Hopsteiner®

Crop Improvement Program

Paul D. Matthews, Ph.D.

2014
NoCal-SoCal
Technical Conference

Humulus IV

IV

Yakima

ISHS

NCPN
National Clean Plant Network
HOPS

USHIPPC

USAHOPS
HOP GROWERS OF AMERICA

ISHS Section on Medicinal and Aromatic Plants in conjunction with the ISHS Commission on Plant Genetic Resources and the ISHS Commission on Fruits and Vegetables and Health presents:

IV International *Humulus* Symposium

to be held 6th – 8th August 2015 in Yakima, WA, USA,

under the auspices of the International Society for Horticultural Science (ISHS) in conjunction with the Hop Research Council (HRC) meetings to be held
4-5th August, 2015 at the same venue.

Hop Research Council

Research Organization

Matthews's Lab
- Mark Coles, chemoanalystics, 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 Meritt, Tom Newhouse, growers

Hopsteiner-funded postdocs and graduate students:
- Dr. Lina Maloukh ILVO – ILVO, Belgium
- Dr. Adam Kavalier – CUNY
- Dr. Shi-Biao Wu- CUNY
- Nicholi Pitra – UNIowa
- Jared Koellling – UNIowa
- Jana Naegel – NRC – Canada
- Dr. Shaun Clark – NRC – Canada
- Alex Feiner – Martin Luther U, IPB-Germany

Collaborating PIs:

Dr. Edward Buckler 4th, 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, UNIowa
Dr. Ryan Weil, Emory U.
Dr. Ludger Wessjohann – IPB – Germany
Dr. Oliver Yu, Danforth Center
And Illumina

Hopsteiner®

Crop Improvement Program

Hopsteiner Fellow, *postdoc position available: statistical genetics, biogeography, haplotype mapping;* ***Institute of Genomic Diversity, Cornell, Ithaca.***

Contact: pmatthews@hopsteiner.com

Tell them what you are going to tell them

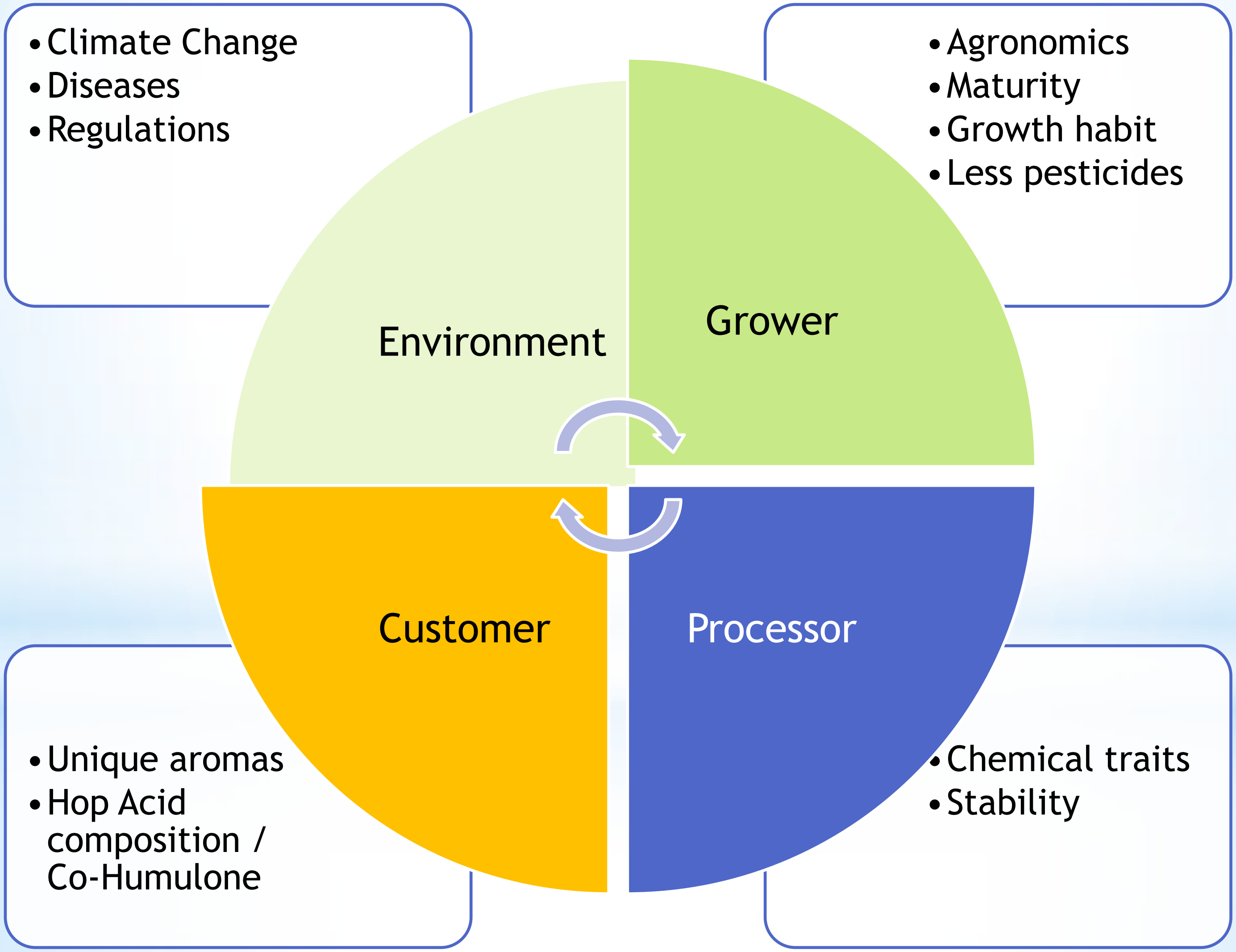
- What do brewers want in a new variety?
- Variety development goals, examples…
- What's needed for Good Breeding Practice:
  - Vertically Integrated Variety Development
  - HTS Phenometrics
  - HTS Chemometrics
  - HTS Genomics
  - Systems approach (Genome-Wide Association Studies)

Breeding Goals

- ✓ Consistency
- ✓ Bio-similarity
- ✓ Novelty
- ✓ Eco-specificty
- ✓ Nefarity
- ✓ Definition
- ✓ Popularity (Availability)
- ✓ Others?

- ✓ Sustainability
  - ☐ Yield gain
  - ☐ Water-use efficiency
  - ☐ Reduced energy input
  - ☐ Reduced carbon footprint
  - ☐ Reduced chemical input
  - ☐ Durability

Hop breeding must be integrated with production



- Climate Change
- Diseases
- Regulations

- Agronomics
- Maturity
- Growth habit
- Less pesticides

Environment

Grower

Customer

Processor

- Unique aromas
- Hop Acid composition / Co-Humulone

- Chemical traits
- Stability

Focus on Agronomy: Disease resistance, yield, pick-ability, processing, and storage, downstream products


JOHN DEERE
2355


2
2


3
8


QUICKPANEL II
DANGER

PROCESSING

PROCESSING

Focus Areas:

1. Chemistry
2. Agronomy (examples)
3. Aroma and Flavor (examples)

Breeding Goals…

Focus on Quality: advanced line profiles

Varieties	Chemical Profiles	Agronomic Traits
✓Delta ✓Calypso ✓Bravo ✓Apollo ✓Super Galena ✓Lemondrop ✓04190 ✓05256 ✓06277 ✓07270 ✓09238	✓Low CoH ✓Novel Aroma ✓High Oil Content ✓Select Polyphenols ✓Storage Stability	✓Durable DMR and PMR ✓Vigor ✓Virus Tolerance ✓Pickability ✓Compact Cones

Focus: Bitter acids and yield

Variety	Galena	Super Galena	07270	Zeus	Apollo	Bravo
Alpha acids % 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 % 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 of α-acids	35 - 40	35 - 40	27-29	27 - 35	24 - 28	29 - 34
Total Oil 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 Mildew*	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
Yield lbs/acre	1,600 - 2,220	2,500 - 2,800	2,500-3,400	2,400 - 3,000	2,600 - 3,000	2,700 - 3,100

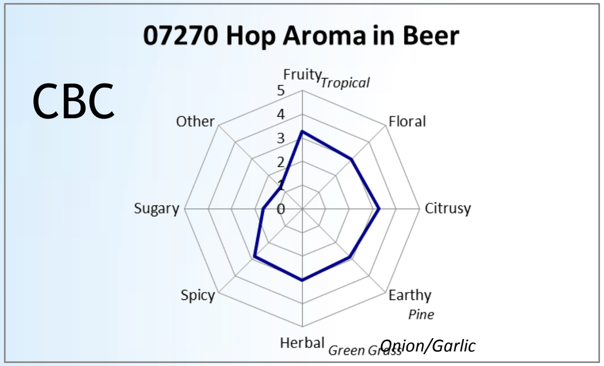
Focus: Aroma and flavor

Variety	Cascade	Calypso	Centennial	Lemondrop	06300	06277
Alpha acids % w/w	4.5-7.0	13.0 - 16.0	9.5-11.5	4.5-6.5	15-18	15-18
Beta acids % 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 of α-acids	33-40	35 - 40	29-30	30-33	45-50	22-26
Total Oil 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 Mildew*	Tolerant	Susceptible	Tolerant	Tolerant	Resistant	Tolerant
Yield lbs/acre	1600-2200	2,500 - 2,800	1,700-2,000	2,000 – 2,800	2,400 – 2,800	2,600 - 3,200

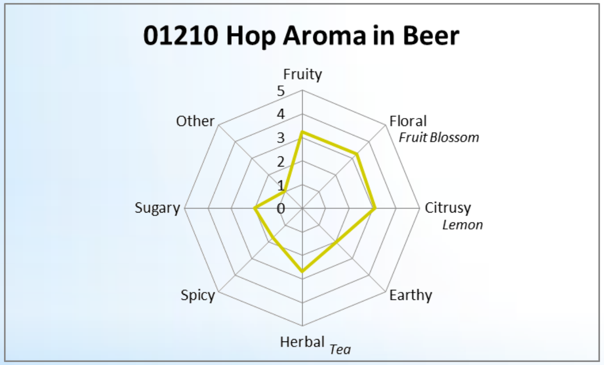
Sensory Evaluation
CBC and Hopsteiner Panel

HOP ID	Panelist 001	Panelist 002	Panelist 003	Panelist 004	Panelist 006	Panelist 007	Panelis 010
Lemondrop Green	spice, nutmeg, sharp	lemongrass	lemon, pine, pear	b g le m			
Lemondrop Dry	lemon, mint, spice	light lemon					

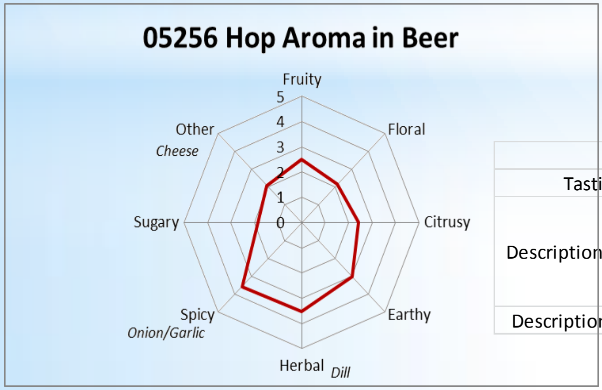
07270: *Onion/Garlic, Pine, Green Grass, Tropical*

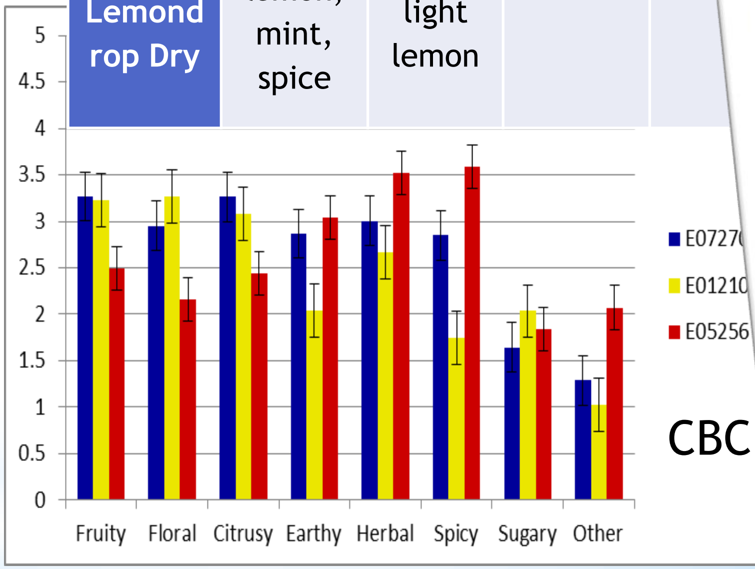


01210: *Lemon, Tea, Fruit Blossom*



05256: *Onion/Garlic, Cheese, Dill*





Lemondrop

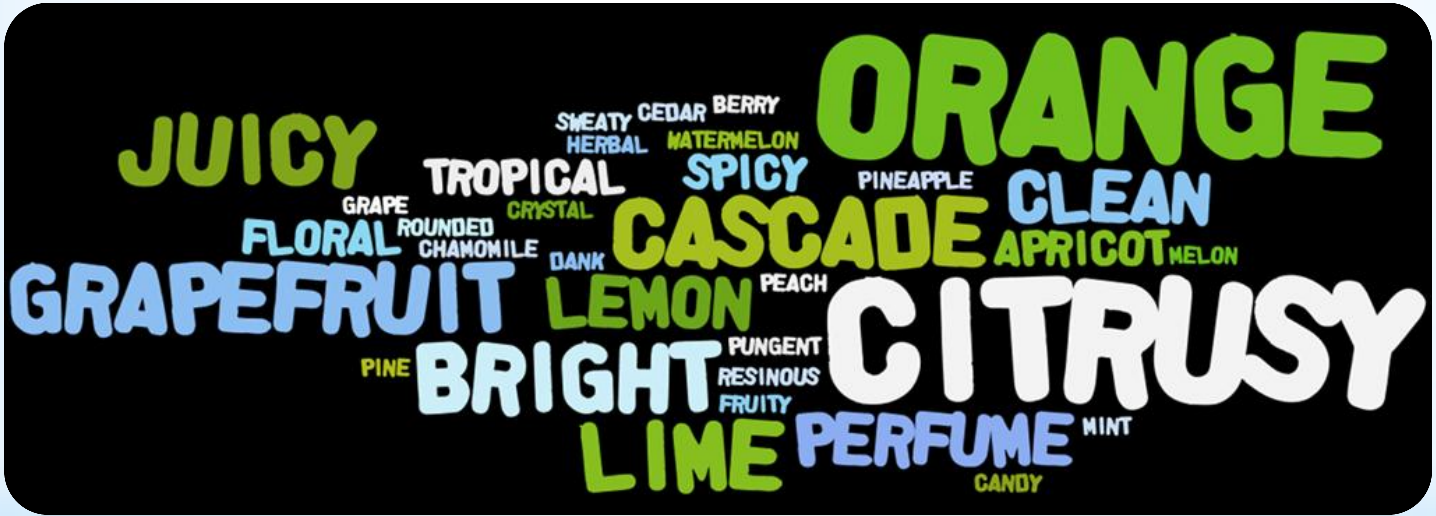
	E07270 (CBC)	E07270 (Panel)	E01210 (CBC)	E01210 (Panel)	E05256 (
Tasting Date	4/9/2014	3/21/2014	4/9/2014	3/21/2014	4/9/
Description of Hop Aroma	*Onion/Garlic, Pine, Grass, Tropical Fruit*	*Rose, Berry, Slight Orange, Spicy*	*Lemon, Tea, Fruit Blossom*	*Apple, Fruit Blossom, Citrus*	*Onion/(Cheese*
Description of Bitterness	*Rounded*	*Rounded*	*Rounded*	*Rounded*	*Linger*

STANDARDID	CATEGORY	False
Clove	Spicy	
Mango	Fruity	
Coriander	Spicy	
Green Grass	Earthy	
Pineapple	Fruity	
Green Tea	Herbal	
Grapefruit	Citrusy	
Pine	Earthy	
Melon	Fruity	
Anise	Spicy	
Orange	Citrusy	
ime	Citrusy	
Cedar	Earthy	
Green Apple	Fruity	
Coconut	Miscellaneou	
Cardamom	Spicy	
Banana	Fruity	
Vanilla	Spicy	
emon	Cirusy	
Rose	Foral	
Pear	Fruity	
Guavea	Fruity	
Grape	Fruity	
Dill	Spicy	
pearmint	Spicy	

Experimental Variety 09326

2014 Most Popular Experimental Hop

(voted 2nd favorite in 2013)



Alpha: 6-7%
Beta: 5-7.5%
CoH: 28-30%
Total Oil: 2.0-3.0
Av. Yield: 2000 lbs/acre
Tolerant to PM in greenhouse
Roza MHN: 80-hills

