Testing the adaptive value of sporulation in budding yeast using experimental evolution

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Saccharomyces yeast grow through mitotic cell division, converting resources into biomass. When cells experience starvation, sporulation is initiated and meiosis produces haploid cells inside a protective ascus. The protected spore state does not acquire resources and is partially protected from desiccation, heat, and caustic chemicals. Because cells cannot both be protected and acquire resources simultaneously, committing to sporulation represents a trade-off between current and future reproduction. Recent work has suggested that passaging through insect guts selects for spore formation, as surviving insect ingestion represents a major way that yeasts are vectored to new food sources. We subjected replicate populations from five yeast strains to passaging through flies, and evolved control populations by pipette passaging. We assayed populations for their propensity to sporulate after resource depletion. We found that ancestral domesticated strains produced fewer spores, and all strains evolved increased spore production in response to passaging through insects, and evolved control populations by pipette passaging. We assayed populations for their propensity to sporulate after resource depletion. We found that ancestral domesticated strains produced fewer spores, and all strains evolved increased spore production in response to passaging through flies, but domesticated strains responded less. Exposure to flies led to a more rapid shift to sporulation that was more extreme in wild-derived strains. Our results indicate that insect passaging selects for spore production and suggest that domestication led to genetic canalization of the response to cues in the environment and initiation of sporulation.

KEY WORDS: Bayesian analysis, dispersal, eco-evolutionary dynamics, experimental evolution, heritability, Saccharomyces, vegetative growth.

Evolutionary theory predicts that natural selection will favor genotypes that maximize the relative fitness of expressing life-history transitions between growth and dispersal stages as a function of the predictability of environmental change and the spatial structure of the populations in question (Olivieri et al. 1995; Tufto 2000). However, theory also predicts that adaptation will be hampered when there are genetic constraints between growth and dispersal due to negative genetic correlations between the relevant traits (Lande 1980), either because of lack of appropriate standing genetic variation or easily accessible de novo mutations.

The budding yeast Saccharomyces cerevisiae can help shed light on the evolution of transitions between growth and dispersal stages in response to environmental conditions. This is because prior work on this organism has established the potential fitness benefits of many life-history traits and because replicate populations can be experimentally evolved in the lab under well-defined ecological conditions. In addition, a set of five genetically defined yeast strains with differing histories of domestication has recently been made available and these can be leveraged to test the influence of different genetic backgrounds on evolution. Specifically, if some strains have loci that have been deleted or become pseudogenes, then point mutations are unlikely to return these loci to their ancestral state.

In nature, populations of S. cerevisiae primarily grow as vegetative mitotic diploid cells and disperse to novel habitats through the guts of insect vectors as meiotic haploid quiescent spores encapsulated within a protective structure called the ascus (Gibbs and Stanton 2001; Coluccio et al. 2008; Stefanini et al. 2012). Sporulation is initiated when diploid cells encounter adverse environments (Neiman 2005). Sporulation can be induced
Yeast cells detect such conditions through the sporulation developmental pathway which funnels environmental information through a master-regulator gene, Ime1, which in turn induces downstream developmental genes that initiate the sporulation program (Madhani 2007). Multiple domesticated yeast strains appear to have lost much of their ancestral sporulation ability, as measured by the fraction of spores produced after exposure to starvation conditions (Gerke et al. 2006; De Chiara et al. 2020). Changes in the sporulation response could thus be due to selection on genotypic variants of detection of environmental information or due to selection on variants enacting the sporulation program. The loss of sporulation ability is presumed to have occurred because these strains have been cultured as growing vegetative cells for many generations without experiencing starvation or ingestion by insects (Ratcliff et al. 2013). Spores and vegetative cells can both survive insect guts, although spores have a much higher survival probability (Coluccio et al. 2008), and only spores are able to cross-fertilize spores from other asci (Reuter et al. 2007).

