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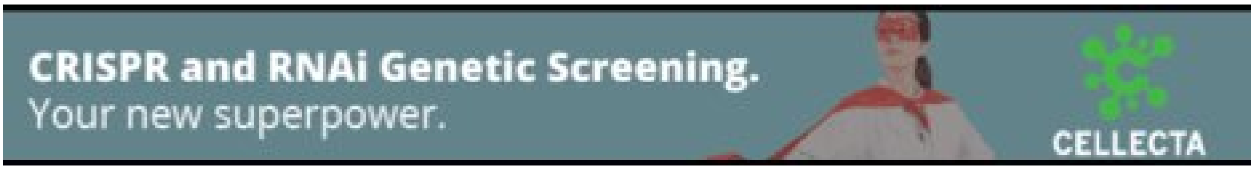

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Extensive sampling of *Saccharomyces cerevisiae* in Taiwan reveals ecology and evolution of pre-domesticated lineages

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ABSTRACT

The ecology and genetic diversity of model yeast *Saccharomyces cerevisiae* prior to human domestication remain poorly understood. Taiwan is regarded as part of this yeast's geographic birthplace where the most divergent natural lineage was discovered. Here, we extensively sampled the broad-leaf forests across this continental island to probe the ancestral species diversity. We found that *S. cerevisiae* is distributed ubiquitously at low abundance in the forests. Whole-genome sequencing of 121 isolates revealed nine distinct lineages that diverged from Asian lineages during the Pleistocene, when a transient continental shelf land bridge connected Taiwan to other major landmasses. Three lineages are endemic to Taiwan and six are widespread in Asia, making this region a focal biodiversity hotspot. Both ancient and recent admixture events were detected between natural lineages and a genetic ancestry component associated with isolates from fruits was detected in most admixed isolates. Collectively, Taiwanese isolates harbor genetic diversity comparable to that of the whole Asia continent, and different lineages have coexisted at a fine spatial scale even on the same tree. Patterns of variations within each lineage revealed that *S. cerevisiae* is highly clonal and predominantly reproduces asexually in nature. We identified different selection patterns shaping the coding sequences of natural lineages and found fewer gene family expansion and contractions which contrast with domesticated lineages. This study establishes that *S. cerevisiae* has rich natural diversity sheltered from human influences, making it a powerful model system in microbial ecology.

INTRODUCTION

The yeast genus *Saccharomyces,* which includes *S. cerevisiae,* is a powerful model system for revealing patterns of genomic variation underlying reproductive isolation and adaptation in eukaryotic microorganisms. Surveys of population genetic data have been used in *S. cerevisiae* to date the origin of key domestication events (Gallone et al. 2016; Duan et al. 2018; Peter et al. 2018), life cycle frequencies in nature(Tsai et al. 2008), the genomic basis of adaptation at continental scale(Duan et al. 2018; Peter et al. 2018), and more recently to establish its geographical origin and dispersal history (Xia et al. 2017). Phylogenomic analyses of the *Saccharomyces sensu stricto* complex and extensive sequencing of collections across the world suggest that *S. cerevisiae* originated in East Asia (Duan et al. 2018; Peter et al. 2018). The 1,011 genome project—the most broad large scale yeast population genomic study—discovered that three wild isolates from Taiwan showed an unprecedented high genetic diversity compared to populations from the rest of the world (Peter et al. 2018). Population genomics of 266 domestic and wild isolates in China revealed six wild lineages from primeval forests. The newly identified CHN-IX group represents the most diverged lineage (Duan et al. 2018). Isolates from this group and the three Taiwanese isolates grouped into a single lineage that exhibited a disjunct geographic distribution (Bendixsen et al. 2021). Although considerable knowledge is available on the biogeography and population genetics of plants and animals across continents (Whittaker et al. 2017), little is known about how eukaryotic microorganisms such as *S. cerevisiae* disperse, establish, reproduce and persist in nature (Liti 2015).

Most *S. cerevisiae* biology has been based on experiments on a handful of laboratory domesticated strains, but comprehensive analyses of the ecology and evolutionary biology of *S. cerevisiae* in the wild are still unavailable. In nature, *S. cerevisiae* have been isolated from the bark, fruits, surrounding soil, and leaves of plants belonging to several different families (Naumov et al. 2013), with early reports suggesting that the yeast is most successfully isolated from the oak Family Fagaceae (Sniegowski et al. 2002; Sampaio and Gonçalves 2008; Wang et al. 2012). *S. cerevisiae* contains high genetic diversity in certain populations, including lineage-specific variants that display clear population structures (Barnett 1992; Wang et al. 2012; Cromie et al. 2013; Strope et al. 2015; Gallone et al. 2016; Gonçalves et al. 2016; Zhu et al. 2016; Duan et al. 2018; Legras et al. 2018; Peter et al. 2018) and explain phenotypic variance similar to common variants (Fournier et al. 2019). Samples from natural habitats tend to be homozygous diploids forming unique populations with minimal genetic admixture, while lineages associated with human activities were likely heterozygous, containing higher ploidy and greater genetic admixture leading to a mosaic genome makeup (Diezmann and Dietrich 2009; Liti et al. 2009; Wang et al. 2012; Almeida et al. 2015). The diverse natural lineages of *S. cerevisiae* present in East Asia provide an excellent opportunity to study the natural diversity of this species, which was previously believed to be fully domesticated (Fay and Benavides 2005).

Taiwan is a continental shelf island with the fifth highest tree density in the world (Crowther et al. 2015). Among the 13 climate-related forests types in Taiwan, five are Fagaceae-dominated natural forests on low- and mid-elevation mountains (Li et al. 2013), thus a potentially ideal natural habitat for *S. cerevisiae*. Taiwan also harbors a high phylogenetic diversity of flowering plants (53 out of 64 angiosperm orders present under the APG IV classification system (Lin and Chung 2017) and endemism compared to other oceanic islands (Hsieh 2002), raising the possibility that the associated microbial populations are genetically different from their continental counterparts. Here, we set out to characterize the intra genetic diversity, relative abundance and distribution of *S. cerevisiae* in Taiwanese forests over four years of broad-sampling. Our study provides novel insights of the pre-domestication phase of *S. cerevisiae* and broaden our understanding of the ecological and biogeographic implications prior to anthropogenic impacts.

RESULTS

Deep sampling of natural *S. cerevisiae* from Taiwanese forests

From July 2016 to October 2020, our sampling strategy consisted of maximizing the number of localities associated with Fagaceae hosts and sampling a broad range of plant families present in Taiwanese broad-leaved forests (**Fig. 1A, Supplemental Table S1**). We surveyed 693 plant hosts belonging to 43 orders, 86 families and 156 genera (**Supplemental Table S2**) collected over 113 non-overlapping 1 km^2 grids. Various substrates (twigs, bark, leaves, flowers, fruits and topsoil around trees) were collected from each tree and subject to selective media enrichments resulting in 5,526 independent incubations (**Supplemental Table S3**). The successful isolation rates of *S. cerevisiae* per sample and per tree host was 1.9 and 10.8%, respectively, higher than from Brazilian forests (Barbosa et al. 2016) and Slovenia oak forests (Dashko et al. 2016), but lower than from North American oaks (Sniegowski et al. 2002) and Chinese wild niches (Wang et al. 2012). These isolates were recovered across altitudes of 0–2,100 meters from 18 plant families (**Fig. 1B**), with a majority from Fagaceae including four genera (27 *Quercus*, nine *Lithocarpus*, eight *Castanopsis*, and one *Fagus* species). Ten plant genera had higher isolation rates than *Quercus,* ranging from 40 to 100% per plant, albeit this recovery rate applied for as few as one tree (**Supplemental Table S2**). Among Fagaceae, *Quercus pachyloma* showed the highest isolation rate (75%; three out of four trees). Of the 339 lichen samples, four yielded successful isolations. Among the types of substrates, litter had the highest isolation rate (8.1%), providing the majority of recovered *S. cerevisiae* isolates (26.2%), followed by fruit, soil, bark and leaves (around 4–5% each). In general, the majority of samples were collected from July to December, and we found the isolation rate to be highest in July (18.9% per host tree), followed by September and October (17.5 and 11.3%, respectively). Isolation rates in other months remained around 0–11% (**Supplemental Table S3**).

