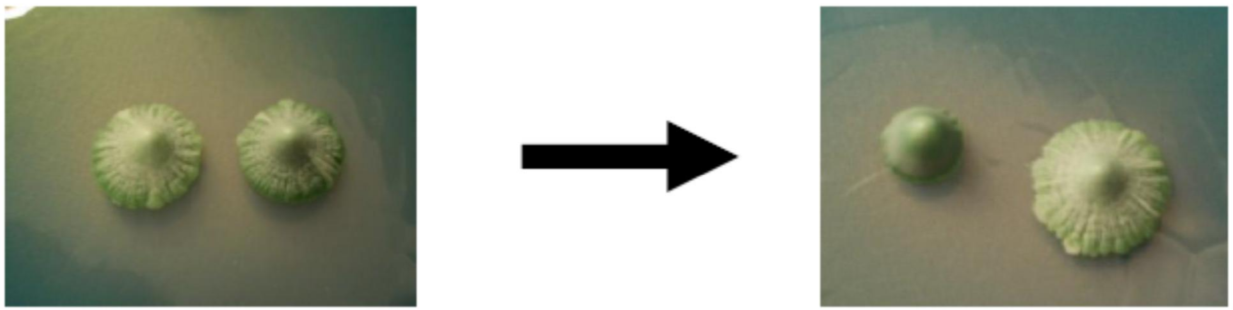
Serial Repitching and Genetic Variation In Brewing Yeast Populations



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Overview

- Origins of brewing yeast cultures
- The yeast genome and fermentation performance
  - The role of yeast
  - Using the 'correct' yeast
- Handling of brewing yeast slurries
  - Use of brewing cultures
  - Drivers for variation/selection
- Genome stability, selection of populations and genetic change in brewing cultures

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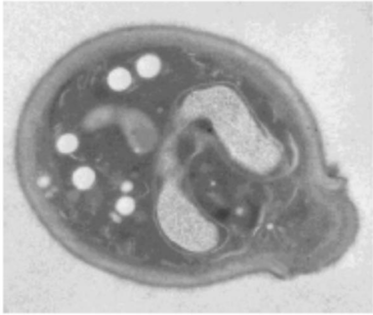
Genetic origins of brewing yeast strains

- Brewing yeast strains selected over many years for desirable fermentation characteristics
- Ale strains are diverse
  - Evolved since Egyptian times from potentially multiple sources
  - Polyploid - *Saccharomyces cerevisiae*
- Lager strains more closely related
  - Originate from 1 or 2 sources
    - Saaz/Frohberg (Dunn and Sherlock, 2008)
  - Aneuploid - *Saccharomyces pastorianus*

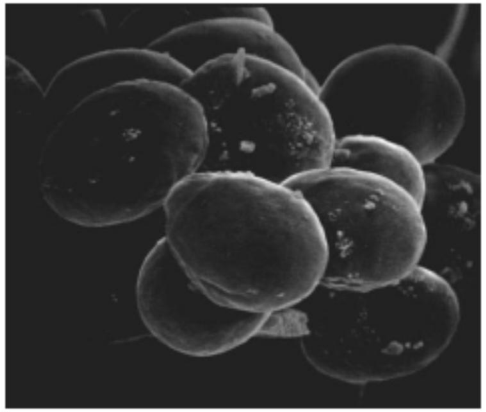


The significance of the yeast genome to fermentation

- Genetic make-up of yeast determines
  - Interaction between yeast and environment
  - Fermentation characteristics
  - Fermentation rate
  - Alcohol production
- Strain dependent flavors
  - Metabolism
  - Uptake of nutrients/nutritional requirements
  - Specific genes (e.g. POF+ yeast)
- It is important to utilize a good yeast strain

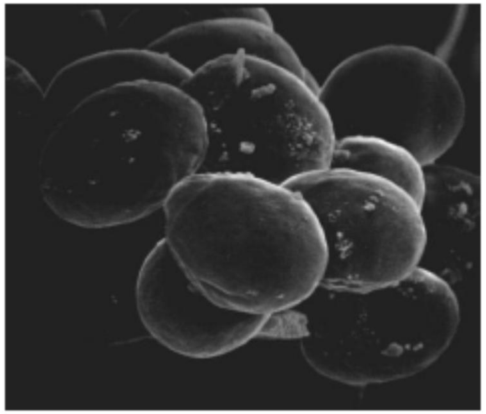
What is a ‘good’ yeast strain

- A good brewers yeast strain should
  - Utilize sugars quickly and efficiently
  - Consistently yield a product with typical and desired characteristics
    - Specific flavors
    - Alcohol yield
  - Flocculate adequately
  - Stress resistant



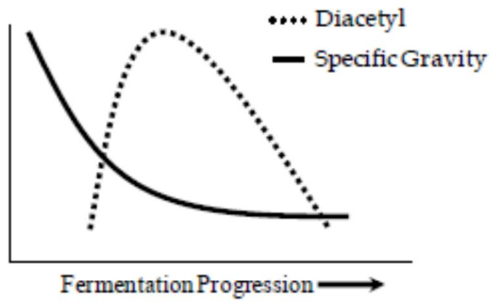
What is a ‘good’ yeast strain

- A good brewers yeast strain should
  - Utilize sugars quickly and efficiently
  - Consistently yield a product with typical and desired characteristics
    - Specific flavors
    - Alcohol yield
  - Flocculate adequately
  - Stress resistant
  - **Genetically stable**



