Chromosome mapping of thermal divergence among *Saccharomyces* yeast species

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Abstract

Identifying the genetic basis of phenotypic divergence between species is key to understanding evolution. While much has been learned from studies of intraspecific variation, these results may not be representative of interspecific divergence. For instance, divergence between species could be caused by numerous small effect changes in the same gene. A major hurdle to unearthing patterns of interspecific divergence is the sterility and inviability of hybrids, which limits genetic mapping of traits to closely related species. We overcome these barriers by using chromosome level loss of heterozygosity to map thermotolerance in hybrids of two distantly related *Saccharomyces* yeast species.

Introduction

Thermotolerant *S. cerevisiae* diverged approximately 15 million years ago from the thermosensitive species, *S. uvarum*. Hybrids of these two species are mitotically stable and retain thermotolerance of the *S. cerevisiae* parent to a certain degree. Genetic mapping of thermal divergence in this hybrid is complicated by failure of meiosis due to incorrect pairing of homologous chromosomes. A previous non-complementation screen failed to identify single genes of large effect that contribute to the hybrid phenotype. However, this screen excluded essential genes and did not test for multiple genes acting together to confer thermotolerance. To overcome these shortcomings, we used hybrid aneuploids to screen the *S. cerevisiae* genome for thermotolerance genes.

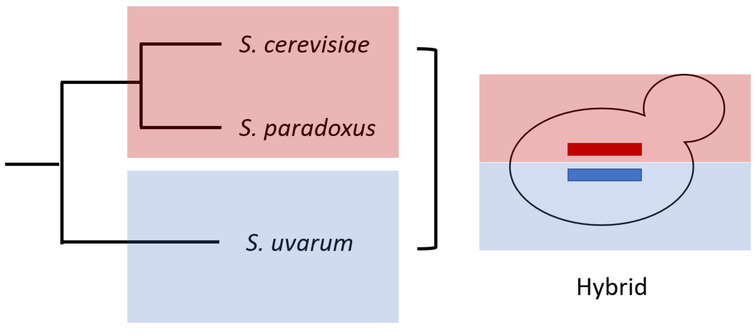


Figure 1: The *Saccharomyces* species group is thermally diverged. *S. cerevisiae* is thermotolerant and can grow at high temperatures like 37°C. Meanwhile, the cryotolerant *S. uvarum* can grow at cold temperatures like 4°C. A hybrid between these two species retains the phenotypes of both parents and can tolerate high as well as low temperatures.

Methods

We use a set of centromere marked *S. cerevisiae* strains (courtesy Dr. Jun-Yi Leu), to generate whole chromosome loss of heterozygosity and measured its effects on growth at different temperatures. These 16 strains of *S. cerevisiae* were crossed to *S. uvarum* to generate 16 hybrids, which were then screened for the loss of the two markers flanking the centromere to ensure deletion of both chromosomal arms. 3-5 isolates of each hybrid aneuploid were sequenced and phenotyped at different temperatures on glucose and glycerol.

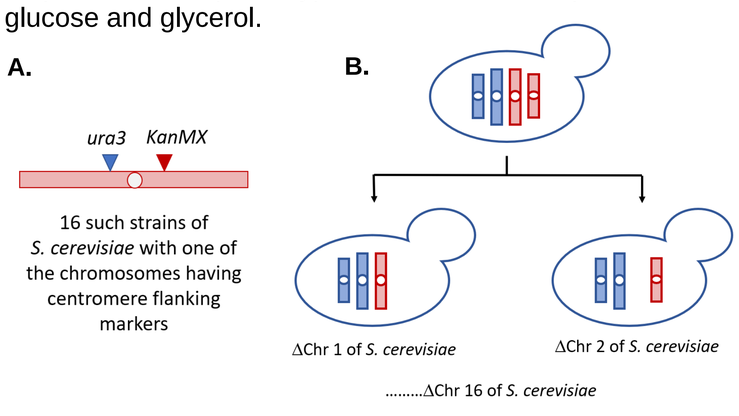


Figure 2: **A)** We used 16 *S. cerevisiae* strains with markers flanking their centromere to generate 16 hybrids. One of these markers was the counterselectable *URA3* and the other was *KanMX*. **B)** We plated these hybrids on 5-FOA to select for loss of *URA3* and screened the colonies for loss of *Kan* resistance as well. This ensured loss of both arms of the marked *S. cerevisiae* chromosome. Aneuploid hybrids were obtained for 12/16 chromosomes.

