



# **Gene dosage influences the functional attributes of *de novo* lager yeast hybrids**

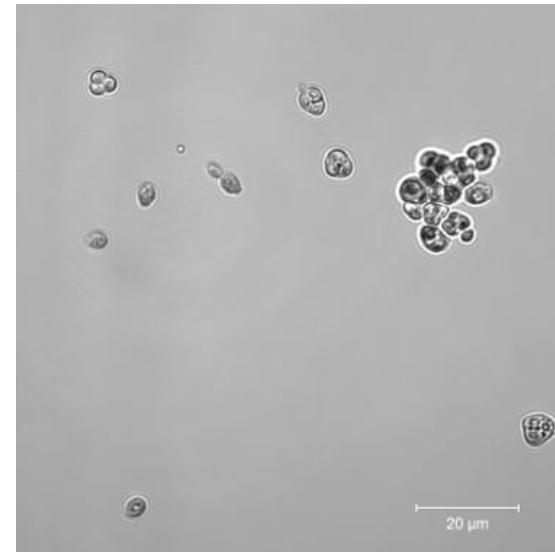
The 5th International Young Scientists Symposium on  
Malting, Brewing and Distilling

April 21-23, 2016 – Chico, California, USA

Kristoffer Krogerus

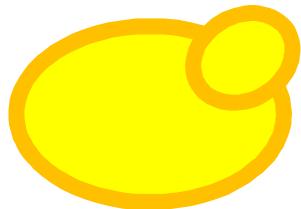
## Background

- Pale lager is the most popular beer style worldwide
- Cold fermentation and clean flavour profile
- Made with lager yeast: *Saccharomyces pastorianus*

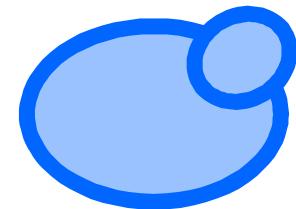


## Background

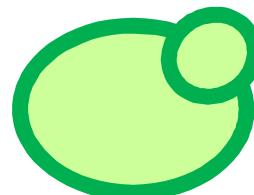
- Lager yeast, *S. pastorianus*, is a natural hybrid between *S. cerevisiae* and *S. eubayanus*:



*Saccharomyces cerevisiae*  
(cold-sensitive, good fermentation)



*Saccharomyces eubayanus*  
(cold-tolerant, poor fermentation)



*Saccharomyces pastorianus*  
(Good fermentation performance at low temperature, lager brewing)

# Background

- Poor diversity among the traditional lager yeasts
- Belong to one of two distinct lineages:
  - Saaz
  - Frohberg



Group 1  
(Saaz)

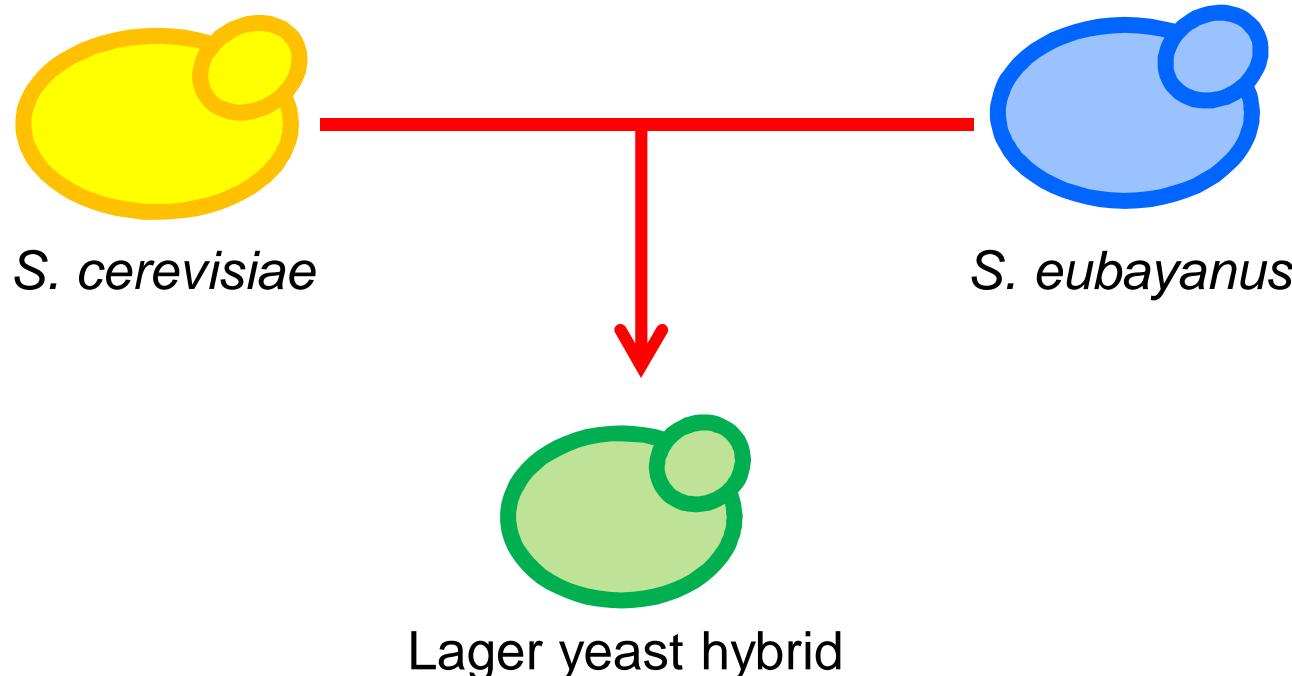


Group 2  
(Frohberg)

- |   |   |
|---|---|
| <ul style="list-style-type: none"><li>■ Weak fermentation</li><li>■ Very cold-tolerant</li><li>■ <i>S. eubayanus</i> genome dominates</li></ul> | <ul style="list-style-type: none"><li>■ Strong fermentation</li><li>■ Cold-tolerant</li><li>■ <i>S. cerevisiae</i> genome dominates</li></ul> |
|---|---|

## Background

- Can we increase the diversity of lager yeast by creating new lager yeast hybrids?
  - Mating *S. eubayanus* with selected *S. cerevisiae* parents?



# Our previous research

- *De novo* lager yeast hybrids can
  - outperform their parent strains during fermentation
  - produce beer with higher concentrations of aroma compounds

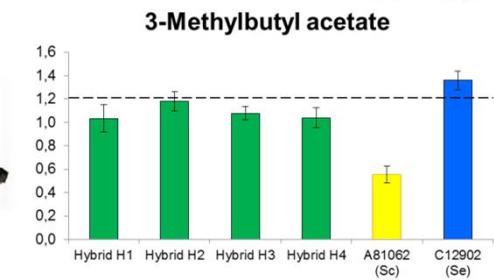
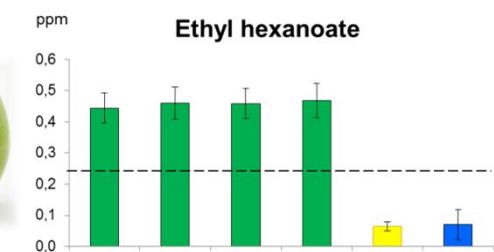
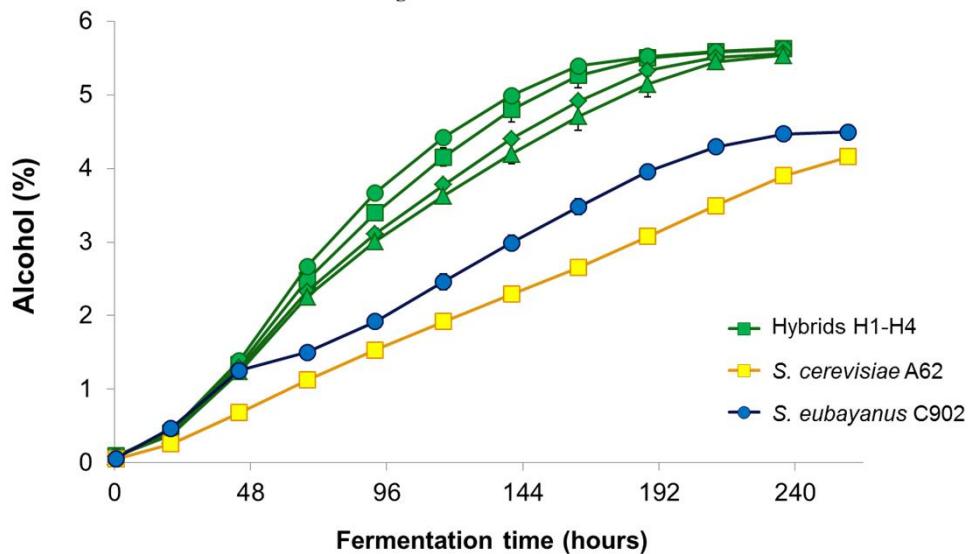
J Ind Microbiol Biotechnol (2015) 42:769–778  
 DOI 10.1007/s10295-015-1597-6

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## New lager yeast strains generated by interspecific hybridization

Kristoffer Krogerus · Frederico Magalhães ·  
 Virve Vidgren · Brian Gibson



# Controlling the properties of the hybrids

- How does the contribution of the parental subgenomes affect important phenotypic traits?
  - Fermentation performance
  - Aroma production
  - Stress tolerance



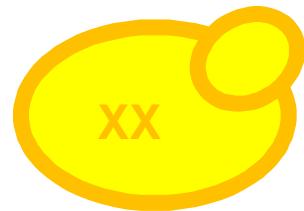
Group 1  
(Saaz)



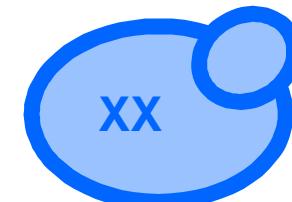
Group 2  
(Frohberg)

- |   |   |
|---|---|
| <ul style="list-style-type: none"><li>▪ Weak fermentation</li><li>▪ Very cold-tolerant</li><li>▪ <i>S. eubayanus</i> genome dominates</li><li>▪ <b>Allotriploid</b></li></ul> | <ul style="list-style-type: none"><li>▪ Strong fermentation</li><li>▪ Cold-tolerant</li><li>▪ <i>S. cerevisiae</i> genome dominates</li><li>▪ <b>Allotetraploid</b></li></ul> |
|---|---|

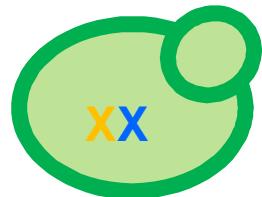
## Controlling the properties of the hybrids



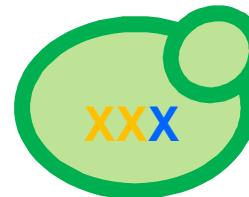
*S. cerevisiae* A62



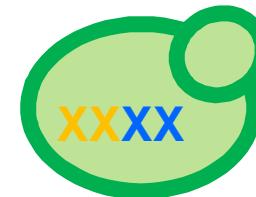
*S. eubayanus* C902



Hybrid A2  
(allo diploid)