Types of brewing yeast mutation

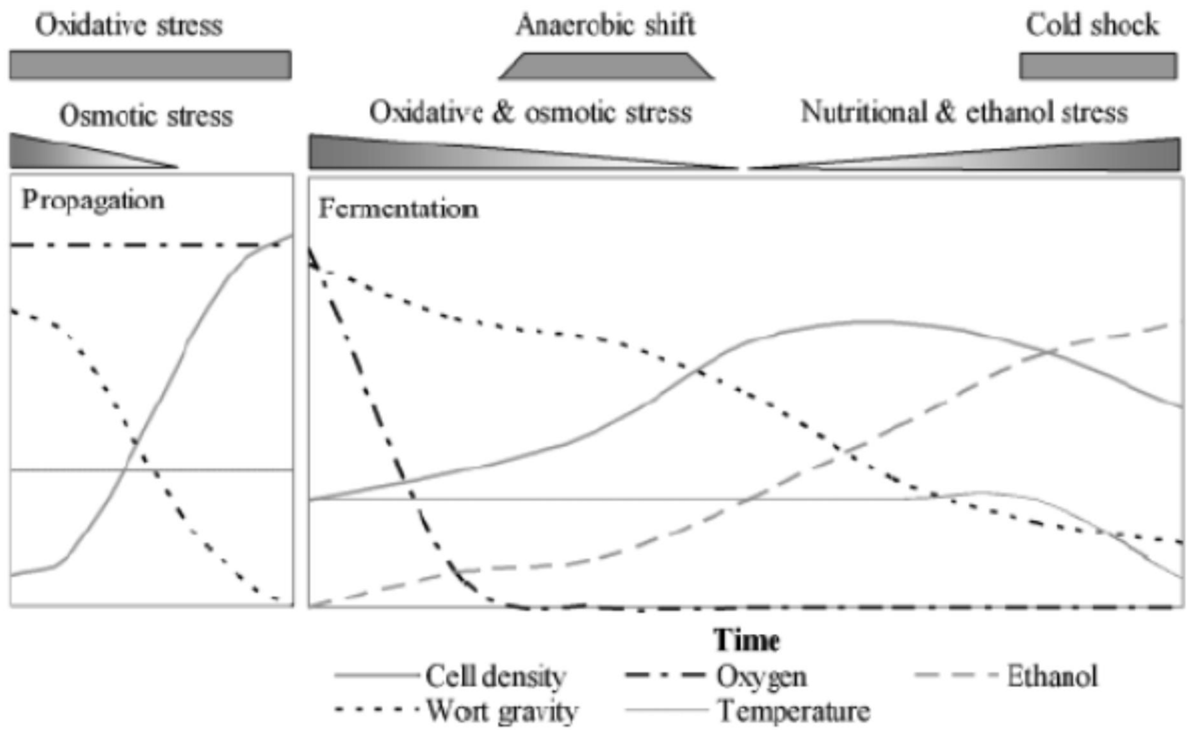
- Strain diversity has been encouraged within the brewing industry
  - However, genetic stability is an increasingly desirable quality
- Mutations can arise during the process
  - Nuclear and/or mitochondrial
- Variety of effects
  - Changes to flocculation
  - Altered sugar uptake rates
    - Loss of ability to ferment maltotriose
  - Inappropriate flavor production (diacetyl reduction)



Why do yeast mutations occur ?

Stress	References
Random changes during division	Many references
Ethanol	Bandas and Zakharov, 1980; Jimenez and Benitez, 1997; Chi and Arneborg, 1999; Gasent-Ramirez, 1999
Hydrostatic pressure	Rosin and Zimmerman, 1977a; 1977b
Temperature stress	Jenkins *et al*, 2001; 2003
Starvation/nutrient limitation	Adams *et al*, 1985; 1982

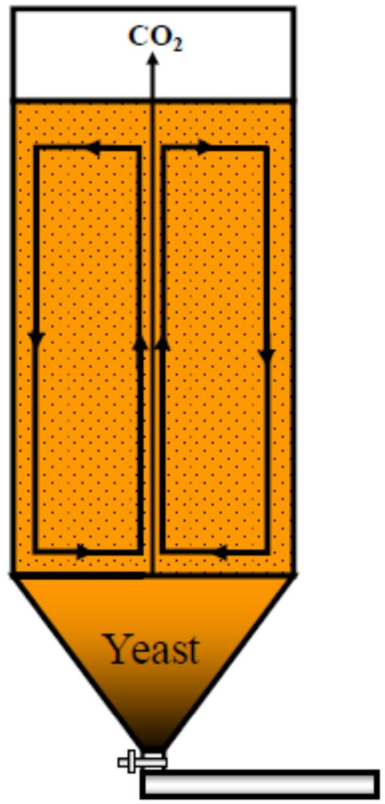
Stresses associated with brewery fermentations



