environmental microbiology reports

Environmental Microbiology Reports (2016) **00**(00), 00–00 doi:10.1111/1758-2229.12446

A systematic forest survey showing an association of *Saccharomyces paradoxus* with oak leaf litter

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Summary

Although we understand the genetics of the laboratory model yeast *Saccharomyces cerevisiae* very well, we know little about the natural ecology and environment that shaped its genome. Most isolates of *Saccharomyces paradoxus*, the wild relative of *S. cerevisiae*, come from oak trees, but it is not known whether this is because oak is their primary habitat. We surveyed leaf litter in a forest in Northern Germany and found a strong correlation between isolation success of wild *Saccharomyces* and the proximity of the nearest oak. We compared the four most common tree genera and found *Saccharomyces* most frequently in oak litter. Interestingly, we show that *Saccharomyces* is much more abundant in oak leaf litter than on oak bark, suggesting that it grows in litter or soil rather than on the surfaces of oaks themselves. The distribution and abundance of *Saccharomyces* over the course of a year shows that oak leaf litter provides a stable habitat for the yeast, although there was significant tree-to-tree variation. Taken together, our results suggest that leaf litter rather than tree surfaces provide the better habitat for wild *Saccharomyces*, with oak being the preferred tree genus. 99.5% of all strains (633/636) isolated were *S. paradoxus*.

Introduction

The fermentation ability of *Saccharomyces cerevisiae* has made it an important component of human culture for thousands of years. In more recent times *S. cerevisiae* has also become one of the best-studied laboratory model organisms, and it was the first eukaryote to have its genome completely sequenced (Goffeau *et al*., 1996). However, its life in the wild remains mysterious (Greig and Leu, 2009) and even its natural habitat is not known. To fully interpret and understand the rich data generated by studying *S. cerevisiae* in the laboratory, it is important to better understand its natural history and to place the species in its ecological and evolutionary context. Moreover, connecting existing lab knowledge with knowledge its ecological and environmental conditions could help biological research in many areas like evolutionary and ecological genomics, population genetics, microbial biogeography, community ecology and speciation (Replansky *et al*., 2008).

S. cerevisiae is readily and consistently found in fermenting wine and other human-made alcoholic fermentations. Many assume, therefore, that its natural habitat must be grapes, or another fruit or sugar source. The unusual tendency of *Saccharomyces* yeast to use inefficient fermentation even when oxygen is present (the Crabtree effect), rather than more efficient respiration, is seen as an evolutionary adaptation to fruit (Piškur *et al*., 2006). But there is actually little direct evidence that the natural habitat of *S. cerevisiae* is fruit, and the large number of places it can be found in low frequency suggest that it may instead be an niche-less generalist (Goddard and Greig, 2015).

It can be difficult to investigate the natural habitat of *S. cerevisiae* because of its long association with humans (Vaughan-Martini and Martini, 1995). Whilst wild, undomesticated examples of *S. cerevisiae* certainly exist (Fay and Benavides, 2005; Wang *et al*., 2012), there is a risk that any individual found in a natural habitat may have recently escaped from a human fermentation or may have mixed ancestry with domesticated strains. Researchers wishing to study wild yeast therefore often look instead at the closest known relative of *S. cerevisiae*, *S. paradoxus* (Replansky *et al*., 2008). The two species are phenotypically and biochemically nearly indistinguishable, share almost the same profiles of assimilation and fermentation of organic compounds and can exist in sympatry in natural habitats (Naumov *et al*., 1998; Sniegowski *et al*., 2002; Sampaio and Gonçalves, 2008), but *S. paradoxus* is not thought to be affected by domestication as it is not found in human fermentations.

