biology letters

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Biol. Lett. 2006 **2**, 553-556
doi: 10.1098/rsbl.2006.0534

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doi:10.1098/rsbl.2006.0534
Published online 12 September 2006

Mate choice assays and mating propensity differences in natural yeast populations

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In sexual microbes, mating occurs by fusion of individual cells. This complete fitness investment suggests that cell behaviour could potentially mediate prezygotic isolation between microbial species, a topic about which very little is known. To investigate this possibility, we conducted individual cell mate choice trials and mass-culture mating propensity assays with isolates from sympatric natural populations of the closely related yeasts *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. Although we found no evidence for active species recognition in mate choice, we observed a marked difference in mating propensity between these two species. We briefly discuss the possibility that this mating propensity difference may contribute to reproductive isolation between *S. cerevisiae* and *S. paradoxus* in nature.

Keywords: *Saccharomyces* spp.; mate choice; mating propensity; prezygotic isolation

1. INTRODUCTION

Saccharomyces cerevisiae is well known as a human commensal and a model organism in cellular and molecular biology. However, wild populations of *S. cerevisiae* are present in nature alongside *Saccharomyces paradoxus* in deciduous woodlands (Naumov *et al.* 1998; Sniegowski *et al.* 2002). Hybrids between *S. cerevisiae* and *S. paradoxus* manifest postzygotic isolation in the form of very low spore viability (Naumov 1996), and genomic comparisons indicate that these species diverged approximately 6 Myr ago (James *et al.* 1997; Naumov *et al.* 2000). Mating in *Saccharomyces* is mediated by a pheromonal signal-transduction cascade, and previous work has shown that individuals within *S. cerevisiae* choose a mating partner based on the level of pheromone production (Jackson & Hartwell 1990). All these observations suggest that prezygotic isolation could well have evolved between *S. cerevisiae* and *S. paradoxus*. Here, we report on assays that test for prezygotic isolation between these yeasts and their initial results.

The electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2006.0534 or via http://www.journals.royalsoc.ac.uk.

2. MATERIAL AND METHODS

(a) *Yeast strains*

Our study utilized strains isolated from sympatric populations of *S. cerevisiae* and *S. paradoxus* at a single woodland site in Pennsylvania, USA (Naumov *et al.* 1998; Sniegowski *et al.* 2002). The *S. cerevisiae* strains (YPS 681 and 670, referred to as Sc1 and Sc2) represent the two distinct genetic haplotypes found at this site (Kuehne 2005). The *S. paradoxus* strains (YPS 664 and 646, referred to as Sp1 and Sp2) were randomly drawn from nine isolates obtained from this site, which are part of a large recombining population (Kuehne 2005). All natural isolates were homothallic diploids.

(b) *Media*

Growth and mating experiments used synthetic oak exudate (SOE), a complex medium that we developed to approximate the composition of oak exudates (Kevan *et al.* 1983; Xu *et al.* 2001), a common habitat from which *Saccharomyces* have been isolated in nature. SOE liquid contains 1% sucrose, 0.5% fructose, 0.5% glucose, 0.1% yeast extract and 0.15% peptone; 1.6% agar is added for solid medium. Sporulation plates contained 1% potassium acetate, 0.005% zinc acetate and 2% agar (Rose *et al.* 1990).

(c) *Construction of heterothallic a and α strains*

We created two complete sets of heterothallic strains with different antibiotic resistances: one set with geneticin resistance and one set with clonat resistance. The natural isolates were transformed (Gietz & Woods 2002) with either a kanMX4 (Wach *et al.* 1994) or a natMX4 (Goldstein & McCusker 1999) cassette targeted to the HO gene. Antibiotic resistant transformants were sporulated and stable haploid **a** and **α** colonies were obtained by tetrad dissection.