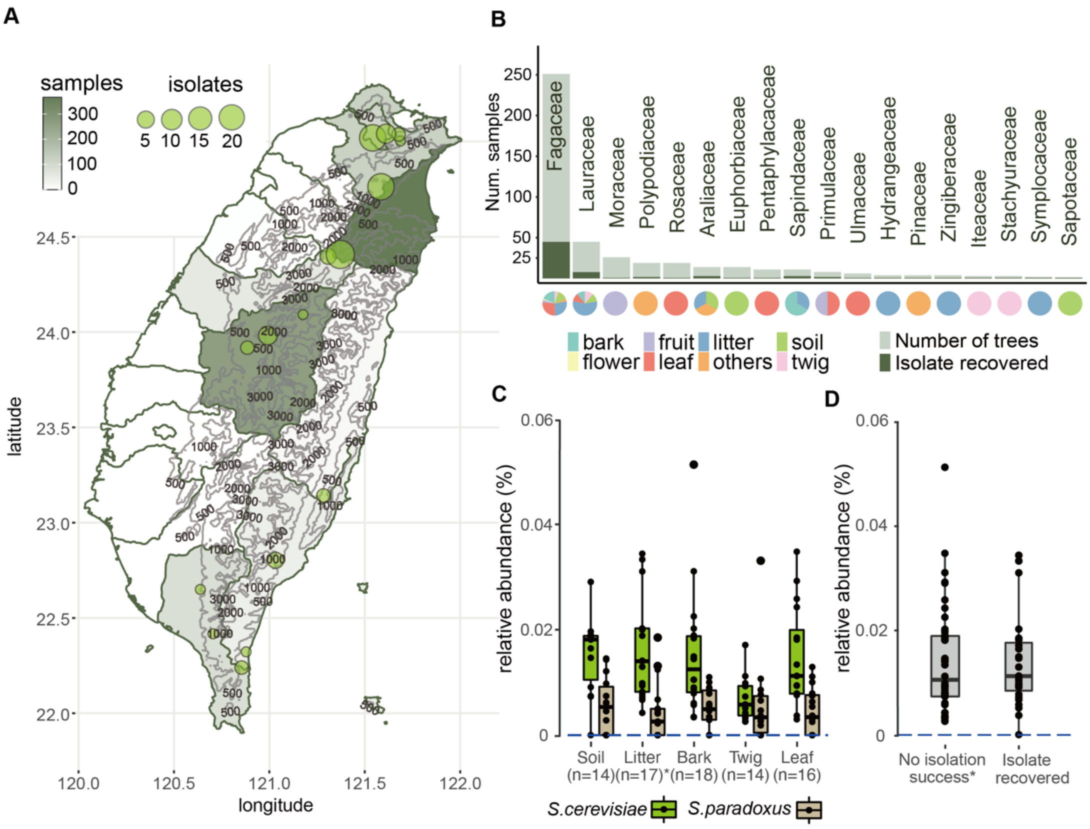

Figure. 1. **Sampling and isolation of *S. cerevisiae* in Taiwan.**

(A) Map of Taiwan showing sampling efforts in each county, with darker shades representing areas with higher number of samples collected and circles denoting the locations where *S. cerevisiae* was successfully isolated. One isolate found on Dongsha Island is not shown on this map. **(B)** Eighteen plant families from which *S. cerevisiae* was isolated. The darker color on each bar corresponds to the number of plants that yielded a successful isolation. Another 73 plant families from which we did not obtain any *S. cerevisiae* isolates are not shown. Pie charts below each bar represent the substrate surrounding plants from which samples were recovered. **(C)** Pairwise comparisons found no differences in the relative abundances of *S. cerevisiae* among bark, leaf, twig (Wilcoxon-rank with Bonferroni correction, bark-leaf, P=1.0; bark-twig, P=0.118, leaf-twig, P=0.461) and **(D)** between samples with or without isolation success.

Recurrent sampling of eight trees over two years showed differential isolation successes (**Supplemental Table S4**), suggesting that *S. cerevisiae* had different abundances in different parts or trees. Focusing on a total of five substrates from 18 trees within ~100 m^2 of this forest (**Supplemental Fig S1 and Table 4**), ITS amplicon sequencing succeeded in detecting just two amplicon sequence variants (ASVs) belonging to the *Saccharomyces* genus—*S. cerevisiae* and *S. paradoxus*. In contrast to surveys in temperate and boreal forests (Charron et al. 2014; Kowallik and Greig 2016; Brysch-Herzberg and Seidel 2017), *S. cerevisiae* had a higher relative abundance calculated as the percentage of the total taxa-classified reads than *S. paradoxus* in the subtropics (**Fig. 1C**). The sequence relative abundance of *S. cerevisiae* was on average 0.012% in these trees belonging to seven families regardless of substrates sampled; this suggested that, despite being ubiquitous in nature, *S. cerevisiae* lives in small populations. The relative abundances of *S. cerevisiae* were found to be constant between pairwise comparisons of bark, leaves and twigs (**Fig. 1C**; Wilcoxon-rank with Bonferroni correction, bark-leaf P=1.0; bark-twig P=0.118; leaf-twig P=0.461); among tree families (**Supplemental Fig S2,** P=1.0); and whether a *S. cerevisiae* isolate was recovered (**Fig. 1D;** P=0.89). In addition, bioclimatic variables extracted from GPS coordinates also exhibited no difference between sites at which isolates were and were not recovered (**Supplementary Info and Supplemental Table S5**). Together, these results imply that the primary habitat of *S. cerevisiae* is unlikely associated with a single tree host.

Multiple natural *S. cerevisiae* lineages in Taiwan

We sequenced the genomes of 121 isolates with a median coverage of 91× depth (**Supplemental Table S6**). All isolates were primarily homozygous (average heterozygosity: 0.01%) diploids, with the exception of isolate PD36A, which was a triploid (**Supplemental Fig S3**) estimated by flow cytometry (**Supplementary Info**). We constructed a maximum likelihood phylogeny based on 765,169 SNPs segregating in 340 isolates (**Fig. 2A**) by including of 219 representative isolates previously studied from multiple habitats (Barbosa et al. 2016; Duan et al. 2018; Peter et al. 2018; Pontes et al. 2019) that sampled all the major world-wide wild and domesticated lineages. The topology of the isolate phylogeny is largely consistent with a previous neighbor joining tree from the 1,011 *S. cerevisiae* genome project (Peter et al. 2018): the natural isolates were mostly grouped according to sampling locations, while industrial isolates were grouped according to fermentation sources. In particular, the Wine/European lineage and Asian Fermentation lineage were separated by a suite of natural isolates, suggesting independent domestication events (Fay and Benavides 2005; Liti et al. 2009; Gonçalves et al. 2016; Gallone et al. 2018). The African Palm Wine lineage was separated from the West African Cocoa lineage and placed near the branch leading to the Asian Fermentation lineage. Furthermore, the CHN-VI/VII lineage, which was collected from fruits, was further separated into two lineages consistently with geographical proximity of its members (designated as CHN-VI/VII.1 and CHN-VI/VII.2 in **Fig. 2A and 2C, Supplemental Table S6**).

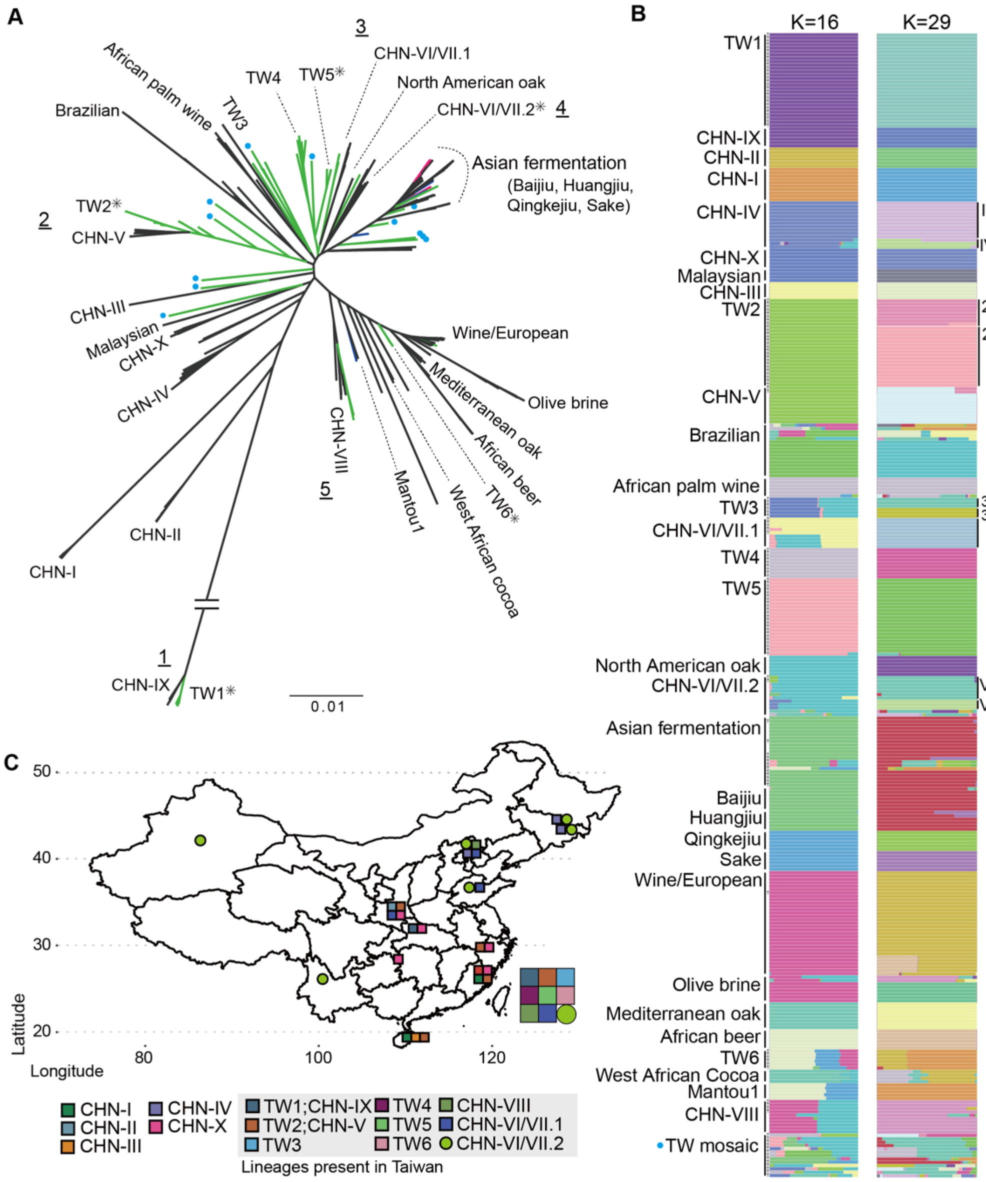

Figure 2. Phylogeny and population structures of 340 *S. cerevisiae* isolates. (A) Unrooted phylogeny based on 765,169 genome-wide SNPs. Bootstrap support was