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Historically, nearly all isolates of *S. paradoxus* have come from oak (*Quercus* spp.). A recent survey of *S. paradoxus* available from culture collections found 81% came from oak, with rest recently isolated from the newly-identified North American habitat of maple trees (Bozdag and Greig, 2014). The earliest isolation recorded in literature was 1914 from Russian oak exudates (Batschinskaya, 1914) and later 1957 from the bark and surrounding soil of oak, as well as from soil surrounding pine (Yoneyama, 1957). Since this time, we find a focus on oak trees, with oak bark as the main source of wild *Saccharomyces* strains (Sniegowski *et al*., 2002; Johnson *et al*., 2004; Koufopanou *et al*., 2006; Sampaio and Gonçalves, 2008; Zhang *et al*., 2010; Wang *et al*., 2012; Charron *et al*., 2014; Sylvester *et al*., 2015). In the Southern Hemisphere, *Nothofagus* trees (southern beeches) inhabit the ecological niche of oaks, and *Saccharomyces* species can be found instead on the surfaces of these trees (Libkind *et al*., 2011).

Although most samples of *S. paradoxus* come from oak trees, it is not clear whether oak trees form a primary habitat to which it is adapted. Only a few recent studies allow comparison of *Saccharomyces* isolation success among different potential habitats (Glushakova *et al*., 2007; Sláviková *et al*., 2007; Sampaio and Gonçalves, 2008; Charron *et al*., 2014; Sylvester *et al*., 2015; Dashko *et al*., 2016; Robinson *et al*., 2016). Most studies about wild *Saccharomyces* isolates focus directly on the oak environment and use enrichment isolation methods which do not give any information about the actual number of yeast cells in a given environmental sample. With enrichment culture, a sample such as oak bark is incubated in a sugar-rich fermentable medium (essentially replicating spontaneous wine fermentation): some samples yield a culture dominated by *Saccharomyces*, but most do not. We recently showed that enrichment culture was sensitive enough to detect single cells, and were therefore able to estimate the average density of *Saccharomyces* cells as less than two cells per square cm of oak bark surface. We also found that the growth of *Saccharomyces paradoxus* on oak bark nutrients was suppressed by the much more abundant members of the oak bark microbial community. Consistent with this low abundance, we found no *Saccharomyces* sequences present among 40,000 ITS sequences from the microbiome of the bark of oak trees, which we knew, by enrichment culturing, carried *S. paradoxus*. Similarly, Dashko *et al*., (2016) found few *Saccharomyces* sequences in the Illumina-sequenced microbiomes of vineyard oak and vineyard soil samples. This low abundance led us to question whether oak bark is really the primary habitat of wild *Saccharomyces*.

Here, we systematically quantify the abundance and distribution of *Saccharomyces* in a mixed forest in Nehmten, Northern Germany. First, we sampled leaf litter along transects and discovered that samples closer to oak trees were more likely to contain yeast than samples from further away. Next, we compared leaf litter samples under four tree genera and found that oak samples were significantly more likely to contain *Saccharomyces*. By quantifying the number of *Saccharomyces* cells, we show that the density of yeast is much higher in oak leaf litter than on oak bark itself, indeed individual cells could be isolated directly from leaf litter samples, without enrichment culture. To determine the effect of season, we sampled transects from six oak trees bimonthly over one year, and quantified the cell numbers of *Saccharomyces* in the litter under each tree. Altogether we sequenced 636 *Saccharomyces* isolates, of which 633 were *S. paradoxus* and only three were *S. cerevisiae*.

Results

Saccharomyces *isolation success decreases with increasing distance from an oak*

The closer you are to an oak tree, the more likely you are to find *Saccharomyces*. Figure 1 shows that the proportion of leaf litter samples containing *Saccharomyces* yeast is significantly negatively correlated with how far from an oak a sample was taken, both for enrichment cultures at 10°C (Spearman's rho $= -0.713$, $P < 0.001$) and at 30°C (Spearman's rho $= -0.553$, $P < 0.001$). There was no significant influence of temperature (GLM; $Z = 0.7$, $P = 0.48$), but distance from the nearest oak was highly significant (GLM; $Z = -7.12$, $P < 0.001$).

