Review

Saccharomyces sensu stricto as a model system for evolution and ecology

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Baker's yeast, *Saccharomyces cerevisiae*, is not only an extensively used model system in genetics and molecular biology, it is an upcoming model for research in ecology and evolution. The available body of knowledge and molecular techniques make yeast ideal for work in areas such as evolutionary and ecological genomics, population genetics, microbial biogeography, community ecology and speciation. As long as ecological information remains scarce for this species, the vast amount of data that is being generated using *S. cerevisiae* as a model system will remain difficult to interpret in an evolutionary context. Here we review the current knowledge of the evolution and ecology of *S. cerevisiae* and closely related species in the *Saccharomyces sensu stricto* group, and suggest future research directions.

The rise of yeast as a model system

Research involving microbial model systems is often criticized for its limited applicability to natural populations (reviewed in Ref. [1]). One answer is to use natural populations of model organisms to conduct experiments and to estimate parameters, but to date only a handful of such studies exist [2–4]. A promising organism for research in ecology and evolution is the yeast *Saccharomyces cerevisiae*, which has been used for decades in genetic and molecular research, resulting in an understanding that is probably unsurpassed in any other eukaryote and includes the first fully sequenced eukaryotic genome (*S. cerevisiae* strain S228C) [5]. The ease of assaying this yeast in the laboratory, as well as its short generation time, readily manipulated sexual system, close relationship to higher eukaryotes and extremely large ecological range, further adds to its attractiveness as a model system [6,7].

Until recently, it was argued that no natural strains of *S. cerevisiae* existed [8,9]. Any strain found at a natural source was thought to have escaped from domestic stocks despite evidence to the contrary [10]. The association of *S. cerevisiae* with humans might have altered its geographic distribution, as well as selected for novel genetic and phenotypic properties [11]. Although recent sequencing of *S. cerevisiae* strains isolated from oak trees has demonstrated that wild *S. cerevisiae* is significantly genetically differentiated from domesticated strains [12,13], the historical relationship of wild *S. cerevisiae* with human activity might never be clearly known. Its sister species, *Saccharomyces paradoxus*, is a better model for ecology and evolutionary biology, as it is nearly phenotypically indistinguishable from *S. cerevisiae* [14,15] and coexists globally with *S. cerevisiae* [16,17] but is not associated with humans.

If results from experiments using *S. cerevisiae* and its siblings as a model system are to be interpretable in the context of existing ecosystems, their basic ecology and biogeography must be well characterized. This knowledge is necessary to provide a reference point for future research where model systems are used to draw conclusions about natural populations. Here we review the current knowledge of the ecology and evolution of *S. cerevisiae* and its closest relatives which form the *Saccharomyces sensu stricto* species complex.

The *sensu stricto* species complex

The *Saccharomyces sensu stricto* group was first proposed on the basis of morphological and physiological properties [18]. Recent advances in molecular identification techniques have divided the *sensu stricto* complex into six species: *S. cerevisiae*, *S. paradoxus*, *Saccharomyces cariocanus*, *Saccharomyces bayanus*, *Saccharomyces mikatae* and *Saccharomyces kudriavzevii*, with *Saccharomyces pastorianus* as a sterile hybrid species (resulting from crosses between *S. bayanus* and *S. cerevisiae*) that is used for the production of lager beer [19] (Figure 1).

Recent estimates suggest that *S. cerevisiae* diverged from the common ancestor of *S. paradoxus* and *S. cariocanus* around 5–10 million years ago (Mya) [20], and *S. cariocanus* and *S. paradoxus* subsequently diverged [19,21]. Estimates of divergence of *S. cerevisiae* from *S. mikatae*, *S. kudriavzevii* and *S. bayanus* suggest that these siblings are much older (diverging from *S. cerevisiae* at 10–15, 15–20 and 20 Mya, respectively) [20]. These dates show that the *sensu stricto* complex is fairly young. There is evidence that new *sensu stricto* species continue to emerge: Eurasian and North American strains of *S. paradoxus* are both genetically divergent [22] and reproductively isolated [23], suggesting that populations on the two continents are in the process of allopatric speciation.