Following ingestion by insects, spores are likely to survive but not vegetative cells (Coluccio et al. 2008; Ratcliff et al. 2013). In this case, relative fitness depends on how many progeny cells survive ingestion, germinate, and establish new populations after the insect defecates. Sporulation timing will also be under selection because ingestion can occur at any time. The sporulation strategy should be considered in the context of a competition-colonization trade-off, where increased investment in sporulation decreases within-habitat competitive ability (Tilman 1994). If, however, ingestion by insects happens after the local population has a chance to consume all the available resources, then selection should favor genotypes that reproduce vegetatively while resources are abundant and then form spores after most nutrients are depleted.

Several predictions can be made when yeast deplete resources before insect ingestion. Vegetative reproduction is favored while resource levels are high. During dispersal, however, selection favors production of cells that survive vectoring. After dispersal, genotypes that detect and respond to suitable growth conditions by initiating germination and mate recognition are favored (Murphy and Zeyl 2012). Thus there are three types of adaptive traits, increased early growth, increased survivorship during vectoring, and a speedy return to growth. Our focus is on the role that the timing and efficiency of spore production plays in increasing the survivorship of cells during gut passing.

Prior work on the adaptive value of sporulation in yeast has focused on determining how insect passing affects outcrossing and the mortality rate of spore cells and vegetative cells. For example, Coluccio et al. (2008) fed cells to fruit flies and then counted surviving and dead cells in the frass. They found that when vegetative cells were fed to flies, a low fraction of cells in the frass were viable (20%), while when spores were fed to flies, a large fraction of spores in the frass were viable (80%). They argued that there is a selective benefit of having a larger fraction of cells in the spore state at the time of fly consumption. Reuter et al. (2007) showed passaging through flies increased mating between spores, leading to greater outcrossing. Although these studies identify potential benefits of producing spores, they do not assess the selective forces across the entire life cycle. These include the effects of sporulation on the total number of vegetative and spore cells present at the time of consumption, biases in fly consumption, the effects of digestion on cell clumping, and the viability and timing of spore and vegetative cell growth following digestion. In particular, for spores to return to the diploid vegetative state they must germinate and begin acquiring resources. Overall, these and other studies have shown that spores may enjoy a survival benefit, but have not assessed the net effect of increased survival and delayed growth once resources are present.

We performed a replicated selection experiment in five S. cerevisiae strains that varied in their level of domestication, and experimentally controlled whether cells were propagated through the digestive tract of Drosophila melanogaster fruit flies or directly propagated by pipette. All strains were initially isogenic and homothallic, such that evolution during the experiment could only occur through selection on de novo mutations. We hypothesized that selection for increased spore production would be mediated by fly ingestion and not by exposure to starvation cues alone. We also hypothesized that the previously domesticated strains would show a lower response to selection because they had previously evolved sporulation pathways under conditions where sporulation was not beneficial.

**Methods**

**YEAST AND FRUIT-FLY STRAINS**

We used a set of five genetically distinct strains of S. cerevisiae that were provided as homozygous diploids with resistance to Geneticin (G418), an ortholog to kanamycin, (Louvel et al. 2014) obtained from the National Collection of Yeast Cultures, UK. These five strains represent a wide range of ecological backgrounds including from an Oak woodland in the Northeast United States (NCYC:3581, North American strain, AM), a palm flower in a Malaysian forest (NCYC:3576, Malaysian, MY), a West African strain from a seminatural wine fermentation (NCYC:3574, West African, WA), a Sake brewery in Japan (NCYC:3579, Japanese Sake, JS), and a winery in Western Europe (NCYC:3570, Wine European, WE). These strains cover a
range of backgrounds from wild to fully domesticated. The AM and MY strains were collected directly from plant exudates in a natural setting. The WA strain was collected from Biliwine production in 1914, a process that involved inoculation of *Osbeckia grandiflora* fruit from naturally occurring yeast. The JS and WE strains have each been maintained in industrial alcohol production for many generations. Although the JS and WE strains have both been heavily domesticated, they are each more closely related to nondomesticated strains than they are to each other (Liti et al. 2009). We follow Liti et al. (2009) and consider the JS and WE strains to be independent origins of domesticated yeast strains, while AM and MY were directly isolated from natural settings and are considered to be wild strains. We designate the WA strain as seminatural because it is genetically more related to nondomesticated strains (Liti et al. 2009) and because it has not experienced multiple generations of selection for properties useful in fermentation. Each strain was previously genetically modified for use in a lab setting by knocking out the mating-type switching locus and adding DNA barcodes with stable diploid strains produced by complementary mating (Louvel et al. 2014). These modifications allow downstream haploid strains to be maintained in laboratory conditions and for strains to be selected using antibiotic media.

