Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast

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Domestication of plants and animals promoted humanity's transition from nomadic to sedentary lifestyles, demographic expansion, and the emergence of civilizations. In contrast to the well-documented successes of crop and livestock breeding, processes of microbe domestication remain obscure, despite the importance of microbes to the production of food, beverages, and biofuels. Lager-beer, first brewed in the 15th century, employs an allotetraploid hybrid yeast, *Saccharomyces pastorianus* (syn. *Saccharomyces carlsbergensis*), a domesticated species created by the fusion of a *Saccharomyces cerevisiae* ale-yeast with an unknown cryotolerant *Saccharomyces* species. We report the isolation of that species and designate it *Saccharomyces eubayanus* sp. nov. because of its resemblance to *Saccharomyces bayanus* (a complex hybrid of *S. eubayanus*, *Saccharomyces uvarum*, and *S. cerevisiae* found only in the brewing environment). Individuals from populations of *S. eubayanus* and its sister species, *S. uvarum*, exist in apparent sympatry in *Nothofagus* (Southern beech) forests in Patagonia, but are isolated genetically through intrinsic postzygotic barriers, and ecologically through host-preference. The draft genome sequence of *S. eubayanus* is 99.5% identical to the non-*S. cerevisiae* portion of the *S. pastorianus* genome sequence and suggests specific changes in sugar and sulfite metabolism that were crucial for domestication in the lager-brewing environment. This study shows that combining microbial ecology with comparative genomics facilitates the discovery and preservation of wild genetic stocks of domesticated microbes to trace their history, identify genetic changes, and suggest paths to further industrial improvement.

beer yeast | next-generation sequencing | yeast ecology | yeast taxonomy

The beginning of agriculture and the domestication of plants and animals are among the most decisive events in human history because they triggered the rise of civilizations and the attendant demographic, technological, and cultural developments (1). The domestication of barley in the Fertile Crescent (2) led to the emergence of the forebear of modern beer in Sumeria 6,000 y ago (3). Beer and other alcoholic beverages may have played a pivotal role in cementing human societies through the social act and rituals of drinking (4) and by providing a source of nutrition, medicine, and uncontaminated water (5). Since the emergence of fermented beverages roughly matches the domestication of plants and animals, it is likely that some yeast lineages with favored traits were also unwittingly domesticated.

In Europe, brewing gradually evolved during the Middle Ages to produce ale-type beer, a process conducted by *Saccharomyces cerevisiae*, the same species involved in producing wine and leavened bread. Lager-brewing arose in 15th century Bavaria, gained broad acceptance by the late 19th century (6), and has since become the most popular technique for producing alcoholic beverages, with over 250 billion dollars of global sales in 2008 (7). Unlike most ales and wines, lagers require slow, low-temperature fermentations that are carried out by cryotolerant *Saccharomyces pastorianus* (syn. *Saccharomyces carlsbergensis*) strains (8); two other cryotolerant *Saccharomyces* spp. have been associated with beer as contaminants (*Saccharomyces bayanus*) and with cider or wine fermented at low temperatures (*Saccharomyces uvarum*) (9). *S. pastorianus* has never been isolated from the wild, depends on humans for its propagation, and appears to be an allotetraploid hybrid species of *S. cerevisiae* and an unidentified species (10, 11). Several hypotheses have been advanced for the source of the non-*S. cerevisiae* genome present in *S. pastorianus*, including the taxonomically and genetically complex species *S. bayanus* (12–14) and an unknown "lager" lineage distinct both from *S. bayanus* and *S. uvarum* (11, 15). Identifying the wild genetic stock of the cryotolerant subgenome of *S. pastorianus* is necessary for resolving the taxonomy and systematics of this important species complex, and for understanding the key events that led to the domestication of lager yeast.

In contrast to extensive investigation into domestication of crops and livestock (2, 16–19), studies of domestication of eukaryotic microbes have been limited (20–24), perhaps because of the inability to conduct direct field studies. Identifying the genetic basis of traits under selection during domestication may clarify the emergence of new traits and show the way toward further improvement. Because domesticated lineages derive from a subset of the original populations, a genetic bottleneck is likely to have caused the disappearance of some alleles (17), especially in microbes, which are often propagated clonally. In an age of accelerated habitat destruction and diminishing biodiversity, discovery of wild genetic stocks of domesticated microbes will facilitate preservation of their genetic resources for strain improvement.

Results and Discussion

Discovery of Wild Populations of Cryotolerant *Saccharomyces*. *Saccharomyces* spp. are associated with oak trees (Fagaceae) in the Northern Hemisphere (25, 26). Because species of the genus *Nothofagus* (Southern beeches, also members of the Fagales) occupy the oak niche in temperate regions of the Southern Hemisphere (27), our survey in Northwestern Patagonia for *Sac-*

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Data deposition: Genome data have been deposited in the National Center for Biotechnology Information's Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) (sequence nos. SRP006155 of SRA030851); individual genes have been deposited in GenBank (accession nos. JF786614–JF786710).

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EVOLUTION

charomyces focused on woodlands containing populations of *Nothofagus antarctica*, *Nothofagus dombeyi*, and *Nothofagus pumilio*, within and near Lanin and Nahuel Huapi National Parks (Argentina) (Fig. S1). We also surveyed stromata of *Cyttaria hariotii* (an obligate ascomycete parasite of *Nothofagus* spp.) because these fruiting structures are rich in simple sugars and provide a favorable yeast habitat (28). A total of 133 samples of *Nothofagus* bark, soil from underneath the trees, and *Cyttaria* stromata, collected from 2006 to 2008, yielded 123 isolates of cryotolerant *Saccharomyces* and two isolates of *S. cerevisiae* (Table S1).