Experimental Variety 06277

2nd Most Popular Experimental Hop

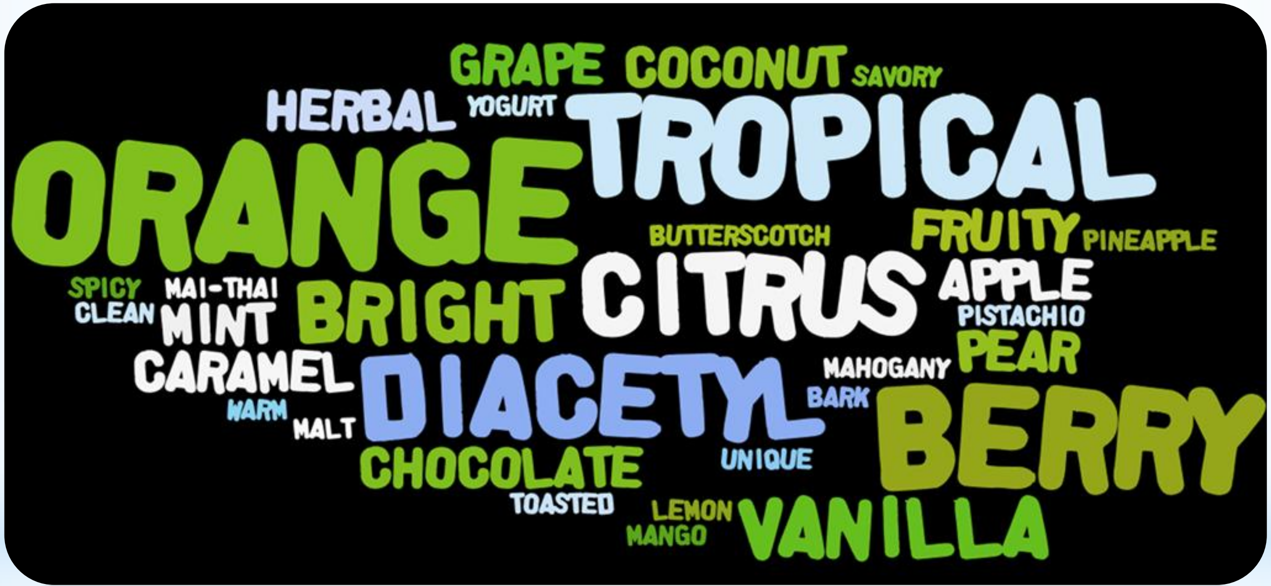
(voted 1st favorite in 2013)

Alpha: 15-17%   Beta: 4-5%
CoH: 22-25%   Total Oil: 4.0-6.0
Av. Yield: 3500 lbs/acre
Susceptible to PM in greenhouse
Emerald 5-acre; Roza MHN: 80-hills

Experimental Variety 06297

3rd Most Popular Experimental Hop

(voted 3rd favorite in 2013)



Alpha: 17-19% Beta: 5.5-7%
CoH: 33-36% Total Oil: 2.0-4.0
Av. Yield: 2600 lbs/acre
Resistant to PM in greenhouse
Roza MHN: 80-hills

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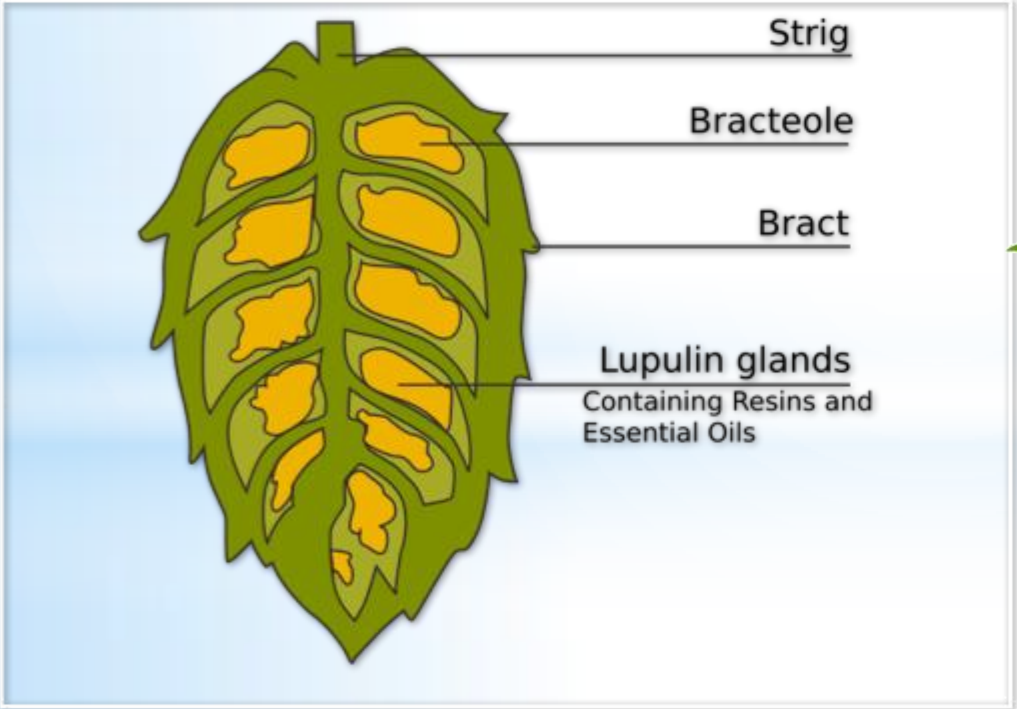
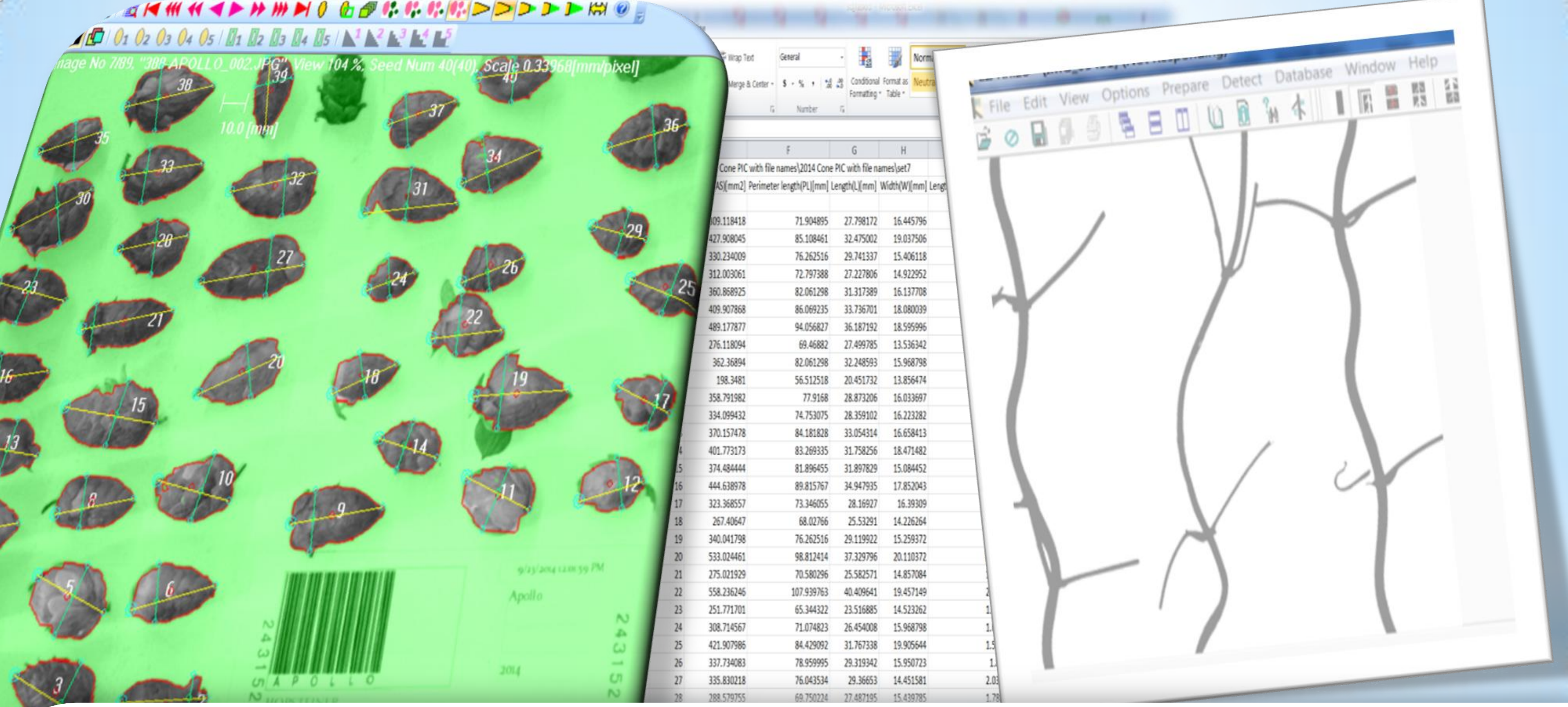
Cone development and variation





Cone PIC with file names\2014 Cone PIC with file names\set7

	AS(mm2]	Perimeter length(PL)[mm]	Length(L)[mm]	Width(W)[mm]	Lengt
	09.118418	71.904895	27.798172	16.445796	
	427.908045	85.108461	32.475002	19.037506	
	330.234009	76.262516	29.741337	15.406118	
	312.003061	72.797388	27.227806	14.922952	
	360.868925	82.061298	31.317389	16.137708	
	409.907868	86.069235	33.736701	18.080039	
	489.177877	94.056827	36.187192	18.595996	
	276.118094	69.46882	27.499785	13.536342	
	362.36894	82.061298	32.248593	15.968798	
	198.3481	56.512518	20.451732	13.856474	
	358.791982	77.9168	28.873206	16.033697	
	334.099432	74.753075	28.359102	16.223282	
	370.157478	84.181828	33.054314	16.658413	
4	401.773173	83.269335	31.758256	18.471482	
15	374.484444	81.896455	31.897829	15.084452	
16	444.638978	89.815767	34.947935	17.852043	
17	323.368557	73.346055	28.16927	16.39309	
18	267.40647	68.02766	25.53291	14.226264	
19	340.041798	76.262516	29.119922	15.259372	
20	533.024461	98.812414	37.329796	20.110372	
21	275.021929	70.580296	25.582571	14.857084	
22	558.236246	107.939763	40.409641	19.457149	2
23	251.771701	65.344322	23.516885	14.523262	1
24	308.714567	71.074823	26.454008	15.968798	1.
25	421.907986	84.429092	31.767338	19.905644	1.5
26	337.734083	78.959995	29.319342	15.950723	1.
27	335.830218	76.043534	29.36653	14.451581	2.03
28	288.579755	69.750224	27.487195	15.439785	1.78

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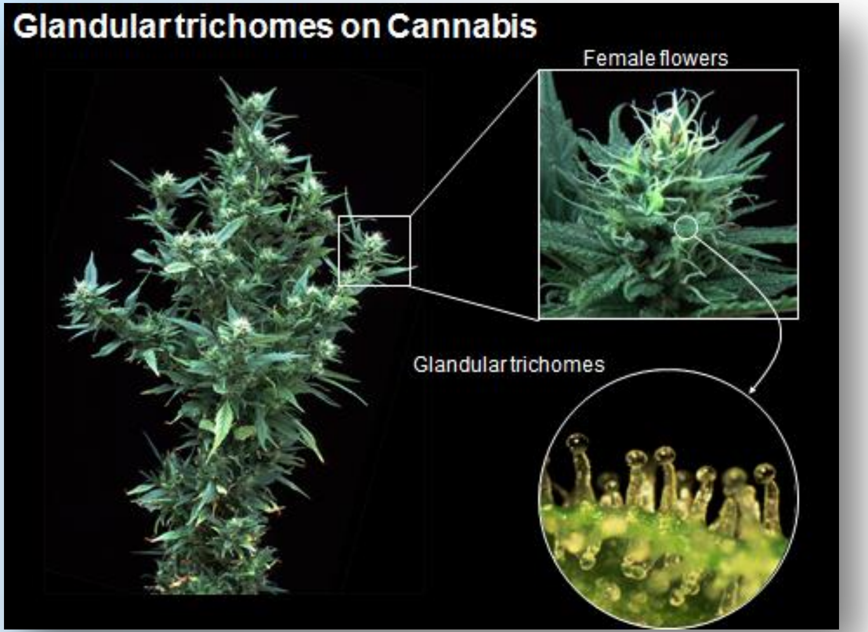
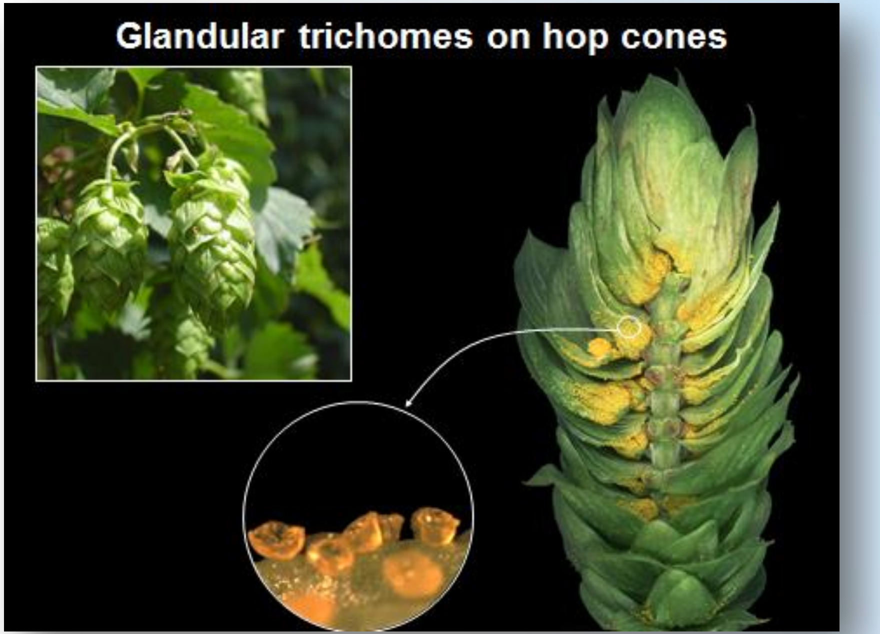
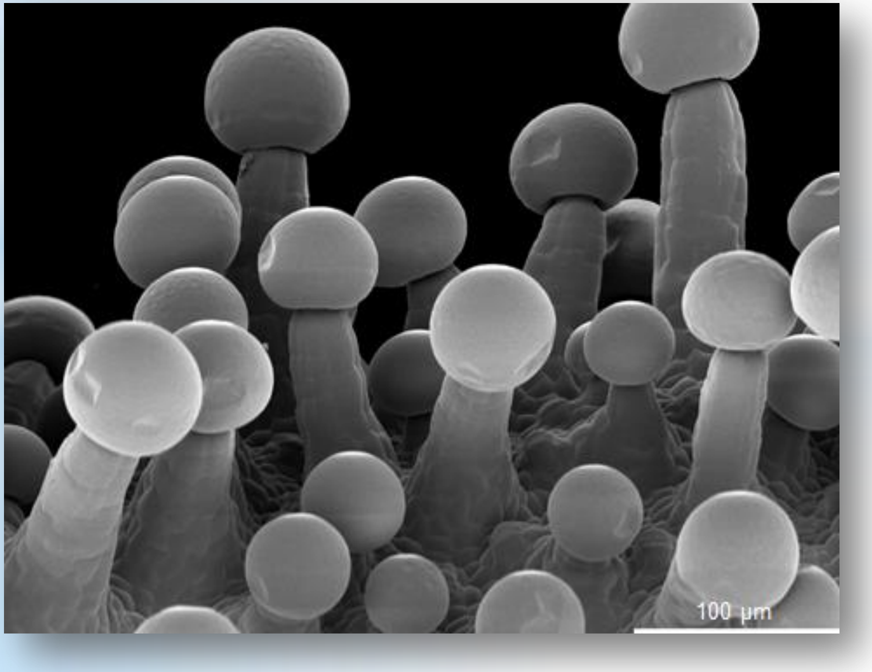
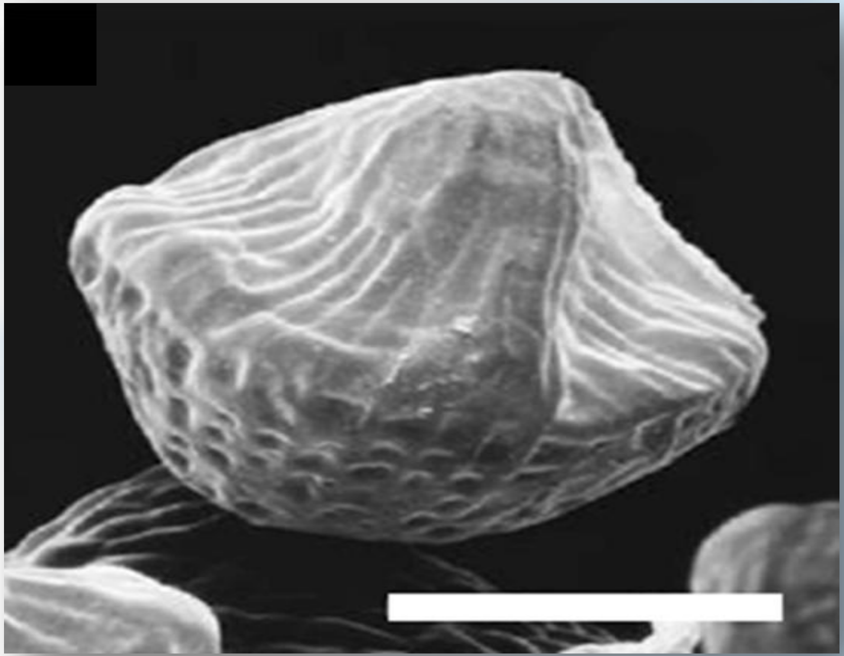


