Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in *Saccharomyces cerevisiae*

Paul M. Magwene[1], Ömür Kayıkçı, Joshua A. Granek, Jennifer M. Reininga, Zackary Scholl, and Debra Murray

Department of Biology and Institute for Genome Sciences and Policy Center for Systems Biology, Duke University, Durham, NC 27708

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We carried out a population genomic survey of *Saccharomyces cerevisiae* diploid isolates and find that many budding yeast strains have high levels of genomic heterozygosity, much of which is likely due to outcrossing. We demonstrate that variation in heterozygosity among strains is correlated with a life-history trade-off that involves how readily yeast switch from asexual to sexual reproduction under nutrient stress. This trade-off is reflected in a negative relationship between sporulation efficiency and pseudohyphal development and correlates with variation in the expression of *RME1*, a transcription factor with pleiotropic effects on meiosis and filamentous growth. Selection for alternate life-history strategies in natural versus human-associated environments likely contributes to differential maintenance of genomic heterozygosity through its effect on the frequency that yeast lineages experience sexual cycles and hence the opportunity for inbreeding. In addition to elevated levels of heterozygosity, many strains exhibit large genomic regions of loss-of-heterozygosity (LOH), suggesting that mitotic recombination has a significant impact on genetic variation in this species. This study provides new insights into the roles that both outcrossing and mitotic recombination play in shaping the genome architecture of *Saccharomyces cerevisiae*. This study also provides a unique case where stark differences in the genomic distribution of genetic variation among individuals of the same species can be largely explained by a life-history trade-off.

The frequency of sex and the nature of breeding systems have a profound effect on genome variation and evolution. For example, inbred populations have an increased frequency of homozygous genotypes (1), lower effective rates of recombination (2), and smaller effective population sizes relative to outcrossed populations with the same number of individuals (3). Likewise, clonal populations are expected to exhibit high levels of heterozygosity coupled with increased allelic diversity but decreased genotypic diversity relative to sexual populations (4).

The budding yeast *Saccharomyces cerevisiae* is one of the best studied model organisms, but relatively little is known about the importance of sexual versus asexual reproduction and inbreeding versus outcrossing in shaping genome evolution in this species. One recent study estimated that outcrossing occurs approximately once every 50,000 generations in *S. cerevisiae* (5), but low rates of outcrossing do not preclude the possibility that outcrossing has an important impact on genetic variation. Studies of the closely related yeast *Saccharomyces paradoxus* suggest that sexual cycles are rare relative to asexual cycles and that when sex does occur it primarily involves inbreeding (6, 7). However, *S. paradoxus* exhibits distinctly different intra- and interpopulation patterns of variation than does *S. cerevisiae* (8), and hence these findings may not be generalizable across the *Saccharomyces* genus.

Patterns of heterozygosity are an important indicator of both outcrossing and clonality (9, 10), and hence a genomic characterization of heterozygosity should prove useful for disentangling these issues. However, all population genomic studies of *S. cerevisiae* to date have been based on the analysis of haploid genomes or diploid strains derived from single spores (8, 11), therefore obscuring heterozygosity.

To provide new insight into the relative impact of asexual/sexual reproduction and outcrossing/inbreeding we conducted a population genomic survey of diploid environmental isolates of *S. cerevisiae*. We present evidence of extensive genomic heterozygosity and large scale loss-of-heterozygosity (LOH) in many yeast strains and we infer from this that outcrossing and mitotic recombination have played important roles in shaping genome architecture in these lineages. Our analysis indicates that heterozygosity is particularly prevalent in yeast isolated from human-associated environments, and we demonstrate that variation in genomic heterozygosity among lineages is correlated with a life-history trade-off that involves how readily yeast switch from asexual to sexual reproduction when faced with nutrient stress. These results suggest that selection for alternate life-history strategies contributes to differential maintenance of genomic heterozygosity in natural and human-associated environments. Our findings provide unique perspectives on the forces that shape genome evolution in *S. cerevisiae* as well as insights into life-history trade-offs and the evolution and maintenance of sex.

