



# Hopsteiner®

Crop Improvement  
Program

Paul D. Matthews, Ph.D.

2016



**WORLD BREWING CONGRESS**

August 13–17, 2016 • Denver, Colorado, U.S.A.

**ELEVATE  
BEER**



# Genomic Estimated Breeding Values and Genomic Predictions

- Motivation for Genomic Estimated Breeding Values
  - Example: The Problems with Male Selection
- Systems
  - Genotyping and Genetics System Parameters
  - Phenotyping and Trait Analyses
  - Genome-wide Association and Mixed Linear Modeling
  - Prediction Accuracy with Machine Learning (Artificial Intelligence)
- Results Examples:
  1. Sex determination genes
  2. Powdery mildew resistance genes

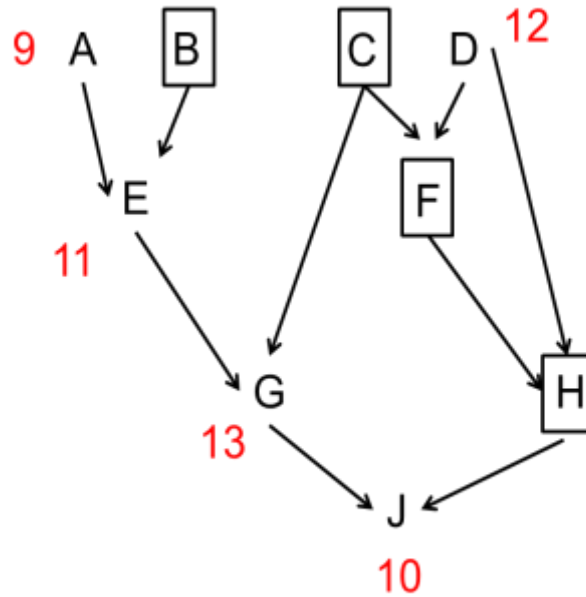
# Breeding systems:

Single Cross  
Select  
Idiotypes



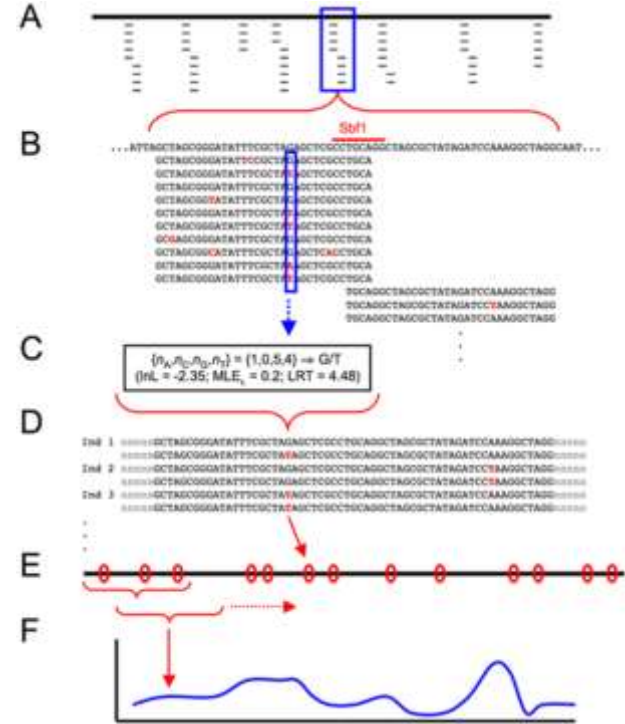
Quantitative Genetics  
Estimated Breeding  
Values (EBV)

Calculate EBVs for Male Alpha



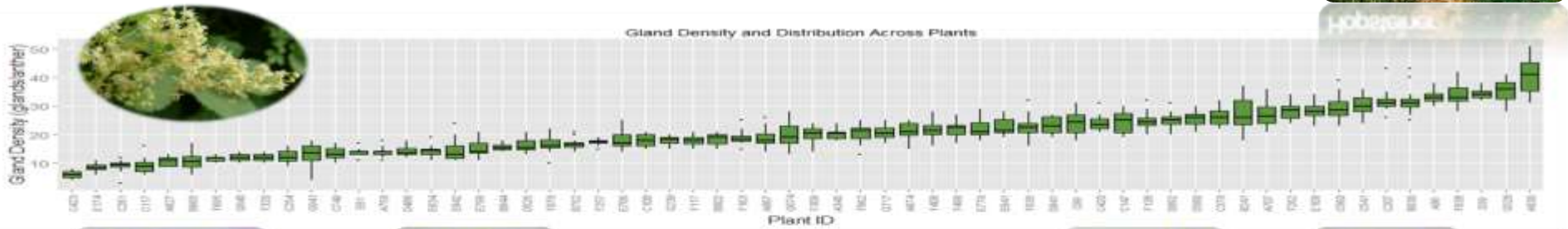
Alpha Acid  $h^2 = 0.5$

Molecular Quantitative  
Genetics  
GEBV



'Omics>>>

# The male selection problem in hops



A627: low gland density (10.8)



E799: medium-low gland density (17.4)



C420: medium-high gland density (24.25)

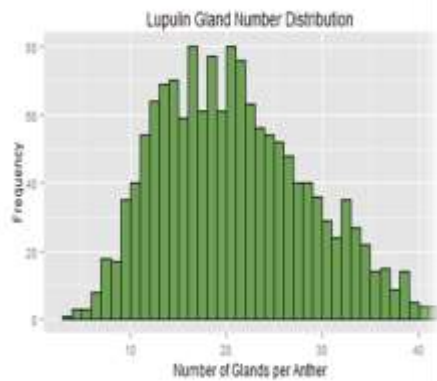
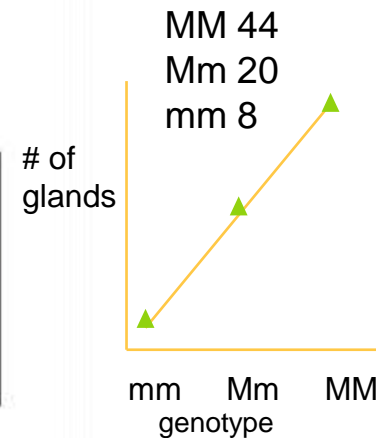


C207: high gland density (31.9)

## Quantitative Traits

- Probably caused by multiple loci
  - Interaction effects
  - Environment

If the mean trait value for individuals with marker state MM is different from the mean trait value of individuals with marker state mm (i.e. the marker is *associated* with the phenotype), then the marker is *linked* to a quantitative trait locus.



# General and Mixed Linear Modeling of Associations Between Traits Variations and Genes Variations (alleles, markers)

## Accounting for Random Effects: Mixed Linear Models

- "Cost" associated with estimating a parameter
- We are not interested in the **value** of the parameter, only the **variance**
- Q-K method (structured association)

$$y = X\beta + S\alpha + Qv + Zu + e$$

**Fixed** effects:

- $\beta$  Vector of fixed effects
- $\alpha$  Vector of SNPs effects
- $v$  Vector of subpopulation effects

**Random** effects:

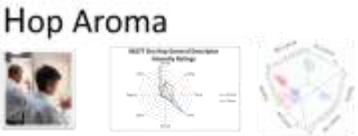
- $u$  Vector of kinship effects
- $e$  Residuals

- $Q$  Matrix of population association (STRUCTURE)
- $X, S, Z$  Incidence Matrices

# Technologies for Breeding

Apollo  
Whole Genome Sequence  
~44,000 contigs

GBSv2 Pipeline

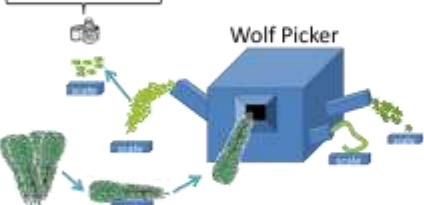


Chemical Content



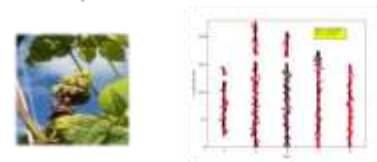
Components of Yield

$$3000 \left( \frac{\sum M_i / n_i}{\sum M_i} \right) = d_c \cdot g / cm^2 \text{ Cone Density}$$

