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Note

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abstract: Gene combinations conferring local fitness may be destroyed by mating with individuals that are adapted to a different environment. This form of outbreeding depression provides an evolutionary incentive for self-fertilization. We show that the yeast *Saccharomyces paradoxus* tends to self-fertilize when it is well adapted to its local environment but tends to outcross when it is poorly adapted. This behavior could preserve combinations of genes when they are beneficial and break them up when they are not, thereby helping adaptation. Haploid spores must germinate before mating, and we found that fitter spores had higher rates of germination across a 24-hour period, increasing the probability that they mate with germinated spores from the same meiotic tetrad. The ability of yeast spores to detect local conditions before germinating and mating suggests the novel possibility that these gametes directly sense their own adaptation and plastically adjust their breeding strategy accordingly.

Keywords: local adaptation, inbreeding, outcrossing depression, germination, fitness.

Introduction

An organism's success depends on its genotype and its environment. Because environments vary, genotypes experience different selection in different places (Kalisz 1986). If the effect of selection is not overwhelmed by drift or migration, then local adaptation can occur (Williams 1966; Lande and Schemske 1985; Kawecki and Ebert 2004). Local adaptation has been demonstrated many times in a wide range of organisms, including plants (e.g., Schemske 1984), animals (e.g., in salmonid fishes; Fraser et al. 2011), and microbes (Belotte et al. 2003), by reciprocal-transplant experiments, which show that the fitnesses of genotypes at their original sites are higher than the fitnesses of genotypes imported from other sites (Hereford 2009).

Local adaptation requires local genetic differentiation (Linhart and Grant 1996). In plants, genetic differentiation among populations is greater for selfing species than for outcrossing species (Hamrick and Godt 1996), and selfing is thought to promote speciation by increasing reproductive isolation between sympatric populations, both in plants (Wendt et al. 2002; Martin and Willis 2007) and in animals (Puritz et al. 2012). Although genetic differentiation may occur as an unselected side effect of selfing, selfing can be directly selected because it reinforces reproductive isolation (e.g., Fishman and Wyatt 1999). Similarly, selfing is expected to be selected in locally adapted populations because it maintains adaptation, reducing the outbreeding depression that would be caused by mating with individuals from other locally adapted populations (Antonovics 1968; Jain 1976; Epinat and Lenormand 2009). This strategy is exemplified by the touch-me-not, *Impatiens capensis*, which not only produces a mixture of chasmogamous (outcrossing) and cleistogamous (selfing) flowers but also sends outcrossed seeds farther, by ballistic dispersal, than selfed seeds (Schmitt et al. 1985). An elegant experiment showed that the fitness of seedlings derived from selfed seeds, relative to that of those derived from outcrossed seeds, decreased as they were moved away from the parental site (Schmitt and Gamble 1990). While there is no generally recognized association between dispersal and outcrossing, it is also observed in amphicarpic plants, which produce subterranean, nondispersing seeds that are selfed and aerial, dispersing seeds that are outcrossed (Cheplick 1987), and in budding yeast, which outcrosses more in response to dispersal by an insect vector (Reuter et al. 2007).

Here we investigate how the rate of inbreeding in yeast is affected by adaptation to the local environment. *Saccharomyces cerevisiae* is widely used for winemaking and as a laboratory model organism, but there is also increasing interest in the natural history, ecology, and evolution of this species and its wild relatives in the genus *Saccharomyces* sensu stricto (Replansky et al. 2008; Greig and Leu

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2009). *Saccharomyces* spp. reproduce mainly by diploid mitosis, but when nutrients become scarce, a diploid cell can enter meiosis and produce a tetrad of four equally sized haploid spores (two of each mating type, *MAT*a and *MAT*α) enclosed within an ascus and joined by interspore bridges (Coluccio and Neiman 2004). Spores are resistant and dormant, but when a spore detects nutrients, it can germinate into a metabolically active haploid cell, mate by fusing with another haploid of the opposite mating type, and produce a new diploid zygote that can begin mitotic growth again. Selfing can occur between haploids from the same meiotic tetrad (equivalent to sporophytic selfing; for clarity, we call this "within-tetrad mating" hereafter), or outcrossing can occur between haploids from different tetrads (hereafter "between-tetrad mating"). Any unmated haploids can divide by haploid mitosis until they find mating partners, but a haploid that has already divided can also switch mating type at the following mitotic division, thus allowing it to mate with its previous clonal daughter cell to produce a homozygous diploid in an extreme form of inbreeding (equivalent to gametophytic selfing; hereafter "autodiploidization").

Measurements of the frequencies of these different forms of mating vary greatly. Tsai et al. (2008) examined the genetic variation within a wild population of *Saccharomyces paradoxus* and calculated 94% within-tetrad mating, 1% between-tetrad mating, and 5% autodiploidization, consistent with the high rate of inbreeding in *S. cerevisiae* estimated from phylogenetic analysis (Ruderfer et al. 2006). In contrast, Goddard et al. (2010) estimated that wild *S. cerevisiae* in New Zealand vineyards outcross in 20% of matings. When inbreeding was measured directly in the laboratory, the frequency of between-tetrad mating was only 0.2%, but this was increased tenfold when tetrad ascospores were fed to *Drosophila melanogaster* (Reuter et al. 2007). This dispersal vector digested the asci and interspore bridges, releasing the resistant spores from their tetrads and increasing outcrossing. Another recent laboratory study measured surprisingly high frequencies of between-tetrad mating of up to 25%, even though the tetrads used were intact and undigested (Murphy and Zeyl 2010).