Models of genetic architecture of thermal divergence

Infinitesimal model: Small effect genes

If small effect genes distributed throughout the genome underlie thermal divergence, every chromosome should have an effect proportional to the number of genes on it. Loss of one *S.cerevisiae* chromosome from the hybrid should reduce high temperature growth by 6% on average in this scenario.

Large effect chromosomes

If a few large effect genes are responsible for thermotolerance, deleting most chromosomes should have no effect unless they carry such a gene. Alternatively, large effect chromosomes can bear multiple small effect genes.

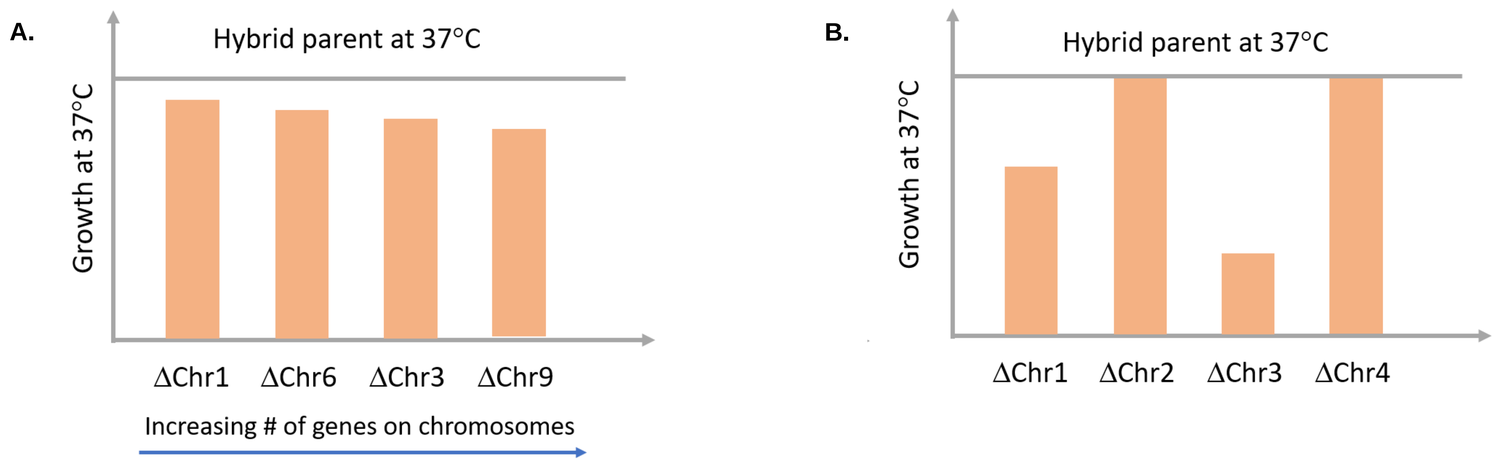


Figure 3: A) Predictions of the infinitesimal model. Loss of each chromosome will have an effect on growth at 37°C proportional to the number of genes on the chromosome.

B) Predictions under the large effect chromosomes model. Loss of certain chromosomes impacts high temperature growth but others have no effect.