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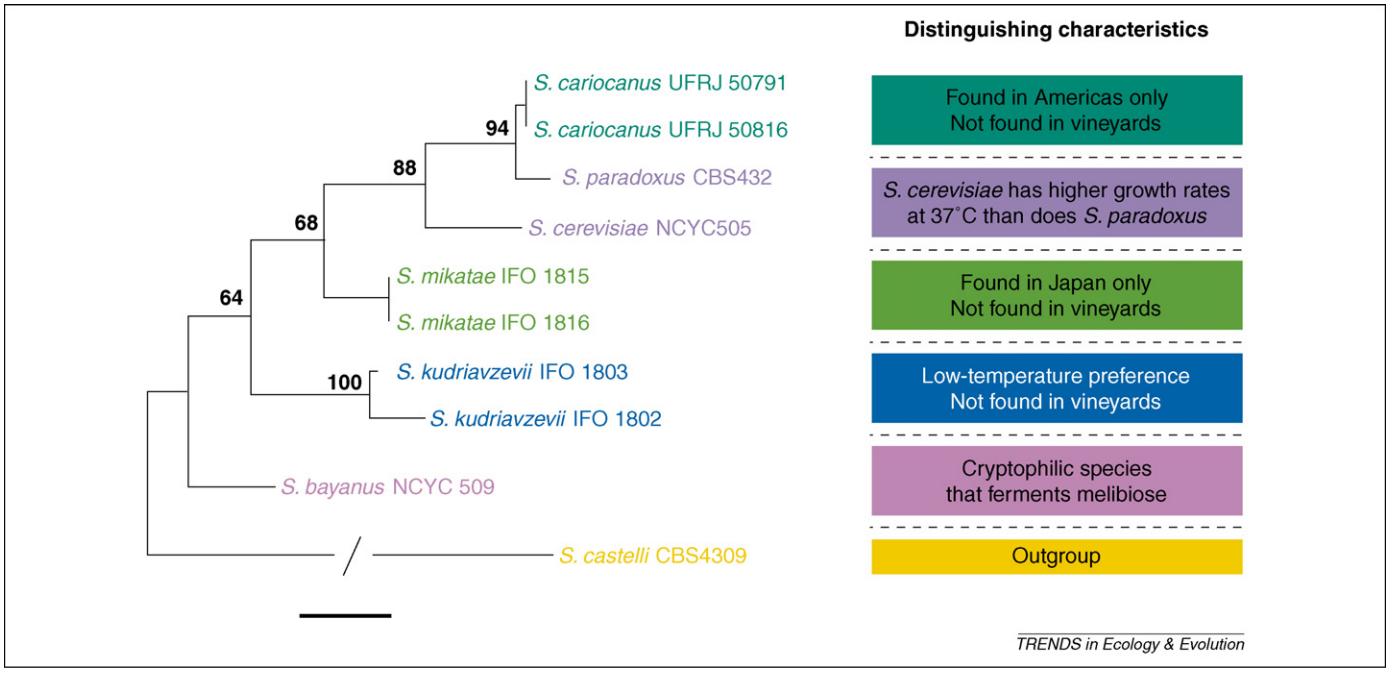

Figure 1. Radiation of *sensu stricto* complex. Phylogenetic tree of the *sensu stricto* species. Information to the right of the tree, under the 'Distinguishing characteristics' heading, summarizes the known phenotypic, ecological and geographical characteristics that differentiate the *sensu stricto* yeast species from each other. The tree is reproduced from Fischer *et al.* [92], with permission. The phylogenetic relationships are based on ITS1 sequence data, and the strains used in the phylogenetic analysis are identified by codes immediately to the right of the species names. The scale bar represents 1 base substitution per 100 nucleotide positions, and the bold numbers refer to bootstrap proportions.

Habitat

The collection of *sensu stricto* yeasts from natural environments began in late 1950s Japan. *S. cerevisiae* var. *tetrasporus* (now known as *S. paradoxus*) was first successfully isolated from the bark and surrounding soil of oak (*Quercus*) species, as well as from soil surrounding pine (*Pinus*) species [24]. Other studies in Japan found *S. paradoxus* growing on the bark of many different tree genera [25], and *S. cerevisiae* was often found on decayed leaves and dung [26], which suggests that *Saccharomyces* yeasts might be found in a wide range of forest microhabitats. There are occasional reports of the *sensu stricto* species being found in mushrooms and insects [27–29], but it is not known how important these habitats are. More recent studies have confirmed *Quercus* and other broadleaf trees as the principal habitat of *S. cerevisiae*, *S. paradoxus* and *S. cariocanus* ([16,17] and T.R. *et al.*, unpublished). Direct evidence that *Saccharomyces* actually grows on the substrates where it is found is lacking, however, although clonal structure (see Biogeography section below) suggests that it does.