Glandular trichomes on Cannabis



Glandular trichomes on hop cones





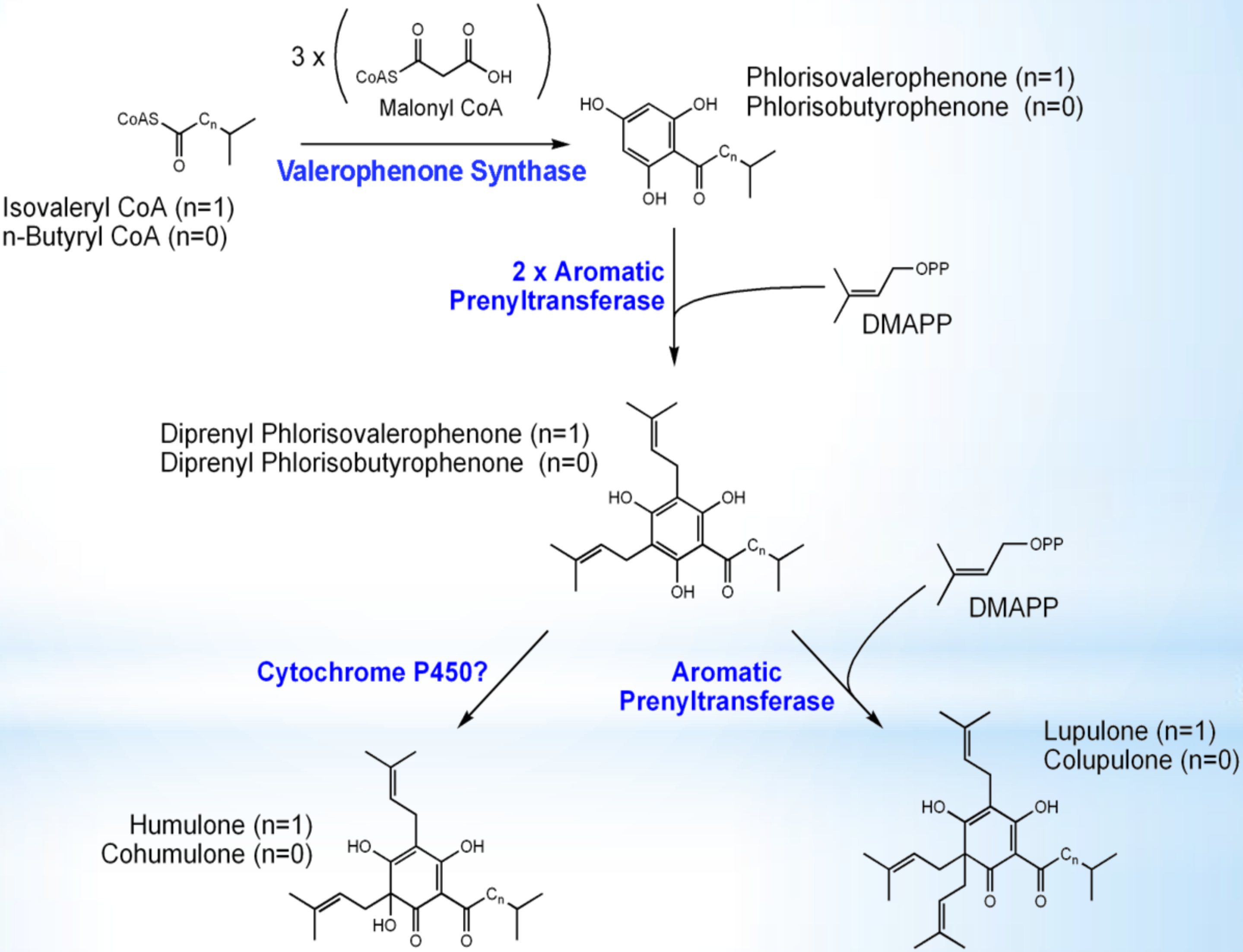
Chemical Diversity: Unique Flavors in Perpetuity

- The bitter acids of hops are terpenophenolics
- Diverse polyphenols contribute to haze, foam stability and bitterness
- Volatile terpenes are aromas
- Many flavors come from:
  - Aldehydes
  - Lactones
  - Norcarotenoids
  - Thiols
  - Esters
  - Flavanol glucosides
  - Many, many others

Roots of Diversity

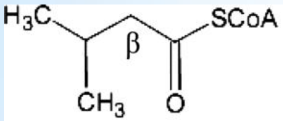
“Bitter” acids

- Humulone
- Prehumulone
- Posthumulone
- Cohumulone
- Adhumulone
- Etc.

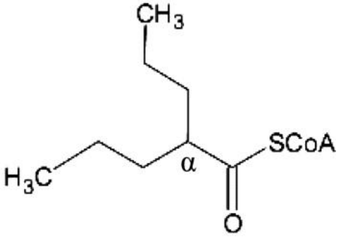


From where do the various forms come?

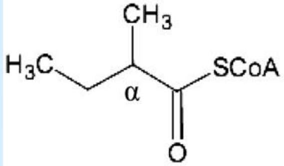
Valerophenone synthase is promiscuous!



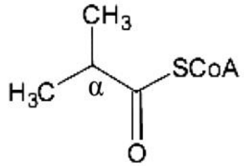
Isovaleryl-CoA



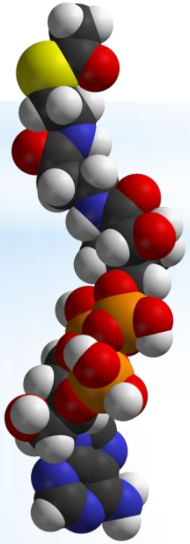
Valproyl-CoA

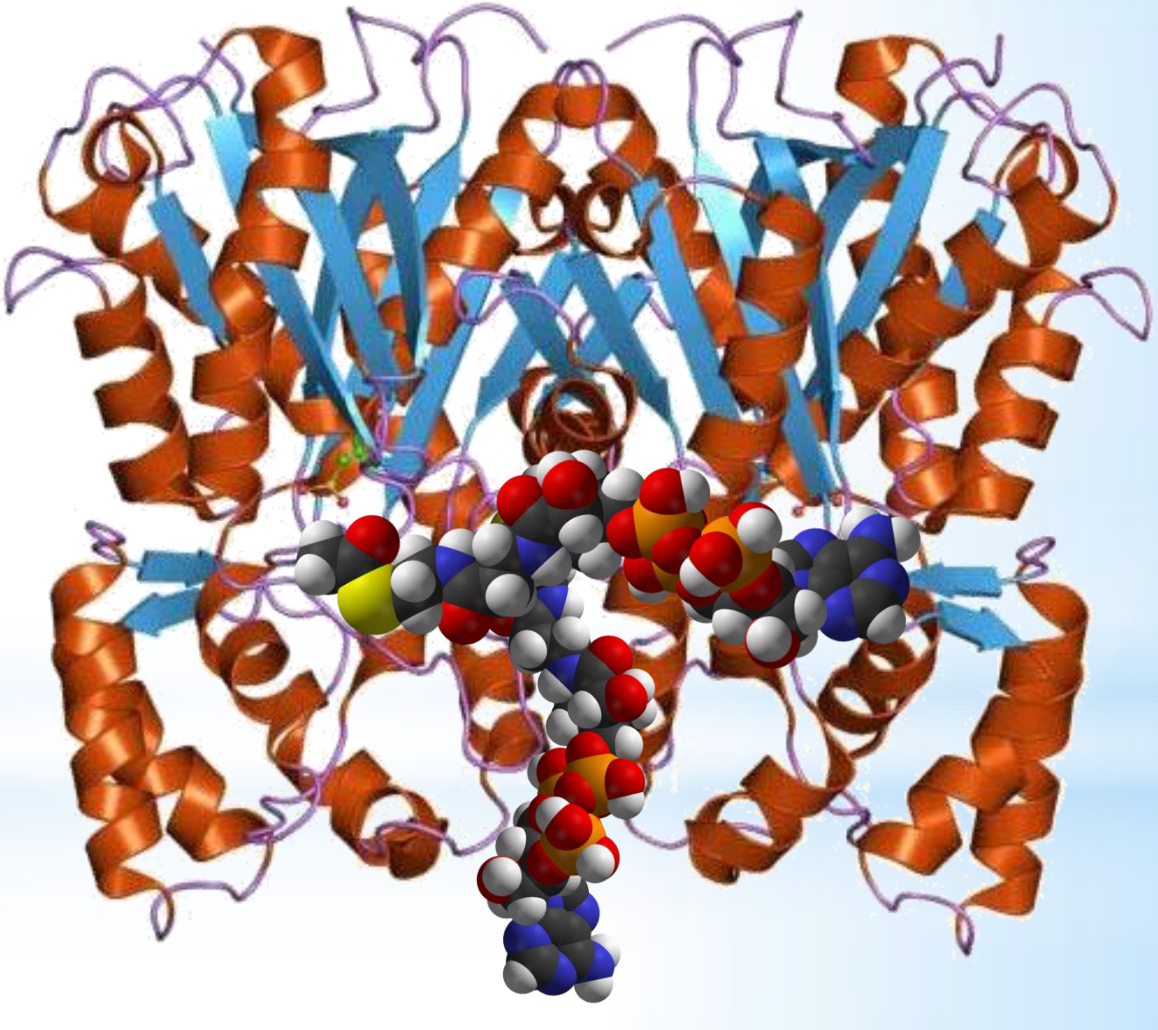


2-Methylbutyryl-CoA



Isobutyryl-CoA





The Plant Cell, Vol. 20: 186–200, January 2008, www.plantcell.org © 2008 American Society of Plant Biologists

EST Analysis of Hop Glandular Trichomes Identifies an *O*-Methyltransferase That Catalyzes the Biosynthesis of Xanthohumol [W][OA]

Jana Nagel,[a,b] Lana K. Culley,[a] Yuping Lu,[a] Enwu Liu,[a] Paul D. Matthews,[c] Jan F. Stevens,[d,e] and Jonathan E. Page[a,1]

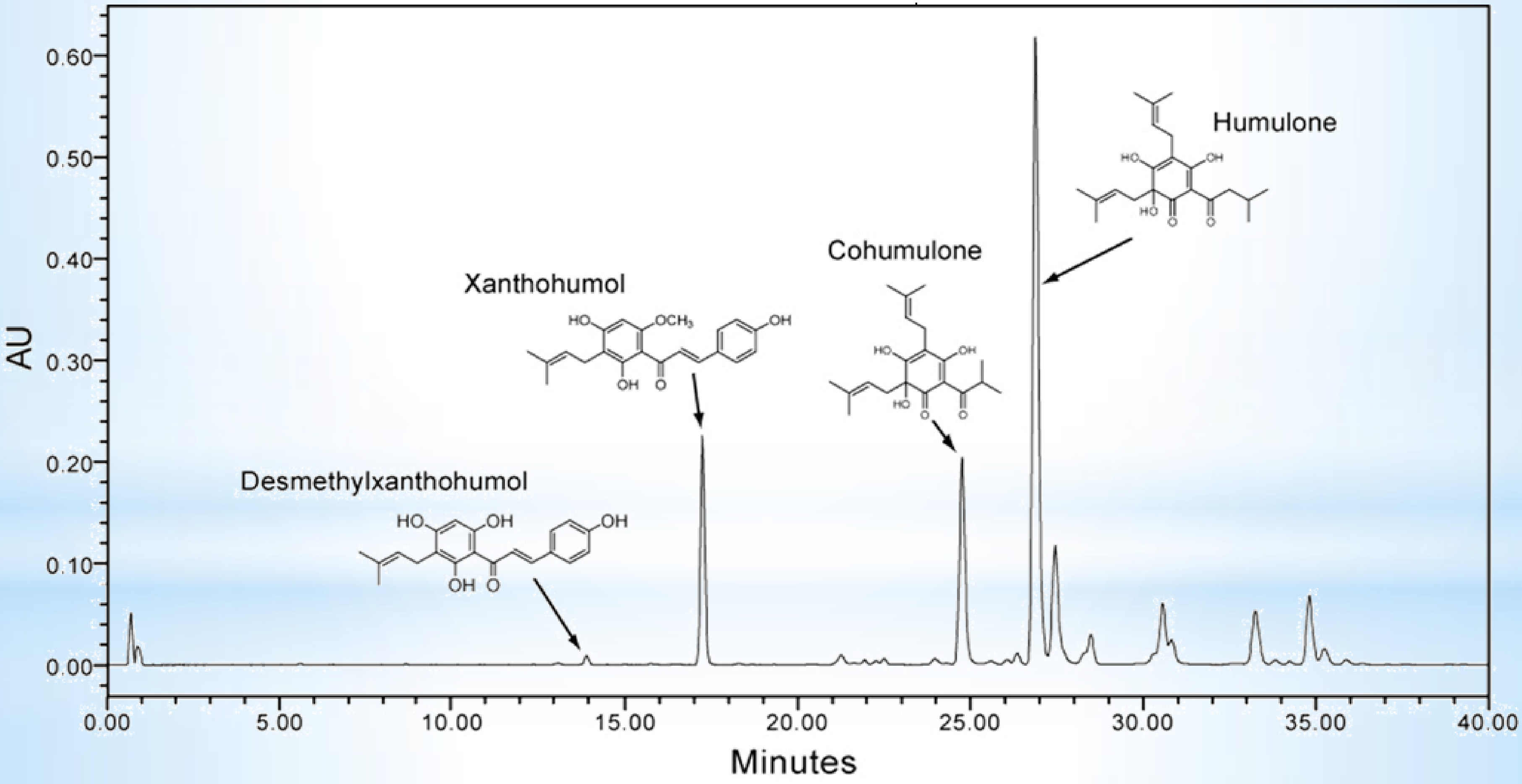
[a] National Research Council–Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada S7N 0W9
[b] Leibniz Institute of Plant Biochemistry, 06120 Halle/Saale, Germany
[c] Hopsteiner, S.S. Steiner, New York, New York 10065
[d] Department of Pharmaceutical Sciences, Oregon State University, Corvallis, Oregon 97331
[e] Linus Pauling Institute, Oregon State University, Corvallis, Oregon 97331

The glandular trichomes (lupulin glands) of hop (*Humulus lupulus*) synthesize essential oils and terpenophenolic resins, including the bioactive prenylflavonoid xanthohumol. To dissect the biosynthetic processes occurring in lupulin glands, we sequenced 10,581 ESTs from four trichome-derived cDNA libraries. ESTs representing enzymes of terpenoid biosynthesis, including all of the steps of the methyl 4-erythritol phosphate pathway, were abundant in the EST data set, as were ESTs for the known type III polyketide synthases of bitter acid and xanthohumol biosynthesis. The xanthohumol biosynthetic pathway involves a key *O*-methylation step. Four *S*-adenosyl-L-methionine–dependent *O*-methyltransferases (OMTs) with similarity to known flavonoid-methylating enzymes were present in the EST data set. OMT1, which was the most highly expressed OMT based on EST abundance and RT-PCR analysis, performs the final reaction in xanthohumol biosynthesis by methylating desmethylxanthohumol to form xanthohumol. OMT2 accepted a broad range of substrates, including desmethylxanthohumol, but did not form xanthohumol. Mass spectrometry and proton nuclear magnetic resonance analysis showed it methylated xanthohumol to 4-*O*-methylxanthohumol, which is not known from hop. OMT3 was inactive with all substrates tested. The lupulin gland-specific EST data set expands the genomic resources for *H. lupulus* and provides further insight into the metabolic specialization of glandular trichomes.

