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

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Short running title: Yeast evolve to anticipate insect gut-vectoring

Keywords: Bayesian analysis, Experimental Evolution, *Saccharomyces*, Eco-evolutionary dynamics, heritability, dispersal, vegetative growth

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Abstract

Saccharomyces yeast can grow through mitotic vegetative cell division while they convert resources in their environment into biomass. When cells encounter specific low nutrient environments, sporulation may be initiated and meiotic division produces 4 haploid cells contained inside a protective ascus. The protected spore state does not acquire new resources but 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 one of the major environmental factors that select for the formation of spores is passaging through insect guts, as this also represents a major way that yeasts are vectored to new food sources. We subjected replicate populations of a panel of 5 yeast strains to repeated, predictable passaging through insects by feeding them to fruit flies (*Drosopila melanogaster*) and then allowing surviving yeast cell growth in defined media for a fixed period of time. We also evolved control populations using the same predictable growth environments but without being exposed to flies. We assayed populations for their sporulation rate, as measured by the percentage of cells that had sporulated after resource depletion. We found that the strains varied in their ancestral sporulation rate such that domesticated strains had lower sporulation. During evolution, all strains evolved increased sporulation in response to passaging through flies, but domesticated strains evolved to lower final levels of sporulation. We also found that exposure to flies led to an evolved change in the timing of the sporulation response relative to controls, with a more rapid shift to sporulation, and that wild-derived strains showed a more extreme response. We conclude that strains that have lost the ability to access genetic variation for total sporulation rate and the ability to respond to cues in the environment that favor sporulation due to genetic canalization during domestication.

Introduction

Most organisms have specialized life-history stages for growth when environmental conditions are favorable or life-history stages for dispersal to novel habitats when environmental conditions become challenging. Evolutionary theory predicts that natural selection will favor genotypes that maximize the relative fitness of expressing these life-history transitions as a function of the predictability of environmental change and the spatial structure of the populations in question (Olivieri *et al.*, 1995; Tufto, 2000). Alternatively, fitness trade-offs between life-history stages can be due to genetic constraints between the relevant growth and dispersal traits, either because of negative genetic correlations if evolution occurs from standing genetic variation or lack of *de novo* mutational options (Lande, 1980).

In nature, populations of the budding yeast *Saccharomyces cerevisiae* are thought to primarily grow as a vegetative mitotic diploid cells and to disperse to novel habitats through the guts of insect vectors as meiotic haploid quiescent spores encapsulated within a protective structure called the ascus (Stefanini *et al.*, 2012; Gibbs and Stanton, 2001; Coluccio *et al.*, 2008). Sporulation in turn is initiated when diploid cells encounter adverse environments (Neiman, 2005). Several strains of *S. cerevisiae* have been domesticated and in these sporulation can be induced by changes in nutrient availability and pH, thought to be correlated with changes in resource availability that indicate starvation

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64 (Neiman, 2005). Domesticated strains appear however to have lost much of their
65 ancestral sporulation efficiency (as measured by sporulation rates), when compared to
66 wild strains (Gerke *et al.*, 2006; De Chiara *et al.*, 2020), presumably because they have
67 been cultured as growing vegetative cells for many generations and maintaining the
68 potential to sporulate is developmentally and physiologically costly when dispersal is no
69 longer assured by insects (Ratcliff *et al.*, 2013). Spores and vegetative cells can both
70 survive insect guts, although with different success rates (Coluccio *et al.*, 2008), and only
71 spores are be able to cross-fertilize spores from other asci which can increase genetic
72 variation available for future adaptation to local environmental conditions (Reuter *et al.*,
73 2007).

74 Explanations about the evolution of sporulation have focused on a scenario where
75 populations periodically experience ingestion by insects that cause high mortality in
76 vegetative cells while allowing survival of spores (Ratcliff *et al.*, 2013; Coluccio *et al.*,
77 2008). In this scenario, the fitness of a genotype depends on how many progeny cells
78 survive ingestion and establish new colonies in the next habitat when the insect defecates.
79 If ingestion by insects happens only rarely, or only after the local resources are depleted,
80 then we expect genotypes that maximally convert resources into spores will evolve. In
81 these circumstances, selection should favor genotypes that sporulate only after most
82 nutrients are depleted and vegetative diploids experience starvation conditions. Ignoring
83 group-level traits (but see Discussion), individual *S. cerevisiae* growth and fitness depends
84 both on traits that confer local adaptation and traits that allow cells to accurately sense
85 unfavorable environmental conditions and only then to initiate and complete sporulation.
86 On the other hand, *S. cerevisiae* dispersal ability depends on the ability to survive insect
87 guts (as spores or vegetative cells) as well as mate recognition and germination once a
88 new suitable growth habitat is reached (Murphy and Zeyl, 2012).