>90% in all major lineages except inner nodes within some lineages indicated by asterisks. Natural, industrial and fermentation-related isolates discovered in Taiwan are in colored in green, blue and magenta, respectively. Mosaic Taiwanese isolates from ADMIXTURE analyses are labelled with blue dots on branch tips. Five cases in which Taiwanese and Chinese isolates were found to be monophyletic are indicated with underscored numbers. The Asian fermentation lineage includes Baijiu-, Huangjiu-, Qingkejiu-, Sake- and fermentation-related isolates from Taiwan, as shown in (b). **(B)** Population structure from ADMIXTURE analysis at K=16 and 29. Labels on the left side of the bars indicate each group from K=16 and some were further separated in K=29 which are annotated on the right side. Natural Taiwanese isolates with admixed genome makeup are shown together in the TW mosaic group. **(C)** Map of China and Taiwan indicating where the *S. cerevisiae* natural lineages were found (colored squares and circle). CHN-IV isolates that were sampled from Japan are not shown on this map.

Previous studies of natural *S. cerevisiae* revealed that most lineages comprise isolates from neighboring geographic origins (Duan et al. 2018; Peter et al. 2018); however, natural Taiwanese isolates are found throughout the phylogeny despite the small size of the island (**Fig. 2A**). The population structure of the 340 isolates used for the phylogeny were analyzed using ADMIXTURE (Alexander et al. 2009) with K from 2 to 30. Cross validation (CV) error was lowest at K=29 (CV error = 0.09025), though it only differed <1% between K=16 and 30 (**Fig. 2B, Supplemental Fig S4**). ADMIXTURE at K=16 was largely consistent with the phylogenetic lineages such as placing CHN-VI/VII into two genetic groups. ADMIXTURE at K=29 further separated two instances in which a group was split into solely either Chinese or Taiwanese isolates, suggesting the presence of lineage-specific segregating sites as a result of geographical isolation **(Fig. 2B and Supplemental Table S7)**. Some groups comprising isolates from a proximate geographical origin were further split into smaller groups, suggesting ongoing genetic differentiation. Based on ADMIXTURE K=29, we reused previously assigned group names (Duan et al. 2018; Peter et al. 2018) and designated these differentiated groups as well as new lineages exclusively found in Taiwan TW1 to TW6 (the most diverged lineage was TW1 and progressively labelled clockwise; **Fig. 2**). Examples include the recovery of 28 TW1 isolates clustered with CHN-IX (Duan et al. 2018; Bendixsen et al. 2021), together representing the most divergent lineage to date, and a new TW4 lineage that did not contain any Chinese strains (**Fig. 2**). This new lineage included isolates sampled from lichens and four isolates sampled from mushrooms that were previously placed in an undefined lineage (Peter et al. 2018), suggesting a possible association with other fungi (Spribille et al. 2016). In other instances, Taiwanese isolates were found in three previously assigned groups such as CHN-VI/VII.1, CHN-VI/VII.2 and CHN-VIII. Isolates of the most diverged TW1/CHN-IX lineage were separated by approximately 1,400 km, with four other natural lineages (CHN-I, V, VI/VII, and X) in between. Twenty-three isolates from northern Taiwan (TW2) clustered with the CHN-V population sampled as far as 1500 km apart. Together, these results suggest that Taiwan harbors the highest number of lineages that exhibit disjunct distributions followed by the Hubei-Shanxi region (nine and five, respectively; **Fig. 2C**)

Evidence of admixture in natural lineages

Both inter- and intra-species spontaneous hybridizations have been documented in *Saccharomyces* species. For instance, the wild *S. paradoxus* SpC* lineage present in North America (Eberlein et al. 2019) and domesticated *S. cerevisiae* Alpechin lineage (D'Angiolo et al. 2020) are classic examples of past hybridizations that played genomic and phenotypic diversities (Barbosa et al. 2016; Duan et al. 2018; Peter et al. 2018; Eberlein et al. 2019). Most Taiwanese isolates tend to have little admixture, with 20 and 5% (27/137, 7/137) of isolates containing at least 10% of the genetic component from two and at least three genetic ancestries (**Fig. 2B, Supplemental Table S7**), respectively. We confirmed the genetic components of domesticated strains' origins in wild isolates from African cocoa (Peter et al. 2018), olive brines and Brazilian forests(Barbosa et al. 2016), and identified an additional TW4 group sharing major genetic components with the steamed buns (Mantou) and Wine/European lineages, albeit recovered from nature. Other Taiwanese admixed isolates were apparent on the phylogenetic tree as isolated branches and had different levels of admixture from domesticated lineages (**Fig. 2A**). Additionally, all Taiwan isolates recovered from fruits contain CHNVI/VII-2a genetic component (**Supplemental Fig S5**); this coincides with the non-admixed CHNVI/VII-2a isolates, which have the widest geographically distribution in Asia (**Fig. 2C**).

To confirm that gene flow occurred between genetic groups, we applied TreeMix (Pickrell and Pritchard 2012) to designated groups from ADMIXTURE K=16 (**Fig. 3A, Supplementary Info and Fig S6**). The TreeMix phylogeny first indicated extensive gene flow among domesticated lineages such as solid- and liquid- state fermentation products and between natural lineages sister to domesticated lineages. Examples include isolates from steamed buns (Mantou) and Asian alcoholic beverages (Sake and Qingkejiu), as well as TW6 forest isolates. Second, the phylogeny also identified gene flow between natural lineages sister to the Wine/European and Asian Fermentation lineages. The CHN-VIII group emerged from both the Wine/European and fruit enriched CHN-VI/VII-2 lineages, which contain isolates from fruits and the natural environment across the Asian continent, including Taiwan. We also recovered hybrids between natural lineages that coexisted in proximity. Two isolates each belonging to TW4 or TW2 lineage came from fallen fruit, while PD38A was isolated from fruit growing on a *Castanopsis fargesii* tree (**Fig. 3a)**. This PD38A hybridization timing was likely to be recent, given the presence of large haplotype blocks not extensively broken down by recombination containing variants identical to each parental lineage (**Supplemental Fig S7**). Overall, these results suggest that hybridizations were common in *S. cerevisiae* and that some admixed lineages have persisted in nature. Reanalysis of the TreeMix phylogeny based on ADMIXTURE group K=29 shows consistent results—recurrent migrations occurred between lineages, leading to the Wine/European and Asian Fermentation lineages (**Supplementary Info, Supplemental Fig S8 and S9**). To incorporate these findings into a comparative resource, we further sequenced the genomes of 24 Taiwanese isolates representing all the natural lineages discovered in Taiwan using Oxford Nanopore reads (**Supplemental Table S8**).

Using molecular calibrations, divergence between different natural lineages as well as Chinese/Taiwanese split was inferred using either pairwise divergence or a phylogenomic approach (**Supplementary Info, Supplemental Figure S10 and Table S9**). A more recent divergence was estimated from the former approach which the lineages were on average diverged 0.03–0.07 million years ago (Ma) (**Fig. 3B**) compared to 0.54–1.11 Ma inferred from the phylogeny (**Supplemental Table S9)**. Together, these estimates fell during the Pleistocene epoch suggesting that the split may represent a vicariant event resulting from the submergence of the Taiwan Strait Land Bridge during interglacial periods and/or uplift of Taiwanese mountains (Teng 1990) during this period.

