and in most cases, they make only one or two cell types (Nagy et al. 2014, Stajich 2017, Shen et al. 2018, 2020, Steenwyk et al. 2019). Their evolutionary history is largely one of loss (Krause et al. 2018, Kiss et al. 2019, Merényi et al. 2023): the ancestor of *Saccharomycotina* appears to have shucked off complexities that had evolved in earlier stages before it branched off from other Ascomycetes and/or during the radiation of the group.

But innovation has not stopped in *Saccharomycotina*. During their diversification, spanning ∼400 million years of evolution (Shen et al. 2018), species in this group have refined traits and evolved new ones. Indeed, the very simplicity of their genetic backgrounds brings these phenotypic gains into relief. In this way, *Saccharomycotina* can serve as an excellent model for the study of evolutionary innovation. We thus have chosen phenotypic variation between species of *Saccharomycotina* from the wild as the subject of the current review.

In compiling this review, our goal has been to complement recent landmark genomic surveys of the subphylum and its variation in genome content, splicing, codon usage, and genetic parasites (Steinberg-Neifach and Lue 2015, Dujon and Louis 2017, Shen et al. 2018, LaBella et al. 2019, 2021, Hurtig et al. 2020, Fredericks et al. 2021, Parker et al. 2023). That is, since *Saccharomycotina* genomes have been covered so thoroughly and so recently, we focus on phenotypes instead. Because the trait of virulence in mammalian hosts has been the subject of incisive recent reviews (Gabaldón et al. 2016, Rokas 2022), we explore other facets of the differences between *Saccharomycotina* species in terms of how cells form and grow, what they metabolize, and how they deal with environmental challenge. We highlight discoveries from the recent literature, including new phenotypes and their mechanisms, and we summarize classic work in the field where it is pertinent.

With an eye to the potential for particularly straightforward evolutionary stories, we have restricted ourselves to qualitative traits from the wild, for which a given clade can be classified as affected or otherwise. Many variable traits that do not meet this description are of keen ecological and applied interest in *Saccharomycotina*, e.g. salt, heat, and cold tolerance (Robert et al. 2015, Gostinčar and Gunde-Cimerman 2018, Segal-Kischinevzky et al. 2022); drug sensitivity (Kuo et al. 2010); DNA damage response (Milo et al. 2019, Steenwyk et al. 2019, Shor et al. 2020, Steenwyk 2021); riboflavin production (Averianova et al. 2020); and the oleaginous phenotype (Ratledge 2013, He et al. 2018, Abeln and Chuck 2021, Salvador López et al. 2022). As we often need specialized phylogenetic methods to infer when and why evolution has built a given quantitative trait, these cases tend to form a topic all to themselves and are not our focus here.

By a similar logic, we have earmarked for this review traits that manifest in well-defined wild *Saccharomycotina* populations and species. We do not cover heterotic phenotypes, which result as long-diverged lineages come together in hybrids; these traits in *Saccharomycotina* and their mechanisms form an exciting literature all their own (Gabaldón 2020).

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Table 1. Cases of regulatory rewiring in *Saccharomycotina*: in (A) each row reports a case in which orthologs of the indicated protein play different roles in the indicated species; and in (B) each row reports a case in which the indicated environmental exposure drives different outputs in the indicated species.