We suspected that the variation in measurements of within-tetrad mating rates was likely to be affected by the local environment, because mating in yeast occurs after spore germination and spore germination depends on sensing local conditions (Palleroni 1961; Savarese 1974). Spores that germinate at different times are unlikely to mate: indeed, differences in germination timing between yeast species contribute to reproductive isolation between species (Maclean and Greig 2008; Murphy and Zeyl 2012). Similarly, hybridization between plant species can be prevented if they have different flowering times (e.g., Young 1996; Pascarella 2007), and locally adapted races can be maintained within a population by differences in their flowering times, most notably in natural *Anthoxanthum odoratum* (McNeilly and Antonovics 1968) as well as in the Park Grass Experiment (Snaydon and Davies 1976; Silvertown et al. 2005). If the four spores within a *Saccharomyces* tetrad germinate at the same time, they can form two mating pairs and mate entirely within the tetrad (as modeled in Tazzyman et al. 2012). But if the spores within a tetrad germinate at different times, then they are less likely to mate together and more likely, therefore, to mate with an available haploid from another tetrad or to autodiploidize if no mates are available. We hypothesized that high-fitness environments would increase the probability of spore germination per unit time, so that spores in the same tetrad would be more likely to germinate at the same time and mate together. In contrast, if low-fitness environments reduce the probability of spore germination per unit time, then the variance in spore germination timing would be greater and germinated spores would be less likely to find mates from the same tetrad, either because potential partners would not yet have germinated or because they would have already germinated and mated. The higher variance in germination timing would reduce within-tetrad mating and increase opportunities for between-tetrad mating. Thus, we expected more within-tetrad mating when a genotype was in an environment to which it was well adapted. Such a mechanism could itself be adaptive, because it would tend to preserve allele combinations that confer high fitness in the local environment but would generate new allele combinations that may allow adaptation to low-fitness environments.

Therefore, we tested five different wild yeast strains to confirm the predicted relationship between adaptation and inbreeding. We measured each strain's competitive fitness and within-tetrad mating rate, relative to a standard laboratory strain, on three different types of media. To investigate whether spore germination could mediate the response of the mating system to the local environment, we measured the overall spore germination rates for each strain on each medium, and we assayed the time course of germination for one of the strains on the three media.

Material and Methods

Strains and Media

We used three *Saccharomyces paradoxus* strains isolated from oak trees in Plön, Germany (SpPlön1, SpPlön2, and SpPlön3); two *S. paradoxus* strains isolated from oak trees in Pennsylvania (Sp1 and Sp2), which were used in a previous inbreeding study (as YPS664 and YPS646, respectively; Murphy and Zeyl 2010); and one *Saccharomyces*

cerevisiae laboratory strain, Y55, as a standard. All six diploid strains were originally derived from single haploid cells that autodiploidized, thereby making homozygous diploids.

From each strain, we constructed two isogenic diploid lines that were homozygous for different dominant antibiotic-resistance markers inserted into the *HO* locus. We used primers HO_SParaF (AAT TTT TTA TCA GTA ACC GTA ACT GAG ACT ATT ACT CAA TCA TTC AAG TAA AGA GAT CAC CAA atc gat gaa ttc gag ctc g) and HO_SParaR (ATA AGC AGC AAT CAA TTT CAT CTA ACT TCA ACA TGC TTT CTG AGA ACA CAA CTA TTC TGA cgt acg ctg cag gtc gac) to amplify either the KANMX4 cassette or the HYGMX4 cassette, which confer antibiotic resistance to G418 and hygromycin, respectively (Goldstein and McCusker 1999). We transformed both of these into all five *S. paradoxus* strains, using a lithium acetate/single-stranded carrier DNA/50% polyethylene glycol transformation (Gietz and Woods 2002), to construct two diploid lines, homozygous for different antibiotic-resistance markers, derived from each strain. We used the same protocol for *S. cerevisiae* strain Y55, but with primers HO_SCereF (ATT AAA TTT TAC TTT TAT TAC ATA CAA CTT TTT AAA CTA ATA TAC ACA TT atc gat gaa ttc gag ctc g) and HO_SCereR (TCT AAA TCC ATA TCC TCA TAA GCA GCA ATC AAT TC cgt acg ctg cag gtc gac). For all primers, lowercase letters indicate overlap with the gene cassette and uppercase letters indicate overlap with the *HO* locus. This procedure eliminated positions 2041–3911 in *S. paradoxus* (strain CBS432 numbering) and positions 155–1932 in *S. cerevisiae* (strain S288c numbering) from the *HO* locus and replaced them with the antibiotic-resistance cassette. Disrupting the *HO* gene prevented mating-type switching and autodiploidization, so that only within-tetrad mating and between-tetrad mating were possible.