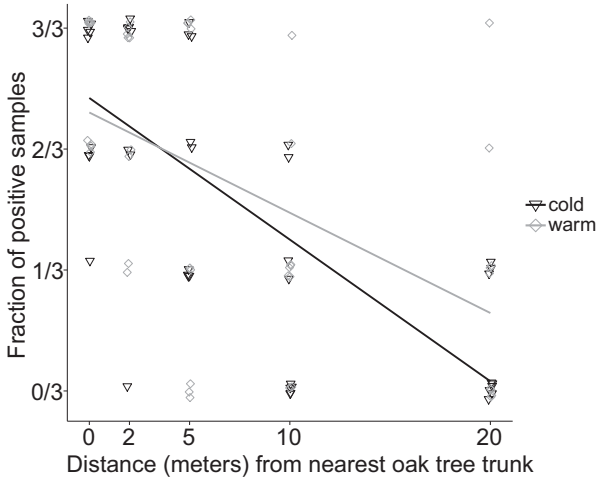

Fig. 1. Relation between distance from oaks and the number of positive samples at 10°C (cold) and 30°C (warm) incubation temperature. The fraction of positive samples for nine transects for each incubation temperature are plotted. The solid lines are linear regression lines.

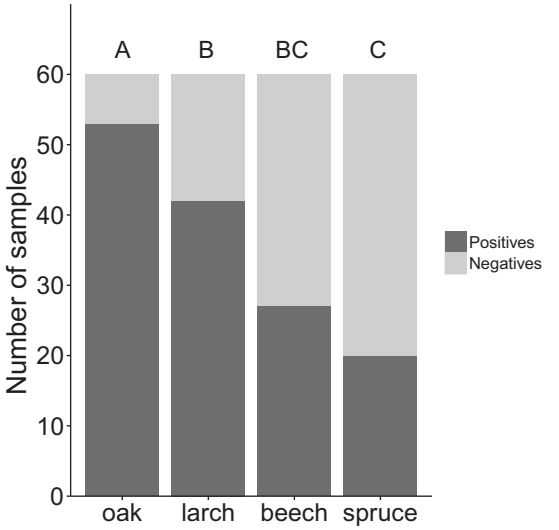

Fig. 2. *Saccharomyces* found in 60 leaf litter samples of oak, larch, beech and spruce. Capital letters indicate significant differences in isolation success between different trees. Columns not sharing a letter are significantly different after Pairwise Fisher tests and FDR correction.

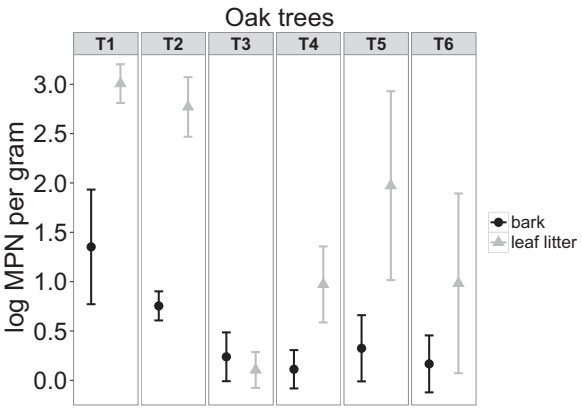

Fig. 3. The log most probable number (MPN) of 1 gram bark and leaf litter samples from each of six oak trees (T1–T6). The mean and standard deviation from each of three samples is plotted.

Oak leaf litter contains more Saccharomyces *than leaf litter from other tree genera*

Saccharomyces was more strongly associated with oak than with the three other common tree types in the Nehmten forest (Fig. 2). 53 out of the leaf litter samples taken under 60 oaks contained *Saccharomyces*, significantly more than the 42/60 positive larch samples, the 27/60 beech samples, and the 20/60 spruce samples (Pairwise Fisher's Exact tests; $P = 0.028$, $P < 0.001$, $P < 0.001$ after FDR correction respectively).