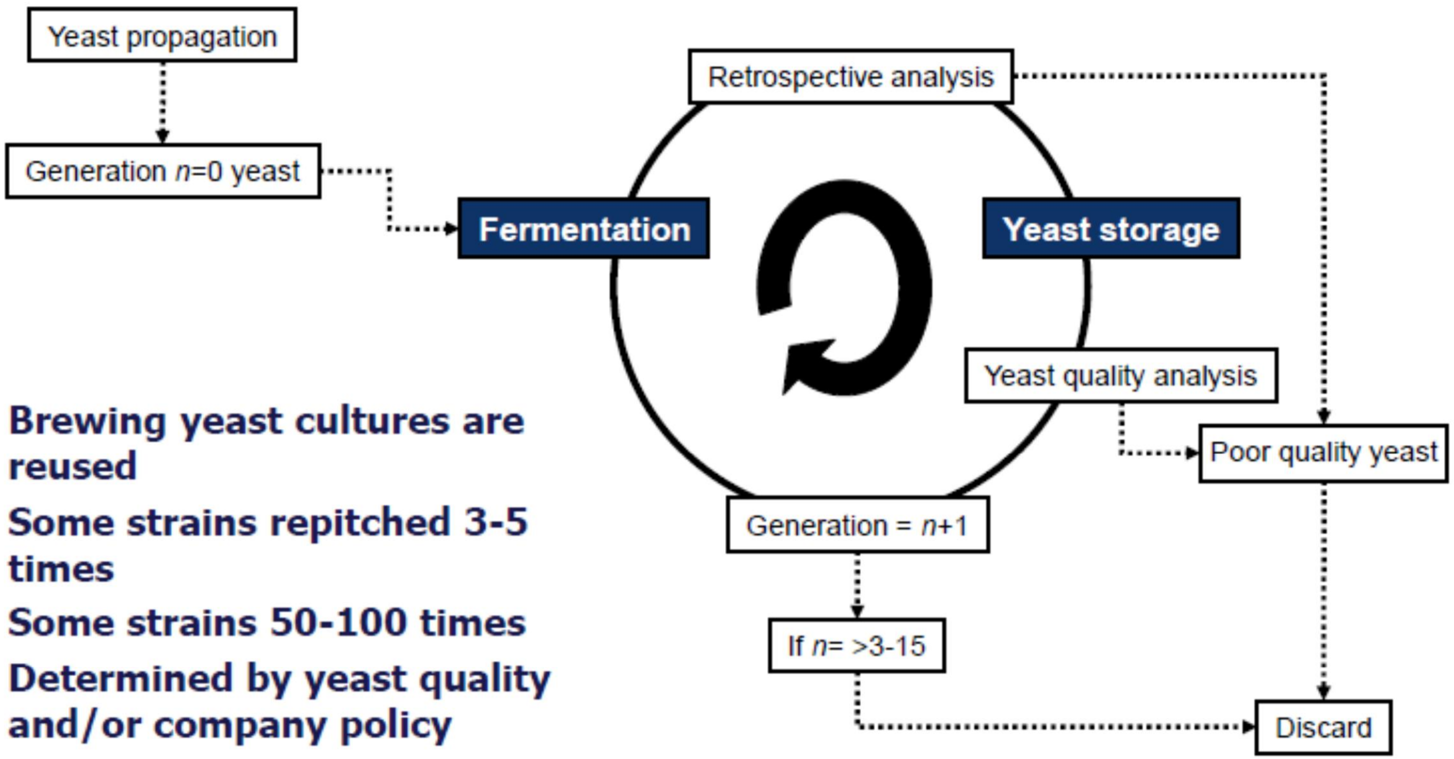
Gibson *et al*, 2007

Yeast fermentation

- During fermentation the yeast population divides ~3 times
- CO_2 production maintains yeast in suspension
- Towards end fermentation CO_2 production slows, vessel is chilled and yeast collects in the cone
- Yeast remains in the cone until fermentation is complete
  - Sugar utilization
  - Diacetyl reduction



Brewery yeast life cycle – Serial repitching of cultures



- **Brewing yeast cultures are reused**
- **Some strains repitched 3-5 times**
- **Some strains 50-100 times**
- **Determined by yeast quality and/or company policy**

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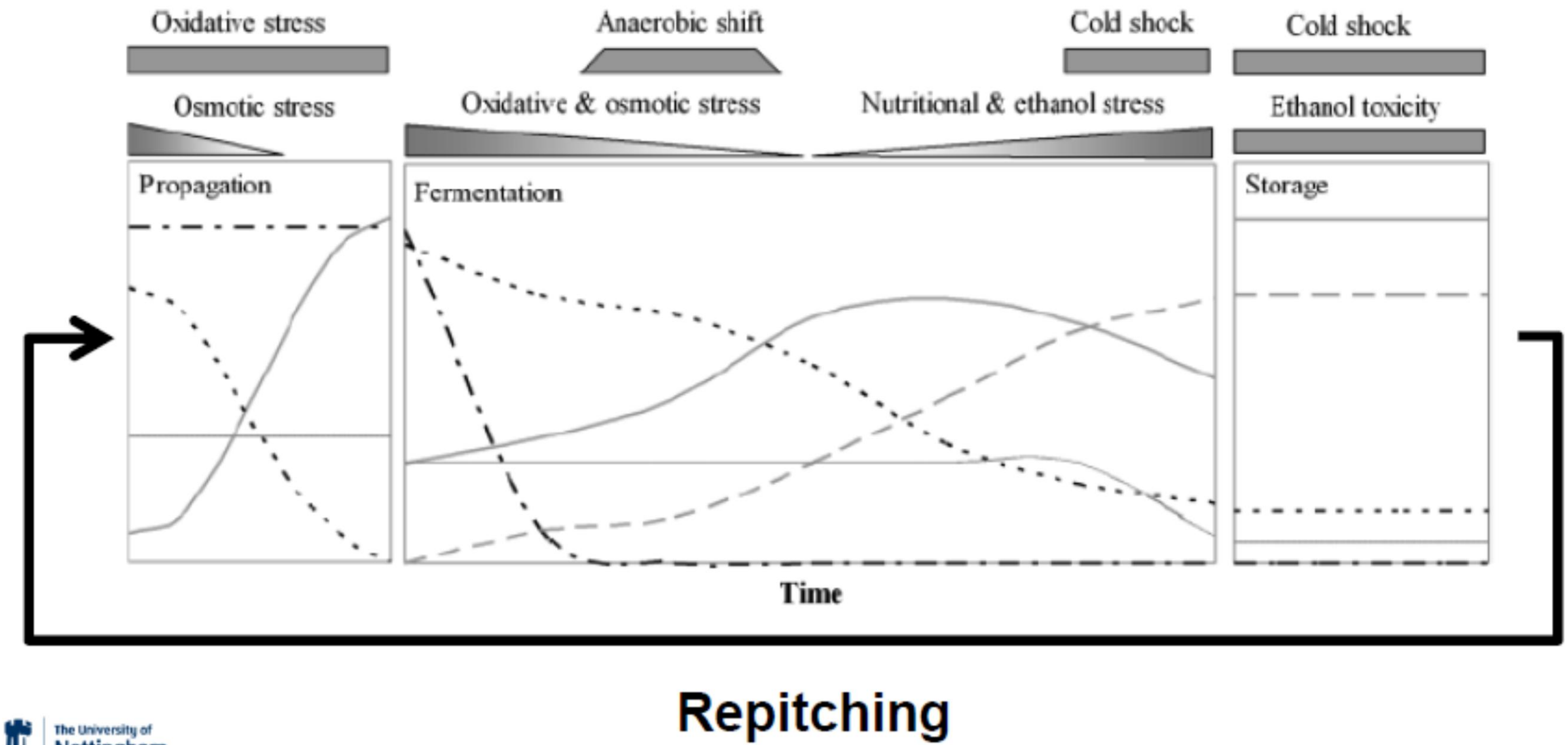
Stresses associated with brewery fermentations



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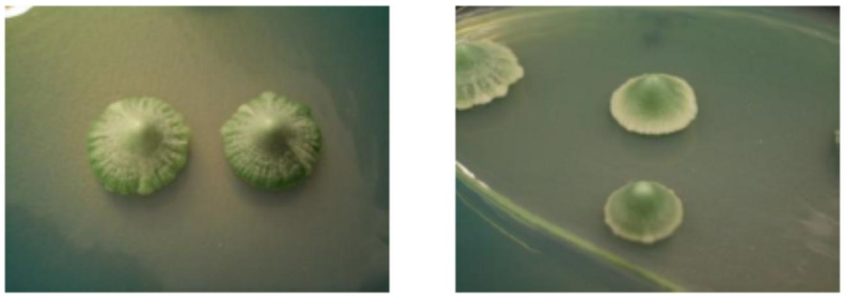
Significance to yeast handling

- Brewers yeast is unique as it is recycled
  - Number of repitchings varies from strain/strain product/product
- What is the optimum number of repitchings ?
  - Why can some strains be recycled more often than others ?
  - Do certain strains undergo genetic change more rapidly than others ?