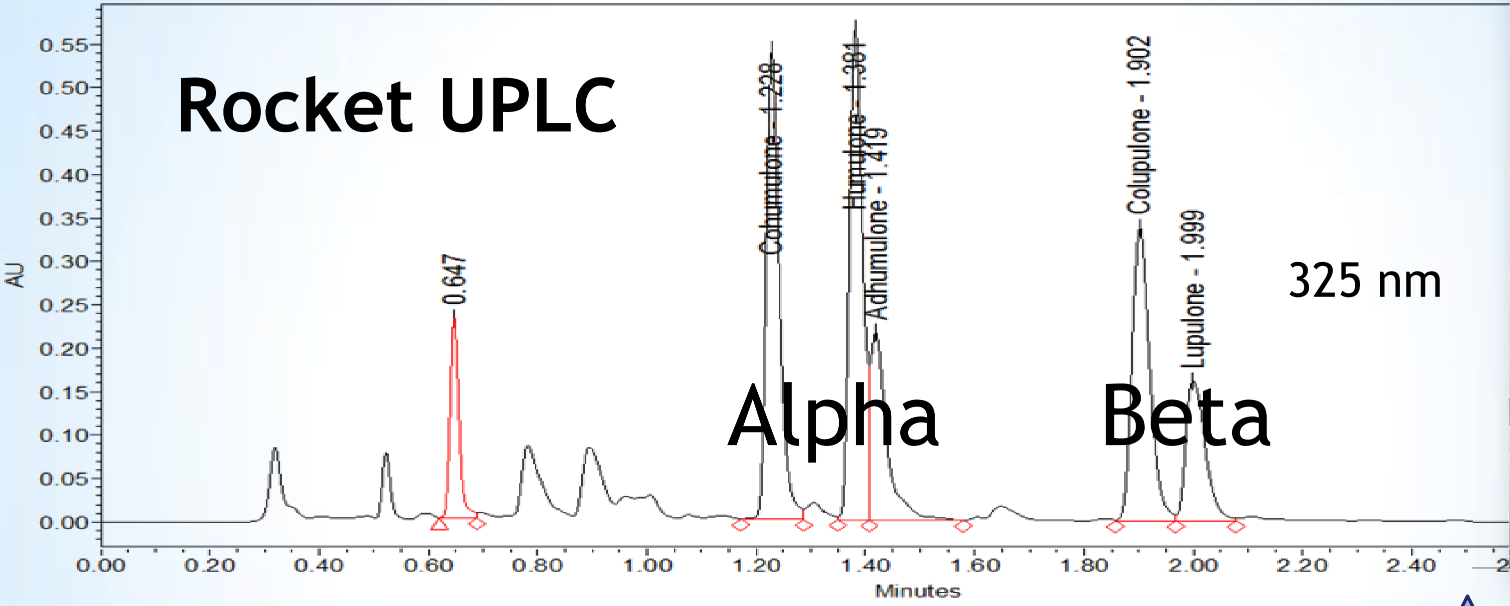
ASBC HPLC of Bitter Acids

Classic Separation

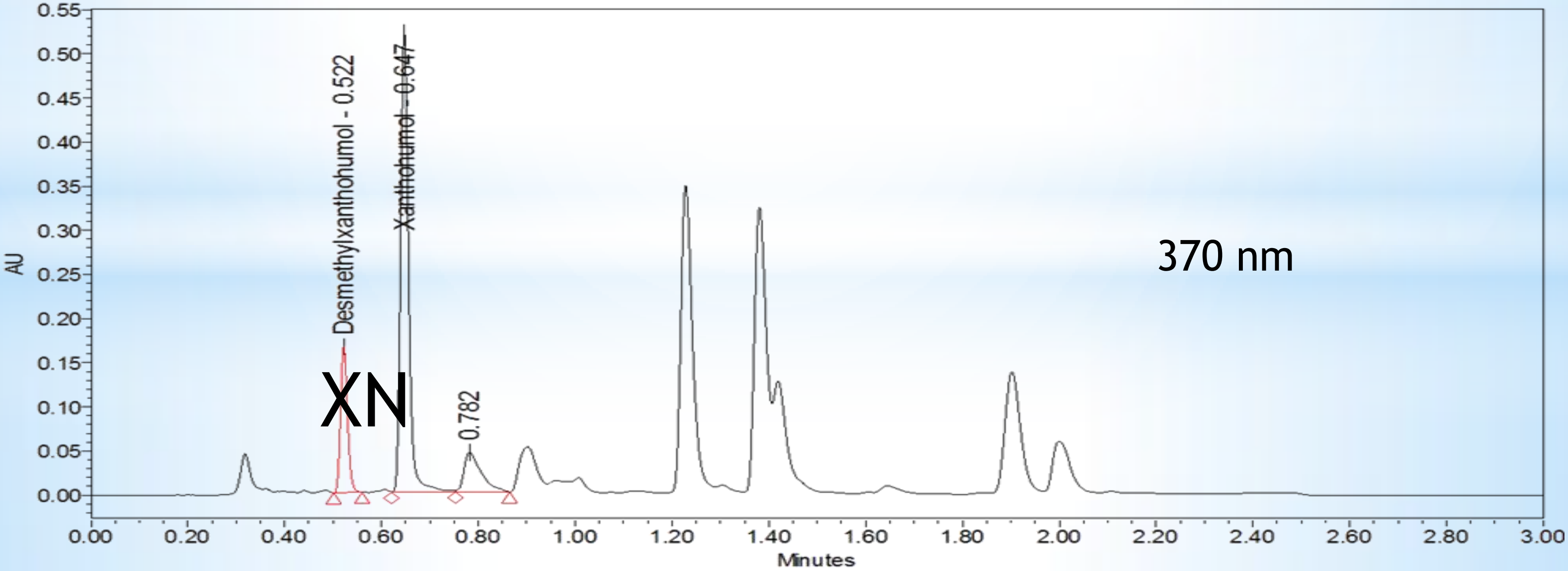
- ❑ Reverse phase, C18
- ❑ Isocratic (MeOH, H3PO4)
- ❑ Long separation time



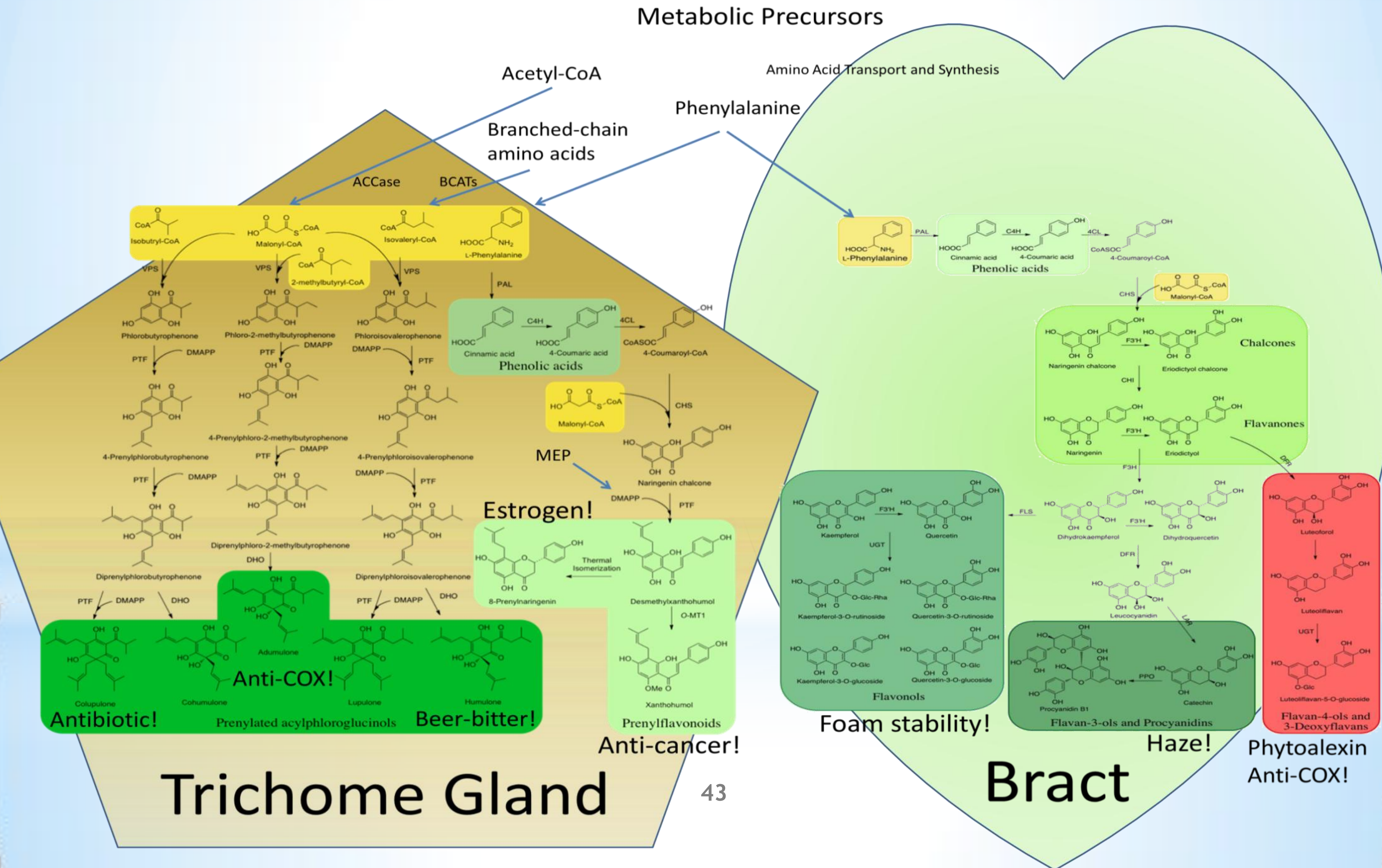
Rocket UPLC



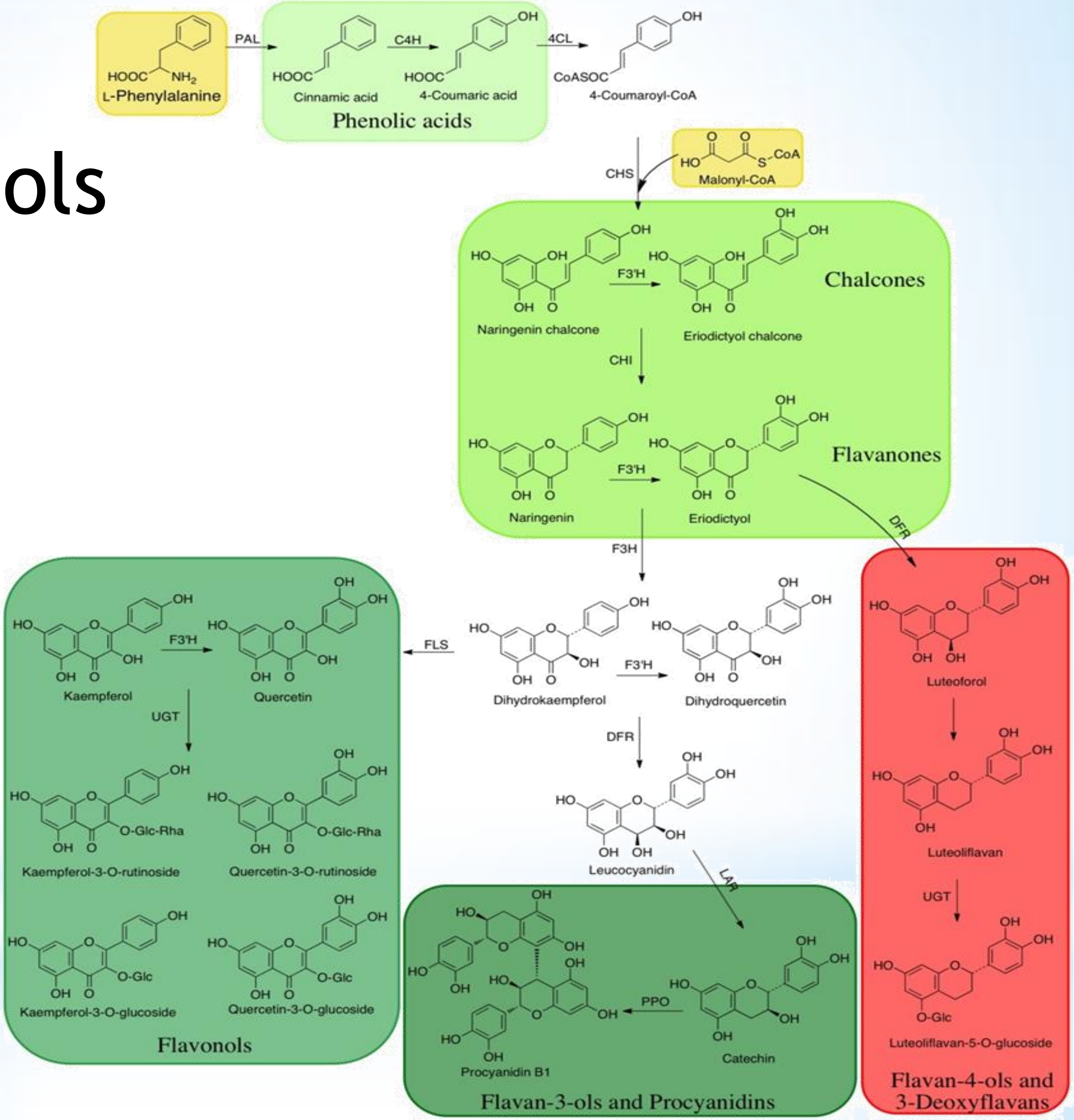
△ 2.5 minutes



Polyphenols

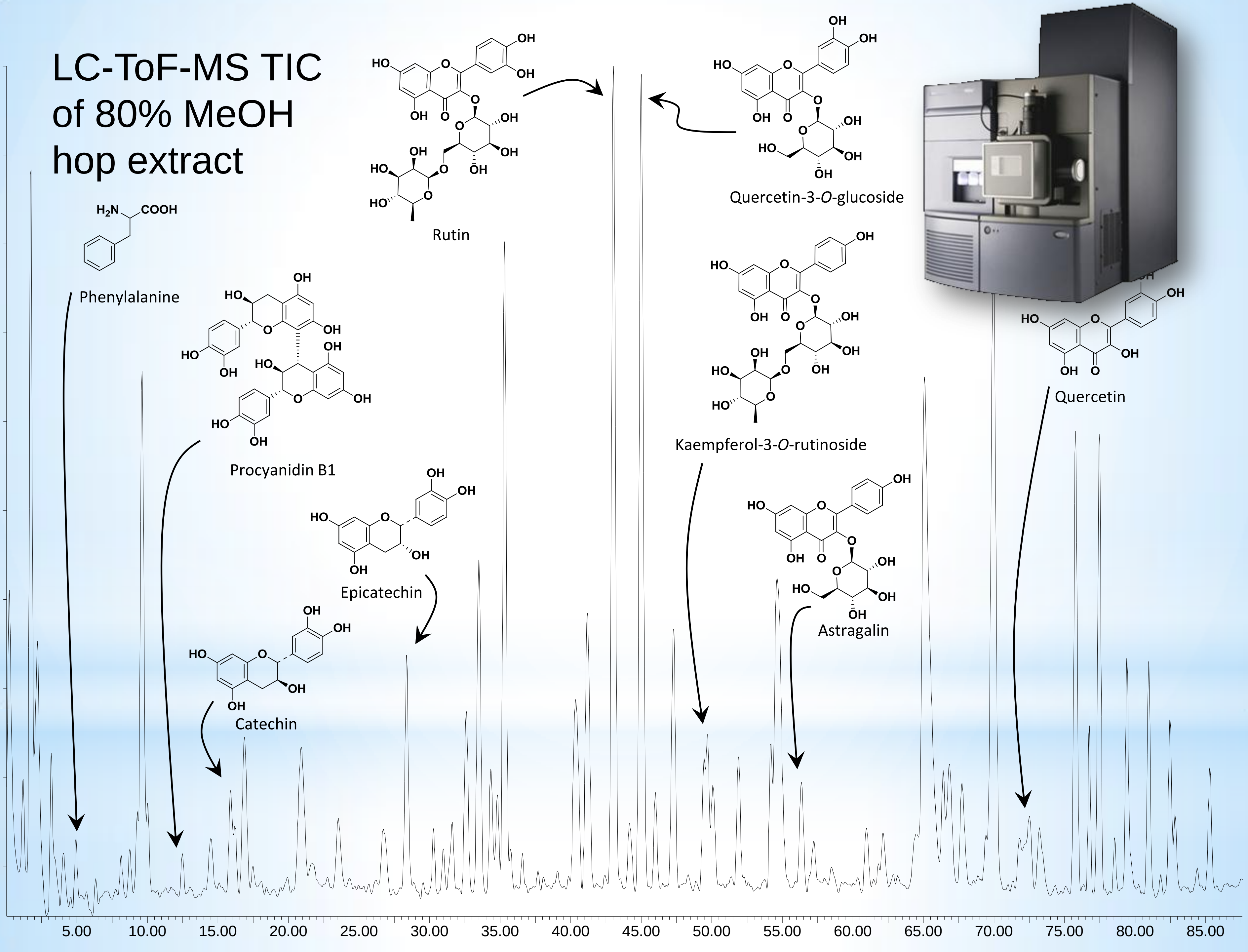


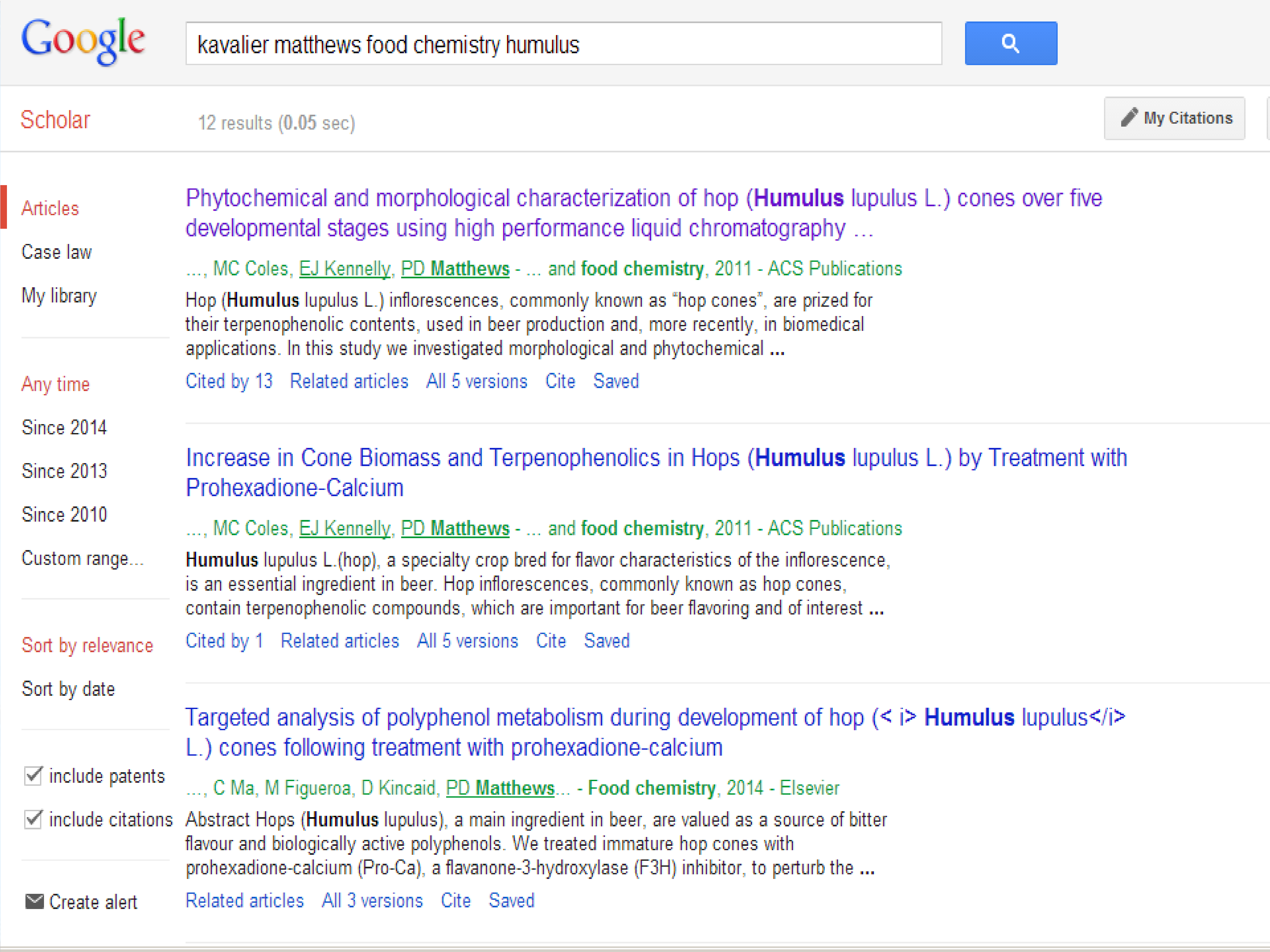
Polyphenols



Tannins

LC-ToF-MS TIC of 80% MeOH hop extract





kavalier matthews food chemistry humulus

12 results (**0.05** sec)

☑ include patents

☑ include citations

Phytochemical and morphological characterization of hop (**Humulus** lupulus L.) cones over five developmental stages using high performance liquid chromatography ...