Unless otherwise stated, we grew cultures in 5 mL YPD (2% dextrose, 2% bactopeptone, 1% yeast extract), and we incubated at 30°C. We shook liquid cultures at 225 rpm, or we added 2.5% agar to make solid plates. We transferred approximately 6×10^7 cells from overnight YPD cultures into 2 mL sporulation medium (2% potassium acetate, 0.22% yeast extract, 0.05% glucose, 0.087% complete amino acid mix) and incubated at room temperature for 2 days, which was sufficient to induce more than 90% sporulation in all strains.

Fitness Assays

We measured the diploid fitnesses of each of the five wild *S. paradoxus* strains relative to Y55 in paired direct competitions (Wu et al. 2006). One antibiotic-resistance-marked line from each of the wild strains (the *ho::HYGMX4/ho::HYGMX4* homozygous diploid line of each wild strain) was mixed with the Y55 line carrying a different marker (i.e., the *ho::KANMX4/ho::KANMX4* homozygous diploid line of Y55), and each mixture was propagated asexually in three different media: YPD, 10% YPD (10% of each component of YPD), and SOE (1% sucrose, 0.5% dextrose, 0.5% fructose, 0.15% bactopeptone, 0.1% yeast extract), which approximates oak tree sap in saccharide and nitrogen content. We inoculated 5 mL of liquid medium with approximately 1.5×10^6 cells of the mixture. Every 24 hours, a fraction of the culture was diluted into 5 mL of fresh medium, and the frequency of the two competing lines was determined by diluting and plating onto YPD agar plates to yield single colonies and replica plating these onto G418 (200 mg/L) and hygromycin (300 mg/L) agar plates to determine their genotype. We used the different antibiotic-resistance markers to determine the numbers of the two strains in each fitness assay at the beginning and end of the growth period, and we calculated relative asexual fitness by using the least squares slope between the dilution rate and the frequency of hygromycin-resistant colonies (Wu et al. 2006). Because fitnesses varied considerably, depending on strain and medium, we optimized the initial frequencies of strains and the daily dilution rate (1 : 10 or 1 : 100), so that the frequency of the tested strain could be accurately determined. All 15 combinations of strain and medium combinations were tested in least three replicate fitness assays.

Mating Assays

The two different antibiotic-resistance-marked lines (i.e., *ho::HYGMX4/ho::HYGMX4* and *ho::KANMX4/ho::KANMX4*) of each strain were sporulated. We mixed intact tetrad asci from both lines together in equal volumes. We concentrated the cells by centrifugation, washed them, and resuspended them in 100 μL of water. We placed a 5-μL spot of each mixture of intact tetrads onto YPD, 10% YPD, and SOE agar plates. After 24 hours of incubation at 30°C to allow spore germination and mating, a sample from each strain's mixture was then tested to determine the ploidy and antibiotic resistance of the individuals in it. We cut out each growth patch from its plate, washed the yeast cells off into 5 mL of water, and diluted and plated each cell suspension separately onto YPD and double-antibiotic (200 mg/L G418 and 300 mg/L hygromycin) agar plates to yield single colonies, which were counted to determine the frequency of double-antibiotic-resistant colonies (i.e., those diploids that resulted from between-tetrad mating). To determine the frequency of unmated haploids, we replicated the YPD agar plates onto sporulation agar plates and incubated them for 1 week at room temperature. We had previously verified that under these conditions and

for all of these strains, haploid colonies (i.e., those that could mate but not sporulate) were whiter and were clearly distinguishable from diploid colonies (i.e., those that could sporulate). We therefore scored all plates using this criterion in order to calculate the frequency of mated, diploid cells.

Two spores mating within the tetrad could produce only a homozygous diploid of the parental genotype, resistant to only one antibiotic. Mating between two spores from different tetrads could produce a diploid that was heterozygous for the antibiotic-resistance alleles inserted at the *HO* locus and thus resistant to both antibiotics. We expected $m(2f_mf_n)$ double-antibiotic-resistant colonies, where f_m and f_n are the initial frequencies of single-resistant colony-forming units and m is the frequency of between-tetrad mating. We corrected for unmated cells by adjusting the expected number of double-antibiotic-resistant colonies by the diploid frequency after mating, f_d, and we calculated the inbreeding frequency $(1 - m)$ from

$$m(2f_mf_n)f_d = f_{mn},$$

where f_{mn} is the frequency of double-antibiotic-resistant colonies. We can rearrange this equation to

$$m = \frac{f_{mn}}{2f_mf_n} \times \frac{1}{f_d},$$

to find the between-tetrad mating frequency, or subtract the between-tetrad mating frequency from 1 to calculate the within-tetrad mating frequency:

$$\text{within-tetrad mating frequency} = (1 - m)$$

$$= 1 - \left(\frac{f_{mn}}{2f_mf_n} \times \frac{1}{f_d}\right).$$

We measured the within-tetrad mating frequency three times for each strain × medium combination.