Hybrid B3  
(allo triploid)



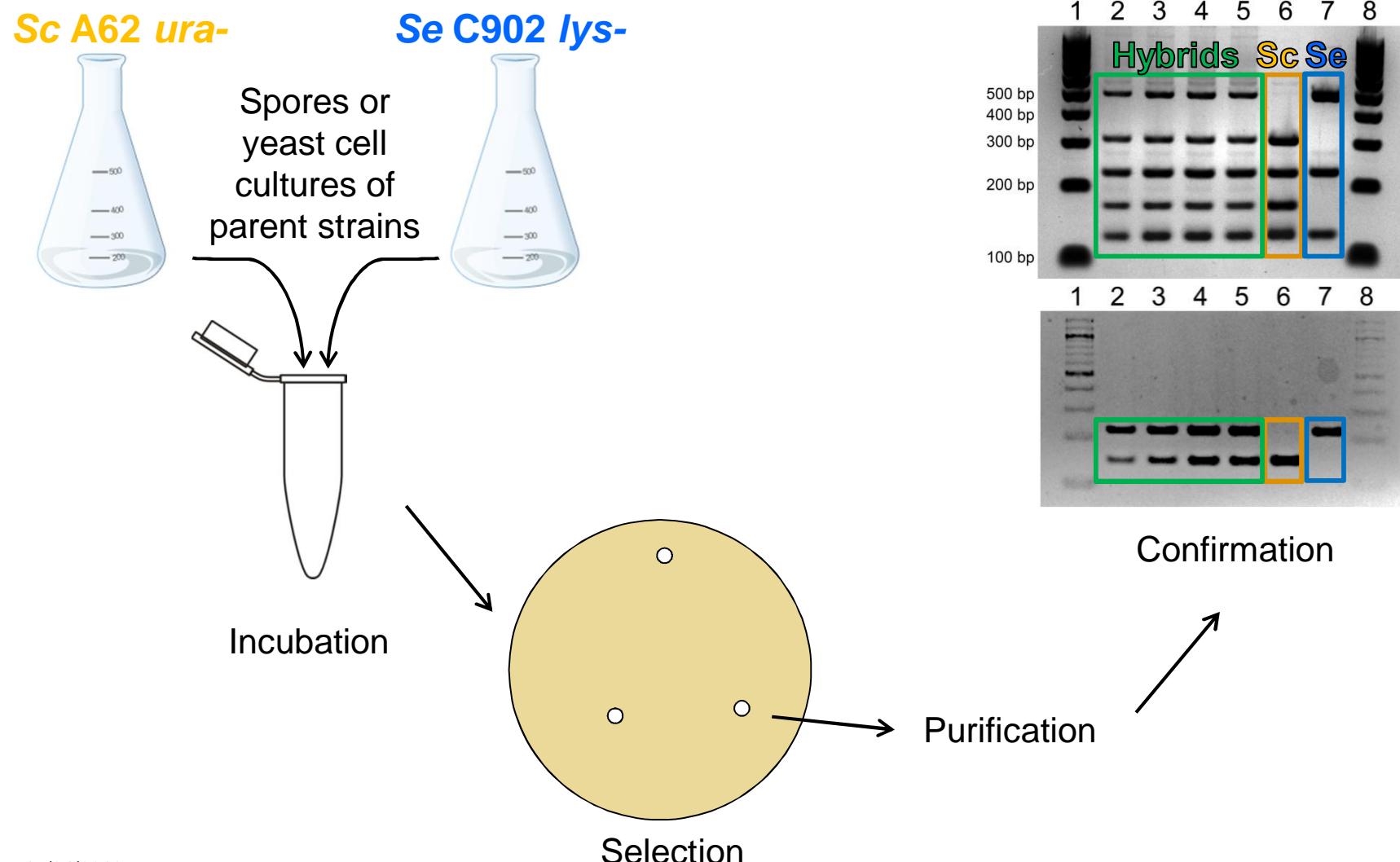
Hybrid C4  
(allo tetraploid)

## Aims

- Compare the performance of these hybrids in 2-litre fermentations using **15** and **25 °P** wort.
- How does the DNA content of the lager yeast hybrids affect
  - **fermentation performance**
  - **aroma production**
  - **resistance to harsh conditions of high gravity wort**
- Elucidate the relationship between **gene expression** and **aroma formation** in the strains.



# Creation



# Ploidy estimation

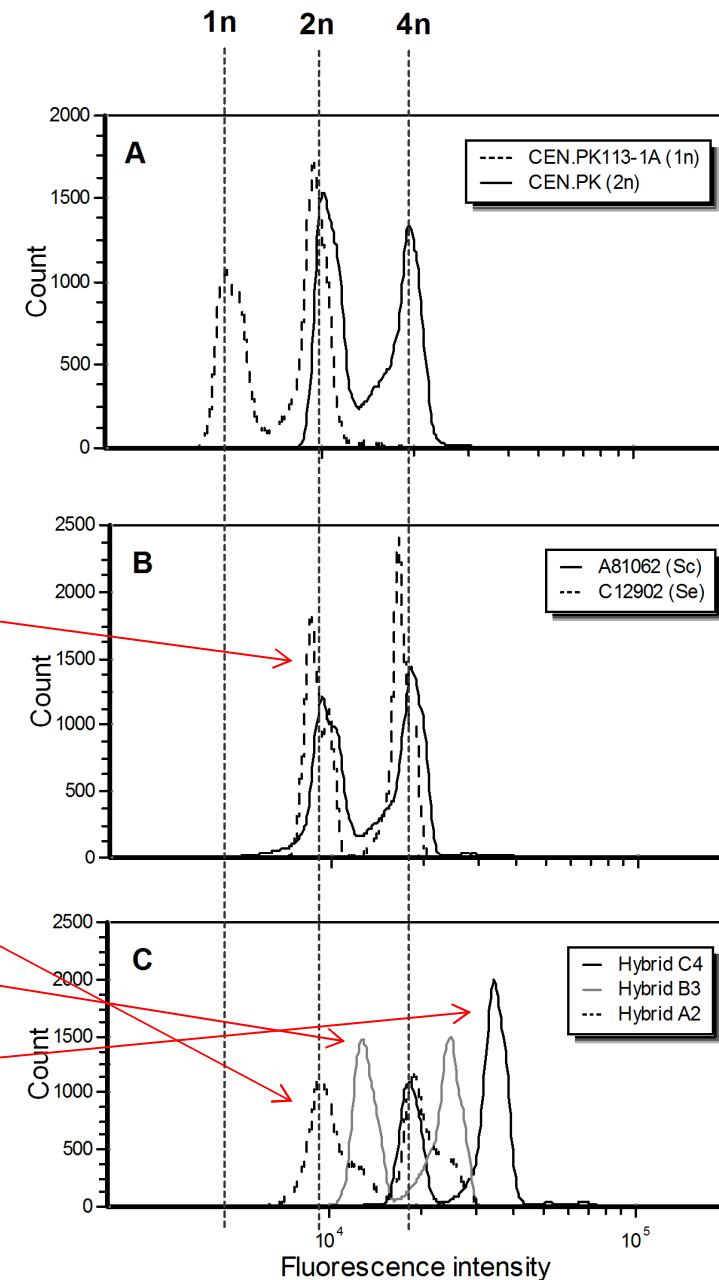
- The DNA content of the hybrids were estimated with flow cytometry

- Parent strains: diploid

- Hybrid A2: diploid

- Hybrid B3: triploid

- Hybrid C4: tetraploid



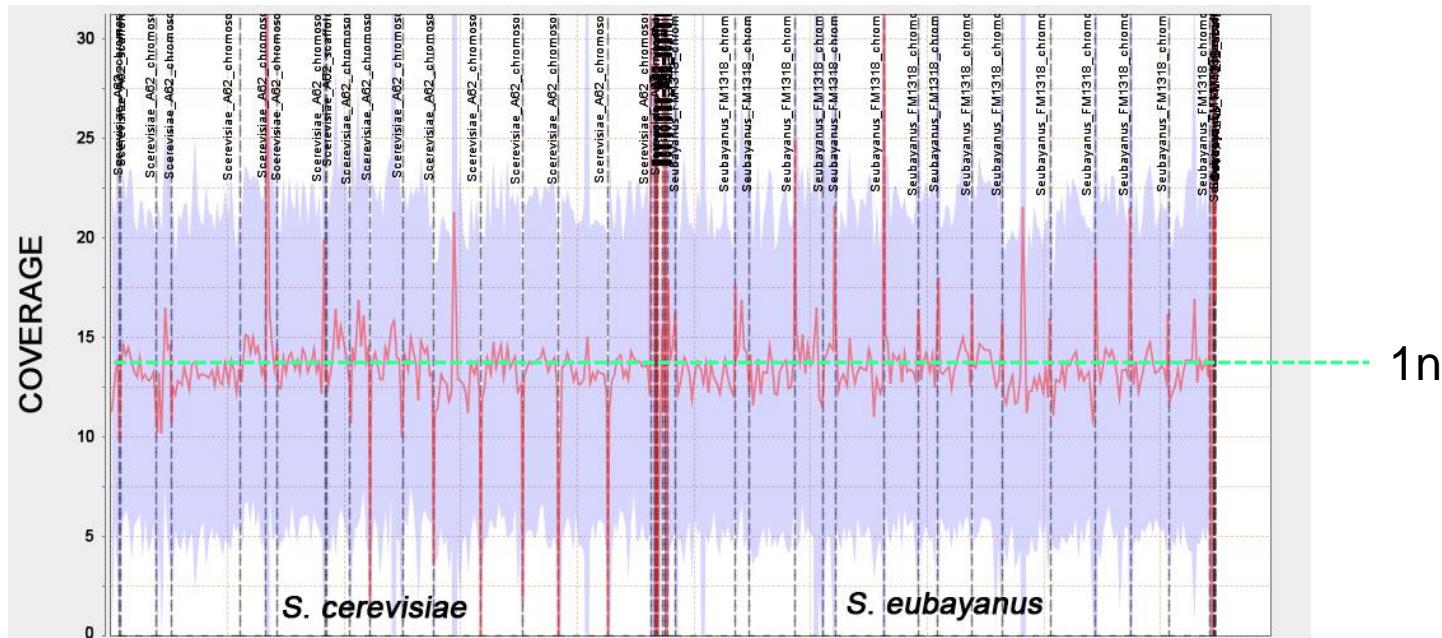
# Sequencing

- Each hybrid strain was analysed with Illumina pair-end DNA sequencing
- These were compared to the parent genomes
  - Sc A62 sequence was obtained through the hybrid assembly of Illumina and PacBio sequencing data
  - Se C902 sequence from previous studies (Baker et al. 2015)