..., MC Coles, EJ Kennelly, PD **Matthews** - ... and **food chemistry**, 2011 - ACS Publications

Hop (**Humulus** lupulus L.) inflorescences, commonly known as "hop cones", are prized for their terpenophenolic contents, used in beer production and, more recently, in biomedical applications. In this study we investigated morphological and phytochemical **...**

Cited by 13 Related articles All 5 versions Cite Saved

Increase in Cone Biomass and Terpenophenolics in Hops (**Humulus** lupulus L.) by Treatment with Prohexadione-Calcium

..., MC Coles, EJ Kennelly, PD **Matthews** - ... and **food chemistry**, 2011 - ACS Publications

Humulus lupulus L.(hop), a specialty crop bred for flavor characteristics of the inflorescence, is an essential ingredient in beer. Hop inflorescences, commonly known as hop cones, contain terpenophenolic compounds, which are important for beer flavoring and of interest **...**

Cited by 1 Related articles All 5 versions Cite Saved

Targeted analysis of polyphenol metabolism during development of hop (< i> **Humulus** lupulus</i> L.) cones following treatment with prohexadione-calcium

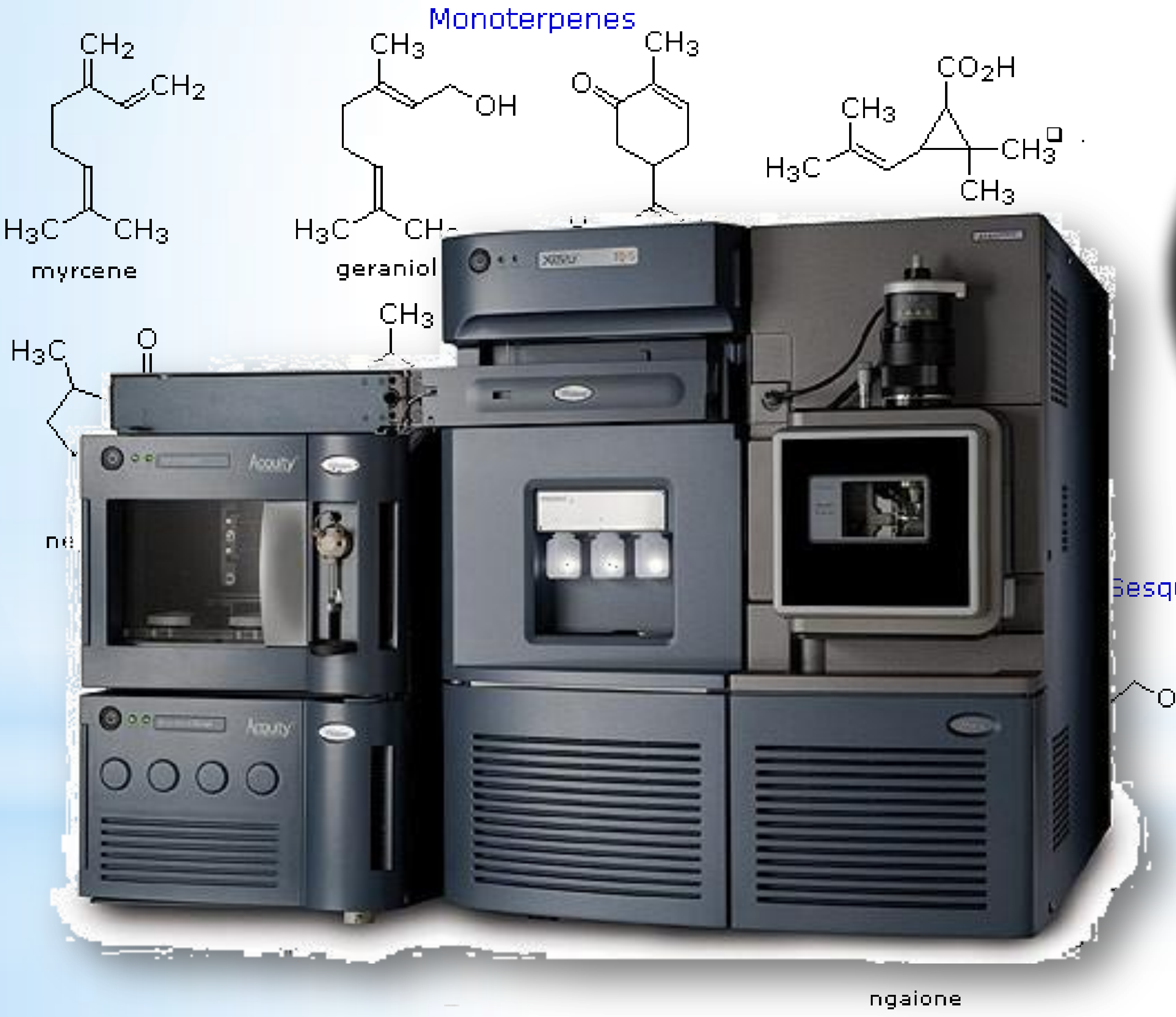
..., C Ma, M Figueroa, D Kincaid, PD **Matthews**... - **Food chemistry**, 2014 - Elsevier

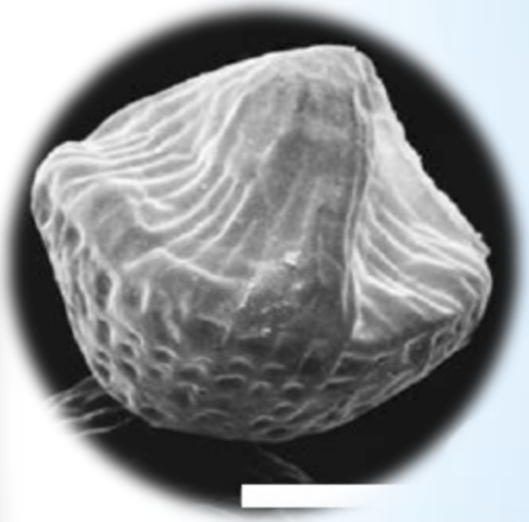
Abstract Hops (**Humulus** lupulus), a main ingredient in beer, are valued as a source of bitter flavour and biologically active polyphenols. We treated immature hop cones with prohexadione-calcium (Pro-Ca), a flavanone-3-hydroxylase (F3H) inhibitor, to perturb the **...**

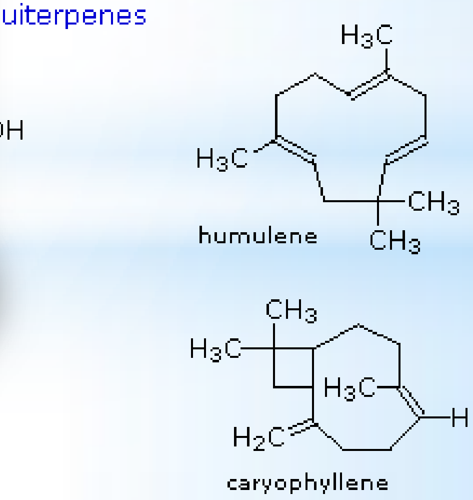
Related articles All 3 versions Cite Saved

Terpenoids

- **Two biosynthetic origins**
- **Many cyclases**
- **Variable oxidative decorations**







Tell them what you are going to tell them

- ✓ What do brewers want in a new variety?
- ✓ Variety development goals, examples…
- ✓ What’s needed for Good Breeding Practice:
  - ✓ Vertically Integrated Variety Development
  - ✓ HTS Phenometrics
  - ✓ HTS Chemometrics
  - ○ HTS Genomics
  - ○ Systems approach (Genome-Wide Association Studies)

Evolution of Breeding Systems Technology

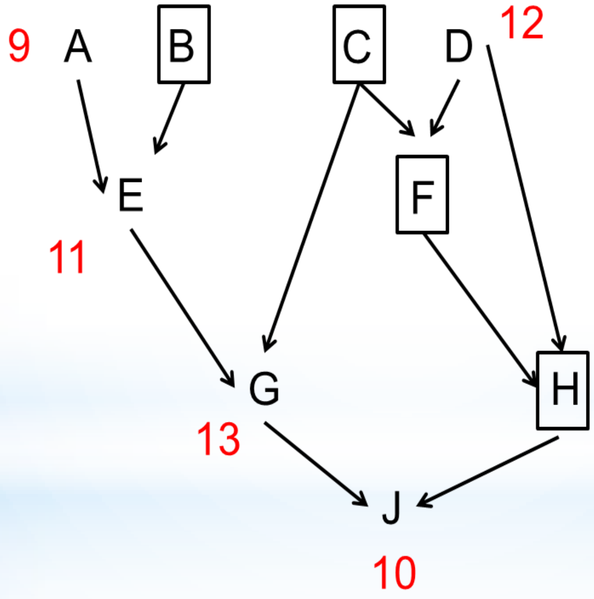
Single Cross

Select

Quantitative

Genetics

Calculate EBVs for Male Alpha

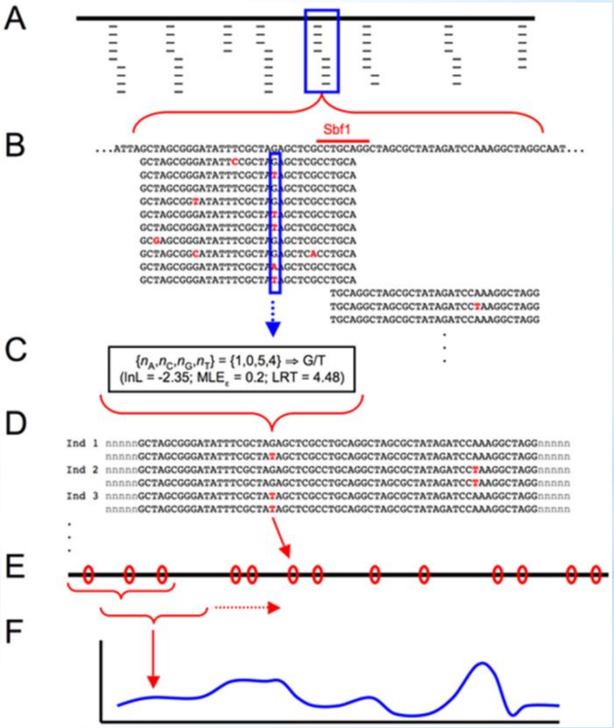


Alpha Acid h2 = 0.5

Molecular

Quantitative

Genetics



‘Omics>>>

Motivation for molecular marker development

- What are markers good for?

  - ✓ Trueness-to-type determination
  - ✓ Parent determination (whose the daddy?)
  - ✓ Variety rights protection
  - ✓ Marker-assisted selection

Accelerated, cost-effective breeding>>>

Time-line of variety development

too slow

Year 0 - 1

- Selection of parents
- Seedling Screening
    - Diseases
    - Marker screening

Year 2 – 3
Single Hill Evaluation

- Disease resistance
- Chemical traits
- Maturity

Year 4 – 6
Multi Hill Evaluation

- Agronomic traits
- Chemical traits
- Different environments
- 1st brewing trials

Year 7 – 10
Semi Commercial

- Confirmation on agronomic and chemical traits
- Extensive brewing tests

50 crosses, ≈25,000 seedlings

≈7,000 plants

100 plants, 20 plants

2 varieties

Development of new microsatellite markers (SSRs) for *Humulus lupulus*

Jared Koelling · Mark C. Coles ·
Paul D. Matthews · Axel Schwekendiek

Received: 21 February 2011 / Accepted: 8 September 2011

Abstract The number of informative markers available for hops currently limits breeding progress. Biotechnological improvement of hops therefore would benefit from a larger number of informative

demonstration of utility, a cluster anal
substantial congruence to previous stu
cultivar's genetic distances. The large
demonstrated SSRs have the potential to

Molecular Marker Development

- ❑ 600 Diversity Array Technology markers (2010)
  - ➢ DNA hybridization micro array

    (give citation)

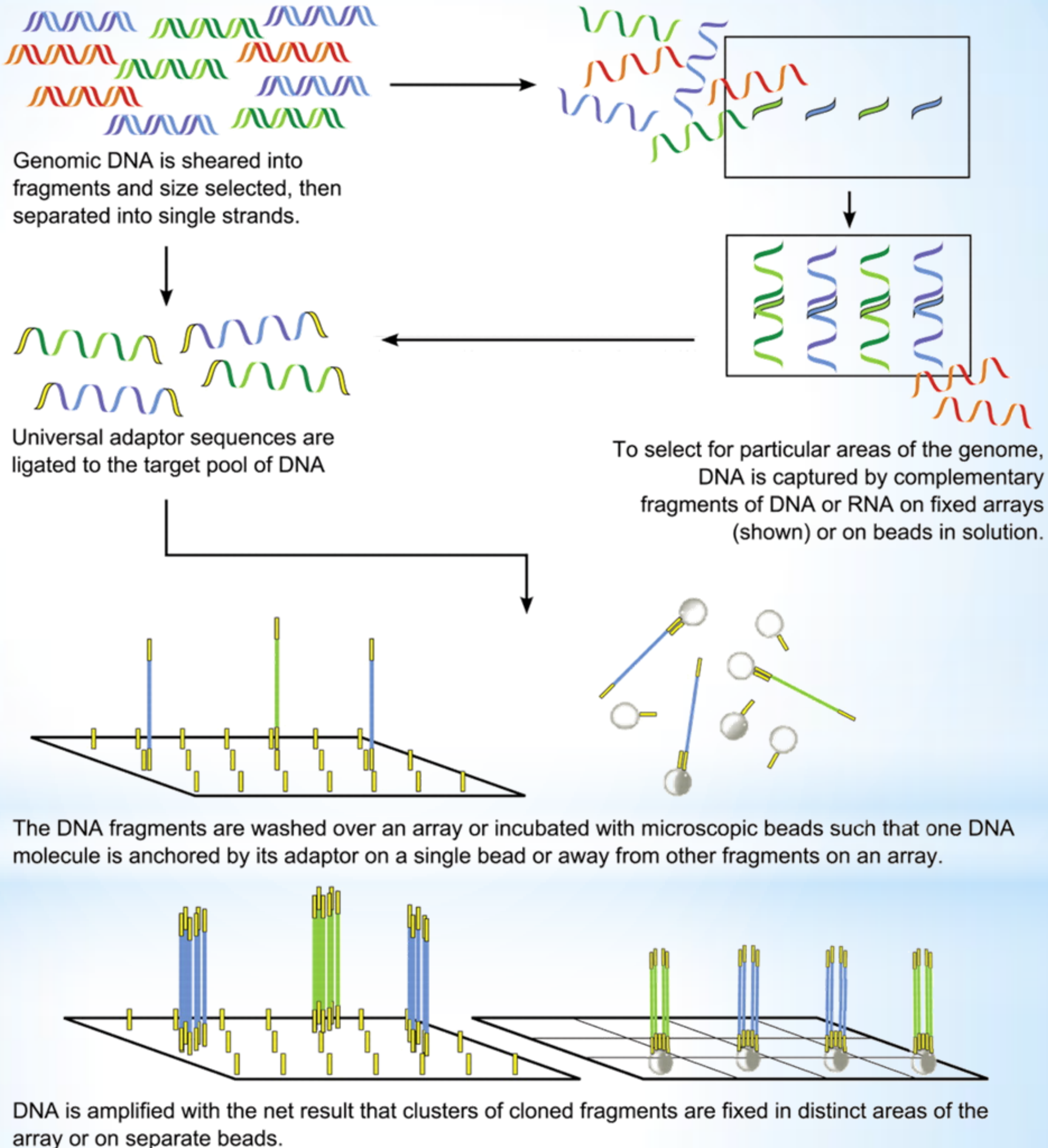
- ❑ 1000 Genic Simple Sequence Repeat markers (2011)
  - ➢ Transciptome mining

    (give citation)

- ❑ 300, 000 Single Nucleotide Polymorphisms (2013)
  - ➢ Genotyping-by-sequencing (give example >)

Next Generation Sequencing provides **Massive Molecular Marker** Data

3-5 million reads of 64 nucleotides per plant



Genomic DNA is sheared into fragments and size selected, then separated into single strands.