Spore Germination Frequency Assays

Within-tetrad mating can occur only if spores of opposite mating type in the same tetrad are available to mate at the same time. We hypothesized that higher frequencies of spore germination would cause higher within-tetrad mating. We used the five wild homozygous diploid strains lacking any drug-resistance markers for this experiment. We streaked each from a YPD agar plate onto sporulation agar. After 2 days of incubation at room temperature, we digested a sample of the resulting tetrads from each strain in 10 μL of 10-U/μL zymolyase for 30 minutes before adding 500 μL sterile water and dissecting the 80 spores from 20 tetrads onto the three media, using a micromanipulator. After 24 hours of incubation at 30°C, we used the microscope to determine whether every spore had germinated or not. We used the spore germination frequency of each set of 80 spores to calculate the expected proportion of tetrads with potential for within-tetrad mating, for all 15 combinations of strain and medium. We calculated the proportion of tetrads with potential within-tetrad mating as the expected frequency of tetrads with three or four germinated spores plus two-thirds of the expected frequency of tetrads with two germinated spores, as one-third of tetrads with exactly two germinated spores will contain only one mating type.

It is formally possible that the germination probability of a spore correlates to that of the other spores in the same tetrad: for example, a single strain might produce some tetrads whose spores all have high germination probabilities and other tetrads whose spores all have low germination probabilities, even though all are genetically identical. This would mean that the overall germination rate would not accurately predict the proportion of tetrads with potential for within-tetrad mating. To eliminate this concern, we examined the distribution of tetrads with no, one, two, three, or four germinated spores, and we compared this to the expected distribution if all spores had the same germination probability for a given strain and environment. We found no evidence that the germination probability of spores varied within each combination of strain and environment ($P > .18$ for all strain and environment combinations; χ^2 test after categories that had fewer than three observed cases were eliminated).

Spore Germination Time Course Assays

In order to investigate the time course of spore germination, we sporulated both antibiotic-resistance-marked lines of SpPlön3 and mixed equal volumes of the tetrads, placing a 5-μL spot of the mixture into the center of agar plates of each of the three media, as with the mating assays. Instead of assaying after 24 hours, we cut out the spots from three replicate plates of each medium at 0-, 1-, 3-, 6-, 9-, and 12-hour time points and resuspended them in 5 mL of water, as for the mating assays. We spun down 1,000 μL from each sample, resuspended the cells in 1,000 μL 1% NaOH, and incubated at 30°C, with 1,000-rpm shaking for 10 minutes. Then we added 1,000 μL 1M HCl to neutralize, inverted the tubes to mix, spun down the cells, and removed 1,500 μL of the solution in order to resuspend the cells in the remaining 500 μL. Previous experiments determined that this treatment kills 0% (95% confidence interval: 6.88%–0%) of spores and more than 99.94%, 99.99%, and 99.99% of log-phase diploid cells in 10% YPD, YPD, and SOE, respectively. We serially diluted and plated these spores to yield single colonies on YPD plates, and we counted the colonies to determine the num-

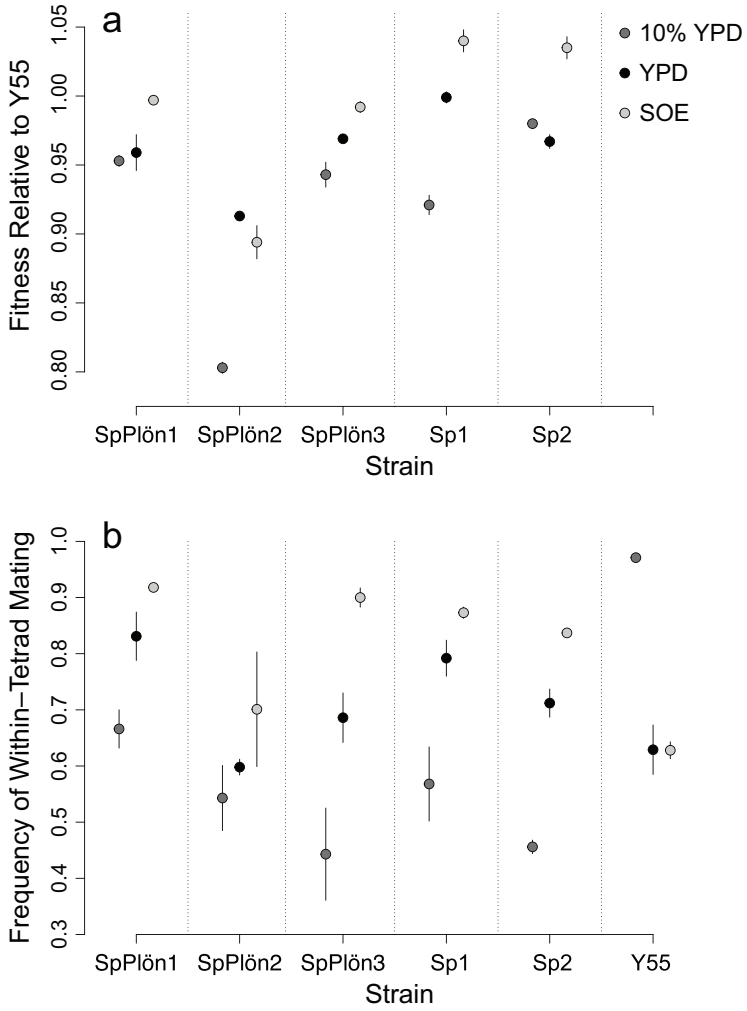

Figure 1: Effect of mating medium on relative fitness (*a*) and within-tetrad mating frequency (*b*). We measured the fitness of G418-resistant lines of five wild *Saccharomyces paradoxus* strains on three media relative to hygromycin-resistant *Saccharomyces cerevisiae* strain Y55; separately, the two antibiotic-resistant derivatives of the six strains were allowed to mate for 24 hours on three different media in order to measure within-tetrad mating frequencies. Error bars show the standard error across three independent assays. Media: YPD = 2% dextrose, 2% bactopeptone, 1% yeast extract; 10% YPD = 10% of each component of YPD; SOE = 1% sucrose, 0.5% dextrose, 0.5% fructose, 0.15% bactopeptone, 0.1% yeast extract.

ber of spores that remained ungerminated at each time point. We did not use a 24-hour time point because by this time the cultures were entering stationary phase, during which vegetative diploids become more resistant to NaOH.