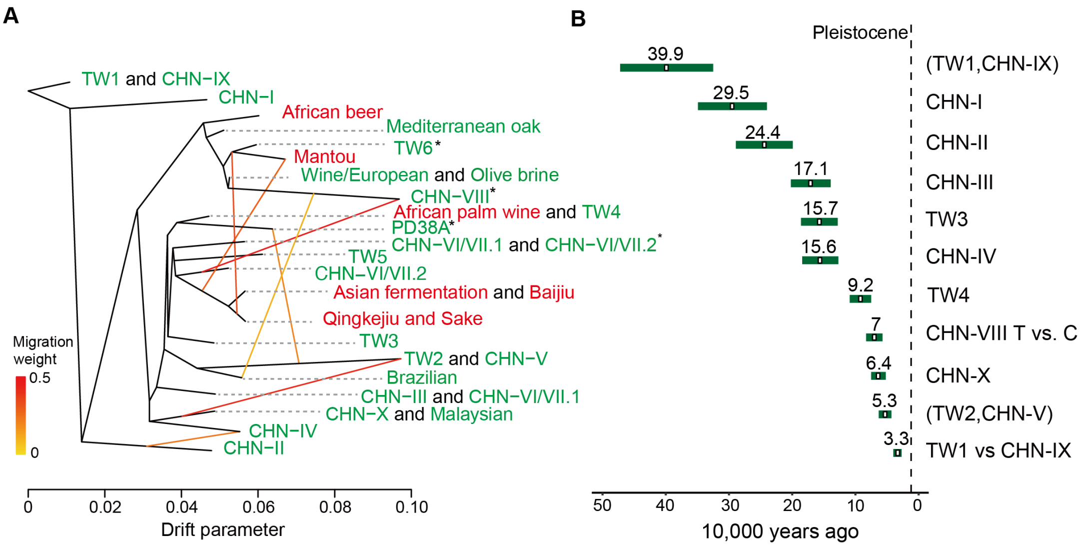

Figure 3. Migration and divergence time between lineages

(A) Migration edges (yellow to red colored lines) estimated by TreeMix showing seven migration edges on the phylogeny. Different edge colors indicate the strength of migration. Lineages were colored according to isolation sources (red and green denote domesticated and wild environment, respectively). Asterisk denotes lineages that contain multiple genetic components from different K from the ADMIXTURE analyses. **(B)** Molecular estimate of time to the most recent common ancestor in different *S. cerevisiae* lineages. The estimates is shown in **Supplemental Table S9A.**

Biogeography of wild *S. cerevisiae* lineages

In nature, single genetically homogenous fungal populations are generally found in distinct geographical regions as a result of isolation by distance (Branco et al. 2017; Chung et al. 2017; He et al. 2022). In contrast, the presence of multiple *S. cerevisiae* lineages at the same locality in Taiwan, even on the same tree, is striking (**Fig. 4A, Supplemental Fig S11 and Table 6**). In one sampling area, four lineages were recovered less than 35 km apart in central Taiwan (TW1-TW4 and mosaics, n=10; **Supplemental Fig S11**). In another sampling site—Fushan Botanical Garden, where we obtained 23 isolates comprising three lineages and admixed isolates were recovered (**Fig. 4A**). Both significant negative and positive correlation between genetic and geographical distance were observed in isolate pairwise comparisons in close distances (P<0.05 with 1,000 permutations; **Supplemental Fig S12**). However, no such association was found of the whole region (Mantel's r = 0.07, P=0.23; **Fig. 4B**), suggesting that in a given region the relationships between isolates was less determined by population structure of single lineages but dictated by the heterogeneity of multiple lineages coexisted at small spatial scale. The admixed isolates did not contain genetic components from adjacent isolates, but instead from CHN-VI/VIII.2a and others (**Supplemental Fig S13**). In addition, these combinations of coexisting lineages were not present in a similar locality range in China (**Fig. 2C**), suggesting coexisting of lineages was established by independent dispersal events.

The overall genetic diversity of Taiwanese isolates was comparable to that of Chinese isolates (Taiwan $\theta_\pi=5\times10^{-3}$ versus China $\theta_\pi=6\times10^{-3}$), even though the samples were only meters to tens of kilometers apart (**Supplemental Fig S14)**. This reinforced that the pattern of *S. cerevisiae* diversity in a geographical region was shaped by the presence of multiple lineages and heterogeneity of metapopulations in the same habitat. Up to a two-fold difference was observed in genetic diversity between lineages, with the aforementioned most widespread CHN-VI/VII.2a group harboring the greatest diversity (**Fig. 4C and Supplemental Table S10**). In contrast, when comparing isolates on the same tree at an extreme microgeographic scale, we found instances of all isolates being clonal or from different lineages with pairwise differences differed by ~35,000-fold (1–35,922 maximum number of pairwise mismatches of isolates recovered on the same tree; $\theta_\pi=8.3\times10^{-8}$–$2.9\times10^{-3}$; **Supplemental Table S11**). Three out of seven lineages have exhibited a linear isolation-by-distance (IBD)(Meirmans 2012) signature including the aforementioned TW2 lineage ($p<0.05$; **Supplemental Fig S15**). TW2 lineage exhibited a central-southern Taiwan discontinuous distribution, where isolates are found as much as 194 km apart. This suggests that the greater the geographical range, the higher the likelihood of genetic differentiation. Indeed, greater sequence divergence was shown when intra-lineage isolates between lineages were >10 km apart ($P<0.001$; Wilcoxon rank-sum test; **Supplemental Fig S16**), which supported genetic differentiation as a result of geographical isolation (Liti et al. 2006).

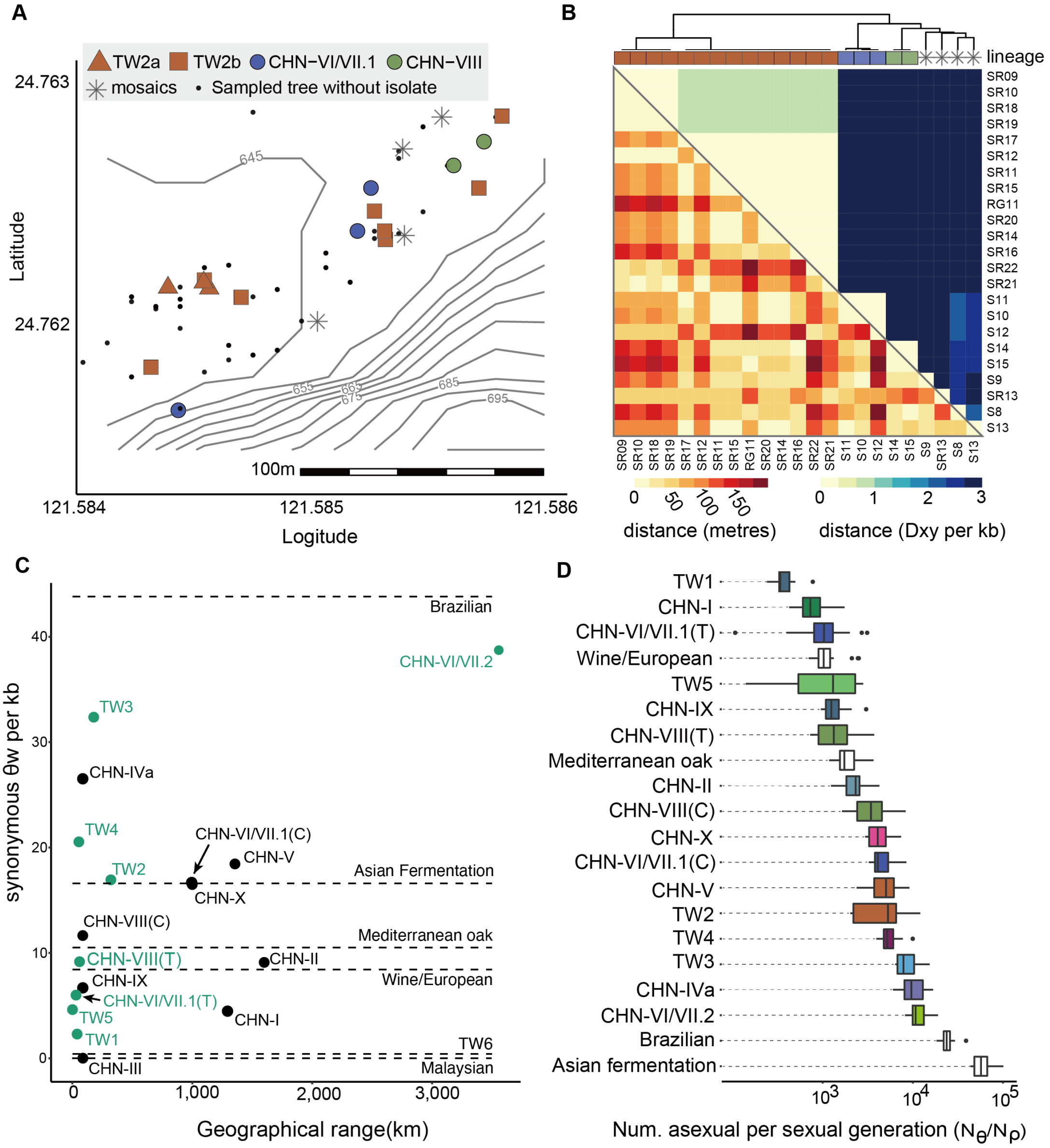

Figure 4. **Patterns of genetic variations and geographical distribution. (A)** Fine-scale geographic sampling at Fushan Botanical Garden in Taiwan. A total of 106 tree sites constituting 286 substrates were sampled in this region. Different colors represent different lineages and filled circle denote sampled trees from which *S. cerevisiae* was not successfully isolated. **(B)** Genetic and geographic distance of isolate pairs identified in (A). **(C)** Lack of correlation between genetic diversity θ_W at synonymous site and geographical range across lineages. Diversity for lineages where geographical range is unavailable is indicated with dashed lines. **(D)** Frequency of asexual per sexual generations across lineages.

