Multiple Changes Underlie Allelic Divergence of CUP2 Between Saccharomyces Species

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ABSTRACT Under the model of micromutationism, phenotypic divergence between species is caused by accumulation of many small-effect changes. While mapping the causal changes to single nucleotide resolution could be difficult for diverged species, genetic dissection via chimeric constructs allows us to evaluate whether a large-effect gene is composed of many small-effect nucleotide changes. In a previously described non-complementation screen, we found an allele difference of CUP2, a copper-binding transcription factor, underlies divergence in copper resistance between Saccharomyces cerevisiae and S. uvarum. Here, we tested whether the allele effect of CUP2 was caused by multiple nucleotide changes. By analyzing chimeric constructs containing four separate regions in the CUP2 gene, including its distal promoter, proximal promoter, DNA binding domain and transcriptional activation domain, we found that all four regions of the S. cerevisiae allele conferred copper resistance, with the proximal promoter showing the largest effect, and that both additive and epistatic effects are likely involved. These findings support a model of multiple changes underlying evolution and suggest an important role of both protein coding and cis-regulatory changes in evolution.

KEYWORDS Saccharomyces CUP2 copper resistance cis-regulatory evolution chimeras

The genetic basis of evolutionary change may involve changes that range from large to small effect. Under the micromutational model, phenotypic divergence predominantly results from the accumulation of numerous small-effect changes (Rockman 2012). However, mapping of quantitative traits has shown that large-effect changes often contribute to phenotypic variation (Orr and Coyne 1992; Bell 2009). Even so, these results may be inherently biased, both by a focus on dramatic phenotypic shifts, such as those that distinguish domesticated species from their wild relatives, and by the limited power of quantitative trait mapping to detect small effects and distinguish between regions with a single large-effect change or many small ones (Orr and Coyne 1992; Rockman 2012). Thus, evaluating the genetic basis of evolutionary change requires accounting for both the context and purview of the evidence.

In genetic studies, both the mapping method and sample size have a strong influence on the results. In contrast to many linkage mapping studies, which tend to find large-effect changes (Fay 2013), genome-wide association studies predominantly detect numerous small-effect associations, e.g., (Wood et al. 2014), and the number of associations depends on sample size (Visscher et al. 2012). Furthermore, evidence for the omnigenic model supports the view that every gene has some slight contribution to a trait (Boyle et al. 2017), and implies that the vast majority of causal variants are not realistically mappable. Knowing the limits of our ability to detect and identify small effect mutations is also relevant to answering questions about the genes, type of changes, and cellular mechanisms underlying phenotypic divergence (Rockman 2012; Boyle et al. 2017).

Limits on our ability to map phenotypic variation are not restricted to a simple tradeoff between effect size and sample size. Mapping interspecific differences often requires different approaches and yields different results compared to studies of intraspecific variation. A prominent limitation of mapping phenotypic differences between species is hybrid sterility and inviability. Consequently, many studies test candidate genes or map traits that differ between closely related, interfertile
species. Based on a review of the literature, interspecific studies find fewer null alleles and more cis-regulatory alleles compared coding alleles (Stern and Orgogozo 2008). Another factor relevant to interspecific studies is that there is enough time for multiple changes to occur at a single locus. These loci are of interest both in regard to why they accumulate multiple changes, but also because they are more readily detected.

Repeated changes at a single locus, termed evolutionary hotspots, are common and relevant to understanding phenotypic divergence (Martin and Orgogozo 2013). Hotspots can be classified as interlineage, involving genes that are repeatedly used during evolution in different lineages, or intralineage, involving the accumulation of multiple changes in a gene along a single lineage (Martin and Orgogozo 2013). In the case of intralineage hotspots, multiple changes within a single gene can be explained by either the unique ability of a gene to affect a trait or pleiotropy, whereby many genes can influence a trait but relative few can do so without adverse effects on other traits (Stern and Orgogozo 2009). The constraints of pleiotropy are also thought to increase the preponderance of cis-regulatory changes in evolution (Carroll 2008). An example of one such hotspot is shavenbaby, which underlies divergence in trichomes between Drosophila species via multiple cis-regulatory changes (McGregor et al. 2007).