For a preliminary identification of the cryotolerant *Saccharomyces* isolates, we determined the DNA sequence of individual genes, performed PCR-fingerprinting, and examined restriction fragment length polymorphisms (RFLPs), using *S. bayanus* CBS 380^T, *S. uvarum* CBS 395^T, and *S. uvarum* CBS 7001 (referred to as *S. bayanus* in genomics literature) as references (Fig. S2). The isolates discretely fall into two groups: group A appears related to *S. bayanus* (78 isolates); group B is closely related to *S. uvarum* (45 isolates). The almost complete occupancy of the *Nothofagus* niche by cryotolerant species contrasts with our ongoing survey of *Saccharomyces* biogeography in the Northern Hemisphere oak niche (North America, Mediterranean, Central Europe, and Japan), where we have isolated ~240 *Saccharomyces* strains from more than 500 oak samples and observed that sympatric species tend to have different growth temperature preferences. For example, *S. cerevisiae* (thermotolerant) and *Saccharomyces kudriavzevii* (cryotolerant) co-occur in Mediterranean regions, but *Saccharomyces paradoxus* (thermotolerant) and *S. uvarum* (cryotolerant) co-occur in temperate Europe and North America (26). Therefore, the detection of a pair of cryotolerant species in Patagonia and the near absence of thermotolerant species suggest the Patagonian ecosystem supporting *Saccharomyces* spp. may be unusual. One potential explanation is that the relatively low annual average temperatures in our Patagonian isolation sites [6/8 °C with mean low temperatures of −1/−2 °C and mean high temperatures of 22/23 °C (29)] may favor cryotolerant over thermotolerant species.

Ecological and Genetic Isolation of Two Cryotolerant Species. The unanticipated detection of two closely related sympatric cryotolerant populations prompted us to investigate the degree of genetic isolation between them by measuring the meiotic sterility of hybrids of the two populations. Spore viability within populations was 89% to 91%, but hybrids produced only 7.3% viable spores. Thus, the two populations exhibit considerable intrinsic postzygotic isolation and can be considered to be two different biological species, although they are phenotypically indistinguishable (Fig. S3). Moreover, population A was found in association with *N. antarctica* and *N. pumilio*, whereas population B was associated with *N. dombeyi* ($P < 10^{-7}$; Fisher's exact test) (Table 1). The evergreen *N. dombeyi* prevails at mesic midelevation sites, but *N. antarctica* and *N. pumilio* are deciduous and tend to replace *N. dombeyi* at high-elevation and xeric sites (27), suggesting that local niche-partitioning at least partially explains the apparent coexistence of these two species. Detailed field and laboratory studies into the causes of isolation are called for.

Table 1. Isolation of cryotolerant *Saccharomyces* in Patagonia

		Isolates	
	No. of samples	Population A	Population B
N. antarctica	47 (9C, 23B, 15S)	**29 (7C, 17B, 5S)**	4 (2C, 2B)
N. pumilio	32 (2C, 15B, 15S)	**25 (1C, 10B, 14S)**	5 (1C, 3B, 1S)
N. dombeyi	54 (12C, 27B, 15S)	8 (5C, 2B, 1S)	**38 (8C, 21B, 9S)**
Total	133 (23C, 65B, 45S)	62	47

The samples used in the isolations correspond to *Cyttaria hariotii* (C), *Nothofagus* bark (B), and soil collected underneath the trees (S). Numerals refer to number of samples or number of isolates. Statistically significantly associations are in boldface ($P < 10^{-7}$).

Genome Sequences Resolve *Saccharomyces* Taxonomy and Systematics. The identification and taxonomy of *S. bayanus, S. pastorianus*, and *S. uvarum* is problematic and controversial because *S. bayanus* and *S. pastorianus* have only been isolated from human-associated fermentations. Indeed, all known representatives of these two species have been suspected (*S. bayanus*) (13) or confirmed (*S. pastorianus*) (10) to be interspecies hybrids. One potential exception is the brewing contaminant NBRC 1948, which was asserted to be a pure strain of *S. bayanus* based primarily on RFLP evidence from all 16 chromosomes (15). A broad survey of several strains previously assigned to *S. bayanus*, *S. pastorianus*, and *S. uvarum* led to the conclusion that an additional "lager" lineage or species exists that contributed its genome to *S. pastorianus* (15). In contrast, the genome sequence of *S. uvarum* CBS 7001 lacks any signs of hybridization, introgression, or horizontal gene transfer events from other *Saccharomyces* spp. (30).

To resolve these taxonomic and systematic issues and conclusively identify our Patagonian strains, we generated draft genome sequences of a representative from each Patagonian species and several key brewing isolates by assembling millions of 36-bp sequences, using the *S. uvarum* genome sequence as a reference. Comparison of these genome sequences allowed us to conclusively test: (*i*) whether our wild Patagonian populations A and B are comprised of pure lineages; (*ii*) whether NBRC 1948 is a pure line or a hybrid; (*iii*) the composition of the type strains of *S. uvarum* (CBS 395^T) and *S. bayanus* (CBS 380^T); and (*iv*) the identity of the non-*S. cerevisiae* moiety of the *S. pastorianus* genome.