Population genomic across lineages

Patterns of segregating sites can be used to infer the relative contributions and frequencies of reproduction modes in nature (Tsai et al. 2008). Wild *S. cerevisiae* isolates were highly inbred: Wright's inbreeding coefficient *F* was an average of 0.99 and clones made up 16–100% of each lineage (**Supplemental Table S6**), suggesting that most generations were mitotic regardless of lineage. We estimated that the effective population size of mutational (N_e) and recombinational (N_ρ) diversity for all chromosomes was 4.1×10^6–7.7×10^7 and 197–12,821, respectively, averaging across chromosomes (**Supplemental Table S12**) of selected lineages (**Supplemental Table S13**). The differences between both N_e estimates equates to approximately 382–61,264 mitotic cell divisions for every meiosis event (**Fig. 4D**). Such estimates overlap with previous estimates of 12,500–62,500 clonal generations based on the decay of heterozygosity during mitosis (Magwene et al. 2011), 1,000–3,000 in two genealogically independent populations of *S. paradoxus* (Tsai et al. 2008) and less in 800,000 generations in the fission yeast *Schizosaccharomyces pombe*(Farlow et al. 2015).

We calculated the mean neutrality index NI_{TG} (Stoletzki and Eyre-Walker 2010) for each lineage using polymorphism data from each lineage and *S. paradoxus* as an outgroup (**Fig. 5A**). NI_{TG} was higher in the domesticated lineages such as Wine/European as well as the most diverged TW1/CHN-IX amongst the natural lineages, suggesting more selection in purging the deleterious alleles in these lineages. We found that variations in NI_{TG} in natural lineages were not due the differences in effective population size inferred from mutational diversity (Kendall's τ= -0.26; P= 0.11, **Supplemental Fig S17**) but from recombinational size (Kendall's τ= 0.33; P=0.047, **Fig. 5B**), suggesting that the selection efficacy was greater when recombination occurred during sexual reproduction, consistent with the results of experimental evolution in a laboratory setting (Goddard et al. 2005). Such a relationship was more significant when lineages with low recombination were removed (Brazilian and the Asian fermentation lineage was excluded; Kendall's τ= 0.52; P=0.002, **Fig. 5B**), indicating similar efficacy of selection in the absence or low recombination.

We next investigated the extent of selection at the gene level within each lineage by conducting the McDonald-Kreitman test (McDonald and Kreitman 1991). Overall, we found 18–503 genes with a neutrality index (NI) >1 in each lineage (**Fisher's exact**

test P < 0.05; Supplemental Table S14), compared to 1–38 genes with NI < 1 (**Fisher's exact test P < 0.05; Fig. 5C and Supplemental Table S15)**, indicating that more genes had an excess of amino acid polymorphisms than were under positive selection (**Fig. 5C and Supplemental Fig S18**). The most genes with NI > 1 in the Asia lineages belonged to the most diverse TW3 (144 genes, **Fig. 5D**). The majority of these genes indicative of departure from neutrality were observed in only one lineage emphasizing the lineages' independent evolutionary history (**Supplemental Fig S19**). These negatively selected genes together were found to be enriched in biological process such as response to stimulus, cell communication and intracellular signal transduction (**Supplemental Table S16**). Within each lineage, no genes under either purifying or positive selection were enriched in any particular biological processes, except for the Brazilian lineage, which contained a sufficient number of genes showing NI>1. Three genes (*CDC10, CIT2* and *SAT4*) in both the Wine/European and Asian Fermentation lineages showed the largest overlap of genes with NI < 1 among the lineages (**Fig. 5c**). *CIT2* encodes a citrate synthase was involved in ethanol tolerance (Kasavi et al. 2014). Similarly, 25 genes under negative selection in these two lineages were the only overlap category found to be enriched in biological processes including Ras protein signal transduction (**Supplemental Table S17**), which were also targets of adaptation across different experimental evolution experiments (Long et al. 2015). Together these results suggest that the common selective pressure from domestication may have driven the adaptations of these genes. It is unlikely that this overlap was the result of stronger divergent selection with the *S. paradoxus* outgroup because the pattern was consistent when we used the McDonald-Kreitman with TW3 and CHN-VIII as outgroups, as they were sister to each of the domesticated lineages (**Supplemental Fig S20**).

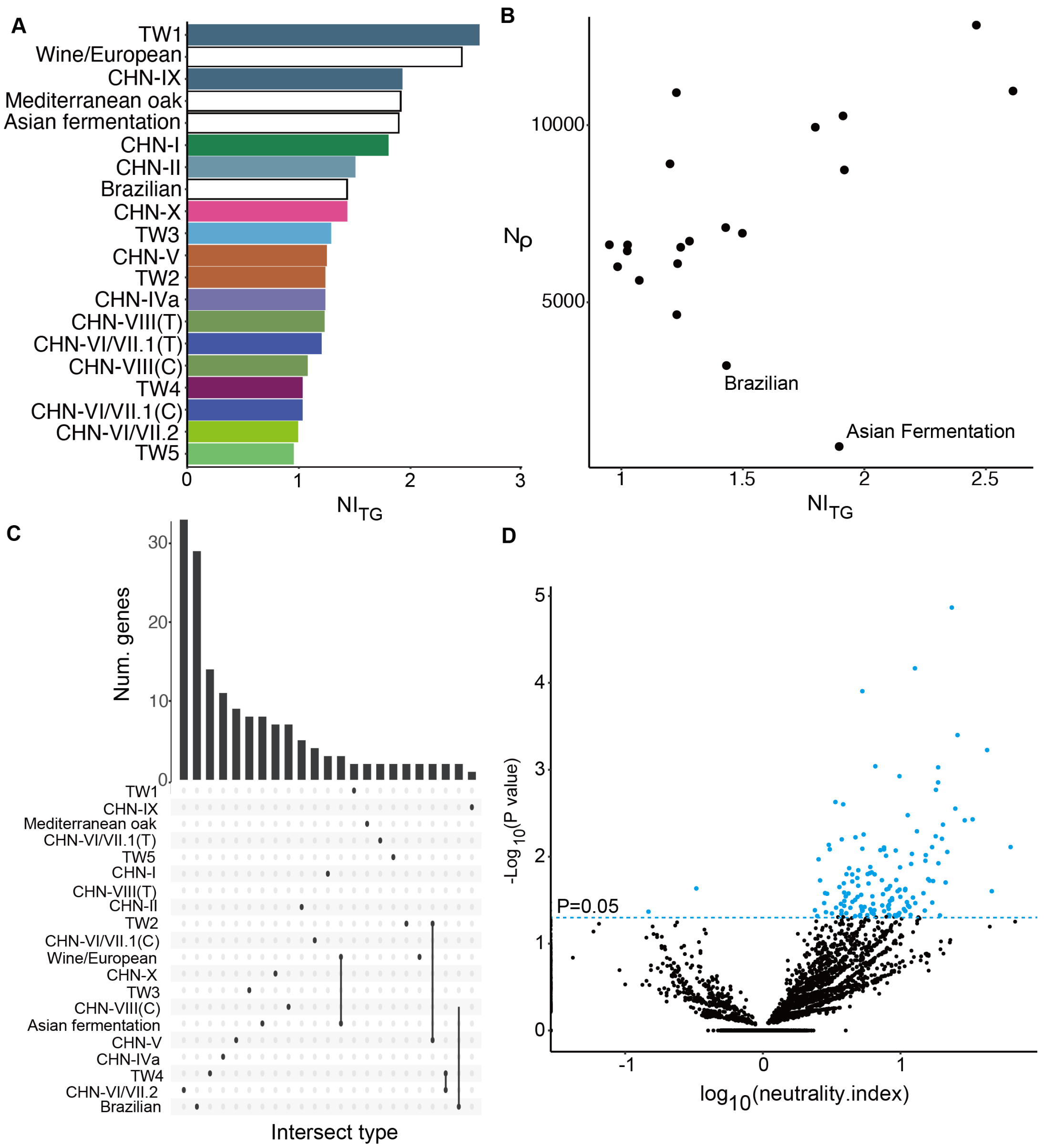

Figure. 5. Population genomics across lineages. (A) NI_{TG} estimates in natural and two domesticated lineages **(B)** Relationship between NI_{TG} and N_{ρ} across natural lineages **(C)** Lineage-specific and shared genes with NI < 1. **(D)** NI from the McDonald-Kreitman test for each gene in the TW3 lineage with *S. paradoxus* as the outgroup. Genes that were significantly different from NI = 0 were highlighted in blue.

Population differentiation dynamics between lineages

The presence of different levels of shared genetic components observed between Chinese and Taiwanese isolates among the five shared lineages suggested a distinct differentiation between the disjunct populations. The average ratio of nonsynonymous to synonymous substitution rates (d_N/d_S) between China and Taiwan isolates across lineages was 0.21 (**Fig. 6A**), suggesting that there was pervasive negative selection acting on the coding sequences of *S. cerevisiae,* with only 40–303 out of 6,572 genes showing signals of positive or balancing selection ($d_N/d_S > 1$) across the Taiwanese lineages. Consistent with observations from NI_{TG}, most of these genes were lineage-specific, with only *AIM21,* involved in mitochondrial inheritance, detected in four out of five lineages (**Supplemental Fig S21 and Table S18**) suggesting that selection acted independently in these lineages.