Results and Discussion

We sequenced the genomes of 11 diploid environmental isolates of *S. cerevisiae*. Our strain panel included six clinical isolates, three agricultural strains, a muscadine grape isolate, and a woodland strain (Table 1). Of particular interest are the ancestors of several widely used strains such as EM93, the primary contributor to the genome of the reference strain S288c (12), and YJM128, the diploid progenitor of YJM789 (13). As a control we also sequenced the genome of a diploid laboratory strain, which should be nearly completely homozygous. Each genome was sequenced using 36- or 50-bp reads on the Illumina GAII platform and reads were mapped to the *S. cerevisiae* reference genome. Average genome coverage was >14× for all strains. To minimize the false positive rate of detecting heterozygous sites we included in our analysis only SNP calls for which two different short-read mapping algorithms agreed (*Materials and Methods* and *SI Appendix, Materials*). Because of the SNP calling criterion we adopted, and based on confirmatory sequencing using conventional Sanger sequencing (*SI Appendix, Materials*), the values

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Data deposition: The sequences reported in this paper have been deposited in the National Institutes of Health Short Read Archive (accession nos. SRX030121–SRX030126, SRX030131– SRX030135, and SRX030579).

[1]To whom correspondence should be addressed. E-mail: paul.magwene@duke.edu.

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Table 1. The number of SNPs and heterozygous sites for *S. cerevisiae* strains sequenced in this study

Strain	Origin	SNPs	Heterozygous sites
EM93	Fig	30,112	24,420
YJM128	Clinical	64,863	33,457
YJM223	Clinical	62,831	37,148
YJM308	Clinical	50,260	22,229
YJM309	Clinical	60,734	22,987
YJM311	Clinical	51,475	23,852
PMY112	Vineyard	43,882	6,480
PMY110	Vineyard	42,768	6,045
PMY093	Grape	55,457	4,086
YJM222	Clinical	48,892	728
YPS670	Oak	58,317	551
Σ1278b	Laboratory	28,477	337

The number of SNPs includes both monoallelic and biallelic (heterozygous) sites.

listed in Table 1 are likely conservative estimates of the true numbers of SNPs and heterozygous sites in these strains.

Nine of the 11 *S. cerevisiae* isolates we sequenced have substantial heterozygosity (>4,000 heterozygous sites). Of these, 6 have >20,000 heterozygous sites. The low heterozygosity strains in our survey had 500–750 heterozygous sites, only slightly higher than the number we estimated for the laboratory strain. Both outcrossing and clonality can contribute to the accumulation of heterozygosity. For the most heterozygous strains, the numbers of heterozygous sites are unlikely to have arisen purely via mutation. Furthermore we find that at the majority of heterozygous sites, the nonreference allele is found in at least one other strain background and that a substantial portion of heterozygous sites are heterozygous in multiple strain backgrounds, contrary to what a purely clonal model predicts. We therefore infer that the high levels of heterozygosity we observe most likely result from outcrossing during their most recent sexual cycle.

Because a large fraction of the strains in our genomic survey are of clinical origin, we expanded our study by genotyping 18 additional strains at nine loci (*SI Appendix*, Table S5). This expanded strain panel included a larger sample of North American oak strains; vineyard strains from Italy, Australia, and North America; and two additional clinical isolates. Of these 18 strains, 9 exhibited patterns of heterozygosity that were comparable to what we observed in the heterozygous genomes at those same loci. In sum, 18 of 29 (62%) of the diploid isolates we examined show evidence of modest to extensive heterozygosity that is consistent with having been generated by outcrossing.

