




Hopsteiner®

**Crop Improvement
Program**
Paul D. Matthews, Ph.D.

**2014
NoCal-SoCal
Technical Conference**





Hop Research Council



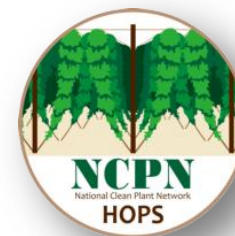
ISHS Section on Medicinal and Aromatic Plants in conjunction with the ISHS Commission on Plant Genetic Resources and the ISHS Commission on Fruits and Vegetables and Health presents:

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.



USHIPPC



Research Organization

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 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 – UNLowa
- ❑ Jared Koelling – UNLowa
- ❑ Jana Naegel – NRC – Canada
- ❑ Dr. Shaun Clark – NRC – Canada
- ❑ Alex Feiner – Martin Luther U, IPB-Germany

Collaborating Pls:

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, UNLowa
 Dr. Ryan Weil, Emory U.
 Dr. Ludger Wessjohann – IPB – Germany
 Dr. Oliver Yu, Danforth Center
 And Illumina



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

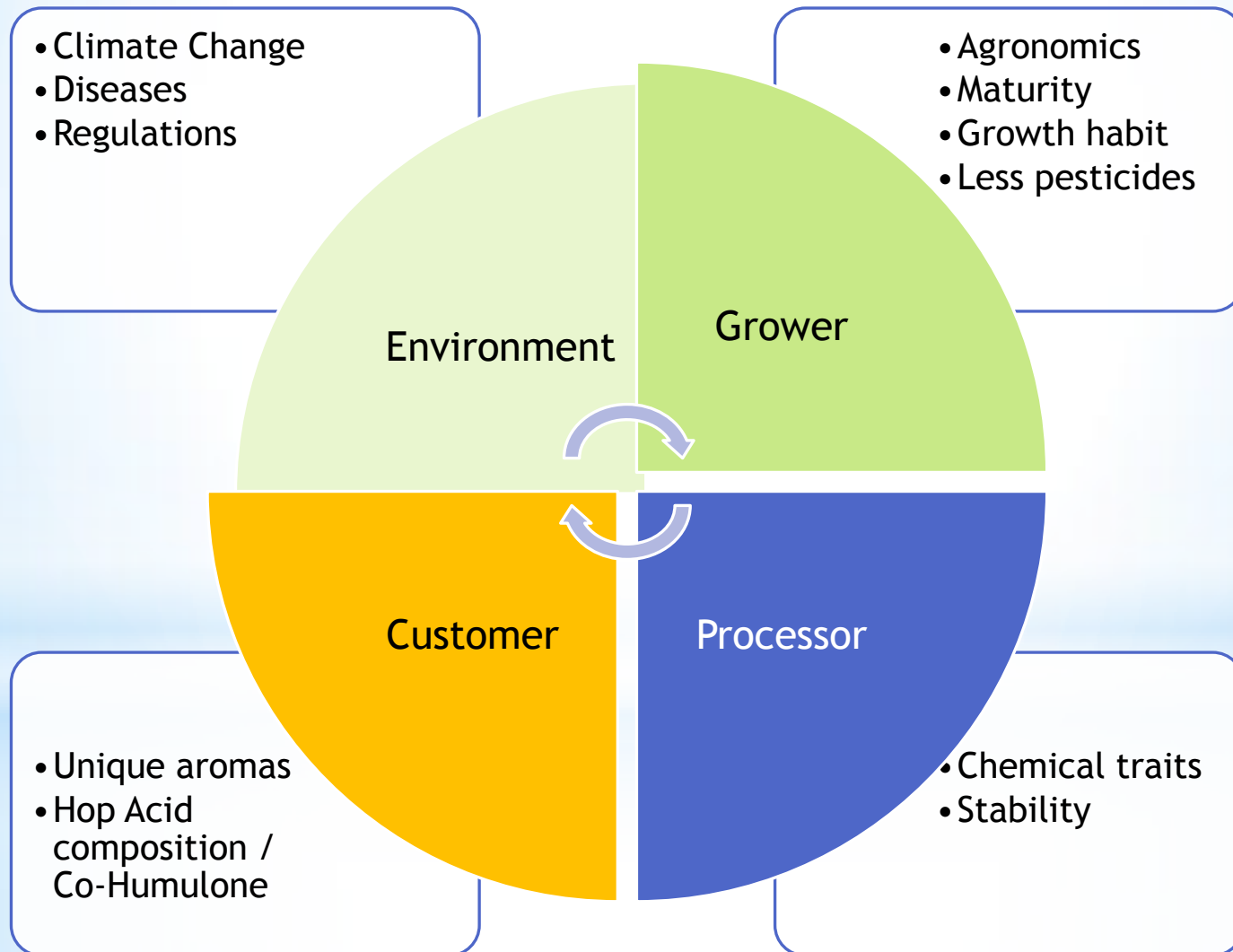
- What do brewers want in a new variety?
- Variety development goals, examples...
- What's needed for Good Breeding Practice:
 - Vertically Integrated Variety Development
 - HTS Phenometrics
 - HTS Chemometrics
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 - Systems approach (Genome-Wide Association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

Breeding Goals

- ✓ **Consistency**
- ✓ **Bio-similarity**
- ✓ **Novelty**
- ✓ **Eco-specificity**
- ✓ **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



