A Characterization of Adaptive Mutations in Yeast

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Adaptive traits enable one species/population/individual to outperform another.
We know a lot about some of the traits that enable adaptation.

Rose Andrew & Kate Ostevik (Rieseberg/Otto)

We know a lot about some of the traits that enable adaptation, but much less about the attributes of beneficial mutations.

Rose Andrew & Kate Ostevik (Rieseberg/Otto)  
Many factors affect the appearance and spread of beneficial mutations
Many factors affect the appearance and spread of beneficial mutations

- Rate new mutations arise
  - Population size \( (N) \)
  - Mutation rate \( (\mu) \)
Many factors affect the appearance and spread of beneficial mutations

- Rate new mutations arise
  - Population size (N)
  - Mutation rate (μ)
- Mutation effect size
Many factors affect the appearance and spread of beneficial mutations

- Rate new mutations arise
  - Population size (N)
  - Mutation rate (\(\mu\))
- Mutation effect size
- Available mutations
Many factors affect the appearance and spread of beneficial mutations

- Rate new mutations arise
  - Population size (N)
  - Mutation rate ($\mu$)
- Mutation effect size
- Available mutations
- Dominance ($h$)
Use microorganisms to study adaption ‘forward’ in time.

*Pseudomonas*

ΦX174 (phage)

*Saccharomyces*
Research Theme 1: The effect of ploidy on adaptation

Haploid – Male honey bee

Diploid – Female honey bee

Haploid/Diploid – *Ulva lactuca*

Haploid – *Mastocarpus papillatus* (gametophyte)

Diploid – *Mastocarpus papillatus* (sporophyte)

Ezov et al. (2006) Genetics
Theme 1: The effect of ploidy on adaptation

![Graph showing the effect of ploidy on adaptation in unstressed and salt-stressed conditions.](image-url)
Theme 1: The effect of ploidy on adaptation

What factor(s) allowed diploid mutants to repeatedly invade haploid populations? [Chapter 2]

Do haploids or diploids adapt faster to mildly stressful environments? [Chapter 3]
Theme 1: The effect of ploidy on adaptation

What factor(s) allowed diploid mutants to repeatedly invade haploid populations? [Chapter 2]

Do haploids or diploids adapt faster to mildly stressful environments? [Chapter 3]
Do haploids or diploids adapt faster to mildly stressful environments?

**HAPLOIDS (one set of chromosomes):**
All mutations immediately “seen” = shorter fixation time.

**DIPLOIDS (two sets):**
Theoretically twice the mutations rate = shorter waiting time for mutations. Most mutations at least partially masked (i.e., recessive) = longer fixation time.

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Zeyl et. al (2003)
Method: evolve haploids and diploids for 200 generations in seven mildly stressful environments.
**Method**: evolve haploids and diploids for 200 generations in seven mildly stressful environments

Measure competitive fitness at 50 and 200 generations

- common competitor
- population of interest
Measure the rate of adaptation for each ploidy $\times$ environment population.

\[
p_0 e^{mt} \over 1 - p_0 + p_0 e^{mt}
\]
Measure the **rate of adaptation** for each ploidy × environment population.
In all environments, haploids adapted faster than diploids.
Haploids adapt faster . . . but diploids take over haploid populations

Why do haploids adapt faster, and why so much variation?

• Initial fitness
• Population size (N)
• Mutation rate (\( \mu \))
• Mutation effect size
• Mutation availability
• Dominance
Why do haploids adapt faster, and why so much variation?

- **Initial fitness**
- Population size (N)
- Mutation rate ($\mu$)
- Mutation effect size
- Mutation availability
- Dominance
Why do haploids adapt faster, and why so much variation?

- Initial fitness
- Population size ($N$)
- Mutation rate ($\mu$)
- Mutation effect size
- Mutation availability
- Dominance
Why do haploids adapt faster, and why so much variation?

- Initial fitness \( \times \)
- Population size (N) \( \times \)
- Mutation rate (\( \mu \))
- Mutation effect size
- Mutation availability
- Dominance
Why do haploids adapt faster, and why so much variation?