89 We set out to test if fitness-trade offs between growth and dispersal traits in *S.*
90 *cerevisiae* are genetically constrained. For this we performed a replicated selection
91 experiment in five *S. cerevisiae* strains that varied in their level of domestication, and
92 where cultures were given time to exhaust their nutrient and were then propagated
93 either through the guts of *Drosophila melanogaster* fruit-flies or directly transferred. All
94 strains were initially isogenic and homothalic, such that evolution during the experiment
95 could only occur through selection on *de novo* mutations and with little opportunity for
96 recombination during the experiment. If fitness trade-offs result from a genetic constraint,
97 then domesticated strains may have lost most of their ability to respond to selection for
98 increased or faster sporulation, when compared with the wild strains. An alternative
99 mode of adaptation to insect passaging would be to evolve increased vegetative cell
100 survival through the *D. melanogaster* gut. The selection response of populations that
101 were directly transferred allow us to disentangle genetic constraints between sensing
102 starvation and initiation of sporulation independently of *D. melanogaster* gut vectoring.

103 Methods

104 Yeast and fruit-fly strains

105 We used a set of five genetically distinct strains of *S. cerevisiae* that were provided as
106 homozygous diploids with resistance to Geneticin (G418), an orthologue to kanamycin,
107 (Louvel *et al.*, 2014) obtained from the National Collection of Yeast Cultures, UK.

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108 These five strains represent a wide range of ecological backgrounds including an Oak
109 woodland in the Northeast united states (North American strain, AM), a palm flower in
110 a Malaysian forest (Malaysian, MY), a West African strain from a semi-natural beer
111 fermentation (West African, WA), a Sake brewery in Japan (Japanese Sake, JS), and
112 a winery in Western Europe (Wine European, WE). These strains cover a range of
113 backgrounds from wild, to partially human associated, to fully domesticated. Each
114 strain was genetially modified for use in a lab setting by knocking out the mating
115 type switching locus and adding DNA barcodes with stable diploid strains produced by
116 complementary mating.

117 Each Ancestor was split into 4 replicate populations, which were then split into
118 control and treatment populations. These 40 experimental populations were evolved
119 for 30 full cycles of population growth, starvation, and passaging. Passaging was
120 through Drosophila feeding in the treatment populations and by pipette for the control
121 populations.

122 The *D. melanogaster* stocks used in one of the selection treatments (see next section)
123 were created by outcrossing strains from isogenic Al-Ral, Taiwanese, Santa Barbarian
124 and Malaysian lines. Flies to be used as vectors during the experiments were allowed to
125 lay eggs on YPD agar plates. Adult flies were then removed and the eggs were bleached
126 using a 10% bleach solution for 40 minutes at 22 °C. Fly eggs were collected by sterile
127 pipette, washed with sterile water, and transferred using sterile technique to clean media.
128 Clean flies were reared and propagated on this media so that other yeasts and fungi
129 were minimized, but also so that the ingestion of antifungal elements did not reduce the
130 viability of living yeasts traveling through the gut.

131 Selection protocol

132 We grew each of our five diploid yeast strains in 2 mL of YPD (Yeast-Peptone-Dextrose)
133 liquid culture over a 5 day period. Samples of these initial strains were then frozen in
134 15% glycerol solution at -80 °C and labelled as the "Ancestral" treatment. The five
135 strains were split into 4 replicate each, and each was then split again into a paired
136 control and treatment population (Figure 1).

137 At the start of each selection cycle, each population was incubated in YPD at 30
138 °C for 5 days without shaking. In order to reduce chances of contamination from other
139 yeasts or bacteria, cultures were always grown in YPD media with G418, tetracycline,
140 and ampicillin added. We performed experiments without shaking to increase the
141 opportunities for haploid cells developing from spores to mate and form diploids. Under
142 these growth conditions, yeast populations usually consume most of the sugar resources
143 within 24 hours, and the 5 day period allowed ample time for spore formation to be
144 initiated and completed.

145 After the 5 day period, each population was adjusted to an optical density of 0.3
146 in an effort to both ensure adequate population size and to prevent blockage in the
147 capillary tube in the CaFe vial caused by high cell density (see below). The dilution
148 process was performed using spent YPD, YPD that had been depleted by culturing
149 yeast for 2 weeks and then filter sterilized. Spent YPD (SYPD) was used to mimic
150 carbon and nitrogen sources in a late growth stage yeast culture population and to
151 minimize population growth or germination of sporulated yeast cells (Madhani, 2007;
152 Neiman, 2005).