Domestication

S. cerevisiae also lives in a quite different habitat, the vineyard. Domestication of vineyard and saké strains is thought to have originally occurred in Africa [12], and likely preceded the domestication of beer or bread strains, because the former processes do not require deliberate inoculation with yeast [30]. Domestic yeast is important for a variety of industries, and has led to the selection of strains for baking [31], brewing [32] and winemaking [33]. These specialized *S. cerevisiae* strains are not readily interchangeable.

Vineyard strains of *S. cerevisiae* are mostly found on damaged grapes, where intense competition with other microbes has led to the evolution of extremely efficient fermentation and the ability to grow at high levels of ethanol, low pH and severe osmotic stress, as well as resistance to preservatives and microbicides such as copper and sulfites (reviewed in Ref. [7]). Traits that affect survival in vineyard environments are often highly variable, suggesting that selection can vary from site to site. Two genes involved in sulfite uptake (*SSU1* and *FZF1*) were found to be highly polymorphic, which was attributed to vineyard-specific selection [13]. Such large changes in the ecological capacity of vineyard strains illustrates the importance of characterizing the habitats of isolates to understand these changes, and of drawing distinctions between assays using strains that were isolated from different environments.

Ecology of killer yeasts

Yeast species that are found in fruit and vineyard environments produce toxins (in the form of small extracellular proteins or glycoproteins) that are lethal to sensitive yeasts and bacteria (Box 1). Toxin-producing strains are known as killers and strains susceptible to the toxin are called sensitive, whereas resistant strains neither produce nor are affected by toxins. It has been suggested that the killer phenomenon provides a competitive advantage against bacteria and other yeasts by preventing competitors from gaining access to resources [34,35]. Killer strains have been shown to exclude sensitive strains both under laboratory conditions [36] and under industrial culture conditions [37].

Experimental studies have examined the ecological factors that determine the success of killers relative to otherwise isogenic sensitive strains. Killer yeasts have the greatest competitive advantage in high-density environ-

Box 1. Killer yeast and competitive interactions

The nature of competitive interactions is a fundamental cornerstone of ecology, and microbial model systems provide an excellent way to study such dynamics [1]. Certain species and strains of yeast have evolved a phenotype that presents an interesting opportunity in further studies of competition; classified as killer yeasts, they have evolved a toxin that is lethal to sensitive strains and to bacteria.

The killer phenomenon was first documented in *S. cerevisiae* [78], and has since been found in a limited number of other yeasts. Killer yeasts have been classified into groups depending on the spectrum of killing activity or crossreactivity of the killer toxin. *S. cerevisiae* is the sole member of the *sensu stricto* group in which killer activity has been documented, and its activity has been grouped into five classes: K1, K2, K3, KT28 and K3GR1 [79–82]. The killer toxin is produced by double-stranded virus-like particles in the cytoplasm (as in the case of *S. cerevisiae*), or plasmids. The peptides (toxins) created by virus-like particles kill sensitive cells by damaging their plasma membranes or by disrupting the membranes' permeability, whereas strains secreting plasmid-made proteins bring about G1 arrest in sensitive cells [83,79]. Killer strains are immune to their own toxins.

The killer phenomenon provides an excellent example of interference competition, where the killer strains kill nearby microbial cells to dominate a culture [34,35]. The fact that killer yeast can target many different types of yeast species, as well as bacteria, makes it an attractive system for studying the nature of competition at various levels of phenotypic and genetic similarity. However, more information is needed for this system to be extensively used, particularly as nothing is known of killer *S. cerevisiae* in forest environments. If killer yeasts can also be located in forests, their interactions could be contrasted with those of strains from vineyards.

ments and in spatially structured rather than well-mixed environments [38]. When grown in isolation, killer strains grow more slowly, presumably because of the metabolic cost of toxin production. Killer and sensitive strains are likely to coexist in natural environments such as grapes [38], sensitive strains having an advantage when grapes are initially inoculated at low cell densities, with killers spreading later [38] (the initial inoculated population of *S. cerevisiae* on vineyard grapes has been estimated as 10^2–10^3 cells per grape [39]). Thus, the killer phenomenon might contribute to the maintenance of genetic diversity in wild yeast. Although the complexity of the killer yeast system provides an excellent model for studies of competition in spatially structured communities, it is important to understand how wild yeast grows to fully characterize its competitive interactions.