If phenotypic divergence between species results from the accumulation of numerous changes of small effect, they may be easier to detect when they form hotspots. However, identifying hotspots between species is also a challenge. Species that are too close may not have enough time to accumulate multiple changes and species that are too distant may be reproductively isolated. Genetic analysis of species’ hybrids provides a means of balancing these limitations. Hybrids, even if infertile, are often viable for distantly related species. Hybrids have been leveraged for deletion mapping of incompatibilities between Drosophila species, e.g., (Coyne et al. 1998; Tang and Pregerasv 2009), and for reciprocal hemizygosity analysis in Saccharomyces species (Weiss et al. 2018; Li et al. 2019). The reciprocal hemizygosity test compares two hybrids each with a different allele deleted, thereby testing for allelic differences while controlling for haploinsufficiency (Steinmetz et al. 2002). Of particular relevance, the test examines the combined effects of all regulatory or coding differences between the two species’ alleles.

In this study we test whether single or multiple changes underlie allelic divergence of CUP2 between Saccharomyces species. Using a genome-wide non-complementation screen, we previously found that divergence of CUP2 contributed to the evolution of copper resistance in Saccharomyces species (Li et al. 2019). S. cerevisiae can tolerate high concentration of copper sulfate, a stress associated with vineyard environments. Although the level of copper resistance is variable among S. cerevisiae strains (Fay et al. 2004; Kvitek et al. 2008; Strope et al. 2015), its relatives, S. paradoxus and S. uvarum, are usually copper sensitive (Kvitek et al. 2008; Warringer et al. 2011; Dashko et al. 2016). Through a non-complementation screen followed by a reciprocal hemizygosity test, we found that the S. cerevisiae CUP2 allele confers higher copper resistance compared to the S. uvarum allele. CUP2 encodes a copper-binding transcription factor and regulates Cup1p, a major copper-activated metallothionein in yeast (Buchman et al. 1989). Previous studies showed that CUP2 is essential for S. cerevisiae’s copper resistance (Thiele 1988; Welch et al. 1989; Jin et al. 2008) and contributes to intraspecific variation in acetic acid (Meijnen et al. 2016) and copper resistance (Chang et al. 2013). Because the sequences of S. cerevisiae and S. uvarum CUP2 are substantially diverged (71.1% identical) we dissected the effect of CUP2 allele divergence using chimeric constructs between the two species. We found that divergence in copper-resistance is caused by multiple nucleotide changes distributed throughout the gene, but with cis-regulatory changes having a larger effect than coding changes.

**MATERIALS AND METHODS**

*S. cerevisiae* strains in the S288C background and *S. uvarum* strains in the CBS7001 background (Scannell et al. 2011) were used in this study. The *S. uvarum* genome sequence and annotations were from Scannell et al. (2011). CUP2 was knocked out with *KanMX4* in *S. cerevisiae* (YJF173, MATα *ho*-ura3-52) and *S. uvarum* (YJF1450, MATα *hoΔ::NatMX*), respectively. Transformations in this study followed a standard lithium acetate procedure (Gietz et al. 1995), with the modification that room temperature and 37°C was used for incubation and heat shock of *S. uvarum*, respectively. Unless otherwise noted, *S. cerevisiae* was maintained at 30°C on YPD (1% yeast extract, 2% peptone and 2% dextrose) while *S. uvarum* and *S. cerevisiae* × *S. uvarum* hybrids were maintained at room temperature.

Chimeric constructs were generated by Gibson assembly (Gibson et al. 2009). Promoters were defined from the end of the upstream gene (PMR1) to the start codon of CUP2. Coding sequence (CDS) was defined from the start codon of CUP2 to the stop codon, and our constructs also included the 3′ non-coding region (until the downstream gene). To further dissect the effects of the promoter and CDS, the promoter was split at nucleotide position -291 for *S. cerevisiae* and its homologous position at -283 for *S. uvarum*. The CDS was split at position +367 for both alleles, based on the previously defined DNA binding domain and transactivation domain (Buchman et al. 1989) (Figure 1A). All positions are relative to the start codon of CUP2.