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For the fruit-fly experimental treatment we employed the Fly Capillary Feeder (CaFe) apparatus, adapted from Ja *et al.* (2007). CaFe tops used four 200μL pipette tips which were cut to increase opening size, rubber stoppers and standard rubber bands. These CaFe tops were then affixed to narrow fly vials each containing 2ml of 3% solidified agarose solution to maintain humidity within the vial. The pipette tips within the CaFe top were fitted to one 5μL capillary tube each. 18 hours prior to treatment, four clean, sexed 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).

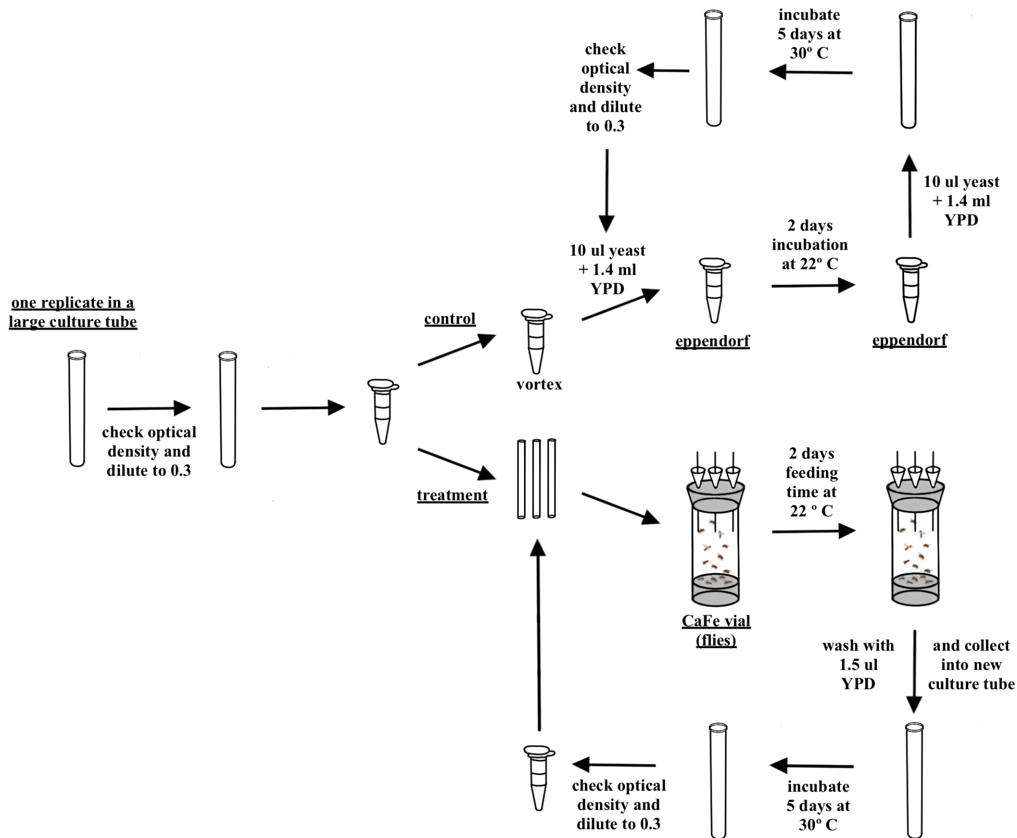

Figure 1: Each lineage was grown in liquid media and the sample was adjusted to an optical density of 0.3 and split into control and treatment tubes. From that point, the control and treatment went through parallel procedures lasting 7 days: 2 days of exposure to treatment (control: 22 °C bench-top incubation; treatment: 22 °C exposure to flies) and 5 days of growth or sporulation in liquid media at 30 °C. This was repeated for 30 cycles.

Yeast cultures were distributed into CaFe feeding apparatus and offered to 3-4 clean flies (see above). Flies were allowed to feed for 48 hours and then removed from the vials. Measurements of total fly food consumption were taken by recording the change in

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165 meniscus of the two capillary tubes. Flies were removed from the vials which were then
166 rinsed with 1.6 mL YPDA media and the supernatant (1.5 mL total volume because
167 some volume is reabsorbed into the agar in the vial) was collected and used as the
168 inoculate for the next round of yeast population growth.
169 For the control selection treatment yeast cultures were not distributed into the CaFe
170 feeding apparatus, being instead placed on the bench-top nearby. After 48h, each control
171 population was vortexed and 10μL of each culture was moved to 1.49 mL of YPDA in a
172 new culture tube (1.5 mL total volume).
173 These selection protocols were repeated for 30 growth cycles. Samples of each
174 population were frozen every other cycle as a backup in case of contamination. After
175 the last cycle all population were frozen and stored at -80 °C.