Life cycle

The life cycle of the *Saccharomyces* yeasts is well documented in the laboratory (Box 2). It is usually assumed that wild yeast has a similar life cycle and, in particular, that the diploid phase predominates. This assumption has never been directly tested, because growth cannot be directly observed in nature. However, evidence suggests that all three modes of reproduction (outcrossing, clonal and inbreeding) occur in a natural population of *S. paradoxus* ranging over an area of 10 km^2 [11]. Repeated isolation of the same genotype gave evidence of clonal

Box 2. The *Saccharomyces* life cycle

Life cycles determine the reproductive capacity of organisms, and thus can reveal much about their growth and population structure in nature. The life cycle of the *Saccharomyces* yeasts is well documented in the laboratory [84] (Figure I). Yeast normally grows as a diploid that reproduces clonally, but will undergo meiosis in response to nitrogen starvation (a common cue for gamete production in many microorganisms), resulting in four haploid spores, two of each mating type (a and α) enclosed within an ascus. Mating type is determined by a single locus, *MAT*. The presence of a *MATa* allele at this locus gives a clone of mating-type a, and a *MAT*α allele gives a clone of mating-type α. Opposite mating types can mate within the ascus upon germination (intratetrad mating), but spores can also reproduce mitotically as haploids (clonal reproduction). Haploid spores can either outcross or undergo a mating-type switch by exchanging types at the *MAT* locus via a gene conversion event mediated by the *HO* gene. Such a mating-type switch allows spores to mate with their clonemates (haplo-selfing or autodiploidization).

Mating between products from a single meiosis is widespread among fungi, but is very rare in most multicellular organisms in which fertilization always occurs between products from different meioses, even in the case of self-fertilization (owing to their requirement for gamete differentiation into males and females) [85,86]. This unusual form of inbreeding has some interesting genetic consequences. For example, intratetrad mating reduces heterozygosity at a much slower rate than normal selfing (1/3 reduction as opposed to 1/2, for intratetrad versus full-sib mating). This is because for each allele within the ascus there is only one potential mate with the same allele and two of the opposite allele, whereas there are two of each type when mating is between products from different meioses.

It has been argued that persistent asci, retaining the four meiotic products in close proximity and enabling intratetrad mating, have evolved as a means to preserve heterozygosity in the face of selection for inbreeding [85,86]. Furthermore, heterozygosity will be most preserved near the mating-type locus, as this is the most highly outbred part of the genome (owing to the self-incompatibility of mating types). Mating with clonemates, or haplo-selfing, by contrast, brings heterozygosity down to zero within one generation, regardless of proximity to *MAT*. This in turn has the consequence of facilitating the purging of deleterious recessive alleles by making them homozygous and thus visible to purifying selection.

Whereas the molecular processes of the *Saccharomyces* life cycle in the laboratory are well characterized, little is known about their significance in natural populations. Recent research has determined which modes of reproduction occur in natural environments [11], which gives us a framework for the evolutionary capacity of these yeasts. However, more ecological data are needed to examine fundamental questions such as the roles and consequences of asexuality and sexuality in nature, and the frequency and purpose of recombination and dispersal in natural microbial populations.

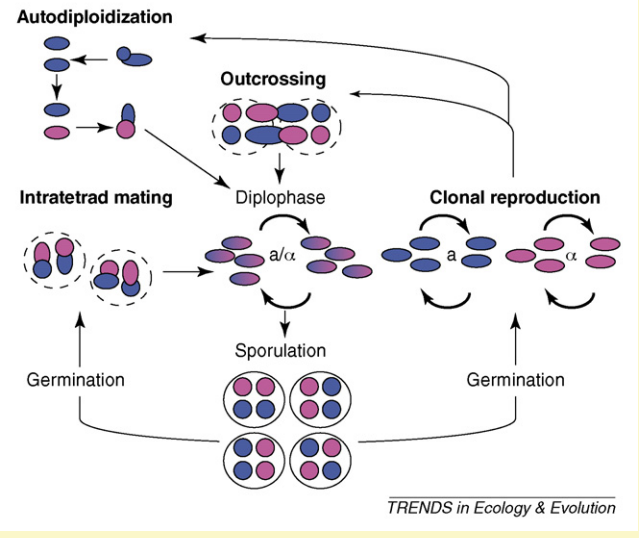

Figure I. *Saccharomyces* life cycle.