A

Protein	Species A	Species B	Function in A	Function in B	Reference
Ndt80	*S. cerevisiae*	*C. albicans*	Regulates sporulation	Regulates biofilm formation	(Nocedal et al. 2017)
Ppr1	*S. cerevisiae*	*C. albicans*	Regulates uracil synthesis	Regulates allantoin breakdown	(Tebung et al. 2016)
Tbf1	*S. cerevisiae*	*C. albicans*	Regulates ribosomal proteins	Player in general gene silencing	(Hogues et al. 2008)
Rtg1/3	*S. cerevisiae*	*C. albicans*	Regulates mitochondrial communication	Regulates galactose metabolism	(Dalal et al. 2016)
Ace2	*S. cerevisiae*	*C. albicans*	Regulates mitosis	Regulates glycolysis and respiration	(Mulhern et al. 2006)
MATa2	*S. cerevisiae*	*C. albicans*	N/A; replaced by α2 for repression of **a**-specific genes in α cells	Activates **a**-specific genes in **a** cells	(Tsong et al. 2003)
Sir2	*K. lactis*	*C. glabrata*	Regulates carbon metabolism, detoxification of arsenic, and iron scavenging	Regulates drug resistance	(De Las Penas et al. 2003; Orta-Zavalza et al. 2013; Humphrey et al. 2020)
Mig1	*S. cerevisiae*	*K. marxianus*	Player in glucose repression	Regulates histidine biosynthesis	(Nurcholis et al. 2019)
Protein kinase A	*S. cerevisiae*	*K. lactis*	Induces stress response genes that have paralogs from whole-genome duplication	N/A; paralogs not present in genome	(Heineike and El-Samad 2021)
Yht1	*S. cerevisiae*	*Y. lipolytica*	Sugar sensor	Sugar transporter	(Palma et al. 2009; Lazar et al. 2017)
Hap1	*S. cerevisiae*	*K. lactis*	Transcriptional activator of respiration, sterol biosynthesis or oxidative stress under hypoxia	Suppressor of the major glucose transporter	(Lamas-Maceiras et al. 2007; Bao et al. 2008)
Rox1	*S. cerevisiae*	*K. lactis*	Regulator of the hypoxic response	Resistance to metals	(Rodríguez Torres et al. 2012)

B

Environmental stimulus	Species A	Species B	Species C	Output of A	Output of B	Output of C	Reference
Hypoxia	*K. marxianus*	*K. lactis* and *S. cerevisiae*	*C. albicans*	Mitophagy	N/A	Hyphal growth	(Hoshida et al. 2020)
Starvation	*K. lactis*	*S. cerevisiae*		Mating	N/A		(Booth et al. 2010)

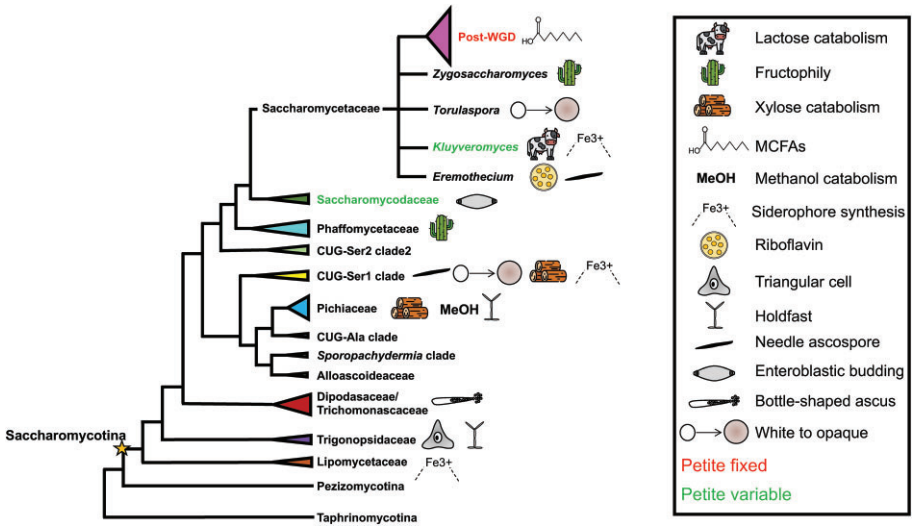

Figure 1. Clade-unique traits in *Saccharomycotina*. Shown is a phylogeny of the subphylum *Saccharomycotina* (with a star denoting its origin) and outgroup relatives, with branch lengths not to scale and clade-unique traits of interest highlighted as icons. WGD, descendants of an ancestor that sustained a whole-genome duplication (really the product of an ancient hybridization) in the family *Saccharomycetaceae*. Lactose catabolism, the ability to convert lactose into galactose and glucose. Fructophily, preferential usage of fructose over other substrates. MCFAs, detectable production of medium-chain fatty acids. Methanol catabolism, the ability to utilize methanol as a sole carbon source. Holdfast, a lifestyle that includes attachment to nematodes. Petite fixed and petite variable indicate clades where, all or some members, respectively, can proliferate without mitochondrial DNA.