176 Sporulation assay

177 The sporulation assay was performed in two blocks defined by the date of assay ex-
178 periment, with each block containing samples of all 40 control and treatment replicate
179 populations as well as the ancestral populations. Each ancestral population was assayed
180 6 times. In each block, two samples were taken from each population and then further
181 divided into two technical replicates each for measurement. For the ancestral populations,
182 three samples were taken in each assay block, also divided into two technical replicates.
183 We first grew thawed population samples on YPD agar plates and then inoculated
184 liquid YPD cultures which grew for 6 hours without shaking at 30°C. Each sample was
185 diluted to an optical density of 1.5 and 2 mL was centrifuged and washed to remove any
186 traces of growth media. Cells were then resuspended in in 2 mL of Potassium Acetate (
187 2% KAc at pH ≈ 6.7), and incubated at 30°C with shaking (230 rpm). Measurements
188 were taken at 2.5 days and at 5 days. Samples were diluted by taking 5μL of sample
189 and 95μ L water. A field of cells was then photographed at $400X$ magnification. Images
190 were processed by adding a grid and counting the number of spores and vegetative cells.

191 Statistical analysis

192 We developed hierarchical models of sporulated cell counts where the observed counts
193 were taken to be binomially distributed. We modeled the logit of the binomial parameter
194 as a linear function of the interaction between selection treatment, and assay time
195 along with a random effect of the replicate population, where we pooled variation
196 among sampling and technical replicates. We performed these analyses separately on
197 the Ancestral populations because they necessarily do not have the same structure of
198 replicate experimental populations. For the experimentally evolved populations, we
199 modelled each strain separately and then performed comparative analyses on the inferred
200 posterior distributions.
201 The main model considers the sporulation probability to be a function of the
202 treatment type (fly treatment or control) and the assay time (2.5 days or 5 days) giving

$$\begin{aligned} \theta_i &= \beta_{T_i,A_i} + \phi_{\text{replicate}_i} \\ C_i &\sim \text{Binomial}(\text{N}_i, \text{logit}^{-1}(\theta_i)) \end{aligned} \quad (1)$$

203 where C_i is the count of sporulated cells in sample i, N_i is the total number of cells
204 counted in sample i. The predictor β depends on the T treatment type and the A assay

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205 time of observation i.

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

$$\begin{aligned} \phi_i &\sim \text{Normal}(0, \sigma^2) \\ \sigma &\sim \text{t}_3(0, 10) \end{aligned}$$

208 meaning that each replicate population is considered to be sampled from a normal
209 distribution and the prior on the standard deviation is a Student's t distribution with 3
210 degrees of freedom, a location of 0 and scale of 10.

211 Here, because we assume that the parameter for each combination of strain, treatment,
212 and assay time is independent, this is similar to a generalized linear model with main
213 effects and interaction terms for each of the three factors. The additional term ϕ
214 represents the random effect of the four replicate populations and effectively allows for
215 overdispersion of the count data relative to a binomial model. Such overdispersion in
216 the count data could arise due to idiosyncratic differences during the creation of the
217 replicate experimental populations from the ancestral strain.

218 We took a Bayesian approach for parameter inference and compared posterior
219 distributions of parameter estimates as a way to evaluate hypotheses regarding the
220 causes of selection responses. We specified the models using `BRMS` (Bürkner, 2017,
221 2018) and `STAN` (Stan Development Team, 2018) using `RStan` which performs Bayesian
222 inference using a Hamiltonian Monte Carlo sampling to calculate the posterior probability
223 of the model parameters given the observed data (`R` version 3.3.2, `RStan` version 2.15.1)
224 (R Core Team, 2019). Convergence of the MCMC chains was checked by ensuring that $\hat{R}$
225 was less than 1.1, where $\hat{R}$ is a metric describing the variability between chains (Gelman
226 *et al.*, 2013).

227 Data and code archiving

228 All data and code for analysis will be submitted to Data Dryad. Complete code to
229 perform the analysis is available in a supplemental RMarkdown file.

230 Results

231 Ancestral sporulation rates

232 Sporulation rates at both assay times differ among the ancestral strains (Figure 2). We
233 also compared the model with factorial effects of strain and assay time to a model with
234 no effect of strain and found that the full model was highly supported (see supplementary
235 rMarkdown file). As expected, this reflects their evolutionary history, with strains coming
236 from natural environments showing higher sporulation rates while strains from industrial
237 alcohol production showing lower sporulation rates (Gerke *et al.*, 2006; De Chiara *et al.*,
238 2020). The African strain WA, which is thought to be only partially-domesticated (see
239 Methods) shows intermediate sporulation rates.