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







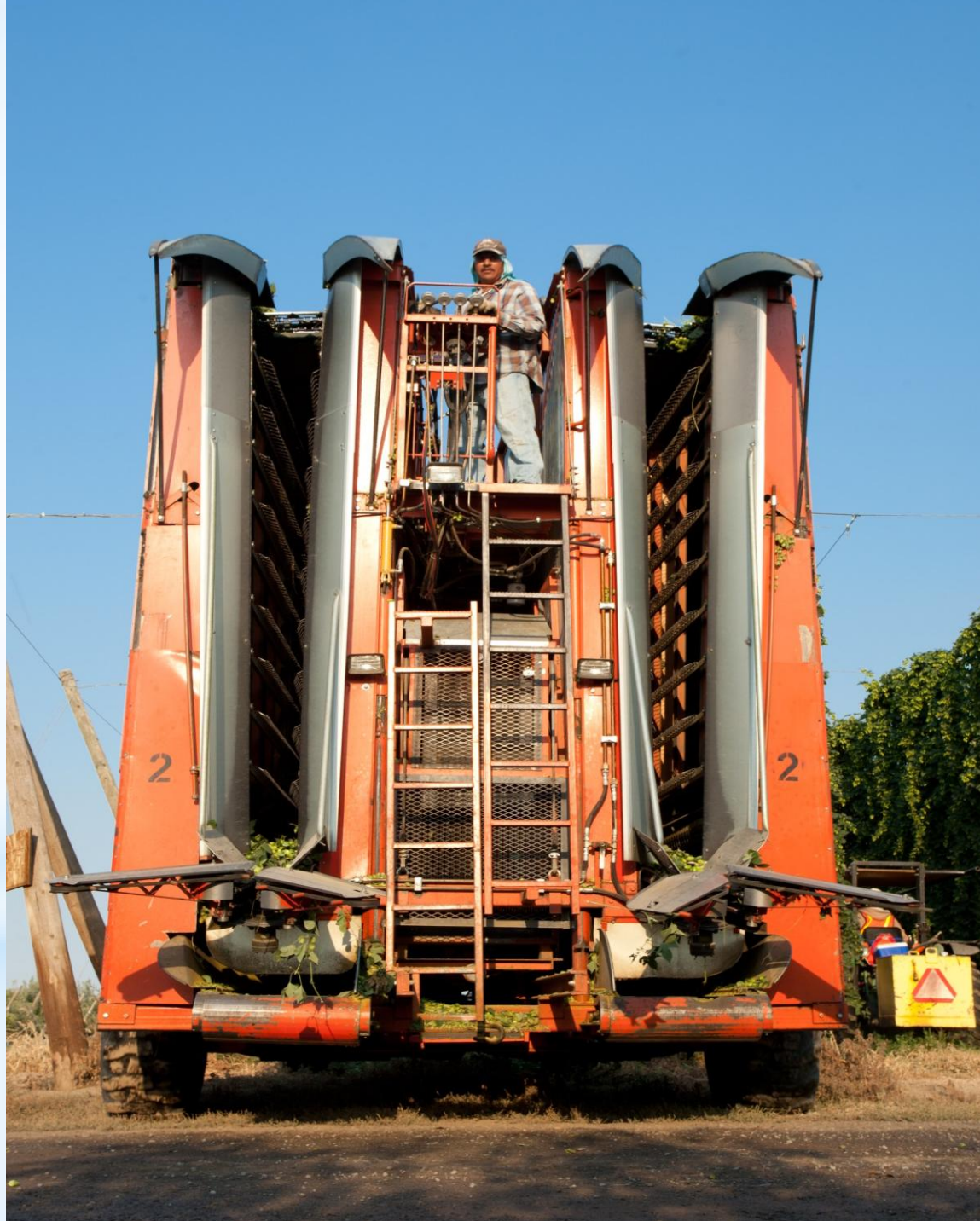






















PROCESSING



EX 2

3

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
<ul style="list-style-type: none"> ✓Delta ✓Calypso ✓Bravo ✓Apollo ✓Super Galena ✓Lemondrop 	<ul style="list-style-type: none"> ✓Low CoH ✓Novel Aroma ✓High Oil Content ✓Select Polyphenols ✓Storage Stability 	<ul style="list-style-type: none"> ✓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

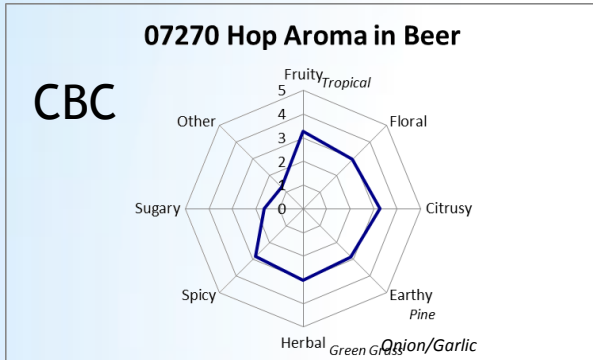


Variety	Cascade	Calypso	Centennial	Lemondrop	06300	06277
Alpha acids % w/w	4.5-7.0	13.0 - 16.0	9.5-11.5	4.5-6.5	15-18	15-18
Beta acids % w/w	4.5-7.0	8.0 - 10.0	3.5-4.5	4.0-6.0	5.0-6.0	4.0-5.0
CoH % w/w of α -acids	33-40	35 - 40	29-30	30-33	45-50	22-26
Total Oil ml/100g	0.8-1.5	1.5 - 2.5	1.5-2.3	1.5-2.0	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