Each ancestor was split into four replicate populations, which were then split into control and treatment populations. These 40 experimental populations were evolved for 30 full cycles of population growth, starvation, and dispersal. Dispersal occurred by passaging through *D. melanogaster* feeding in the fly treatment evolution populations and by pipette for the control evolution populations.

The *D. melanogaster* stocks were created by outcrossing strains from isogenic Al-Ral, Taiwanese, Santa Barbarian and Malaysian lines. Flies to be used as vectors during the experiments were allowed to lay eggs on yeast-peptone-dextrose (YPD) agar plates. Adult flies were then removed and the eggs were bleached using a 10% bleach solution for 40 minutes at 22°C. Fly eggs were collected by sterile pipette, washed with sterile water, and transferred using sterile technique to sterile media. Although flies are typically reared on media that contains an antifungal compound, we did not use antifungal compounds in the media for our experimental flies so that we would not reduce viability of the yeast being passaged. Although these flies were reared to minimize fungal gut flora, they were not reared in a way to reduce bacterial gut flora because axenic conditions have been shown to reduce health (Broderick and Lemaitre 2012) and change behavior (Wong et al. 2014) in *D. melanogaster*.

**SELECTION PROTOCOLS**

During the experimental treatment, we grew each of our five diploid yeast strains in 1.5 ml of YPD liquid culture over a five-day period. The initial growth culture was inoculated with four 3 mm diameter colonies from a G418 plate of single colony isolates and grown in 2 ml of YPDA to ensure adequate volume for each of the four treatments. Samples of these initial strains were then frozen in 15% glycerol solution at −80°C and labeled as the “ancestral” treatment. The five strains were split into eight replicates each, and four of these replicates were labeled “control” while the other replicates were labeled “experimental treatment.” Each of the experimental replicates were subjected to the Fly Capillary Feeder (CaFe) vial feeding treatment and all sequential treatments then followed the complete selection cycle described next (Fig. 1).

Before each selection cycle, each population was incubated in YPD at 30°C for five days without shaking. The inoculate size of each experimental treatment was determined by the amount of surviving yeast collected by the postdigestion wash, while the inoculate size of the control was 10 μl of vortexed control sample into 1.49 ml of YPDA. To reduce chances of contamination from other yeasts or bacteria, cultures were always grown in YPD media with G418, tetracycline, and ampicillin added.

We performed experiments without shaking to increase the opportunities for haploid cells developing from spores to mate and form diplods. Because yeast mate by growing mating projections toward opposite mating-type cells, mating is not usually successful in shaken media. Under these growth conditions, yeast populations typically consume sugar resources within 24 h, and the five-day period allowed ample time for spor formation to be initiated and completed.

After the five-day period, each population was adjusted to an optical density of 0.3 in an effort to both ensure that the population size was large enough for selection, rather than drift, to shape the evolutionary trajectory, but small enough to prevent blockage in the capillary tube in the CaFe vial caused by high cell density. Based on these optical densities and estimates of yeast survival during *Drosophila* passaging, we estimate that approximately 10^6 diploid cells were transferred into the CaFe vials, and that no more than 10^5 cells survived passaging (Amberg et al. 2005; Coluccio et al. 2008), thus the effective population size would be on the order of 10^4–10^5.

The dilution process was performed using spent YPD (SYPD), YPD that had been depleted by culturing yeast for two weeks and then filter sterilized. SYPD was used to mimic carbon and nitrogen sources in a late growth stage yeast culture population and to minimize population growth or germination of sporulated yeast cells (Neiman 2005; Madhani 2007). This SYPD was consistently produced by inoculating standard YPD with one colony each of all five ancestral strains, and allowing the culture to grow for two weeks. This media was then filter sterilized and reinoculated with one colony each of the five regional strains again to ensure the media was maximally depleted. After another
week of growth, this SYPD was then filter sterilized again and stored at 20°C until use.