Morphology, vegetative growth, and sex

Yeast morphology and budding

A mycologist on the street, asked for a defining feature of *Saccharomycotina*, is likely to answer, "Yeasts." Species growing only as yeasts, with no hyphal morphology, crop up again and again in the phylogeny (*Saccharomyetaceae, Pichiaceae, Saccharomycodaceae, Phaffomycetaceae,* and *Ascoideacae*), likely representing independent losses of the inferred ancestral hyphal program. Interspersed between these yeast clades are plenty of lineages that have retained hyphal growth [e.g. *Blastobotrys, Candida*, *Eremothecium*, *Yarrowia, Dipodascaceae*, *Alloascoidea,* and *Saccharomycopsis* (Philippsen et al. 2005, Nagahama et al. 2008, Stajich et al. 2009, Kurtzman and Robnett 2013, Sipiczki and Hrabovszki 2023)]. The polyphyletic pattern of yeasts across *Saccharomycotina*, which also manifests elsewhere in fungi (Nagy et al. 2014, Naranjo-Ortiz and Gabaldón 2019), is a testament to the likely benefit of the yeast lifestyle across niches (Ivarsson et al. 2020), and to the evolutionary accessibilility of its mechanisms. Phylogenetic inference points to a role in the latter for the loss of Zn-cluster transcription factors (Nagy et al. 2014). Interestingly, in *Saccharomycotina*, programs to produce asexual spores show up for the most part in groups that also form hyphae [e.g. arthroconidia in *Dipodascaceae* (Kurtzman et al. 2011), chlamydospores in *Candida albicans* and *C. dublinensis* (Citiulo et al. 2009), and conidia on condiophores in *Blastobotrys* (Kurtzman et al. 2011)], suggesting a molecular and ecological link between the characters.

Apart from the hyphal-yeast dichotomy, more nuanced morphologies as they vary across *Saccharomycotina* have caught the eye of researchers in the field—not for hyphae so much [barring a few observations of septal pore structure (Van Der Klei et al. 2011)] but in great depth for budding yeasts. During vegetative growth, most yeasts adopt an ovoid-to-apiculate (lemon-shaped) cell form, either with planktonic, completely septated cells or those that remain attached to form pseudohyphae. A few more striking exceptions are known. *Trigonopsis* grow as curious triangular and tetrahedral cells as a function of nutrient availability (Fig. 1; Senthe-shanmuganathan and Nickerson 1962, Kurtzman et al. 2011), for which no ecological drivers have yet come to light. The ability to grow a foot-like holdfast, attaching fungal cells to the body of a living invertebrate animal, has arisen at least twice in *Saccharomycotina* (Fig. 1), in beetle-associated *Pichia stipitis* (Suh et al. 2004) and *Botryozyma* spp. (Kerrigan et al. 2001). The latter also has a specialized cell wall and a mucilage secretion program to help them stick to the cuticles of nematodes (Kerrigan and Rogers 2013). Plausibly, these associations with animals could promote nutrient exchange (Petersen et al. 2016) and/or dispersal for the yeasts, analogous to models that have emerged for bacterial symbionts (Bayer et al. 2009, Shu et al. 2018). *Metschnikowia* spp. produce a thick-walled pulcherrima cell, considered a chlamydospore, with a red color owing to pulcherrimin production (Fig. 1 and see below) and high lipid titer (Pitt and Miller 1968); this marks an exception to the general trend that only hyphal-forming species in *Saccharomycotina* make asexual spores. Plastic white and opaque yeast cell programs (Fig. 1), which differ in cell axis length, metabolism, mating, and interactions with the host immune system (Lohse and Johnson 2009), have long appeared to be a unique property of *C. albicans, C. dublienensis,* and *C. tropicalis* (Pujol et al. 2004, Turner and Butler 2014) but were recently reported in a phylogenetically distant species, *Torulaspora microellipsoides* (Brimacombe et al. 2020).