240 The sporulation rates of the strains are distinct as is the transition between days 2.5
241 and day 5, inferred by comparing models that do not include the strain or day of assay
242 effect (see supplementary R markdown file). The strain effect can also be visualized by

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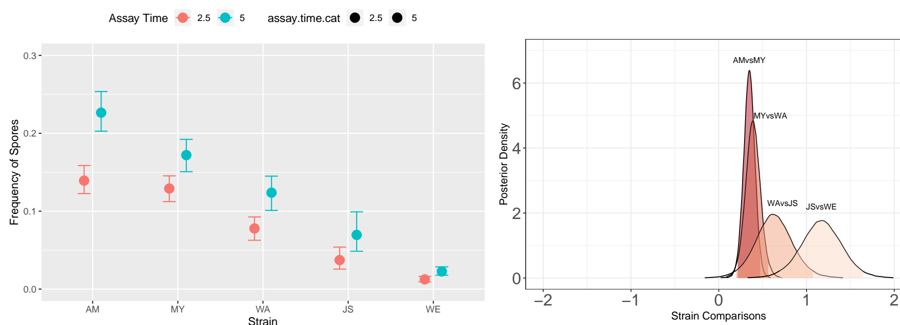

Figure 2: Sporulation rates in the ancestral strains. Panel A shows the fitted values for sporulation rate measured at 2.5 and 5 days (see equation 1), with the solid circles showing the median posterior value and the bars are the 95% posterior credible interval. The strains are arranged by decreasing ancestral sporulation rate, which also reflects the the degree of domestication (AM and MY, wild; WA, partially-domesticated; JS and WE, industrial). The difference between sporulation rate at 2.5 and 5 days can be interpreted as the speed at which vegetative cells enter and complete sporulation. Wild strains therefore show a faster transition to sporulation than domesticated strains. Panel B shows the pairwise comparisons between successive strains at the 5 day assay time point. The posterior probability of the difference being less than 0 is less than 0.001 for all comparisons

243 comparing the posterior distributions of the day five assay in a pairwise fashion between
244 each successive strain to show that each strain has a distinct sporulation rate (figure 2)
245 The difference between sporulation rate at 2.5 and 5 days represents the speed
246 at which vegetative cells enter and complete sporulation once they sense starvation
247 conditions while in the culture tubes (Figure 1). For this trait, wild strains show
248 a sharper change in completed sporulation between the time points as compared to
249 domesticated strains (Figure 2). Note also that the strains are inferred to have distinct
250 sporulation rates, as the posterior distributions between strains do not overlap at the
251 95% level.

252 Selection responses in the fruit-fly treatment

253 After 30 growth cycles involving fruit-fly gut dispersal the evolved populations showed
254 higher sporulation rates (Figure 3 and figure 4). For all strains, the posterior distribution
255 is well away from 0 (the posterior probability of the difference from the ancestral being
256 less than zero is less than 10^{-3}), with sporulation rates of the evolved populations at
257 the assay time of day 5 ranging from 30% to 95%. However, the magnitude of this effect
258 is independent of the degree of domestication: for example, one of the wild strains (AM)
259 shows the maximum response relative to its ancestral state, while another wild strain
260 (MY) shows an intermediate response.
261 The rate of sporulation completion, as measured by the assay time effect, varies
262 with the degree of domestication (Figure 5). Wild strains showed a large response to
263 fly vectoring in that there was a quicker evolved onset of sporulation (the posterior
264 probability of the difference from the ancestral being less than zero is less than 10^{-3}).
265 The other three strains showed more modest responses to selection and the posterior

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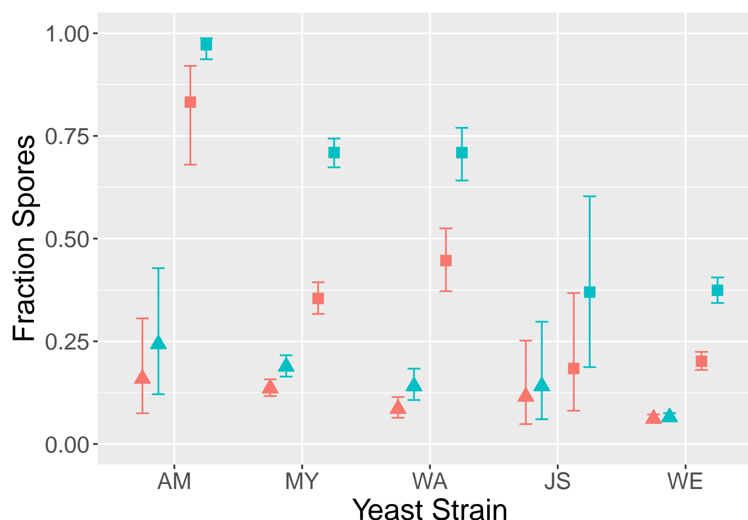

Figure 3: Inferred sporulation frequencies in the experimentally evolved strains strains. The triangles represent control population, while squares represent fly treatment populations. The red fill is for the 2.5 day assay point, while the blue fill is for the 5 day assay point. Replicate populations in each treatment are modeled hierarchically.

266 probablity of a difference from the ancestor overlapped with 0.