- Initial fitness
- Population size (N)
- Mutation rate ($\mu$)
- Mutation effect size
- Mutation availability
- Dominance
Research Theme 2: Characterizing single adaptive mutations in a eukaryote

Tenaillon et. al 2012
*Escherichia coli*
115 lines, 2000 generations

Schenk et. al 2012
TEM-1 β-lactamase
48 mutations (mutagenesis)
Theme 2: Characterizing single adaptive mutations

virus (too simple)

maize (too complex)
Theme 2: Characterizing single adaptive mutations

- virus (too simple)
- yeast (just right)
- maize (too complex)
Theme 2: Characterizing single adaptive mutations

What is the genomic and phenotypic breadth of the first adaptive mutations acquired in response to a novel stressor? [Chapter 4]
Theme 2: Characterizing single adaptive mutations

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Do adaptive mutations have the same effect size in haploids and homozygous diploids? [Chapter 5]
Theme 2: Characterizing single adaptive mutations

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Do adaptive mutations have the same effect size in haploids and homozygous diploids? [Chapter 5]

What is the effect of zygosity on adaptive mutations? [Chapter 6]
**Method:** Isolate the first-step mutations that arise in response to nystatin stress.
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1) What is the genomic and phenotypic breadth of the first adaptive mutations?

Characterize **genomic breadth** by having a really small & well-annotated genome, luck, and magic.
1) What is the genomic and phenotypic breadth of the first adaptive mutations?

Characterize **phenotypic breadth** as tolerance to the stressor (IC$_{50}$).

![Graph showing the relationship between optical density (OD) and environment (uM nystatin).](image)
Genomic breadth – Every single line has a mutation in the ergosterol biosynthesis pathway.

Acetyl-CoA → squalene → \textit{ERG7} → lanosterol → zymosterol → \textit{ERG6} → fecosterol → episterol → \textit{ERG3, ERG5} → ergosterol
Phenotypic breadth – Tolerance is similar among different mutations within an *ERG* gene, but different among mutations in different genes.
Theme 2: Characterizing single adaptive mutations

1) What is the genomic and phenotypic breadth of the first adaptive mutations?
   - narrow genomic breadth (four genes in one pathway)
   - broad phenotypic breadth (large differences in primary environment, gene-environment interactions)
Theme 2: Characterizing single adaptive mutations

1) What is the genomic and phenotypic breadth of the first adaptive mutations?

2) Do adaptive mutations have the same effect size in haploids and homozygous diploids?

3) What is the effect of zygosity on adaptive mutations?
**Method:** Make heterozygous and homozygous diploids from haploid nystatin mutants.

- **Haploid mutant** × **Haploid mutant** = **Diploid homozygous mutant**
- **Haploid wildtype** × **Haploid mutant** = **Diploid heterozygous mutant**

\( \text{V} = \text{adaptive mutation} \)
**Method:** Make heterozygous and homozygous diploids from haploid nystatin mutants.

\[
\text{Haploid mutant} \times \text{Haploid mutant} = \text{Diploid homozygous mutant}
\]

\[
\text{Haploid wildtype} \times \text{Haploid mutant} = \text{Diploid heterozygous mutant}
\]

\[
\text{= adaptive mutation}
\]
2) Do adaptive mutations have the same effect size in haploids and homozygous diploids? 

- \( N = 2N \) 
- \( N < 2N \) 
- \( N > 2N \) 

![Graph showing the effect of nystatin on optical density in haploids and diploids](chart.png)
Nystatin adaptive mutations generally confer larger tolerance on haploids than homozygous diploids.

Chapter 5: submitted to Biology Letters
Nystatin adaptive mutations generally confer larger tolerance on haploids than homozygous diploids.
Theme 2: Characterizing single adaptive mutations

2) Do adaptive mutations have the same effect size in haploids and homozygous diploids?
   - not these mutations
Theme 2: Characterizing single adaptive mutations

1) What is the genomic and phenotypic scope of the first adaptive mutations?

2) Do adaptive mutations have the same effect size in haploids and homozygous diploids?

3) What is the effect of zygosity on adaptive mutations?
Method: Make heterozygous and homozygous diploids from haploid nystatin mutants.