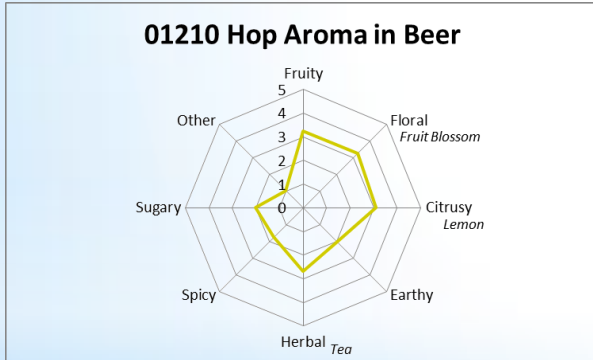
Sensory Evaluation

CBC and Hopsteiner Panel

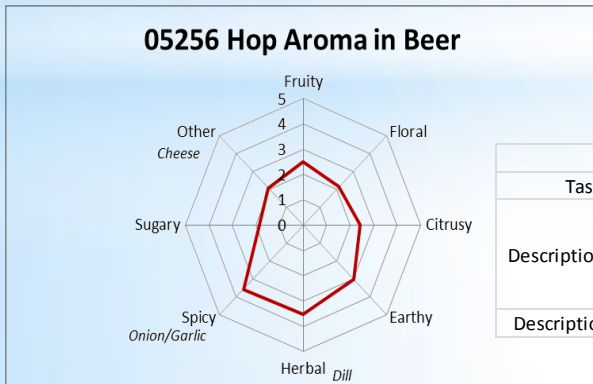
07270: Onion/Garlic, Pine, Green Grass, Tropical



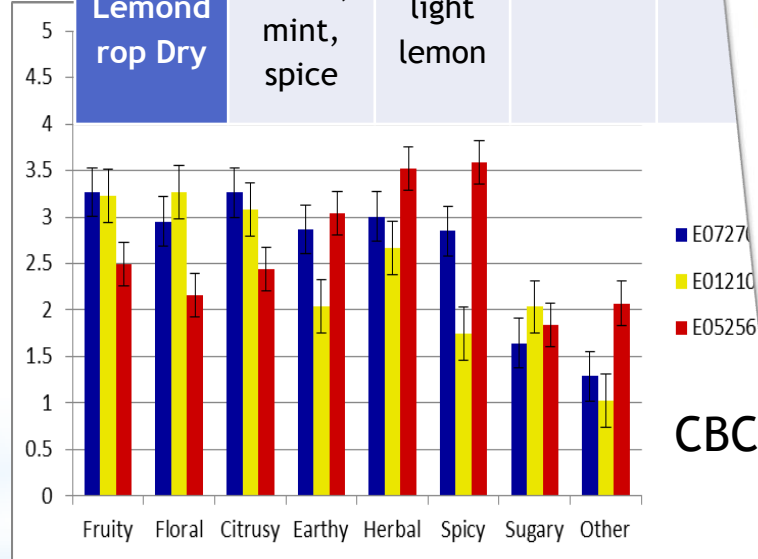
01210: Lemon, Tea, Fruit Blossom



05256: Onion/Garlic, Cheese, Dill



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



	Lemondrop				
	E07270 (CBC)	E07270 (Panel)	E01210 (CBC)	E01210 (Panel)	E05256 (CBC)
Tasting Date	4/9/2014	3/21/2014	4/9/2014	3/21/2014	4/9/2014
Description of Hop Aroma	Onion/Garlic, Pine, Grass, Tropical Fruit	Rose, Berry, Slight Orange, Spicy	Lemon, Tea, Fruit Blossom	Apple, Fruit Blossom, Citrus	Onion/Garlic, Cheese, Dill
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	
Lime	Citrusy	
Cedar	Earthy	
Green Apple	Fruity	
Coconut	Miscellaneous	
Cardamom	Spicy	
Banana	Fruity	
Vanilla	Spicy	
Lemon	Citrusy	
Rose	Foral	
Pear	Fruity	
Guavea	Fruity	
Grape	Fruity	
Dill	Spicy	
Peppermint	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