(d) *Mate choice assays*

All strains were grown in SOE liquid at 30°C to the point at which they yielded the most mating-proficient cells. Fresh medium was inoculated with culture at stationary phase (1 : 50 dilution) and grown approximately 18 h for *S. cerevisiae* and approximately 36 h for *S. paradoxus*. For each trial, a focal cell (**a** or **α** mating type) was offered two cells of the opposite mating type with different antibiotic resistances: one derived from the same ascus and the other from an ascus of a different strain. (Pilot experiments showed that the antibiotic/mating-type combination had no effect on mate choice.) We used a micromanipulator to set-up potential maters in a triangle formation on agar with each cell touching the other two, as illustrated in figure 1*a*. After 3 h at 30°C, the mating cells and the haploid budding cell were separated (see electronic supplementary material for video); 24 h later, the mating plate was replica plated to antibiotic plates in order to determine with which partner the focal cell had mated. Only trials in which the third (unmated) cell was budding were scored to ensure that all three cells in scored trials had been viable and at approximately the same point in the cell cycle.

(e) *Mating propensity assays*

All strains were first grown in SOE liquid as described previously. Cultures were centrifuged, washed and resuspended in water, and briefly sonicated to break up clumps. The cell suspensions were analysed on a Beckman Z2 Coulter Counter to determine density. For each replicate assay, an equal number of **a** and **α** cells with different antibiotic resistances were combined and poured onto a 47 mm SOE plate so as to form a single layer of cells. The mating plate was incubated at 30°C and random portions of it were sampled at 30 min intervals for the first 5 h and once at 24 h. The samples were suspended in water, spread on SOE plates and replica-plated to SOE+geneticin+clonat plates after 24 h to determine the proportion of cells in the sample that were mated diploids.

3. RESULTS

Figure 1*b* shows results from intraspecific mate choice trials. Both species mated at random in these trials (figure 1; $\chi^2_3 = 3.6$, $p = 0.32$). Figure 1*c* shows results from interspecific trials. *S. cerevisiae* mated intraspecifically far more often than expected in these trials, whereas *S. paradoxus* appeared to mate preferentially with *S. cerevisiae*. Overall, the hypothesis of random mating was rejected in the interspecific mate choice trials ($\chi^2_7 = 41.7$, $p < 0.0001$).

To test whether contributions from the mating propensities of both species could explain the observed discrepancy in mate choice between