Our survey indicates that a large proportion (75%) of strains isolated from human-associated environments such as vineyards and clinical settings are heterozygous whereas none of the woodland isolates are highly heterozygous. This finding suggests that outcrossing might play an important role in human-associated lineages. To explore this, we estimated inbreeding values (F_{IS}), treating the strains as coming from two or three subpopulations (14) (*SI Appendix*, *Materials*). The two-group model we considered represents a division into "natural" and "domesticated" (human-associated) isolates; the three-group model further subdivided the domesticated isolates into "vineyard" and "clinical" subgroups. In the two-group model, the estimated F_{IS} values were natural = 0.95 and domesticated = 0.47. In the three-group model F_{IS} estimates were natural = 0.95, clinical = 0.44, vineyard = 0.31. On the basis of F_{IS} we calculated the effective outcrossing rate within these groupings, using the formula $t = (1 - F_{IS})/(1 + F_{IS})$ (9). For the natural subpopulation, $t = 0.02$, indicating a very low rate of outcrossing. For the domesticated subpopulations t is in the range 0.36–0.52. Higher rates of outcrossing in clinical and vineyard environments are consistent with recent studies that have estimated higher rates of recombination among fruit and clinical strains compared with woodland and soil isolates (15, 8, 16). For example, in a survey of 103 *S. cerevisiae* strains (surveyed at five loci), Diezmann and Dietrich (16) found that between 33 and 88% of human-associated isolates exhibited heterozygosity whereas none of the soil isolates they examined showed any evidence of heterozygosity. Human-associated environments might increase opportunities for outcrossing by bringing diverse strain backgrounds into proximity (17), by creating mass mating conditions (18), or by creating greater opportunities for spore dispersal by insect vectors (19). Alternatively, human-associated environments may have selectively favored genomically mixed strains.

Frequent Loss-of-Heterozygosity. Heterozygous sites are distributed genome-wide but most of the heterozygous strains also exhibit multiple, large (>200 kb) chromosomal regions that are nearly completely homozygous (Fig. 1*A* and *SI Appendix*, Figs. S1–S12). Because these regions of homozygosity are found against a genomic background of high heterozygosity, we attribute such features as due to LOH events. Large-scale LOH is thought to primarily result from mitotic recombination initiated by double-stranded breaks during replication (20). Meiotic recombination can also lead to LOH but the size of such regions is generally smaller and they are more uniformly distributed across chromosomes (21).

Several chromosomal regions appear to be particularly prone to LOH. For example, four of the eight heterozygous genomes have large LOH regions on the right arm of chromosome XII (Fig. 1*B*). In three strains the LOH border is in close proximity to a replication origin, ARS1216, just upstream of the tandem rDNA array. This region on chromosome XII is the portion of the yeast genome that is most sensitive to DNA lesions caused by perturbations to origin firing (22) and hence is likely to be a hot spot for frequent mitotic recombination events. Other chromosomal regions, for example on the right arms of chromosomes II and IV, also exhibit large LOH blocks in multiple strain backgrounds.

Recent studies support the idea that mitotic recombination and LOH play an important role in fungal genome evolution. In *S. cerevisiae* LOH has been noted as occurring frequently in wine fermentations (23). In *Candida albicans*, LOH events occur during infection (24) and contribute to the acquisition of antifungal resistance (25). Loss-of-heterozygosity has also been proposed to contribute to genotypic diversity in the chytrid fungus *Batrachochytrium dendrobatidis*, the causal agent of the emerging amphibian disease chytridiomycosis (26).

A Life-History Trade-Off Correlates with Genomic Heterozygosity. What contributes to the maintenance of heterozygosity in some lineages but not others? One possibility is that heterozygosity itself is advantageous (heterosis or hybrid vigor). Several studies have suggested a link between heterozygosity and fitness under conditions relevant to fungal virulence (27, 28). Another possibility, and the one we explore here, is that differences among lineages in the relative frequencies of sexual and asexual cycles might contribute to variation in the maintenance of heterozygosity. None of the strains we examined are obligate asexuals (they all sporulate and produce viable spores); we therefore considered the propensity to sporulate, a trait that should influence the frequency of sexual cycles. We measured sporulation efficiency (the percentage of sporulated cells after 48 h in sporulation medium) in our panel of environmental isolates and found that heterozygous strains tend to sporulate inefficiently whereas homozygous strains are strong sporulators (Fig. 2*A*; P value <0.0001, Mann–Whitney U test). There is an approximately sevenfold difference in the mean sporulation efficiency between heterozygous and homozgous strains. We hypothesize