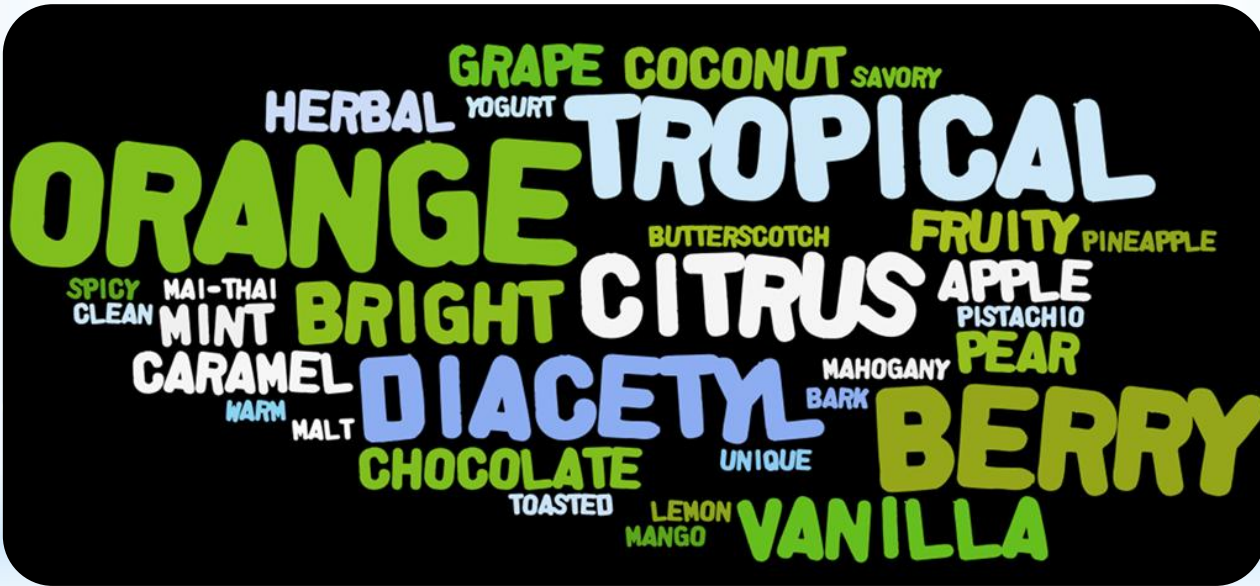
Susceptible to PM in greenhouse

Emerald 5-acre; Roza MHN: 80-hills

EXDGLIUGURGI AGLIGRA NOVA

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

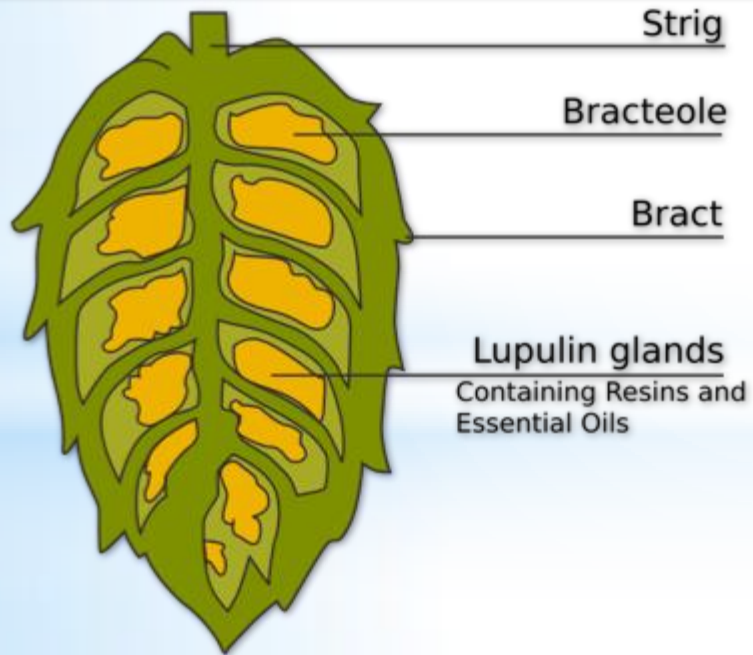
Resistant to PM in greenhouse

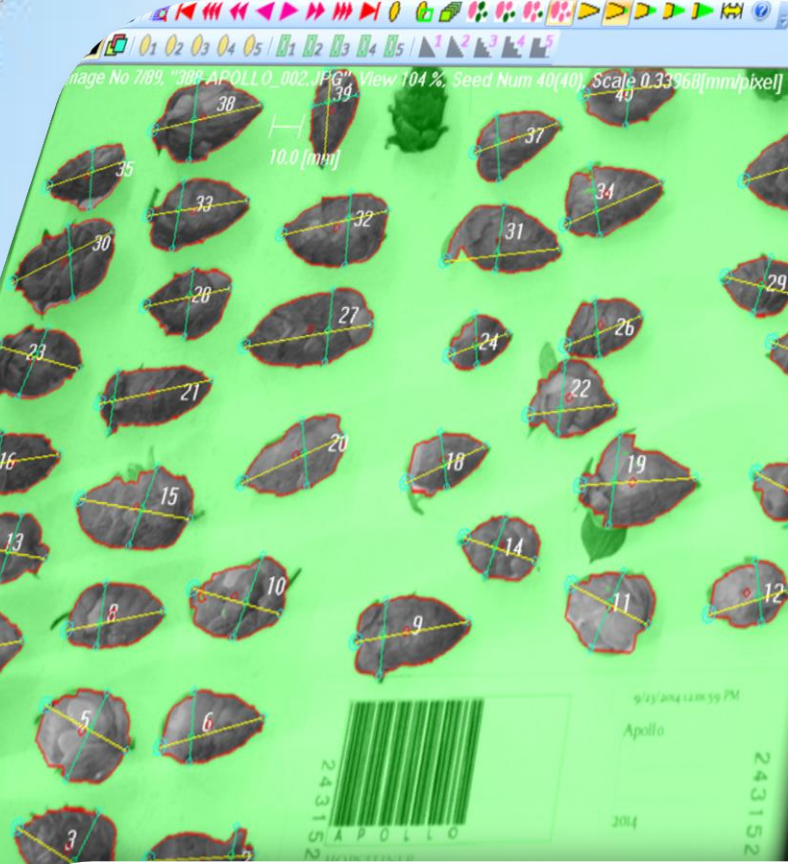
Roza MHN: 80-hills

Tell them what you are going to tell them

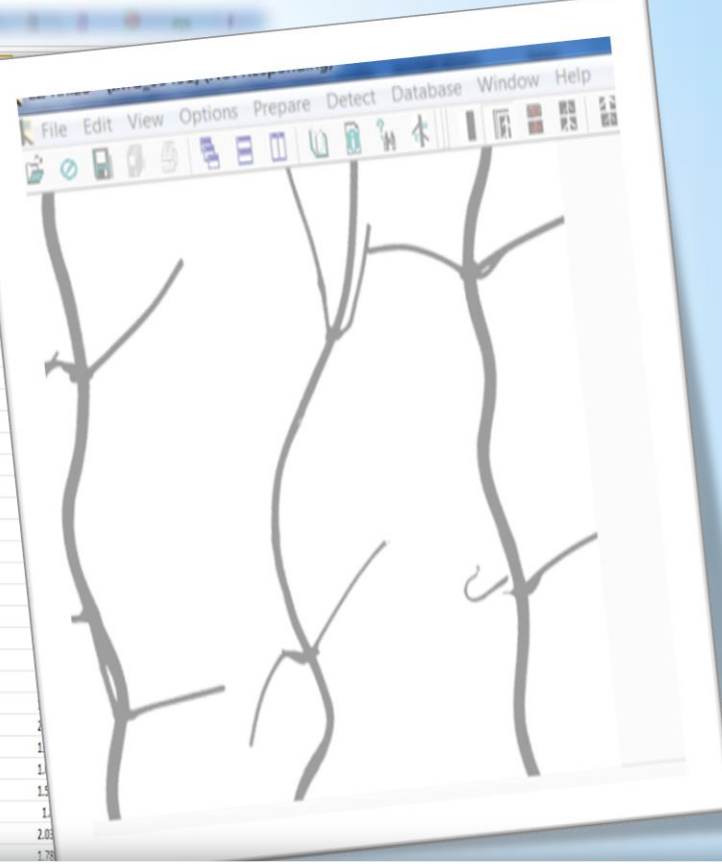
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Cone development and variation