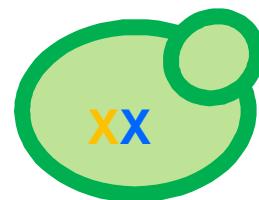


ATGTGACTTACCCATGTAGGCATAGCATGGATCATTAGCGCATGGGCATACGA

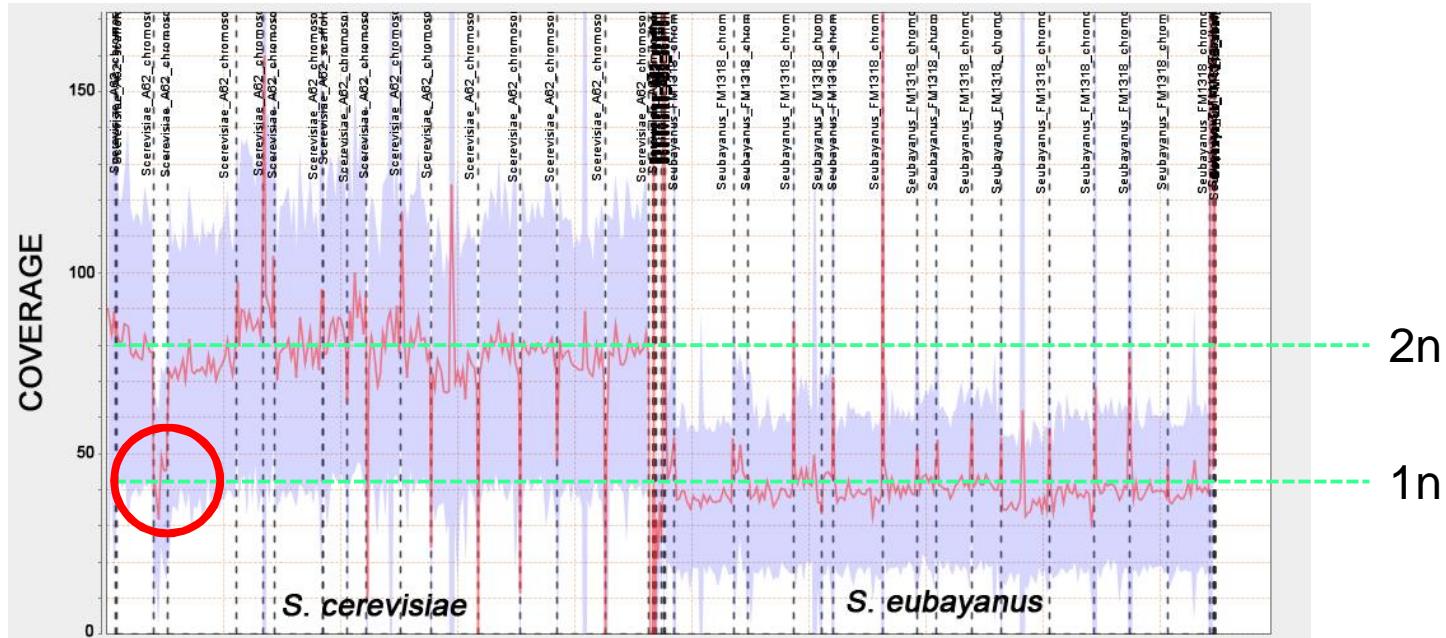
# Sequencing – allo diploid Hybrid A2



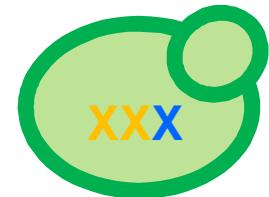
- 1 copy of each chromosome from both subgenomes



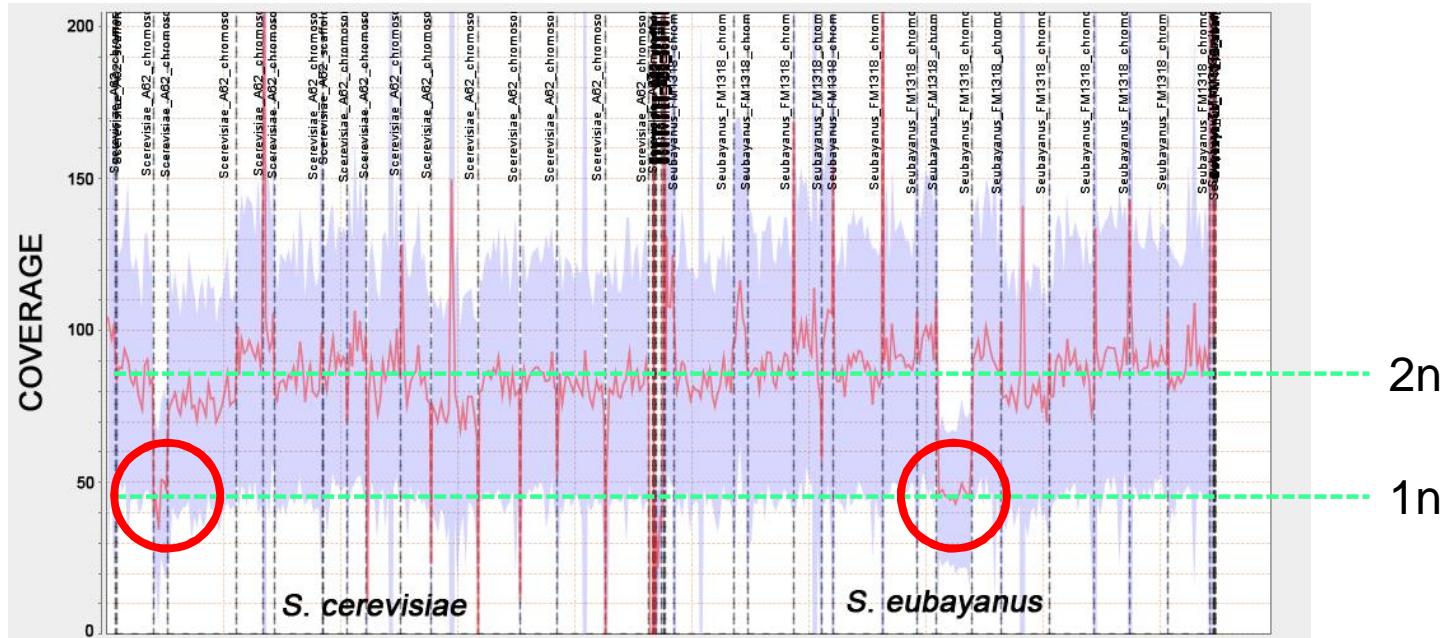
# Sequencing – allotriploid Hybrid B3



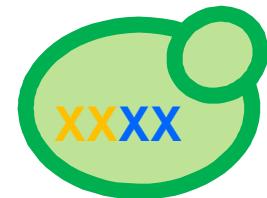
- 2 copies of each chromosome from *S. cerevisiae*
  - Exception in chromosome III (1 copy)
- 1 copy of each chromosome from *S. eubayanus*



# Sequencing – allotetraploid Hybrid C4

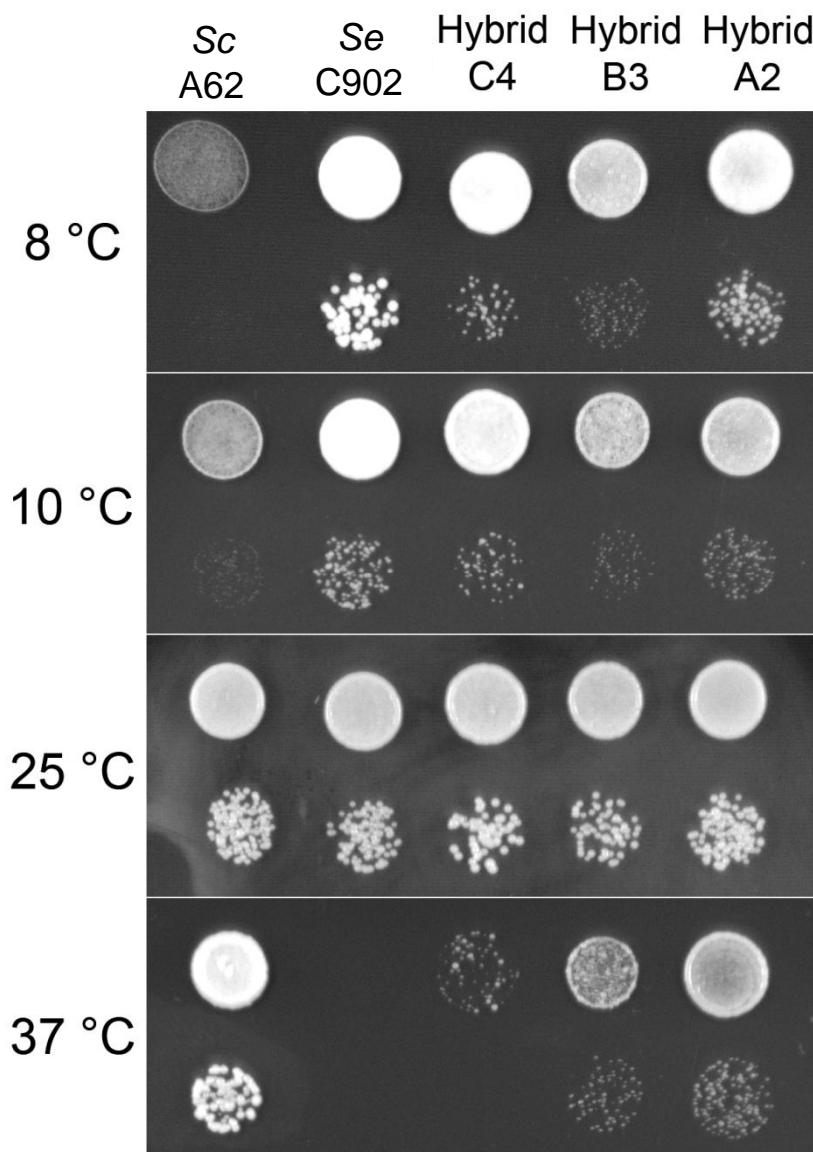


- 2 copies of each chromosome from *S. cerevisiae*
    - Exception in chromosome III (1 copy)
  - 2 copies of each chromosome from *S. eubayanus*
    - Exception in chromosome X (1 copy)