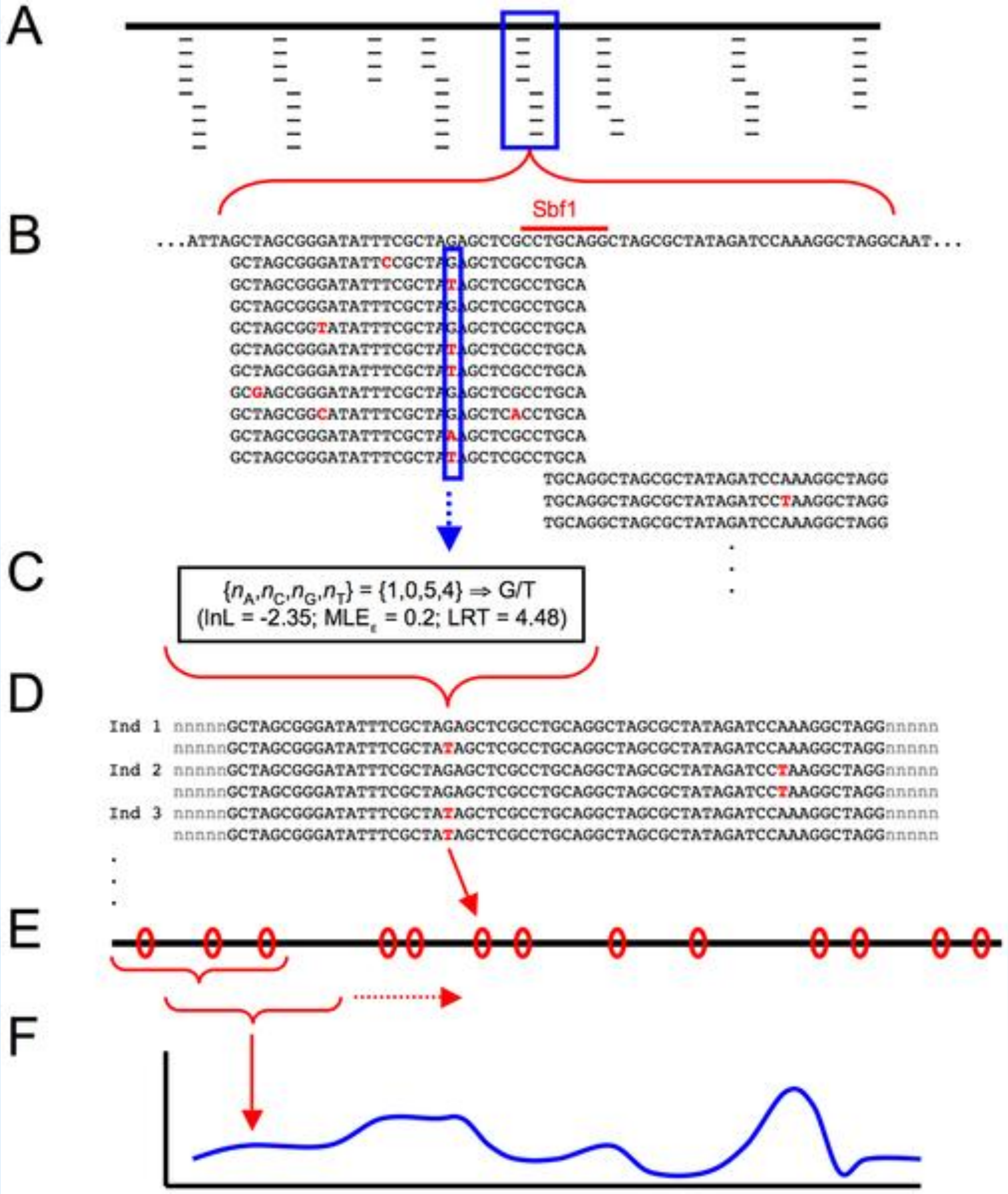
To select for particular areas of the genome, DNA is captured by complementary fragments of DNA or RNA on fixed arrays (shown) or on beads in solution.

Universal adaptor sequences are ligated to the target pool of DNA

The DNA fragments are washed over an array or incubated with microscopic beads such that one DNA molecule is anchored by its adaptor on a single bead or away from other fragments on an array.

DNA is amplified with the net result that clusters of cloned fragments are fixed in distinct areas of the array or on separate beads.

Genotyping-by-sequencing



GBS Revolution:

•2-5 million makers per analysis & plant

•>1000 analyses completed

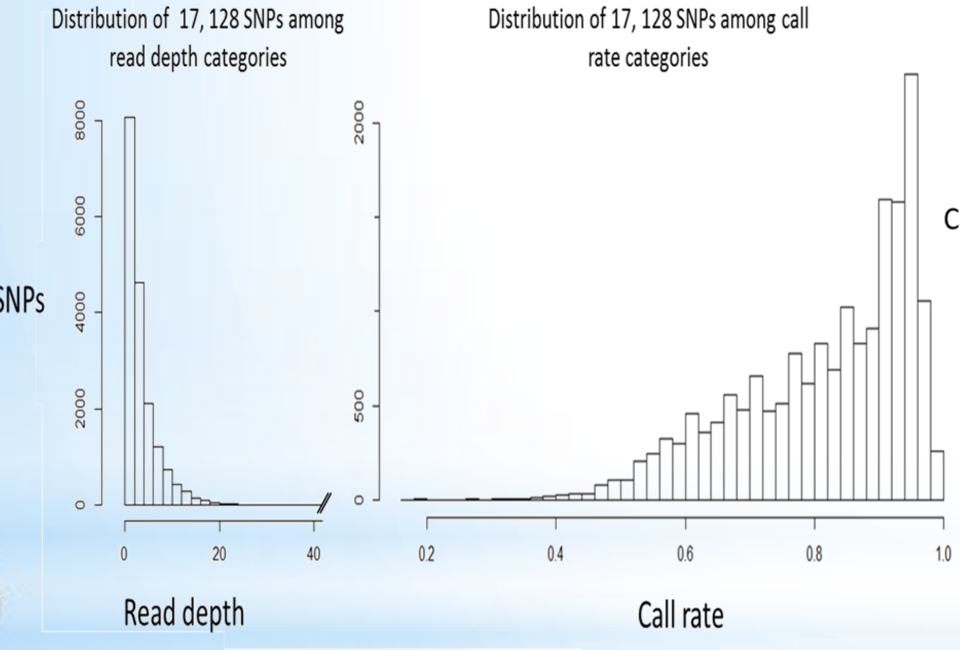
•3 billion markers scored

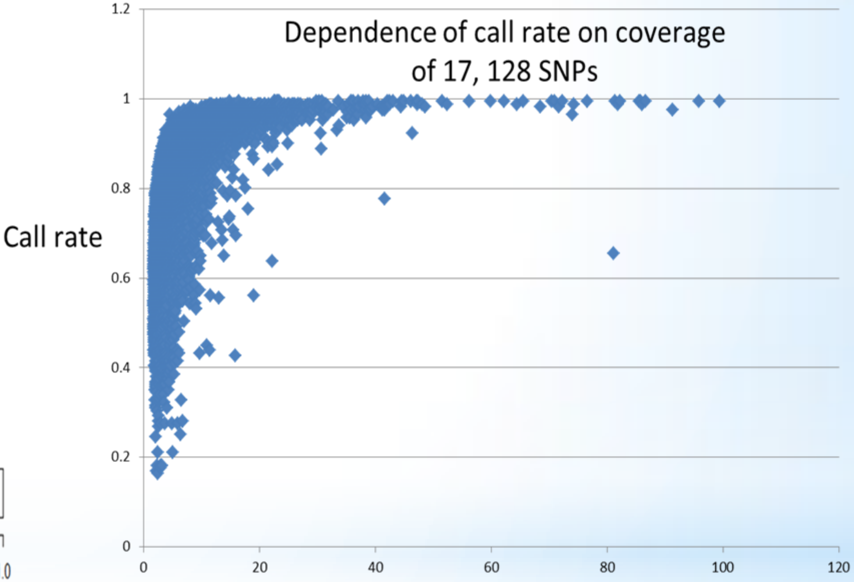
•64 bp gene tags around each marker

Mark Coles
Nicholi Pitra

Genotyping-by-sequencing

96 plants (genotypes) per lane of Illumina HiSeq 2000 gives appropriate whole genome coverage



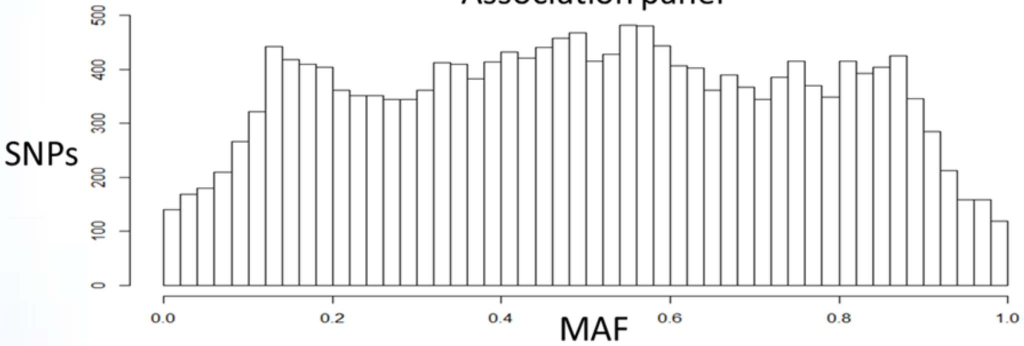


Genotyping-by-sequencing

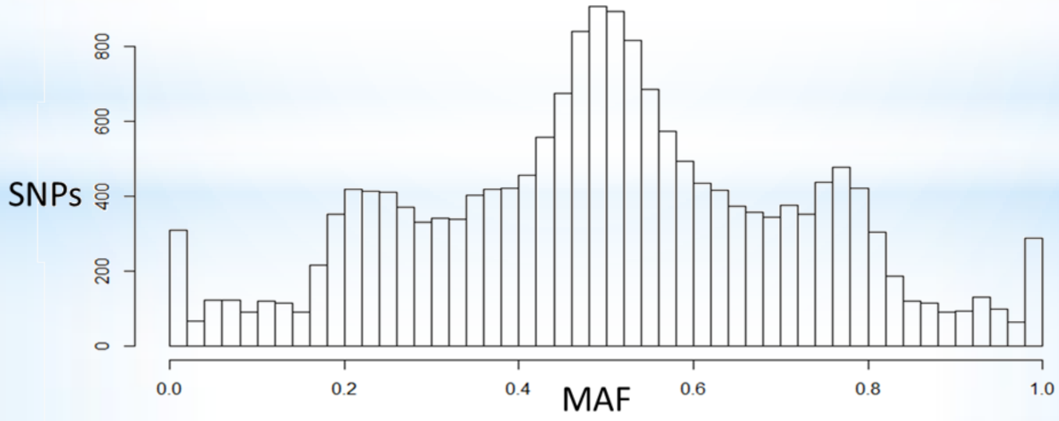
GBS UNEAK TASSEL markers behave well genetically

Distribution of SNPs among minor allele frequency categories

Association panel



Full-sib family



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1.

2.

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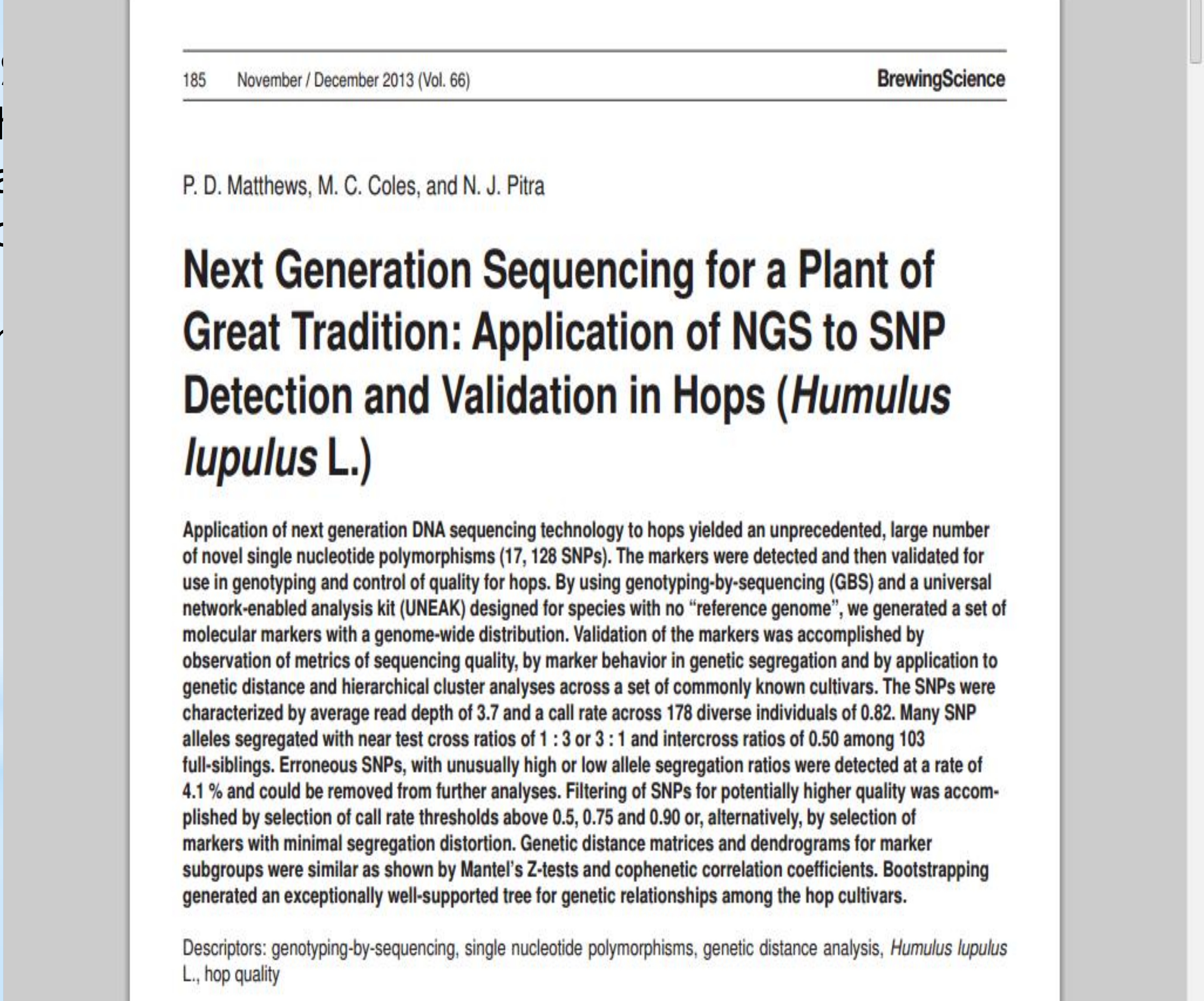


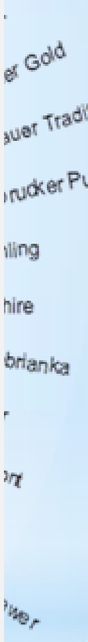
185 November / December 2013 (Vol. 66) **BrewingScience**

P. D. Matthews, M. C. Coles, and N. J. Pitra

Next Generation Sequencing for a Plant of Great Tradition: Application of NGS to SNP Detection and Validation in Hops (*Humulus lupulus* L.)

