The Cost of Gene Expression Underlies a Fitness Trade-off in Yeast

Gregory I. Lang (glang@princeton.edu)[1], Andrew W. Murray[2], and David Botstein[1]

[1] Lewis-Sigler Institute for Integrative Genomics and the Department of Molecular Biology, Princeton University, Princeton, NJ

[2] FAS Center for Systems Biology and the Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA

Abstract

Natural selection optimizes an organism's genotype within the context of its environment. Adaptations to one environment can decrease fitness in another, revealing evolutionary trade-offs.

We show that the cost of gene expression underlies a trade-off between growth rate and mating efficiency in the yeast, *Saccharomyces cerevisiae.* During asexual growth, mutations that eliminate the ability to mate provide a ~2% per generation growth-rate advantage.

Some strains, including most laboratory strains, carry an allele of *GPA1* (an upstream component of the mating pathway) that increases mating efficiency by ~30% per round of mating at the cost of a ~1% per generation growth-rate disadvantage.

In addition to demonstrating a trade-off between growth rate and mating efficiency, our results illustrate differences in the selective pressures defining fitness in the laboratory versus the natural environment, and show that selection, acting on the cost of gene expression, can optimize expression levels and promote gene loss.

Evolutionary Significance

It is frequently observed that traits not maintained by selection will be lost

Examples of regressive evolution:

- Loss of olfactory receptors in primates (1)
- Loss of pigmentation and vision in *Astyanax* cavefish (2)
- Loss of the galactose utilization pathway in yeast (3)
- Reduction in catabolic breadth in *E. coli* (4)
- Sterility in *S. cerevisiae* (5)

Importance of gene loss in evolution:

- Extensive gene loss in proto-mitochondria and *Mycobacterium leprae* may have fostered the transition from facultative to obligate intracellular parasites (6).
- Reciprocal gene loss following whole-genome duplication has reinforced species barriers by establishing Dobzhansky-Muller incompatibilities (7).
- The loss of key developmental regulators early in vertebrate evolution has been suggested to have played a role in the establishment of modern phyla (8).

Two mechanisms could account for gene loss during evolution:

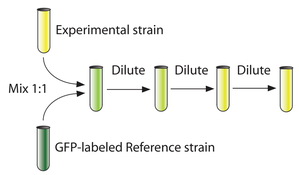
(1) One possibility is that in the absence of selection, genes are lost due to the neutral accumulation of mutations.
(2) Alternatively, gene loss events could be driven by selection.

Here we set out to directly test whether selection drives yeast to become sterile by determining whether mutations conferring sterility provide a selective advantage.

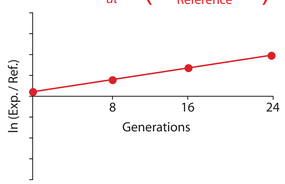
Fitness Assay

To measure fitness (growth rate) we employed a FACS-based competitive growth-rate assay.

- Mix experimental strain 1:1 with a GFP-labeled reference strain
- Propagate mixed population through ~25 generations

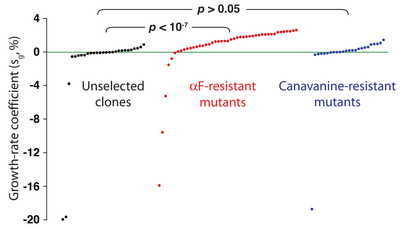


- Monitor the population by flow cytometry
- Calculate growth rate advantage = $\frac{d}{dt} \ln\left(\frac{\text{Experimental}}{\text{Reference}}\right)$

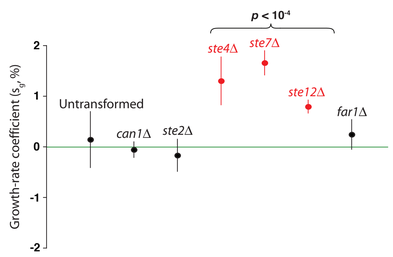


Sterility Increases Growth Rate by Eliminating Unnecessary Gene Expression

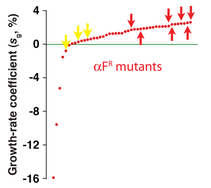
We isolated sterile strains by screening for spontaneous αF^R mutations and by targeted gene disruption. We measured the growth rate of sterile strains using the FACS-based fitness assay. Our results demonstrate that a subset of αF^R mutations provide a competitive growth advantage.



- The growth-rate coefficients (s_g) 45 **αF^R mutants** show greater variation and a positive growth-rate advantage (**s_g = 1.48% ± 0.85%**) compared to 27 **unselected clones** (**s_g = 0.08% ± 0.35%**, $p < 10^{-7}$, Wilcoxon rank sum test).
- The distribution of growth-rate coefficients for 24 **Can^R mutants** (**s_g = 0.36% ± 0.48%**) is indistinguishable from the distribution of 27 **unselected clones** ($p > 0.05$, Wilcoxon).
- Eight outliers have a growth-rate disadvantage of at least 1%, suggesting that these strains may have acquired a deleterious mutation or have become mitochondrial deficient.