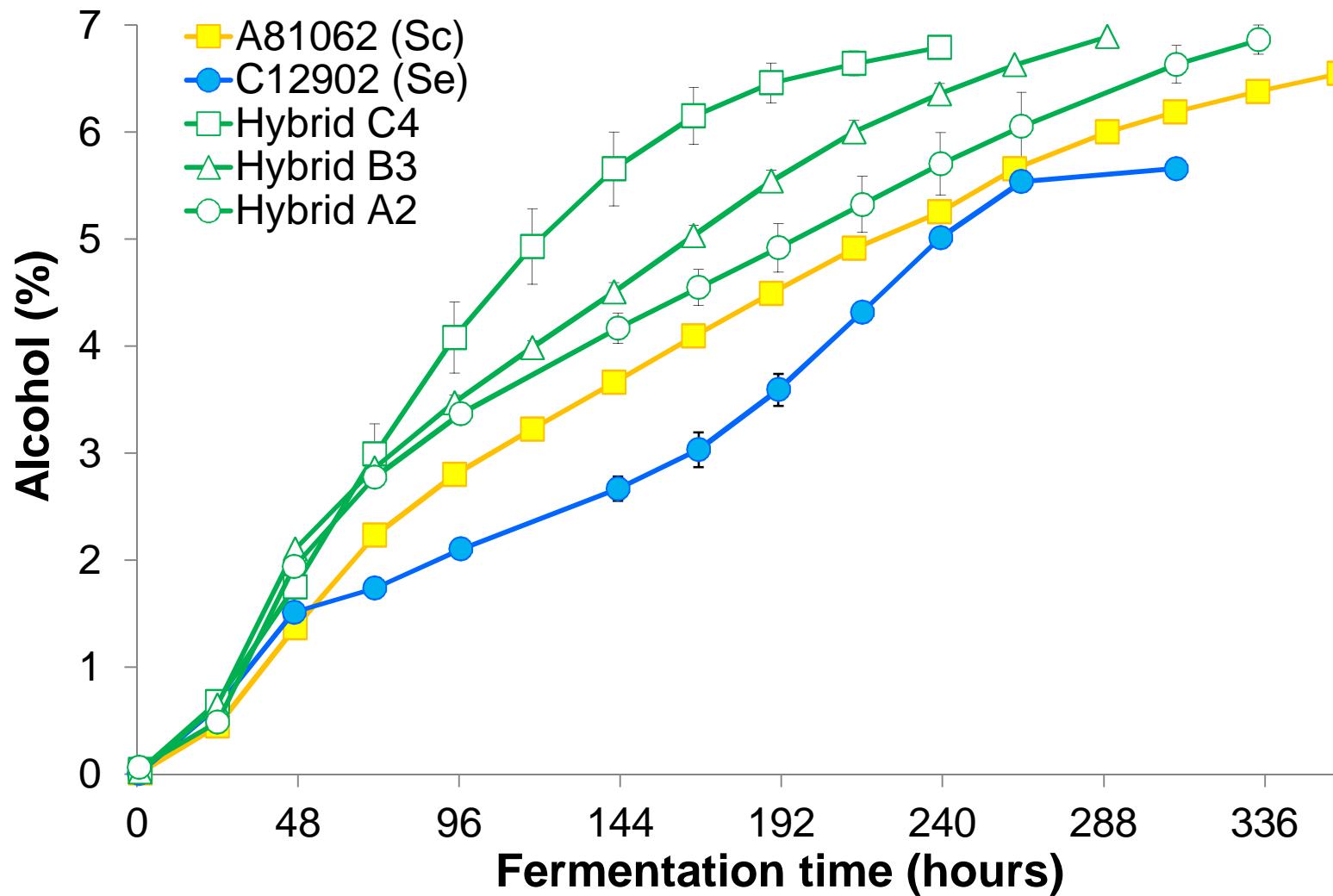


## Growth at various temperatures

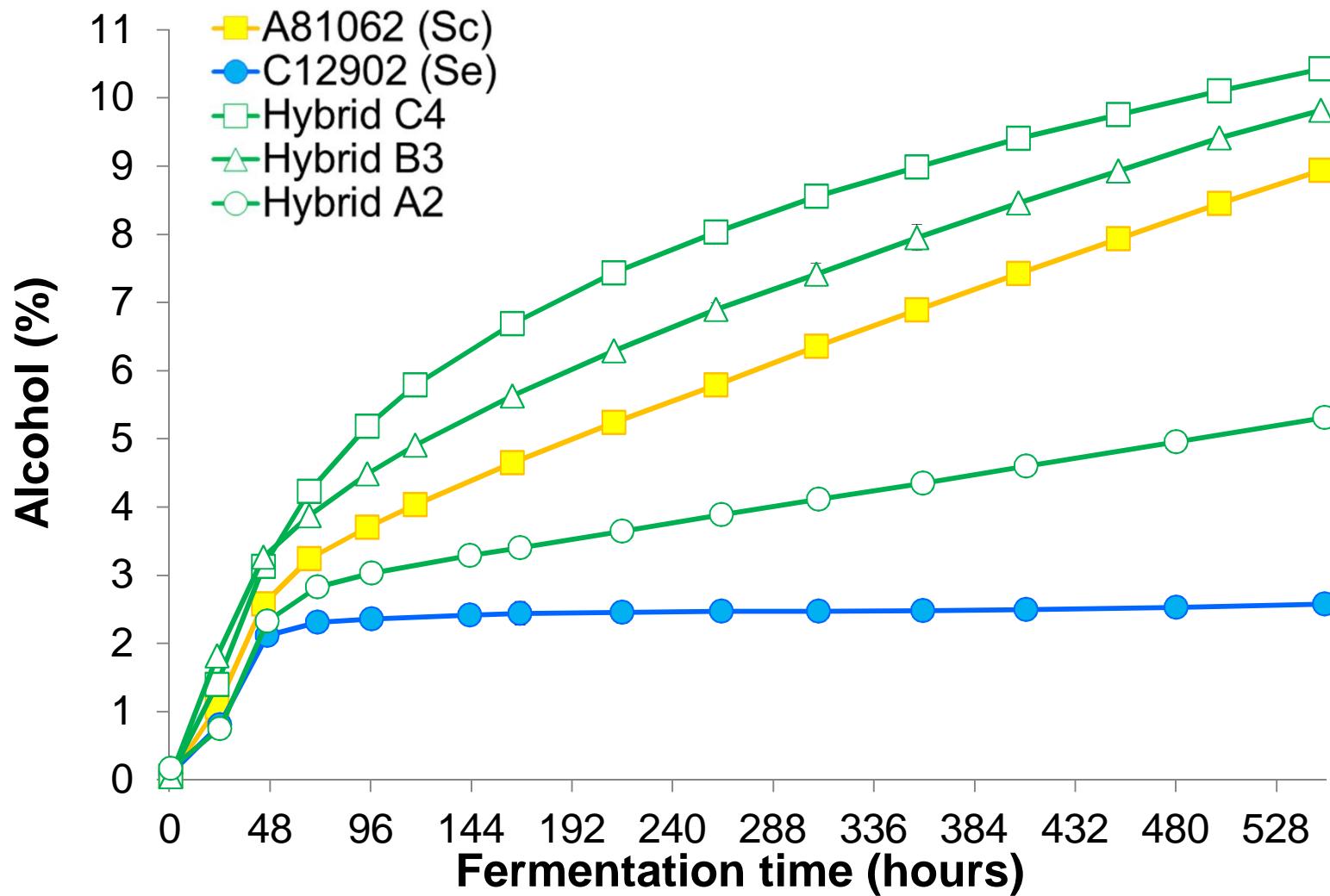
- Hybrids have a broader temperature range of growth
- The same strain can be used for low and high temperature fermentations?



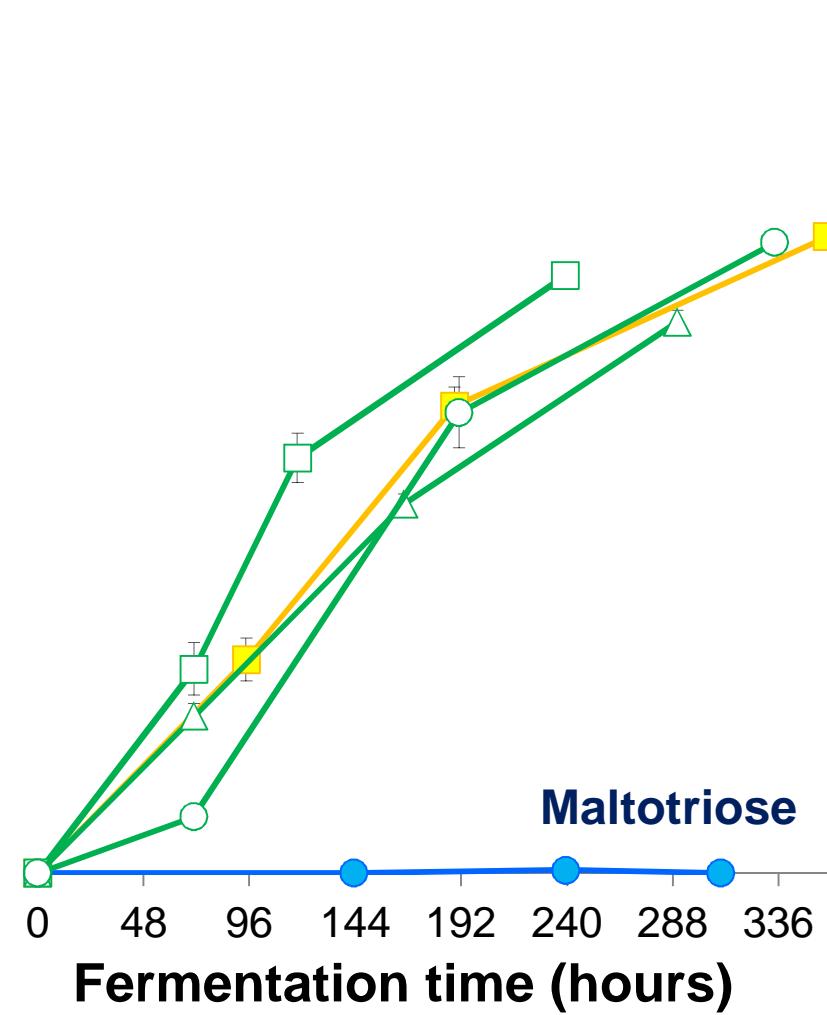
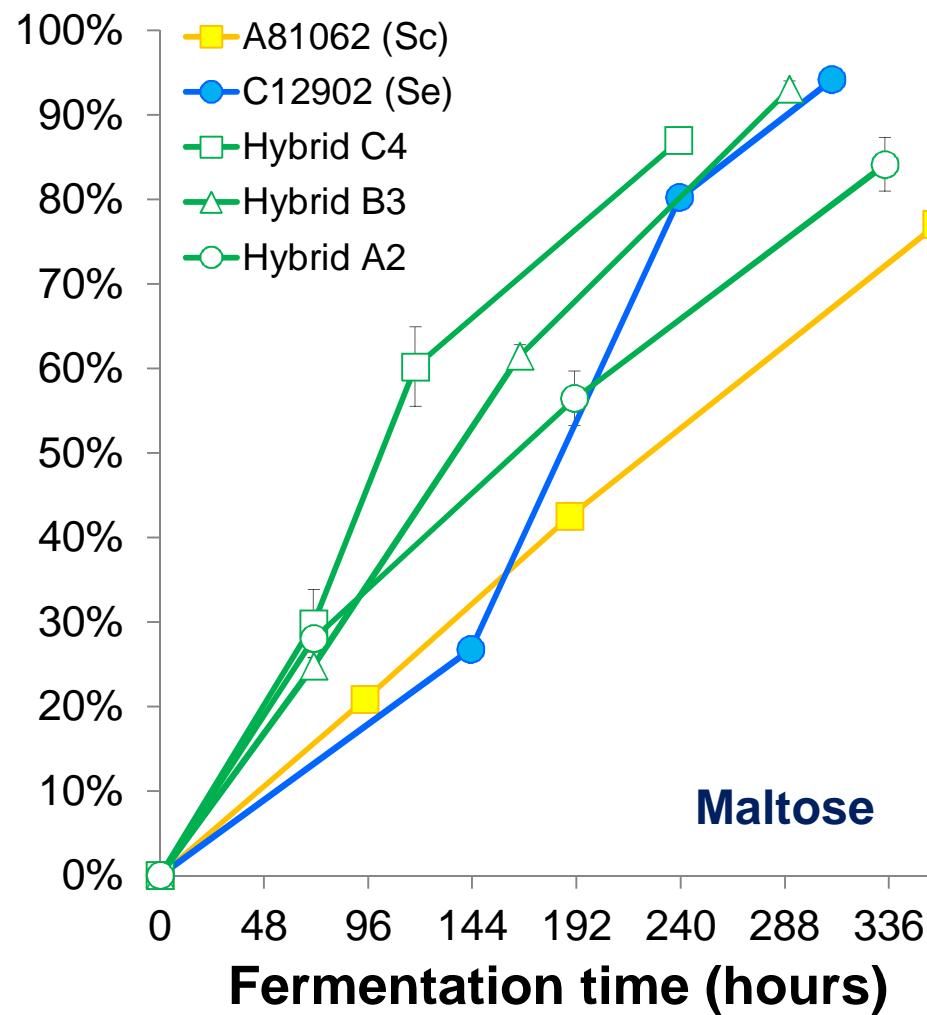
## Fermentation performance (15 °C, 15 °P)