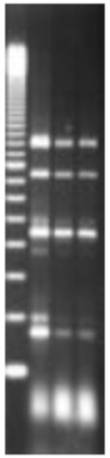


Yeast mutation detection by giant colony analysis

What are the effects of serial repitching on brewing yeast cultures

- Serial repitching can influence yeast viability and vitality
  - Such changes may not be permanent
- Genetic changes in asexual populations are usually irreversible
  - Muller's ratchet
- Formation of genetic variants
  - Most genetic change yields inferior cells which are outcompeted
  - Some mutations can lead to new individuals which can compete or co-exist within the population

Serial Repitching and Yeast Mutation



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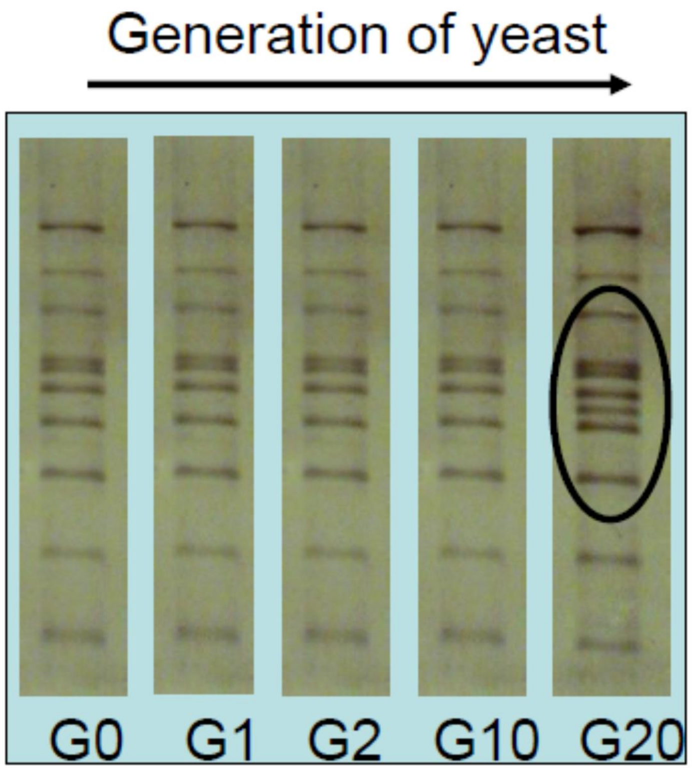
Methods for identifying mutants

- Analysis of fermentation characteristics
  - Physiological tests
  - Phenotype assessment
- Large scale changes to DNA
  - Chromosome analysis by karyotyping
  - Identification of CLP's (Chromosome Length Polymorphisms)
- Small changes to DNA
  - Analysis of location/frequency of transposons by RFLP
  - Characterisation of specific or repeated sequences
    - Inter-delta sequences, Microsatellites/minisatellites
  - Analysis of mtDNA by RFLP

Identifying mutants during repitching of an ale yeast

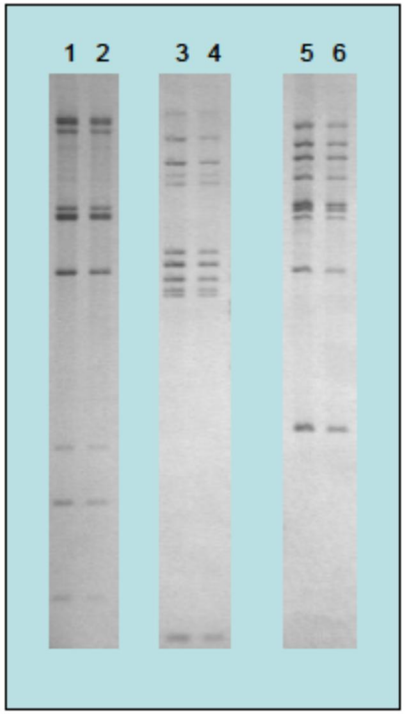
- Regularly noticed 'strange' fermentation patterns in 'older' yeast cultures
- Altered flocculation characteristics
  - One of the most common mutations observed in brewers yeast slurries
- Altered Ty RFLP profile was observed



Analysis of yeast transposon 1 by RFLP

Analysis of change during extended repitching of an ale yeast

- Yeast serially repitched more than 100 times compared to original stocks
  - No change in fermentation performance/flocculation



Analysis of yeast transposon 1 using 3 different restriction enzymes

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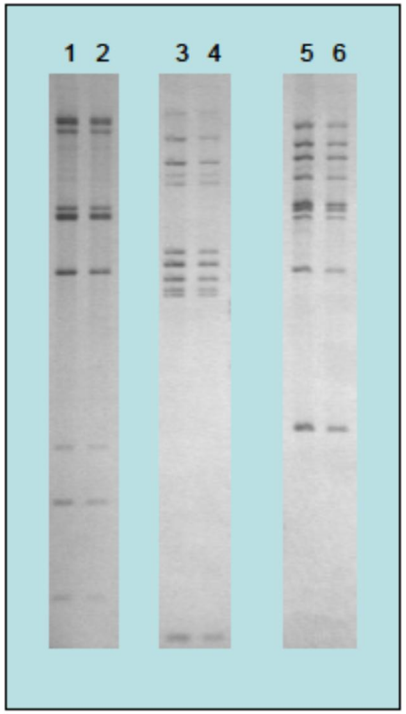
Analysis of change during extended repitching of an ale yeast

- Yeast serially repitched more than 100 times compared to original stocks
  - No change in fermentation performance/flocculation
  - No change to yeast transposons
  - No change in inter-delta sequences
  - No change in karyotype
- Some yeast strains are particularly stable over time