Saccharomyces *is more abundant in oak leaf litter than on bark*

Saccharomyces cell density was much higher in the leaf litter under six oak trees than on the bark of the trees themselves (Fig. 3). We found an average of 350 cells per gram of oak leaf litter (range 0 to 1319 cells), compared to just 7 cells per gram of oak bark (range 0–70 cells), a significant difference (Nested ANOVA; $df = 1$, $F = 38.5$, $P < 0.001$; Supporting Information Fig. 3). The cell density also varies significantly from tree to tree (Nested ANOVA; $df = 5$, $F = 13.54$, $P < 0.001$), and we find a significant interaction between source of sample (litter or bark) and tree (Nested ANOVA; $df = 5$, $F = 2.66$, $P = 0.048$) (Fig. 3).

Saccharomyces *abundance and distribution varies between trees and over season*

To characterize seasonal changes in distribution (Supporting Information Figs 1 and 4) and abundance (Supporting Information Fig. 2) of wild *Saccharomyces*, we took bimonthly samples of six oak tree transects over the course of a year. For the distribution data a Generalized Linear Mixed Model (GLMM) on binary data – using *distance* as fixed effect, and *tree* and *season* as random effects – explained a significant amount of variance in isolation success (Supporting Information Table 1). No overdispersion was found. Consistent with the earlier transect study (see above), distance from the nearest oak tree trunk (factor *distance*) had a significant effect on isolation success ($X^2 = 94.664$, $P < 0.001$). Consistent with the tree-to-tree variation in cell number in our bark and leaf litter comparison above, we found that transects from different trees (factor *tree*) differed significantly in isolation success ($X^2 = 35.99$, $P < 0.001$). And finally, we found that the month of the year (factor *season*) also affected isolation probability across the transects ($X^2 = 7.4624$, $P = 0.006$). There was no significant interaction between *distance* and *season*, nor between *distance* and *tree*, nor between *tree* and *season*.

Consistent with the significant tree-to-tree variation in distributions across transects, different trees also varied in the abundance of *Saccharomyces* cells under them (ANOVA: df= 5, $F = 54.1$; $P < 0.001$). However, the month (factor *season*) did not affect overall abundance of cells under the six trees (ANOVA: $df = 6$, $F = 1.46$; $P = 0.203$), inconsistent with its affect on the transects. There is a significant interaction between *season* and *tree* (ANOVA: $df = 30$, $F = 2.35$; $P = 0.0012$) which may be explained by different trees responding differently to the seasons.

Given these minor inconsistencies between the two forms of data (abundance and distribution), we compared the seasonal variation in total positive samples found across each transect (Supporting Information Fig. 1) with the seasonal variation in absolute cell number under the trees (Supporting Information Fig. 2), and found a significant positive correlation (Supporting Information Fig. 5,

Pearson's $r = 0.343$, $P = 0.026$), giving confidence that these two independent methods accurately reflect the *Saccharomyces* in leaf litter. Testing the influence of seasonal temperature on our sampling data showed no significant influence on the abundance (Spearmańs correlation coefficient rho $= -0.036$, $P = 0.964$) nor the distribution (Spearmańs correlation coefficient rho $= 0.286$, $P = 0.556$) of *Saccharomyces*.