Cone PIC with file names\2014 Cone PIC with file names\set7			
AS (mm2)	Perimeter length(PL)(mm)	Length(L)(mm)	Width(W)(mm)
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
401.773173	83.269335	31.758256	18.471482
374.484444	81.896455	31.897829	15.084452
444.638978	89.815767	34.947935	17.852043
323.368557	73.346055	28.16927	16.39309
267.40647	68.02766	25.53291	14.226264
340.041798	76.262516	29.119922	15.259372
533.024461	98.812414	37.329796	20.110372
275.021929	70.580296	25.582571	14.857084
558.236246	107.939763	40.409641	19.457149
251.771701	65.344322	23.516885	14.523262
308.714567	71.074823	26.454008	15.968798
421.907986	84.429092	31.767338	19.905644
337.734083	78.959995	29.319342	15.950723
335.830218	76.043534	29.36653	14.451581
288.579755	69.750224	27.487195	15.438785



Tell them what you are going to tell them

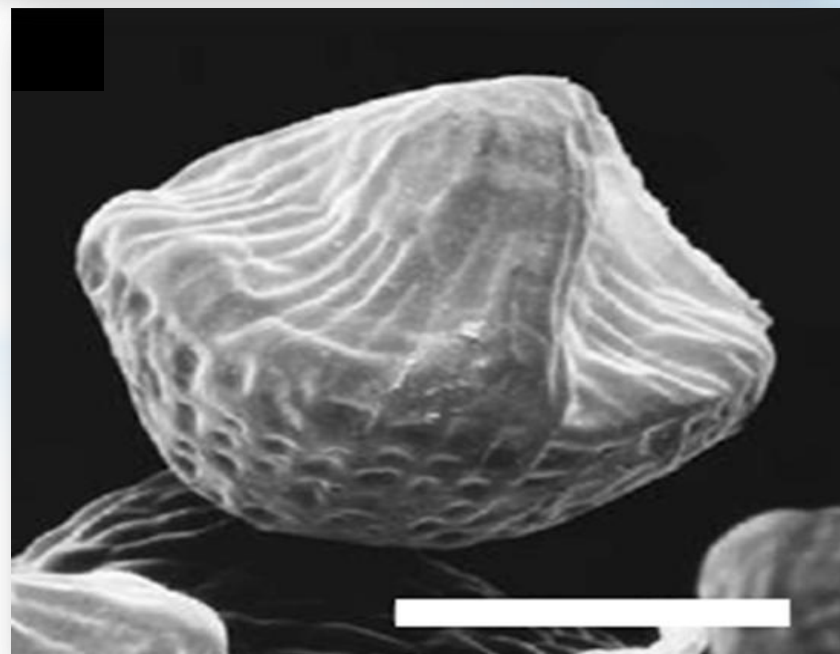
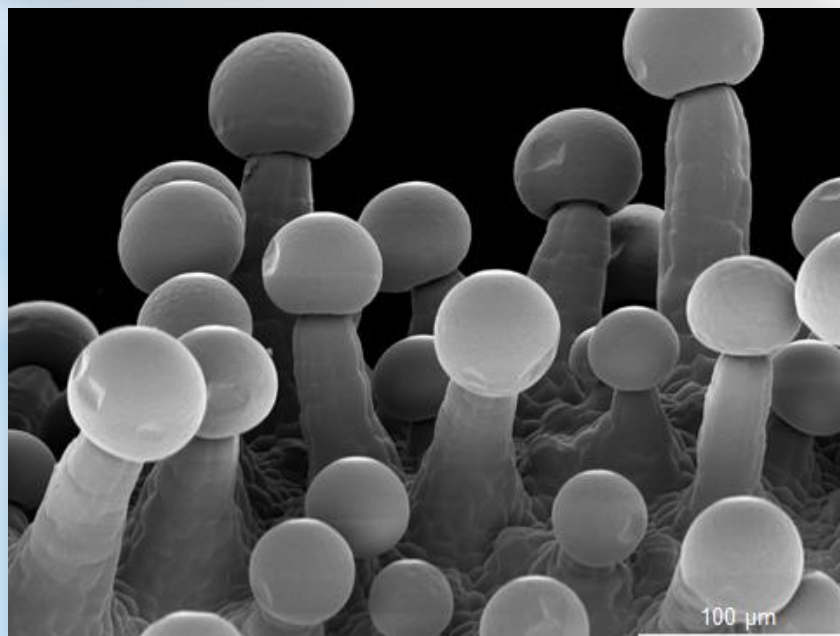
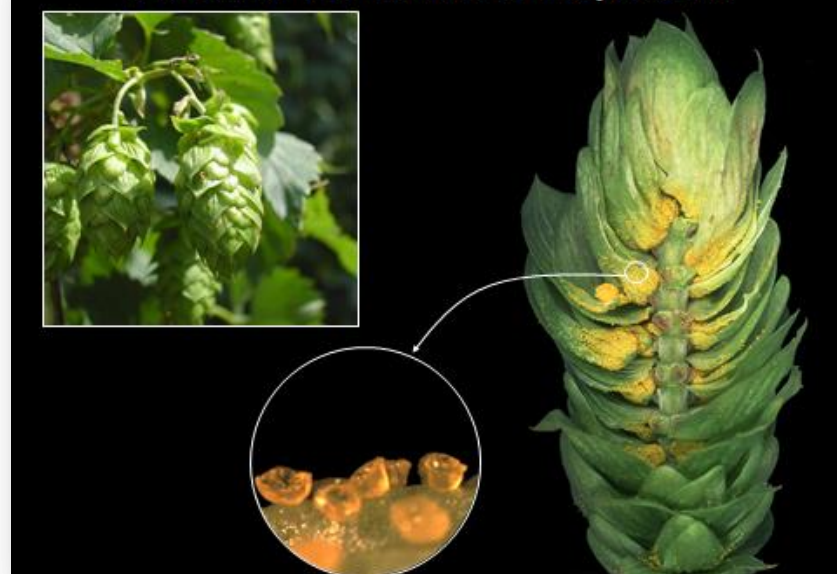
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Glandular trichomes on Cannabis