For the fruit-fly experimental treatment we employed the CaFe apparatus, adapted from Ja et al. (2007). CaFe tops used four 200 μl pipette tips that were cut to increase opening size, rubber stoppers, and standard rubber bands. These CaFe tops were then affixed to narrow fly vials each containing 2 ml of 3% solidified agarose solution to maintain humidity within the vial. The pipette tips within the CaFe top were fitted to one 5 μl capillary tube each. Eighteen hours prior to treatment, four clean flies were added to each of four vials per replicate. This starvation period was added to ensure sufficient consumption of yeast by the flies during treatment (Reuter et al. 2007).

Yeast cultures were distributed into CaFe feeding apparatus and offered to three to four clean flies because pilot experiments showed that three to four flies per vial maximized yeast passing. Adding more flies led to rapid consumption of all the presented yeast (20 μl) after which flies fed on frass, which reduced the amount of yeast we were able to collect from the vial. Flies were allowed to feed for 48 h and then removed from the vials. Measurements of total fly food consumption were taken by recording the change in meniscus of the two capillary tubes. Flies were removed from the vials that were then rinsed with 1.6 ml YPDA media to collect fecal material on the tube surfaces, and the supernatant (1.5 ml total volume because some volume is re-absorbed into the agar in the vial) was collected and used as the inoculate for the next round of yeast population growth.

For the control selection treatment, 400 μl of similarly diluted (OD 0.3) yeast cultures were placed on the bench at ambient temperature. After 48 h, each control population was vortexed and 10 μl of each culture was moved to 1.49 ml of YPDA in a new culture tube (1.5 ml total volume).

These selection protocols were repeated for 30 growth cycles, which would include about 300 cell divisions. Samples of each population were frozen every other cycle as a backup in case of contamination. After the last cycle all populations were frozen and stored at −80°C.

**SPORULATION ASSAYS**

Measurements of sporulation were performed in two blocks defined by the date of the assay, with each block containing samples of all 40 control and treatment replicate populations as well as the ancestral populations. In each block, two samples were taken from each population and then further divided into two
technical replicates. For the ancestral populations, three samples were taken in each assay block, also divided into two technical replicates.

We first plated frozen population samples on YPD agar plates and then collected yeast from these plates to start liquid YPD cultures that grew for 6 h without shaking at 30°C. Each sample was diluted to an optical density of 1.5 and 2 ml was centrifuged and washed to remove any traces of growth media. Cells were then resuspended in 2 ml of potassium acetate (2% KAc at pH ≈ 6.7), and incubated at 30°C with shaking (230 rpm). We resuspended cultures in potassium acetate because it is known to induce sporulation and to reduce the possibility that the evolved populations achieved different degrees of sporulation because they modified the media environment. Measurements were taken at 2.5 days and at 5 days postinoculation. Samples of 5 μl were taken from cultures and diluted with 95 μl distilled water. One field of cells was then photographed at 400× magnification. Images were processed in photoshop by adding a grid and counting the number of spores and vegetative cells.

**STATISTICAL ANALYSIS**

We used a Bayesian approach to estimate the effect of the evolution treatment (fly and control) on the fraction of spores produced among the five yeast strains. We considered replicate populations as a random effect nested within each of the five strains. All of our analyses are done on the log-odds, or logit, scale that allows the parameters to take on values over the range from negative infinity to positive infinity. The odds can be interpreted as the ratio of the fraction of sporulated to unsporulated cells, in other words they represent how much more likely a cell is to sporulate than to remain in the vegetative state. The log-odds transformation converts multiplicative differences into additive differences, which is how generalized linear models are constructed. We used the posterior distributions of parameters from the Bayesian analysis to ask if yeast strains had different ancestral sporulation programs and to ask if the strains responded to selection in the same way.