Discussion

S. paradoxus *is the dominant* Saccharomyces *species*

For this study 1536 leaf litter samples were collected and 636 tetrad-forming strains detected, from which we sequenced the ITS regions and confirmed 633 as being *S. paradoxus* and just three as *S. cerevisiae*. We also sequenced 36 strains with colonies that looked like *Saccharomyces* but which could not sporulate, hoping to find non-sporulating *Saccharomyces* isolates, but all were non-*Saccharomyces* genera (*Wickerhamomyces*, *Saccharomycodes*, *Debaryomyces*, *Cryptococcus, Torulaspora*, *Zygosaccharomyces*, *Hanseniaspora*, *Citeromyces, Metschnikowia*, *Candida*). Thus, we can conclude that enrichment sampling combined with tetrad-screening is a very efficient way to isolate *Saccharomyces*, and that *S. paradoxus* is by far the most dominant *Saccharomyces* species in this forest. Other studies have also shown that *S. paradoxus* is the main wild yeast species, with *S. cerevisiae* notably absent from northern latitudes (Johnson *et al*., 2004; Charron *et al*., 2014; Sylvester *et al*., 2015). *S. cerevisiae* has a higher optimum and maximum growth temperature than *S. paradoxus* (Sweeney *et al*., 2004; Salvadó *et al*., 2011; Leducq *et al*., 2014) and the locations where wild *S. cerevisiae* could be found are consistent with the geographic distribution of its optimal growth temperature and most *S. cerevisiae* strains isolated outside this range are human-associated strains (Robinson *et al*., 2016).

Three hypothesis about the oak as a habitat for S. paradoxus

Most samples of wild *Saccharomyces* have been isolated from oak trees, but few studies have compared oak trees to other potential habitats and there is no conclusive evidence supporting the claimed *Saccharomyces*/oak association. We find that the proximity of an oak tree has a strong positive effect on the occurrence of *S. paradoxus* in surrounding forest leaf litter. This effect has a relatively short range, extending about eight metres from the trunk (Supporting Information Fig. 4). This range is consistent with at least three possible, non-exclusive, mechanisms by which oak trees might promote the local abundance of *S. paradoxus*. First, the yeast cells might grow primarily on the surface of oaks, and be dispersed by rainwater or insects to the surrounding area. A second possibility is that *S. paradoxus* primarily grows on resources released by the decomposition of fallen oak tree leaves, and that cells are dispersed from the litter to the tree by insects or rainwater spray. A third possibility is that the yeast benefits directly or indirectly from oak root exudates, and cells then disperse from the soil up to the litter layer and onto tree surfaces. It was observed as long ago as 1904 (Hiltner, 1904) that the areas under trees harbored higher microbial densities, and this is due to the influence of tree roots on the surrounding soil (the rhizosphere). We will consider the evidence for these three possible models.

There is more Saccharomyces *in leaf litter than on bark*

Other researchers have previously isolated *Saccharomyces* from soil (e.g. Sniegowski *et al*., 2002; Sylvester *et al*., 2015) but here we show that the cell density per unit mass is much greater on the ground under oak trees than on their trunks (Fig. 3 and Supporting Information Fig. 4). This discovery challenges the idea that *Saccharomyces* grow primarily on the trees themselves, and suggests that yeast found on the trunk may actually originate from the ground. Leaf litter contains abundant complex polysaccharides derived from lignocellulose which cannot be utilized directly by *Saccharomyces*, but which are digested by extracellular enzymes from other fungi and bacteria to yield simple sugars (Sinsabaugh and Linkins, 1990; Steffen *et al*., 2007) which the yeast might consume. However, we note that other scientists have isolated *Saccharomyces* from fresh oak leaves (Glushakova *et al*., 2007; Sláviková *et al*., 2007) which are presumably well isolated by distance from the ground, raising a question as to whether yeast might also grow on the leaves. Further, we must note that leaf litter is of course a very different substrate than bark, with a much higher surface area per unit mass. We cannot rule out the possibility, therefore, that *Saccharomyces* grows primarily on a tree, but cells are washed down the trunk and trapped in the leaf litter, where they accumulate. One way to test whether the tree or the ground is the source of the yeast would be to perform vertical transects, up the tree, and see whether the abundance changes with increasing distance from the ground. However, if insects, rather than rainwater, disperse the yeast across the surface of the tree, no such pattern would be found. Another method would be to catch falling leaves before they contact the litter, for example with a tarpaulin, to identify the primary source of the yeast and how its abundance changes with time. What we can say is that researchers seeking wild yeast can expect to find more in leaf litter than on bark. Indeed, the

abundance of yeast in leaf litter is so high that we were able to sample it directly by plating, without enrichment culture. This allows for individual genotypes to be sampled at their true frequency, without introducing potential biases by artificial selection in enrichment culture.