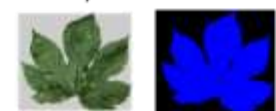


Disease Resistance

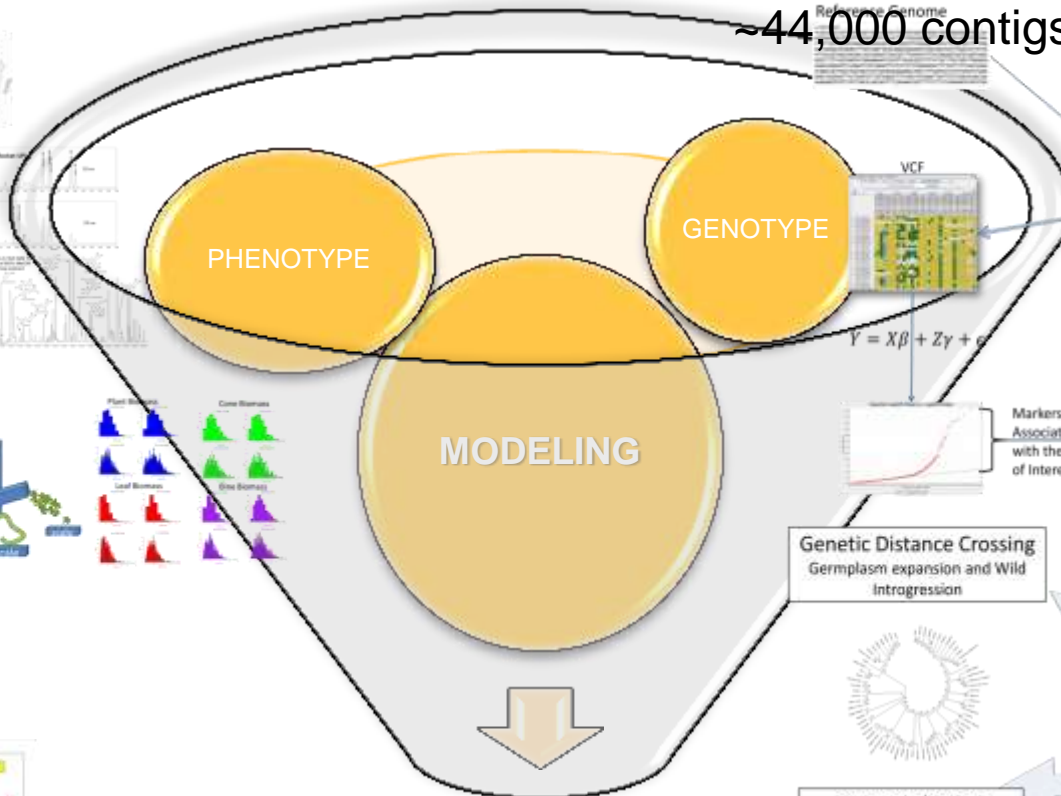
Downey Mildew



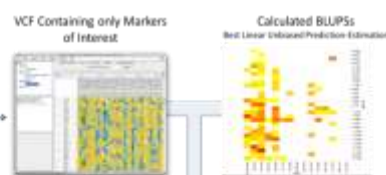
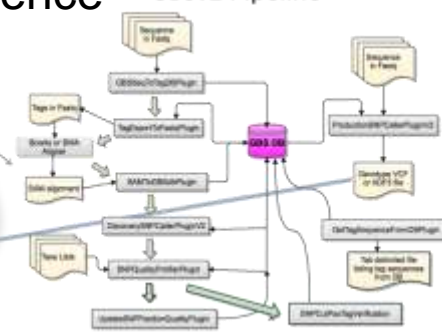
Powdery Mildew



R1, R2, R3, R4, R6



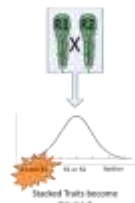
$$Y = X\beta + Z\gamma + e$$



Genetic Distance Crossing  
Germplasm expansion and Wild  
Introgression



Parent Selection



Male Selection



Female Traits are "Visible"  
in males that do not express them

Germplasm Screening



Computing



Molecular Selection Tools

# Specific system components

**bioRxiv**  
beta  
THE PREPRINT SERVER FOR BIOLOGY

“5000  
Genome  
Project”

$3.9 \times 10^{13}$   
Gene:Trait  
Associations

## Genomes

5

Teamaker 1.8 Gb  
ShinsuWase 2.05 Gb  
Apollo 2.28 Gb

## SNPs

1,235,148

## Germplasm

4396

cultivars

116 wilds

22 families

5,572,988,000  
SNP calls  
X  
70,128 traits

## Traits

(70,128)

Targeted Chemical  
(113)

Untargeted Chemical  
(70,000)

Morphological (15)  
Disease (7)