Received 16 June 2006
Accepted 14 August 2006

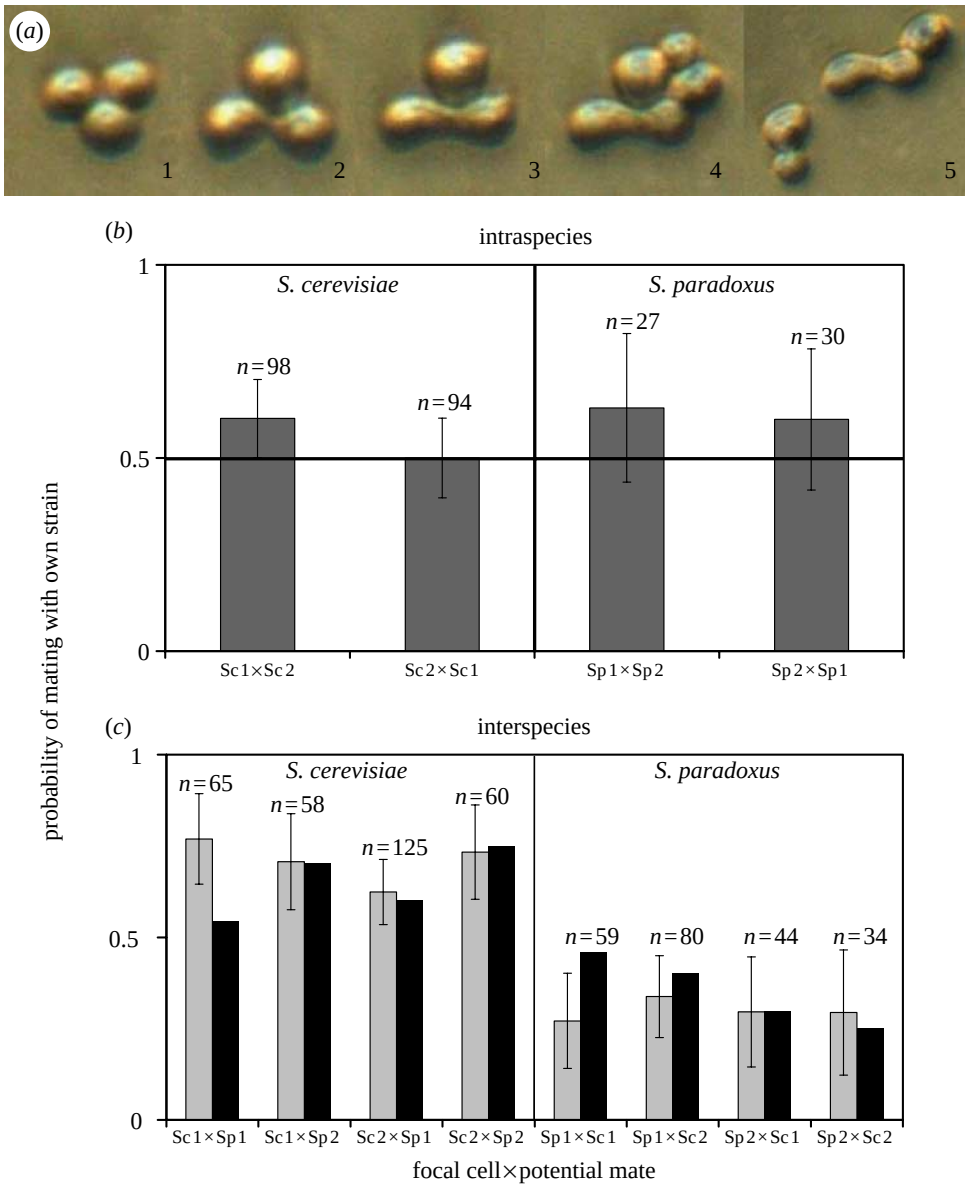

Figure 1. Mate choice trials in *Saccharomyces*. (*a*) Representative photos of the mating experiment. (1) Three cells placed together; (2) two cells beginning to mate; (3–4) both the haploid cell and the mating cells budding; (5) the haploid cell and the mated cells separated. (*b*) Results of intraspecific mate choice trials. Sc, *S. cerevisiae*; Sp, *S. paradoxus*. The horizontal line represents the random mating expectation (0.5). (*c*) Results of interspecific trials. Bars with errors in (*b*) and (*c*) show observed proportion of trials in which the focal cell mated with the cell from its own strain ± 2 s.e.m. Standard errors were calculated using the binomial variance for the specified number of trials, with $\pi = 0.5$. There was no difference between trials with **a** or **α** focal cells ($p = 0.68$, paired *t*-test), so results were pooled. Bars without errors in (*c*) show expected proportion of matings based on the ratios of observed mating propensities shown in figure 2; for example, the expected proportion of trials in which strain 1 mates with itself when also given the choice of strain 2 would be slope 1/(slope 1 + slope 2).

S. cerevisiae and *S. paradoxus*, we quantified the mating propensities of the individual strains in mass mating assays (figure 2). *S. cerevisiae* mated more quickly with itself than did *S. paradoxus*, and at 24 h a greater proportion of *S. cerevisiae* cells than *S. paradoxus* cells had mated (70 versus 47%, $F_{1,12} = 5.13$, $p = 0.04$). The relative mating propensities observed within the two species (the ratios of the slopes illustrated in figure 2) provided a good predictor of the proportion of mate choice trials that resulted in mating within strains ($\chi^2_7 = 11.8$, $p = 0.11$; figure 1*c*).

To test whether the mating propensities of each species contributed equally to courtship, we examined the kinetics of interspecific mass matings in experiments identical to those previously conducted to measure mating propensity within each species (data not shown; see electronic supplementary material for details). Surprisingly, the rate of interspecific mating was statistically indistinguishable from the rate of *S. paradoxus*/*S. paradoxus* mating and significantly slower than the rate of *S. cerevisiae*/*S. cerevisiae* mating ($p = 0.41$ and 0.04, respectively, repeated measures two-way ANOVA with planned comparisons). This result suggests that *S. paradoxus* has a stronger influence on the rate of mating, or that *S. cerevisiae* actively avoids mismating, or some combination of these possibilities. An aspect of our mate choice trials also supports this interpretation; we observed that trials involving one *S. cerevisiae* cell and