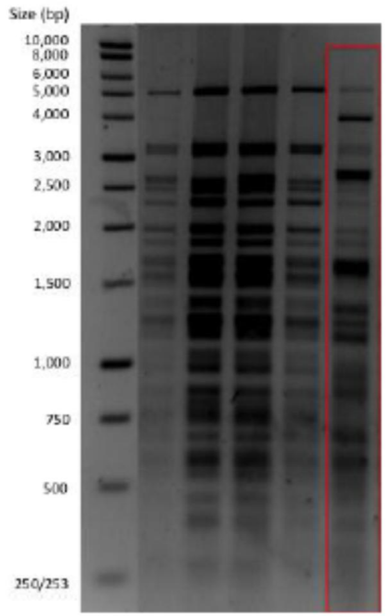


Analysis of yeast transposon 1 using 3 different restriction enzymes

Changes to mtDNA during lager yeast recycling

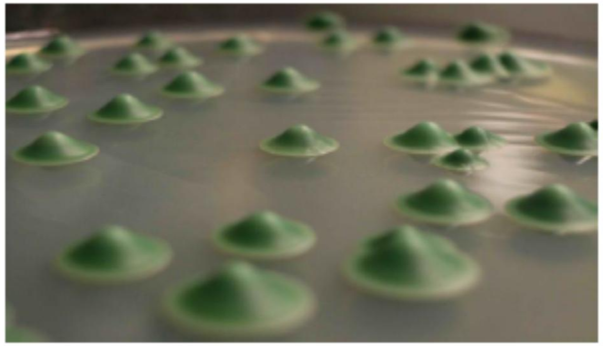
- Brewing yeast strains serially repitched for 20 generations
  - Artificial wort environment with ethanol (5%)
- Analyzed for genomic and mtDNA integrity
- One strain exhibited a significant number of petites and mtDNA change after 10-15 generations

Petites	Strain 1	Strain 2	Strain 3	Strain 4
G0	<0.1%	<0.1%	<0.1%	<0.1%
G5	<0.1%	<0.1%	1%	<0.1%
G10	<0.1%	<0.1%	<0.1%	<0.1%
G15	7.2%	<0.1%	<0.1%	<0.1%
G20	34.8%	<0.1%	2.4%	0.7%

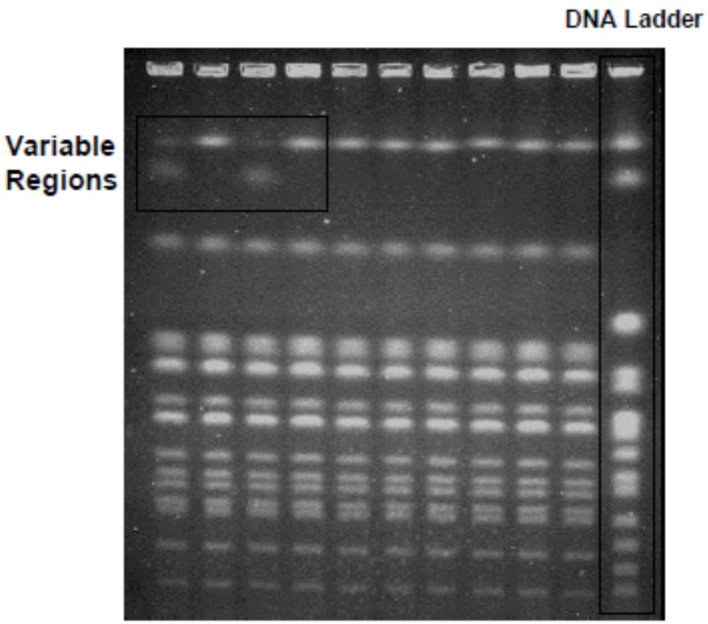


Strain 1 mtDNA RFLP

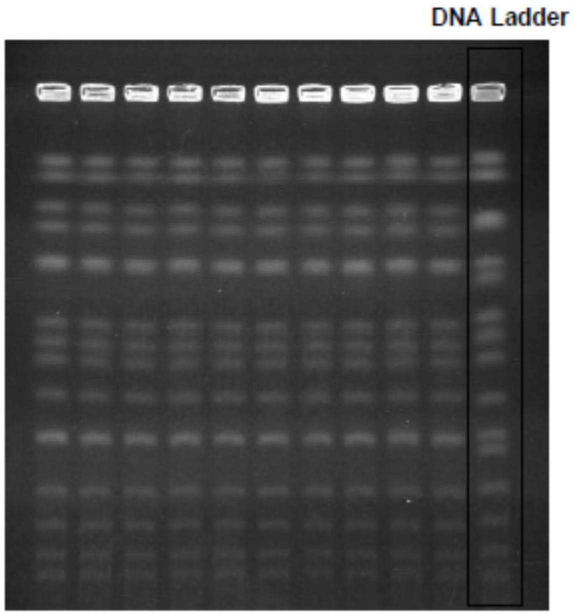
Simple Analysis of Yeast Innate Genetic Stability



Genetic stability trait analysis by karyotyping



10 colonies of an *S. cerevisiae* yeast strain showing genetic variability in chromosome profiles (CLP's)



10 colonies of an *S. cerevisiae* yeast strain showing identical chromosome profiles

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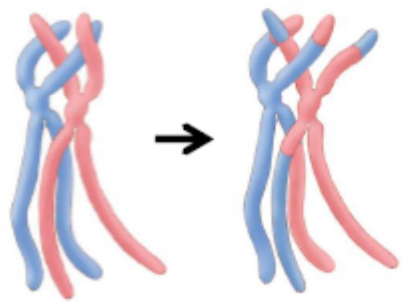
Powell and Van Zandycke 2009

Chromosome Rearrangements

- Genome 'Hot Spots'
  - DNA repeat sequences such as Ty elements and mini-satellites may act as sites of chromosomal crossover
  - In wine yeast variants, >100 genomic regions have been identified which induce CLP's **(Infante *et al*, 2003)**
  - Recombination sites common to both wine and beer strains have been identified **(Bond *et al*, 2004)**
- There is still much unknown about brewing yeast hot spots
  - Possibly linked to flocculation genes
  - Other genes associated with fermentation
    - Sugar transport genes – HXT6/7