With respect to proliferation, one mechanism dominates *Saccharomycotina* yeasts during vegetative growth: holoblastic budding, by which a scar forms during each budding event, blocking re-use of the bud site during subsequent rounds of mitosis (Kurtzman and Sugiyama 2015). By contrast, a few species within *Saccharomycotina*—those of *Phaffomycetaceae* and *Saccharomycodaceae* (Streiblová et al. 1964, Phaff 1998, Imanishi et al. 2009, Jindamorakot et al. 2009; Smith 2011a, b), and *Hanseniaspora* spp. (Boekhout et al. 1994, Jindamorakot et al. 2009)—divide by enteroblastic budding, with a given cell reusing the identical bud site again and again at one or the other of its poles (Fig. 1). This appears to mark an independent acquisition of enteroblastic budding relative to yeasts of *Basidiomycota*, which also use the mechanism (McLaughlin et al. 2001). The ecological drivers remain unknown, though in principle enteroblastic budding could

confer an advantage if it helps minimize defects in membrane function from bud scars.

Sex and mating type

Most *Saccharomycotina* species go through sexual reproduction via fusion of individuals of opposite mating type, followed by meiosis and gamete release (Wolfe and Butler 2017). A few species-unique modifications have appeared, most notably parasex (fusion without meiosis) in *C. albicans,* covered by a longstanding literature (Mishra et al. 2021), and a program of meiosis without recombination, recently discovered in *Saccharomycodes* (Papaioannou et al. 2021). A handful of *Saccharomycotina* species may have truly lost sex altogether (Krassowski et al. 2019). Overall, though, as in other fungi, sex is the rule rather than the exception across the subphylum—likely driven by the fitness benefits of genetic reshuffling; the pleiotropic "side effects" of sexual programs (Otto 2021); and/or the influence of selfish elements (Hanson and Wolfe 2017).

Apart from the mechanics of sex *per se*, the ability for a given individual to switch mating types has evolved repeatedly in *Saccharomycotina* yeasts (Krassowski et al. 2019). Expert reviews have explored the potential adaptive roles of switching, namely that a given clone, even in the absence of genetically distinct mating partners, could still access the benefits of sex, diploidy, and meiotic spore formation (Hanson and Wolfe 2017). In one line of support for the latter model, filamentous *Saccharomycotina* species with mitotic spore forms (conidia, chlamydospores, *etc.*; see Section "*Yeast morphology and budding*") tend not to exhibit mating-type switching. This would make sense if mitotic programs met the needs for dormancy in such species and obviated the need for an extra route to meiosis of the kind that switching provides. Recent work has pinned down the origin of one switching mechanism, that of the *Saccharomycetaceae*, to the domestication of a homing element (Coughlan et al. 2020).

Morphology of sexual structures

As in all Ascomycetes, *Saccharomycotina* species that do have sex retain the products of meiosis in a sac called an ascus. In terms of ascus morphologies, the prize for complexity and uniqueness may go to the bottle shape in *Dipodascus* (Fig. 1; Vanheerden et al. 2005, Van Heerden et al. 2007, Olivier et al. 2013), which enables active spore release from turgor pressure, the only case known in all of *Saccharomycotina*. Also salient from the literature is the long, thin ascus form in *Metschnikowia* and *Eremothecium/Ashbya* (Fig. 1; Lachance 2016, Wendland 2020), which accommodates barbed, needle-like ascospores that may play a role in infection for some pathogens and dispersal in other species. As for the rest of *Saccharomycotina*, though the ascus structure is roughly globose, ascospore shape and ornamentation vary widely. Cell biologists have noted ascus morphologies from spherical [*Saccharomyces* (Kurtzman et al. 2011)], warty [*Kazachstania, Debaryomces,* and, rarely, *Saccharomyces* (Mrak and Bonar 1938, Moens et al. 1974, Bilinski and Miller 1980, Klapholz and Esposito 1980, Imanishi et al. 2007)], crescent-like [*Ascobotryozyma* (Kerrigan et al. 2001)], and hat-like [*Glactomyces geotrichum*, *Pichia membranigaciens*, and *Ascoidea asiatica* (Kurtzman et al. 2011, Kurtzman and Robnett 2013)] to one report of helical spores [*Tortispora ganteri* (Lachance and Kurtzman 2013)]. Essentially all this variation is polyphyletic, consistent with the volatility in characters of sexual spores across fungi more broadly (Calhim et al. 2018); their ecological roles, if any, remain unknown (Money 2016). Likewise, *Saccharomycotina* species can produce anywhere from one spore [*Torulaspora* and *Debaryomyces* (Suzuki et al. 2011)] to tens of spores [e.g. in *Vanderwaltozyma* (Chang et al. 2020), *Dipodascus* (Vanheerden et al. 2005, Van Heerden et al. 2007, Olivier et al. 2013), and *Lipomyces* (Kurtzman et al. 2011)] within the ascus. The latter may have evolved in species with little other recourse to effective dispersal mechanisms: when a given spore very rarely finds its way far from the parent, making large numbers of spores can be one way to beat the numbers game (Money 2016).