***S. eubayanus* sp. nov. Is the Missing Wild Genetic Stock of *S. pastorianus*.** Comparison of the draft genome sequences revealed that the two Patagonian species differ at ~6% to 8% of nucleotides across the genomes of surveyed strains [Fig. 1 (explained in detail in the legend), and Datasets S1 and S2] (average divergence 6.89%). The uniformity of sequence divergence suggests that there has been little, if any, recent introgression between the Patagonian species, consistent with the low spore viability of the hybrid cross. (Introgression of regions that are small, subtelomeric, difficult to assemble, or missing from some strains cannot be excluded.) The species B strain is indeed closely related to the reference strain of *S. uvarum* (average divergence 0.52%) and to its type strain CBS 395^T, but the species A strain is closely related to the non-*S. cerevisiae* moiety of the *S. pastorianus* lager yeast genome (average divergence 0.44%). In contrast to these essentially pure strains, the main component of the genome of the type strain of *S. bayanus* (CBS 380^T) is *S. uvarum* (67%), although there are substantial contributions from species A (33%), including several large heterozygous regions (19% of the genome). Similarly, large portions of the genome of NBRC 1948 are derived from *S. uvarum* (37%), but the majority of these portions are derived from species A (63%).

We also screened the short sequence reads for evidence of introgression, hybridization, or horizontal gene transfer using two different methods and the available *Saccharomyces* spp. reference genomes (see *SI Materials and Methods*). We found no evidence of foreign genes in either Patagonian strain or in the *S. uvarum* type strain. Although these analyses cannot exclude foreign contributions among genes missing from the *Saccharomyces* spp. ortholog set, they are sensitive enough to readily detect multiple subtelomeric contributions from *S. cerevisiae* in the two strains of *S. bayanus* (CBS 380^T and NBRC 1948) (Dataset S3).

Given the clear differences in ecological background and in genomic constitution between the hybrid species *S. bayanus* and the essentially pure species A, we propose regarding the wild Patagonian lineage as a distinct species: *S. eubayanus* sp. nov. Moreover, these genome sequence analyses firmly establish *S. eubayanus* as the donor of the non-*S. cerevisiae* subgenome of *S. pastorianus*, and exclude the contribution by an unknown "lager" species substantially divergent from *S. cerevisiae* and *S. eubaya-*

