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The genomics of microbial domestication in the fermented food environment

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Shortly after the agricultural revolution, the domestication of bacteria, yeasts, and molds, played an essential role in enhancing the stability, quality, flavor, and texture of food products. These domestication events were probably the result of human food production practices that entailed the continual recycling of isolated microbial communities in the presence of abundant agricultural food sources. We suggest that within these novel agrarian food niches the metabolic requirements of those microbes became regular and predictable resulting in rapid genomic specialization through such mechanisms as pseudogenization, genome decay, interspecific hybridization, gene duplication, and horizontal gene transfer. The ultimate result was domesticated strains of microorganisms with enhanced fermentative capacities.

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Introduction

Domestication refers to the genetic modification of a species by breeding it in isolation from its ancestral population in an effort to enhance its utility to humans [1]. The domestication of plants and animals lay at the core of the Neolithic Agricultural Revolution, a transition period that witnessed a rise of settled societies, an acceleration of technological innovations, and the establishment of organized systems of governance [1]. During this time, early farmers began selectively breeding plants and animals to become increasingly reliable and nutritive sources of sustenance. For instance, many crops were bred to increase the size and number of seeds, the loss of seed shattering, and a minimization of seed dormancy [2,3]. Similarly, favor was given to livestock which displayed increased passivity or docility, reductions in teeth size and number, alterations to body morphology, and reductions in brain size [4,5]. The genomic basis underlying many of these developmentally related phenotypes has been intensely studied due to their anthropological significance, applied agricultural importance, and suitability as a model system for evolutionary, genetic, and medical studies [2,6–9].

Less appreciated is the fact that a wealth of archeological, molecular, and genetic evidence supports the parallel domestication of microbes along with that of plants and animals. Traditional artisanal food production practices such as back-slopping (the serial reinoculation of new foods with material from previous products) resulted in the continuous and long-term passage of isolated populations of microbes under specialized environmental conditions, leading to adaptation and genetic differentiation. The domestication of bacteria, yeasts, and molds was probably the unwitting result of Neolithic humans harnessing the metabolic capabilities of microbes in an effort to control the digestibility, palatability, and longevity of their newly abundant foods [10]. For example, cheese and yogurt appear very early on [11,12] and rely on the cooption of select microbes to break down lactose into lactic acid thereby making milk both more digestible and resistant to spoilage. Fruits and grain were similarly transformed and preserved by microbes, with evidence for wine, beer and bread dating back to at least 9000 years ago [13••,14]. The impact of this relationship is still observable in the form of traditional fermented food products such as wine, beer, cheese, bread, kefir, yogurt, shoyu, miso, and tempeh.