267 Selection responses in the control treatment

268 There is evidence that some of the control evolution treatment strains show a directional 269 shift in sporulation rate (Figure 4). For the two domesticated strains (WE and JS) 270 the posterior distributions for sporulation rates and assay time changed significantly 271 after evolution. Both WE and JS increased their total sporulation rate in response to 272 control evolution conditions (posterior probability of a change less than 0 of less than 273 10^{-3} for WE and of less than 0.05 for JS). In contrast, each of the other strains broadly 274 overlapped with 0 change from the ancestor. In terms of the timing of sporulation, 275 only WE was strongly diverged from the ancestor, with a decrease in the effect of assay 276 time on sporulation (the posterior probability of the difference from the ancestral being 277 greater than zero is less than 10^{-3}).

278 Comparison between the selection response in the fruit-fly and control 279 conditions

280 Comparisons between the fly evolved treatment populations and the control evolved 281 treatment populations show a consistent effect of exposure to fly vectoring that involved 282 and increase in the total sporulation rate and an increase in the assay timing effect (see 283 figures 4 and 5). In all cases the posterior probability of the difference between the 284 treatment and control being less than zero is less than 10^{-3}.

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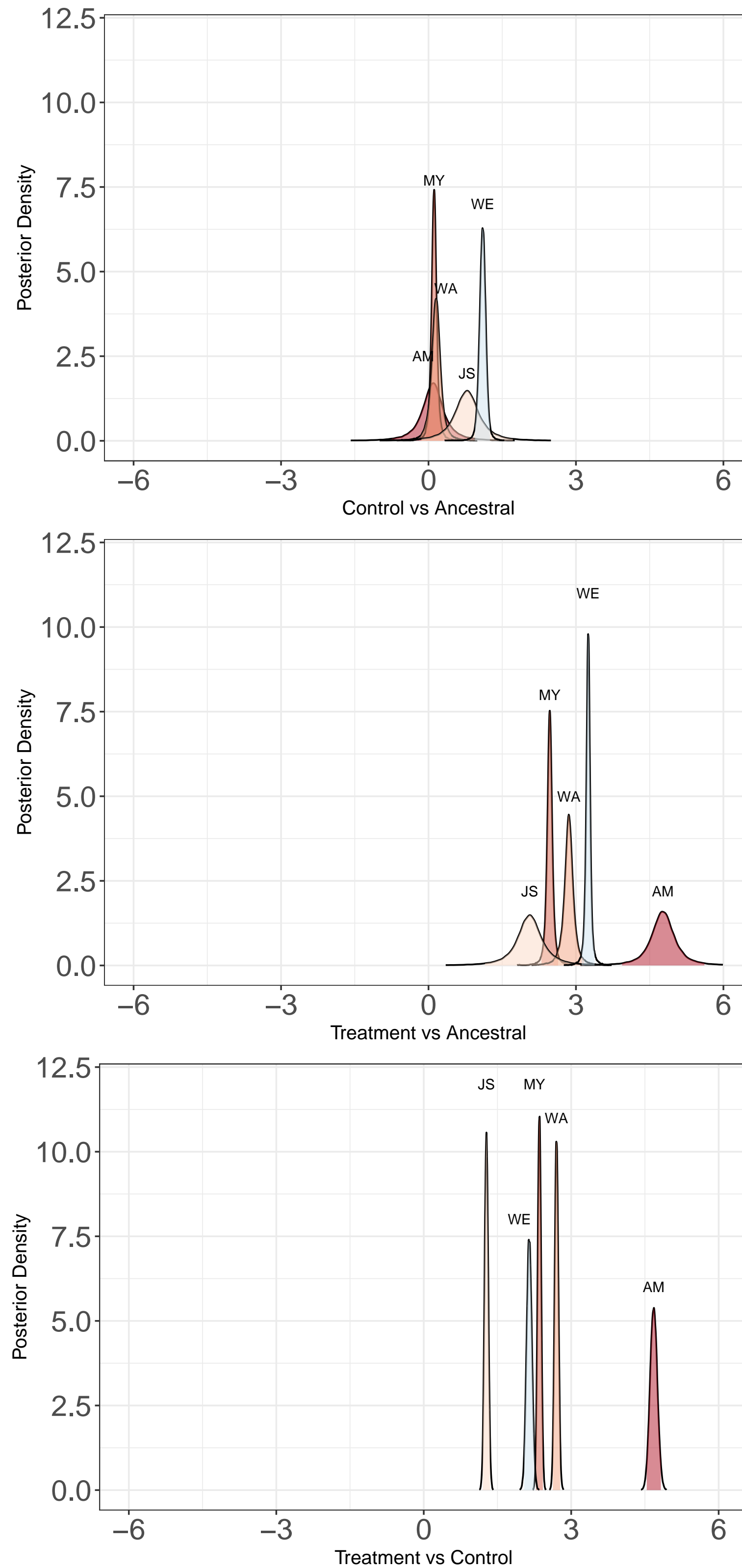

Figure 4: Three representations of the effect of treatment on sporulation rate. The top two panels compare the posterior distributions from the strain-specific models to the mean of the ancestral inference for that strain. The bottom panel compares the treatment to control for each strain.