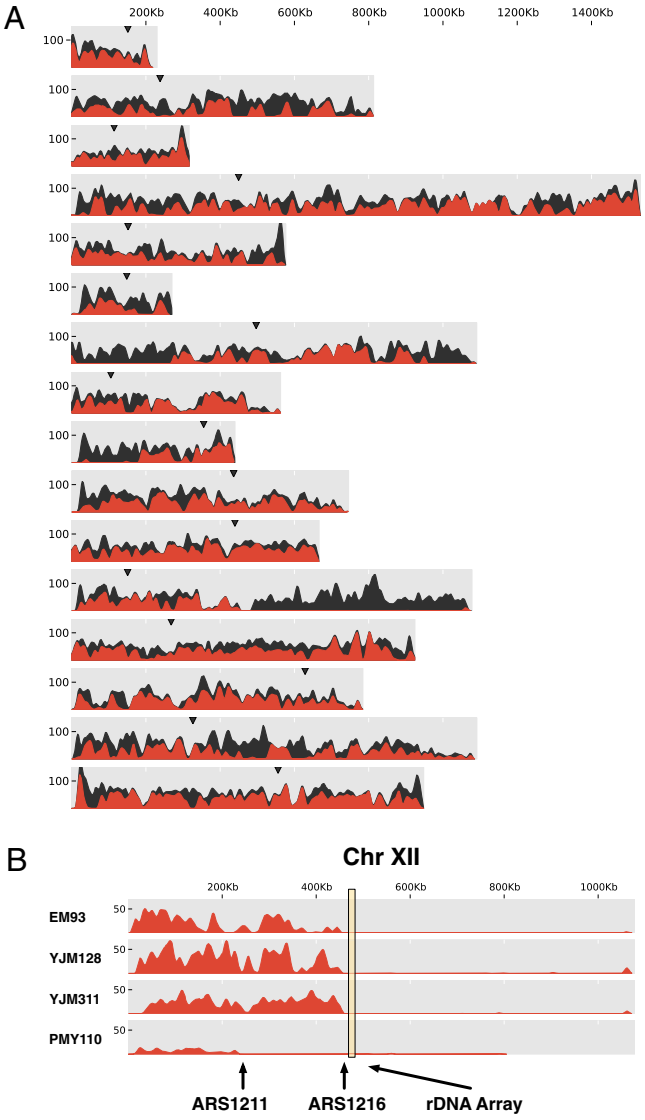

Fig. 1. (*A*) The distribution of SNPs and heterozygous sites across the 16 nuclear chromosomes of strain YJM128. The black curves indicate the number of SNPs (both homozygous and heterozygous) per 10-kb window relative to the reference yeast genome. The red curves indicate the number of heterozygous SNPs per 10 kb. The inverted triangles indicate the location of the centromeres. (*B*) The distribution of heterozygous sites on chromosome (Chr) XII for four yeast strains. The red curves indicates the number of heterozygous sites per 10-kb window. The yellow box depicts the approximate position of the repetitive rDNA array on Chr XII.

that poor sporulation efficiency facilitates the maintenance of heterozygosity because selective forces favoring heterozygosity can be weak and yet effective as long as opportunities to inbreed are rare. A decrease in the relative number of sexual cycles would also serve to magnify the relative importance of mitotic recombination (see *Estimating Clonal Generations Using LOH*).

Because sporulation is a developmental response to nutrient limitation (29), we also explored the relationship of heterozygosity to pseudohyphal growth, another nutrient-induced developmental response. Pseudohyphal growth is a form of diploid mitotic growth that is characterized by unipolar budding, cell elongation, increased adhesion, and invasive growth (30). The signals that induce pseudohyphal growth and sporulation partially overlap; nitrogen starvation and a brief G1 arrest are necessary to induce either pathway (31, 32). However, fermentable carbon sources, like glucose, inhibit sporulation (29) whereas pseudohyphal growth is robust in the presence of glucose (although perhaps even stronger in the presence of sucrose) (33).