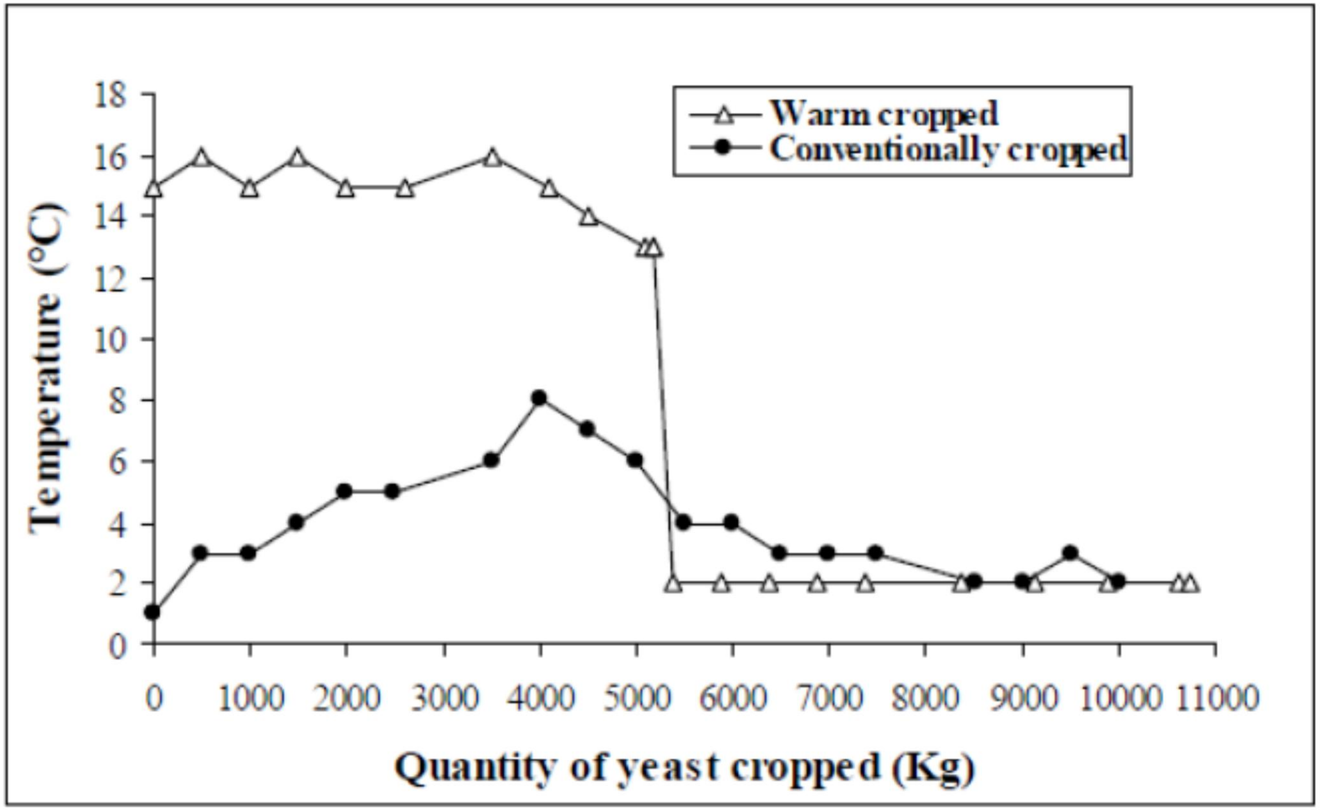


Why do Changes Arise and Accumulate During Serial Repitching?

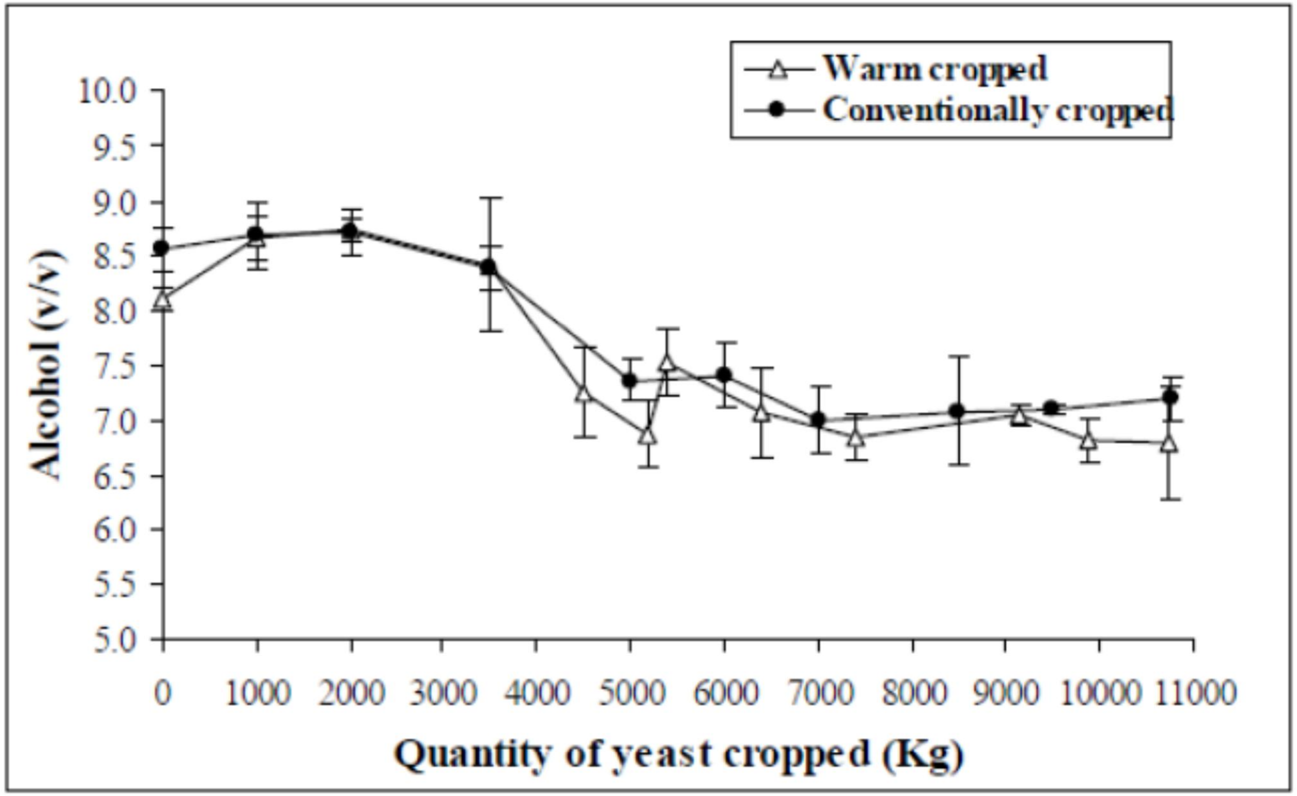
The 'cone environment'

- Towards the end of the fermentation yeast settles into the cone of the vessel
  - Time at which yeast separates is strain dependent
  - Can occur relatively early during fermentation
  - Cone is typically cooled to ~4°C
  - Yeast in the cone is considered to be 'dormant'
  - Conditions within the cone are considered to be stable
- What are the environmental conditions within the cone ?