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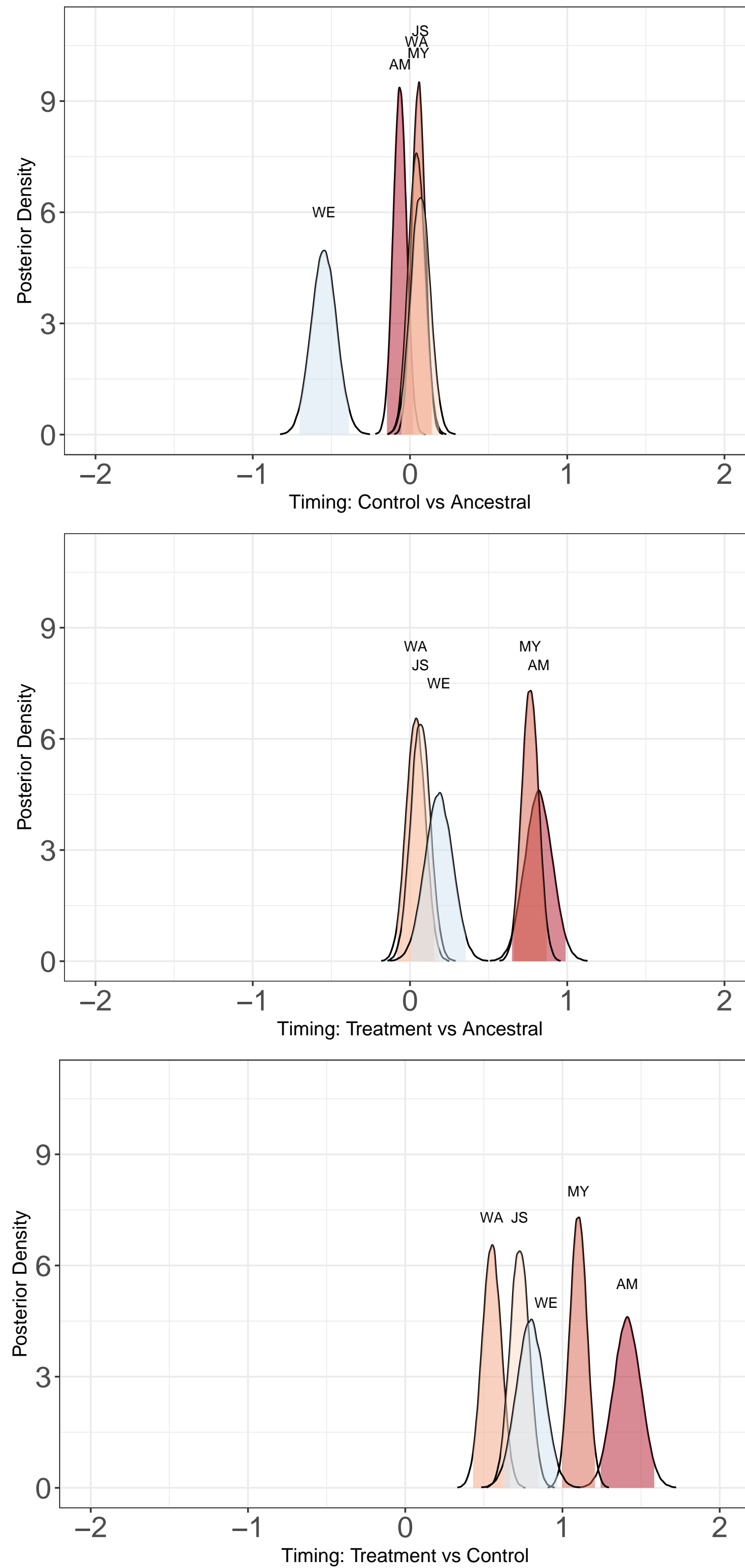

Figure 5: The effect of the fly treatment on the timing of sporulation. Larger positive values indicate sharper transition to sporulation between the time point at day 2.5 and at day 5. Comparisons between the treatment and control evolution experiments show that the transition to sporulation is sharper for populations evolving in the presence of flies.