Sporulation and pseudohyphal growth represent two distinct strategies to cope with environmental stress: continued asexual growth (pseudohyphal growth) or induction of the sexual cycle (sporulation). Life-history theory (34, 35) predicts the existence of trade-offs between components of asexual versus sexual fitness or traits that contribute to reproduction versus survival. We therefore predicted that there should be a negative relationship between sporulation and pseudohyphal growth, and as a correlate of this relationship pseudohyphal strains would be more likely to be heterozygous.

As predicted, among the strains included in our survey there is a phenotypic trade-off: Strongly pseudohyphal strains tend to be inefficient sporulators and vice versa (Fig. 2*B*; P value <0.001, Mann–Whitney U test). Also as predicted, most pseudohyphal strains exhibit substantial heterozygosity (P value <0.001, G-test with Williams correction). To further explore the generality of this finding we expanded our phenotyping to include a larger panel of strains. As in our genotyping panel we observed a strong, negative association between sporulation and pseudohyphal growth, although the relationship is nonlinear (*SI Appendix*, Fig. S13). Except in one case (the strain SK1), *S. cerevisiae* strains are strong sporulators and weak pseudohyphal growers, strongly pseudohyphal but weakly sporulating, or poor performers in both assays. Many of the strongest sporulators were isolated from nonagricultural plant sources. The most strongly pseudohyphal strains include vineyard, distillery, and clinical isolates.

To summarize, the genomic and phenotypic data suggest that *S. cerevisiae* environmental isolates fall into one of two classes: *i*) strains that are efficient sporulators, weakly or nonpseudohyphal, and homozygous or *ii*) inefficient sporulators with strong pseudohyphal growth and substantial heterozygosity. We hypothesize that these two genotypic/phenotypic classes result from selection for alternative responses to nutrient stress, perhaps associated with differences in the quality or patchiness of resources in different niches (36). We predict that pseudohyphal growth is most likely to be an effective strategy in environments where the characteristic scale of resource patchiness is short (either spatially or temporally). On the other hand, efficient sporulation might be selected for in environments where resource patchiness has a longer characteristic scale, thus favoring a "hunker down" strategy. We hypothesize that human association both increases opportunity for outcrossing among yeast lineages and provides niches that favor the pseudohyphal response, which in turn leads to facultative asexuality as a result of antagonistic pleiotropy between pseudohyphal growth and sporulation. Facultative asexuality in turn contributes to the maintenance of genomic heterozygosity.

Estimating Clonal Generations Using LOH. A prediction that follows from the facultative asexuality hypothesis is that facultatively asexual lineages are likely to experience a large number of clonal generations between sexual cycles. We used the number of LOH regions in each strain (*SI Appendix*, Table S3) and published estimates of rates of mitotic recombination (37, 38) to estimate the number of clonal generations that each lineage experienced since outcrossing on the basis of a molecular clock analysis (*SI Appendix*, *Materials*). Among the eight strains for which we were able to carry out this analysis our estimates ranged from 12,500 to 62,500 clonal generations, with an average across strains of between 25,000 and 35,000 clonal generations. These estimates are compatible with the estimated rate of outcrossing (1 in 50,000 generations) that Ruderfer et al. (5) arrived at on the basis of estimates of the frequency of recombination between lineages. The ubiquity of large LOH regions among the heterozygous strains is thus consistent with long periods of clonality predicted by the facultative asexuality hypothesis.