growth. High levels of homozygosity, indicative of high levels of inbreeding, were also found, with only a few rare instances of outcrossing. Only one auxotrophic mutation was documented, which is consistent with the belief that non-vineyard environments are nutritionally poor and thus could not support strains lacking the ability to produce their own nutrients, such as the majority of laboratory strains.

Recent population genomic data from a resequencing project in *S. paradoxus* have allowed more precise estimates of different aspects of the life cycle [40]. For instance, the nucleotide or mutational diversity as well as the recombinational diversity along the same chromosome were calculated for the European and Far Eastern populations. Either measure should give the same estimate of population size in an idealized obligately sexual and panmictic population where all evolution is neutral. A much larger estimate was derived from mutational diversity, however, implying an excess of ~1000-fold of mitotic over meiotic cell divisions in both populations [40]. This excess of mitotic divisions indicates that the population of *S. paradoxus* is primarily asexual, with mating and sporulation occurring only about every 1000 vegetative generations.

Similarly, vineyard populations of *S. cerevisiae* are predominantly diploid, carry no auxotrophic mutations [41] and are homozygous for the homothallism gene (*HO*/*HO*) [42] (Box 2), suggesting that yeast can switch mating types and autodiploidize. Moderate levels of heterozygosity (10%) indicate that outcrossing is much more common in vineyard populations of *S. cerevisiae* than in *S. paradoxus* [41]. Lastly, analysis of genomic data indicates a high rate of clonal reproduction relative to outcrossing, similar to *S. paradoxus* [43], which suggests that the different *sensu stricto* yeasts might all grow similarly in the wild and that this growth is predominantly clonal.

Dispersal

Dispersal could play a very important role in controlling the frequency of outbreeding, particularly over larger spatial scales. Unfortunately, yeast dispersal is poorly understood in both vineyard and non-vineyard environments. Early work found *Saccharomyces* (including *S. paradoxus*) to be the most abundant yeasts isolated from intestinal tracts of wild *Drosophila* species [44]. In an effort to trace yeast environments following potential vectors, subsequent studies surveyed *Drosophila* breeding sites [45,46]. These failed to detect *Saccharomyces* strains. Interestingly, yeasts isolated from the crop of young *Drosophila* flies differed repeatedly and markedly from yeasts found on suspected feeding sources of adult flies [45,46], suggesting that tracing yeast environments by following potential vectors is not straightforward.

It has been recently demonstrated that outcrossing rates increase 10-fold when *S. cerevisiae* spores pass through the intestinal tract of *D. melanogaster* [47]. In this study, marked homothallic strains were mixed and sporulated, kept in the presence or absence of flies and then cultured. Outbred heterozygotes were found to be more than ten times more frequent when flies were present. Flies were also fed tetrads, after which their feces were spread onto plates so that the yeast colonies within the feces could germinate. A large proportion of colonies sampled were haploids capable of mating with tester strains. This suggests that the ascus protects the spores during their passage through the insect's digestive tract, but is partly digested by enzymes during this passage, which then facilitates outcrossing by liberating spores from their tetrad partners [47]. As yeast populations have been found to be clonal over small spatial scales, but well mixed at a scale of kilometers [11,22], it is likely that insects facilitate long-distance dispersal. The increased rate of outcrossing mediated by insect dispersal might thus play an adaptive role for the transmitted yeast population, as higher levels of recombination in dispersed spores might increase their fitness in distant and novel habitats [47].

Biogeography and genetic structure

The study of microbial ecology has been influenced by the 'everything is everywhere' hypothesis, first advanced decades ago [48] and recently revived [49,50]. According to this hypothesis, large population sizes and high migration rates of microbes prevent biogeographical differentiation and speciation among them [49,51].