Application of next generation DNA sequencing technology to hops yielded an unprecedented, large number of novel single nucleotide polymorphisms (17, 128 SNPs). The markers were detected and then validated for use in genotyping and control of quality for hops. By using genotyping-by-sequencing (GBS) and a universal network-enabled analysis kit (UNEAK) designed for species with no "reference genome", we generated a set of molecular markers with a genome-wide distribution. Validation of the markers was accomplished by observation of metrics of sequencing quality, by marker behavior in genetic segregation and by application to genetic distance and hierarchical cluster analyses across a set of commonly known cultivars. The SNPs were characterized by average read depth of 3.7 and a call rate across 178 diverse individuals of 0.82. Many SNP alleles segregated with near test cross ratios of 1 : 3 or 3 : 1 and intercross ratios of 0.50 among 103 full-siblings. Erroneous SNPs, with unusually high or low allele segregation ratios were detected at a rate of 4.1 % and could be removed from further analyses. Filtering of SNPs for potentially higher quality was accomplished by selection of call rate thresholds above 0.5, 0.75 and 0.90 or, alternatively, by selection of markers with minimal segregation distortion. Genetic distance matrices and dendrograms for marker subgroups were similar as shown by Mantel's Z-tests and cophenetic correlation coefficients. Bootstrapping generated an exceptionally well-supported tree for genetic relationships among the hop cultivars.

Descriptors: genotyping-by-sequencing, single nucleotide polymorphisms, genetic distance analysis, *Humulus lupulus* L., hop quality



Application of GBS to molecular marker discovery and marker-assisted selection

Genotyping: trueness-to-type

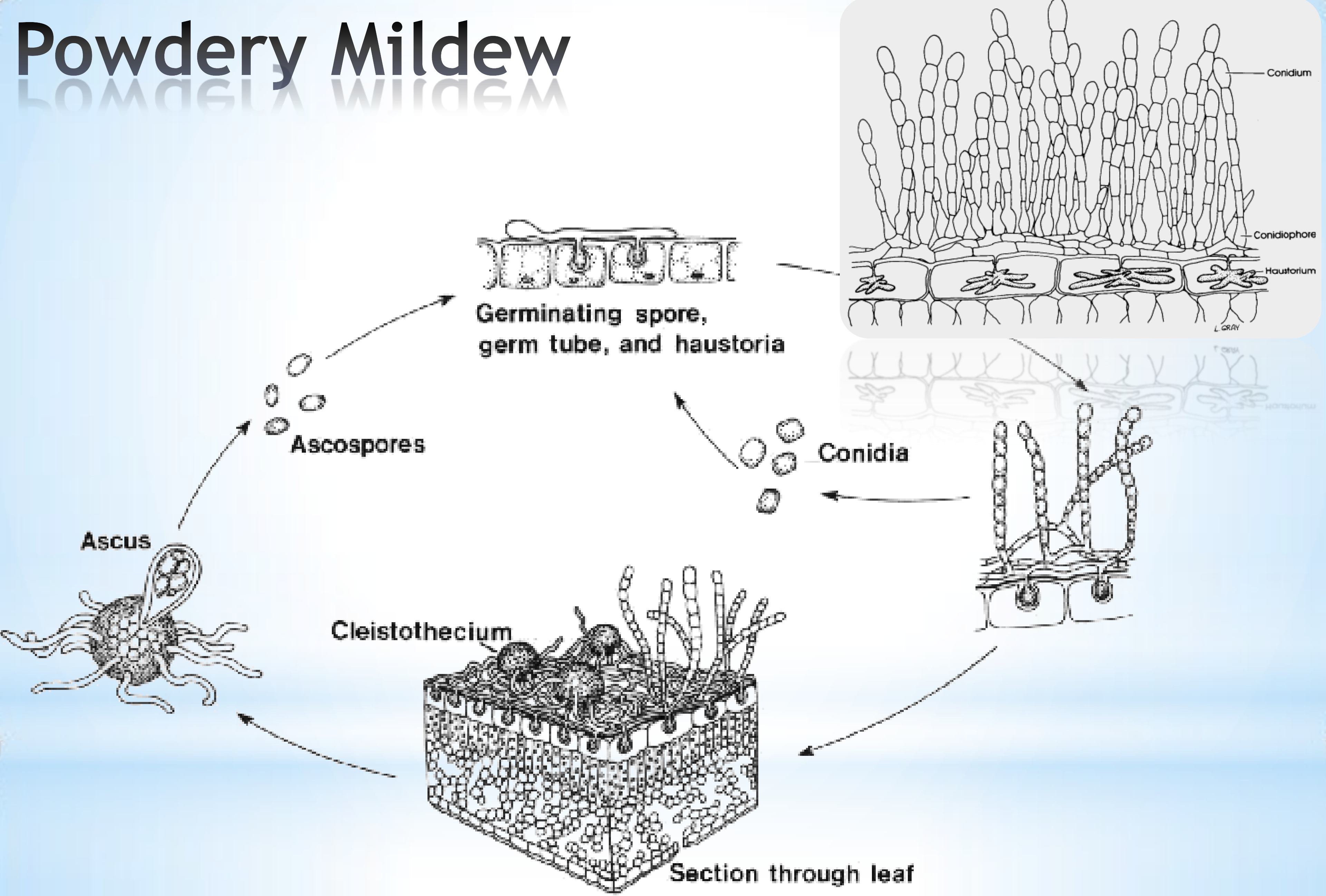
Genome-wide association genetics

for Disease resistance
for Flavor

The problem:
80% of breeding efforts are Breeding for disease resistance

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

Powdery Mildew



http://pnwhandbooks.org/plantdisease/hop-humulus-lupulus-powdery-mildew

Powdery mildew resistance breeding

Powdery mildew resistance genes

- Against evolving fungal virulence strains

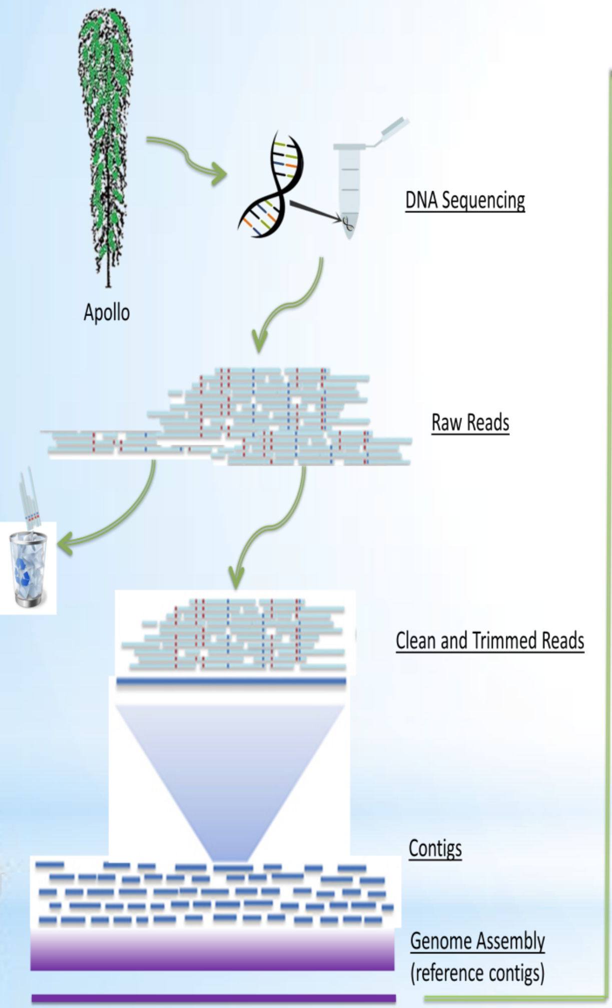
Gene	Source	Status in USA
R1, R3, Rb	Zenith	Tolerance
R2	Wye Target	Resistance
R4	Early Choice	Tolerance
R5	Cascade	Tolerance
R6	Nugget	Broken
19058mR6		Broken
Kazak 2000R	Kazak 2000	Resistant, HSR

Might stacked resistance genes confer durable resistance/tolerance?

Genome-Wide Association Studies (GWAS)

Genome Wide Association Studies

1. Making a Reference Genome



3. Genome Based Selection

Mapping Reads to Reference

Single Nucleotide Polymorphism Detection

…………..CTCCGTAGCTAGCTTCGTGAGCGACTTCGGCGATTCGGGATC**G**ATCGTACGTACGTGACGGTAGCGTGATCGATGGCTGGATCGGCTATCATCTGCGGCGATTAATATATATTCTCTTTTCATATA…………….

CTTCGGCGATTCGGGATC**G**ATCGTACGTACGTGACGGTAGCGTG

CTTCGGCGATTCGGGATC**A**ATCGTACGTACGTGACGGTAGCGTG

CTTCGGCGATTCGGGATC**G**ATCGTACGTACGTGACGGTAGCGTG

Mixed Liner Modeling

SNP Matrix

	SNP1	SNP2	SNP3	SNP4	………
Plant_1	A	G	T	C	………
Plant_2	A	A	C	C	………
Plant_3	T	G	T	T	………
…………	…..	…..	…..	…..	………

Matrix of Relatedness

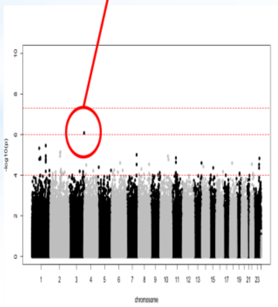
	Plant_1	Plant_2	Plant_3	………
Plant_1	1.0	0.825	0.356	………
Plant_2	0.825	1.0	0.074	………
Plant_3	0.356	0.074	1.0	………
…………	…..	…..	…..	………

Phenotype

	Resistant
Plant_1	yes
Plant_2	no
Plant_3	yes
………	…..

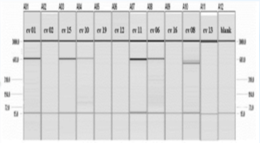
$$y = X\beta + Zu + e$$

$$h^2 = \frac{Var(A)}{Var(P)}$$



Apply the Marker

Analyze Results and Advance Breeding Program



R S R R S S R R S R S

R = resistant

S = susceptible

2. Generating GBS Markers

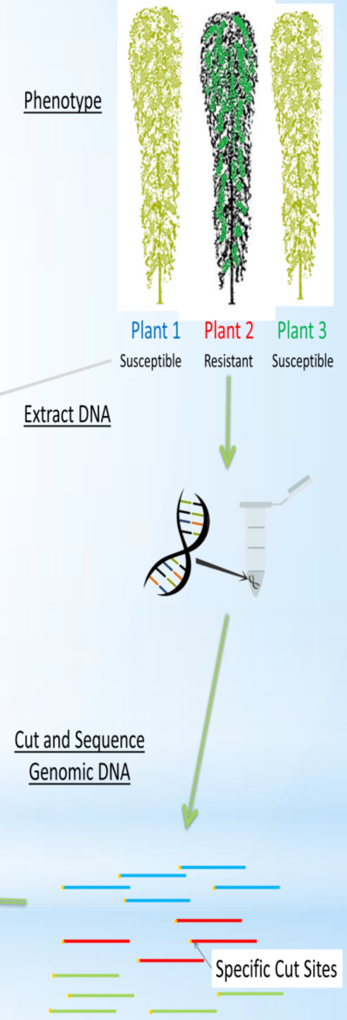
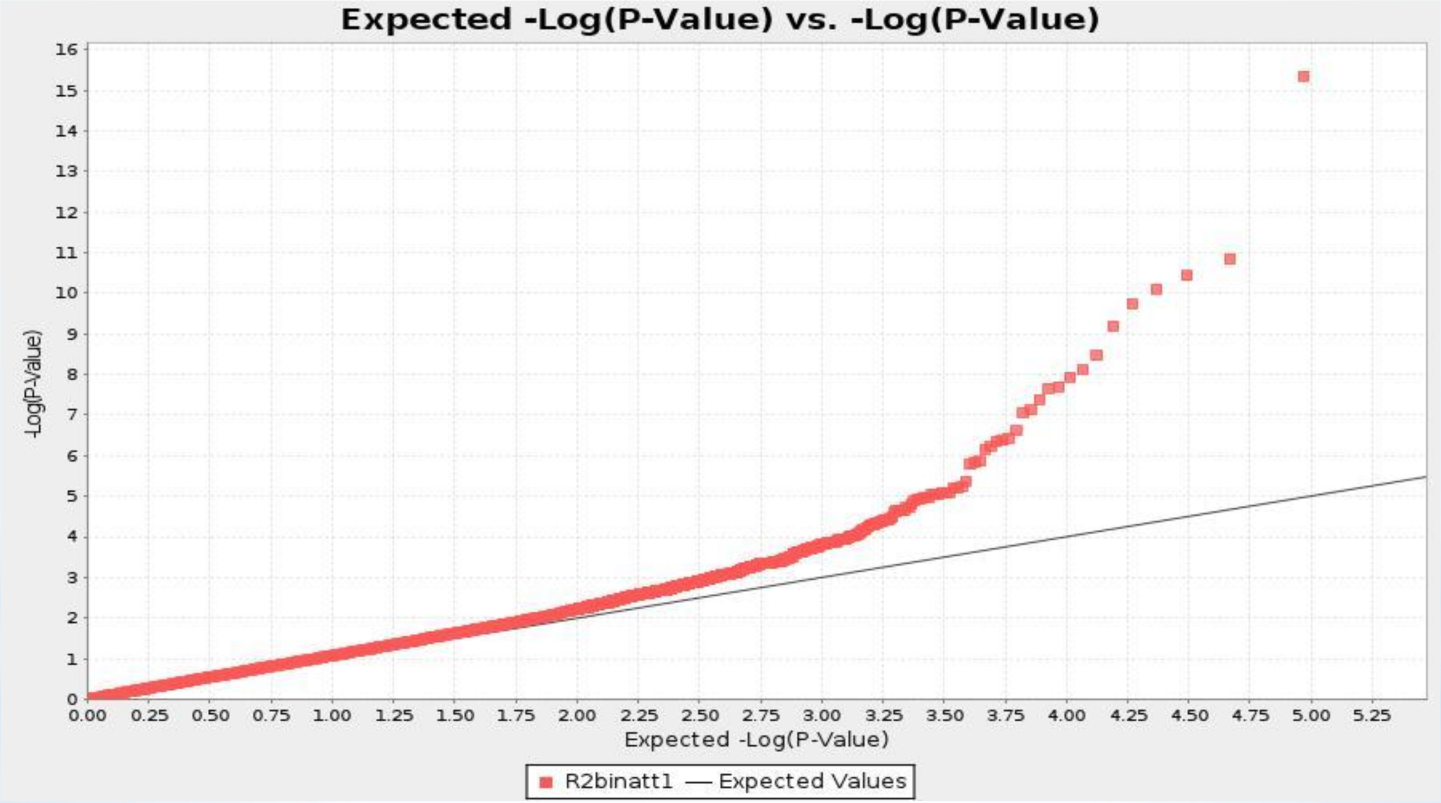


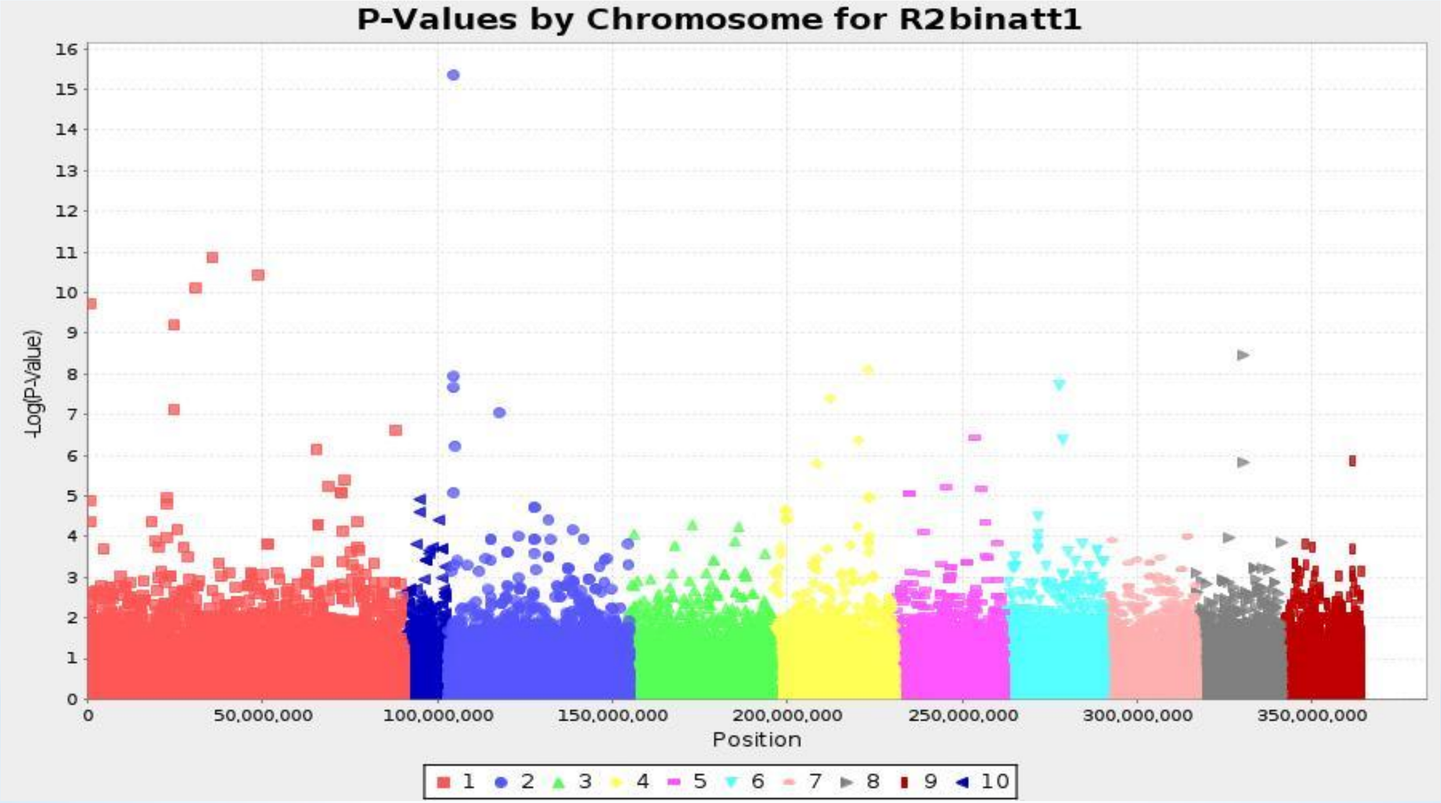
Figure 1. DNA is extracted from Apollo hop variety and is run on Next Generation Sequencing machines. The raw reads produced are then groomed and trimmed. The cleaned reads are then assembled using massive computing into a reference genome.