Seasonal changes in Saccharomyces *abundance and distribution in oak leaf litter*

In temperate zones, fall occurs once a year, so if fallen leaves are the primary resource for *Saccharomyces* we would expect to see strong seasonal variation in abundance. There is indeed a significant seasonal effect on distribution along transects, suggesting that the zone influenced by a tree changes according to the season (Supporting Information Fig. 1). But the effect is not as pronounced as we would expect if fallen leaves were the primary resource, and, surprisingly, no significant effect of season on overall abundance was detected. On the other hand, we found a significant interaction between season and tree suggesting that trees respond individually to season and the reason for that might be that the leaf litter of each tree will decay slightly differently according to differences in biomass (more or less leaf litter around) or surrounding environmental factors. Even if *S. paradoxus* grows primarily on another resource provided by the tree, either on its surface or into the soil via its roots, seasonal changes in weather would presumably affect both the provision of this resource by the tree (e.g. Grayston *et al*., 1997), and the ability of yeast to grow. It has been shown previously that success of isolating *Saccharomyces* over a year in northeast America increased continually from April to August/September and decreased at the end of summer (Charron *et al*., 2014). The relatively weak seasonal effects are perplexing, but on balance perhaps suggest that the yeast we have isolated from the leaf litter surface have migrated from a population deeper in the leaf litter or soil, where they are less affected by season. The yeast might also mainly be present as spores which would make it also unaffected by seasonal changes.

Tree-to-tree and sample-to-sample variation in S. paradoxus *abundance*

For the six transects there was a significant effect of tree, indicating that some trees support more yeast and others less (Supporting Information Fig. 1). The abundance data also confirm that some oak trees are in general better habitats for *S. paradoxus* than others, consistently over a whole year (Supporting Information Fig. 2). *S. paradoxus* appears to be a relatively minor member of microbial communities, so it is possible that these differences are due to underlying stochasticity in, for example, community assembly. However, the differences may be due to selective effects, such as differences in tree age (e.g. see Robinson *et al*., 2016), tree location with respect to exposure to abiotic or biotic factors, or tree genotype, which is known to affect microbial composition in the ectomycorrhizal (Morris *et al*., 2009) and soil (Schweitzer *et al*., 2008) community. We also see variation among the three samples taken from three sides of each tree (Fig. 3), consistent either with environmental exposure, such as weather or the proximity of a tree root, being important, or with a patchy distribution of the yeast. It is well known that resources are patchily distributed in soils (Hodge, 2006; Rennert, 2012) and the same is true for the microbial life, which tends to live in aggregates and to form spots of activity (Nunan *et al*., 2003; Ling *et al*., 2011). This variation offers the possibility that a larger survey might reveal the factors affecting *S. paradoxus* abundance, and, therefore, provide information about the resources that the yeast exploits.

S. paradoxus *found more often under oaks than under larches, beeches or spruces*

Consistent with the established tradition of collecting wild yeast from oaks, we find that *S. paradoxus* is significantly more likely to be found under oak than other tree species. Surprisingly, the second 'best' habitat is litter from larch, a conifer with needle leaves, and not beech, which has broad leaves like oak. The differences between tree species are small enough that they might be explained by minor factors, for example perhaps the consistency or surface area of leaf litter from different species differs in such a way that it carries more or less yeast per unit mass. We also cannot rule out the possibility that *Saccharomyces* grows in association with oaks but disperses to other trees. What we can rule out is that the yeast is ubiquitous in forest leaf litter as its presence strongly declines with transect distance (Fig. 1). The most likely explanation is that oak promotes *S. paradoxus* growth more than other tree genera, either directly or by affecting other members of the community. Different leaf litters encourage development of distinct microbial communities (Bray *et al*., 2012). Oak plant material provides especially high amounts of tannins and other bioactive substances which can suppress the growth of certain microbes (Scalbert, 1991) – indeed oak material has been used as traditional antibacterial medicine (Brantner and Grein, 1994).