We developed hierarchical models of sporulated cell counts where the observed counts were taken to be binomially distributed. We modeled the log-odds (logit) of the binomial parameter as a linear function of the interaction between yeast strain, selection treatment, and assay time (2.5 and 5) with an additional random effect of the replicate population, where we pooled variation among sampling and technical replicates. We performed these analyses separately on the ancestral populations because they necessarily do not have the same replicate structure.

Specifically, the main model considers the sporulation probability to be

\[
\theta_i = \beta_{S,T,A} + \Phi_{\text{ReplicateID}},
\]

\[
C_i \sim \text{Binomial}(N_i, \text{logit}^{-1}(\theta_i)),
\]

where \(C_i\) is the count of sporulated cells in sample \(i\), \(N_i\) is the total number of cells counted in sample \(i\). The predictor \(\beta\) depends on the S yeast strain, the T selection treatment, and the A assay time of observation.

Effects are modeled as a linear combination of parameters:

\[
\beta_{S,T,A} = \beta_S + \beta_{T,T}I_T(T) + \beta_{S,A}I_A(A) + \beta_{S,T,A}I_T(T)I_A(A),
\]

where \(I_T\) is an indicator function that takes on 1 for the fly treatment and 0 for the control, and \(I_A\) is an indicator function that takes on 1 if the assay is done at day 5 and 0 if it is done at day 2.5. We used mean-zero normal prior distributions for each \(\beta\), which helps control the magnitude of the coefficients. This “partial pooling” approach leverages data from all group while still allowing effects to vary by \(S, T,\) and \(A\) (McElreath 2020).

\[
\beta \sim \text{Normal}(0, 1).
\]

We modeled the variation that comes from replicate populations sharing the same ancestry as

\[
\phi_i \sim \text{Normal}(0, \sigma^2)
\]

\[
\sigma \sim \text{half-Cauchy}(0, 2),
\]

meaning that each replicate population is considered to be sampled from a normal distribution and the prior on the standard deviation is a half-Cauchy distribution with location at 0 and a scale of 2.

Because we assume that the parameter for each combination of strain, treatment, and assay time is independent, this is a generalized linear model with main effects and interaction terms for each of the three factors and a logit link function (Pinheiro and Bates 2000). The additional term \(\phi\) represents the random effect of the four replicate populations and effectively allows for overdispersion of the count data relative to a binomial model. Such overdispersion in the count data could arise due to idiosyncratic differences during the creation of the replicate experimental populations from the ancestral strain. By constructing this generalized linear model on the log-odds scale, we are able to infer the additive effects of each element of the model. This allows us to ask how a factor, such as the time of the assay, alters the odds of sporulation.

Models were specified with **BRMS** (Bürkner 2017, 2018) and **STAN** (Stan Development Team 2018) using **RStan** which performs Bayesian inference using a Hamiltonian Monte Carlo sampling to calculate the posterior probability of the model parameters given the observed data (R version 3.3.2, RStan version 2.15.1) (R Core Team 2019). Convergence of the MCMC chains was checked by ensuring that \(\bar{R} \) was less than 1.1, where \(\bar{R}\) is a metric describing the variability between chains (Gelman et al. 2013).
Figure 2. Inferred fraction of cells as spores following experimental evolution. The circles represent ancestral populations, the triangles represent control populations, while squares represent fly treatment populations. The red fill is for the 2.5-day assay point, while the blue fill is for the 5-day assay point. Points represent the estimates for the mean effect of each strain/treatment combination, while the credible interval bars show where 95% of the posterior parameter estimate distribution lies. The two fully domesticated strains are Japanese Sake (JS) and Wine European (WE), the seminatural strain is West African (WA), while the naturally isolated strains are North American (AM) and Malaysian (MY); see Figure S1 for the raw sporulation data.

Results

ANCESTRAL POPULATION SPORE PRODUCTION

The fraction of cells that had formed spores differ at both assay times among the ancestral strains (Fig. 2). We compared a model with factorial effects of strain and assay time to models lacking an effect of strain or assay time and found that both a strain effect and an assay time effect were highly supported (see Supporting Information RMarkdown File (https://doi.org/10.25349/D9FK6Q)). As expected, this reflects evolutionary history of the ancestral strains, with strains coming from natural environments showing a larger fraction of cells as spores than strains from industrial alcohol production (Gerke et al. 2006; De Chiara et al. 2020). The African strain, WA, which is thought to be only partially domesticated (see Methods), had an intermediate fraction of spores. All strains show unique levels of spore production as they do not overlap at the 95% credible level (see Supporting Information RMarkdown File (https://doi.org/10.25349/D9FK6Q)).