Segments of CUP2 were PCR-amplified from *S. cerevisiae* or *S. uvarum* genomic DNA with Q5 polymerase (New England Biolabs). Promoter and CDS segments from different species were Gibson-assembled into pRS306 to generate promoter-swaps. Full-length *S. cerevisiae* and *S. uvarum* CUP2 alleles were assembled in parallel for controls. An *S. cerevisiae* allele from a copper sensitive oak tree strain was included for comparison, and was amplified from genomic DNA of YJF153 (MATα *hoΔ::dsdAMX*), a YPS163 derivative. To split the promoter or CDS, the segments of interest were assembled into pRS306-derived plasmids pXLO7 or pXLO5, respectively carrying the full-length *S. cerevisiae* or *S. uvarum* allele. All constructs were Sanger-sequenced; one of the chimeras (CCUC) carried a deletion of a single adenine nucleotide in a stretch of 14 As in the *S. cerevisiae* promoter, but it did not seem to cause deleterious effects in the phenotypic assays.

The plasmids were linearized with BstBI (CUP2 constructs) or Stul (vector control) and integrated into the *ura3* locus of an *S. cerevisiae* CUP2 knockout strain YJF2872 (MATα *ho*-ura3-52 *cup2Δ::KanMX4*). The integrated strains were backcrossed to an *S. cerevisiae* strain YJF175 (MATα *ho*-ura3-52) and sporulated to remove any second-site mutations. The resulting haploid *S. cerevisiae* strains carrying the CUP2 deletion and chimeric constructs were then crossed to an *S. uvarum* CUP2 knockout YJF2917 (MATα *hoΔ::NatMX cup2Δ::KanMX4*). The final interspecific hybrid was null for both *S. cerevisiae* and *S. uvarum* alleles at their endogenous loci and carried chimeric or full-length constructs at the *ura3* locus. The hybrids were genotyped by PCR (Li et al. 2019) and found to carry *S. cerevisiae* mitochondrial DNA.

Growth curves in copper-supplemented media were recorded by a BioTek microplate reader. Three biological replicates were used for each strain. Overnight cultures were diluted 1:100 into 200 ul complete media (CM, 0.3% yeast nitrogen base with amino acids, 0.5% ammonium sulfate, 2% dextrose) supplemented with 0, 0.2 or 0.5mM copper sulfate in a 96-well plate. The plate was incubated at room temperature (25-26°C),
with the optical density (OD) at 600 nm taken every 10 min for 40 h. The plate was shaken for 20 s before each OD reading. To quantify growth, the spline differences, area under the curve (AUC) was measured as the integral of the spline fit of growth curves using the grofit package (Kahm et al. 2010) in R. Copper resistance was represented by normalized AUC (nAUC), the AUC of copper treatments divided by the mean AUC of the same strain in CM without copper.

Linear models were used to analyze the effects of each region. Data from the oak allele and the vector control were excluded from the models. The sum of nAUC across the two concentrations (snAUC) was used to represent copper resistance of each strain. The data were fit to two models: 1) \( \text{snAUC} \sim R_1 + R_2 + R_3 + R_4 \), to analyze the additive effects of region 1 to 4 (R1 to R4); 2) \( \text{snAUC} \sim (R_1 + R_2 + R_3 + R_4)^2 \), to analyze both additive and pairwise epistatic effects. R1 to R4 were categorical variables (C or U representing \( C.\) cerevisiae alleles and \( S.\) uvarum alleles, respectively). P-values were extracted from the models and were adjusted by false discovery rate (Benjamini and Hochberg method) to correct for multiple comparisons.

Data availability

Strains and plasmids are available upon request. File S1 contains all AUC and nAUC values. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplemental material available at FigShare: https://doi.org/10.25387/g3.9782801.