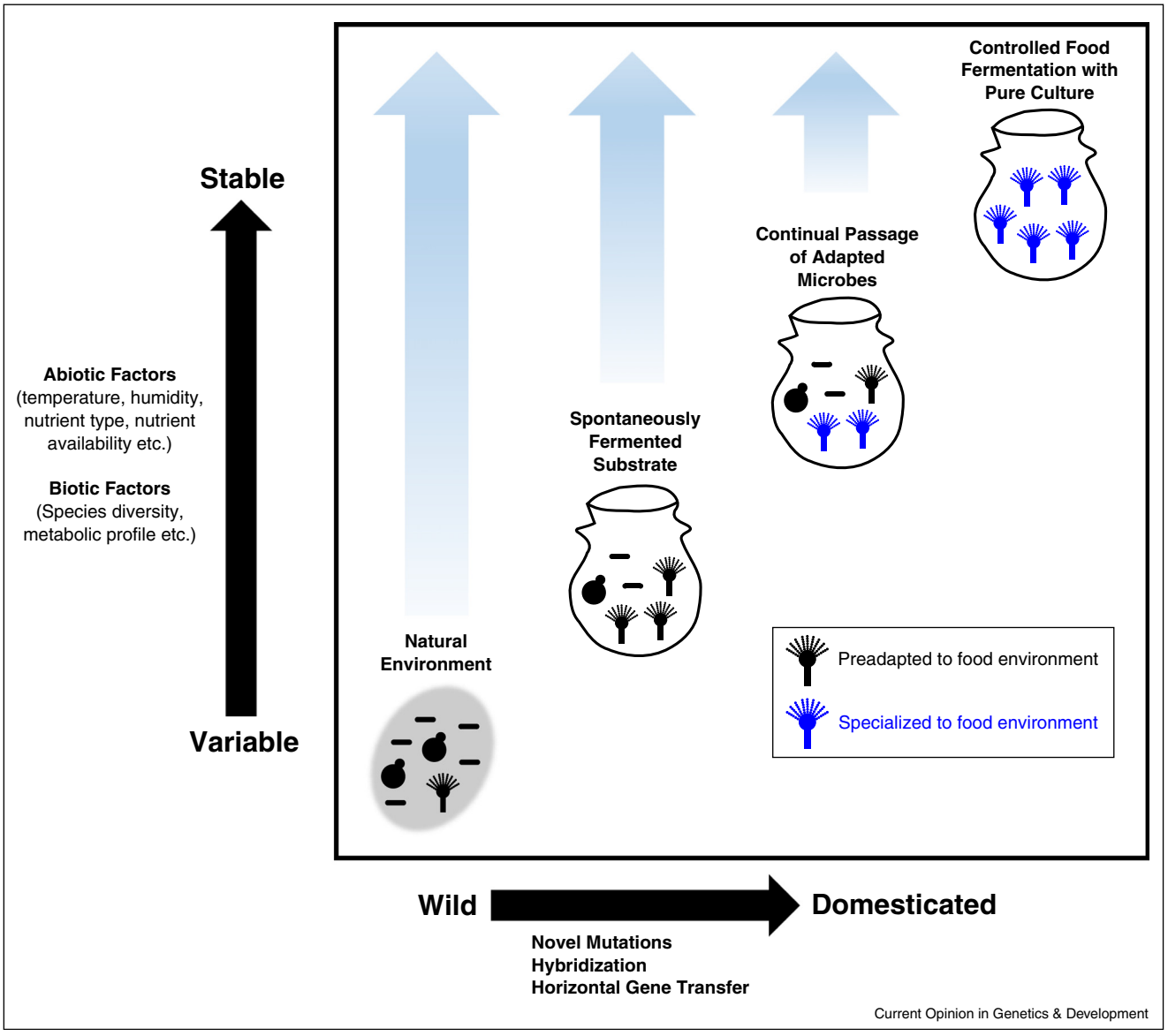
Until relatively recently, substantially less focus has centered on the genetics and genomics of microbial domestication in comparison with plant and animal domestication models, despite the crucial role microbes assume in food preservation, nutritional quality, consistency and flavor [15,16]. In this review, we focus on recent progress made toward firstly, elucidating the origins of domesticated microbes in the fermented food environment; secondly, understanding the impact of domestication on genome architecture and function; and thirdly, discovering of the complex microbial community dynamics responsible for a variety of fermented food products.

The origin of domesticated microbes

Much like domesticated plants and animals, it has been proposed that wild bacteria, yeast, and mold were 'tamed'

into the industrial organisms we use today [15,16]. The shift from variable and complex natural environments to more stable and relatively simple agrarian substrates favored specialized adaptations in these microbial isolates (Figure 1). Properly identifying the progenitors of domesticated species is vital to comparative genomics studies and to searching for target genes affected by artificial selection. In plant and animal examples the progenitor species are typically easily identifiable because of highly similar morphologies (with some exceptions such as maize/teosinte [17]) and overlapping geographical histories [18]. In contrast, identifying the progenitors of domesticated microbes it is often more challenging because phenotypic relationships between microbial species are

Figure 1



Model of the progression toward microbial domestication. In this model, a species of filamentous fungi has been domesticated (likewise, bacteria or yeast could have alternatively been depicted). The *Y* axis represents abiotic and biotic variability while the *X* axis reflects the progression from wild to domesticate. The natural environment is typically more variable than the food environment (depicted by stylized fermentation vessel) and the range of relative variability is depicted by light blue arrows in each stage. In the 'spontaneously fermented substrate' stage (e.g. kimchi and cocoa), microbes preadapted to the food substrate and fermentation environment dominate the microbial community. Long-term continual passage of preadapted microbes (i.e. either through intentional back-slopping or through persistence in the food processing environment) in the more controlled food environment promotes adaptation and specialization (blue filamentous fungi in this case) which can be achieved through various mechanisms including novel mutations, hybridization, and horizontal gene transfer. Finally, in particular cases, such as industrial food production, abiotic and biotic factors are carefully regulated, and pure cultures of the domesticated microbe are used to ensure the quality, homogeneity, and safety of food product.

not always apparent, and many microbial species are globally distributed [19]. Nevertheless, several studies have used genomic data to identify the source of domesticated microbial species.