Biochemistry and metabolism

Biochemistry and secondary metabolites

Many secondary metabolite pathways ancestral to Ascomycetes were lost wholesale in *Saccharomycotina* (Kroken et al. 2003, Arvas et al. 2007, Bushley and Turgeon 2010, Khaldi et al. 2010, Chen et al. 2014, Krause et al. 2018, Linder 2019a). Against this backdrop, a few small-molecule production traits have emerged in *Saccharomycotina* as evolutionary gains of potential ecological relevance. Several compelling stories center on the synthesis of iron-uptake compounds (Fig. 1). In one example, a survey of *Saccharomycotina* found that only *Kluveromyces* spp. and *Metschnikowia* spp. make the siderophore pulcherrimin, identifying the causal gene cluster and inferring its ancient origin and repeated losses elsewhere in the subphylum (Krause et al. 2018). Recent work suggests a role for pulcherrimin in microbial competition and stress protection (Kregiel et al. 2022, Charron-Lamoureux et al. 2023). Meanwhile, *Lipomycetaceae* spp. appear to be unique in their synthesis of a separate siderophore, ferrichrome (Van Der Walt et al. 1990), presumably for iron transport and iron storage as in *Schizosaccharomyces pombe* (Schrettl et al. 2004). The latter results, and the wealth of *Saccharomycotina* species harboring genes for siderophore uptake (Kluyver et al. 1953, Araujo and Hagler 2011, Lachance 2016), suggest a landscape of interactions by *Saccharomycotina* in communities based on iron nutrients (Thanh et al. 2002), analogous to well-studied dynamics in bacteria (Kramer et al. 2020).

Another line of investigation has found a lineage-unique biochemical character in *Saccharomyces, Naumovozyma,* and *Nakaseomyces,* which are so-called post-whole-genome duplication species of the family *Saccharomycetaceae* [really the product of an ancient hybridization (Marcet-Houben and Gabaldón 2015)]: this clade, and no other *Saccharomycotina* species yet tested, make fatty acids 10–14 carbons in length (Fig. 1; Froissard et al. 2015). Plants, other fungal phyla, and bacteria also produce such medium-chain fatty acids (Liu et al. 2017, Stamatopoulou et al. 2020), which are of relevance for industrial production of fuels, lubricants, detergents, and cosmetics. In *Saccharomycotina,* our understanding of the ecology and evolution of these lipids remains rudimentary. Aside from making medium-chain fatty acids at low titer, *Saccharomycetaceae* species can assimilate and catabolize them from growth media (Nakagawa et al. 2000, McDonough et al. 2002), but one recent study suggests that these lipids are not stored in lipid droplets (Funk et al. 2017). Medium-chain fatty acids in *Saccharomycetaceae* could represent the product of truncation errors in the biosynthesis of long-chain molecules. Alternatively, they could be synthesized to serve biological functions in their own right, including intracellular signaling (Van Roermund et al. 2000) or interactions with animal hosts, e.g. fruit flies, which sense them directly (Brown et al. 2021).