RESULTS

The \( S.\) cerevisiae allele of \( CUP2 \) confers higher copper resistance than the \( S.\) uvarum allele (Li et al. 2019). The two alleles share 71.1% sequence identity, with hundreds of nucleotide substitutions across the coding and non-coding regions. To test whether the allele differences in copper resistance are caused by multiple nucleotide changes and whether they occur in coding or cis-regulatory regions, we generated chimeric constructs between \( S.\) cerevisiae and \( S.\) uvarum \( CUP2 \) alleles (Figure 1) and integrated them into the \( uRA3 \) locus in \( S.\) cerevisiae. Copper resistance was measured in a hybrid of \( S.\) cerevisiae and \( S.\) uvarum, in which the endogenous \( CUP2 \) alleles were knocked out. The hybrid background was used in accordance with the previously conducted reciprocal hemizygosis test (Li et al. 2019), but the effects of chimeras were the same in \( S.\) cerevisiae (Fig. S1).

All four of the regions showed a significant effect on copper resistance using an additive model (Table 1). Across two different concentrations of copper, the resistance of chimeras generally increased with the number of \( S.\) cerevisiae segments in the constructs (Figure 2). Relative to the \( S.\) uvarum allele, substituting in the \( S.\) cerevisiae promoter conferred higher resistance than substituting the \( S.\) cerevisiae CDS (gray). The chimeras that split the promoter or CDS regions further mapped the largest effect to the proximal half of the \( S.\) cerevisiae promoter (the UCUU construct), while the other three \( S.\) cerevisiae regions tested also conferred low-to-moderate levels of resistance when inserted into the \( S.\) uvarum allele (light blue, left panel), suggesting that multiple nucleotide changes underlie the allele effect of \( CUP2 \). While the combination of any three \( S.\) cerevisiae segments was sufficient to confer resistance to the 0.2 mM copper treatment (orange), these chimeras showed various levels of sensitivity to 0.5 mM, also consistent with a model of multiple changes.

Using a linear model, we also tested whether there are epistatic interactions between the regions (Table 1). We found that the model accounting for epistatic effects explained the data better than the model with only additive effects (0.974 vs. 0.839 for adjusted R-squared, \( P = 1.94E-10 \) in ANOVA), and this holds true when the two concentrations were analyzed separately (Table S1). In the epistatic model, all four \( S.\) cerevisiae regions retained significant effects on copper resistance, with region 2 showing the largest effect. Positive epistasis was detected between region 1 and 4. At high copper concentration, substitution of \( S.\) cerevisiae region 1 or 4 into the \( S.\) uvarum background had little effect (Figure 2, right panel, CUUU and UUUC compared to UUUU), but showed much larger effects when the other region was also present (CCUU to CUCU and UUUC to CUCC). Regions 1-2 and 2-3 showed modest negative interactions. These findings suggest that both changes with additive and epistatic effects contributed to the divergence of \( CUP2 \) alleles.

We also included a full-length \( CUP2 \) allele from a copper-sensitive \( S.\) cerevisiae oak isolate for comparison. The oak allele has 12 nucleotide
differences from the S288C allele used in the chimeras. While the oak allele showed similar levels of resistance as the S288C allele at 0.2 mM copper, it was more sensitive than the S288C allele at 0.5 mM. This suggests that a portion of the divergence between the \textit{S. cerevisiae} S288C allele and \textit{S. uvarum} may be caused by recent changes (polymorphism).

However, of the 572 differences between the S288C and \textit{S. uvarum} allele (out of a 1586 bp alignment, including gaps), only 4 of these can be explained by polymorphism between the two \textit{S. cerevisiae} strains and only 57 of these are polymorphic in other \textit{S. cerevisiae} strains (Peter et al. 2018).

**DISCUSSION**

Evolution can occur through accumulation of many small-effect changes, but mapping small-effect changes can be technically challenging (Orr 2001; Rockman 2012). In the present study, we tested whether a relatively large effect on copper resistance caused by \textit{CUP2} allele divergence is a consequence of multiple nucleotide changes. By splitting the \textit{CUP2} gene into four regions and measuring their effects via chimeric constructs, we found that the \textit{CUP2} allele difference was caused by accumulation of multiple small-to-medium effect changes, with the proximal promoter region showing the largest effect.