## Transcriptomes

36 cultivars

5 tissues

39,000 genes

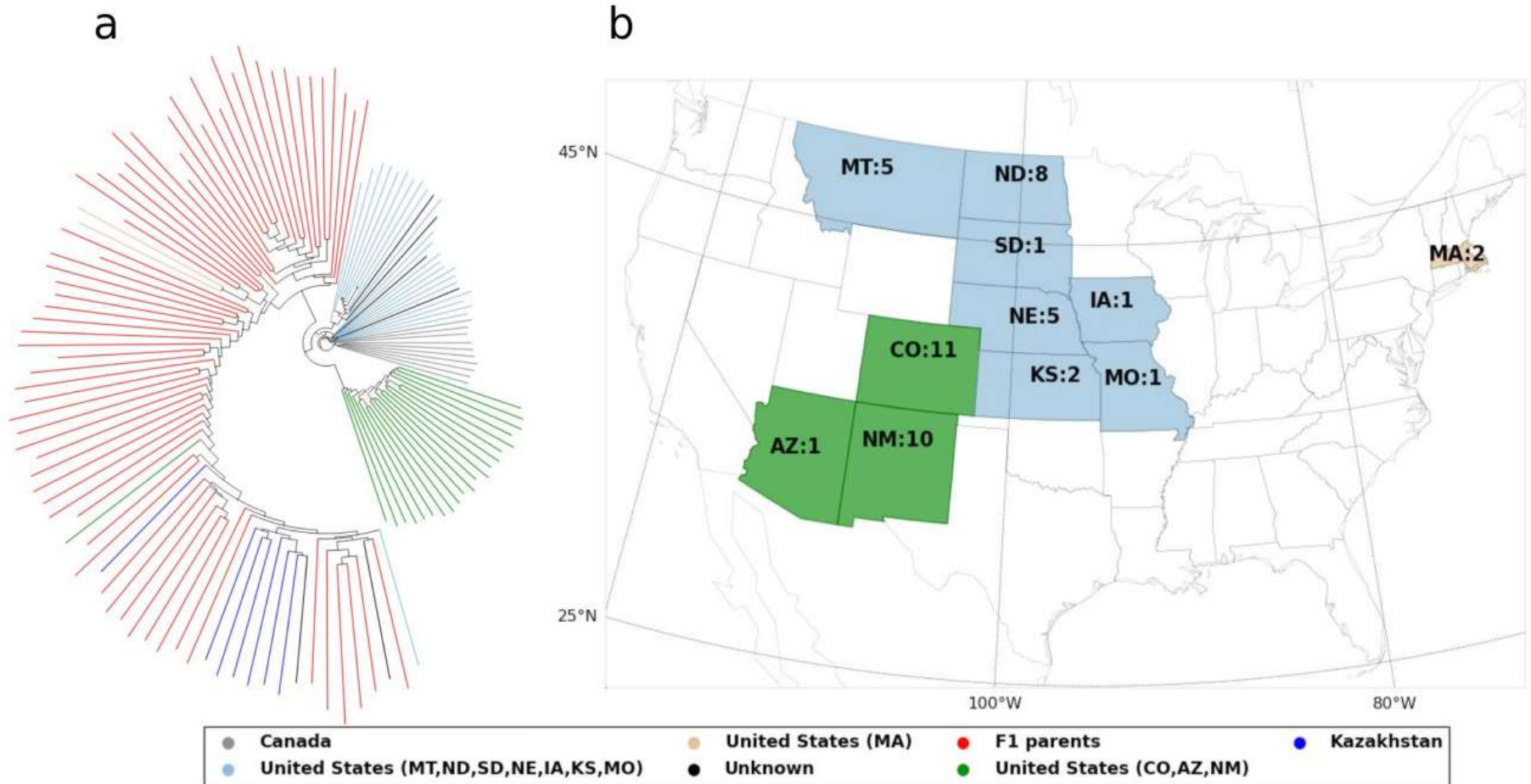




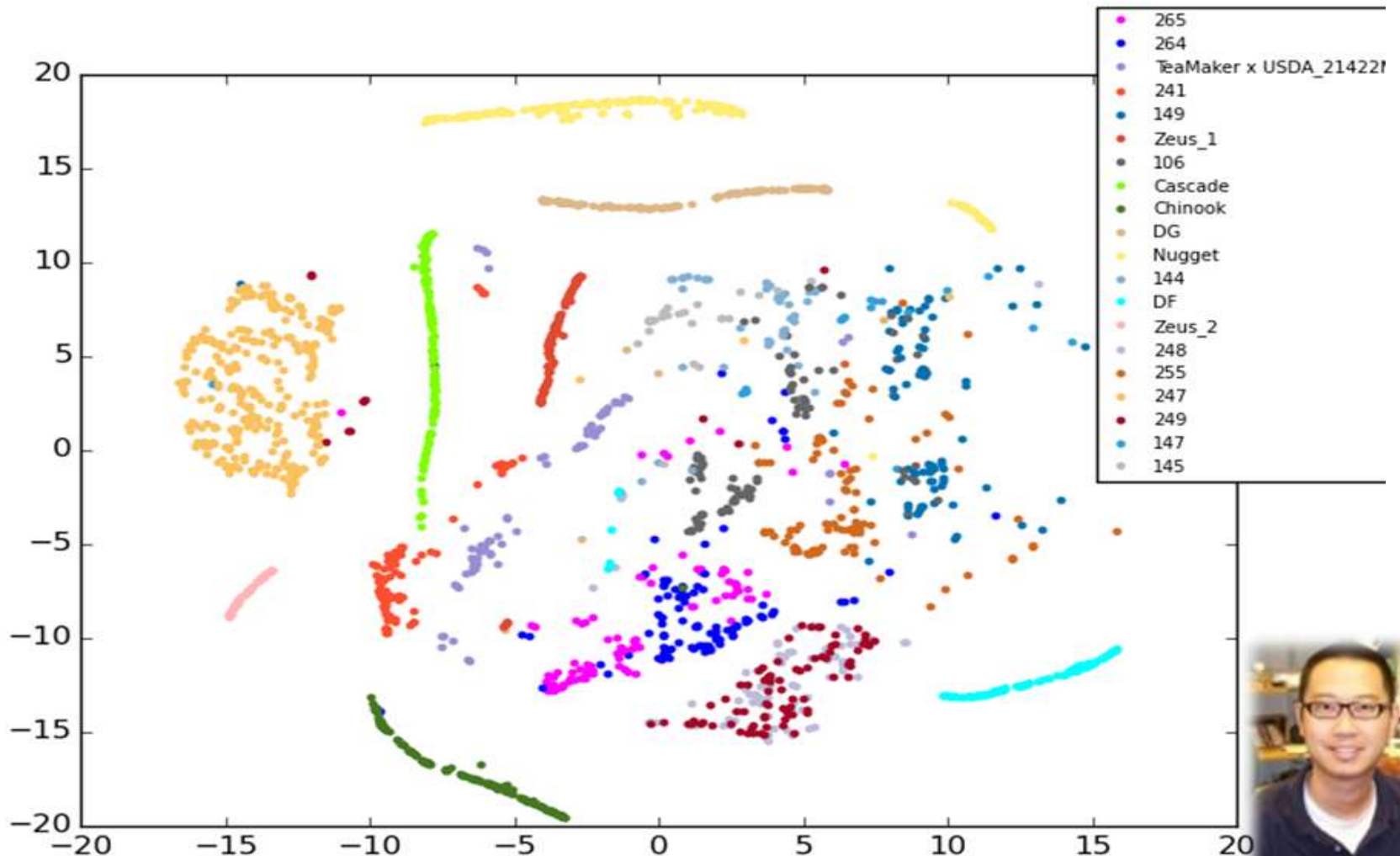
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# Phylogeny of domestic and wild hops



# Genetic Distance Principle Component Analysis

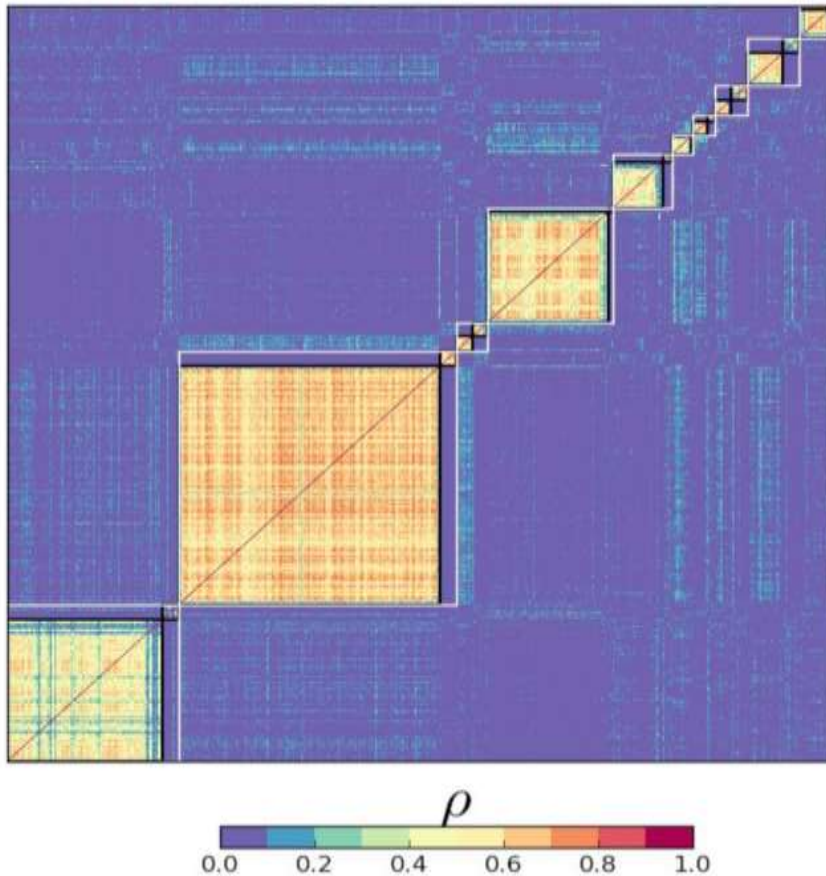


Dong Zhang, Nicholi J. Pitra, Mark C. Coles, Edward S. Buckler, Paul D. Matthews

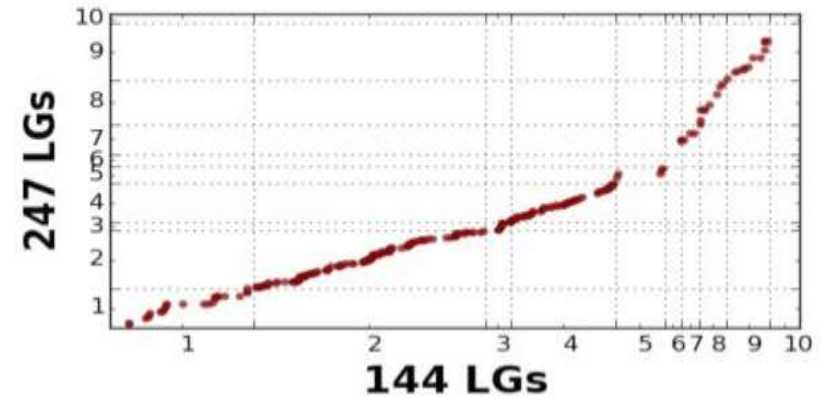
Non-Mendelian inheritance of SNP markers reveals extensive chromosomal translocations in dioecious hops. 2016 BioRxiv.