Glandular trichomes on hop cones



Chemical Diversity: Unique Flavors in Perpetuity

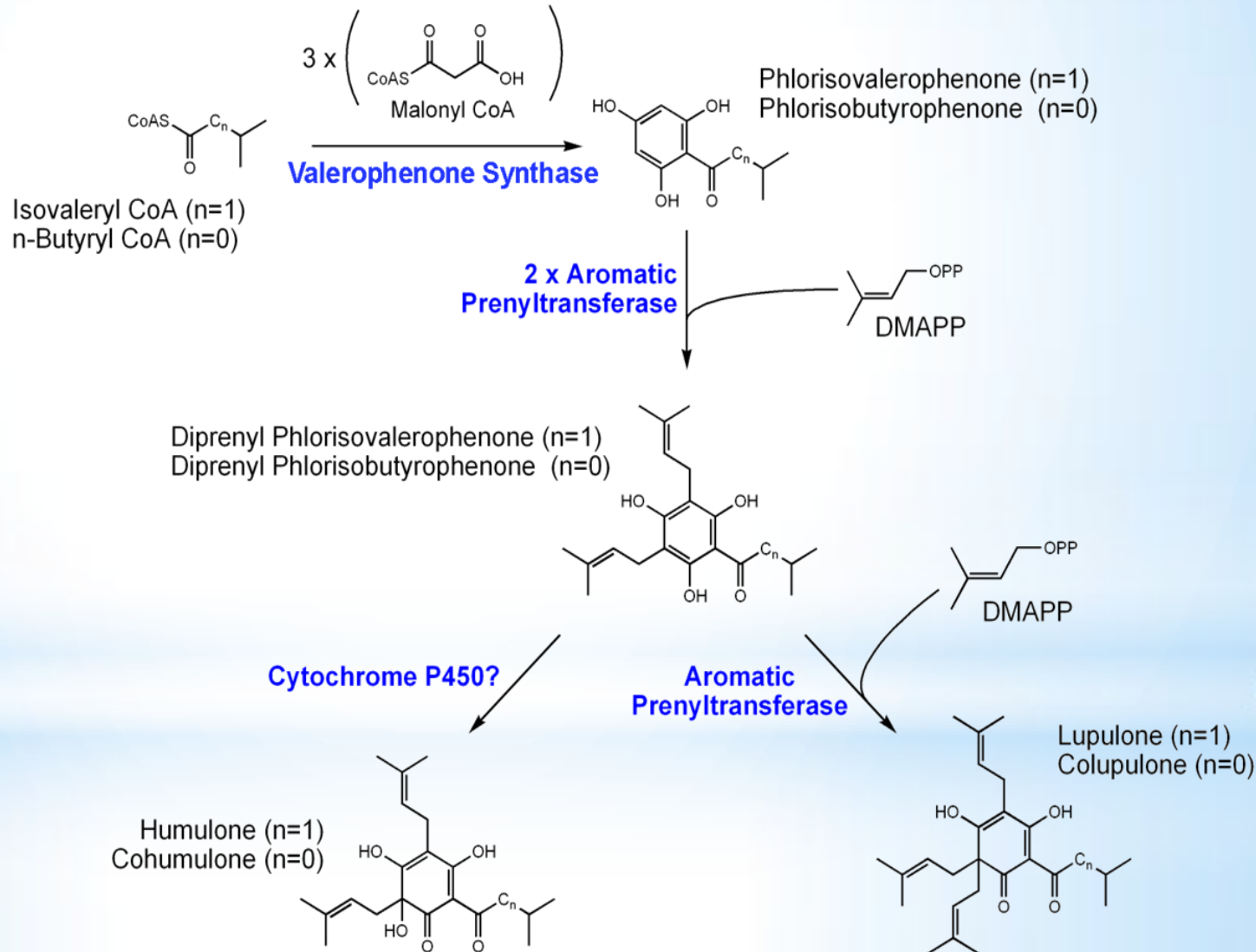
- The bitter acids of hops are **terpenophenolics**
- Diverse **polyphenols** contribute to haze, foam stability and bitterness
- Volatile **terpenes** are aromas
- Many flavors come from:
 - ❖ Aldehydes
 - ❖ Lactones
 - ❖ Norcarotenoids
 - ❖ Thiols
 - ❖ Esters
 - ❖ Flavanol glucosides
 - ❖ Many, many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

Roots of Diversity

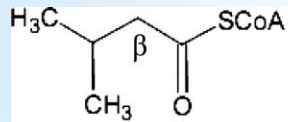
“Bitter” acids

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

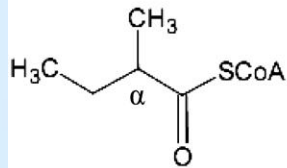
From where do
the various
forms come?



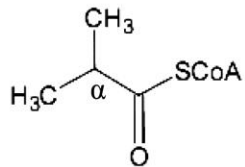
Valerophenone synthase is promiscuous!