Results

All raw data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.r0g9m (Miller and Greig 2014).

Relationship between Fitness and Within-Tetrad Mating

We measured the fitnesses and the within-tetrad mating frequencies of the five wild strains in three different environments, relative to those of the standard laboratory strain Y55 (fig. 1*a*). Two-way ANOVA on relative fitness showed that strain ($F_{4, 51} = 130.46$, $P < .001$), medium ($F_{2, 53} = 137.82$, $P < .001$), and their interaction ($F_{8, 47} = 14.11$, $P < .001$) affected their relative fitnesses (fig. A1, available online). Two-way ANOVA on within-tetrad mating showed that strain ($F_{4, 40} = 7.76$, $P < .001$) and medium ($F_{2, 42} = 57.2$, $P < .001$), but not their interaction ($F_{8, 36} = 1.79$, $P = .118$), also had significant effects on the fre-

quencies of within-tetrad mating frequency. Figure 1*b* shows that the within-tetrad mating frequency in wild strains was lowest on 10% YPD and highest on SOE. Y55 had significantly different inbreeding frequencies on SOE and on 10% YPD, compared to the wild *S. paradoxus* strains ($P < .001$ for both comparisons; Student's *t*-test corrected for multiple comparisons), but not on YPD ($P = .147$; Student's *t*-test). Thus, in wild strains and in laboratory strain Y55, inbreeding frequencies depended on strain identity and the environment that they mated in.

As we predicted, there was a significant correlation between fitness and the frequency of within-tetrad mating, both relative to Y55 ($r_{15} = 0.585$, $P = .022$; Student's *t*-test; fig. 2). To determine whether this was caused by plasticity of the mating system in response to the environment and not simply by strain effects, we examined linear regressions between fitness and within-tetrad mating across media for each strain separately. As expected, the slope of the regression line for each strain was positive (although not always significantly positive); these slopes from the five wild strains as a group are significantly positive ($P = .005$; Student's *t*-test).

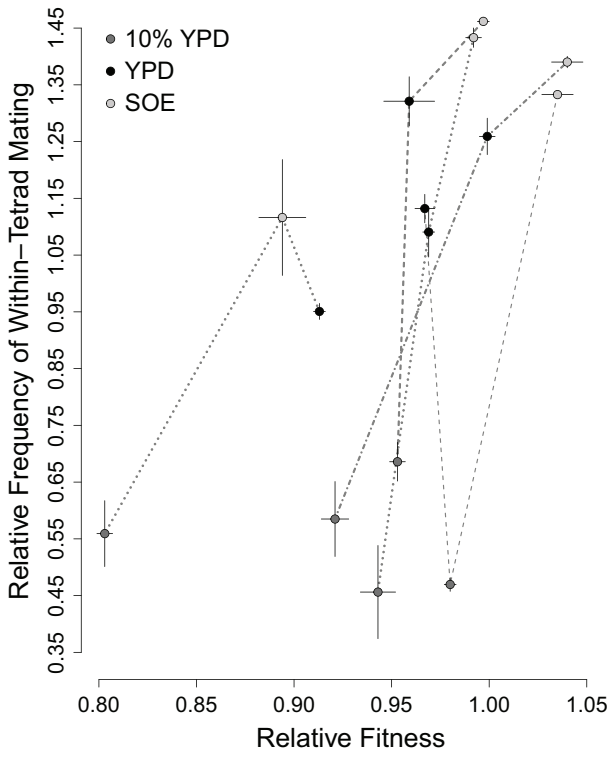

Figure 2: Relationship between fitness and within-tetrad mating frequency for five wild strains, relative to those for a common laboratory strain, in three environments. We measured the fitnesses of the G418-resistant derivatives of the five wild *Saccharomyces paradoxus* strains relative to those of the hygromycin-resistant common competitor Y55 growing asexually on 10% YPD, YPD, and SOE. We plotted these relative fitnesses against within-tetrad mating frequency relative to that for Y55. Lines connect the values of each strain across the three media. Error bars show the standard error across at least three independent assays. Environments: YPD = 2% dextrose, 2% bactopeptone, 1% yeast extract; 10% YPD = 10% of each component of YPD; SOE = 1% sucrose, 0.5% dextrose, 0.5% fructose, 0.15% bactopeptone, 0.1% yeast extract.