Fermentation, anaerobic growth, and petiteness

If the main morphological character we associate with *Saccharomycotina* is yeast growth, metabolically the subphylum has achieved the most renown for fermentation. Fermentation and

respiration can be complementary programs to generate energy; early work motivated by applications in the brewing industry (Eliodório et al. 2019) identified *Saccharomycotina* species that are hard-wired to favor fermentation even in the presence of oxygen, accumulating ethanol at high titer (the Crabtree effect; De Deken 1966). As the field advanced, two independent acquisitions of the Crabtree effect became apparent within *Saccharomycotina*, in the families *Saccharomycetaceae* [*Saccharomyces*, *Kazachstania*, *Naumovozyma*, *Nakaseomyces*, and *Vanderwaltozyma* spp. (Merico et al. 2007)] and *Pichiaceae* [*Dekkera/Brettanomyces* spp. (Rozpędowska et al. 2011, Hagman et al. 2013)], respectively. (The Crabtree effect also manifests in other fungi and in some animal tissues (Gojković et al. 2004, Hagman et al. 2013).) The avid fermentation lifestyle likely benefits these species by virtue of its rapid rate of ATP synthesis and the ability to kill off competitors via ethanol release (Pfeiffer and Morley 2014). Genetic mechanisms of the Crabtree effect, and their evolutionary dating, within *Saccharomycotina* remain an area of active research (Schüller 2003, Thomson et al. 2005, Piskur et al. 2006, Lin and Li 2011, Rozpędowska et al. 2011, Hagman et al. 2013, Dashko et al. 2014, Pfeiffer and Morley 2014, Williams et al. 2015).

In the family *Saccharomycetaceae*, among the clades that exhibit the Crabtree effect, many species also have the ability to proliferate without mitochondrial DNA, referred to as the petite phenotype (Fig. 1; Chen and Clark-Walker 1999). The two traits manifest together in most of the post-whole-genome duplication clades of this family [*Saccharomyces, Kazachstania, Naumovozyma,* and *Nakaseomyces* spp., though not in all *Tetrapisispora* or *Vanderwaltozyma* spp. (Fekete et al. 2007, Merico et al. 2007, Procházka et al. 2010)]. Furthermore, not only can most of the post whole-genome duplication species of *Saccharomycetaceae* live without respiration, and routinely avoid it, but they can also grow in the absence of oxygen. The latter phenotype appears to have arisen earlier in the history of *Saccharomycetaceae* than the date of the whole genome duplication *per se*, since it has also been noted in more basal *Saccharomycetaceae* clades [*Lachancea, Torulaspora, Zygotorulaspora,* and *Zygosaccharomyces* spp. (Merico et al. 2007, Hagman et al. 2013, Krause and Hittinger 2022)]. The spotty appearance of each trait—the Crabtree effect, petite formation, and low-oxygen growth—suggests that selective pressures for these behaviors have come and gone repeatedly, even within the family *Saccharomycetaceae*. That said, broadly speaking, the phenotypic syndrome has led to a model of ecological specialization by most *Saccharomycetaceae* to low-oxygen but not strictly anaerobic niches (Krause 2023)—e.g. the environment within ripening and rotting fruit.

Complementing this extensive literature on respiration-related traits in *Saccharomycetaceae*, similar trends have cropped up in other clades of the *Saccharomycotina*. Hypoxia tolerance arose independently in *Dekkera/Brettanomyces* spp. (Rozpędowska et al. 2011), mirroring the Crabtree metabolism in these species (see above). And petite positivity appears to have been reinvented by evolution in *Kluyveromyces wickerhamii, C. glabrata,* and *Hanseniaspora osmophila* (Merico et al. 2007, Hagman et al. 2013, Guo et al. 2016). Such changes may well reflect ecological constraints similar to those shaping *Saccharomycetaceae*. By contrast, yet another independent gain of hypoxia tolerance, in *C. albicans,* likely had a distinct ecological driver, namely the ability to proliferate inside animal hosts (Ernst and Tielker 2009).