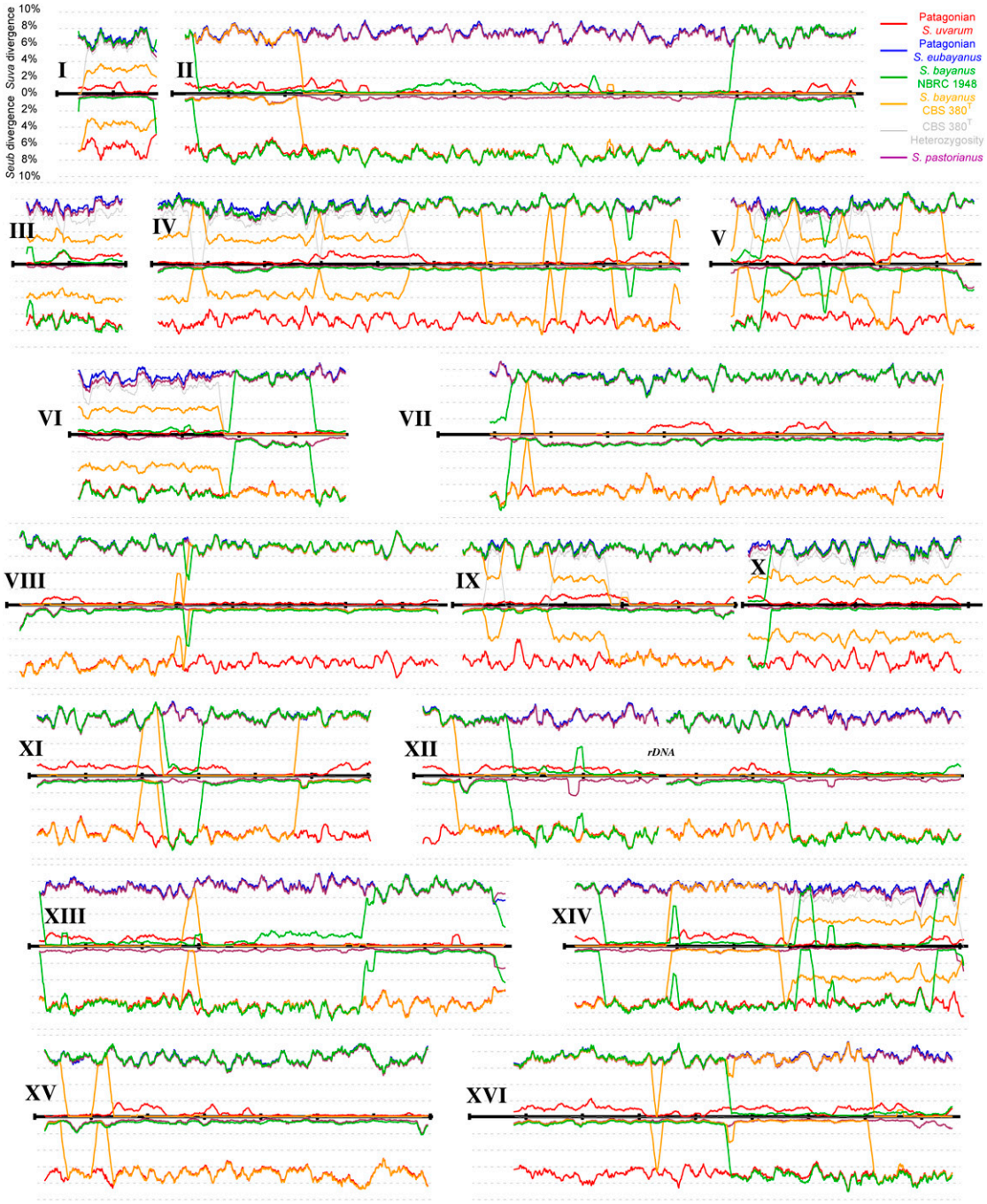


Fig. 1. *S. eubayanus* sp. nov. is distinct from *S. uvarum* and donated its genome to *S. pastorianus*. All 16 *S. uvarum* chromosomes are shown as *x* axes, with hash marks every 100 kbp; divergence (uncorrected) relative to *S. uvarum* CBS 7001 (30) is plotted above each chromosome; divergence relative to *S. eubayanus* is plotted (inverted) below each chromosome. Strains are color-coded according to the key. Each strain appears above and below the chromosomes to represent both comparisons. *S. uvarum* CBS 395^T is only shown in Dataset S1; the Patagonian *S. eubayanus* reference strain is only shown above the chromosomes. To avoid overlaps of the comparisons, some strains are offset from the *x* axis as follows: *S. eubayanus*, 0.3%; NBRC 1948, 0.2%; CBS 380^T, 0.1%. CBS 380^T contains both *S. uvarum* and *S. eubayanus* alleles in several regions, so its percentage heterozygosity (of base pairs) is also shown in thin gray above the chromosomes. The divergence plots for a strain generally move in parallel above and below the chromosomes because nearly all regions of each strain are closely related to either the *S. uvarum* or the *S. eubayanus* reference (within 2% of the axis on one side and 6–8% on the other side). Quantitative differences reflect regions with different evolutionary rates, heterozygous regions (CBS 380^T), or regions that may harbor older alleles because of incomplete lineage sorting. For example, on the left arm of chromosome VI, CBS 380^T is heterozygous, but NBRC 1948 contains only *S. uvarum* alleles; moving right, CBS 380^T becomes homozygous *S. uvarum*, and NBRC 1948 contains a large section from *S. eubayanus* before returning to *S. uvarum* characteristics toward the telomere. Across the filtered draft genome sequences (~80% of 1-kb windows), *S. pastorianus* is comprised of *S. eubayanus* alleles (>99.9%), and the Patagonian *S. uvarum* is closely related to the *S. uvarum* reference (>99.9%).

nus (Dataset S4). Instead, a broad survey of strains (Fig. S2) and the genome sequences of both of our representative strains of *S. bayanus* (Fig. 1 and Datasets S1 and S3) suggest that the diversity of this hybrid species can be explained by the contribution of mixtures of alleles from *S. uvarum* and *S. eubayanus*, along with some genes contributed by *S. cerevisiae*.

Evidence of Domestication. Domestication of crops and livestock selects for desirable characteristics through directional breeding so that domesticated lineages become genetically distinct from their wild ancestors in ways that make them more useful to humans (16, 17). To determine which genetic changes might have been favored in brewing, we searched for differences between the genome of *S. eubayanus* and three domesticated strains associated with brewing (*S. pastorianus* and the triple hybrids CBS 380^T and NBRC 1948).

The disaccharide maltose is one of the most abundant sugars in wort, so its utilization is a strongly selected trait in the brewing environment (11, 31). Like *S. pastorianus*, the triple hybrid strains associated with brewing contain subtelomeric maltose (*MAL*) gene clusters horizontally transferred from *S. cerevisiae*, as well as the *S. cerevisiae SUC4* gene for processing the disaccharide sucrose (Dataset S3). Surprisingly, we discovered that these three strains share an identical chromosome translocation breakpoint within *ZUO1* (*YGR285C*) that fuses the right arm of *S. eubayanus* chromosome VII to subtelomeric sequences on the right arm of *S. cerevisiae* chromosome VII (Fig. 2 *A* and *B*). The transferred fragment carries the *S. cerevisiae IMA1* (*YGR287C*) gene that encodes isomaltase, which catalyzes cleavage of the disaccharide isomaltose (32). The identical breakpoints indicate a common origin for this *S. cerevisiae* sugar-processing gene in all three hybrid strains and suggest strong selection for optimal sugar utilization during brewing.

Sulfite formation is also important in lager-brewing because sulfite is an antioxidant and flavor stabilizer (31). *S. pastorianus* Weihenstephan 34/70 was previously noted to carry inactive copies of both the *S.cerevisiae* and *S. eubayanus SUL1* genes, while retaining functional versions of both *SUL2* genes (11), which encode the high affinity transporters of sulfate, the metabolic precursor of sulfite (33). Surprisingly, we found that both triple hybrid strains contain the same frame-shift mutation as *S. pastorianus* in their copies of *S. eubayanus SUL1* (Fig. 2*C*). Interestingly, selective expression of *SUL2*, especially the *S. eubayanus* allele, has been shown to improve sulfite production (34). Because we found that the *SUL1* gene is intact in *S. eubayanus*, it is likely that the inactivation of *SUL1* is a consequence of artificial selection in the brewing environment. The losses of *SUL1* and some *MAL* transporters suggest a tendency to selectively discard less-efficient nutrient transport systems and retain or acquire others that are more efficient under brewing conditions, a trade-off possibly resulting from the 2D space constraints of the plasma membrane.