Spore Germination

We measured spore germination frequencies to test our hypothesis that germination timing is the mechanism underlying the response of the mating system to the environment. For each strain × medium combination, we measured the frequency of spores that germinated after 24 hours (table A1, available online) in order to estimate the proportion of tetrads with potential for within-tetrad mating (i.e., with at least two germinated spores of opposite mating type). The frequency of spores that germinated within 24 hours was lowest on 10% YPD and highest on SOE ($P < .001$ for both comparisons; Fisher's exact test corrected for multiple comparisons). As predicted, the proportion of spores with the potential for within-tetrad mating was significantly correlated with both the measured frequency of within-tetrad mating ($r_{13} = 0.797$, $P < .001$; fig. A1) and relative fitness ($r_{13} = 0.516$, $P = .049$). Furthermore, the results of the germination time course (fig. 3) give direct support for the hypothesis that higher-fitness environments promote spore germination, as the NaOH treatment kills growing vegetative cells but not spores. One-way ANOVA, with time point as a blocking variable, showed that the medium that the spores germinated on had a significant effect on the overall number of resistant cells (i.e., spores) remaining across time points ($F_{2,38} = 14.53$, $P < .0001$), with significantly fewer resistant cells remaining on SOE than on either 10% YPD ($P < .0001$; Tukey's honest significant difference test) or YPD ($P < .001$; Tukey's honest significant difference test), which did not differ significantly ($P = .91$) in this assay.

Discussion

These results support our prediction that the environment in which yeast spores germinate and mate affects the frequency of within-tetrad mating (i.e., selfing; fig. 1). The underlying mechanism determining mating-system plasticity is spore germination (fig. 3). Further, we find that the direction in which inbreeding changes in response to environmental differences should help maintain adaptation to the environment in which mating occurs: that is, the modulation of inbreeding is consistent with it being an adaptation itself. We predicted that within-tetrad mat-

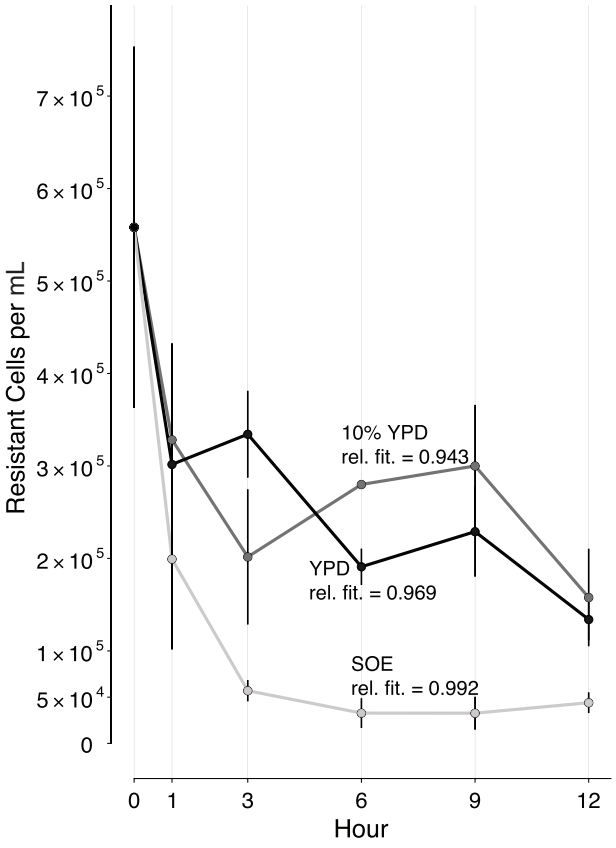

Figure 3: Spore germination time course for strain SpPlön3. Vegetatively growing cells are killed by 1% NaOH, but spores are resistant. This graph shows how the number of resistant cells decreases as spores germinate into vegetative cells in the three different media. Average fitnesses relative to strain Y55 (rel. fit.) in the three environments are also shown. Error bars show the standard error across three independent assays. Environments: YPD = 2% dextrose, 2% bactopeptone, 1% yeast extract; 10% YPD = 10% of each component of YPD; SOE = 1% sucrose, 0.5% dextrose, 0.5% fructose, 0.15% bactopeptone, 0.1% yeast extract.

ing frequencies should be highest under conditions of high fitness, so that the combinations of genes conferring local adaptation are preserved, and this is what we observe (fig. 2).

There are evolutionary and ecological costs and benefits to both selfing and outcrossing, which have been well studied in plants that can do both. Theoretically, an allele that increases self-fertilization should spread because it is transmitted to all the plant's own seeds by selfing as well as to the seeds of other plants by cross-pollination (Fisher 1941); in other words, it pays a reduced cost of meiosis (Maynard Smith 1971). However, if pollen is limited so that the pollen that is used for selfing is not available for outcrossing, then the transmission advantage of a selfing allele is reduced (i.e., "pollen discounting"; Lloyd 1979). Unlike plants, *Saccharomyces* yeast species produce equal numbers of equally sized haploid gametes of both mating types, so they are not expected to pay the cost of meiosis (Maynard Smith 1978). Therefore, the high level of within-tetrad mating in yeast cannot be explained simply by an intrinsic transmission advantage of selfing, because an allele that increased selfing in yeast would suffer a corresponding decrease in its transmission by outcrossing (Charlesworth 1980). Another potential benefit of selfing is mating assurance (Lloyd 1979; reviewed in Busch and Delph 2012), but, unlike plants, yeast have mating assurance independent of within-tetrad mating because of their ability to autodiploidize. The most likely benefit of within-tetrad mating over between-tetrad mating is therefore the preservation of beneficial combinations of alleles, that is, the avoidance of outbreeding depression. Both theory (Lynch 1991; Schierup and Christiansen 1996) and experiments (Moll et al. 1965; Price and Waser 1979; Willi and Van Buskirk 2005) in plants show that mating between individuals from geographically and genetically distant populations can result in outbreeding depression. Like plants, yeast populations adapt to their local environment, with life-history trade-offs promoting phenotypic divergence (Spor et al. 2008), and, as in plants, genetic differentiation between subpopulations increases with physical distance (Koufopanou et al. 2006). Crosses between individuals from different continents show such strong outbreeding depression that they appear to be diverging into different species (Greig et al. 2003; Kuehne et al. 2007). We therefore consider that the disruption of combinations of alleles conferring high fitness in a certain environment is the major factor selecting for inbreeding in yeast.