# Linkage Groups in Families

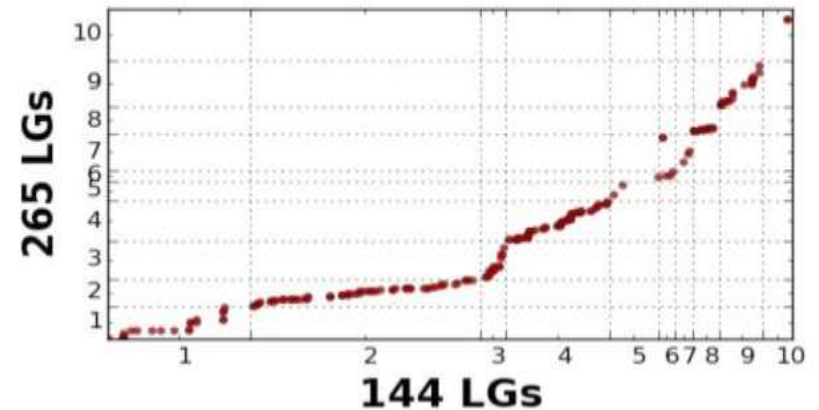
a



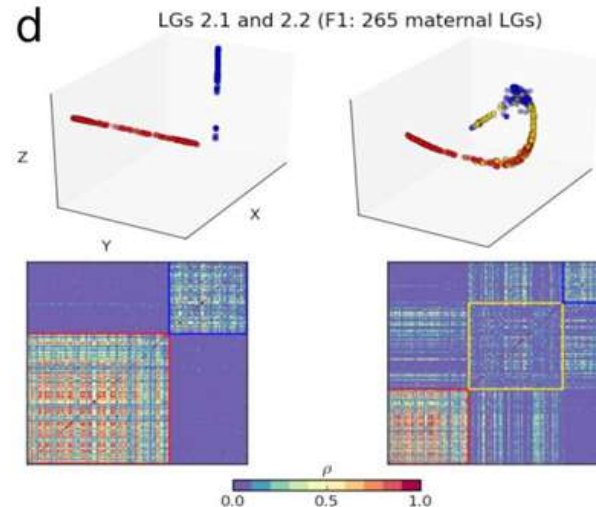
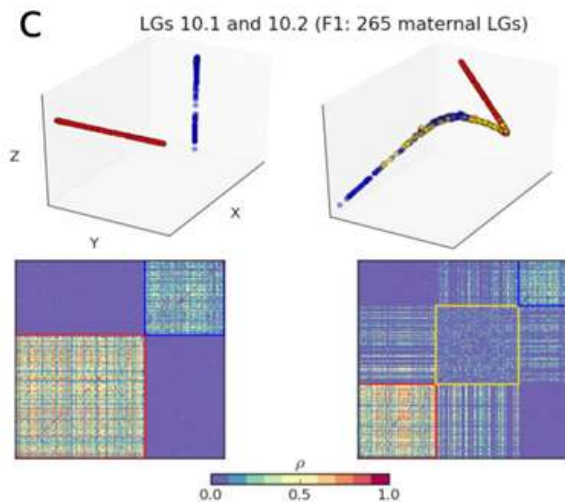
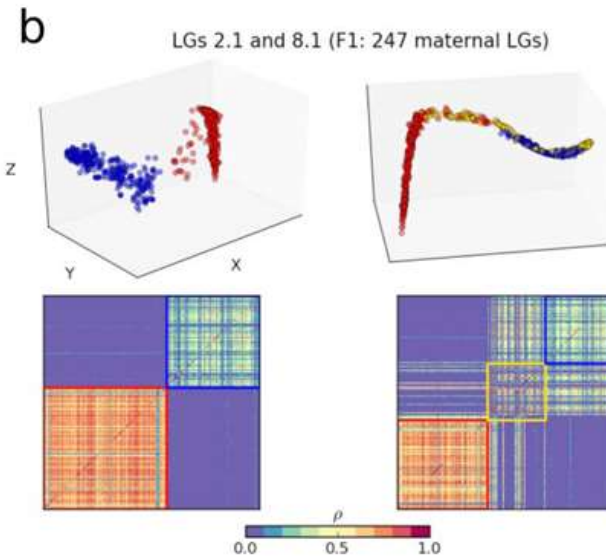
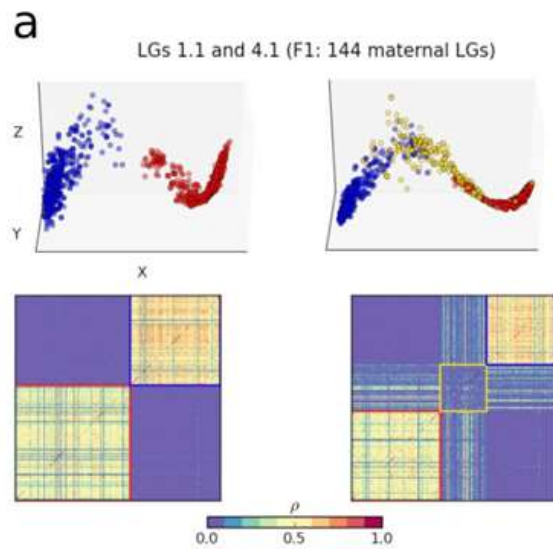
b