Evolution of *S. pastorianus* and *S. bayanus* Under Domestication. Based on our ecological and comparative genomic analyses, *S. bayanus* encompasses a set of hybrid strains known only from the industrial brewing environment, whereas *S. eubayanus* exists as an essentially pure lineage in natural conditions in Patagonia. According to our model (Fig. 3), in the lager-brewery environment of the 15th century (6) wild *S. eubayanus* genomes began fusing with ale-type *S. cerevisiae* genomes to give rise to allotetraploid hybrids, rare events that seem to have happened at least twice (10). In these ancestors of modern-day *S. pastorianus*, mitotic crossovers and other DNA repair mechanisms fused some *S. cerevisiae* chromosomes to *S. eubayanus* chromosomes, making

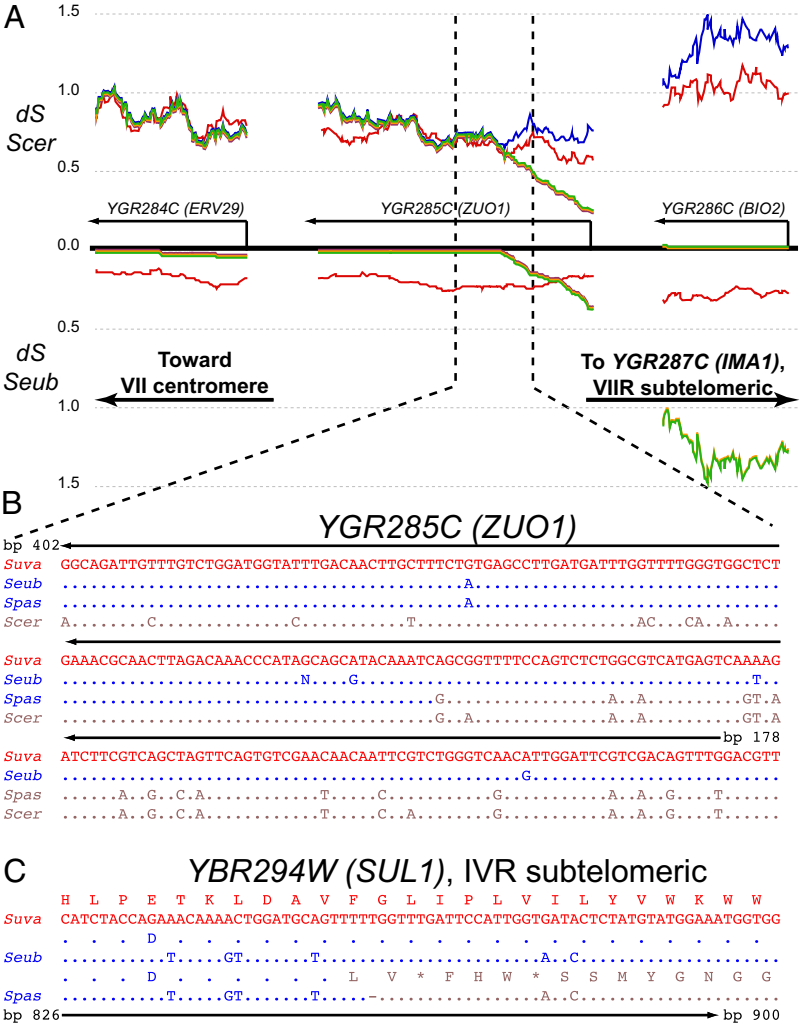


Fig. 2. *S. pastorianus* and two triple hybrid strains associated with brewing share identical domestication alleles. (*A*) Sliding window analysis of synonymous site divergence (*dS*, Jukes-Cantor corrected) for subtelomeric coding regions with arrows marking direction of transcription and with the color scheme and *y* axis as in Fig. 1, except the *dS* shown is to *S. cerevisiae* (*Scer*), rather than to *S. uvarum* (*Suva*); the *y*-value offsets necessary for visualization are 10-fold larger than in Fig. 1 because of the different scale; 2,000 sites are plotted with a 100-site window, a step of one, and an arbitrary intergenic spacer of 200 sites. (*B*) Close-up of partial coding sequences with dots denoting identity. Note the breakpoint fusing the 3′ portion of *S. eubayanus* (*Seub*) *ZUO1* (blue) to the 5′ portion of *ScerZUO1* (brown) and distal genes, including *ScerBIO2* and *ScerIMA1*. [*S. pastorianus* clearly possesses *ScerIMA1* and the *ZUO1* breakpoint, but there is a gap across *ScerBIO2* in the published assembly (11)]. (*C*) Partial coding sequence and translation of *SUL1* highlighting a 1-bp deletion in *SpasSUL1* that causes an inactivating frame-shift mutation. *S. pastorianus* (*Spas*) also represents NBRC 1948 and CBS 380^T in *B* and *C* because all called base pairs are identical in the coding sequences of *ERV29*, *ZUO1*, *BIO2*, and *SUL1* in those strains.