Conversely, the major genetic force opposing the evolution of selfing in general is thought to be inbreeding depression due to the unmasking of recessive deleterious mutations in homozygous self-fertilized progeny (reviewed in Charlesworth and Charlesworth 1987). It is likely that *Saccharomyces* grows as a diploid for most of its life cycle (Greig and Leu 2009), allowing it to accumulate recessive deleterious mutations that are masked in the heterozygous state. Within-tetrad mating can make such mutations homozygous, exposing them to selection but causing costly inbreeding depression. Recent theory has examined how inbreeding depression due to within-tetrad mating is affected by within-tetrad mate choice (Tazzyman et al. 2012) and how heterozygosity is maintained by genetic linkage to the mating-type locus despite inbreeding (Knop 2006). Although inbreeding depression has not been measured in natural yeast populations, we consider that it is probably the major factor opposing the evolution of pure selfing (i.e., within-tetrad mating).

In organisms that can both self and outcross, the evolution of the mating system should be affected by the

balance between inbreeding depression (due to homozygous deleterious mutations) and outbreeding depression (due to disrupting locally adapted or coadapted gene complexes). Plant mating-system models that balance these opposing factors typically predict that heterogeneous selection pressures will select for intermediate (but constant) rates of selfing (for reviews, see Goodwillie et al. 2005 and Karron et al. 2012), rather than for plastic strategies such as increasing outbreeding in response to dispersal (Reuter et al. 2007). While the touch-me-not apparently optimizes its mixed mating system further by dispersing outcrossed seeds more than selfed seeds (Schmitt et al. 1985; Cheplick 1987; Schmitt and Gamble 1990), the effect of phenotypic plasticity in the ratio of outcrossed and selfed seeds is not considered. But there is considerable plasticity in the inbreeding rates of amphicarpic grasses (Campbell et al. 1983), with selfing typically increasing under stress (Cheplick 2007), and there are similar examples in insect-pollinated plants. For example, a plastic decrease in the ratio of chasmogamous (outcrossing) and cleistogamous (selfing) flowers of *Viola praemorsa* allows that species to compensate for a lack of pollinators (Jones et al. 2013). Reduction of herkogamy (i.e., stigma-anther separation) and increased pollen self-compatibility in response to stress appear to aid colonization of new habitats by promoting selfing (Levin 2010), which is likely to be important when humans disturb plant habitats (Eckert et al. 2010). Such cases are expected to decrease offspring variation and reduce outcrossing depression; mating with unknown gametes might lead to the offspring losing the ability to thrive in the challenging environment.

However, a key difference between these examples of mating-system plasticity in plants and the yeast system we have described here is that yeast gametes sense their local environment directly before mating. This makes modulation of the mating system according to local genotype × environment (G × E) interaction an intriguing possibility, because haploid yeast gametes express their genomes and interact with the environment in nearly the same way that yeast diploids do. Thus, by increasing inbreeding among gametes with high fitness, this mechanism could potentially help keep adaptive combinations of genes together and help break up low-fitness combinations that do not work well together, responding dynamically as environmental conditions vary in space or time. Similar systems may exist in animals and plants, such as whether expression and modification of self-incompatible S-alleles in pollen (reviewed in Takayama and Isogai 2005) are influenced by G × E interactions (as has been proposed in Levin 1996, 2010; Good-Avila et al. 2008).