Figure 2. DNA is extracted from a group of plants selected to optimize the power of the experiment. This example shows three plants, but a typical population size for an association study is ~200. Using an enzyme the DNA is then cut at specific sites. These fragments are then sequenced.

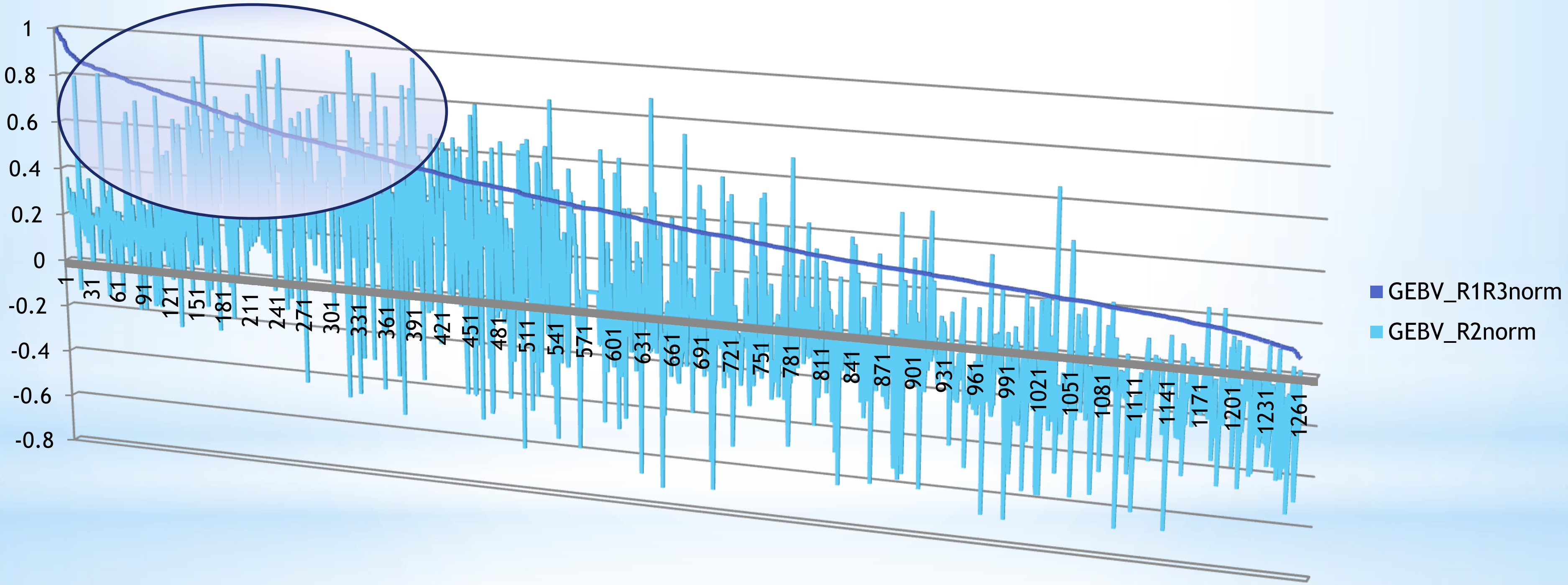
Q-Q plot of MLM associations

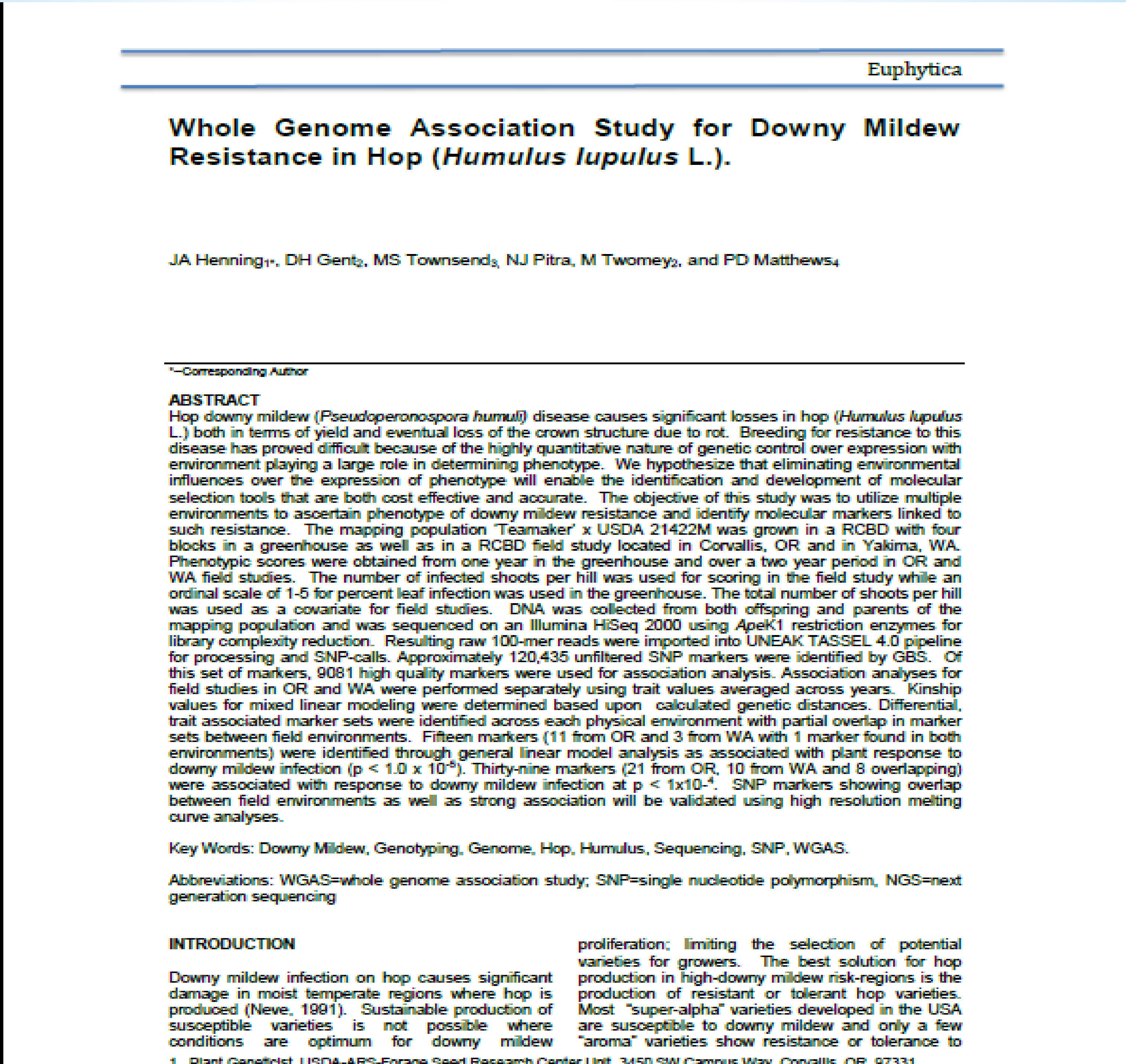


Manhattan Plot of MLM associations



Normalized Breeding Values Showing Stackedness for Two Traits: R1,R3 and R2


High GEBV for both sets of markers



Euphytica

Whole Genome Association Study for Downy Mildew Resistance in Hop (*Humulus lupulus* L.).

JA Henning[1]*, DH Gent[2], MS Townsend[3], NJ Pitra, M Twomey[2], and PD Matthews[4]

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*–Corresponding Author

ABSTRACT

Hop downy mildew (*Pseudoperonospora humuli*) disease causes significant losses in hop (*Humulus lupulus* L.) both in terms of yield and eventual loss of the crown structure due to rot. Breeding for resistance to this disease has proved difficult because of the highly quantitative nature of genetic control over expression with environment playing a large role in determining phenotype. We hypothesize that eliminating environmental influences over the expression of phenotype will enable the identification and development of molecular selection tools that are both cost effective and accurate. The objective of this study was to utilize multiple environments to ascertain phenotype of downy mildew resistance and identify molecular markers linked to such resistance. The mapping population 'Teamaker' x USDA 21422M was grown in a RCBD with four blocks in a greenhouse as well as in a RCBD field study located in Corvallis, OR and in Yakima, WA. Phenotypic scores were obtained from one year in the greenhouse and over a two year period in OR and WA field studies. The number of infected shoots per hill was used for scoring in the field study while an ordinal scale of 1-5 for percent leaf infection was used in the greenhouse. The total number of shoots per hill was used as a covariate for field studies. DNA was collected from both offspring and parents of the mapping population and was sequenced on an Illumina HiSeq 2000 using *ApeK*1 restriction enzymes for library complexity reduction. Resulting raw 100-mer reads were imported into UNEAK TASSEL 4.0 pipeline for processing and SNP-calls. Approximately 120,435 unfiltered SNP markers were identified by GBS. Of this set of markers, 9081 high quality markers were used for association analysis. Association analyses for field studies in OR and WA were performed separately using trait values averaged across years. Kinship values for mixed linear modeling were determined based upon calculated genetic distances. Differential, trait associated marker sets were identified across each physical environment with partial overlap in marker sets between field environments. Fifteen markers (11 from OR and 3 from WA with 1 marker found in both environments) were identified through general linear model analysis as associated with plant response to downy mildew infection ($p < 1.0 \times 10^{-5}$). Thirty-nine markers (21 from OR, 10 from WA and 8 overlapping) were associated with response to downy mildew infection at $p < 1\text{x}10^{-4}$. SNP markers showing overlap between field environments as well as strong association will be validated using high resolution melting curve analyses.

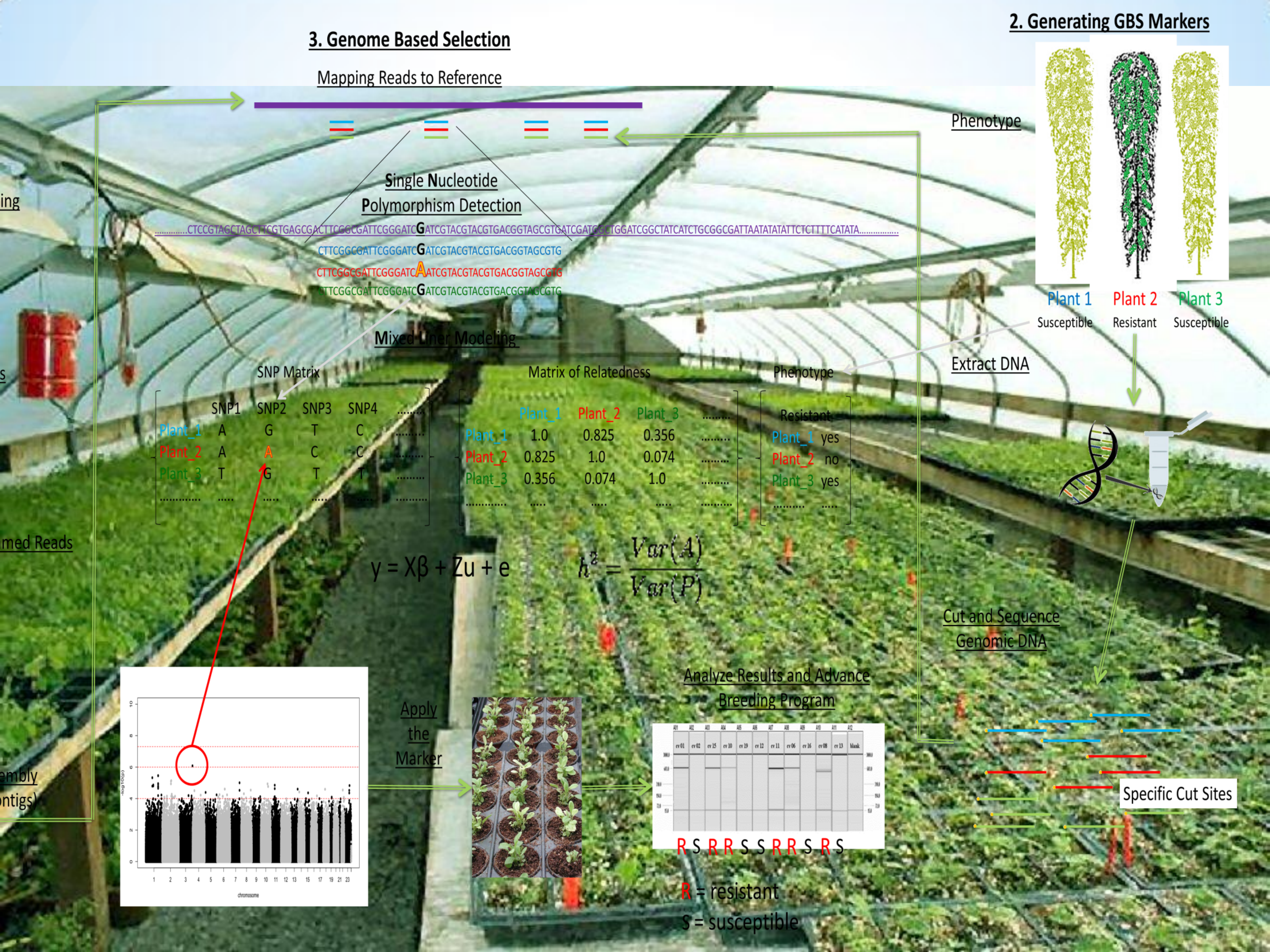
Key Words: Downy Mildew, Genotyping, Genome, Hop, Humulus, Sequencing, SNP, WGAS.

Abbreviations: WGAS=whole genome association study; SNP=single nucleotide polymorphism, NGS=next generation sequencing

INTRODUCTION

Downy mildew infection on hop causes significant damage in moist temperate regions where hop is produced (Neve, 1991). Sustainable production of susceptible varieties is not possible where conditions are optimum for downy mildew proliferation; limiting the selection of potential varieties for growers. The best solution for hop production in high-downy mildew risk-regions is the production of resistant or tolerant hop varieties. Most "super-alpha" varieties developed in the USA are susceptible to downy mildew and only a few "aroma" varieties show resistance or tolerance to

1. Plant Geneticist, USDA-ARS-Forage Seed Research Center Unit, 3450 SW Campus Way, Corvallis, OR 97331



2. Generating GBS Markers

Phenotype

Extract DNA

Cut and Sequence Genomic DNA

Specific Cut Sites

3. Genome Based Selection

Mapping Reads to Reference

Single **N**ucleotide **P**olymorphism Detection

```
..............CTCCGTAGCTAGCTTCGTGAGCGACTTCGGCGATTCGGGATCGATCGTACGTACGTGACGGTAGCGTGATCGATGGCTGGATCGGCTATCATCTGCGGCGATTAATATATATTCTCTTTTCATATA.................
CTTCGGCGATTCGGGATCGATCGTACGTACGTGACGGTAGCGTG
CTTCGGCGATTCGGGATCAATCGTACGTACGTGACGGTAGCGTG
CTTCGGCGATTCGGGATCGATCGTACGTACGTGACGGTAGCGTG
```

Mixed Liner Modeling

SNP Matrix

	SNP1	SNP2	SNP3	SNP4	....
Plant_1	A	G	T	C	....
Plant_2	A	A	C	C	....
Plant_3	T	G	T	T	....
....	.....	.....	.....	.....	....

Matrix of Relatedness

	Plant_1	Plant_2	Plant_3	....
Plant_1	1.0	0.825	0.356	....
Plant_2	0.825	1.0	0.074	....
Plant_3	0.356	0.074	1.0	....
....	.....	.....	.....	....

Phenotype

	Resistant
Plant_1	yes
Plant_2	no
Plant_3	yes
....	.....

$$y = X\beta + Zu + e$$

$$h^2 = \frac{Var(A)}{Var(P)}$$

Apply the Marker

Analyze Results and Advance Breeding Program

R S R R S S R R S R S

R = resistant

S = susceptible

Summary

- ✓ Hops exhibit great morphological, genetic and chemical and diversity
- ✓ Hop breeding must take advantage of diversity with available technologies
- ✓ Chemo-analytic methods have improved allowing deep chemical profiles
- ✓ Next generation DNA sequencing has revolutionized marker development
- ✓ New markers have been successfully applied to selection of disease resistance
- ✓ Complicated traits, such as aroma and flavor, are now feasible breeding targets

Hopsteiner®

Thank you very much.

Simon H. Steiner, Hopfen, GmbH.

S.S. Steiner Inc.

Steiner Hops Limited

Steiner Asia Limited