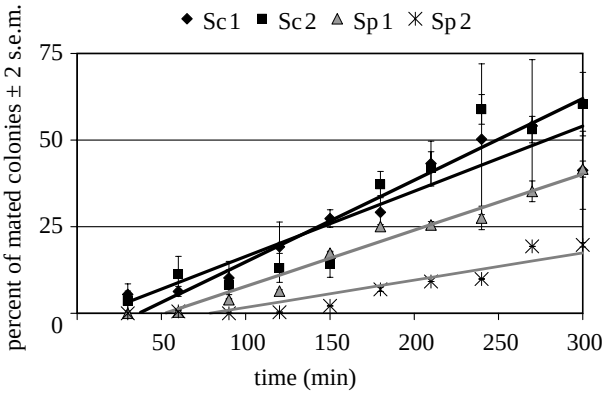

Figure 2. Mating propensities measured in intraspecific mass mating assays. Diamonds, Sc 1 ($n=4$, slope$=0.0019$). Squares, Sc 2 ($n=4$, slope$=0.0024$). Triangles, Sp 1 ($n=4$, slope$=0.0016$). Crosses, Sp 2 ($n=2$, slope$=0.0008$). Error bars represent ± 2 s.e.m. The two *S. paradoxus* strains are significantly different from each other and the *S. cerevisiae* strains; the *S. cerevisiae* strains are not significantly different from each other (Tukey's honestly significant difference test, $\alpha=0.05$). All analyses were done on log-transformed data; the results from the transformed and untransformed data were qualitatively the same. Untransformed data are presented in the graph.

two *S. paradoxus* cells were significantly more likely to result in no mating than trials with two *S. cerevisiae* cells and one *S. paradoxus* cell (6.5 versus 1.8%, $n=577$ trials, $p=0.008$, Fisher exact test).

4. DISCUSSION

We have demonstrated the feasibility of assaying prezygotic isolation in a sexual microbe using the closely related yeasts *S. cerevisiae* and *S. paradoxus*. In our assay, *S. cerevisiae* mates with itself more frequently than with *S. paradoxus*, whereas *S. paradoxus* makes the 'wrong' choice more frequently than expected. This difference appears to be driven by mating kinetics: *S. cerevisiae* has a higher mating propensity than *S. paradoxus*, and the ratio of mating propensities is a good predictor of the outcome of the mating assays.

Our results are reminiscent of earlier studies in which differences in mating propensity could explain apparent asymmetric mate choice in *Drosophila* (reviewed in Barton & Charlesworth 1984). However, unlike in *Drosophila*, mating in *Saccharomyces* is a one-time commitment; fusion of two maters results in either fertile offspring or a genetic dead end. Thus, we are tempted to speculate that disparity in mating propensity could itself act as a passive prezygotic isolating barrier in yeast, because individuals who mate quickly will mate with each other and not be available later for those who mate slowly. This very form of prezygotic isolation has been shown to evolve within a few tens of generations in laboratory experiments with *S. cerevisiae* when the cost of mismating is severe (Leu & Murray 2006).

A limitation of our results is that we have conducted our tests using haploid vegetative cells rather than haploid spores. The mating behaviour of *Saccharomyces* yeasts in nature is as yet uncharacterized; some mating events may involve interactions between spores rather than vegetative cells. The use of cells has a practical advantage in that we are able to ascertain mating type status before the assays and thus can confidently predict the possible outcomes of any given assay. In addition, by using cultures of cells of known mating type and genotype, it will be possible to characterize evolved differences in gene expression and pheromone expression levels related to mating behaviour divergence in these species.

Another limitation is that we have only assayed behaviour in two strains of each species. We stress that the strains used here were isolated in sympatry and that the *S. cerevisiae* strains represent the total genetic diversity at our study site. Nonetheless, it is possible that the mating differences are properties of the strains rather than that of the species themselves. Further work incorporating larger numbers of isolates from each species is underway and will definitively address whether the mating behaviour variability that we have observed can be extrapolated to the species level, the population level, or only the individual level. Regardless, our assays have documented substantial variability in mating behaviour in natural populations of a sexual microbe, and this variability certainly has implications for the evolution of prezygotic isolation.

We are grateful to Barak Cohen for strains and primers, Peter Petraitis for statistical advice and Clifford Zeyl for access to photographic equipment. We also thank P.P. and C.Z. for comments on the manuscript. Supported by grants from the National Science Foundation (DEB-0351445) to P.D.S. and (DDIG-DEB-0308895) to H.A.M. and P.D.S.

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