At the global scale, *S. cerevisiae* and *S. paradoxus* are ubiquitous, *S. mikatae* and *S. kudriavzevii* are endemic to Japan, *S. bayanus* has been isolated in Europe and the Far East and *S. cariocanus* has not been found outside of the Americas. A recent study, however, reports multiple isolations of *S. kudriavzevii* from oak bark in Portugal, in sympatry with *S. cerevisiae* and *S. paradoxus* [52], pointing to our limited knowledge of geographic ranges for these species. *S. kudriavzevii* was isolated at 10 °C, compared with the usual 30 °C for the other two species, revealing a low-temperature preference for *S. kudriavzevii*. This also shows that growth-temperature preferences might be important in determining sympatric associations of different species of yeast.

A study of a single population of *S. paradoxus* showed that the probability that isolates are clonal decreased as distance increased between them, both for isolates sampled from the same tree and for those from different trees. This implies that the local dispersal of clones is limited [22]. By contrast, the genomes of isolates from populations around Europe appear well mixed, suggesting at least a moderate gene flow over long distances. At a global scale, European isolates differ from Far East Asian and Canadian strains by 1.5% and 5% sequence divergence, respectively, with no shared polymorphisms, which suggests at least three independent lineages of *S. paradoxus* [22]. Recently, a few Eurasian strains were also sampled from North America, presumably following a recent migration to North America [23]. This maintenance of genetic isolation in sympatry reinforces the current model of *S. paradoxus* biogeography.

The population structure of *S. cerevisiae* has also been studied. Multilocus sequence typing of 27 strains from around the globe distinguished vineyard strains from forest strains, suggesting that ecological factors might play a larger role than geographic factors in shaping the genetic structure of this species [13], in agreement with an earlier

study that also included clinical strains [12]. *S. cerevisiae* strains are less divergent than strains of *S. paradoxus*, implying a more recent common ancestor or more thorough population mixing [17], perhaps as a consequence of the association of *S. cerevisiae* with humans. More studies of the population genetic structure of the *sensu stricto* yeasts should allow for interesting comparisons of the biogeography of closely related species. Demonstrating that similar species have very different global structure and occurrence would disprove any theories concerning the ubiquity of microbes.

Genome evolution

The use of *S. cerevisiae* as a simple genetic model system has created much interest in studying the evolution of the *Saccharomyces* genome. The ancestor of *S. cerevisiae* underwent a loss of transposons and a reduction in the number of introns. The appearance of centromeres in their current form might have facilitated segmental duplication, and led to the creation of the *HMR*/*HML* silent mating-type cassette pair (summarized in Ref. [53]). This was followed by the acquisition of *HO*, which encodes an endonuclease, from a mobile genetic element [54,55]. Such changes allowed *Saccharomyces* yeasts to switch from an ancestral obligate heterothallic system to a mating-type switching system, and greatly changed the sexual capacity of these yeasts. Comparison of the *S. cerevisiae* genome to that of the pre-duplication species *Kluyveromyces waltii* reveals ~500 paralogs among 5500 genes, which suggests that an ancestor bearing 5000 genes underwent duplication but subsequently lost 90% of the extra copies [56]. Divergent evolution between paralog pairs has given rise to several important changes in *Saccharomyces* yeasts (Box 3).

Studies of glycolytic genes, which remain duplicated in *S. cerevisiae*, suggest that the ancestor of *S. cerevisiae* could have benefited from an immediate selective advantage of increased copy number of these genes by linking the duplication event to the same time period as the emergence of a new food source, the radiation of flowering plants [57,58]. It might also be possible to use the yeast genome to elucidate environmental conditions and stresses that wild yeast is faced with by studying the correlation between gene expression patterns and codon usage bias in different environmental conditions [59]. Such an approach benefits from a fully sequenced genome, and might facilitate answering questions about yeast's ecology without the need for extensive ecological studies. A well-characterized environment would thus help characterize many of the remaining uncharacterized genes in the *Saccharomyces* genome. Such knowledge is desirable, as much of our knowledge of the eukaryotic cell comes from studying the yeast genome.

A large number of gene knockout experiments in different eukaryotes have shown little or no phenotypic effects in the laboratory [60–62]. It is believed that genes lacking a phenotype might be important in conditions that are not encountered in the laboratory, and thus a better knowledge of yeast ecology would allow characterization of genes of unknown function by testing them in environments that are closer to the yeast's natural habitat [63].