c



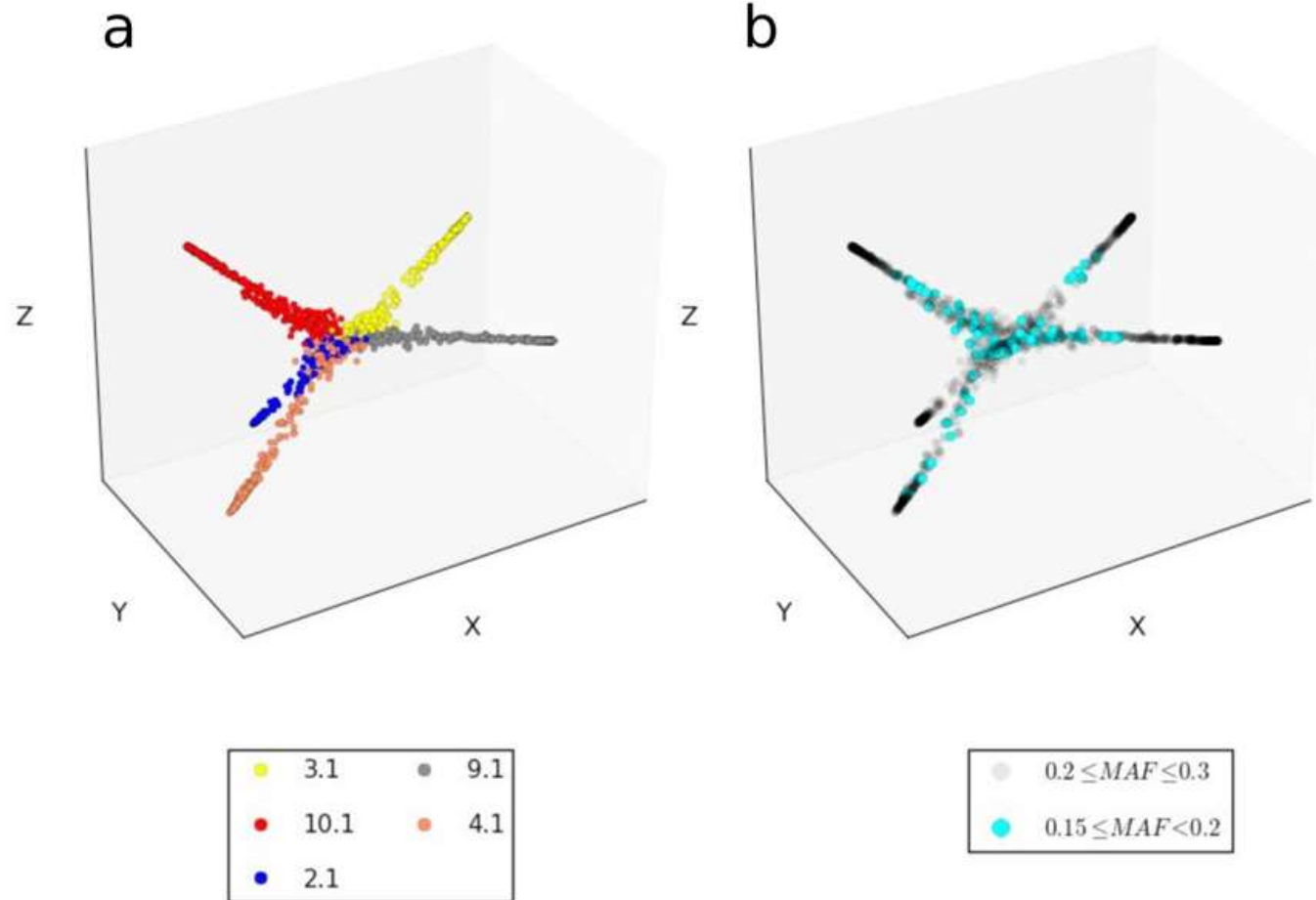
# Linkage groups in correlation projections



SD markers  
convergence

...

# Linkage groups “sharing”

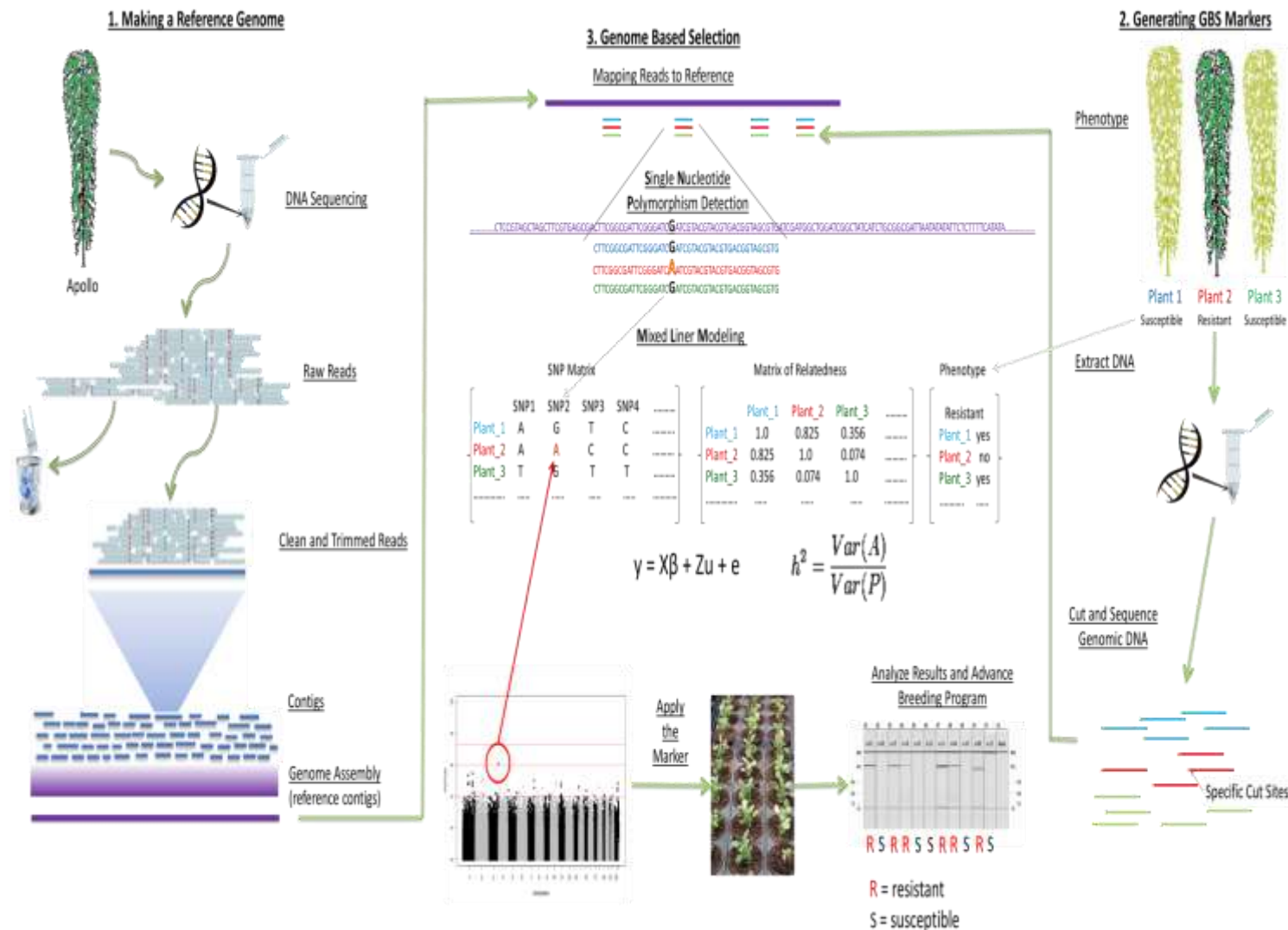


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 Non-Mendelian inheritance of SNP markers reveals extensive chromosomal translocations in dioecious hops. 2016 bioRxiv.

