Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts

Brigida Gallone[1,2,3,4,5], Stijn Mertens[1,2,5], Jonathan L Gordon[1,2,5], Steven Maere[3,4], Kevin J Verstrepen[1,2,5] and Jan Steensels[1,2,5]

Yeasts have been used for food and beverage fermentations for thousands of years. Today, numerous different strains are available for each specific fermentation process. However, the nature and extent of the phenotypic and genetic diversity and specific adaptations to industrial niches have only begun to be elucidated recently. In *Saccharomyces*, domestication is most pronounced in beer strains, likely because they continuously live in their industrial niche, allowing only limited genetic admixture with wild stocks and minimal contact with natural environments. As a result, beer yeast genomes show complex patterns of domestication and divergence, making both ale (*S. cerevisiae*) and lager (*S. pastorianus*) producing strains ideal models to study domestication and, more generally, genetic mechanisms underlying swift adaptation to new niches.

Addresses
[1] Laboratory for Genetics and Genomics, Centre of Microbial and Plant Genetics (CMPG), KU Leuven, Kasteelpark Arenberg 22, B-3001 Leuven, Belgium
[2] Laboratory for Systems Biology, VIB Center for Microbiology, KU Leuven, Bio-Incubator, Gaston Geenslaan 1, 3001 Leuven, Belgium
[3] Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, 9052 Ghent, Belgium
[4] VIB Center for Plant Systems Biology, Technologiepark 927, 9052 Ghent, Belgium
[5] Leuven Institute for Beer Research, KU Leuven, Bio-Incubator, Gaston Geenslaan 1, B-3001 Leuven, Belgium

Corresponding authors: Verstrepen, Kevin J (kevin.verstrepen@kuleuven.vib.be), Steensels, Jan (jan.steensels@kuleuven.vib.be)

Current Opinion in Biotechnology 2017, **49**:148–155

This review comes from a themed issue on **Food biotechnology**

Edited by **Maria Marco** and **Eddy Smid**

http://dx.doi.org/10.1016/j.copbio.2017.08.005

Introduction

'Domestication' is a term that refers to artificial selection and breeding of wild species to obtain cultivated variants with enhanced desirable features that thrive in man-made environments, often at the cost of suboptimal fitness in natural settings. Several genotypic and phenotypic signatures of domestication have been described in crops, livestock and pets. These include genome decay, polyploidy, chromosomal rearrangements, gene amplifications and deletions, horizontal gene transfer and loss of genetic diversity due to bottlenecking [1,2]. Interestingly, similar phenomena are also observed in various microbial species, both prokaryotic and eukaryotic, that are linked to human food production.

Perhaps the most well studied model is the common brewer's and baker's yeast, *Saccharomyces cerevisiae*, which is the main driver in many industrial fermentations. However, studies focusing on the evolution of industrial *Saccharomyces* strains often use the terms 'adaptive evolution' or 'domestication' too freely. For example, both terms are commonly used to explain phenotypic divergence from wild ancestors, overlooking alternative explanations such as random genetic drift [3]. Only recently, more elaborate studies have reported clear genome-wide signatures of domestication as well as convergent evolution of industrially relevant traits in separate lineages. These observations provide conclusive evidence that industrial yeast diversity is not solely shaped by genetic drift caused by bottlenecking and small isolated populations, but also as a result of selection and niche adaptation. In wine yeasts for example, adaptive horizontal gene transfer events [4,5,6•] and copy number variations [7,8,9,10] have been described that increase sugar and nitrogen metabolic activity, conferring competitive advantages during grape must fermentation and providing better tolerance to chemicals used in vineyards (e.g. copper sulphate) and in wine [11] (e.g. sulphite) (For a review see [7]). Interestingly however, the strongest genetic and phenotypic signatures of domestication are found in yeasts used for beer production. Several distinctive features make traditional beer production an ideal setting for microbial domestication. Firstly, beer yeasts are harvested and re-used after the fermentation process to initiate the next fermentation batch, a process called 'backslopping'. This continuous growth in a very specific industrial niche has resulted in continuous selection imposed by the brewing environment. Secondly, beer is produced year-round, causing a near-complete isolation from wild isolates. In contrast, wine is seasonal and wine yeasts spend most of the year in and around the vineyards or in the guts of insects, where nutrient limitation can trigger sexual cycles and hybridization with wild yeasts [12]. Therefore, present-day beer yeasts can be considered the result of a centuries-long evolution experiment in a highly selective niche. In this review, we will

highlight new insights into beer yeast evolution and domestication. We will discuss *S. cerevisiae* and *S. pastorianus*, both involved in production of specific beer types, which underwent a different route to domestication.