Temperature in the cone



Alcohol in the cone



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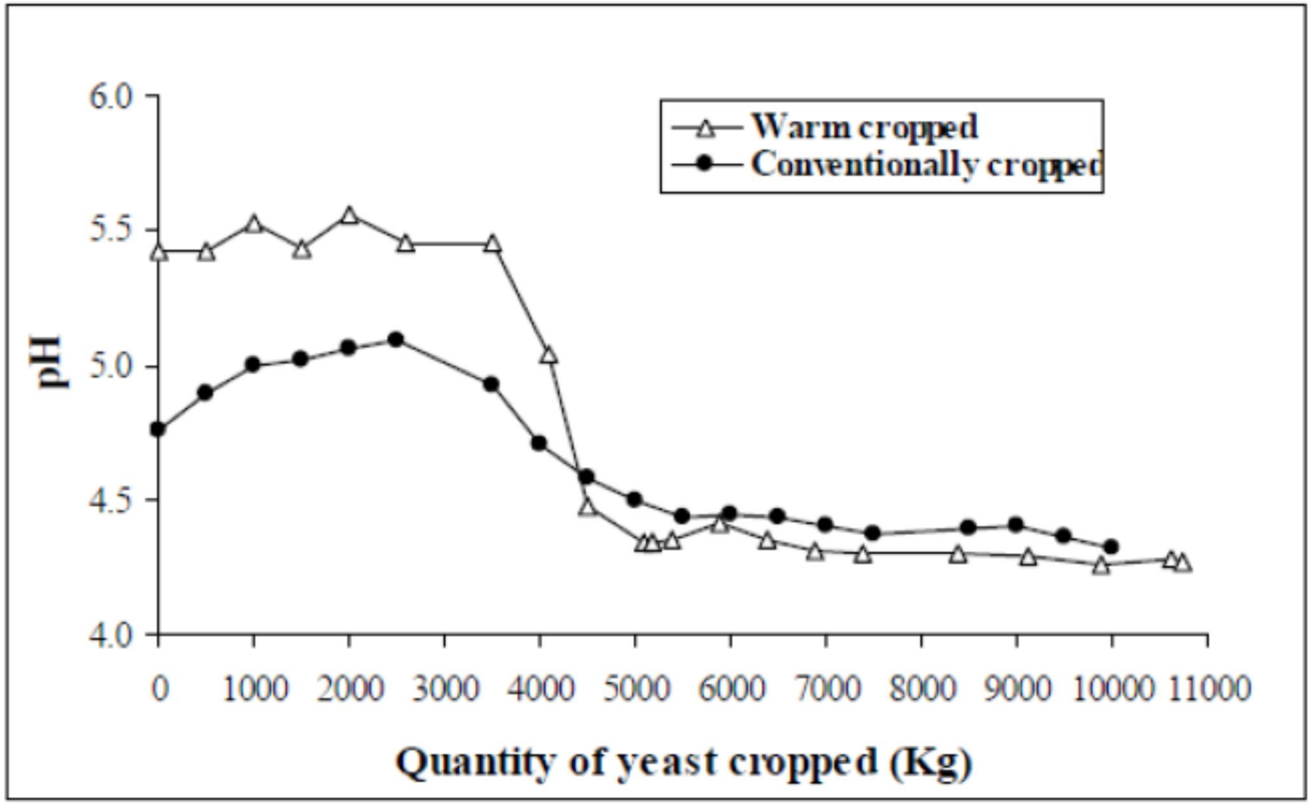
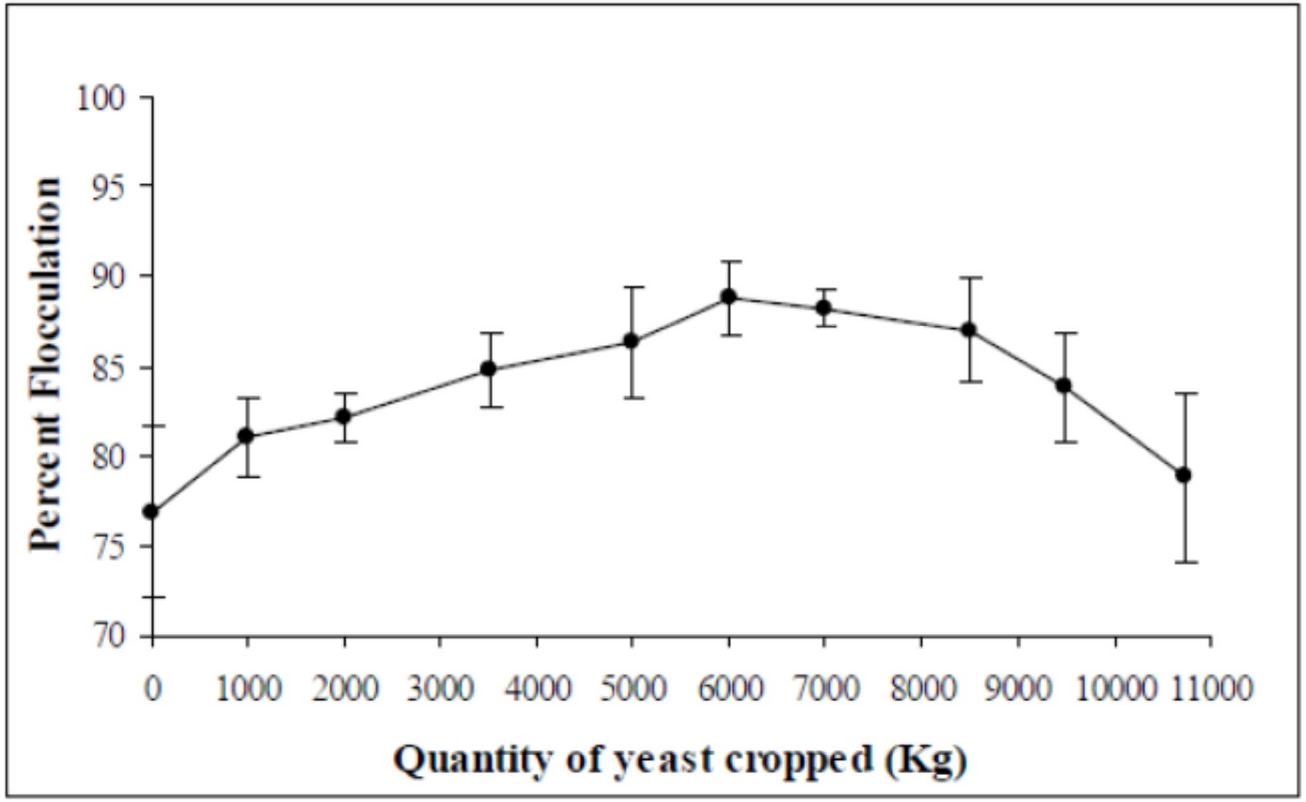
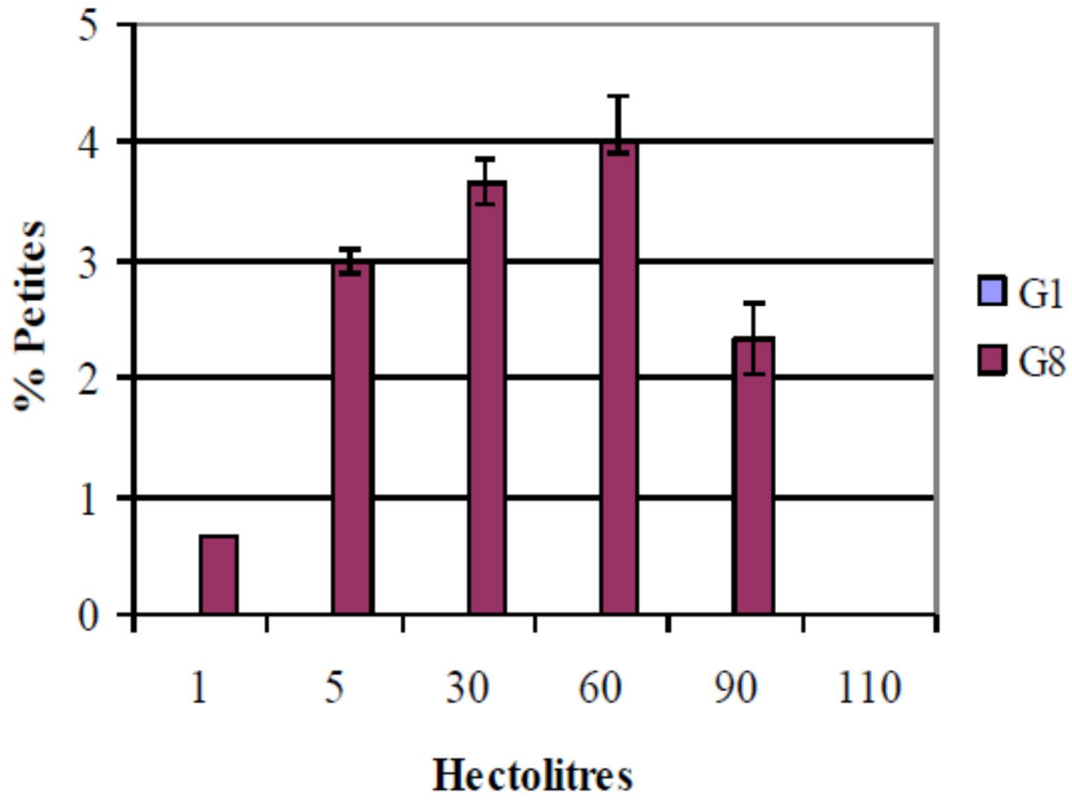
pH in the cone