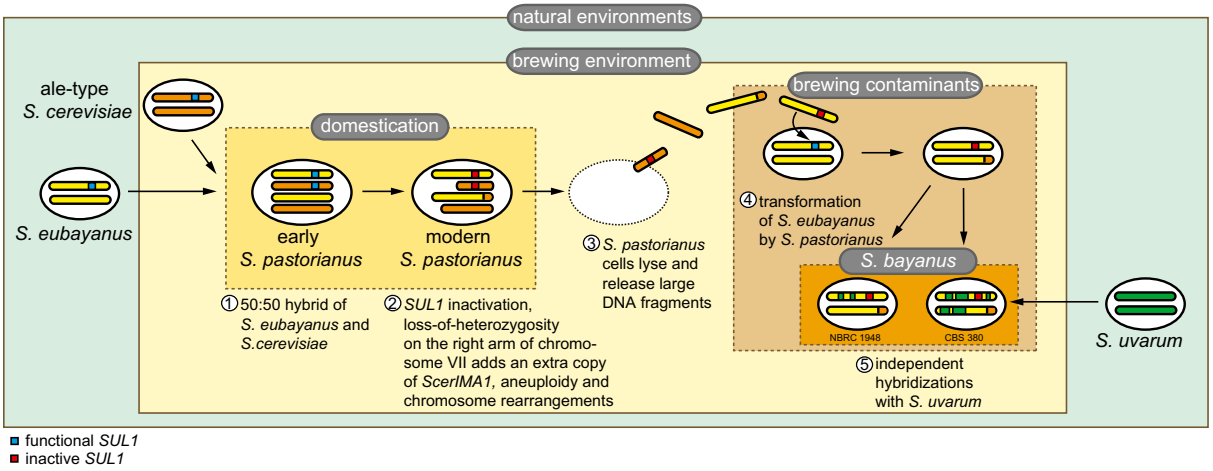


Fig. 3. A model of the formation of *S. pastorianus* and the hybrid strains of *S. bayanus*. First, wild *S. eubayanus* and ale-type *S. cerevisiae* hybridized to form an allotetraploid that gave rise to *S. pastorianus*. Second, domestication imposed strong selective pressure for strains with the most desirable brewing properties. Third, in the brewing vats with high densities of *S. pastorianus*, cell lysis releases large DNA fragments that occasionally transform, fourth, contaminating wild strains of *S. eubayanus* because of the lack of pure culture techniques. Fifth, multiple hybridization events with wild strains of *S. uvarum* gave rise to CBS 380^T and NBRC 1948. This model does not exclude prior or parallel involvement of *S. uvarum* in brewing or contamination.

some portions of the genome homozygous and other regions aneuploid (10, 11). The *S. eubayanus* portions of these chromosomal fusions that occurred in *S. pastorianus* would have provided ample nearly identical sequence to seed the recombination needed to introduce *S. cerevisiae* DNA into other *S. eubayanus* and *S. uvarum* strains arriving in the brewing environment, thus gradually giving rise to the complex *S. bayanus* genome. Alternatively, the tremendous population sizes achieved in the brewing environment may have allowed for the recovery of extremely rare viable hybrid spores or for strains to return to euploidy via a parasexual cycle. Because all *S. cerevisiae* genes detected in the triple hybrids are subtelomeric in the *S. uvarum* or *S. cerevisiae* reference genomes, we believe transformation is the more likely mechanism because it requires only one crossover proximal to the subtelomeric gene under selection, and it explains the absence of widespread transfer of *S. cerevisiae* proximal genes that would be expected under the other models. Regardless of the mechanism of gene transfer, the identical frame-shift mutations (*SUL1*), chromosome breakpoints (*IMA1*), and identical hitchhiking sequences suggest that strong positive selection was imposed by the brewers choosing the best strains or by the competitive brewing environment itself to spread these alleles to *S. pastorianus* and both *S. bayanus* triple hybrids.

It is surprising that European isolates of *S. eubayanus* have never been found, despite records of yeast isolation since the late 19th century, including a recent emphasis on sampling more natural arboreal environments (26). The facile recovery of this species from Patagonia suggests that *S. eubayanus* may have been absent in Europe until it was imported from overseas after the advent of trans-Atlantic trade. In sharp contrast, its sister species, *S. uvarum*, has been repeatedly isolated in Europe from both artificial (e.g., brewing) and natural substrates (e.g., insects, bark). Although additional environmental sampling is called for worldwide, it seems likely that any natural European niches suitable for *S. eubayanus* are occupied by other species, but that it found great success through hybridization in the new artificial environment of lager breweries.

The ~7% genome-wide sequence divergence between *S. eubayanus* and *S. uvarum* is the lowest level observed within the *Saccharomyces* genus to result in genetic and ecological isolation (Table S2). The geographic distribution and apparent niche differentiation of these sister species make them a genetically tractable system to study fungal speciation in allopatry with secondary contact, or in sympatry because of ecological factors.

Taxonomy of *S. eubayanus*, *S. bayanus*, and *S. uvarum*. The nomenclature of *S. bayanus* and *S. uvarum* has been confusing and controversial for decades. High DNA-DNA reassociation values between the type strains of these two species (35) led Naumov to defend their merger, with *S. uvarum* initially considered a synonym of *S. bayanus* (36), and later a variety (12). Other studies employing molecular methods documented two well-separated groups (37, 38), leading to proposals for the reinstatement of *S. uvarum* as a separate species (13, 14). Unfortunately, the absence of a clear resolution of these taxonomic issues caused CBS 7001, a strain selected for genome-sequencing (30), to be referred to as *S. bayanus* (instead of *S. uvarum*) in most of the ensuing genomics literature and databases. However, all known strains of *S. bayanus* (including the type strain CBS 380^T) appear to be hybrids of *S. eubayanus* and *S. uvarum* that contain contributions from *S. cerevisiae* in at least some cases (Fig. S2). Therefore, like *S. pastorianus*, *S. bayanus* is not a "species" in the ecological and evolutionary sense and is best viewed as a product of the artificial brewing environment with no occurrence in nature. Because it is now evident that the varietal and other designations adopted by some researchers lack scientific support, we propose that "*S. uvarum*" and "*S. eubayanus*" be used as descriptors of biologically meaningful species, whereas "*S. bayanus*" and "*S. pastorianus*" should be restricted to the domesticated and hybrid lineages.