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# Loading the MLM

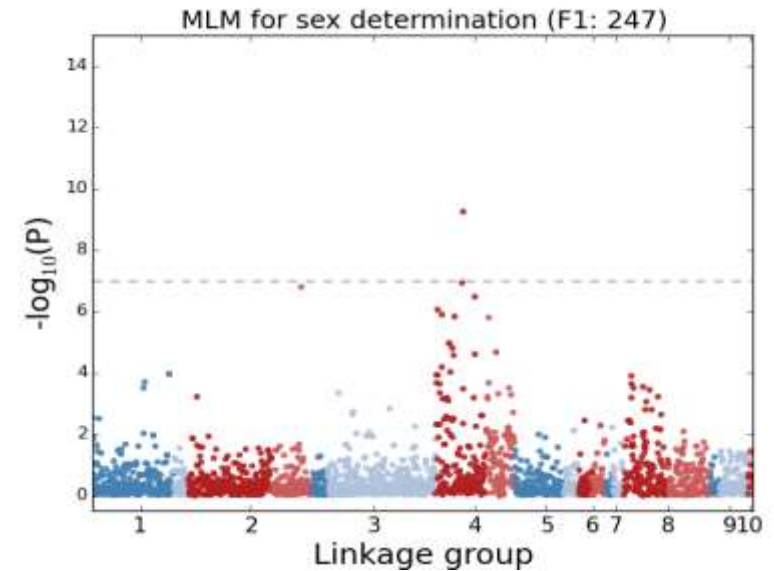
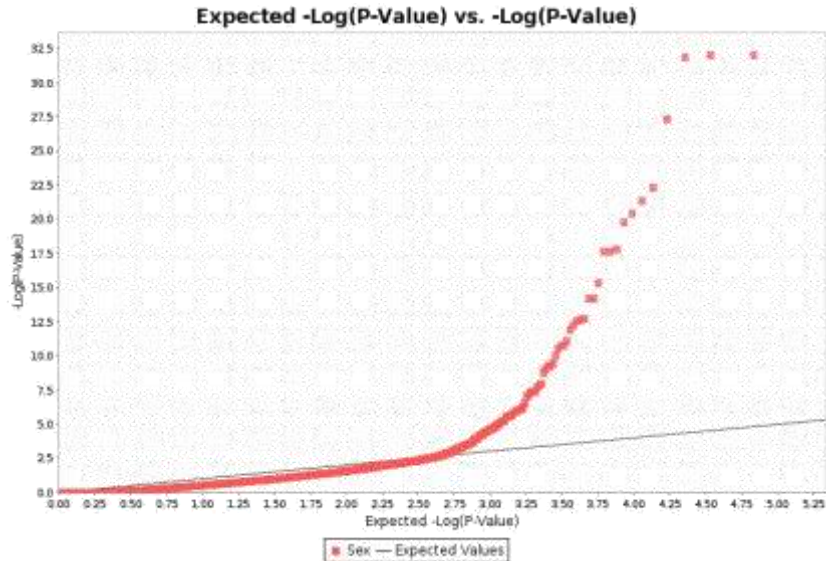




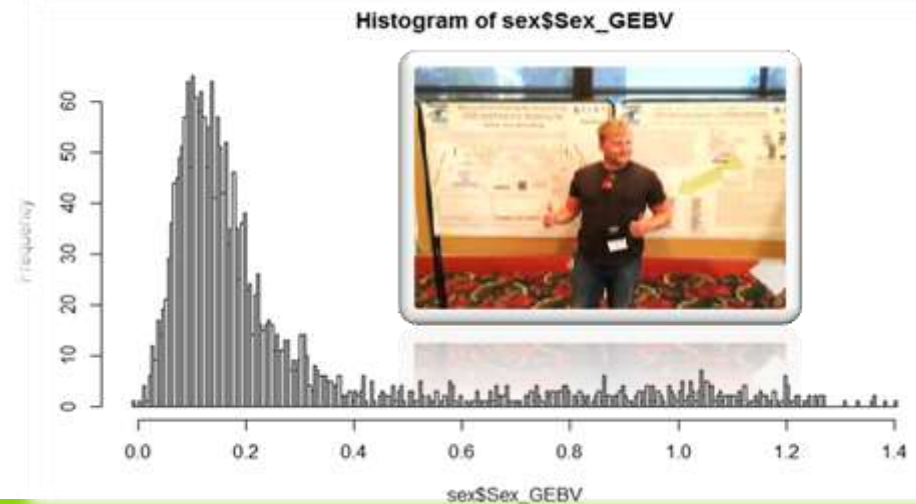
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# MLM and rrBLUP (GEBV) for sex\$Sex

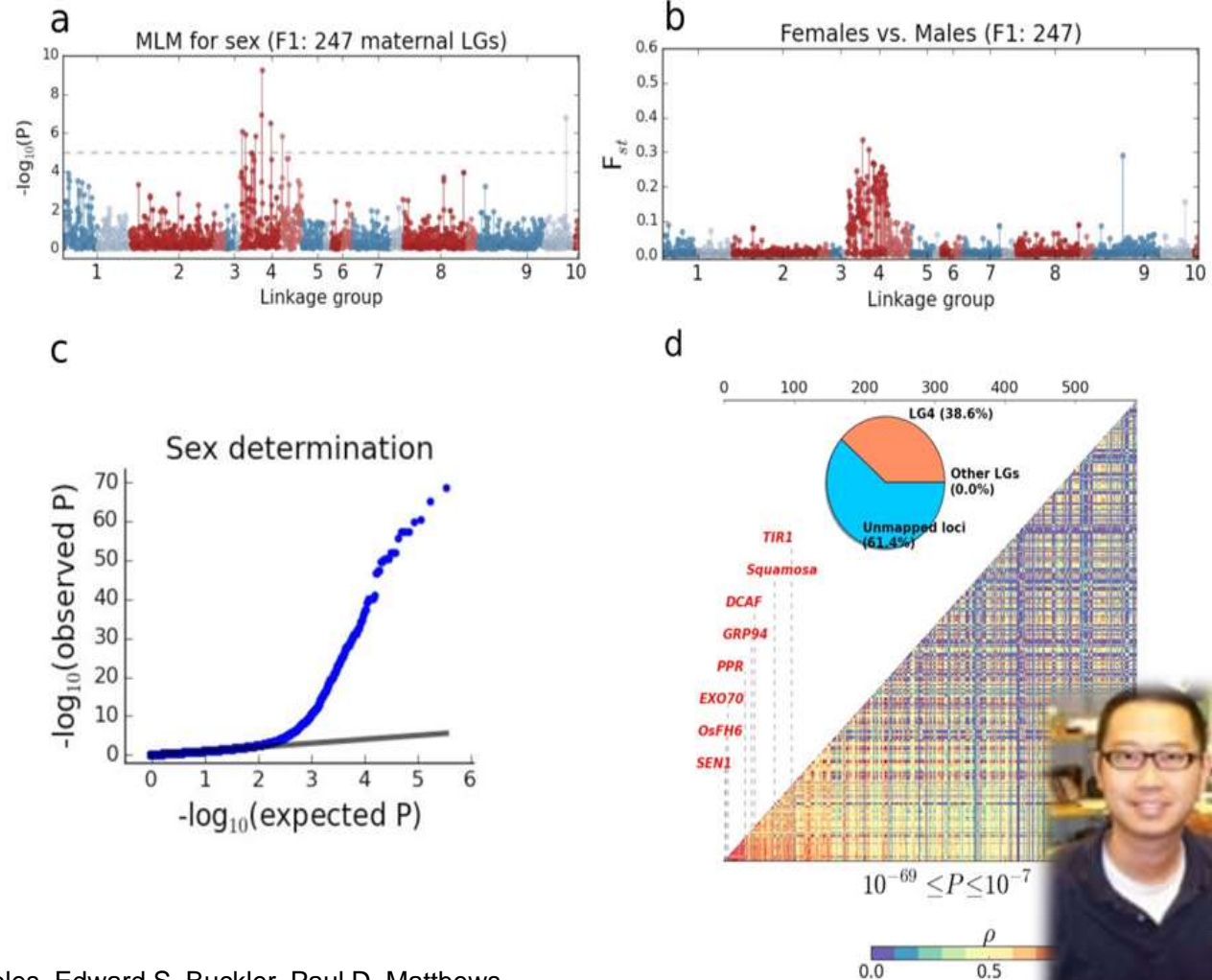


- REFERENCES**
- Matthews PD, Coles MC, Pitra NJ, [Next Generation Sequencing for a Plant of Great Tradition: Application of NGS to SNP Detection and Validation in Hops \(\*Humulus lupulus\* L.\)](#), 2013, *Monatsschrift für Brauwissenschaft*, 66:8
- Glaubitz JC, Casstevens TM, Lu F, Harriman J, Elshire RJ, Sun Q, Buckler ES (2014) TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PLoS One* 9(2):e90346
- Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6:e19379
- Rawlings JO, Pantula SG, Dickey DA (1998) *Applied regression analysis: A research tool*, 2nd edn. Springer, New York
- Whittaker JC, Thompson R, Denham MC (2000) Marker-assisted selection using ridge regression. *Genet Res* 75:249–252



# Candidate Genes for Male Flower Development Found

1. Glucose-regulated protein 94 (GRP94)-like protein on scaffold LD152823 that is known in *Arabidopsis* affecting shoot apical meristems, floral meristems and pollen tube elongation
2. Squamosa-like protein, identified on scaffold LD147778, has essential roles in vegetative phase change and flower development in multiple plants



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Non-Mendelian inheritance of SNP markers reveals extensive chromosomal translocations in dioecious hops. 2016 bioRxiv.