Haploid mutant × Haploid mutant = Diploid homozygous mutant

Haploid wildtype × Haploid mutant = Diploid heterozygous mutant
In an unstressful environment, heterozygotes generally grow better than homozygous mutants.
In nystatin, heterozygotes grow stochastically.

[Graph showing growth patterns under different conditions]
**Method:** isolate heterozygous replicates that showed growth in nystatin, re-sequence the known mutation locus.
Heterozygotes are not staying heterozygous in nystatin.

- **Grown in YPD**:
  - (2/9/4/13)

- **Grown in YPD+2μM nystatin**:
  - (0/1/1/3)

- **Grown in YPD+4μM nystatin**:
  - (1/0/0/2)

**Frequency of zygosity post-growth**

- wildtype/wildtype
- wildtype/mutation
- mutation/mutation
Heterozygotes are not staying heterozygous in nystatin.

All lines have (previously seen) homozygous secondary mutations in \textit{ERG3} or \textit{ERG6}, indicative of between-well contamination.

- \textbf{Grown in YPD}:
  - (2/9/4/13)
- \textbf{Grown in YPD}:
  - (0/1/4*/0)
- \textbf{Grown in YPD+2} µM nystatin:
  - (0/1/1/3)
- \textbf{Grown in YPD+4} µM nystatin:
  - wildtype/wildtype: (1/0/0/2)
  - wildtype/mutation: (3/6/0/7)
  - mutation/mutation: (3/6/0/7)
Rapid loss-of-heterozygosity.

**Grown in YPD**
- Frequency of zygosity post-growth
  - Wildtype/wildtype: 0/0/1/2
  - Wildtype/mutation: 2/9/4/13
  - Mutation/mutation: 0/1/4/0

**Grown in YPD+2μM nystatin**
- Frequency of zygosity post-growth
  - Wildtype/wildtype: 0/1/1/3
  - Wildtype/mutation: 6/0/0/5
  - Mutation/mutation: 2/6/0/3

**Grown in YPD+4μM nystatin**
- Frequency of zygosity post-growth
  - Wildtype/wildtype: 1/0/0/2
  - Wildtype/mutation: 3/6/0/7
  - Mutation/mutation: 3/6/0/7
3) What is the effect of zygosity on adaptive mutations?
   - Mutations tended to be less deleterious in heterozygotes
   - Heterozygotes grew stochastically under nystatin stress
     - Rapid loss-of-heterozygosity of adaptive mutations
   - Dominance of mutations changed between environments
Conclusions

1) Haploids adapted faster than diploids to mildly stressful environments.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Rate of adaptation (/generation)</th>
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<tbody>
<tr>
<td></td>
<td>YPD</td>
</tr>
<tr>
<td></td>
<td>HCl</td>
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<tr>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
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<tr>
<td></td>
<td>Nystatin</td>
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<tr>
<td></td>
<td>NaCl</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
</tr>
</tbody>
</table>

● haploid

* diploid
Conclusions

2) The first acquired nystatin adaptive mutations have a narrow genomic breadth and broad phenotypic breadth.

- Nystatin tolerance (IC50 in µM)
- Acetyl-CoA ➔ squalene ➔ lanosterol ➔ zymosterol ➔ fecosterol ➔ episterol ➔ ergosterol

- ERG7
  - 1 nonsynonymous SNP
  - 3 premature stop codons
  - 1 small deletion

- ERG6
  - 3 nonsynonymous SNPs
  - 6 premature stop codons
  - 1 small deletion

- ERG3
  - 1 duplication

- ERG5
  - 3 nonsynonymous SNPs
  - 6 premature stop codons
  - 1 small deletion

- Acetyl-CoA ➔ squalene ➔ lanosterol ➔ zymosterol ➔ fecosterol ➔ episterol ➔ ergosterol

Graph showing Nystatin tolerance (IC50 in µM) for different strains: wt, erg7, erg6, erg3, erg5.
Conclusions

3) The first acquired nystatin adaptive mutations confer a larger tolerance to haploids than homozygous diploids.
Conclusions

4) Heterozygotes grow better than homozygous mutants in YPD
Conclusions

4) Heterozygotes grow better than homozygous mutants in YPD, yet must become homozygous to grow under nystatin stress.
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