Gene duplication played an important role in the evolution of domesticated *S. cerevisiae* strains exhibiting more rapid copy number variation than wild strains (Bergström et al. 2014; Yue et al. 2017; Duan et al. 2018). To investigate the extent to which gene families differed between sister natural lineages, we *de novo* assembled, annotated and inferred orthogroup of non-clonal isolates using OrthoFinder (Emms and Kelly 2015). Compared to domesticated lineages (116 CHN-VIII vs Wine/European and 111 TW3 vs Asian fermentation; **Supplemental Fig S22**), only 17-49 orthogroup (OGs) were found to differ between the Chinese and Taiwanese lineages since their split (Wilcoxon rank sum test P <0.05; **Fig. 6B and Supplemental Figure S23**). A large fraction (36.7-94.7%) were single copy expansion or contractions (**Supplemental Table S19**), lineage specific and enriched in subtelomeres (**Supplemental Table S20**). The category that overlapped the most comprised seven OGs that were significantly different in two coexisting lineages: CHN-VIII and TW2 (**Fig. 4A** and **Supplemental Figure S24**). In addition, the largest orthogroup inferred was made up of hexose transporter genes (HXT), which are involved in polyol transport; this orthogroup was significant in four out of seven lineage comparisons **(Fig. 6C).** Copy numbers differed both between domesticated and natural lineages and among the natural lineages (**Supplemental Figure S25)**. The Taiwanese lineages typically exhibited expanded HXT copies compared to Chinese or domesticated lineages and inspecting isolates with long read assemblies revealed these copies were colinear regardless of lineage (**Fig. 6D and Supplemental Figure S26**). Together these results suggest that the Taiwanese isolates may have maintained a larger HXT repertoire, perhaps allowing them to utilise different sugar types or concentrations.

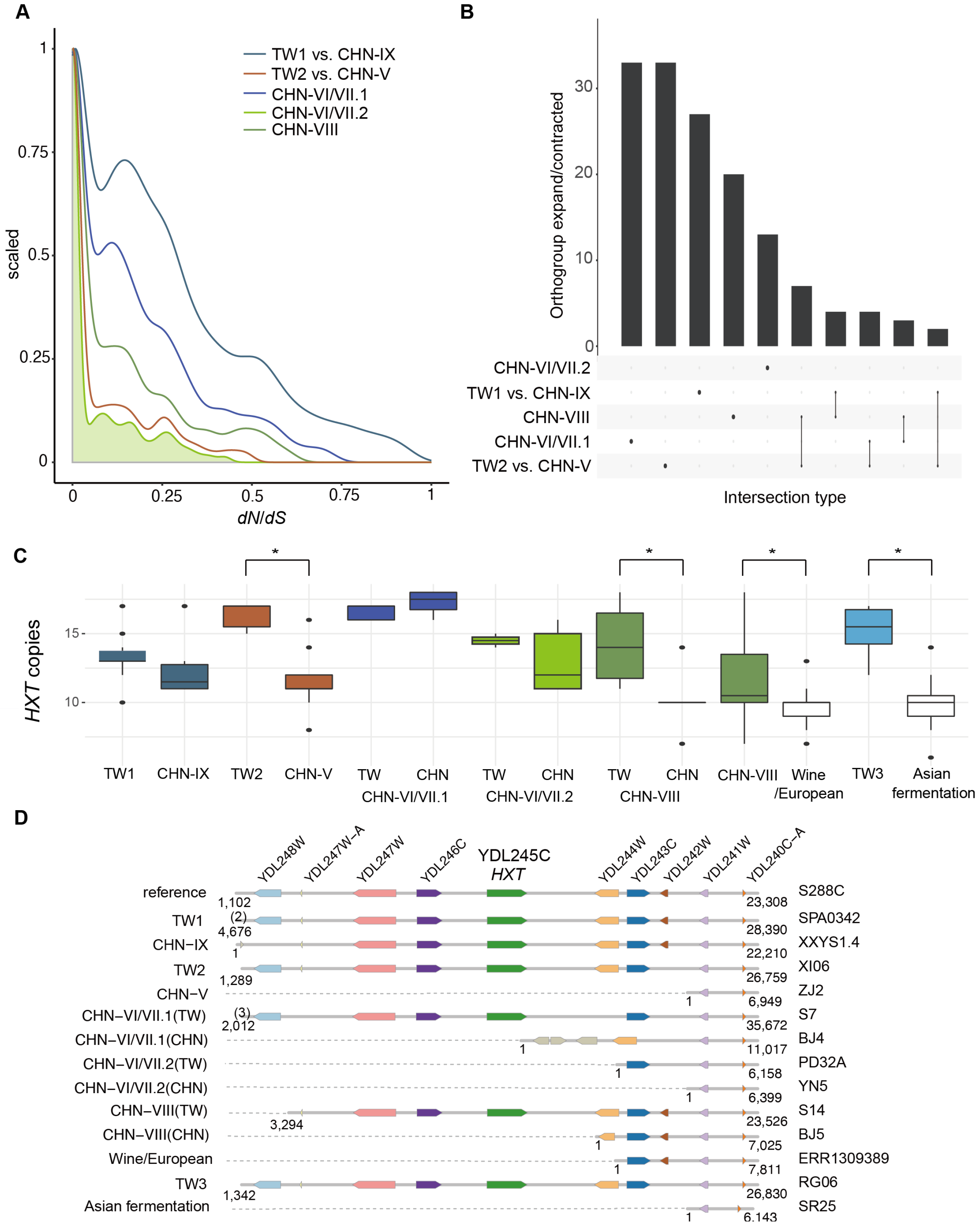

Figure 6. Dynamics between lineages (A) Density plot of d_N/d_S showing the majority of genes with d_N/d_S < 1. **(B)** Number of specific and shared orthogroups showing significant difference between pairwise lineage comparisons. **(C)** Distribution of *HXT* genes in each lineage **(D)** Synteny of *HXT* and adjacent genes on Chr IV 5' subtelomere. One representative *S. cerevisiae* isolate in each lineage was chosen. Numbers denote genome coordinates. Numbers in bracket were annotated genes until chromosome end.

DISCUSSION

A comprehensive understanding of the natural history of the budding yeast *S. cerevisiae* is key to further utilizing one of the most human-exploited microorganisms. In this study, we leveraged a four-year extensive sampling in Taiwan and combined metabarcoding approach to uncover *S. cerevisiae*'s ubiquitous presence but low abundance in broadleaf forests. We isolated and whole-genome sequenced 121 isolates to confirm the presence of the most diverged lineage TW1 (Bendixsen et al. 2021) and uncover five additional lineages that shared ancestries with lineages found in China as well as four new lineages exclusively found in Taiwan. We show that sympatric lineages coexist in different parts of Taiwan and identified introgressions between lineages. We found that the population structure of *S. cerevisiae* can be explained by a markup of different lineages that each outcrossed on average once in every 382–61,264 mitotic generations. These differences resulted in different selection efficacies across the lineages. The availability of high-quality *S. cerevisiae* assemblies presented here, in addition to genetic, molecular tools and genome resources such as the 1,011 genomes collection (Peter et al. 2018) already available in this model organism provides an exciting new platform to study microbial ecology.

Although *S. cerevisiae* has repeatedly been recovered from oak bark in the Northern Hemisphere (Sniegowski et al. 2002; Robinson et al. 2016) and being the only substrate of isolation in recent studies (Goddard and Greig 2015; Duan et al. 2018), our findings shows that *S. cerevisiae* is present as a generalist occurring at low abundance in a variety of broadleaf forest substrates. In addition to temperature, we speculate that isolation success for *S. cerevisiae* was shaped by co-existing microbial communities (Kowallik et al. 2015) competing with *S. cerevisiae* in the enrichment media. In addition, at a lineage level *S. cerevisiae* was found to be associated with particular environments, suggesting that it may have had an ecological niche (Goddard and Greig 2015): TW4 was isolated only from fungal fruiting bodies and lichens though further work is needed to conclude a possible symbiotic relationship, and a CHN-VI/VII.2 genetic component was present in many lineages and enriched in isolates recovered from the tree fruit substrate. Higher frequencies of admixed isolates observed in fruits may simply be a result of increased contacts with other lineages. Alternatively, fruits and organisms associated with those fruits such as frugivorous animals and vectors may represent niches that promote hybridization; for instance, sporulation has been suggested to be an adaptation that allows cells to survive in nutrient-depleted conditions such as insects' intestines during experimental passaging (Thomasson et al. 2021). Notably, the presence of CHN-VI/VII.2 genetic components in many natural lineages across the world, as well as in admixed isolates found in fruits, raises the possibility that the common ancestors dispersed from East Asia were from this lineage. In addition to abiotic factors, we speculate that such dispersal events of fruits may be aided by insects and human foraging.