Lactic acid bacteria (LAB) were probably the first microbes used in food fermentation by humans and are known to enhance food flavor and texture, while also functioning to prevent spoilage by producing antimicrobial peptides and by lowering the pH of the food environment [20]. Characterized by their ability to convert hexose sugars to lactic acid [21], LAB remain a diverse and prominent starter culture for many types of fermented foods and, reflecting their industrial importance, many LAB genomes have been sequenced [22–24]. Plants represent the original habitat of some LAB species used in dairy fermentation, yet isolates of plant and dairy derived *Lactococcus lactis* display divergent metabolic and growth characteristics on plant derived carbohydrates [25]. This probably reflects the vastly different classes of carbohydrates found in plant and dairy sources and suggests that some dairy derived LAB lost ancestral metabolic pathways during specialization. Additionally, dairy strains show signatures of genome reduction and pseudogenization in loci involved in the metabolism of plant carbohydrates [26••].

Yeasts are perhaps the most well-known domesticated microbes and are at the workhorse of fermentation during the production of beer, wine, and bread [27,28••]. Species of *Saccharomyces* (mainly *S. cerevisiae*) are particularly well suited for food fermentation because they do not secrete toxic secondary metabolites, but they do produce high levels of alcohol as well as desirable flavor molecules including esters and phenols [16]. Lager-type beer originated in Bavaria around the 15th century and relies on a specialized, cryotolerant strain of *Saccharomyces* (*S. pastorianus*) that is especially amenable to cooler production temperatures of that region. Although it has been surmised that *S. pastorianus* was an interspecies hybrid of *S. cerevisiae* and a cold tolerant species, identifying the unknown species remained a mystery for several decades [29,30]. In 2011, Libkind and colleagues isolated two crytolerant yeast species from Patagonian forests, sequenced their genomes, and in doing so, identified the *S. pastorianus* missing hybridization donor species, which they named *Saccharomyces eubayanus* [31••]. Subsequent work has identified *S. eubayanus* from sources in North America [32] as well as in Asia [33•] and strongly suggests an East Asian, rather than Patagonian, origin [33•,34•].

In addition to bacteria and yeast, molds have also served as an essential catalyst in fermented products [15]. Species of *Rhizopus* are used in the production of different alcoholic drinks as well as tempeh, *Monascus purpureus* is used to make red yeast rice, *Penicillium* species are used during cheese making, and *Aspergillus* species are utilized during the production of traditional alcoholic drinks, sauces, and condiments. *Aspergillus oryzae* is used in the saccharification of rice during sake production where the newly created sugars are then fermented to alcohol by *S. cerevisiae* [35]. It is well established that *A. oryzae* was domesticated from the aflatoxin producing agricultural pest, *Aspergillus flavus* [36], but specific details concerning the evolution of *A. oryzae* remained vague. To elucidate the origins of *A. oryzae*, Gibbons *et al.* [37••] sequenced the whole genomes of a diverse collection of 14 *A. flavus* and *A. oryzae* isolates. Population, phylogenetic, and functional analysis confirmed that *A. oryzae* was probably the product of a single domestication event from an atoxigenic lineage of *A. flavus* [36,38,39], which may have been selected by sake makers because of its cooperation with yeast [37••].

Genome optimization of domesticated microbes

In their natural environment, microbes contend with labile abiotic conditions and intense competition for nutrients that are often heterogeneously distributed and intermittently available (Figure 1). In contrast, the human designated food milieu represents a stable, abundant, simplified, and less competitive niche in which artificial selection could rapidly drive microbial genome optimization. In an elegant experiment highlighting the speed at which selection may work in microbial systems, Bachmann *et al.* continually propagated a plant derived isolate of *L. lactis* on milk [26••]. After only 1000 generations the authors observed increased growth rates, increased acidification, and transcriptional profiles similar to dairy derived isolates of *L. lactis*. Below, we discuss several different mechanisms of genome optimization to the food environment.

The transition from generalist to specialist can lead to pseudogenization driven by relaxed selection for genes no longer useful or, in some cases, positive selection against genes now detrimental in the new environment [40]. For example, *A. oryzae* and *Aspergillus sojae* have accumulated various inactivating mutations in the cyclopiazonic acid and aflatoxin gene clusters [37••,41–46], and the *shochu* brewing species *Aspergillus kawachii*, has lost a 21 kb region of the polyketide synthase gene that drives the production of ochratoxin A [47]. Extensive loss of genes associated with primary and secondary metabolism was also observed in the *Monascus pururеus* genome in comparison with other Eurotiales species [48]. In the food environment, metabolic defense mechanisms may be energetically inefficient or detrimental to interdependent microbial relationships. In *S. pastorianus*, both copies of the *SUL1* sulfate transporters have become inactivated in favor of retaining the function of the two *SUL2* genes which are more efficient under fermentation conditions [31••]. LAB species have experienced comparable fates [24], exemplified in the *Streptococcus thermophilus* genome

where more than 10% of genes, many associated with pathogenicity and carbon metabolism, have been pseudgenized or lost [49,50].