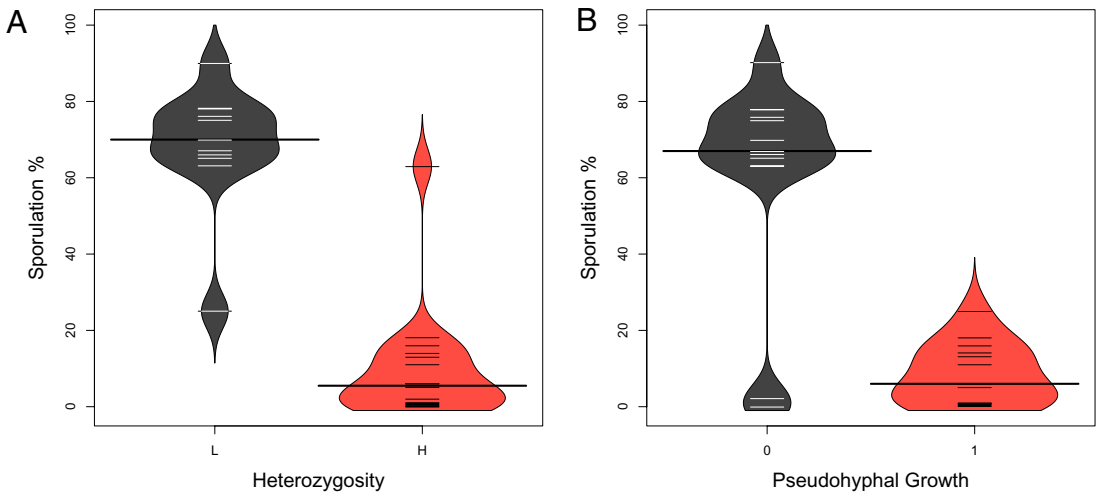

Fig. 2. (*A*) Sporulation efficiency as a function of heterozygosity class and (*B*) sporulation efficiency as a function of pseudohyphal growth for the 28 *S. cerevisiae* strains included in the genome sequencing/genotyping panel. In *A*, L and H indicate low and high heterozygosity, respectively.

The prevalence of LOH is particularly interesting in the context of the facultative asexuality model. A recent theoretical analysis by Mandegar and Otto (37) showed that LOH driven by mitotic recombination can substantially reduce the time between the appearance of a beneficial mutation in a heterozygote and the production of a mutant homozygote. They predicted that mitotic recombination "might thus facilitate the persistence of diploid asexuals" and that higher mitotic recombination rates would occur among organisms that are predominantly asexual (ref. 37, p. 1306). Our findings are consistent with these predictions and suggest that different lineages of *S. cerevisiae* may be an ideal platform to test this and other theoretical models for the evolution and maintenance of sex.

Variation in RME1 Expression Contributes to the Phenotypic Trade-Off Between Sporulation and Pseudohyphal Growth. Given the striking trade-off we observed between sporulation and pseudohyphal growth, we sought to identify genetic loci that might contribute to this interaction. A promising candidate locus that we predicted should have antagonistic pleiotropic effects on sporulation and pseudohyphal growth is RME1 (Repressor of Meiosis). Rme1p is a zinc-finger transcription factor involved in inhibiting meiosis by repressing the expression of *IME1*, a meiotic initiator (39, 40). Allelic variation in the promoter region of RME1 has been shown to affect sporulation ability (41–43). The polymorphic region falls within a predicted binding site for the heterodimeric a1/α2 transcription factor complex that regulates key transcriptional differences between haploid and diploid cells (44). One allele (the S288c allele) correlates with higher levels of expression of RME1 and consequently a lower sporulation percentage. Strains with the alternate allele (the SK1 allele) have lower RME1 expression and sporulate more proficiently (42). In addition to its role as a negative regulator of meiosis, van Dyck et al. (45) showed that Rme1p has a positive effect on invasive growth, by regulating the transcription of *FLO11*, a cell-wall–expressed glycoprotein important for cell adhesion. On the basis of these previous studies, we predicted that strains with weak sporulation/strong pseudohyphal growth would be characterized by higher levels of RME1 expression than strains that exhibit the strong sporulation/weak pseudohyphal growth phenotype.