Standard description. Standard description of *Saccharomyces eubayanus* Sampaio, Libkind, Hittinger, P. Gonçalves, Valério, C. Gonçalves, Dover et Johnston sp. nov.

Etymol.: The epithet is chosen to refer to the pure and natural lineage that is related to the hybrid species *Saccharomyces bayanus* Saccardo.

Cells globose to ovoidal (2.5–5 × 5–7 μm). Pseudomycelium absent. Asci oval and persistent containing two to four round ascospores. The main diagnosis characteristic is the genome sequence deposited in NCBI database. Salient physiological properties are listed in Dataset S5. Strain CBS 12357^T (CRUB 1568^T, PYCC 6148^T) is designated as the type strain.

Latin description. Latin description of *Saccharomyces eubayanus* Sampaio, Libkind, Hittinger, P. Gonçalves, Valério, C. Gonçalves, Dover et Johnston sp. nov.

Species generis Saccharomycetis. Cellulae globosae ad ovoideae. Pseudomycelium nullum. Asci ovales, persistentes, 2–4 ascosporis globosis. Sequentia genomae totae in collectione sequentiarum acidi nucleici NCBI numero SRP006155 in SRA030851 deposita. Cultura typica CBS 12357^T.

Domestication Processes Across Taxonomic Kingdoms. Domestication is an inherently evolutionary process, the understanding of which requires the inference of ancestral states, either by comparison with wild progenitor populations or by archaeological investigations. The characteristics of the previously undescribed species *S. eubayanus* explain the instantaneous formation of *S. pastorianus* by hybridization with *S. cerevisiae*. Selection imposed by the brewing environment further refined lager yeast, and we pinpointed several genetic changes attendant to lager production. Given the diverse mechanistic natures of genetic changes that occurred during crop and livestock domestication (2, 17, 18), we anticipate that additional genetic changes that are not obvious from sequence comparisons alone will be uncovered in the future, such as changes in gene regulation and mechanisms to integrate two genomes separated by millions of years of evolution. Identification of these evolutionary changes and access to the previously unknown wild stock promise to illuminate the role that fermented beverages have played in human civilization and provide new strategies for improving yeasts for brewing and biofuel production.

Materials and Methods

Yeast Isolation, Preliminary Characterization, and Crosses. The selective protocol used for *Saccharomyces* isolations was previously described (26). Preliminary identification was based on confirmation of *Saccharomyces*-type ascospore production; DNA-sequencing of *FSY1*, *FUN14*, *HIS3*, *MET2*, *RIP1* (Dataset S6), and the ITS region of *rDNA*; PCR-fingerprinting with primers $(GTG)_5$ and M13 (39); and PCR-RFLP using *FUN14* digested with BanII, *RIP1* digested with PstI, and *HIS3* digested with RsaI (15) (Fig. S2). Isolated ascospores of strains *S. eubayanus* CRUB 1568 and *S. uvarum* CRUB 1595 were crossed to obtain interspecies hybrids. Hybridization was confirmed with PCR-RFLP as above. Interspecific spore viability was determined by examining 362 ascospores produced by three independent hybrid strains. For the assessment of intraspecific spore viability, ~100 ascospores of each of the two species (strains CRUB 1568, CRUB 1595) were studied.

Genome Sequencing and Analyses. The draft genome sequences of monosporic-derivatives of CRUB 1568 (FM1318), CRUB 1595 (FM1317), and NBRC 1948 (FM1309) were determined by assembling single-pass 36-bp Illumina sequence reads to the *S. uvarum* CBS 7001 reference genome sequence (30), as described previously (40) with modifications (*SI Materials and Methods*). Viable spores of CBS 380^T and CBS 395^T were not recovered, so the genomes of these strains were sequenced without further manipulation and shown to be aneuploid, providing a likely explanation for their sterility. Genome sequence analyses were performed using custom PERL scripts and standard software.