While mating-system plasticity can, in principle, be an adaptation to dynamically balance inbreeding depression and outbreeding depression, alternative explanations are possible (van Kleunen and Fischer 2005). We consider three alternative adaptive explanations for the mating-system plasticity we have identified in yeast. First, the primary cue for spore germination is a fermentable carbon source (Savarese 1974), and it is likely that the germination system evolved to stimulate spores to germinate when there are enough nutrients present to reestablish the growth phase of the life cycle. We note, however, that carbon availability is not the only factor that influences within-tetrad mating frequencies. SOE has the same amount of fermentable carbon and less nitrogen than YPD, yet SOE stimulates more germination and more inbreeding than YPD in wild strains (figs. 1*b*, 2; table A1). It is interesting that the mating system of the *S. cerevisiae* laboratory strain Y55 responds very differently, compared to that of wild strains, perhaps because it has adapted to the rich media of the laboratory. The effects of strain and medium on the yeast mating system can explain the apparent disparities in different experimental measurements of yeast inbreeding (e.g., Reuter et al. 2007; Murphy and Zeyl 2010), and specific nutrient availability clearly influences spore germination (on YPD vs. that on 10% YPD; table A1). Second, certain levels of nutrients may provide enough energy to allow germination but may not reliably indicate that enough nutrients are present to allow diploid asexual growth. Under such circumstances, it may be beneficial to allow some spores to germinate to exploit the available (but poor) resource but others to remain dormant so that they are available to exploit a future, potentially better, resource (Philippi 1993; Danforth 1999; Clauss and Venable 2000), that is, "bet hedging" in poor-quality environments (Cohen 1966). The correlation between a strain's fitness and its inbreeding frequency might not be an adaptation to optimize the mating system but could instead be the indirect result of selection for bet hedging. Third, sexual selection could cause strains with lower fitness to be less attractive and therefore less likely to mate immediately within the tetrad; this then increases the possibility that they will mate later with a partner from another tetrad (Smith and Greig 2010). These different forms of selection are not mutually exclusive, and all may contribute to the evolution of the organism.

It is also possible that the mating-system plasticity we have identified is not an adaptation at all. Our simplified experimental system cannot fully capture the complexity of a wild yeast population in its natural environment. Simplification was necessary to allow us to make the measurements we wanted, but we should be careful not to overinterpret our results. In our experiment, the two lines derived from each strain were identical except for the antibiotic-resistance markers they carried, so there was no actual difference in the genotypes produced from within-tetrad and between-tetrad matings; both types of matings

produced diploids identical to their parents, apart from their markers. Thus, there could be no inbreeding depression or outbreeding depression in our experiments. In nature, however, we would expect opportunities for mating between tetrads from genetically distinct lineages to occur (Tsai et al. 2008), and the mechanism we have uncovered could optimize the probability of these matings. In our experiments, all haploid spores within a tetrad were also genetically identical, except for their mating-type alleles. But spores within natural tetrads are expected to differ, especially for recessive deleterious mutations, which may have accumulated in the heterozygous state during the previous diploid clonal expansion. The modulation of outcrossing we have proposed could also, in principle, act within a single tetrad to allow a pair of spores with high fitness to germinate at the same time and mate together. Potentially, the remaining two low-fitness spores in the same tetrad could germinate later at different times and outcross. A similar mechanism of assortative mating between the fittest pair of spores within a tetrad, mediated by honest pheromone signaling, has recently been proposed (Tazzyman et al. 2012). Mating systems like these that allow high-fitness alleles to preferentially assort, even within a single meiotic tetrad, could potentially add a new dimension to the evolutionary enigma of sex (Otto 2009).

Another way that our system was simplified was that our strains were unable to autodiploidize because we deleted their *HO* genes. This modification was necessary to allow us to determine within-tetrad mating frequencies: if unmated haploids could have autodiploidized, the resulting diploids would have been indistinguishable from the diploids resulting from within-tetrad mating. The ability to autodiploidize would have affected not the within-tetrad mating frequencies that we report here but only our ability to measure them. It would, however, have reduced the between-tetrad mating frequencies, because any unmated haploids that divided could have autodiploidized instead of continuing to divide until they found mates from other tetrads. A consequence of modulating the within-tetrad mating frequencies by reducing the germination of less adapted spores is that newly germinated haploids may find no available mates in extremely low fitness environments, although the ability to switch mating type and autodiploidize provides mating assurance. This leads to the interesting prediction that within-tetrad mating (i.e., sporophytic selfing) should predominate in environments conferring very high fitness, between-tetrad mating (i.e., outcrossing) should predominate in intermediate environments, and autodiploidization (i.e., gametophytic selfing) should predominate in very low fitness environments.

In this article, we have focused on how local adaptation might contribute to the balance between two mating strategies, within-tetrad mating and between-tetrad mating. But these are just two possibilities in a broader range of reproductive strategies available to yeast, which also includes autodiploidization and asexual reproduction, both in diploids and haploids. We presume that each of these different reproductive strategies will have different effects on local adaptation and that local adaptation is just one of several possible benefits that will vary among the different strategies. Understanding all the benefits and costs of sexual and asexual reproduction is one of the most elusive problems in evolutionary biology (Otto 2009), but it is reasonable to expect that natural selection will maximize fitness by optimizing the balance between sexual and asexual strategies. The existence of "intermediate" strategies, such as autodiploidization and within-tetrad mating, which combine some features of both sexual and asexual reproduction, could be evidence of that optimization, as could plastic changes in reproductive systems in response to environmental and ecological cues.

Conclusion

We show that the environment in which yeast spores germinate has a strong effect on their inbreeding rate, such that genotypes that are well adapted are more likely to inbreed. This is consistent with the hypothesis that the plasticity of the yeast mating system evolved to promote local adaptation. Such plasticity could also be beneficial in environments that change in time as well as in space, and indeed it could help preserve gene combinations that are adapted to each other (i.e., coadapted gene complexes). The lack of understanding of yeast natural history makes it difficult to determine the contribution of these mating strategies to yeast evolution, but the advantages of yeast for genetic manipulation and experimental evolution make it an excellent organism to complement the plant and animal models that are traditionally used by ecologists.

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