Domestication of *Saccharomyces cerevisiae* ale beer yeasts

Saccharomyces cerevisiae is the main microbial workhorse for the production of ale beers, which includes beer styles such as stouts, pale ales, doubles, triples and quadruples. As with all domesticated organisms, in *S. cerevisiae* the phenotypes of domesticated strains are a combination of enhanced selectable traits inherently present in *S. cerevisiae* (e.g. adaptation to sugar-rich, oxygen-limited environments and high tolerance to ethanol), and traits acquired during interaction with humans (e.g. efficient maltotriose utilization). In this review, we will expound on the latter aspect, and we refer to other review papers for the former [13,14].

Phylogenetics and population structure

Many studies of *S. cerevisiae* population structure focused on wine, wild and/or clinical strains, neglecting the broad diversity of beer yeasts. However, two recent studies, sequencing more than 100 ale beer strains, have provided the first comprehensive insight into their evolution and diversification [15••,16••]. Both studies found that the majority of beer yeasts are genetically distinct from known wild stocks, and cluster into two independent lineages (Figure 1). It has been estimated that the last common ancestor (LCA) of each lineage occurred around 1600–1700AD, well after the first reported beer production (3000–4000 BC), but before the discovery of microbes in the 19th century. Interestingly, the estimated period of occurrence of these LCAs coincides with the gradual switch from domestic brewing in private households, to more professional large-scale brewing, first in pubs and monasteries, and later in breweries. This suggests that true domestication of yeast occurred far more recently than the first leveraging of yeast for the production of fermented food and beverages, which likely happened several thousand years ago [17].

Genome structure

Variation in genome structure, including changes in ploidy and large segmental duplications or copy number variations (CNVs), have repeatedly been found in association with adaptation to specific niches in experimentally evolved microbes [18••,19]. Perhaps not surprisingly, similar chromosomal changes are also a recurrent theme in domestication of higher organisms, especially in crop species, for which polyploidization promoted the proper genetic circumstances to domestication [20]. Similarly, *S. cerevisiae* beer yeasts show large-scale genome structure variations. While most wild *S. cerevisiae* strains are clean diploids with very few large segmental duplications, the ploidy of the vast majority of beer strains exceeds 2n, with most close to 4n (Figure 2a). However, most ale yeasts also show aneuploidies, and are almost never perfectly diploid or tetraploid (Figure 2b). Previous studies have shown that aneuploidies and polyploidies can provide an adaptive advantage under selection [18••], but that they are often transient and are maintained until a more cost-effective adaptive strategy has evolved [21]. It has also been shown that small structural genome variations (e.g. duplication, deletion, recombination, gene conversion and rearrangement) are frequently located in telomeric and subtelomeric regions, which are known hotspots for evolution. These regions are functionally enriched for genes involved in nitrogen and carbon metabolism, ion transport and flocculation, and likely play a role in niche adaptation [15••,22,23•].

Brewing phenotypes

The most obvious sign of adaptation to a specific industrial niche is arguably the accentuation of traits desirable for humans that are a burden for the organism in a natural setting. Closer examination of the genetic underpinnings of specific traits have provided strong evidence that human selection indeed underlies certain industrially relevant traits in beer yeasts.

A prime example of a domestication trait in beer yeast is their ability to ferment maltotriose, an important carbon source in beer wort, but not generally found in high concentrations in natural yeast environments. Efficient metabolism of maltotriose imposes a selective advantage in brewing environments where it is present at high concentrations because it opens the door to a previously under- or poorly utilized energy source. This trait has evolved independently and through different genetic pathways in the two main beer lineages, suggesting strong selection pressure [15••] (Figure 1a). In one of the beer groups a homolog of the maltose transporter (called *AGT1*) with an increased affinity for maltotriose is present [24]. Interestingly, in the second beer group, the *AGT1* allele is non-functional, but the majority of isolates are able to efficiently ferment maltotriose, suggesting the presence of a distinct, yet unknown mechanism for the maltotriose uptake in this lineage. Another well-documented domestication trait is the selection against production of 4-vinyl guaiacol (4VG), an unpleasant aroma-active compound that is derived from ferulic acid, a cell wall component of barley. Yeast requires two genes for decarboxylation of ferulic acid to 4VG: *PAD1* and *FDC1*. Various independent nonsense mutations in these genes have been found in many industrial (and especially beer) yeasts, but not in biofuel or non-industrial isolates, suggesting that the selection of 4VG-free fermentations has favoured the spread of domesticated beer yeasts unable to produce this specific off-flavour (Figure 1b) [15••,16••].