We genotyped a panel of 30 yeast strains at the RME1 promoter site and measured the relative expression of RME1 in those same strains under nitrogen starvation conditions using qPCR (*Materials and Methods* and *SI Appendix*, *Materials*). Strains that are homozygous for the S288c allele (S288c/S288c) or heterozygous (S288c/SK1) show an approximately threefold higher mean RME1 expression than strains homozyogus for the SK1 allele (SK1/SK1) (*SI Appendix*, Fig. S14*A*). The mean sporulation efficiency of S288c/S288c and S288c/SK1 strains is 18% whereas that of SK1/SK1 genotypes is 60%, although there are outliers in both groups (*SI Appendix*, Fig. S14*B*). A linear regression of sporulation efficiency on RME1 is significant with a modestly large coefficient of determination (R^2 = 0.5; *SI Appendix*, Fig. S14*C*). To test whether RME1 expression is predictive of the trade-off we coded both sporulation efficiency and pseudohyphal growth as binary variables and regressed each trait on RME1 expression using a logistic regression model. The model coefficients for RME1 expression are significant for both phenotypes (sporulation, P = 0.005; pseudohyphal growth, P = 0.007). Fig. 3 combines the predictions from the two logistic regression models; it suggests that strains that have RME1 expression values less than ~ −8.8 have a >50% probability of being strong sporulators whereas strains with RME1 expression > −8.6 are most likely to be pseudohyphal. For RME1 expression values in the range −8.8 to −8.6, the models predict probabilities of <0.5

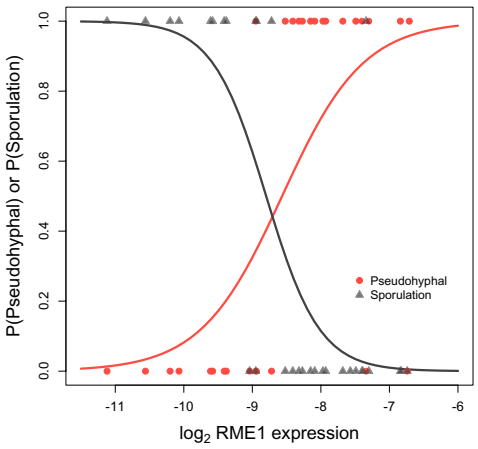

Fig. 3. Logistic regressions of sporulation (black) and pseudohyphal growth (red) on RME1 expression. The predicted probabilities are shown of a strain being strongly sporulating or strongly pseudohyphal as a function of relative RME1 expression.

for either phenotype, suggesting that this range of relative expression values represents a threshold region for the trade-off.

In sum, variation in the expression of RME1 appears to be an important contributor to the phenotypic trade-off between sporulation and pseudohyphal growth. However, there is significant residual variation in both sporulation and pseudohyphal growth ability that is not correlated with RME1 expression. A promising direction for future studies is to identify additional genetic and mechanistic interactions that contribute to this trade-off.

Summary and Conclusions

Our genome-wide survey of heterozygosity in *S. cerevisiae* demonstrates that outcrossing and mitotic recombination, although numerically rare, have a significant impact on genome architecture and evolution. Whereas there may be tens of thousands of generations between outcrossing events, the impact of outcrossing tends to persist in facultatively asexual lineages. This is attested to by the prevalence of heterozygous strains isolated from human-associated environments. Loss-of-heterozygosity, driven by mitotic recombination, may facilitate the persistence of asexual lineages. Recent reports suggest that *S. cerevisiae* is not the only fungus whose genome bears unexpected evidence of (para)sexual cycles and mitotic recombination (26, 46, 47). The genetic variation generated by both of these processes is likely to influence adaptation to novel environments (48) and is particularly relevant to understanding the variation and evolution of traits of clinical, agricultural, and industrial interest.

Our study also sheds new light on the evolution and maintenance of sex. Our findings support a model that posits that preferential maintenance of heterozygosity in some yeast lineages relates to a life-history trade-off between asexual and sexual modes of reproduction. We hypothesize that this trade-off is driven by selection for alternative developmental responses to nutrient starvation that are favored in different niches. Life-history theory predicts that the trade-off should be due to loci with antagonistic pleiotropic effects on sporulation and pseudohyphal growth, such as the case of RME1 illustrated here. The availability of rapid QTL mapping methods (49) and an increasingly detailed knowledge of gene networks in yeast (50) hold the promise that a detailed understanding of the genetic and mechanistic basis of this life-history trade-off is within reach.