SELECTION RESPONSES IN THE FRUIT-FLY TREATMENT

After 30 growth cycles involving fruit-fly gut dispersal, the evolved populations produced a larger fraction of spores than their ancestors (Fig. 3A). For all strains, the effect of the fly treatment on the fraction of spores is greater than 0 (the posterior probability of the difference from the ancestral being less than 0 is below 10^{-3}), with the evolved populations producing between 30% to 95% spores.

We measured rate of change in the fraction of spores between the assay time at 2.5 days and the assay time at 5 days. We calculated the timing effect of the treatment as the difference in this rate of change between the treatment and the ancestral populations (Fig. 3B). The slope of the timing response is calculated as a change in the effect size on the logit scale between assay time points, and the difference of this measure between treatments is a measure of the change in timing due to evolution treatment,

\[
\zeta_{S,T} = (\beta_{S,5} + \beta_{T,5}) - (\beta_{S,2.5} + \beta_{T,2.5})
\]

(3)

\[
\zeta_{S,T,T} = \zeta_{S,T} - \zeta_{S,T_0}.
\]

(4)

Here \(\zeta_{S,T,T}\) is a measure for strain \(i\) of the difference in sporulation timing between treatment \(j\) and treatment \(k\). This is all performed on the logit scale.

All strains showed a large response to fly vectoring in that logit scale effect of the time of assay is greater than 0 (the posterior probability of the difference from the ancestral being less than 0 is below 10^{-3}). This effect is largest for the wild-derived strains (Fig. 3 and Supporting Information RMarkdown File (https://doi.org/10.25349/D9FK6Q)).

Discussion

Previous studies have suggested that sporulation in budding yeast is an adaptation allowing lineages to survive passage through insect vectors (e.g., Neiman 2005; Coluccio et al. 2008). These arguments were based on observations of differential survival by vegetative cells and spores in Drosophila frass. Other work has suggested that selection for dispersal traits, such as mating ability and germination, may also favor sporulation because insect digestion breaks up the ascus, freeing nonrelated spores to
mate following deposition of frass on fresh food sources (Reuter et al. 2007). Selection on sporulation onset and completion may however also depend on the timing of resource competition between unrelated genotypes of vegetative cells and the mortality effects of challenging environments (Ratcliff et al. 2013).

We found that strict passaging through the fly digestive tract resulted in the evolution of both a sharper transition to the spore state and an increase in the fraction of cells that are spores. For each strain, the fraction of spores after five days was higher than in the ancestor, and the effect of assay day was larger. Because the experiments encompassed on the whole life cycle of growth and dispersal, these results thus show that an increase in fitness is associated with increased production of spores.

The domesticated strains started with a deficit in their tendency to sporulate, so these lineages could have adapted by increasing vegetative cell survival through the gut. A recent analysis of a large set of yeast isolates has shown that some strains have evolved increased survival of quiescent vegetative cells (De Chiara et al. 2020). This was not the case here, where even the domesticated strains showed a large increase in spore production. However, domesticated strains evolved to lower levels of spore production than the wild strains. This result suggests that domestication led to the loss of mutational options that allow the cells to modify the sporulation program. Even without knowledge of the specific genetic changes responsible for the observed differences in sporulation, and assuming similar mutation rates across genetic backgrounds, the fact that the domesticated isolates achieved a lower response to selection implies that the mutations that did occur had lower total effects on sporulation. We suspect that this pattern could occur even if similar mutations in the exact same regulatory genes caused the difference in sporulation and other differences in the genetic background of the isolates created epistatic interactions canalizing such mutations (Flatt 2005; Proulx and Phillips 2005). We use the definition provided in Flatt (2005) for genetic canalization as a reduction in the phenotypic response to genetic variation, which is a form of epistasis. In general, our results are reminiscent of those of Kvitek and Sherlock (2013), where experimental evolution in a constant environment led to the loss of developmental and physiological programs involved in the sensing of environmental variation.