Results

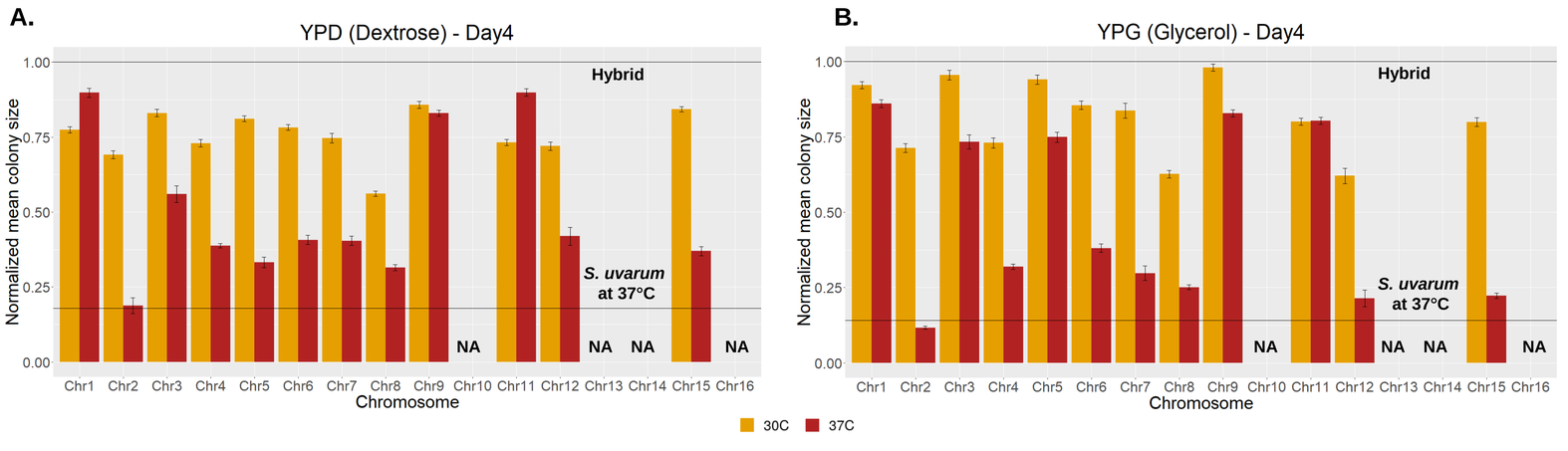
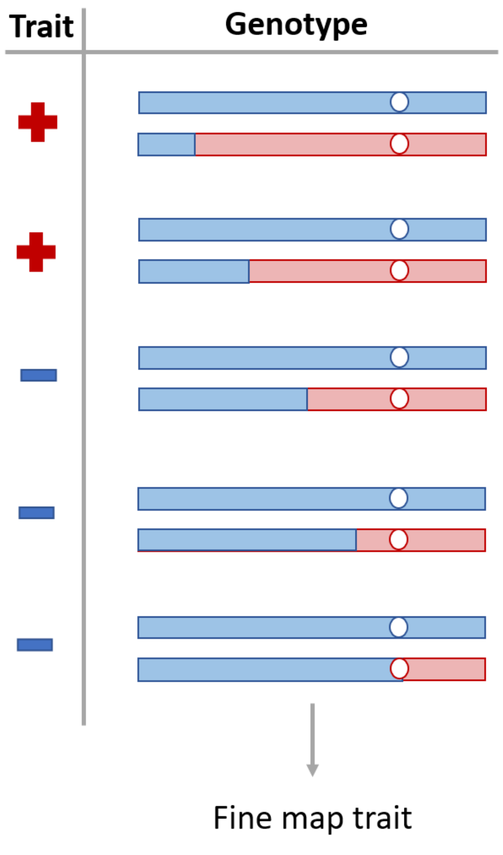


Figure 4: A) Hybrid aneuploids grown on dextrose at 30°C and 37°C. **B)** Hybrid aneuploids grown in glycerol, a non-fermentable carbon source. All strains have been normalized by the average hybrid parent size (= 1) and the horizontal line marks the growth of *S. uvarum* at 37°C. 12/16 aneuploids were obtained. Decrease in growth at 30°C relative to the hybrid parent can be attributed to growth defects caused by aneuploidy, while decrease in growth at 37°C could be due to loss of temperature tolerance genes. (NA – Not available)

Inferences and future directions



- Loss of certain *S. cerevisiae* chromosomes leads to large defects in high temperature growth on both fermentative and non-fermentative substrates. Whereas, other chromosomes have a small to moderate effect and loss of chromosome 11 in particular, has no impact on high temperature growth.
- Our results support a mixture of large effect and small effect chromosomes, pointing to a composite influence of both small and large effect genes.
- The observation of multiple large effect (>30% drop at 37°C) chromosomes, is consistent with epistasis whereby multiple *S. cerevisiae* chromosomes are necessary but not sufficient for thermotolerance.

To resolve these effects to the genic level, we will use CRISPR to generate loss of heterozygosity along chosen chromosomes in the hybrid and monitor thermotolerance.

- CRISPR directed double strand breaks along a chromosome will be followed by marker selection to pick isolates with loss of heterozygosity that spans from the cut site to the end of the chromosomal arm.
- This panel of isolates will be phenotyped to narrow down the region responsible for thermotolerance and the process will be repeated till the causal genes are identified and confirmed by the reciprocal hemizygosity test.

Figure 5: Loss of heterozygosity panel generated using CRISPR to direct double strand breaks along *S. cerevisiae* chromosomes in hybrids. Change in phenotype across this panel can be used to narrow the mapping interval. Multiple iterations of this process will allow us to identify causal genes.

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References

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