Conclusion

Here we confirm oak trees as a primary habitat of *S. paradoxus*. We find higher abundance in leaf litter under oak trees than on the surface of the trees themselves, but abundance declines with distance from a tree. We

propose therefore that *S. paradoxus* grows primarily in leaf litter or soil associated with oaks. Further, we find the oak leaf litter contains more *S. paradoxus* than the litter under other tree genera.

Experimental procedures

Leaf litter transects

Between July 11, 2014 and September 18, 2014, we sampled 18 transects in an old mixed forest in Nehmten, Northern Germany. Each transect was a straight line 20 metres long, starting at the trunk of an oak tree and ending at least 20 m away from the next nearest tree. Each sample composed of 2 cm^3 sample of compressed leaf litter (Supporting Information). We took three samples at five different points on each transect: 0 m (directly next to the trunk), 2 m, 5 m, 10 m, and 20 m. All 270 samples were taken immediately back to the lab and processed (Supporting Information). To determine whether incubation temperature had any effect on isolation success, we incubated the samples from nine of the transects at 30°C for ten days, and those from the other nine transects at 10°C for 28 days, without shaking. We then streaked the sample onto a solid, second selective medium (Supporting Information) and after incubation, the plates were examined for yeast colonies.

The method to identify candidate colonies as *Saccharomyces* was built of two steps; tetrad screening and Sanger sequencing (Supporting Information). To determine how effective a pre-selection of candidate colonies based on forming *Saccharomyces* specific tetrads, was for identification, all 146 candidate colonies were Sanger sequenced and all were identified as *S. paradoxus* and all these colonies formed tetrads. Additionally, 21 yeast-like colonies that did not sporulate or formed very different spores compared to *Saccharomyces* have been sequenced to determine whether the ability to sporulate was a useful characteristic to identify *Saccharomyces*, or whether non-sporulating *Saccharomyces* might also exist. These 21 samples were identified as belonging to seven other non-*Saccharomyces* yeast species. The perfect congruence between tetrad-formation and *Saccharomyces* identity persuaded us to use tetrad screening as pre-selection for the later parts of this study, greatly increasing the scale of experiments.

The data from the 18 transects were pooled and the effect of distance from an oak tree on *Saccharomyces* isolation probability was tested with a simple logistic regression model in R, with the additional factor 'incubation temperature'. To test if the incubation temperature has a significant effect on *Saccharomyces* isolation success we tested the difference between the two regression lines using a Generalized Linear Model (GLM) on binary data in R.

Comparison of leaf litter under four tree genera

To test for a tree genus specificity of *Saccharomyces*, we compared the four most common tree genera in the Nehmten forest in terms of isolation success of the yeast. In September 2014, we took one 2 cm^3 sample of compressed leaf litter directly next to each of the trunks of 60 oaks (*Quercus* spp.), 60 beeches (*Fagus* spp.), 60 larches (*Larix* spp.) and 60 spruces (*Picea* spp.) and processed them as described in Supp. Information, except that all samples were incubated in PIM1 at 30°C for 10 days. All sporulating yeast colonies were sequenced and again all pre-selected colonies belonged to *Saccharomyces*. As a control to test whether there might be missing *Saccharomyces* isolates that could not sporulate, we also tested the ITS sequences of 15 other candidate colonies that resembled *Saccharomyces* but did not form *Saccharomyces* like spores – all contained ITS sequences from other yeast species. We performed pairwise Fisher tests on the isolation success among all four tree types. To correct for multiple testing, the resulted *P*-values were adjusted using the false discovery rate ('fdr') option of the R function p.adjust() (R-Team, 2015).