In our experiments, there was ample time for populations to deplete nutritional resources, sense the change in the environment, and respond by initiating spore formation. In the control experiments, where there was only selection for starvation resistance, we found that sporulation did not evolve to high levels. We did find some evidence for selection on sporulation, in that the domesticated strains (JS, the sake strain, and WE, the wine strain) did evolve small but well-supported increases in spore frequency. All strains, however, showed significantly higher spore frequency in their fly passaging treatment compared to the control treatment. Because there appears to be some selection for sporulation under starvation conditions, perhaps the adaptive value in the ancestor yeast where sporulation first evolved was the ability to cope with challenging nutritional environments. If so, sporulation may have then been co-opted as a dispersal strategy because of ecological opportunities, namely that small fruit flies preferentially feed on yeast (Schiabor et al. 2014) and are small enough to vector them between favorable habitats (Gibbs and Stanton 2001; Tsai et al. 2008; Stamps et al. 2012).
The process of sporulation includes both sensing of the changing environment and the processes of meiosis and spore wall formation. Assessing changes in traits that are based on the time course of a response is challenging because such traits are necessarily multivariate. In our statistical approach, we considered a model where the rate of sporulation was defined as the difference in spore fraction on the logit scale. The use of the logit scale for this type of measurement is a standard method for generalized linear models, and allows us to separately assess the mean level of spore production and the rate of sporulation (McElreath 2020). We found that in the control evolution experiments, only the domesticated strains showed a response in sporulation rate, and they responded by evolving a more uniform fraction of spores across time, implying that producing a small and stable fraction of spores is adaptive.

In the fly passaging experiments all strains evolved a larger timing effect. For the wild North American strain (AM), this effect is not particularly evident in Figure 2 in that the difference between the fraction sporulated at assay time 2.5 and 5 is similar for the ancestor and the fly-passaged treatment. On the logit scale, however, the experimental treatment group shows a much larger effect because the proportion of spores is so close to 1 at assay day 5. Biologically, this means that the change in the odds of a cell being in the sporulated state is much higher for the evolved AM populations than the ancestral AM populations. For the other nondomesticated strains (MY and WA), there were large positive changes in the rate of spore production both on the logit scale and in terms of the difference in mean spore percentage, as can be seen in Figure 2. The domesticated strains showed increases in the rate of sporulation, albeit more modest. We conclude that the fly passaging environment selected for sharper transition to the sporulated state, a conclusion that is consistent with selection favoring genotypes that consume resources until they are depleted and then quickly develop into spores.

In natural, spatially heterogeneous conditions, the ecological dynamics of resources and the insect ingestion can be seen as creating a competition-colonization trade-off (Tilman 1994). Yeast strains that sporulate at higher frequency and earlier during growth suffer a within-deme competition cost, but gain a between-deme colonization benefit. Competition-colonization trade-offs can generally allow for coexistence of alternative strategies, sometimes allowing many strategies to coexist at once (Snyder and Adler 2011). We speculate that such competition-colonization trade-offs led to coexistence of yeast species in nature. In line with this, S. cerevisiae and S. paradoxus have similar developmental and physiological profiles for surviving adverse environments but, tellingly, differing sporulation and germination programs (Murphy and Zeyl 2010, 2012). A possible explanation for this coexistence therefore is that they occupy distinct positions in the competition-colonization space.

**AUTHOR CONTRIBUTIONS**

KMT and SRP conceived the experiments. KMT performed the experiments. KMT, HT, AF, and SRP performed the analysis. KMT, HT, and SRP wrote the paper.

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**DATA ARCHIVING**

All data and code for analysis has been submitted to Data Dryad. Complete code to perform the analysis is available in a Supporting Information RMarkdown File. The data and code are available at https://doi.org/10.25349/D9FK6Q.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S.1:** Observed sporulation fraction for each strain studied.