- Targeted gene disruptions show that loss the G_β subunit (**Ste4**), the MAP kinase kinase (**Ste7**), or the transcription factor (**Ste12**) increases growth rate ($p = 2.8 \times 10^{-5}, 5.8 \times 10^{-9}, 2.2 \times 10^{-6}$, respectively, t-test); however, loss of the receptor (**Ste2**) or **Far1** does not ($p = 0.23, 0.03$, respectively, t-test).
- This suggests that a growth rate advantage exists for the subset of sterile strains that abolish basal signaling through the mating pathway (therefore eliminating basal expression of the mating genes), which is dependent upon **Ste4**, **Ste7**, and **Ste12**, but not **Ste2** or **Far1**.

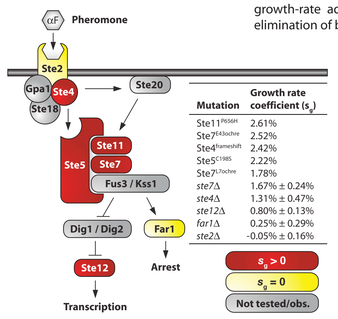


We identified the mutations in six αF^R mutants from the higher end of the growth rate distribution by hybridizing genomic DNA to microarrays.

- Five of the six faster-growing spontaneous αF^R mutants contain a single mutation that reduces basal-signaling-induced gene expression: Ste11^{P656H}, Ste7^{E3ochre}, Ste4^{frameshift}, Ste5^{C198S}, and Ste7^{L7ochre}.

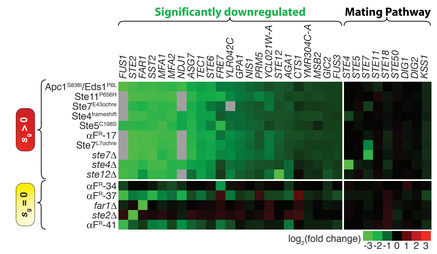
We assayed for changes in gene expression for seven αF^R mutants from the upper end of the growth rate distribution ($s_g > 0$), three from the lower end of the distribution ($s_g = 0$), and the targeted gene disruptions.

- The seven spontaneous αF^R mutants from the upper end of the growth rate distribution significantly decrease the expression of 23 genes while not significantly increasing the expression of any other genes.
- The three spontaneous αF^R mutants from the lower end of the growth rate distribution and the *far1* and *ste2* deletions do not decrease the expression of these genes to the degree seen in strains with a growth-rate advantage, consistent with the hypothesis that the growth-rate advantage is due to elimination of basal expression of the mating pathway genes.

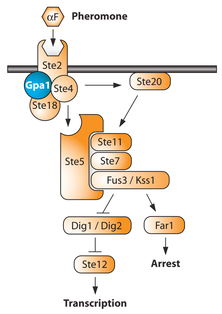


Mutation	Growth rate coefficient (s_g)
Ste11^{P656H}	2.61%
Ste7^{E43ochre}	2.52%
Ste4^{frameshift}	2.42%
Ste5^{C198S}	2.22%
Ste7^{L7ochre}	1.78%
ste7Δ	1.67% ± 0.24%
ste4Δ	1.31% ± 0.47%
ste12Δ	0.80% ± 0.13%
far1Δ	0.25% ± 0.29%
ste2Δ	-0.05% ± 0.16%

$s_g > 0$
$s_g = 0$
Not tested/obs.



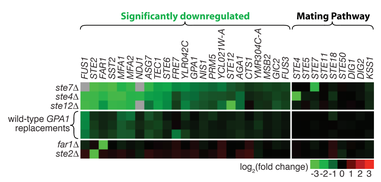
Selection for Mating Efficiency Can Increase the Burden of Gene Expression



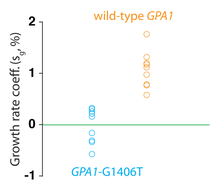
Although expressing the mating genes decreases growth rate, some strains (including most laboratory strains) carry an allele of the G_β subunit (*GPA1*-G1406T) that increases basal expression of these genes.

The derived (laboratory) allele contains a T at position 1406 in place of the ancestral G (found in multiple wild isolates and the other sequenced *sensu stricto* species) resulting in the missense mutation in a region of the protein thought to interact with the receptor; other mutations in this region constitutively activate the mating pathway (9).

We re-introduced the wild-type *GPA1* allele into our laboratory strain and measured the effect of this mutation on gene expression, growth rate, and mating efficiency.



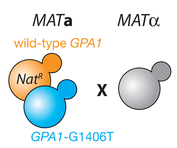
The wild-type *GPA1* allele reduces expression of genes downstream of Ste12 relative to the *GPA1*-G1406T allele, though these genes are not reduced to the levels seen for sterile mutations (*ste7Δ*, *ste4Δ*, and *ste12Δ*), which eliminate signaling through the mating pathway.

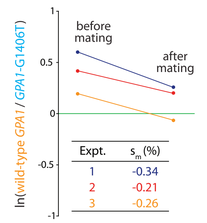


Strains with the wild-type *GPA1* allele have a significant growth rate advantage over strains with the *GPA1*-G1406T allele. (s_g = 0.92% ± 0.35%, $p = 2.57 \times 10^{-6}$, t-test).