Materials and Methods

Genomic Sequencing and Short-Read Mapping and SNP Calling. Library construction followed Illumina sample prepration kit instructions, and we used ≈5 ng extracted DNA (Genomic-Tip; Qiagen) as starting material. We cloned a portion of each library and sequenced it for further validation. Libraries were processed on the Illumina GAII Genome Analzyer at Duke University's DNA Sequencing Facility. Short reads were mapped to the genome of the standard *S. cerevisiae* reference genome (obtained from the *Saccharomyces* Genome Database, January 2010), using the short-read mapping software MAQ, version 0.7.1 (51) and BWA, version 0.5.0 (52). For MAQ, SNP calls were made using the default parameters of the "easyrun" option of the maq.pl script. For BWA, SNP calls were made using the "pileup" and "varFilters" options of SAMtools, version 0.1.7 (53). Custom Python scripts were used to filter the SNP calls on the basis of the read depth and quality of the aligned reads and to identify the subset of SNP calls that were called identically by the MAQ and BWA algorithms.

Sanger Sequencing. To verify calls from the whole-genome sequence data, we selected 20 loci from 14 chromosomes, each with either two or three predicted heterozygous sites. Primers were designed in regions lacking polymorphic sites. We then sequenced these regions in five highly heterozygous strains at the DNA Sequencing Facility at Duke University on an Applied Biosystems 3730xl DNA Analyzer. For the broader survey of heterozygosity, we selected 18 additional strains collected from woodland, vineyard, and clinical settings (*SI Appendix*, Table S2) and sequenced nine genes of interest. Fragments that failed to produce clean sequences from PCR amplicons were cloned using the StrataClone PCR cloning kit from Stratagene. We sequenced at least three clones per fragment as described above. Sequences were assembled and edited, and heterozygous calls were made using CodonCode Aligner v2.0.

Media and Phenotypic Assays. Yeast strains were grown overnight in YPD medium to a density of 2×10^7 cells/mL. The cells were washed several times in sterile water and transferred to either liquid media (sporulation; density 10^5 cells/mL) or agar plates (pseudohyphal growth; plating density 5×10^3 cells/plate). The sporulation media [low-ammonium sporulation (LA-SPO)] used is composed of 0.17% yeast nitrogenous base without amino acids and ammonium sulfate (YNB − AA/AS), 1% potassium acetate, and 50 μM ammonium sulfate. To study pseudohyphal growth we used a modified SLAD medium (30) consisting of 0.17% YNB − AA/AS, 1% glucose, 50 μM ammonium sulfate, and 2% Noble agar. Sporulation and pseudohyphal growth were assayed 48 h after transfer to the appropriate medium. Sporulation was quantified as the percentage of sporulated cells (including tetrads, triads, and dyads). Pseudohyphal growth was treated as a binary trait that was scored on the basis of the appearance of elongated cells, unipolar budding, and agar invasion.

Gene Expression Measurement by qPCR. Total RNA was isolated from cells grown in SLAD media (54). cDNA was generated using an iScript cDNA synthesis kit (BioRad). Gene expression was measured by quantitative RT-PCR using the iQ SYBR Green kit (BioRad). qPCR reactions were carried out on a Rotor-Gene 6000 real-time cycler (Corbett Research). We used the ACT1 gene as a "housekeeping" gene to normalize RME1 expression values. For these strains and growth conditions ACT1 exhibits relatively small interstrain variation and thus is a good basis for normalization. Primers for the qPCR reactions are as follows: (*i*) ACT1, forward primer, CCTTCTGTTTTGGGTTTGGA; (*ii*) ACT1, reverse primer, AGCGGTGATTTCCTTTTGC; (*iii*) RME1, forward primer, TCCAACAAGCAACTGGTCTG; and (*iv*) RME1, reverse primer, TTTGCGCCTAAACGTTTTC. Primers were synthesized by Sigma-Aldrich. Relative expression of RME1 was calculated as $\log_2(E_R^{Ct_R} - E_T^{Ct_T})$, where E_R and E_T are the amplification efficiencies of the reference (ACT1) and the target gene (RME1) and Ct_R and Ct_T are the measured cycle thresholds for each gene during the qPCR reaction.

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