A trait syndrome ties cell morphology to glycolysis across the yeast subphylum

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Summary

Traits that co-vary across species can provide fundamental insights into the trade-offs and constraints that govern their evolution. In a recent article in *Current Biology*, Li et al.[1] reported that glucose uptake rates (GUR) are inversely correlated with the cell surface area-to-volume (SA:V) ratio across 11 yeast species. Here we substantially expand this analysis to 282 species to test whether the GUR-SA:V correlation generalizes across the ancient Saccharomycotina yeast subphylum and to determine the contribution of shared evolutionary history to the co-variation of these two traits. Using regression models that account for co-variation due to phylogeny, we found that extracellular acidification rates (ECAR, which we used as a proxy for GUR) had a significant correlation with SA:V across Saccharomycotina. In contrast to Li et al.[1], our increased sample sizes provided statistical power to reveal additional significant correlations of ECAR with genome sizes and growth rates. Our findings dramatically extend and expand those of Li et al.[1] and suggest that a trait syndrome governs several metabolic, genomic, and morphological traits across yeasts.

Main Text

The Saccharomycotina yeast subphylum includes over a thousand species and displays impressive genetic and phenotypic diversity.[2,3] The ability of most yeast species to grow well in axenic culture enables both the characterization of many traits and the exploration of biological principles that govern their variation.[3] Such studies have revealed that certain traits are consistently associated with each other and may collectively contribute to trait syndromes.[4] These trait syndromes can be driven by intrinsic factors (e.g. genomic characteristics) or extrinsic factors (e.g. varying resource availability).[3,5]

Recently, Li et al.[1] reported the interesting finding that glucose uptake rate (GUR) is inversely correlated with surface area-to-volume ratio (SA:V) across 16 yeast strains in 10 Ascomycota species (subphyla Saccharomycotina and Taphrinomycotina) and 1 Basidiomycota

species (subphylum Pucciniomycotina). Parallel to and independently from Li et al., we have measured glucose-induced extracellular acidification rates (ECAR) as a proxy for glycolytic rates[6] and characterized yeast phenotypic variation by compiling data on asexual budding cell size and shape[7] of 282 diverse yeast species within Saccharomycotina. Our dataset is uniquely suited to test whether the correlation reported by Li et al.[1] holds true across an entire yeast subphylum. We also considered two additional traits, genome size and rates of growth on glucose, which we previously collected for these yeast species as part of the Y1000+ Project.[3]

First, we calculated the surface area, volume, and SA:V of each yeast species (Table S1). We then calculated the phylogenetic signal of each trait using Pagel's lambda (Supplemental methods) and found that all traits had high and significant phylogenetic signal values (ECAR: $\lambda = 0.815$, p-value = $1.14E^{-49}$; SA:V ratio: $\lambda = 0.846$, p-value = $1.23E^{-19}$; genome size: $\lambda = 0.975$, p-value = $1.07E^{-42}$; and growth rate on glucose: $\lambda = 0.725$, p-value = $5.78E^{-7}$). The high phylogenetic signal values suggest that some of the variation in each trait can be attributed to shared evolutionary history. Thus, we applied phylogenetically-corrected regression models (Supplemental methods) to determine the relationships that exist between ECAR and SA:V and genome size (n = 282 species), as well as between ECAR and growth rate on glucose (n = 231 species). We found that ECAR was positively correlated with SA:V (phylogenetic signal λ of the model = 0.808, $R^2 = 0.565$, p-value = 0.0001, Figure 1A). Since ECAR is negative (faster glucose-induced acidification) when GUR is high, these results support the main finding of Li et al.[1] and reveal that the inverse correlation between GUR and SA:V holds across a much larger group of yeast species and that it cannot be explained solely through phylogenetic co-variance.