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285 # Discussion

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

296 Our results mostly support the latest view about the adaptive value of sporulation.
297 We found that strict passaging through the *Drosophila* digestive tract resulted in the
298 evolution of both faster sporulation and higher sporulation rates. Since both of these
299 traits are properties of the growing vegetative cells, we might have expected that
300 domesticated strains would show partial loss of sensitivity to starvation as well as
301 reduced initiation and completion of sporulation. Given that these strains are starting
302 from a deficit in their tendency to sporulate, these lineages could have adapted to
303 passaging through *Drosophila* guts by increasing vegetative cell survival through the
304 gut. Indeed, analysis of a large set of yeast isolates has shown that some strains have
305 evolved increased survival of quiescent vegetative cells (De Chiara *et al.*, 2020). This
306 was not the case here, where even the domesticated strains showed a strong response to
307 the fly treatment in terms of their total sporulation rate. However, domesticated strains
308 evolved a lower overall sporulation rate and lower speed of sporulation, as compared
309 with the wild strains. This suggests that domestication led to the loss of mutational
310 options that allow the cells to sense and respond to environmental changes. These
311 results are reminiscent of those of Kvitek and Sherlock (Kvitek and Sherlock, 2013),
312 where experimental evolution in constant environment led to the loss of developmental
313 and physiological programs involved in the sensing of environmental variation.

314 Because our experiments allowed populations to grow for a fixed time period before
315 ingestion, we expected selection for a steep change in sporulation rates associated
316 with that timing or alternatively for vegetative cell starvation sensing. In particular,
317 there was ample time before ingestion to deplete nutritional resources, sense it and
318 respond appropriately. The results observed in the control populations, which were
319 not passaged through the *Drosophila* gut but that were also subjected to selection for
320 starvation resistance are instructive. In them, we found that only those derived from
321 the domesticated isolates showed an increase in total sporulation rate. We might have
322 expected all populations to evolve decreased sporulation if vegetative cell resistence
323 instead evovled. Mutational options towards vegetative cell resistance thus appear
324 to be fewer than those of sporulation. Selection responses in both of our treatments
325 further indicate that the adaptive value at the origin of sporulation was not to survive
326 insect vectoring, or outcrossing, but the ability to cope with challenging environments.
327 Sporulation was perhaps just co-opted as a dispersal strategy because of ecological
328 constraints, namely that small Drosophilids may preferentially diet on yeast (Schiabor
329 *et al.*, 2014) and are small enough to vector them between favorable habitats (Gibbs
330 and Stanton, 2001; Stamps *et al.*, 2012; Tsai *et al.*, 2008).

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331 We focused on selection for sporulation based on individual selection. Natural yeast
332 populations are strongly spatially-structured and thus group-level selection must have also
333 be at work in nature. Theoretical expectations for the evolution of sporulation in these
334 circumstances depends on the way that cells gain resources from the environment and
335 are passaged to future demes. In our experiments, there was strict vertical transmission
336 of populations through insect ingestion and selection was strongest on surviving the
337 passaging event itself. In spatially-heterogeneous conditions, sporulation by an individual
338 cell should evolve in response to the pattern of variability in terms of both resource
339 availability and timing of insect ingestion. Prior theory on this idea treats the evolution of
340 the sporulation as a kin selection problem, asking how an individual cell that sporulates
341 affects clone-mates in terms of resource availability (Ratcliff *et al.*, 2013), because cells
342 that sporulate will stop taking up resources, which are then available both to genetically-
343 related and genetically-unrelated cells in the same environment. The degree of mixing
344 between cell lineages in the founding of new demes probably limits kin selected benefits
345 and will likely select for increases in the total number of viable emigrants each genotype
346 produces from an existing demes; but such theory remains to be fully developed.
347 In the context of a competition-colonization trade-off (Tilman, 1994), a yeast strain
348 that sporulates earlier or at a higher frequency before ingestion by insects is more likely
349 to survive the process of ingestion, digestion and transfer. However, if the period of
350 competition within a deme is long, the higher sporulating genotype will reproduce more
351 slowly and eventually be displaced by genotypes that have lower sporulation rates. In
352 contrast, once insect ingestion occurs, genotypes that have a higher fraction of cells
353 in the sporulated state will have higher survivorship during vectoring and therefore
354 increased representation in newly founded demes. Competition-colonization trade-offs
355 can thus allow coexistence of alternative strategies, sometimes allowing many strategies
356 to coexist (Snyder and Adler, 2011). We speculate that such competition-colonization
357 trade-offs lead to coexistence of yeast species with similar physiological niches but
358 differing sporulation and germination programs. In particular, *S. cerevisiae and* S.
359 paradoxus have similar developmental and physiological profiles for surviving adverse
360 environments but, tellingly, differing sporulation and germination programs (Murphy
361 and Zeyl, 2010, 2012). A possible explanation for this coexistence therefore is that they
362 occupy distinct positions in the competition-colonization space. And in general, such
363 competition-colonization trade-offs may explain much of the biological diversity found
364 in microbes.

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