## Fermentation performance (15 °C, 25 °P)

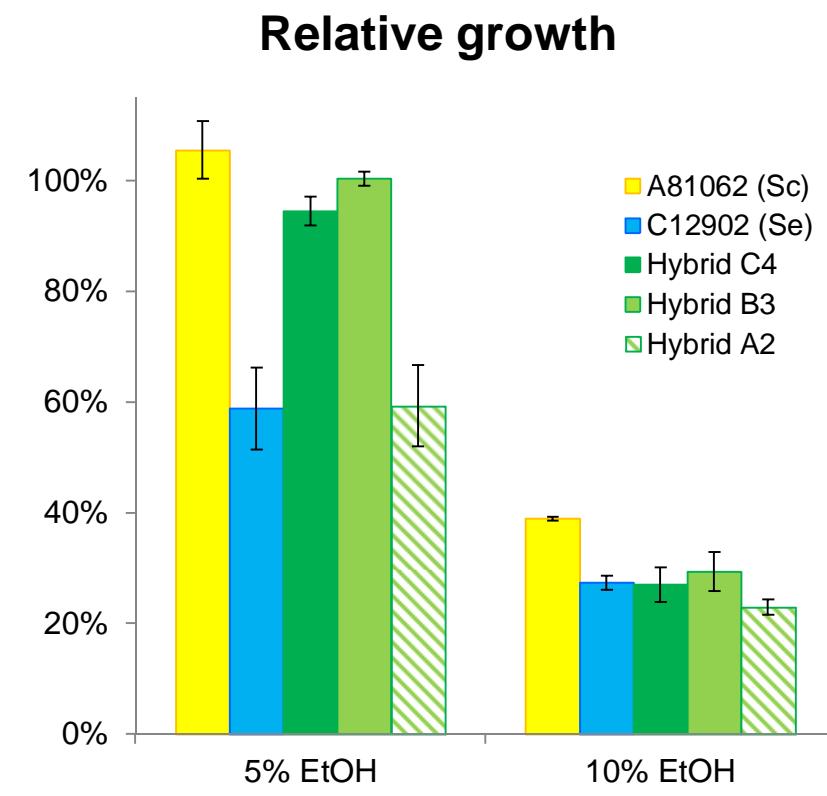
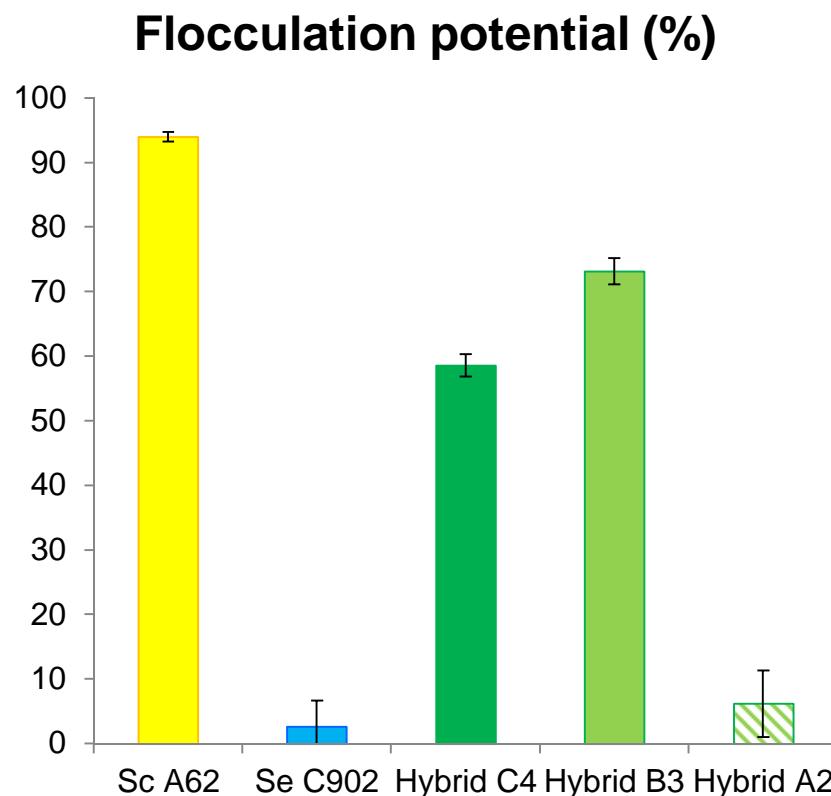


## Sugar use (15 °P)



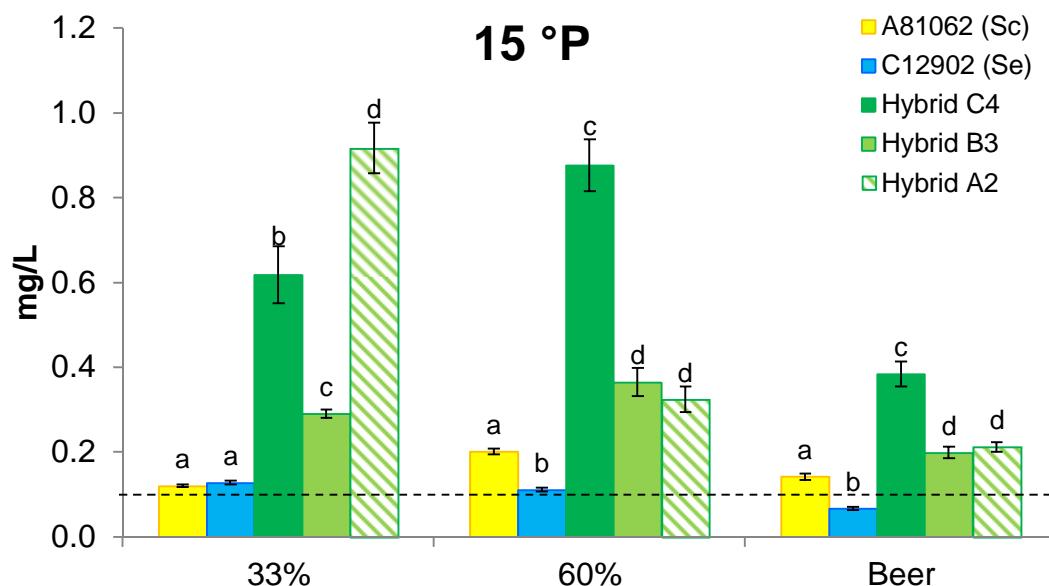
# Flocculation & ethanol tolerance

- Loss of flocculation ability in diploid Hybrid A2



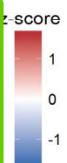
## Diacetyl

- Highest diacetyl peaks were observed for diploid Hybrid A2 and tetraploid Hybrid C4
  - No clear pattern between hybrid strains and the parent strains
  - More rapid diacetyl removal in diploid hybrid A2