# 80% of breeding effort: Breeding for disease resistance

## ➤ Plant microbial diseases

- ❖ Powder mildew (*Podosphaera macularis*)
- ❖ Downy mildew (*Pseudoperonospora humuli*)
- ❖ Viruses and viroids (stunt viroid)



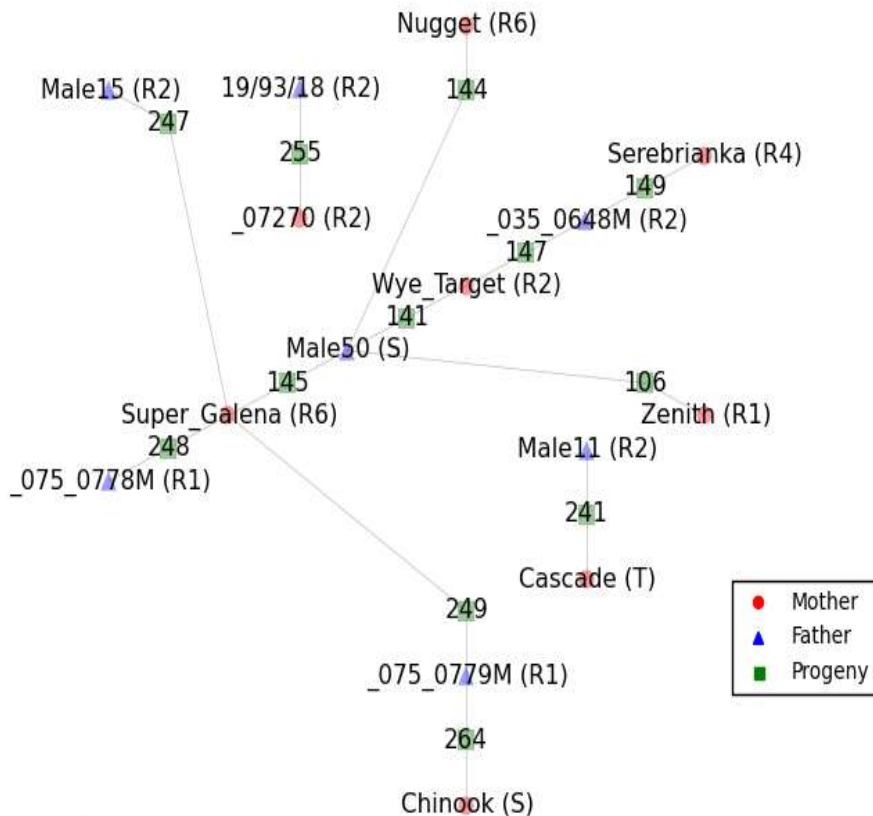
# Powdery Mildew GEBVs

A set of 1224 half-siblings were used a nested association panel across six known powdery mildew resistance genes

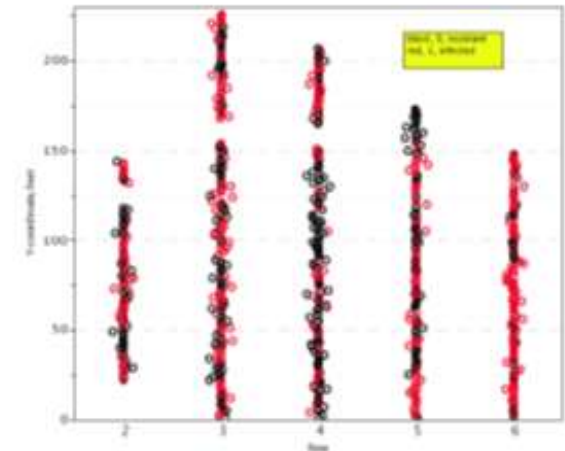
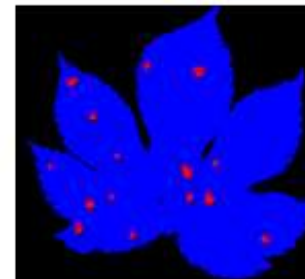
| Gene        | Source       | Status in USA  |
|-------------|--------------|----------------|
| R1, R3, Rb  | Zenith       | Tolerance      |
| R2          | Wye Target   | Resistance     |
| R4          | Early Choice | Tolerance      |
| R5          | Cascade      | Tolerance      |
| R6          | Nugget       | Broken         |
| 19058mR6    |              | Broken         |
| Kazak 2000R | Kazak 2000   | Resistant, HSR |

Might stacked resistance genes confer durable resistance/tolerance?

# Nested Powdery Mildew Mapping Families

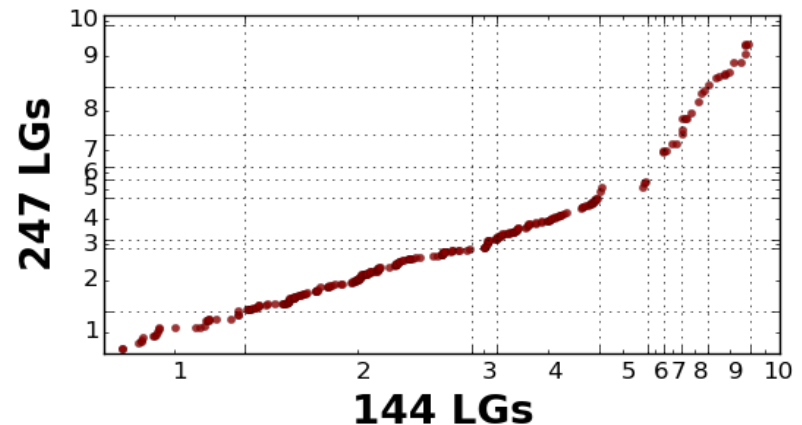
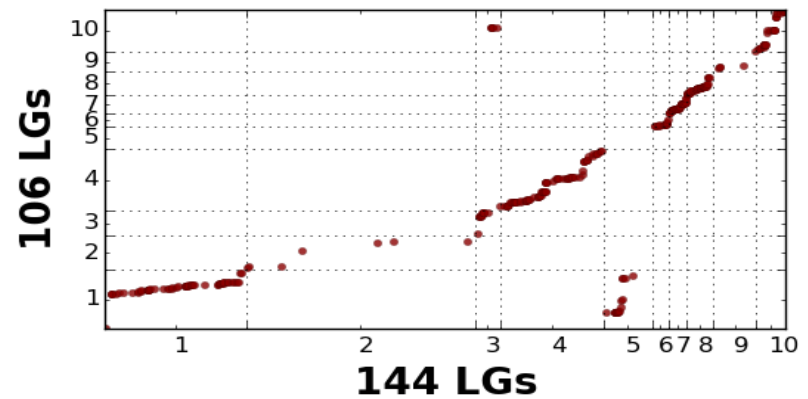
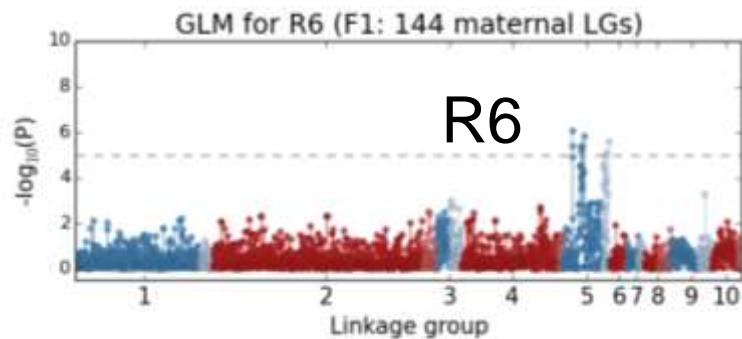
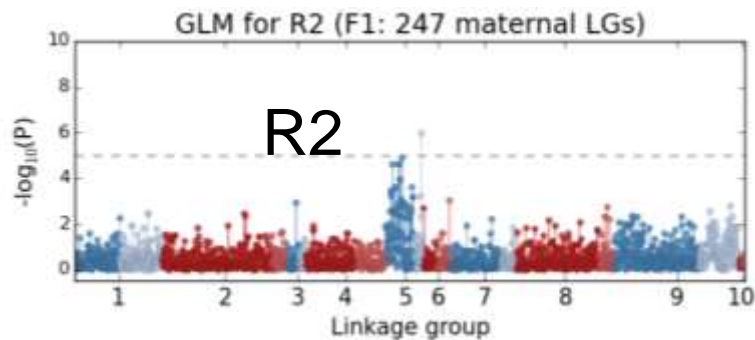
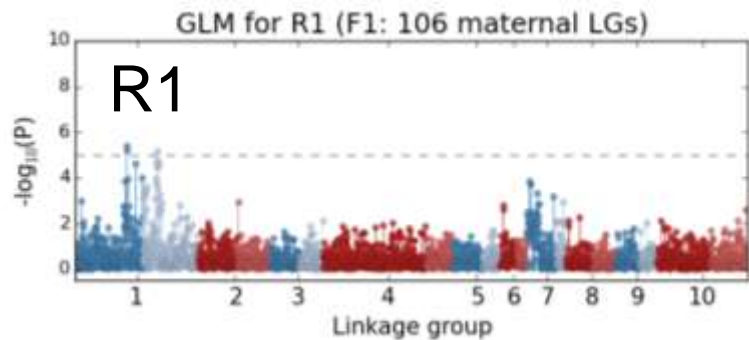