Copy number variation (CNV) is a rapid source of genotypic and phenotypic variation, is an effective strategy to alter levels of transcription and translation [49], and allows for quick adaptation to new environments in domesticated microbes. For example, *A. oryzae* is valued for its ability to digest rice starches and while growing on rice, the alpha-amylase gene appears as the most highly expressed gene and protein of the *A. oryzae* genome, but not in *A. flavus* [37••]. Examination of the species' respective genomes reveals the alpha-amylase gene is present in two or more copies in *A. oryzae* but found as only a single copy in *A. flavus* genomes [51]. In *S. cerevisiae* a number of studies observed an increased number of hexose transporter genes in strains from low glucose environments which resulted in heightened expression and increased glucose transport into the cell [52–54]. Additionally, diverse industrial strains exhibited adaptive CNV in genes functioning to assimilate different amino acids as their primary source of nitrogen [55].

Adaptive genetic variation can also be acquired via horizontal gene transfer (HGT), defined as 'the non-genealogical transmission of genetic material from one organism to another' [56]. HGT is common in prokaryotes and a collection of studies over the past several years suggest it is also widely prevalent in microbial eukaryotes [57,58] and can facilitate rapid adaptation to novel food niches [59]. As HGT in industrial prokaryotes has been extensively reviewed elsewhere [59–61], here we focus on several examples of HGT events in domesticated microbial eukaryotes.

S. cerevisiae has been the recipient of several laterally transferred loci from bacteria [62–64], as well as other yeasts. Bolstered by phylogenetic and syntenic support, analysis of the wine making isolate *S. cerevisiae* EC1118 genome revealed several loci that appear to have been laterally transferred from the wine spoilage yeast species *Zygosaccharomyces bailii* [65•]. Remarkably, many of these genes are relevant to the wine making process and are found almost entirely in isolates used to make wine. *A. niger*, used for its polysaccharide metabolizing capacities, recently acquired a 72 kb locus from *A. oryzae* that contained the gene encoding for the starch degrading enzyme alpha-amylase [66]. The cheese environment was the stage for a massive lateral transfer of a genomic region containing nearly 250 genes between *Penicillium camemberi* and *Penicillium roqueforti* [67••]. The HGT region contained genes involved in conidiation and secondary metabolite production, and may have conferred a competitive advantage in the multi-microbe cheese environment. Importantly, the occurrence of HGT in domesticated microbes underscores the complex community dynamics and close organismal associations that take place in the production of most fermented foods [68,69]. As more genomes become publicly available, it is probably that additional cases of HGT will be discovered between domesticated microbes and their ecological neighbors.

Microbial community dynamics of fermented food

The interspecific interactions central to both HGT and hybridization events were the result of taxonomically heterogeneous fermentation environments. Such mixed environments were effectively unavoidable before the advent of the pure culture and sanitary techniques of the mid-nineteenth century, and all instances of historic, microbial domestication occurred within the context of broader microbial communities.

Traditional fermentations, which depend upon autochthonous (spontaneous) elements, are the simplest example of microbial communities being employed in human food production. Kimchi and cocoa are examples of food products derived entirely from spontaneous fermentations. Kimchi is a Korean food product resulting from the fermentation of raw vegetables in closed, un-sanitized vessels and relies solely upon the heterogeneous and variable collection of microbes present in the starting materials. While kimchi fermentations inevitably end up being predominated by just a few strains of LAB, the dominate genera can vary between given fermentations (reviewed in [70]). Moreover, in *dongchimi* (watery kimchi) metagenomic studies have shown the dominating microbes to vary dynamically with changing abiotic fermentation conditions (temperature, substrate, pH, free sugar, etc.) [71,72•,73]. Like kimchi, the fermentation of cocoa beans is also purely spontaneous but more dynamic and complex as it involves native LAB, dozens of yeast species, and acetic acid bacteria. However, cocoa fermentations appear to differ from those of kimchi in that individual fermentations show consistently similar bacterial profiles and progressions in the predominating species of wild yeast (reviewed in [15,16]). Therefore while the dominating microbial profiles within separate kimchi fermentations may be the result of both variation within the starting materials and their associated microbiomes, both kimchi and coca fermentations seem to derive a certain degree of batch-to-batch consistency through the human management of the food production milieu even in the absence of any inoculum (Figure 1).