Box 3. Ecological and evolutionary consequences of genome duplication

The *Saccharomyces* genome has undergone several events that have shaped it into its current form. Most notably, the ancestor of the *sensu stricto* complex is thought to have undergone a whole-genome duplication, followed by a loss of 90% of the duplicated genes. The remaining 500 paralogous gene pairs fall into three major groups, one of which has striking asymmetries in evolutionary rates between copies. This group is composed of 115 gene pairs in which one paralog has evolved at least 50% faster than the other. The more slowly evolving copy might have a function more similar to that of the pre-duplication gene, which allows us to elucidate the gene's ancestral function, whereas the faster-evolving paralog attains a new function, and tends to be specialized in its localization, expression and function [20].

It has been suggested that the rise of faster-evolving paralogs in the *Saccharomyces* genome has provided many new opportunities for the evolution of the *Saccharomyces* yeasts. For example, the ability to grow anaerobically might be a consequence of genome duplication, because transcription of each gene of a paralogous pair is differentially controlled by oxygen availability [87]. The low- and high-affinity glucose systems in *S. cerevisiae* are also likely differentiated following the creation of redundant genes by duplication [88]. This new flexibility in glucose and oxygen use might have coincided with the radiation of fruit-bearing plants 100–200 Mya [89]. It has also been argued that the ability to grow anaerobically and to produce ethanol might provide a competitive advantage against bacteria and other microorganisms [90].

It is likely that gene duplication also fueled the development of a bipolar budding pattern in *Saccharomyces* yeasts; *Bud8* and *Bud9* are paralogous genes that have been shown to differentially mark the poles of yeast cells [91]. Polar differentiation also allows *Saccharomyces* yeasts to bud asymmetrically (producing small daughter cells from large mothers) from either pole, in contrast to pre-duplication species such as *K. waltii*, which bud symmetrically (mitosis is delayed until the daughter cell reaches the size of the mother) from the end opposite the previous mother–daughter junction.

Genetic changes throughout the evolution of *Saccharomyces* yeasts have greatly shaped the ecological and evolutionary capacities of these yeasts. Knowledge of genome evolution in a system as well-characterized as *Saccharomyces* should allow a better understanding of the types of processes that might play a role in the evolution of other organisms, particularly microbes, and how these changes could relate to an organism's ecology.

The relative roles of structural versus regulatory mutations in adaptive evolution are of great interest in evolutionary biology. Gene expression analyses that have been performed using natural strains of wine yeasts have found genome-wide variation in gene expression, including metabolic genes (reviewed in Ref. [7]). Such variation is likely to be incredibly important in affecting fitness, and suggests a large role for regulatory variation in adaptive evolution. The mechanisms of the evolution of gene expression are not yet fully understood, but recent work shows that genes comprising certain structural elements are highly sensitive to mutation, environmental perturbations and stochastic noise, and that these genes have a greater potential to undergo regulatory change [64]. Understanding how genetic diversity is created and maintained in a natural environment is an enormous challenge in evolutionary biology. Such questions can be addressed using the vast amount of diversity present in natural yeast populations, as well as the genetic knowledge and tools that are available for *S. cerevisiae* (reviewed in Ref. [7]).

Sexual isolation

As the *sensu stricto* complex is young, it holds much interest for studying processes of speciation; reproductive barriers between the *sensu stricto* species might be weaker than those in older species complexes, and might leave open the possibility of the formation of a new hybrid species. Species within the *sensu stricto* complex can mate with each other and form F1 hybrids, although hybridization tends to be avoided in laboratory experiments with *S. cerevisiae* and *S. paradoxus* in which individual spores are offered a choice of their own species or the other species as mates [65]. F1 hybrids are viable and can grow asexually, but they are sexually sterile because the gametes they produce are inviable [66,67], a condition that has been attributed to several mechanisms including the mismatch repair system and chromosomal rearrangements (reviewed in Ref. [7]).