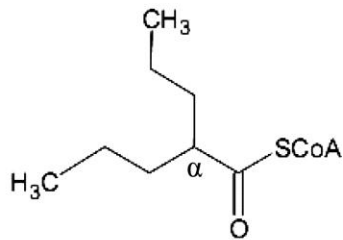
Isovaleryl-CoA



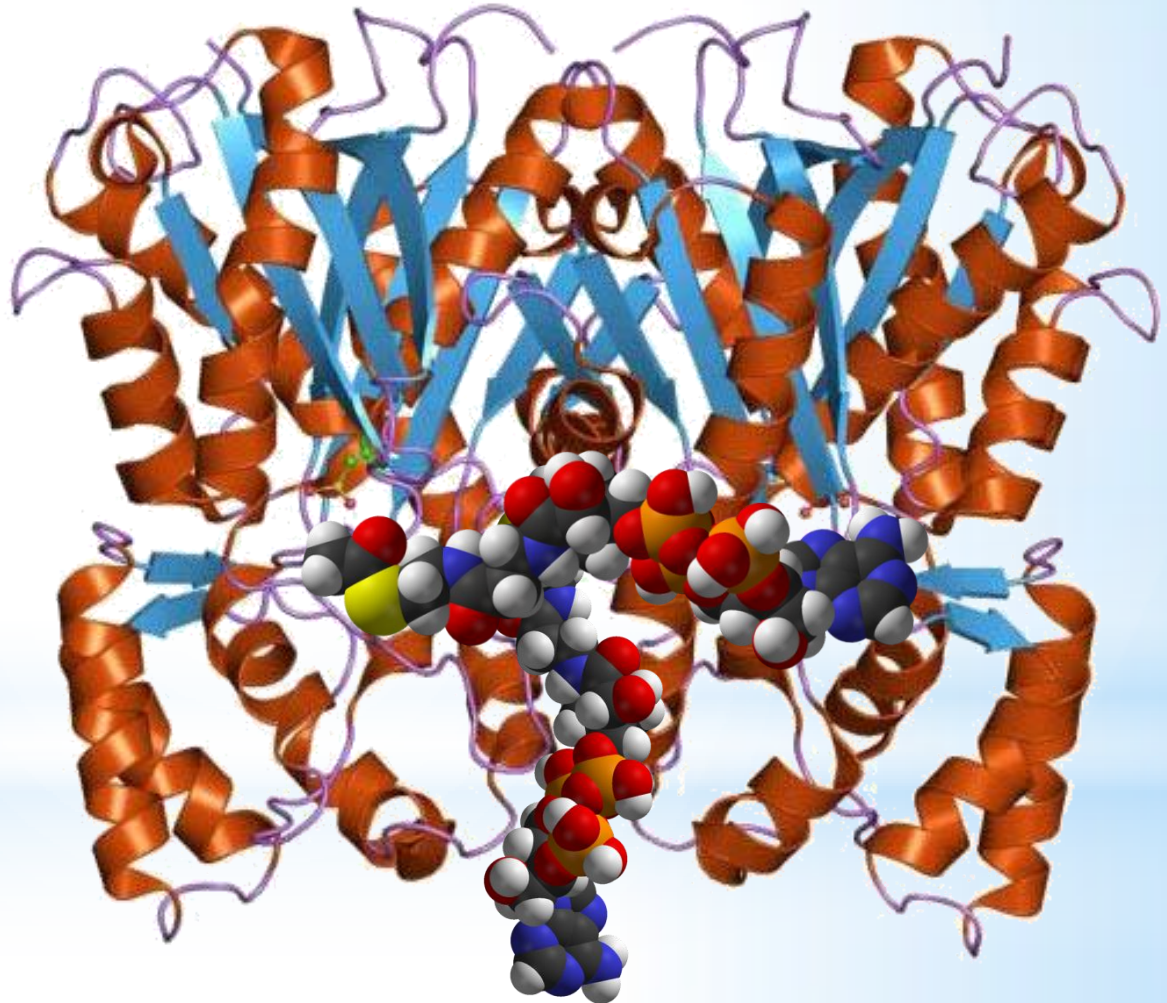
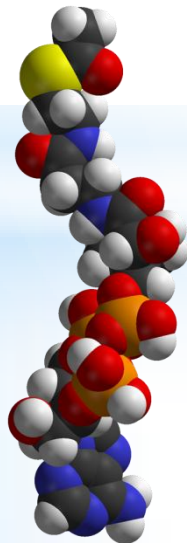
2-Methylbutyryl-CoA



Isobutyryl-CoA



Valproyl-CoA



EST Analysis of Hop Glandular Trichomes Identifies an O-Methyltransferase That Catalyzes the Biosynthesis of Xanthohumol