Similar microbial progressions are present in the spontaneously fermented style of Belgian beer called lambic which is fermented by native microbes from both the local air and harbored in wooden fermentation and aging vessels. Successive molecular characterization of the evolving microbiome over the course of lambic fermentations have identified qualitatively distinct, semi-overlapping phases dominated at first by a variety of enterobacteria and

non-*Saccharomyces* yeasts followed by increasing levels of *Saccharomyces* including *S. cerevisiae* and *S. bayanus* [74,75•], then LAB, and finally by *Brettanomyces bruxellensis* [75•,76•]. Similar microbial communities and progressions have been documented in other types of spontaneously fermented beer [28••,77] and in traditionally produced kimoto-style sake [78]. Again, while no overt inoculation of microbes occurs during the production of these beverages, there are nonetheless microbes resident to the brew house environment which take part in the fermentation process and show signatures of adaptation [28••,77,79•].

In contrast to such liquid and semi-solid growth conditions, fermented foods can also harbor microbial communities in the form of biofilms. Despite, their diverse structure, formation, and community composition, biofilms embody the predominate form of microbial life and confer significant fitness advantages in competitive environments. In pure-culture domestication environments (including food and laboratory) the loss of biofilm formation has been observed, reflecting its dispensability in low stress [80,81]. Cheese rinds, kombucha, and vinegar all rely on the properties of biofilms or pellicles to produce and preserve their associated food products [68]. The communities present on the exposed cheese rind are distinct from those found in the core of the cheese and are dominated by molds, yeasts, and aerobic bacteria but their interactions are equally complex and affected by milk processing and geographic location [82]. In contrast, the composition of the microbial communities of the rind appears 'strikingly similar' between geographic location and harbor co-evolving communities of microbes [83••]. This then suggests that the domestication signatures seen in some cheese microbes [67••,84] probably arose under similar communal conditions.

Overall, the implication appears to be that the diversity of native, regional microbial communities can become wholly subsumed by the powerfully selective environment created by the food milieu. Once constrained by these human contrived niche environments, preadapted genera rapidly predominate and lay the essential ground work upon which the domestication process can further build (Figure 1).

Future directions

Identifying the progenitor species or lineages of domesticated microbes remains a challenging and largely unexplored area of research that is fundamental to the reconstruction of genotypic and phenotypic evolution. To this end, extensive sampling combined with population genomic and phylogenomic approaches can be used to resolve the origin and number of domestication events [33•,37••,85,86]. Moreover, metagenomic sequencing of ecological niches that share firstly, abiotic characteristics similar to the fermented food environment in question (i.e. those potentially promoting preadaptations such as temperature tolerance, flavor molecule production, carbon metabolism, and spoilage control [16]), and secondly, geographical proximity to the putative origins of the specific fermented food (e.g. cocoa producing regions), offers a useful approach for better understanding how preadapted species initially entered the food milieu. Advances in ancient DNA metagenomics also afford promising potential for identifying progenitors from preserved food [87]. Understanding the history of microbial domestication not only provides many instructive ethnomicrobiological and evolutionary insights, but also yields the promise of novel industrial applications. For example, the identification of the lager yeast progenitor species, *S. eubayanus*, has enabled the generation of new *S. pastorianus* strains [88,89••].

Cheaper and more efficient DNA sequencing and genotyping technologies have substantially improved our ability to finely catalog genetic variation at single nucleotide resolution and to identify the genomic underpinnings of phenotypes. These approaches have recently been applied to yeast to map quantitative trait loci (QTL) responsible for industrially important traits such as sulfite resistance [90], aromatic compound production [91], flocculation [92], and thermotolerance [93]. Future work on other domesticated microbes would first have to focus on establishing conditions to induce sexual reproduction in cryptically sexual food-related eukaryotic microbes [94], as has been initiated in *A. oryzae* [95,96] and *P. roqueforti* [97,98]. This would enable QTL analysis, and is promising for breeding strains with combinations of desirable traits.

For primarily (or solely) asexually reproducing species, experimental evolution followed by whole-genome sequencing (i.e. an evolve and resequence approach) would be a powerful strategy to select for desired phenotypes and then to track the mutational landscape in real time. Microbes offer rapid generation times, streamlined genomes, established phenotypic measurement assays, and long-term storage and viability, and are thus powerful models of experimental evolution to examine the speed of adaptation, rates of parallel evolution, and the spectrum of adaptive mutations in conditions similar to those experienced during domestication [26••]. In recent years, experimental evolution systems have been developed around several species of filamentous fungi [99–102] and are reflective of the potential these approaches offer to rapidly select for desirable traits in domesticates and to model the impact of microbial domestication in progenitors.

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