**Multiple changes with small effects**

Our findings support the micromutationism view that evolution involves many small-effect changes. All four regions tested conferred copper resistance with various effect sizes, suggesting that the copper-resistant nucleotide substitutions are distributed throughout the \textit{CUP2} gene. The largest effect was mapped to the proximal promoter. The promoter effect was unlikely to be caused by changes in transcription factor binding sites: there is only one putative \textit{REB1} binding site in the \textit{CUP2} promoter (YeTFaSCo database, de Boer and Hughes 2012, Figure 1A), and it is conserved across the \textit{Saccharomyces} species. The large effect of the \textit{CUP2} promoter supports the previously suggested prominent role of cis-regulatory changes in long-term evolution (Stern and Orgogozo 2008). While cis-regulatory changes were often found to underlie morphological evolution, the example of \textit{CUP2} along with several prior studies demonstrated that they are also important to physiological traits in yeast (Gerke et al. 2009; Engle and Fay 2012; Roop et al. 2016).

\textit{Cup2p} consists of an N-terminal DNA binding domain (region 3) and a C-terminal transcriptional activation domain (region 4) (Buchman et al. 1989), with the former being more conserved (Figure 1A). We found that the DNA binding domain of \textit{S. cerevisiae} conferred moderate copper resistance when inserted into the \textit{S. uvarum} allele. The gain of copper resistance could be due to changes in binding affinity to the \textit{CUP1} promoter, the major target of \textit{Cup2p}. The N-terminal of \textit{Cup2p} is suggested to bind DNA via a zinc module and a copper-regulatory domain (Graden et al. 1996) (Figure 1A), both of which contain amino acid differences between the two species.

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**Table 1 Additive and epistatic effects of \textit{S. cerevisiae} CUP2 regions on copper resistance**

| Region⁴ | Additive model | Epistatic model |
|---------|---------------|----------------|
|         | Effect size   | P-value†       | Effect size | P-value†       |
| (Intercept) | 0.138 | 0.0841 | 0.197  | 0.000445 |
| 1       | 0.479 | 3.11E-06 | 0.314  | 9.37E-05  |
| 2       | 0.515 | 1.33E-06 | 0.801  | 1.29E-11  |
| 3       | 0.274 | 0.00267 | 0.333  | 5.59E-05  |
| 4       | 0.527 | 1.33E-06 | 0.211  | 0.00369  |
| 1²/2    | −0.339 | 0.000292 |                   |                   |
| 1²/3    | 0.0754 | 0.370  |                   |                   |
| 1²/4    | 0.594  | 1.47E-07 |                   |                   |
| 2²/3    | −0.232 | 0.00679 |                   |                   |
| 2²/4    | NA     | NA     |                   |                   |
| 3²/4    | 0.0381 | 0.622  |                   |                   |

⁴Regions were defined as in Fig. 1A. The asterisks indicate interactions. †P-values were adjusted by the false discovery rate (Benjamini and Hochberg method).

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**Figure 2** Copper resistance of chimeric constructs. \textit{S. cerevisiae} × \textit{S. uvarum} hybrids carrying the chimeric constructs were grown in labeled copper concentrations and their resistance was measured by area under curve (AUC) of OD₆₀₀ growth curves, normalized to their growth in complete media. Points represent the mean of three biological replicates and error bars represent 95% confidence interval. The colors are based on the number of \textit{S. cerevisiae} segments in the chimeras (red = 4, orange = 3, gray = 2, light blue = 1, blue or black = 0).
Further dissection of this region would help understand the molecular mechanism of CUP2-mediated copper resistance. However, these dissections are expected to become increasingly difficult under the micromutational model.