# Aroma compounds

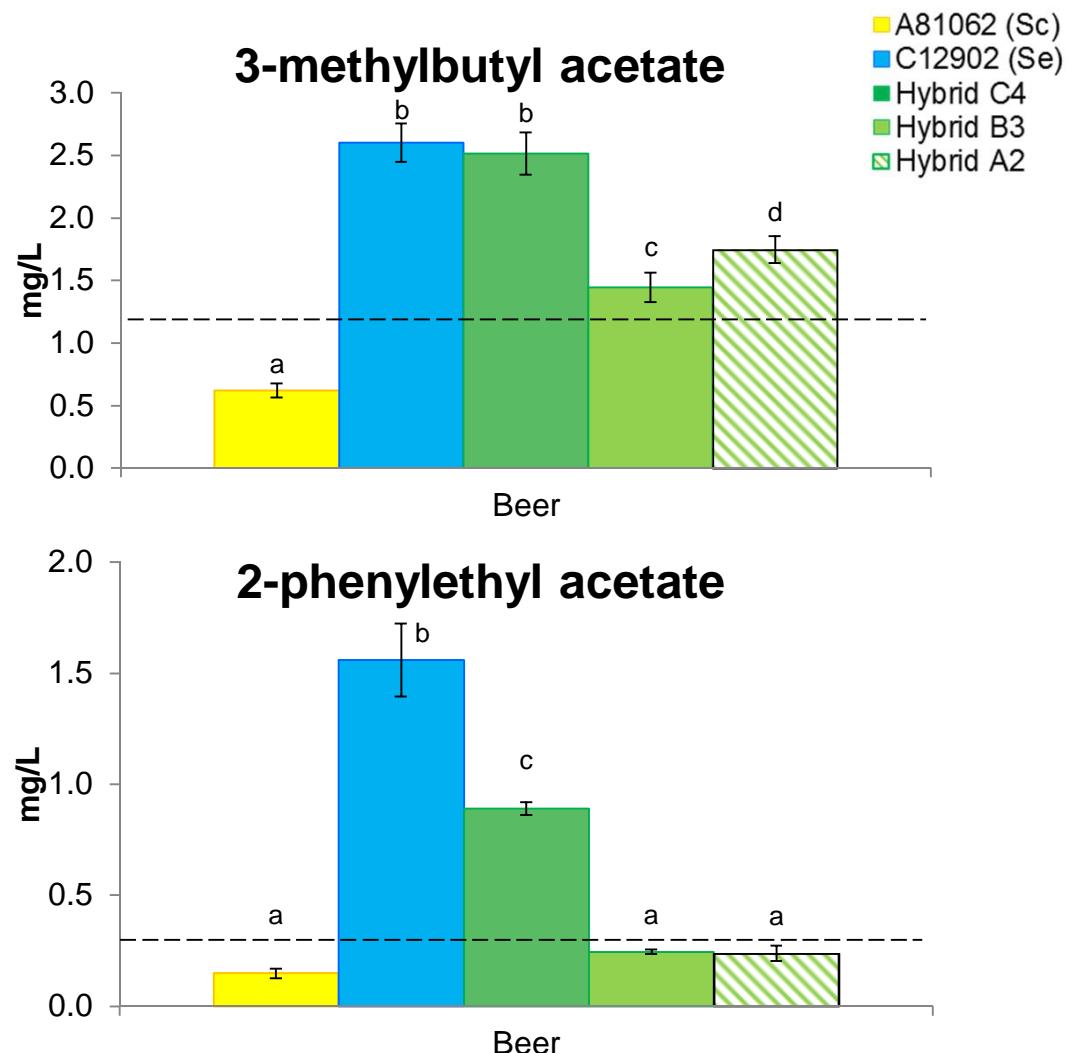
(mg L <sup>-1</sup> )	Sc A81062	Se C12902	Hybrid C4	Hybrid B3	Hybrid A2	Sc A81062	Se C12902	Hybrid C4	Hybrid B3	Hybrid A2
Acetaldehyde (10 mg L <sup>-1</sup> )	21.31 (±1.48) <sup>a</sup>	8.34 (±0.57) <sup>b</sup>	30.20 (±2.80) <sup>c</sup>	15.34 (±1.65) <sup>d</sup>	4.99 (±0.30) <sup>e</sup>	14.24 (±1.33) <sup>a</sup>	20.86 (±2.08) <sup>b</sup>	31.94 (±1.21) <sup>c</sup>	16.23 (±1.63) <sup>a</sup>	5.19 (±0.31) <sup>d</sup>
1-Propanol (800 mg L <sup>-1</sup> )	16.38 (±0.44) <sup>a</sup>	13.76 (±0.96) <sup>b</sup>	16.95 (±0.56) <sup>a,c</sup>	18.08 (±0.36) <sup>c</sup>	25.34 (±1.13) <sup>d</sup>	30.16 (±1.15) <sup>a</sup>	6.67 (±0.43) <sup>b</sup>	34.85 (±1.17) <sup>c</sup>	29.02 (±1.94) <sup>a</sup>	17.90 (±1.33) <sup>d</sup>
2-Methylpropanol (200 mg L <sup>-1</sup> )	11.40 (±0.51) <sup>a</sup>	31.05 (±1.67) <sup>b</sup>	24.95 (±1.02) <sup>c</sup>	21.45 (±0.50) <sup>d</sup>	36.54 (±1.36) <sup>e</sup>	16.59 (±0.88) <sup>a</sup>	14.01 (±0.64) <sup>b</sup>	51.34 (±1.16) <sup>c</sup>	35.59 (±1.97) <sup>d</sup>	23.96 (±0.88) <sup>e</sup>
2-Methylbutanol (65 mg L <sup>-1</sup> )	17.59 (±0.64) <sup>a</sup>	28.09 (±1.40) <sup>b</sup>	32.86 (±1.01) <sup>c</sup>	28.41 (±0.60) <sup>b</sup>	32.44 (±1.56) <sup>c</sup>	13.31 (±0.62) <sup>a</sup>	11.39 (±0.49) <sup>b</sup>	42.67 (±0.79) <sup>c</sup>	31.34 (±1.63) <sup>d</sup>	20.72 (±0.59) <sup>e</sup>
3-Methylbutanol (70 mg L <sup>-1</sup> )	21.09 (±0.99) <sup>a</sup>	73.27 (+3.44) <sup>b</sup>	54.05 (+2.10) <sup>c</sup>	45.62 (+1.05) <sup>d</sup>	89.42 (+3.81) <sup>e</sup>	36.00 (+1.31) <sup>a</sup>	36.30 (+1.37) <sup>a</sup>	81.81 (+1.37) <sup>b</sup>	57.43 (+2.62) <sup>c</sup>	57.02 (+1.69) <sup>c</sup>
3-Methylbutyl acetate (1.2 mg L <sup>-1</sup> )	0.62 (±0.06) <sup>a</sup>	2.60 (±0.15) <sup>b</sup>	2.51 (±0.17) <sup>b</sup>	1.45 (±0.12) <sup>c</sup>	1.75 (±0.11) <sup>d</sup>	0.84 (±0.07) <sup>a</sup>	0.49 (±0.04) <sup>b</sup>	2.14 (±0.10) <sup>c</sup>	1.51 (±0.12) <sup>d</sup>	1.14 (±0.11) <sup>e</sup>
2-Phenylethyl acetate (0.3 mg L <sup>-1</sup> )	0.15 (±0.02) <sup>a</sup>	1.56 (±0.16) <sup>b</sup>	0.89 (±0.03) <sup>c</sup>	0.24 (±0.01) <sup>a</sup>	0.24 (±0.03) <sup>a</sup>	ND	0.34 (±0.02) <sup>b</sup>	0.39 (±0.03) <sup>c</sup>	0.15 (±0.01) <sup>d</sup>	ND
Ethyl acetate (30 mg L <sup>-1</sup> )	29.30 (±2.42) <sup>a</sup>	30.83 (±2.03) <sup>a</sup>	32.90 (±2.08) <sup>a</sup>	41.42 (±2.72) <sup>b</sup>	60.15 (±2.72) <sup>c</sup>	55.40 (±4.60) <sup>a</sup>	7.49 (±0.53) <sup>b</sup>	62.84 (±1.62) <sup>c</sup>	78.20 (±4.16) <sup>d</sup>	44.24 (±1.67) <sup>e</sup>
Ethyl hexanoate (0.21 mg L <sup>-1</sup> )	0.40 (±0.02) <sup>a</sup>	0.10 (±0.01) <sup>b</sup>	0.48 (±0.03) <sup>c</sup>	0.42 (±0.05) <sup>a</sup>	0.38 (±0.02) <sup>a</sup>	0.58 (±0.06) <sup>a</sup>	0.06 (±0.01) <sup>b</sup>	0.33 (±0.03) <sup>c</sup>	0.36 (±0.05) <sup>c</sup>	0.21 (±0.02) <sup>d</sup>
Ethyl octanoate (0.9 mg L <sup>-1</sup> )	0.36 (±0.18) <sup>a,b</sup>	0.20 (±0.04) <sup>a,c</sup>	0.44 (±0.12) <sup>b</sup>	0.22 (±0.08) <sup>a,c</sup>	0.17 (±0.04) <sup>c</sup>	0.57 (±0.15) <sup>a</sup>	0.41 (±0.06) <sup>b</sup>	0.24 (±0.03) <sup>c</sup>	0.15 (±0.02) <sup>c</sup>	0.14 (±0.02) <sup>c</sup>
Ethyl decanoate (1.5 mg L <sup>-1</sup> )	0.04 (±0.02) <sup>a</sup>	0.12 (±0.02) <sup>b</sup>	0.16 (±0.03) <sup>b</sup>	0.04 (±0.01) <sup>a</sup>	0.07 (±0.02) <sup>a</sup>	0.11 (±0.04) <sup>a</sup>	0.21 (±0.02) <sup>b</sup>	0.12 (±0.01) <sup>a</sup>	0.04 (±0.01) <sup>c</sup>	0.05 (±0.01) <sup>c</sup>



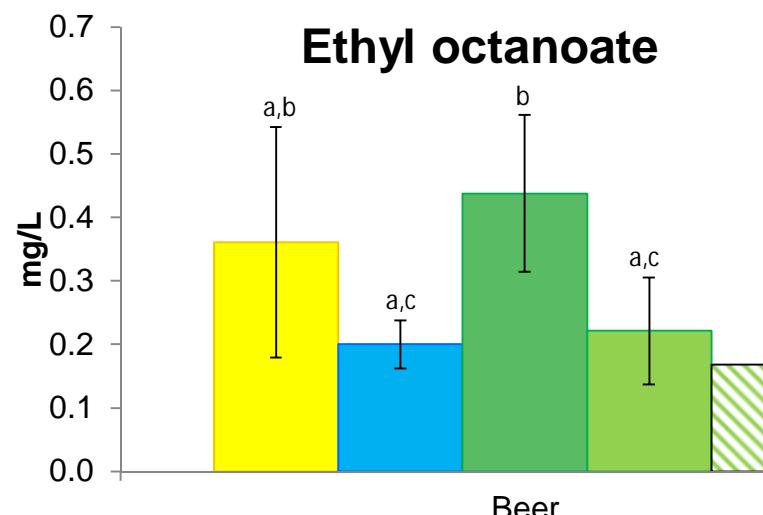
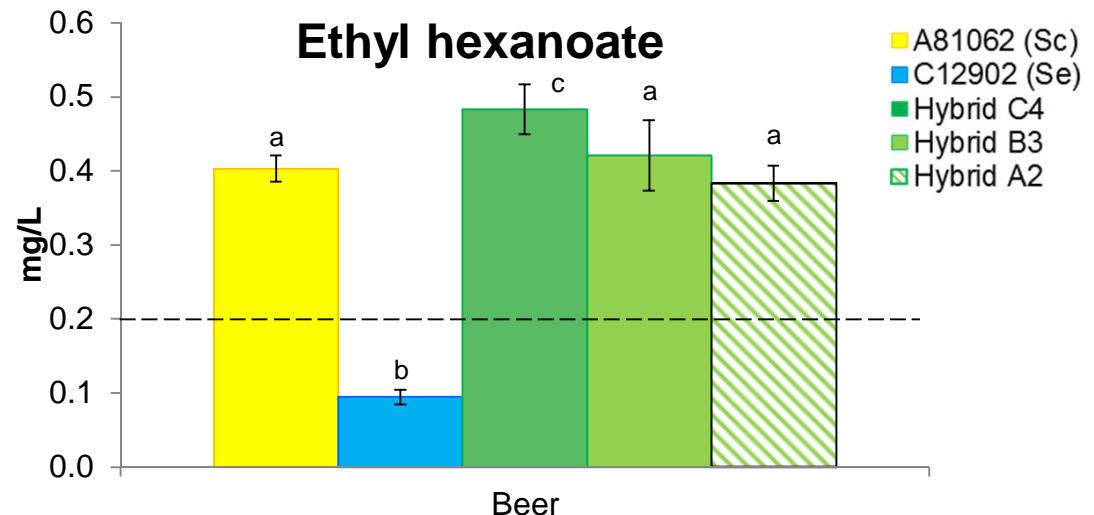
15P