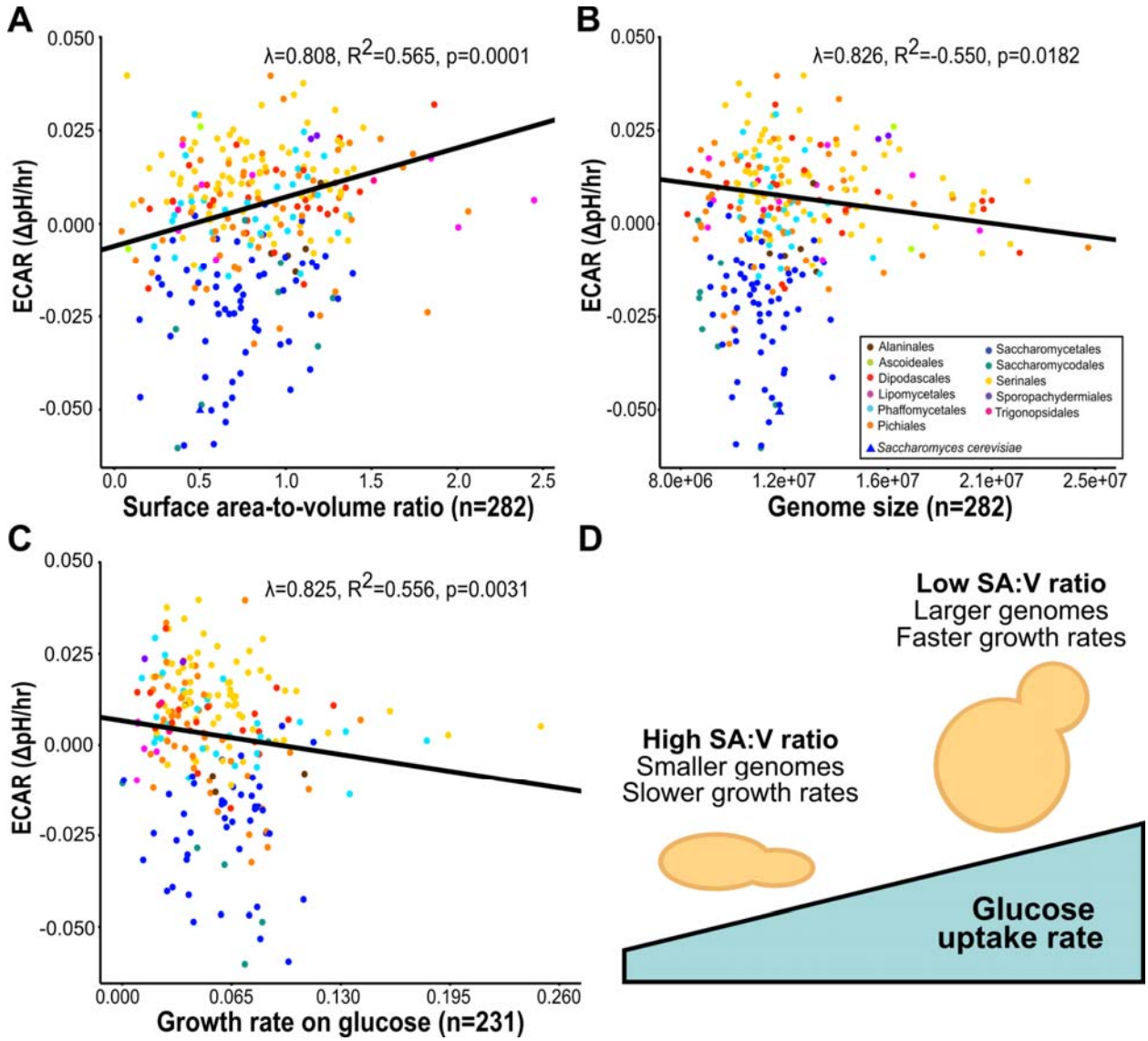

Figure 1. Glycolytic rate is significantly associated with cell morphology, genome size, and growth across the Saccharomycotina yeasts. (A-C) Scatter plots show significant correlations between ECAR (extracellular acidification rates, measured as a proxy for glycolytic rates) and: (A) cell surface area-to-volume ratio; (B) genome size; and (C) growth rate on glucose. Each dot represents a single yeast species colored according to its taxonomic order as labeled (B). *S. cerevisiae* is represented by a triangle in panels (A) and (B). The R^2 and p-values are based on a phylogenetic generalized least squares model, which accounts for correlation stemming from shared phylogenetic history. (D) A schematic model proposes a trait syndrome associated with a rapid glucose uptake rate among diverse yeast species.