Enumeration of the Saccharomyces *cell number in oak leaf litter and on oak bark*

In January 2015, we collected three oak leaf litter samples and three oak bark samples each from six different oak trees. Each 2 cm^3 sample of compressed oak leaf litter was collected as described in the Supp. Information. Bark was sampled by cutting all the bark from a 10 cm by 5 cm patch at head-height using a sterile scalpel, and placing the bark pieces into a 50 ml Falcon tube (approximately 15 cm^3 of loose bark pieces). All 36 samples were immediately taken to the laboratory, weighed and transferred into 50 ml Falcon tubes. The number of viable *Saccharomyces* cells was determined in each of these 36 samples using the most probable number (MPN) technique which is an estimation of organisms by noting growth in successive dilutions (McCrady, 1915) (Supp. Information). The MPN of *Saccharomyces* cells in a sample was divided by the number of grams/sample to determine the most probable number of cells per gram of leaf litter or oak bark material. We analysed the Box-Cox transformed MPN data using a nested ANOVA with *sample type* (leaf litter or bark) nested in *tree*.

Over-the-year sampling study

To test for changes over season in yeast distribution we sampled transects away from six oak trees. We took leaf litter samples as described but one sample every metre (from direct to 20 metre distance). We changed

between starting from the trunk going the 20 metres away and starting 20 metres away and going in tree direction to dilute the bias that we might introduce by walking the transects. We pre-screened candidate colonies as before by their ability to form tetrads, and confirmed all 348 tetrad-forming strains as *Saccharomyces* by ITS sequencing. We analysed the distribution data with a GLMM (Supp. Information) using the lme4 package in R (Bates *et al*., 2014).

To additionally test for seasonal changes in abundance, we took three leaf litter samples at different sites directly under the trunk of each tree at the same time as we collected the samples for the transects. We estimated the MPN of *Saccharomyces* per leaf litter sample as described before. We analysed the Box-Cox transformed MPN data using a two-factor ANOVA with *transect* and *season* as factors.

Connecting the collected distribution and abundance data of *Saccharomyces* across the year, we compared the seasonal variation in total positive samples found across each transect with the seasonal variation in absolute cell number under the trees for each sampling time point by a linear regression model in R.

To test if temperature has an effect on the distribution and abundance of *Saccharomyces* in this data set, we determined correlation coefficients in R between the average temperature of the sampling months with the average MPN as well as the average numbers of isolated strains from all six transects per month.

Acknowledgements

The authors thank Gunda Dechow-Seligman for help with the sequencing of the *Saccharomyces* strains and also Nadja Gogrefe and Sarah Gaugel for great help during forest sampling tours, with DNA extractions and PCRs. The authors are thankful to the owner of the Nehmten forest Christoph Freiherr von Fürstenberg-Plessen who kindly agreed on our sampling studies. The manuscript was greatly improved thanks to the efforts of the editor and two anonymous reviewers. Funding was provided by the Max Planck Society.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Fraction of positive samples of *Saccharomyces* per whole transect (T1-T6) over the year. The dashed red line shows the average of the six transects.
Fig. S2. Log most probable number (MPN) of three samples per transect tree and month. The lines connect the mean of these three data points and the red dashed line represents the average MPN of all six transect trees per sampling time point.
Fig. S3. The most probable number (MPN) of *Saccharomyces* in one gram of bark or leaf litter material from 18 samples (three samples from each of six oak trees). Plotted dark points are the means with added standard errors and the single data points are shown using open symbols.
Fig. S4. Average distribution of *Saccharomyces* isolates from all six transects at different months. The solid lines are local polynomial regression fittings.
Fig. S5. Relation between fraction of positive samples for each of the six transects per each sampling month and the average log MPN for this tree at the same sampling time

point. The solid line represents the linear regression with 95% conficence interval.

Table S1. GLMMs on the effects of distance, tree and season (which month sampled) as well as on interactions between effects (characterized with:) on the isolation success of *S. paradoxus*. Akaikes information criterion (AIC) describes the quality of fit of each model (higher AIC = information loss). To evaluate the significance of fixed and random effects and interactions, alternative models without the variable or interaction of interest were compared to the full model (bold) using likelihood ratio tests in R.