25P

## Aroma compounds (15 °P) – acetate esters



# Aroma compounds (15 °P) – ethyl esters



# Formation of aroma compounds

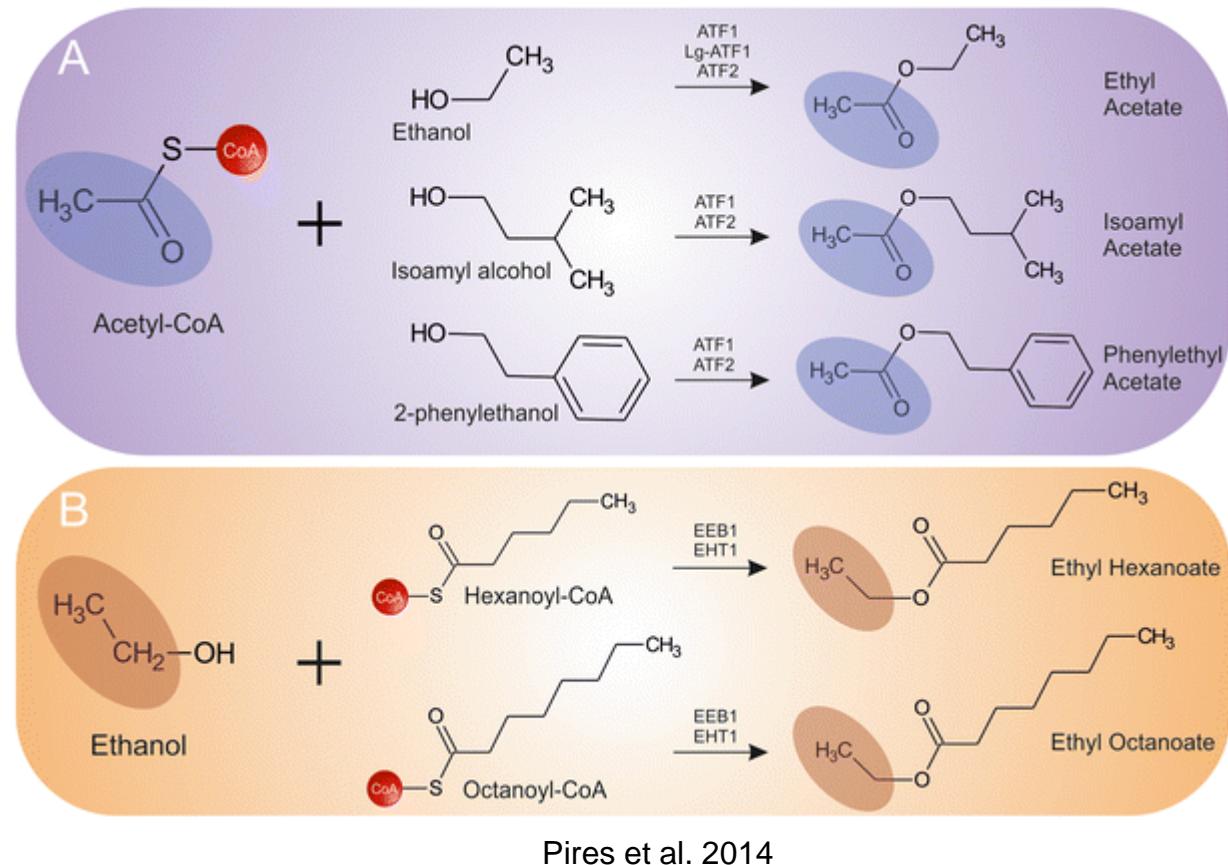
- Condensation reaction between an alcohol and acyl-CoA

- Acetate esters

- ATF1*
- ATF2*

- Ethyl esters

- EEB1*
- EHT1*



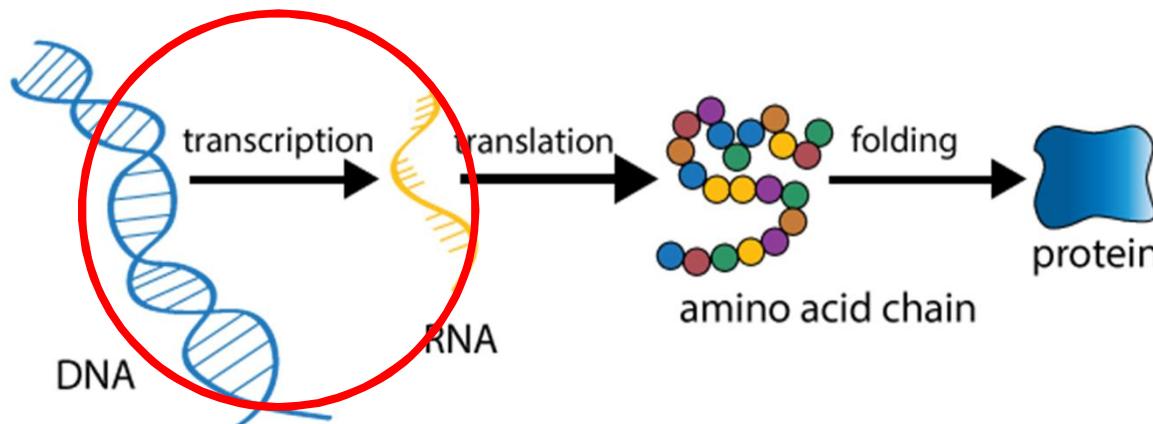
# Gene copy numbers

Chromosome	Genes located on chromosome		Hybrid A2		Hybrid B3		Hybrid C4	
	Scer	Seub	Scer	Seub	Scer	Seub	Scer	Seub
II	Sc-EHT1		1	1	2	1	2	2
IV		Se-EHT1	1	1	2	1	2	2
VII	Sc-ATF2	Se-ATF2	1	1	2	1	2	2
VIII	Sc-BAT1	Se-ATF1	1	1	2	1	2	2
XV	Sc-ATF1	Se-BAT1	1	1	2	1	2	2
XVI	Sc-EEB1	Se-EEB1	1	1	2	1	2	2

- Estimated from the sequence coverage
- Supported by qPCR analysis

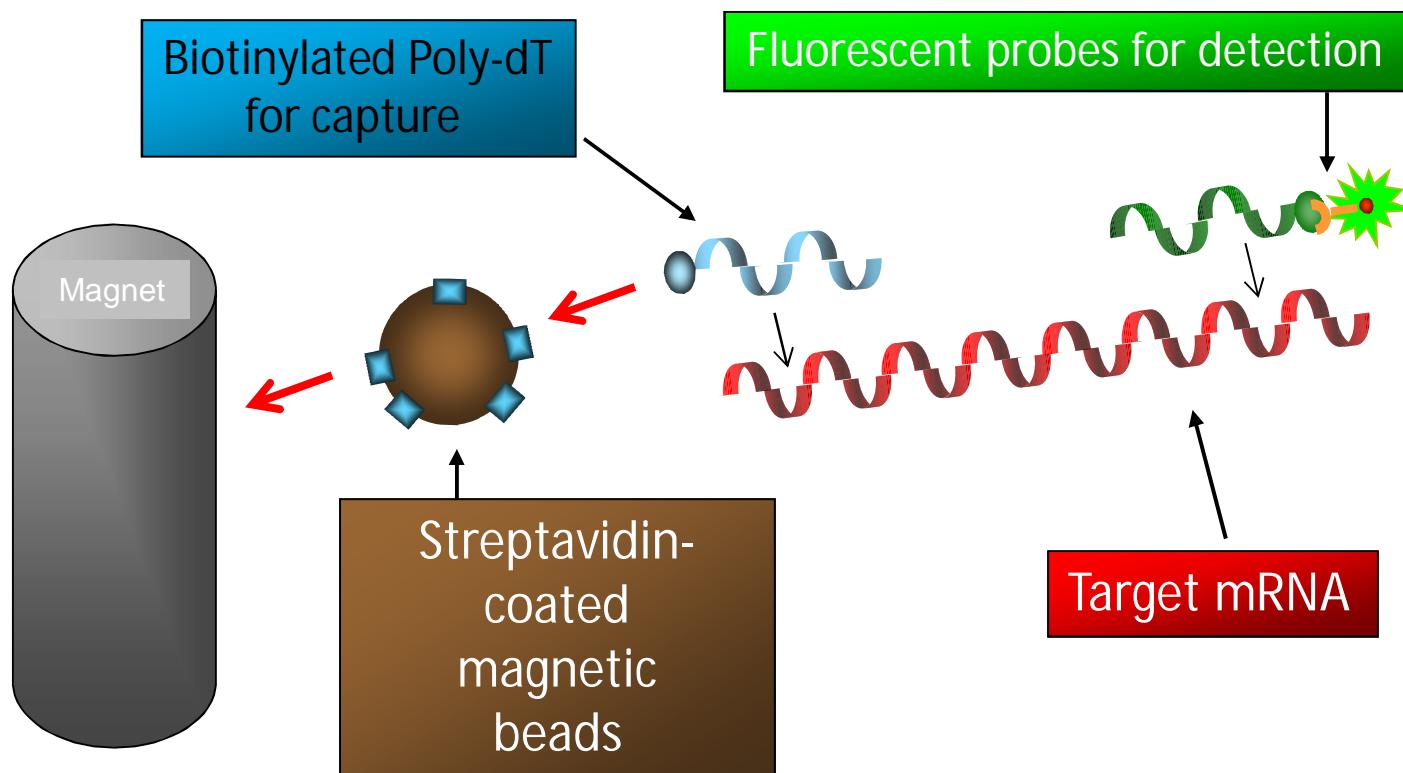
# Transcriptional analysis

- Gene expression: information from a gene is used in the synthesis of a functional gene product.
  - Transcription is the first step of gene expression, in which a particular segment of DNA is copied into mRNA

