Genetic interactions involving five or more genes contribute to a complex trait in yeast

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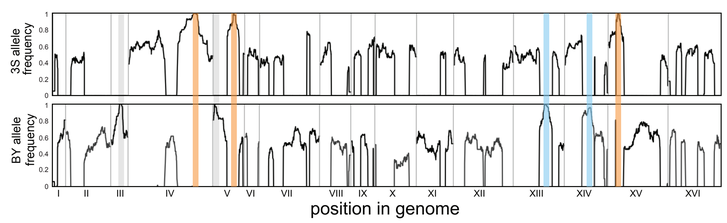
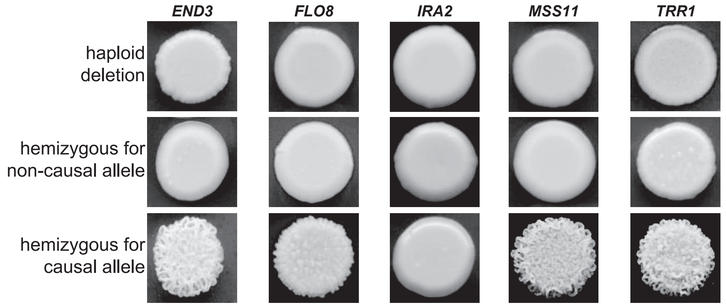
Introduction

Many phenotypes of biological interest vary among individuals due to the effects of alleles at different loci. Although it is well known that these alleles can interact, the forms of these interactions have not been fully characterized. Most work on this problem to date has focused on cases involving only two loci. However, higher-order interactions involving three or more loci can also occur, and may have significant effects on the relationship between genotype and phenotype. In this paper, we dissect a colony morphology trait that segregates in a cross of two yeast strains and is caused by genetic interactions among five or more loci. Our work demonstrates that higher-order interactions can have major phenotypic effects, and provides novel insights into the genetic and molecular basis of these interactions.

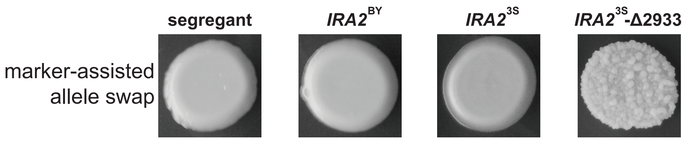
Although both BY (A) and 3S (B), as well as most of their haploid offspring (C), form smooth colonies, ~2% of their progeny exhibited rough colonies (D) when we examined 250 segregants. Previous work has shown that such heritable variation in colony morphology in *S. cerevisiae* can arise due to naturally occurring polymorphisms or spontaneous mutations at chromosomal loci, aneuploidies, and prions. Unlike chromosomal loci, which should show stable inheritance across generations, aneuploidies and prions can be gained or lost, resulting in phenotypic switching.



Sequencing of hundreds of backcross progeny revealed 7 fixed or near-fixed loci– two selecrable markers used for generation of haploid progeny, 3 regions from 3S (top, chrIV, V, and XV), and 2 regions from BY (bottom, chrXIII and XIV).



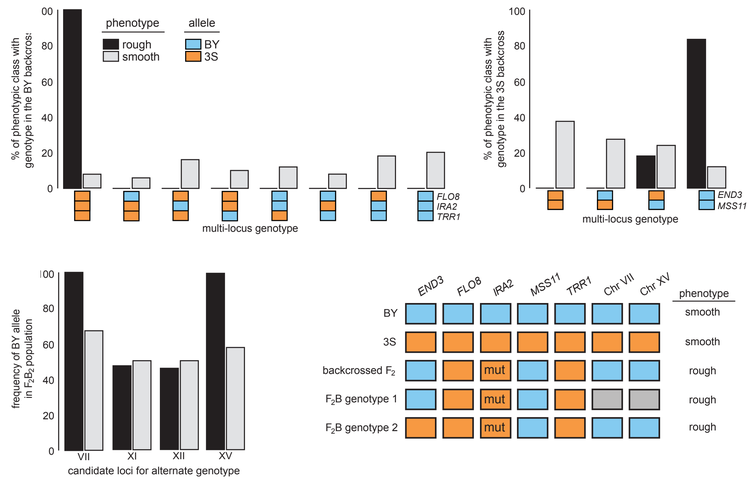
Every gene within the detected loci was deleted in a rough individual. Deletion of one gene in each interval resulted in a loss of rough morphology. These deletion strains were mated to appropriate rough and smooth strains to produce hemizygotes that suggested functional variation in 4/5 of these genes.



Our sequencing data showed that all rough individuals possessed a new mutation in IRA2: a deletion that truncates the tail end of the protein. Engineering this deletion into the appropriate smooth genetic background led to expression of the rough morphology phenotype.

chromosome	contributing parent	causal gene	function of candidate gene
IV	3S	TRR1	Cytoplasmic thioredoxin reductase
V	3S	FLO8	Transcription factor that regulates cell-cell adhesion
XIII	BY	MSS11	Transcription factor involved in regulation of invasive growth
XIV	BY	END3	EH domain-containing protein involved in endocytosis
XV	3S	IRA2	GTPase-activating protein; negatively regulates RAS

Genes w/ functional variation fall in to a number of disparate molecular pathways, and most combinations of genes have not been shown to have a direct functional relationship



Tetrad dissections from backcross populations showed that rough individuals all possess *IRA2*^{3S}-Δ2933, *FLO8*^{3S}, *MSS11*^{BY}, *TRR1*^{3S}, and either *END3*^{BY} or *END3*^{3S}. Rough individuals with END3^{3S} also possessed BY alleles at loci on chromosomes VII and XV.

Conclusion

In summary, we have demonstrated that sets of five or more genetic variants can synthetically interact to produce major phenotypic effects. Alleles involved in these higher-order interactions may either be polymorphisms that segregate in natural populations or spontaneous mutations. Our work also shows that rather than functioning in a single biochemical pathway, protein complex, or regulatory circuit, the genes involved in higher-order interactions can play roles in a number of cellular processes. This finding implies that characterizing higher-order interactions using data from screens and annotations focused solely on reference genomes may be a challenge, and highlights how genetic variation can serve as a tool for detecting previously unidentified functional relationships among genes. Further, we have shown that different sets of alleles can interact to produce the same phenotypic effect..

Acknowledgements

Thank you to the Ehrenreich Lab for their help in developing this project, and to Oscar Aparicio, Susan Forsburg, Steve Finkel, Sergey Nuzhdin, and Peter Ralph for input. This work was funded by startup funds to IME.