While all four regions showed different levels of additive effects, the context-dependent effect sizes of individual regions suggest epistasis. The S. cerevisiae region 1 and 4 showed small effects when inserted into the S. uvarum allele (Figure 2, CUUU and UUUC constructs) but large effects when replaced by the S. uvarum regions (Figure 2, UCCC and CCCU). It is possible that these two regions of S. cerevisiae contain large-effect copper-resistant changes that depend on the presence of other S. cerevisiae regions. Alternatively, the S. cerevisiae region 1 and 4 may only contain small-effect changes, and the sensitivity of the UCCC and CCCU constructs was caused by deleterious effects of the S. uvarum regions. Our data could not distinguish these two possibilities, although the linear model suggested that synergistic epistasis between the S. cerevisiae region 1 and 4 could be the best explanation (Table 1).

**Evolution of copper resistance**

The evolutionary history of CUP2 provides some insight into the evolution of copper resistance. The CUP2 coding sequences do not exhibit signatures of positive selection according to site-specific dN/dS models (Scannell et al. 2011) or McDonald-Kreitman tests (Doniger et al. 2008). However, the coding sequences do show significant heterogeneity in the dN/dS ratio across Saccharomyces lineages (P = 0.00523 compared to a model of fixed rates), indicating variation in selection pressure across lineages, with the S. cerevisiae lineage showing the highest ratio (0.562) (Scannell et al. 2011). The gain of copper resistance of S. cerevisiae has been associated with its adaptation to vineyard environments, where copper has been used as a fungicide (Mortimer 2000). While this trait is variable within S. cerevisiae, suggesting recent adaptation, most tested strains of S. paradoxus and S. uvarum are sensitive (Kvitek et al. 2008; Warringer et al. 2011; Dashko et al. 2016). Therefore, S. cerevisiae might have acquired copper-resistant changes prior to adaptation of wine strains to the vineyard. This view is supported by the observation that the S. cerevisiae oak allele, which is from one of the most copper sensitive S. cerevisiae strains (Fay et al. 2004), showed much higher copper resistance than the S. uvarum allele of CUP2. While variation in copper resistance within S. cerevisiae strains is largely attributed to copy number variation of CUP1 and CUP2 (Fogel and Welch 1982; Chang et al. 2013), the interspecific divergence may have a more complex genetic architecture. We showed that multiple changes in CUP2 contribute to copper resistance in the present study, but the sum of their effects did not account for the total difference between S. cerevisiae and S. uvarum (Li et al. 2019). Fully elucidation of the genetic basis of copper resistance would require further genetic analysis between Saccharomyces species.