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1. Bar-Yosef O (1998) The Natufian culture of the Levant, threshold to the origins of agriculture. *Evol Anthropol* 6:159–177.
2. Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the near east. *Nat Rev Genet* 3:429–441.
3. Hornesey I (2003) *A History of Beer and Brewing* (RSC paperbacks, Cambridge, UK).
4. Joffe AH, et al. (1998) Alcohol and social complexity in ancient western Asia. *Curr Anthropol* 39:297–322.
5. McGovern PE (2009) *Uncorking the Past: The Quest for Wine, Beer, and Other Alcoholic Beverages* (University of California Press, Berkeley, CA).
6. Corran HS (1975) *A History of Brewing* (David and Charles, London, UK).
7. Beer: Global industry guide. http://www.researchandmarkets.com/reports/53577 (accessed 15 January 2011).
8. Bond U (2009) The genomes of lager yeasts. *Advances in Applied Microbiology*, eds Laskin AI, Sariaslani S, Gadd GM (Academic Press, Burlington), Vol 69, pp 159–182.
9. Rainieri S, Zambonelli C, Kaneko Y (2003) *Saccharomyces* sensu stricto: Systematics, genetic diversity and evolution. *J Biosci Bioeng* 96:1–9.
10. Dunn B, Sherlock G (2008) Reconstruction of the genome origins and evolution of the hybrid lager yeast *Saccharomyces pastorianus*. *Genome Res* 18:1610–1623.
11. Nakao Y, et al. (2009) Genome sequence of the lager brewing yeast, an interspecies hybrid. *DNA Res* 16:115–129.
12. Naumov G (2000) *Saccharomyces bayanus* var. *uvarum* comb. nov., a new variety established by genetic analysis. (Translated from Russian) *Mikrobiologiia* 69:410–414.
13. Nguyen H-V, Gaillardin C (2005) Evolutionary relationships between the former species *Saccharomyces uvarum* and the hybrids *Sacharomyces bayanus* and *Saccharomyces pastorianus*; reinstatement of *Sacccharomyces uvarum* (Beijerinck) as a distinct species. *FEM Yeast Res* 5:471–483.
14. Pulvirenti A, et al. (2000) *Saccharomyces uvarum*, a proper species within *Saccharomyces sensu stricto*. *FEMS Microbiol Lett* 192:191–196.
15. Rainieri S, et al. (2006) Pure and mixed genetic lines of *Saccharomyces bayanus* and *Saccharomyces pastorianus* and their contribution to the lager brewing strain genome. *Appl Environ Microbiol* 72:3968–3974.
16. Diamond J (2002) Evolution, consequences and future of plant and animal domestication. *Nature* 418:700–707.
17. Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. *Cell* 127:1309–1321.
18. Larson G, et al. (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proc Natl Acad Sci USA* 107:7686–7691.
19. Zeder MA (2008) Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proc Natl Acad Sci USA* 105:11597–11604.
20. Fay JC, Benavides JA (2005) Evidence for domesticated and wild populations of *Saccharomyces cerevisiae*. *PLoS Genet* 1:66–71.
21. Legras J-L, Merdinoglu D, Cornuet J-M, Karst F (2007) Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Mol Ecol* 16:2091–2102.
22. Liti G, et al. (2009) Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
23. Rokas A (2009) The effect of domestication on the fungal proteome. *Trends Genet* 25: 60–63.
24. Schacherer J, Shapiro JA, Ruderfer DM, Kruglyak L (2009) Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature* 458:342–345.
25. Sniegowski PD, Dombrowski PG, Fingerman E (2002) *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. *FEM Yeast Res* 1:299–306.
26. Sampaio JP, Gonçalves P (2008) Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. *Appl Environ Microbiol* 74:2144–2152.
27. Veblen TT, Donoso C, Kitzberger T, Rebertus AJ (1996) Ecology of southern Chilean and Argentinean Nothofagus forests. *The Ecology and Biogeography of Nothofagus forests*, eds Veblen TT, Hill RS, Read J (Yale University Press, New Haven, CT), pp 293–351.
28. Libkind D, Ruffini A, van Broock M, Alves L, Sampaio JP (2007) Biogeography, host specificity, and molecular phylogeny of the basidiomycetous yeast *Phaffia rhodozyma* and its sexual form, *Xanthophyllomyces dendrorhous*. *Appl Environ Microbiol* 73: 1120–1125.
29. Paruelo JM, Beltrán A, Jobbágy E, Sala OE, Golluscio RA (1998) The climate of Patagonia: General patterns and controls on biotic processes. *Ecologia Austral* 8:85–101.
30. Cliften PF, Fulton RS, Wilson RK, Johnston M (2006) After the duplication: Gene loss and adaptation in *Saccharomyces* genomes. *Genetics* 172:863–872.
31. Donalies UE, Nguyen HTT, Stahl U, Nevoigt E (2008) Improvement of *Saccharomyces* yeast strains used in brewing, wine making and baking. *Adv Biochem Eng Biotechnol* 111:67–98.
32. Brown CA, Murray AW, Verstrepen KJ (2010) Rapid expansion and functional divergence of subtelomeric gene families in yeasts. *Curr Biol* 20:895–903.
33. Cherest H, et al. (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* 145:627–635.
34. Nakao Y, Kodama Y, Shimonaga T (2006) Sulfate ion transporter gene and use thereof. US Patent Appl No. 11/922,219.
35. Rosini G, Federici F, Vaughan AE, Martini A (1982) Systematics of the species of the yeast genus *Saccharomyces* associated with the fermentation industry. *Appl Microbiol Biotechnol* 15:188–193.
36. Naumov GI (1996) Genetic identification of biological species in the *Saccharomyces* sensu stricto complex. *J Ind Microbiol* 17:295–302.
37. Nguyen HV, Gaillardin C (1997) Two subgroups within the *Saccharomyces bayanus* species evidenced by PCR amplificatiom and restriction polymorphism of the non-transcribed spacer 2 in the ribosomal DNA unit. *Syst Appl Microbiol* 20:286–294.
38. Rainieri S, Zambonelli C, Hallsworth JE, Pulvirenti A, Giudici P (1999) *Saccharomyces uvarum*, a distinct group within *Saccharomyces* sensu stricto. *FEMS Microbiol Lett* 177: 177–185.
39. Sampaio JP, et al. (2001) Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *Rhodosporidium kratochvilovae* and related anamorphic species. *Int J Syst Evol Microbiol* 51:687–697.
40. Hittinger CT, et al. (2010) Remarkably ancient balanced polymorphisms in a multi-locus gene network. *Nature* 464:54–58.