Typically, smaller cells with larger SA:V ratios have been found to exhibit higher metabolic rates due to increased transport capacity at the cell surface.[8] However, an alternative model suggests that efficient scaling of biosynthesis and sufficient protein production are key to

cell enlargement and rapid metabolism.[9] Our findings are largely consistent with this second model as cells with lower SA:V ratios generally had higher metabolic rates. We speculate that, in yeasts, larger cells have more space in the cytosol for the catabolism of glucose, thereby enabling a stronger pull through glycolysis and consequently rapid GUR and growth rates. This glycolytic effect may be particularly applicable to Crabtree/Warburg-positive yeasts, which ferment in the presence of oxygen and have been proposed as an exception to the classic view that high SA:V is necessary for high GUR.[8] Overall, this model proposes that the metabolic rate of yeasts growing on glucose is more influenced by enhanced glycolytic flux enabled by increased cytosolic volume than by nutrient import.

In contrast to the findings of Li et al.[1], our much larger dataset also showed significant negative correlations between ECAR and genome size ($\lambda = 0.826$, $R^2 = 0.550$, p-value = 0.0182, Fig 1B), and between ECAR and growth rate on glucose ($\lambda = 0.825$, $R^2 = 0.556$, p-value = 0.0031, Fig 1C). The contrast between our findings and those of Li et al.[1] is likely explained by the enhanced statistical power of our dataset. Previous work showed that a larger genome size was associated with a generalist phenotype in Saccharomycotina yeasts, wherein generalists grew on a larger range of carbon sources and had faster growth rates.[3] Our data further show that yeast species with larger genomes have increased glucose uptake rates and faster rates of growth on glucose. Therefore, yeasts with larger genomes, such as those with whole genome duplications[10], could be promising for industrial applications requiring rapid metabolism.

Altogether our results and previous work lead us to propose a trait syndrome in which glycolytic rate is associated with multiple morphological and genomic features across the yeast subphylum. Furthermore, the association between metabolism and genome size suggests that gene acquisitions, gene family expansions, or genome duplications may be, at least in part, influencing glucose uptake rates and cell size. Finally, our results raise important questions with respect to the mechanisms that link primary carbon metabolism to the regulation of cell morphology, whether genomic features other than size contribute to the relationship between

these traits, and the environments that select for this trait syndrome. The findings reported here and by Li et al.[1], alongside the rich genomic dataset available for the Saccharomycotina subphylum[3], are poised to guide experiments that will uncover these mechanisms.

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Author contributions

L.C.H, C.M.C, A.R., and C.T.H. conceptualized this project. L.C.H and C.M.C. developed the methodology, curated and analyzed the data, and prepared the figures under the supervision and guidance of A.R. and C.T.H. L.C.H and C.M.C wrote the manuscript, and A.R. and C.T.H. reviewed edited and approved the manuscript. L.C.H., A.R., and C.T.H. acquired funding for this project.

Conflicts of Interest

A.R. is a scientific consultant of LifeMine Therapeutics, Inc. and a member of the Advisory Board of *Current Biology.* All other authors declare no competing interests.

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Supplemental methods

To investigate the role of shared evolutionary history in the variation of each trait, we calculated Pagel's lambda for each trait using the *R* package phytools[1] version 2.5-2 phylosig() with a set seed. Since these traits each had high phylogenetic signal values, we applied phylogenetically corrected regression models to determine their relationships using the *R* package nlme version 3.1-168 gls() function with model set to 'corPagel' from the package ape version 5.8-1.[2-4]

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