We found that, unlike the general expectation in biogeographic studies that an island only contains a subset of genetic diversity from the mainland population, the genetic diversity of *S. cerevisiae* populations from Taiwan can be as diverse as those found in the Asia continent. The persistence of ancestral lineages may be a result of Taiwan being a high environmentally heterogeneous region (Ali 2018; Lin et al. 2020) and its prolonged bioclimatic stability (Tsukada 1966) than that of nearby eastern China. Alternatively, the geographic scale for distinguishing island and mainland populations and the importance of habitat diversity may differ between microorganisms (Davison et al. 2018) and other macroorganisms, such as animals and plants. The biogeography of *S. cerevisiae* appears to be similar to that of its associated flora in East Asia. Disjunct distributions of plants between Taiwan and different parts of China are common(Jianfei et al. 2013). The phylogeography of representative herbaceous and woody plants indicates that these representatives originated in mainland China, then migrated to Taiwan and the Ryukyu Archipelago during the Pleistocene as sea level fluctuations yielded recurring landbridges (Chiang and Schaal 2006; Niu et al. 2018; Jiang et al. 2019). We note that the Pleistocene was also the period when several tree species extinctions first took place across both the Americans (Seersholm et al. 2020) and Europe (Magri et al. 2017); this was followed by a rapid migration of *Quercus* that made it the dominant tree genus (Magri et al. 2017), which may have played a role in the restricted *S. cerevisiae* lineages observed outside East Asia. A systematic sampling of *S. cerevisiae* in the mainland continent—especially regions containing flora records exhibiting a disjunct distribution like in Taiwan, e.g., the Himalaya-Hengduan mountains (Niu et al. 2018), as well as plate boundaries—may help us better understand the biogeography of *S. cerevisiae*.

Our findings of rampant hybridization events between wild, wild with domesticated and domesticated lineages bring new perspectives to the ongoing debate over whether *S. cerevisiae* domestication happened once (Duan et al. 2018; Han et al. 2021) or multiple times(Almeida et al. 2015; Peter et al. 2018). By revealing frequent hybridizations between natural lineages, we show that isolates used in Asian and European fermentations may have been domesticated independently from the lineage CHN-VI/VII.2, and the single-domestication-event notion may be confounded by admixed isolates. Isolates from Asian fermentations were sister to the CHN-VI/VII.2 lineage, and subsequent genetic differentiations of this group have led to independent lineages such as the North American oak group, or the Mediterranean oaks group which is sister to the European/Wine isolates (**Fig. 2A and 3A**). Isolates outside of East Asia likely bear genetic components of this group. This may result in the placement of these isolates in or close to this group in a phylogeny. Ongoing hybridizations also complicate the inference; for instance the Brazilian rum population is a result of hybridization between European/Wine and North American groups (Almeida et al. 2015). Efforts to identify signatures of domestication environments (Han et al. 2021) may also be challenging when admixture is detected between these lineages. Isolation and recording the frequencies of these admixed isolates in nature could provide further insights into the conditions in which new lineages emerge.

Inferring population history in *S. cerevisiae* with different frequencies of asexual and sexual generations (Tsai et al. 2008) is challenging when using population genetics methods designed around human heterozygosity and recombination rates (Li and Durbin 2011). Disagreement in the divergences estimated from the phylogeny and pairwise divergence between isolates were observed. The phylogenomic method assume no gene flow and recombination with different lineages, and although *S. cerevisiae* is a predominantly asexual organism, recombination ρ was still detected and thus inflate divergence (Schierup and Hein 2000; Li et al. 2019). Conversely, estimates from pairwise divergences were more consistent to other reports (Leducq et al. 2016) but may underestimate the true divergence as we do not know the extent of quiescence of different *S. cerevisiae* lineages (Gray et al. 2004). Recent advances in directly tracking genotype evolution across natural habitats (Xia et al. 2017; Rudman et al. 2022) may lead to more accurate inferences once some of the fundamental parameters such as average generation time can be obtained in nature.

To conclude, we combined deep sampling, metabarcoding, isolate collection and whole-genome resequencing to illuminate the pre-domestication phase of *Saccharomyces cerevisiae* at an unprecedented resolution. The roles of *S. cerevisiae* in temperate forest environment have been studied in details (Mozzachiodi et al. 2022), and we reveal that multiple natural lineages of *S. cerevisiae* persist in the subtropic and tropical broadleaf forests in Taiwan, indicating the species is found everywhere but some genetically differentiated lineages prefer certain substrates. These observations help us to revisit our understanding of eukaryotic microorganism evolution—for instance, an alternating life cycle seems to be a convenient life history trait when genetically diverged partners are around. As more and more ecosystems—e.g., tropical cloud forests (Karger et al. 2021)—and biodiversity are lost, actions should be taken to conserve and reveal the ecology and evolution of not just *S. cerevisiae* but species with a proposed geographical origin. The availability and gene flow between these lineages also allow future experiments such as on hybrid fitness to be designed to resemble the subject's natural scenarios rather than relying on domesticated strains.

METHODS

Sampling and isolating *Saccharomyces cerevisiae*

From September 2016 to October 2020, we collected a total of 2,461 environmental samples from various substrates (bark *n*=340, twigs *n*=328, leaf *n*=528, litter *n*=320 and fruit *n*=78) surrounding 693 plant hosts (**Supplemental Table S2**). A total of 339 lichen samples, aliquots from six fermentation practices, and 68 from other sources (insect corpse *n*=43, fruiting body *n*=14, industrial strains *n=5* and others where biomaterial was sampled only once *n=6*) were also collected. Collection time and GPS coordinate in gpx format of host plants was recorded on the day of collection. Leaves, flowers of host plants were photographed. Bioclimatic variables of sampling sites were retrieved from CHELSA(Karger et al. 2017) database (v1.2) using recorded GPS coordinates. Digital terrain models (DTM) of sampling sites were retrieved from Taiwan's Open Government Data website (https://data.gov.tw/dataset/35430). Environmental samples were collected using alcohol sterilized tweezers or spoons and stored in zip bags. Whenever possible during the sampling trips metadata such as the identity of the host plant, lichens and altitude were recorded. Samples were redistributed into 50ml falcon tubes and stored at room temperature. Each sample was divided into two proportions and immersed in two enrichment media: a liquid medium made up of either i) 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L sucrose, 7.6% EtOH, 1 mg/L chloramphenicol, and 0.1 % of 1-M HCl as used in ref(Sniegowski et al. 2002) or ii) YPD containing 10% dextrose and 5% ethanol adjusted to pH 5.3 as used in (Hyma and Fay 2013). Samples were incubated at 30℃ until signs of microbial growth and fermentation were detected, such as white sediment and effervescence. Sediments were then streaked onto YPD agar plates. Single colonies were picked out and incubated in potassium acetate medium 23°C for 7-10 days(Liti et al. 2017). Single colonies with ascus-like (four spores) structures under microscope were picked out and streaked onto YPD agar plates. Sanger sequencing and gel electrophoresis of ITS1-5.8S-ITS2 region PCR amplified with ITS1F/ITS4 primer set were performed to identify the species of isolates(White et al. 1990; Gardes and Bruns 1993). Pilot sampling, modification and rationale during the course of sampling strategies are further provided in **Supplementary Info**. Sampling efforts were visualized using the R's package ggplot2 (v.3.3.5) and annotated with metR (v.0.10.0; https://github.com/eliocamp/metR) and ggspatial (v.1.1.5; https://paleolimbot.github.io/ggspatial/). In order to determine ploidy levels for our isolates, we carried out flow cytometry analysis for the 105 Taiwanese isolates from this study using propidium iodide (PI) staining assay using previously established protocols (Todd et al. 2018) (**Supplementary Info**).

DNA extraction

Field-collected environmental samples can vary, so we preprocessed these samples and extracted their DNA differently (see **Supplementary Info** for details). For whole genome sequencing of *Saccharomyces cerevisiae,* isolates taken from frozen stocks were streaked out onto YPD plates and incubated in 30℃ until colonies became visible. Single colonies were then incubated in 5ml YPD liquid medium at 30℃ in a shaker at 200 rpm overnight. High molecular weight genomic DNA was extracted using protocol described in (Denis et al. 2018). DNA quality was determined by Qubit readings, A260, A280, A260/280 ratios on NanoDrop and gel electrophoresis.

Library construction and whole-genome sequencing

For Illumina sequencing, paired-end libraries were constructed using the Illumina Nextera or NEB Next Ultra DNA library preparation kit with the manufacturer's protocol. The first 91 isolates were sequenced by Illumina HiSeq 2500 and the remaining 30 were sequenced by Novaseq to produce 125- and 150-bp paired-end reads, respectively. Oxford Nanopore libraries were prepared using SQK-LSK109 with 12 isolates multiplexed by EXP-NBD104 and EXP-NBD114 barcoding kit (ver NBE_9065_v109_revV_14Aug2019) and sequenced by a R9.4.1 flow cell on a GridION instrument. A total of 24 isolates were run on two flow cells. Nanopore fast5 files were basecalled using Guppy (v4.0.11).

Amplicon sequencing and analysis

Amplicon libraries were constructed as previously described(Tedersoo et al. 2014) from 89 environmental samples (18 bark, 18 twig, 18 leaf, 18 litter, 17 soil), three positive controls (*Saccharomyces cerevisiae* S288C, *S. paradoxus* YDG197 and lab isolate *Pseudocercospora fraxinii*); and DNA from two *Escherichia coli* as a template to confirm primer specificity towards only fungal species. The ITS3ngs (5-CANCGATGAAGAACGYRG-3') and ITS4ngsUni (5'-CCTSCSCTTANTDATATGC-3') primer pair(Tedersoo et al. 2015) was used. Two no template controls were included during the PCR step to confirm that amplicon generation was free of contaminating DNA. To determine the background amplicon noise from experimental pipeline, a sterile filter was treated and processed as one the field samples. Amplicons were normalized using the SequalPrepTM Normalization Plate Kit (Thermo Fisher Scientific, ID: A1051001), then pooled and concentrated using AMPure XP (Beckman Coulter, ID: A63881). Finished DNA libraries were sequenced on the Illumina MiSeq platform using 2x300 bp pair-end sequencing chemistry.