Digital metrics of powdery mildew disease index lesion area/leaf area used as quantitative trait



- ✓ Qualitative field scores were corrected for infection hotspots
- ✓ Among six nested families- all showed expected Mendelian segregation ratios

# Powdery Mildew Resistance Genes, R1, R2, R6 mapped



# Conclusions:

- ✓ GEBVs could be a new evaluation tool for hop breeding potential, especially in male selection
- ✓ An efficient, high density molecular marker system has been qualified and validated for hops
- ✓ Segregation distortion in hops is extensive and real
- ✓ GEBVs have been developed for sex and powdery mildew tolerance
- ✓ Candidate genes have been identified for sex and PMT





# Advance and sustainable bitter acids and yield

| Variety                         | Galena           | Super Galena  | 07270       | Zeus             | Apollo           | Bravo            |
|---------------------------------|------------------|---------------|-------------|------------------|------------------|------------------|
| Alpha acids<br>% w/w            | 10.0 - 13.5      | 13.0 - 16.0   | 18-20       | 12.0 - 16.5      | 15.0 - 19.0      | 14.0 - 17.0      |
| Beta acids<br>% w/w             | 7.0 - 9.0        | 8.0 - 10.0    | 4.5-6.0     | 4.0 - 6.0        | 5.5 - 8.0        | 3.0 - 5.0        |
| CoH % w/w<br>of $\alpha$ -acids | 35 - 40          | 35 - 40       | 27-29       | 27 - 35          | 24 - 28          | 29 - 34          |
| Total Oil<br>ml/100g            | 0.9 - 1.2        | 1.5 - 2.5     | 3.0         | 1.0 - 2.0        | 1.5 - 2.5        | 1.6 - 2.4        |
| Stability                       | 75 - 80%         | 75 - 80%      | 85%         | 50 - 60%         | 80 - 90%         | 60 - 70%         |
| Powdery<br>Mildew*              | Susceptible      | Resistant     | Resistant   | Susceptible      | Resistant        | Resistant        |
| Yield<br>lbs/acre               | 1,600 -<br>2,220 | 2,500 - 2,800 | 2,500-3,400 | 2,400 -<br>3,000 | 2,600 -<br>3,000 | 2,700 -<br>3,100 |



# Advanced sustainable aroma and flavor

| Variety                         | Casc.     | Calypso       | Cent.       | Lemondrop     | EUREKA!          | Denali           |
|---------------------------------|-----------|---------------|-------------|---------------|------------------|------------------|
| Alpha acids<br>% w/w            | 4.5-7.0   | 13.0 - 16.0   | 9.5-11.5    | 4.5-6.5       | 15-18            | 15-18            |
| Beta acids<br>% w/w             | 4.5-7.0   | 8.0 - 10.0    | 3.5-4.5     | 4.0-6.0       | 5.0-6.0          | 4.0-5.0          |
| CoH % w/w<br>of $\alpha$ -acids | 33-40     | 35 - 40       | 29-30       | 30-33         | 45-50            | 22-26            |
| Total Oil<br>ml/100g            | 0.8-1.5   | 1.5 - 2.5     | 1.5-2.3     | 1.5-2.0       | 1.5-2.0          | 4.0              |
| Stability                       | 48%       | 75 - 80%      | 45-55%      | 65%           | 80%              | 80%              |
| Powdery<br>Mildew*              | Tolerant  | Susceptible   | Tolerant    | Tolerant      | Resistant        | Tolerant         |
| Yield<br>lbs/acre               | 1600-2200 | 2,500 - 2,800 | 1,700-2,000 | 2,000 - 2,800 | 2,400 -<br>2,800 | 2,600 -<br>3,200 |

## Matthews's Lab

- ❑ Mark Coles, chemoanalytics, DNA
- ❑ Tiffany Pitra, sensory evaluation, administration, pathology
- ❑ Nicholi Pitra, Genomics, computing
- ❑ Rachel Jones, greenhouse, tissue culture

## Agronomy

- ❑ Roger Jeske, agronomist
- ❑ Ann Petro, propagation, field collections
- ❑ Danny Hallman, Paul Merritt, Tom Newhouse, growers

## Past Hopsteiner-funded postdocs and graduate students:

- ❑ Dr. Lina Maloukh ILVO – ILVO, Belgium
- ❑ Dr. Adam Kavalier – CUNY
- ❑ Dr. Shi-Biao Wu- CUNY
- ❑ Nicholi Pitra – UNLowa
- ❑ Jared Koelling – UNLowa
- ❑ Jana Naegel – NRC – Canada
- ❑ Dr. Shaun Clark – NRC – Canada
- ❑ Alex Feiner – Martin Luther U, IPB-Germany

## Collaborating PIs:

Dr. Edward Buckler 4<sup>th</sup>, IGD, Cornell  
Dr. Arne Heyerrick – UGhent – Belgium  
Dr. Edward Kennelly – CUNY  
Dr. Dwight Kincaid - CUNY  
Dr. Jonathan Page –NRC- Canada  
Dr. Fred Stevens, L. Pauling Institute  
Dr. Axel Schwekendiek, UNLowa  
Dr. Ryan Weil, Emory U.  
Dr. Ludger Wessjohann – IPB – Germany  
Dr. Oliver Yu, Danforth Center  
Dr. John Henning, ARS-USDA  
And Illumina



## Crop Improvement Program

### Current Hopsteiner Fellows:

- ❖ *Taylan Morcol, CUNY, Kennelly Lab*
- ❖ *Dr. Dong Zhang, Cornell, Buckler Lab*
- ❖ *Jenna Kahn, Harvey Mudd College*
- ❖ *Aurélie Muntzel, Binghamton, SUNY*

  
Hopsteiner®

Thank you very much.

*Simon H. Steiner, Hopfen, GmbH*

*S.S. Steiner, Inc.*

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