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**LITERATURE CITED**

Bell, G., 2009 The oligogenic view of adaptation. Cold Spring Harb. Symp. Quant. Biol. 74: 139–144. https://doi.org/10.1101/sqb.2009.74.003
Boyle, E. A., Y. I. Li, and J. K. Pritchard, 2017 An Expanded View of Complex Traits: From Polygenic to Omnicogenic. Cell 169: 1177–1186. https://doi.org/10.1016/j.cell.2017.05.038
Buchman, C., P. Skroch, J. Welch, S. Fogel, and M. Karin, 1989 The CUP2 gene product, regulator of yeast metallothionein expression, is a copper-activated DNA-binding protein. Mol. Cell. Biol. 9: 4091–4095. https://doi.org/10.1128/MCB.9.9.4091
Carroll, S. B., 2008 Evo-Devo and an Expanding Evolutionary Synthesis: A Genetic Theory of Morphological Evolution. Cell 134: 25–36. https://doi.org/10.1016/j.cell.2008.06.030
Chang, S. L., H. Y. Lai, S. Y. Tung, and J. Y. Leu, 2013 Large-Dynamic Scale Chromosomal Rearrangements Fuel Rapid Adaptation in Yeast Populations. PLoS Genet. 9: e1003232. https://doi.org/10.1371/journal.pgen.1003232
de Boer, C. G., and T. R. Hughes, 2012 YeTFaSCo: A database of evaluated yeast transcription factor sequence specificities. Nucleic Acids Res. 40: D169–D179. https://doi.org/10.1093/nar/gkr993
Doniger, S. W., H. S. Kim, D. Swain, D. Corcuer, M. Williams et al., 2008 A catalog of neutral and deleterious polymorphism in yeast. PLoS Genet. 4: e1000183. https://doi.org/10.1371/journal.pgen.1000183
Engle, E. K., and J. C. Fay, 2012 Divergence of the Yeast Transcription Factor FZF1 Affects Sulphite Resistance. PLoS Genet. 8: e1002763. https://doi.org/10.1371/journal.pgen.1002763
Fay, J. C., 2013 The molecular basis of phenotypic variation in yeast.Curr. Opin. Genet. Dev. 23: 672–677. https://doi.org/10.1016/j.gde.2013.10.005
Fay, J. C., H. L. McCullough, P. D. Sniegowski, M. B. Eisen, E. Schadt et al., 2004 Population genetic variation in gene expression is associated with phenotypic variation in Saccharomyces cerevisiae. Genome Biol. 5: R26. https://doi.org/10.1186/gb-2004-5-4-r26
Fogel, S., and J. W. Welch, 1982 Tandem gene amplification mediates copper resistance in yeast. Proc. Natl. Acad. Sci. USA 79: 5342–5346. https://doi.org/10.1073/pnas.79.17.5342
Gerke, J., K. Lorenz, and B. Cohen, 2009 Genetic interactions between transcription factors cause natural variation in yeast. Science 323: 498–501. https://doi.org/10.1126/science.1166426
Gibson, D. G., L. Young, R.-Y. Chung, J. C. Venter, C. A. Hutchison, III et al., 2009 Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods 6: 343–345. https://doi.org/10.1038/nmeth.1318
Gietz, R. D., R. H. Schiestl, A. R. Willems, and R. A. Woods, 1995 Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast 11: 355–360. https://doi.org/10.1002/yea.10408
Graden, J. A., M. C. Posewitz, J. R. Simon, G. N. George, I. J. Pickering et al., 2016 Changes in the mitochondrial electron transport chain of wine yeast isolates. PLoS Genet. 5: e1003232. https://doi.org/10.1371/journal.pgen.1003232
Jin, Y. H., P. E. Dunlap, S. J. McBride, H. Al-Refai, P. R. Bushel et al., 2008 Global transcription and deletion profiles of yeast exposed to transition metals. PLoS Genet. 4: e1000053. https://doi.org/10.1371/journal.pgen.1000053
Kahn, M., G. Hasenbrink, H. Lichtenberg-frate, J. Ludwig, and M. Kechischo, 2010 Grofit: Fitting biological growth curves. J. Stat. Softw. 33: 1–21. https://doi.org/10.18637/jss.v033.i07
Kvitek, D. J., J. L. Will, and A. P. Gasch, 2008 Variations in stress sensitivity and genomic expression in diverse S. cerevisiae isolates. PLoS Genet. 4: e1000223. https://doi.org/10.1371/journal.pgen.1000223
Li, X. C., D. Peris, C. T. Hittinger, E. A. Sia, and J. C. Fay, 2013 The molecular basis of phenotypic variation in yeast. Current Biology 23: 14583–14589. https://doi.org/10.1016/j.cub.2013.10.042
Martin, A., and V. Orgogozo, 2013 The loci of repeated evolution: A catalog of genetic hotspots of phenotypic variation. Evolution 67: 1235–1250.
McGregor, A. P., V. Orgogozo, I. Delon, J. Zanet, D. G. Srinivasan et al., 2007 Morphological evolution through multiple cis-regulatory
mutations at a single gene. Nature 448: 587–590. https://doi.org/10.1038/nature05988

Meijnen, J.-P., P. Randazzo, M. R. Foulquié-Moreno, J. van den Brink, P. Vandecruys et al., 2016 Polygenic analysis and targeted improvement of the complex trait of high acetic acid tolerance in the yeast Saccharomyces cerevisiae. Biotechnol. Biofuels 9: 5. https://doi.org/10.1186/s13068-015-0421-x