At the opposite extreme, some *Saccharomycotina* species get their energy exclusively from respiration, and have lost their ability to ferment. So-called oxidative yeasts show up across the subphylum, including *Kluyveromyces nonfermentans*, *Kazachstania turicensis,* and some *Botryozyma* and *Debaryomyces* spp.; others are members of fungal groups outside *Saccharomycotina* (Nagahama et al. 1999, Kerrigan et al. 2001, Kurtzman et al. 2011, Paleo-López et al. 2016). The prevalence of oxidative yeasts in sampled seawater (Kutty and Philip 2008, Libkind et al. 2017) raises the possibility that extra-avid respiration has been adaptive, or fermentation subject to relaxed selection, in marine niches. In a separate story, recent work has traced events by which ancestors of the *Wickerhamiella/Starmiella* group lost the ability to ferment and then gained it back, through the horizontal acquisition of bacterial homologs (Gonçalves et al. 2018).

Carbon source utilization

Across the tree of life, organisms from specialized niches often make use of unique substrates for energy, and this logic has of course borne out in *Saccharomycotina*. The ability to break down methanol as a carbon source is a case in point (Fig. 1): methanotrophy arose once in the family *Pichiaceae* [likely originally in wood-associated niches (Kurtzman and Robnett 2010), and is now observed in *Komagataella/Pichia* spp., *Ogataea* spp., and *Kuraishia* spp. (Kurtzman 2005, Suh et al. 2006, Limtong et al. 2008, Yurimoto and Sakai 2019)]; classic genetic dissection has revealed the underlying pathway (Gellissen 2010, Yurimoto et al. 2011).

Another line of the classic literature has pursued galactose catabolism in *Saccharomycotina*, namely its repeated gains and losses (Opulente et al. 2018) and their genetic basis in metabolic enzymes and transporters (Rokas and Hittinger 2007, Shen et al. 2018, Haase et al. 2021, LaBella et al. 2021). Similarly, a small fraction of *Saccharomycotina* species can split the disaccharide lactose into its component parts, galactose and glucose (Fig. 1), and rigorous proof for lactase as a causal gene has emerged in *Kluyveromyces* spp. domesticated for milk fermentations (Sreekrishna and Dickson 1985, Varela et al. 2019).

Xylose utilization, also a longstanding interest in *Saccharomycotina*, is polyphyletic (Fig. 1; Nalabothu et al. 2023), with growth on this wood sugar noted in *Komagataella/Pichia* spp. and species from the guts of wood-eating insects, *Scheffersomyces* and *Spathaspora* spp. (Toivola et al. 1984, Lee et al. 1986, Nguyen et al. 2006, Koivistoinen et al. 2008, 2008). Here the molecular basis remains incompletely understood, since primary xylose catabolic enzymes are insufficient for xylose growth *per se* in *Saccharomycotina* species (Jeffries and Kurtzman 1994).

Glucose repression

In a complex environment, a given fungus needs to make a choice—should it try to make use of multiple carbon sources simultaneously, or should it prioritize just one pathway? Classic work focused on the shutdown of other catabolic pathways in the presence of glucose, as it manifests in the model species *S. cerevisiae* (Kayikci and Nielsen 2015). But we now know of evolutionary tweaks across *Saccharomycotina* that have yielded quite different metabolic logic. Extensive literature has focused on organisms that ignore glucose, *i.e.* shut down glucose catabolism pathways when there is fructose around. This fructophily trait (Fig. 1) seems to have been invented twice in *Saccharomycotina*, once in *Zygosaccharomyces* spp. (Leandro et al. 2011) and again in species of the *Wickerhamiella/Starmerella* clade that thrive in fructose-rich flower nectar (Baek et al. 2010, Magyar and Tóth 2011). The mechanism of the latter has been partly pinned down to a specialized fructose transporter (Pina et al. 2004, Gonçalves et al. 2020). A separate field has focused on clades of *Saccharomycotina* that metabolize multiple sugars at the same time. These

include activation of galactose catabolic pathways in the presence of glucose, in a domesticated milk lineage of *S. cerevisiae* (Duan et al. 2019) and some *K. lactis* (Breunig 1989); simultaneous glucose and mannose breakdown by *Lipomyces* (Yang et al. 2014); and glucose and lactose co-utilization by *C. albicans* (Sandai et al. 2012).