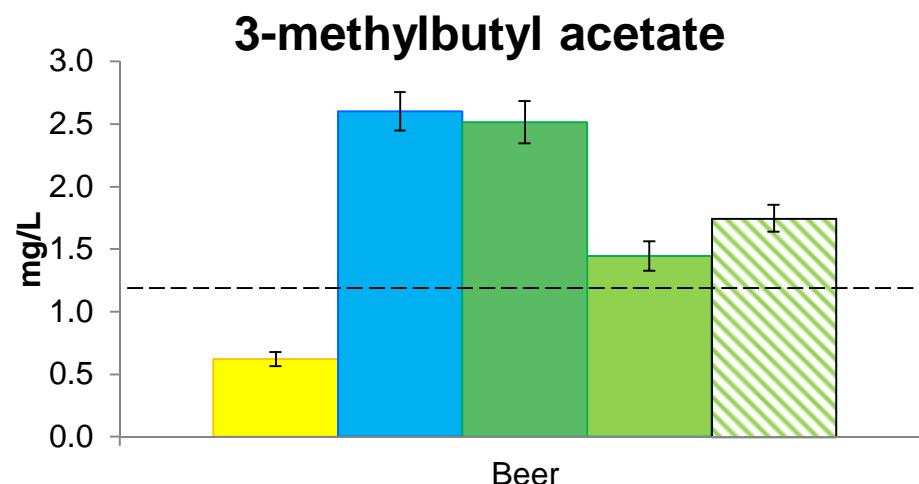
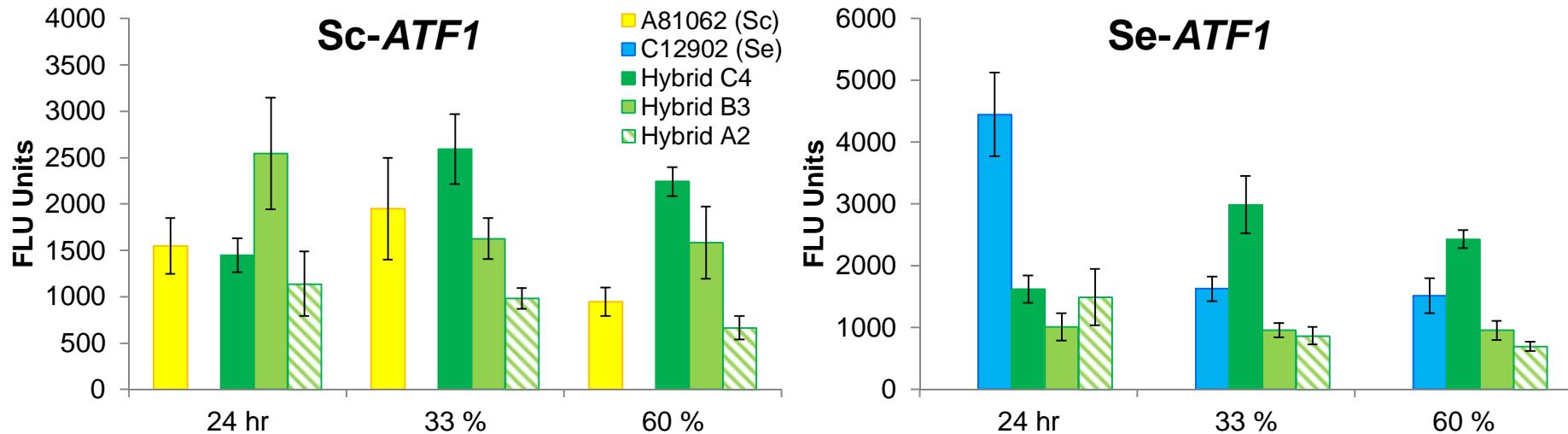


# Transcriptional analysis (TRAC)

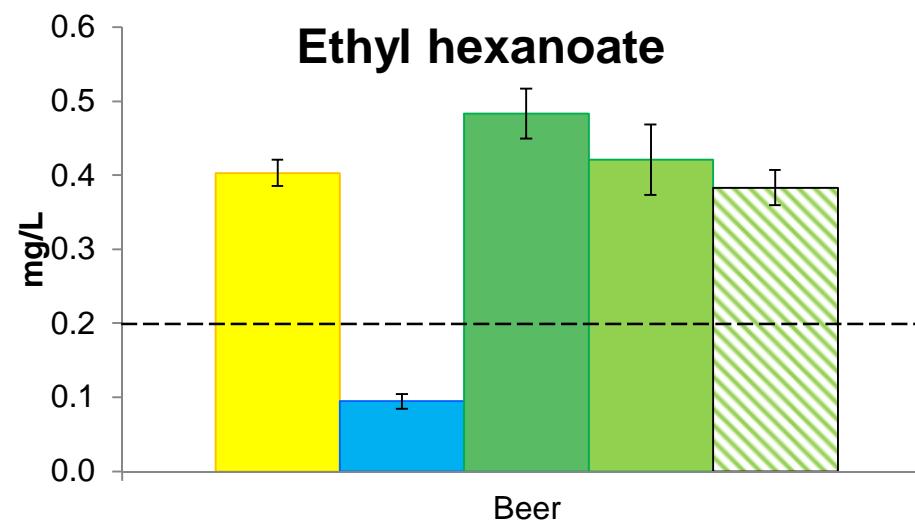
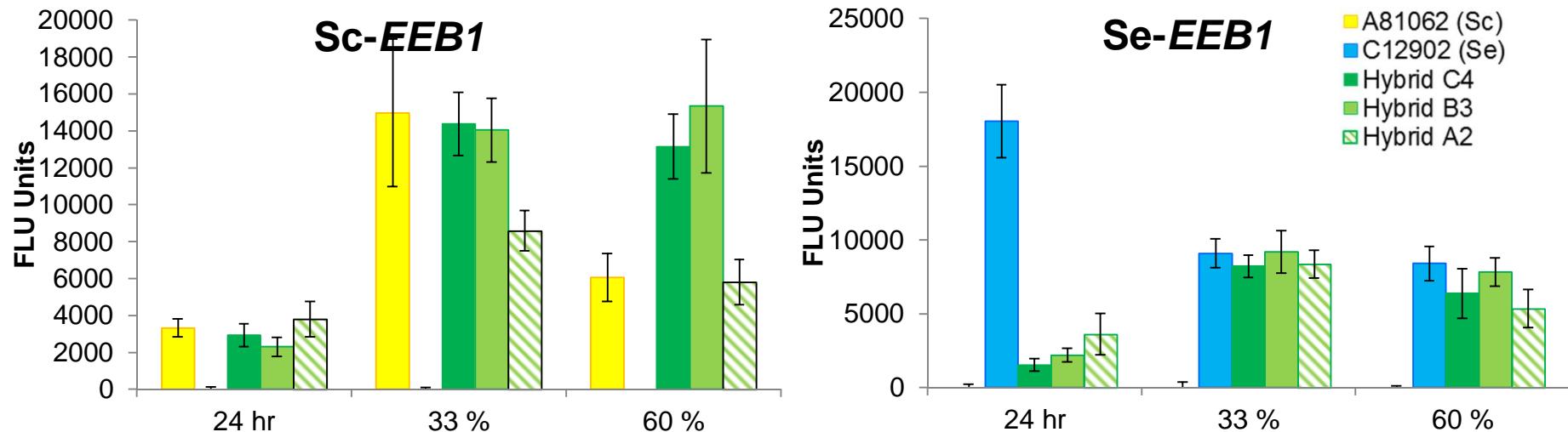
- Transcriptional analysis with the aid of **affinity capture**



# Transcriptional analysis (TRAC)

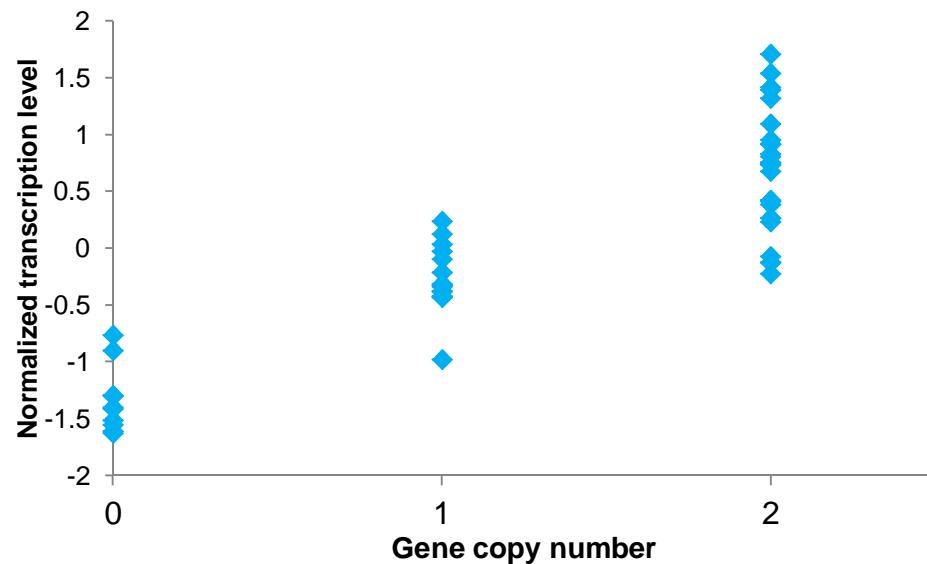


# Transcriptional analysis (TRAC)



## Correlation – Transcription vs Gene Copies

- Positive correlation between gene copy numbers and transcription levels

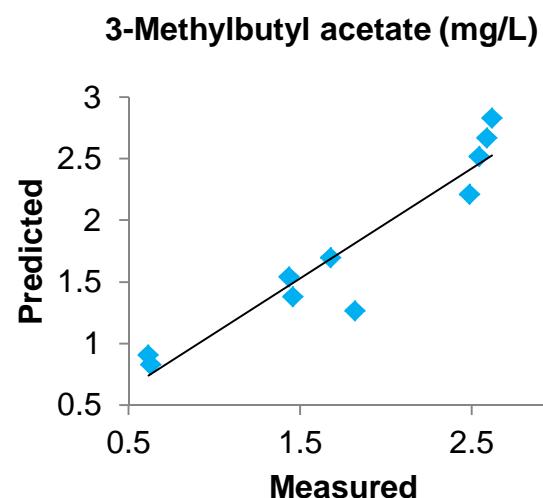


Pearson correlation:  $r = 0.88$

## Correlation – Transcription vs Aroma

- Multiple linear regression between maximum transcription levels and aroma concentrations
  - Positive correlation observed for several genes and esters

Gene	3-methylbutyl acetate	2-phenylethyl acetate	Gene	Ethyl hexanoate
Sc-ATF1	NS	NS	Sc-EHT1	NS
Se-ATF1	$4.8 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$	Se-EHT1	$-3.8 \cdot 10^{-5}$
Sc-ATF2	NS	NS	Sc-EEB1	$1.6 \cdot 10^{-5}$
Se-ATF2	$5.8 \cdot 10^{-4}$	$3.7 \cdot 10^{-4}$	Se-EEB1	NS



# Conclusions

- Physiological properties of hybrid strains can be controlled to some extent with their ploidy and subgenome inheritance
- We observed an increased fermentation performance and stress tolerance with increased ploidy level
- Ester formation linked to genome contribution from parent strains
  - Higher transcription levels of orthologous genes linked to aroma synthesis when present in higher copy numbers.



## Take-home message

- Yeast hybrids often **perform better** than the parents
- Create strains with increased flavour diversity
- Endless possibilities for creating new lager yeasts
- No genetic engineering involved



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# Acknowledgements

## VTT:

- Brian Gibson
- Mikko Arvas
- Sirpa Jylhä
- Frederico Magalhães
- Merja Oja
- Virve Vidgren
- Annika Wilhelmson
  
- Aila Siltala
- Arvi Wilpolä
- Eero Mattila

## IRCAN:

- Gianni Liti
- Matteo De Chiara
- Jia-Xing Yue

## Funding:

- Alfred Kordelin Foundation
- Svenska Kulturfonden
- PBL Brewing Laboratory
- SABMiller
- Academy of Finland
- FP7 Marie-Curie ITN  
YEASTCELL