Mortimer, R. K., 2000 Evolution and variation of the yeast (Saccharomyces) genome. Genome Res. 10: 403–409. https://doi.org/10.1101/gr.10.4.403

Orr, H. A., 2001 The genetics of species differences. Trends Ecol. Evol. 16: 343–350. https://doi.org/10.1016/S0169-5347(01)02167-X

Orr, H. A., and J. Coyne, 1992 The genetics of adaptation: a reassessment. Am. Nat. 140: 725–742. https://doi.org/10.1086/285437

Peter, J., M. De Chiara, A. Friedrich, J.-X. Yue, D. Pflieger et al., 2018 Genome evolution across 1,011 Saccharomyces cerevisiae isolates. Nature 556: 339–344. https://doi.org/10.1038/s41586-018-0030-5

Rockman, M. V., 2012 The QTN Program and the Alleles That Matter for Evolution: All That’s Gold Does Not Glitter. Evolution 66: 1–17. https://doi.org/10.1111/j.1558-5646.2011.01486.x

Roop, J. I., K. C. Chang, and R. B. Brem, 2016 Polygenic evolution of a sugar specialization trade-off in yeast. Nature 530: 336–339. https://doi.org/10.1038/nature16938

Scannell, D. R., O. A. Zill, A. Rokas, C. Payen, M. J. Dunham et al., 2011 The Awesome Power of Yeast Evolutionary Genetics: New Genome Sequences and Strain Resources for the Saccharomyces sensu stricto Genus. G3 (Bethesda) 1: 11–25. https://doi.org/10.1534/g3.111.002273

Steinmetz, L. M., H. Sinha, D. R. Richards, J. I. Spiegelman, P. J. Oefner et al., 2002 Dissecting the architecture of a quantitative trait locus in yeast. Nature 416: 326–330. https://doi.org/10.1038/416326a

Stern, D., and V. Orgogozo, 2009 Is genetic evolution predictable? Science 323: 746–751. https://doi.org/10.1126/science.1158997

Stern, D. L., and V. Orgogozo, 2008 The loci of evolution: how predictable is genetic evolution? Evolution 62: 2155–2177. https://doi.org/10.1111/j.1558-5646.2008.00450.x

Strope, P. K., D. A. Skelly, S. G. Kozmin, G. Mahadevan, E. A. Stone et al., 2015 The 100-genomes strains, an S. cerevisiae resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. Genome Res. 25: 762–774. https://doi.org/10.1101/gr.185538.114

Tang, S., and D. C. Presgraves, 2009 Evolution of the Drosophila Nuclear Pore Complex Results in Multiple Hybrid Incompatibilities. Science 323: 779–782. https://doi.org/10.1126/science.1169123

Thiele, D. J., 1988 ACE1 regulates expression of the Saccharomyces cerevisiae metallothionein gene. Mol. Cell. Biol. 8: 2745–2752. https://doi.org/10.1128/MCB.8.7.2745

Turner, R. B., D. L. Smith, M. E. Zawrotny, M. F. Summers, M. C. Posewitz et al., 1998 Solution structure of a zinc domain conserved in yeast copper-regulated transcription factors. Nat. Structural Biol. 5: 551–555. https://doi.org/10.1038/805

Visscher, P. M., M. A. Brown, M. I. McCarthy, and J. Yang, 2012 Five Years of GWAS Discovery. Am. J. Hum. Genet. 90: 7–24. https://doi.org/10.1016/j.ajhg.2011.11.029

Welch, J., S. Fogel, C. Buchman, and M. Karin, 1989 The CUP2 gene product regulates the expression of the CUP1 gene, coding for yeast metallothionein. EMBO J. 8: 255–260. https://doi.org/10.1002/j.1460-2075.1989.tb03371.x

Wood, A. R., T. Esko, J. Yang, S. Vedantam, T. H. Pers et al., 2014 Defining the role of common variation in the genomic and biological architecture of adult human height. Nat. Genet. 46: 1173–1186. https://doi.org/10.1038/ng.3097

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