Consistent with the hypothesis that the growth-rate advantage is due to reduction in gene expression, we find that, like expression levels, the growth-rate advantage of the wild-type *GPA1* allele replacement strains is greater than that observed for strains that do not effect signaling (*far1Δ* and *ste2Δ*) and less than that observed for strains that eliminate signaling (*ste7Δ*, *ste4Δ*, and *ste12Δ*) through the mating pathway.

To determine if the 1% growth-rate disadvantage of strains carrying the *GPA1*-G1406T allele is offset by an increase in mating efficiency, we measured competitive mating efficiency of strains carrying the two alleles of *GPA1* using an assay analogous to the competitive growth rate assay.

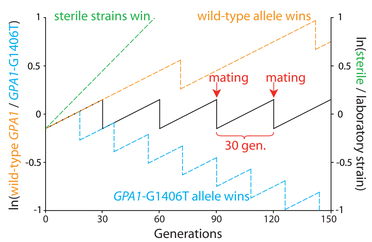




Expt.	s_m (%)
1	-0.34
2	-0.21
3	-0.26

The mating coefficients are all negative indicating that strains carrying the wild-type *GPA1* allele, which have a growth-rate advantage, have a disadvantage in mating relative to the *GPA1*-G1406T allele (s_m = -27.2% ± 6.5%).

The *GPA1*-G1406T allele increases the basal expression of genes in the mating pathway thus increasing mating efficiency by ~30% at a growth-rate cost of ~1% per generation.



Under what conditions will each allele be favored?

- If strains carrying these two alleles are mixed and propagated in a regime where one round of mating occurs every 30 generations, they would be **equally fit** (black trace).
- If mating is less frequent than every 30 generations the **wild-type allele** is favored.
- If mating is more frequent than every 30 generations the ***GPA1*-G1406T allele** is favored.
- During long-term evolution, strains are typically propagated asexually. In this regime, **sterile strains,** which eliminate basal signaling through the mating pathway, will out-compete mating-proficient strains.

Conclusions & Discussion

The cost of gene expression underlies a fitness trade-off.

- *Mutations that eliminate the expression of 23 genes in the mating pathway provide a 2% growth-rate advantage but result in sterility.*

Our results support the hypothesis that there is a general cost to gene expression.

- *Assuming that each of the 23 genes contributes equally to the 2% growth-rate advantage, the growth rate advantage attained by eliminating a single dispensable gene is ~0.1%. Therefore, for populations sizes greater than ~10^3, such as panmictic microbial populations, selection will oppose unnecessary gene expression.*

Our results provide an explanation for the existence of the *GPA1*-G1406T allele.

- *Given that gene expression is costly, it is surprising that some strains carry an allele of GPA1 that increases expression of the mating genes.*
- *This polymorphism is one of the strongest trans-acting regulatory polymorhphisms between laboratory and wild strains (10).*
- *We show that this mutation increases the expression of genes in the mating pathway thus increasing mating efficiency by ~30% at a growth-rate cost of ~1% per generation.*

The *GPA1*-G1406T allele is a naturally-occurring allele that is common in lab strains.

- *The prevalence of this allele in laboratory populations may result from the "founder effect" since laboratory strains are derived from a small number of wild isolates.*
- *Alternatively, laboratory cultivation may select for the GPA1-G1406T allele. In the laboratory, cells are mated en masse; a condition that strongly selects for cells that produce more pheromone (11).*
- *This raises the question: To what degree is laboratory domestication the result of de novo mutation versus the selection of favorable combinations of naturally occurring alleles.*

Notes & References

- This work was published as an open-access article in *PNAS* (2009) 106:5755-5760.
- The complete laboratory notebook describing these experiments is available at http://www.genomics.princeton.edu/glang/notebooks.htm
- This work was supported by two NIGMS Centers of Excellence grants (GM068763 [A.W.M.] and GM071508 [D.B.]) and individual NIH grants (GM43987 [A.W.M.] and GM046406 [D.B.])

(1) Gilad Y, Przeworski M, Lancet D (2004) *PLoS Biol* 2:E5.
(2) Protas ME, *et al.* (2006) *Nat Genet* 38:107-111.
(3) Hittinger CT, Rokas A, Carroll SB (2004) *PNAS* 101:14144-14149.
(4) Cooper VS, Lenski RE (2000) *Nature* 407:736-739.
(5) Zeyl C, Curtin C, Karnap K, Beauchamp E (2005) *Evolution* 59:2109-2115.
(6) Cole ST, *et al.* (2001) *Nature* 409:1007-1011.
(7) Scannell DR, *et al.* (2006) *Nature* 440:341-345.
(8) De Robertis EM (2008) *Cell* 132:185-195.
(9) Hirsch JP, Dietzel C, Kurjan J (1991) *Genes Dev* 5:467-474.
(10) Yvert G, *et al.* (2003) *Nat Genet* 35:57-64.
(11) Jackson CL, Hartwell LH (1990) *Cell* 63:1039-1051.