Raw sequencing reads containing the Illumina sequencing index were demultiplexed using *sabre* (v1.0; https://github.com/najoshi/sabre). Sequencing quality was determined using *FastQC* (v0.11.7; https://github.com/s-andrews/FastQC). Reads were quality filtered based on a Qscore >20 and 50 base pairs were trimmed from the 3'end end using usearch(Edgar 2010) (v11.0.667). Filtered reads were processed following the UPARSE (Edgar 2013) pipeline. In brief, paired reads were merged and dereplicated into unique sequences. Unique sequences were filtered using usearch default settings. Filtered sequences were denoised into zero-radius operational taxonomic unit (zOTUs) using the unoise2 (Edgar 2016b) algorithm. The taxonomy of zOTUs was classified using the SINTAX (Edgar 2016a) algorithm (Edgar 2016) against the UNITE (Nilsson et al. 2018) Fungal database (v8.2). Merged reads were assigned into zOTUs with 100% sequence identity and tabulated using the *usearch_global* function. Processed reads were analyzed in the R-Studio environment (v 1.2.5033). Sequencing data were analyzed with phyloseq(McMurdie and Holmes 2013) (v1.34). Statistical significance was tested for using *kruskal.test* from the *stats* package in R (R Core Team 2021).

Variant calling

To determine the evolutionary history of new Taiwanese isolates, we collected a total of 219 published genomes representing established *S. cerevisiae* industrial and natural populations: 102 isolates from the 1,011 genome project(Peter et al. 2018) (31 Wine/European, 8 Mediterranean oak, 6 African beer, 6 African palm wine, 4 West African cocoa, 4 Malaysian bertam palm nectar, 6 North American oak, 6 Sake, 11 Asian fermentation, 1 CHN-I, 1 CHN-III, 4 CHN-IV, 1 CHN-V, 6 Mixed origin groups and 7 other isolates of Taiwanese origin), 93 isolates from the Chinese population(Duan et al. 2018) (69 CHN-I to CHN-X isolates excluding those previously sequenced in the 1,011 genome project, 5 isolate from Mantou1, 6 Huaugjiu, 7 Baijiu and 6 Qingkejiu), 16 isolates from the Brazilian wild lineage(Barbosa et al. 2016) and eight isolates from olive brine(Pontes et al. 2019). This combined with the 121 isolates from this study yielded a total of 340 individuals, 30% of which originated from industrial sources and 70% from the natural environment (**Supplemental Table S6**). Read quality was examined with FastQC (v.0.11.9; https://github.com/s-andrews/FastQC). Read quality and adaptor trimming was performed using Trimmomatic(Bolger et al. 2014) (v0.36; Pair end mode, ILLUMINACLIP;LEADING:20;TRAILING:20;SLIDINGWINDOW:4:20;MINLEN:150). For the 340 samples, 64-95% of raw paired reads from the 340 samples were kept after trimming. Trimmed reads were each mapped to the S288C reference genome version R64-2-1 using Burrows-Wheeler Aligner(Li and Durbin 2009) (v 0.7.17-r1188) and the mapping rate was 91-99%. Duplicate reads were marked using GATK (McKenna et al. 2010) MarkDuplicates (v.4.1.9.0) . Variants were first called in multi-sample manner and filtered using BCFtools(Danecek et al. 2021) v1.8 (-d 1332; QUAL 30, MQ 30, AC >=2 and 50% missingness; genotype-filtered with minDP 3). 88% (1,150,658/1,306,082) of variants were retained. Second, variants were also called and filtered with FreeBayes (Garrison and Marth 2012) and VCFtools (Danecek et al. 2011) (v. 1.3.2 and v 0.1.15, respectively; minDP 3, QUAL

30, MQ 30, AC >=2 and 50% missingness, sites with 0.25<AB<0.75 and 0.9<MQM/MQMR<1.05 were retained). 56% of sites were retained based on these criteria (818,025/1,443,685). Finally, 808,864 intersecting variants discovered from both callers were used for further analysis. The functional effects of variants were annotated with SnpEff(Cingolani et al. 2012) (v.4.3t).

Assembly, annotation and ortholog identification

Nanopore reads of each isolate were assembled using Canu (Koren et al. 2017) (v1.9). For isolates without long reads, Illumina paired-end reads were assembled using SPAdes (Bankevich et al. 2012) (v. 3.14.1, options *k*-mer size 21, 33, 55, 77 and --careful). Consensus sequences of the assemblies were polished with four rounds of Racon (Vaser et al. 2017) (v1.4.11), one round of Medaka (v1.0.1) using nanopore raw reads and five rounds of Pilon using Illumina reads. The assemblies were further scaffolded using RagTag (Alonge et al. 2019) against the S288C genome reference. Annotations were then transferred using Liftoff (Shumate et al. 2021), with additional *de novo* annotations using AUGUSTUS (Stanke et al. 2006) on regions without any transferred annotations. Orthogroup was inferred using OrthoFinder (Emms and Kelly 2015) (v.2.5.4). Orthogroup that differentially abundant between assemblies produced using different sequencing technologies were excluded from further analyses. The assembly metrics and description of the nanopore assemblies are shown in **Supplemental Table S8**.

Phylogenomic analyses

After removing 43,695 invariant sites resulting from ambiguous nucleotide codes among all isolates, the remaining 765,169 variable sites were used to construct a phylogeny for the 340 isolates. The resulting best-fit model was indicated by BIC to be TVMe+R3 first with IQ-TREE. In addition, a maximum likelihood phylogeny was inferred using IQ-TREE with the TVMe+R3+ASC model and a 1,000 ultrafast bootstrap approximation (Hoang et al. 2018; Minh et al. 2020). A separate *S. cerevisiae* lineage phylogeny was inferred and used in MCMCtree method of the PAML (Yang 2007) package to estimate the divergence time among the *S. cerevisiae* lineages (**Supplementary Info**).

Diversity, population structure and demography estimates

For the population structure estimate, biallelic SNPs were kept and filtered based on linkage disequilibrium. Sites that are linked were filtered out using PLINK(Chang et al. 2015) (v1.90b4), excluding pairs of loci with $r^2 > 0.5$ (--indep-pairwise 50 10 0.5 --r2). The remaining 482,161 sites were used for ancestry estimation by ADMIXTURE (Alexander et al. 2009) using K=2 to K=30 with five-fold cross validation from five runs of different seed numbers. CV errors for each K value in five runs were compared to choose the representative number of clusters. Migration signals on the phylogeny were estimated with TreeMix using 1000 bootstrap for natural populations according to clusters in K=16. The numbers of migration edges were estimated, aided by the optM (v. 0.1.5) package (Fitak 2021) and presented in **Supplementary Info**.

A consensus genome sequence containing variants for each isolate was generated from the SNPs matrix using BCFtools (Danecek et al. 2021) consensus (v.R64-2-1) with the S288C reference genome sequence. For the IBD analysis, geographical distance between isolates was measured using the sf package in R for Taiwanese isolates with GPS records. For Chinese isolates, since GPS records were not available, we used approximate coordinates for each sample site (Duan et al. 2018) as recommended by the authors. To estimate the maximum geographical distance within the Chinese lineage, we chose sample sites that were the furthest apart. For instance, in CHN-V, the distance between Shanxi and Hainan was used. For lineages sampled from only one site (CHN-II, CHN-IX), the largest range of the site was used. Diversity estimates for 16 nuclear chromosomes and corresponding coding/noncoding regions were examined by VariScan (Vilella et al. 2005) with RunMode 11 (n<4) and 12 (n>=4). These diversity estimates were used to infer frequency of sex according to (Tsai et al. 2008) and detailed in **Supplementary Info**.

DATA ACCESS

The sequencing data of the 121 *S. cerevisiae* isolates and ITS amplicon sequences of 89 samples generated in this study have been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA755173. The accession numbers of the isolates are also shown in **Supplemental Table S6**. The zOTU table for the amplicon data and all of the scripts written to carry out this study was deposited in https://github.com/tjleez/popgen.methods and are also available as **Supplemental Code**.

COMPETING INTEREST STATEMENT

The authors declare no competing interests.

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AUTHORS CONTRIBUTION

I.J.T. conceived and led the study. T.J.L, Y.C.L and W.A.L carried out the sampling and isolation of *Saccharomyces cerevisiae*. J.P.H, C.L.H and K.F.C helped with the sampling and identified the lichen and plant samples. T.J.L, W.A.L, Y.F.L and H.M.K conducted the experiments. Y.F.L carried out the amplicon analyses. H.H.L., H.M.K and I.J.T. performed the sequencing and assemblies of the *S. cerevisiae* genomes. T.J.L, Y.C.L, H.H.L and I.J.T. carried out the population genomic analyses. Y.C.L, H.H.L and I.J.T. carried out the comparative genomics, phylogenomics analyses and the divergence time estimation. T.J.L and I.J.T. wrote the manuscript with substantial input from J.P.H, K.F.C and G.L.

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