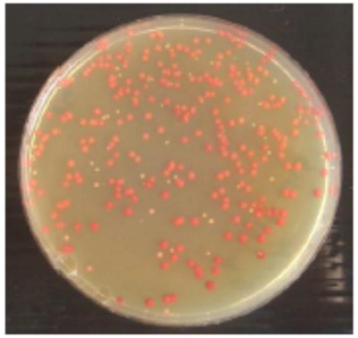


Yeast flocculation potential



Petite cells in the cone





Detection of petite colonies

Jenkins *et al*, 2009

The 'cone environment'

- The cone is not a constant environment
  - Alcohol
  - pH
  - Temperature
- Yeast within the cone is not homogenous
  - Flocculation properties
  - Yeast viability
  - Yeast size
  - Petites

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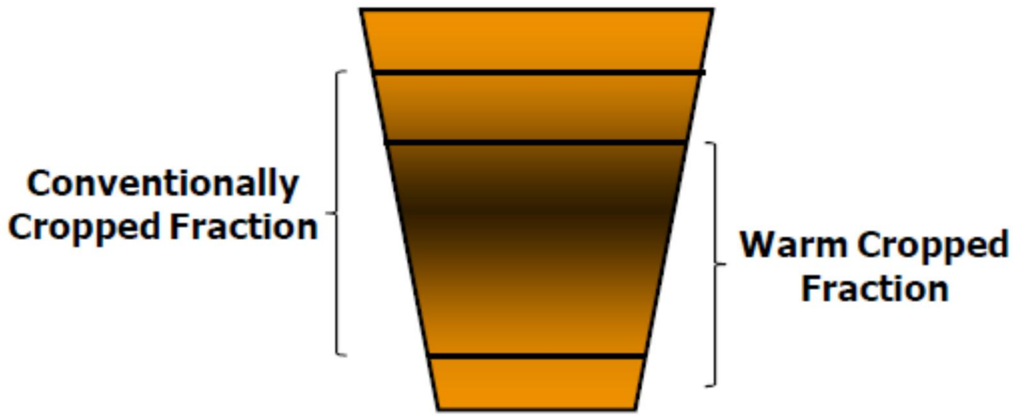
The significance of cone variation

- Yeast recovery methods may favor selection
  - Increased use of early or 'warm cropping'
- Continual selection of individuals could potentially lead to genetic drift
  - Populations with altered fermentation properties



Conclusions

- Genetic integrity is essential for a yeast culture to function consistently
- Yeast stress during fermentation and yeast handling may contribute towards genetic change
  - Stress resistance properties of the strain may be linked to the frequency of mutation
- Mutation accumulation rate varies between brewing strains
  - May be dependent on intrinsic genetic stability as well as process conditions
- The environment and population dynamics within the vessel cone are not homogenous
  - Selection during cropping may provide a vehicle for genetic change

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Miller Coors US

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Thank You For Your Attention