Nutrient utilization: a wider view

Having devoted years to targeted case studies of metabolic variation across *Saccharomycotina*, the field has now come to appreciate how many more substrate-preference stories there are to tell. Species compendia (Kurtzman et al. 2011) started this trend by enabling a broader, but anecdotal, view of nutrient specialization as it comes and goes in the subphylum, from proline to ammonia and from maltose to inulin. More recently, high-throughput methods have allowed well-controlled surveys of growth on hundreds of substrates. The latter has led to new insights into the evolutionary history of metabolic gains and losses across *Saccharomycotina*, and the discovery of associations with ecology and genome content (Novo et al. 2009, Gonçalves et al. 2016, Opulente et al. 2018). Among the many advantages of this approach, it has put the evolutionary study of nitrogen utilization well within reach (Wang et al. 2015, Filteau et al. 2017, Linder 2019b) as a complement to the classic focus on variation in carbon source preferences.

Regulatory rewiring

Alongside studies of the attributes of cells and their growth as they vary across *Saccharomycotina*, a sizeable literature has catalogued species divergence in gene expression (Tsankov et al. 2010, Tirosh et al. 2011, Thompson et al. 2013, Brion et al. 2016). The approach here is often to consider mRNA levels of interest for their own sake, as molecular phenotypes that serve as models for the discovery of evolutionary principles. Most proximally, the field has focused on how and why gene regulation changes between species; within *Saccharomycotina*, the major advances have come in a few model systems, whose results we have condensed into a list of references in Table 1.

For much of the field, the term "regulatory rewiring" means changes between species in the targets of regulatory proteins. In the simplest cases, two species have more or less the same complement of regulators and downstream targets but with distinct relationships. Thus, the division of labor between regulators—which genes they induce or repress—has changed over evolutionary time. In Table 1A, we lay out examples of this kind of divergence in *Saccharomycotina*, and they are legion. A salient conclusion from the literature has been how little such changes may have to do with macroscopic traits: they may have arisen and been maintained by genetic drift with no functional consequences (Nocedal and Johnson 2015).

In another version of regulatory rewiring, two species subject to the same environmental stimulus trigger different expression outcomes. This may be mediated by new regulatory proteins altogether or, as above, by factors of ancestral origin that one species has adjusted to achieve a new logic. We list two observations of this type from the *Saccharomycotina* literature in Table 1B. In each, as species set off different expression programs in response to the environmental exposure, their ultimate cellular phenotypes also change—establishing a compelling potential link between divergent genetics, gene regulation, and organismal fitness. In landmark cases, similar stories have emerged from comparative transcriptomics in higher organisms (Dalal and Johnson 2017).

Conclusions and outlook

From morphology to metabolism, we have laid out a range of examples in which *Saccharomycotina* species evolved divergent phenotypes. Though our knowledge even of the best-characterized traits is not complete, we can infer with confidence that many represent true evolutionary novelties. Some represent phenotypic gains in just one lineage, and others have recurred in many independent groups across the subphylum or, more broadly, in the fungi. For a few such traits we know the underlying genes, driven in large part by powerful genome sequencing and analysis efforts. The latter have underscored the importance of gene presence/absence, copy number polymorphisms, horizontal gene transfer, and codon optimization as major modes of evolution. Many more of the cases we have covered here are ripe for future genetic dissection. Indeed, as the field proceeds, *Saccharomycotina* will keep serving as a flagship for comparative biology and genetics, thanks to their compelling ecology, small haploid genomes, and genetic tractability. Discoveries from *Saccharomycotina* will continue to shed new light on when and how nature has built new traits—and to help forge an understanding of evolutionary principles from the wild, with relevance across Eukarya.

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