In other taxa, especially *Drosophila*, hybrid sterility is believed to be caused by incompatibilities known as 'speciation genes' [68]. Previous work has described how geographically separated populations might fix beneficial alleles at different loci that could be incompatible if the populations were reunited and formed hybrids [69]. For example, male hybrids of *Drosophila mauritania* and *Drosophila simulans* are viable but sterile and probably have around 100 genetic incompatibilities [70]. However, converting sterile F1 *Saccharomyces* hybrids from diploids into tetraploids restores their sexual fertility, allowing them to produce viable diploid gametes and showing that dominant speciation genes are not responsible for yeast hybrid sterility [71]. Recessive incompatibilities could not affect (heterozygous) F1 diploid hybrids directly, but could potentially sterilize them by killing the (hemizygous) haploid gametes they produce. These incompatibilities were screened by testing for the ability of *S. paradoxus* chromosomes to substitute for their *S. cerevisiae* homologs in *S. cerevisiae* haploids [72]. None of the chromosomes tested were lethal to gametes, showing that they lacked recessive speciation genes capable of causing F1 hybrid sterility. It is remarkable that genomes that are at least 10% diverged [20] are still so compatible, something that would not have been predicted from *Drosophila* studies of speciation.

Experimental speciation

Selection can mitigate the inferiority of hybrids. If the surviving gametes produced by F1 hybrids are allowed to switch mating-type and self-fertilize, the homozygous F2 hybrids they produce are themselves very fertile, producing on average 84.4% viable gametes [73]. Furthermore, because different F2 hybrids had different combinations of chromosomes from the two parental species, they were sexually isolated both from the parent species and from each other, producing few viable gametes in these crosses and backcrosses. Thus, fertile and sexually isolated novel hybrid species can be produced readily in the laboratory. Inbreeding is very common in wild yeast populations [11], and hybrid winemaking and brewery strains have been identified [74,75]. Thus, it is possible that wild *S. cerevisiae* and *S. paradoxus* can naturally give rise to novel hybrid species.

Conversely, divergent selection in stressful environments can give rise to specialized strains that produce inferior hybrid offspring when crossed [76]. Selection lines were grown in high-salt and low-glucose media in laboratory microcosms for 500 generations. The offspring of crosses between lines from different environments had reduced fitness in either environment relative to the parent selected in that environment, as expected. They also tended to have lower sporulation efficiency, however, suggesting that they were undergoing the initial stages of speciation. In another experiment, mating discrimination between two selection lines was observed [77]. Analysis of the evolved populations suggested that different mutations had changed the speed of mating between cells. Such differences could create a reproductive barrier and eventually contribute to speciation. Hence, *Saccharomyces* provides a model for experimental speciation, either by hybridization or by allopatric ecological divergence, under laboratory conditions.

Concluding remarks and future perspectives

Unique among all other model systems, more than 74% of *S. cerevisiae* genes have been characterized [63], and this offers a great opportunity to study the genetic basis of adaptation. As a result of the very compact nature of the yeast genome, multiple genomes of closely related yeast have been sequenced (see http://www.yeastgenome.org), offering great opportunities for studies in comparative genomics. In addition, genome resequencing projects providing multiple genomes of the same species allow, for the first time, a close and detailed examination of the evolutionary process at the genomic level (see http://www.sanger.ac.uk/Teams/Team71/durbin/sgrp). Finally, many new large-throughput technologies use yeast as a testing ground (reviewed in Ref. [7]), providing genetic and molecular methods that are unavailable for other organisms.

Already the most-used system in genetics, yeast is now becoming a model system in ecology and evolution. Filling in the ecological knowledge for this system is much easier than bringing a different model organism's genetics up to the level of yeast, yet we still know very little about yeast's natural history. To interpret the vast amount of information that is being generated with this system, we need to better understand the context against which yeast has been evolving, and there is now renewed interest in the ecology and biogeography of yeast (e.g. [11,15,22] and T.R. *et al.*, unpublished). Further sampling would define the natural habitat and biogeography of these yeasts, which would in turn help clarify the types of selection and interactions that they face naturally. For instance, understanding the kinds of resources that yeast is commonly faced with could guide more accurate experiments into the function and expression of genes, as researchers would be better equipped to simulate the natural environment. Knowledge of the biotic interactions of yeasts would provide an excellent starting point for community ecology experiments using yeast.

The development of this model system is extremely powerful because we can use laboratory and genomic tools to approach largely field-based questions. We have the opportunity to create the first 'general' model organism

in biology: a complete, defined system, suitable for study from any biological perspective. The ability to integrate genetic, ecological and evolutionary research, as well as the importance of the *Saccharomyces sensu stricto* complex to so many fields, is perhaps what makes it the most exciting of all model systems to work with.

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