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

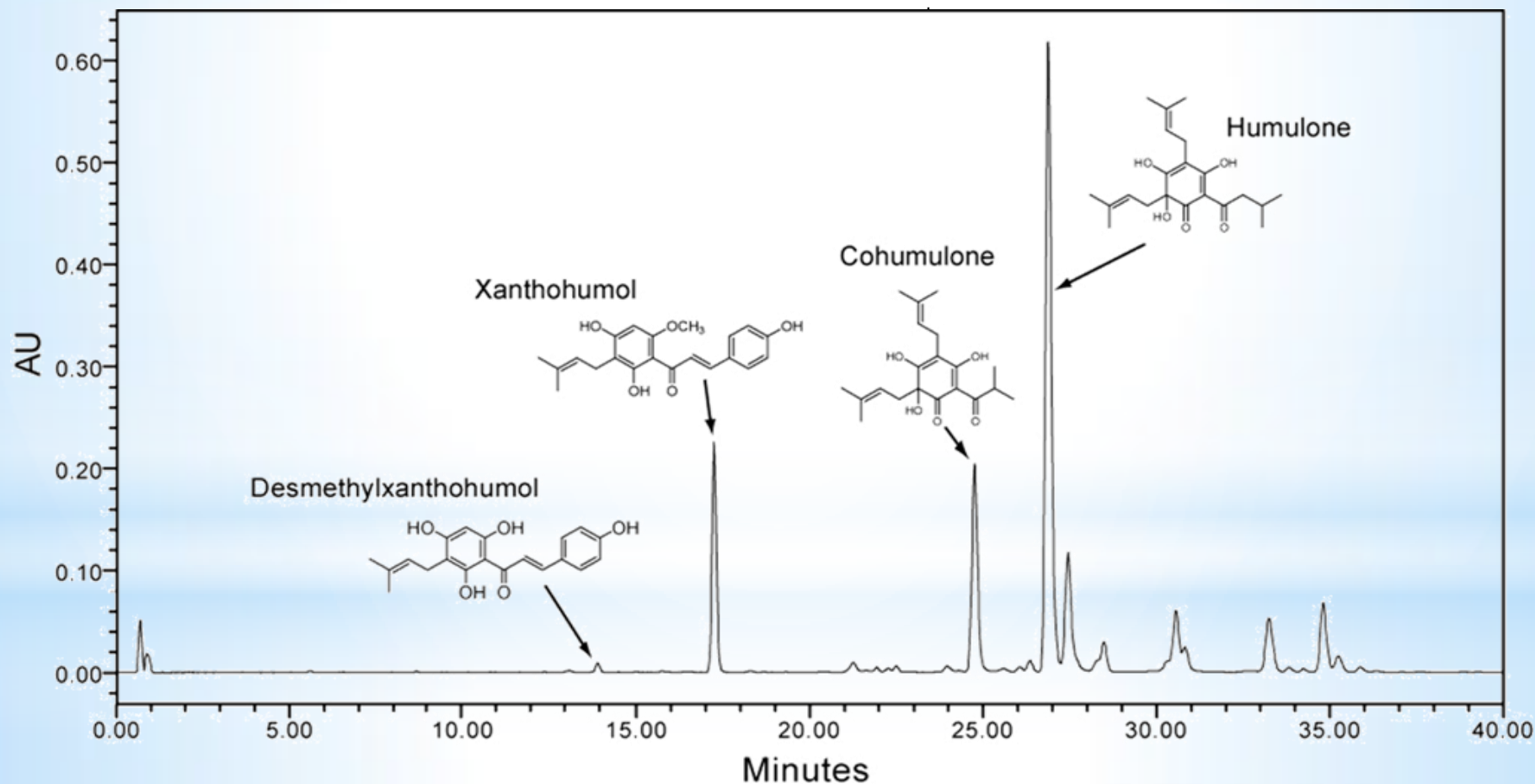
^e Linus Pauling Institute, Oregon State University, Corvallis, Oregon 97331

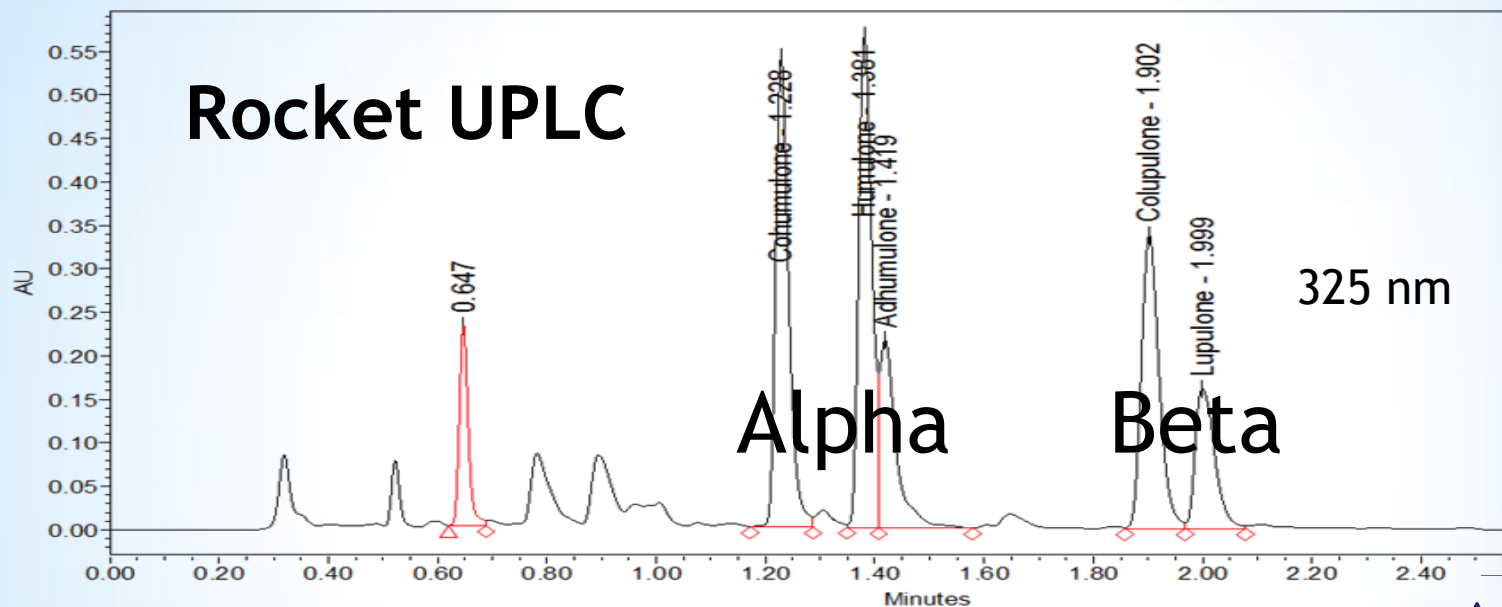
The glandular trichomes (lupulin glands) of hop (*Humulus lupulus*) synthesize essential oils and terpenophenolic resins, including the bioactive prenylflavonoid xanthohumol. To dissect the biosynthetic processes occurring in lupulin glands, we sequenced 10,581 ESTs from four trichome-derived cDNA libraries. 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

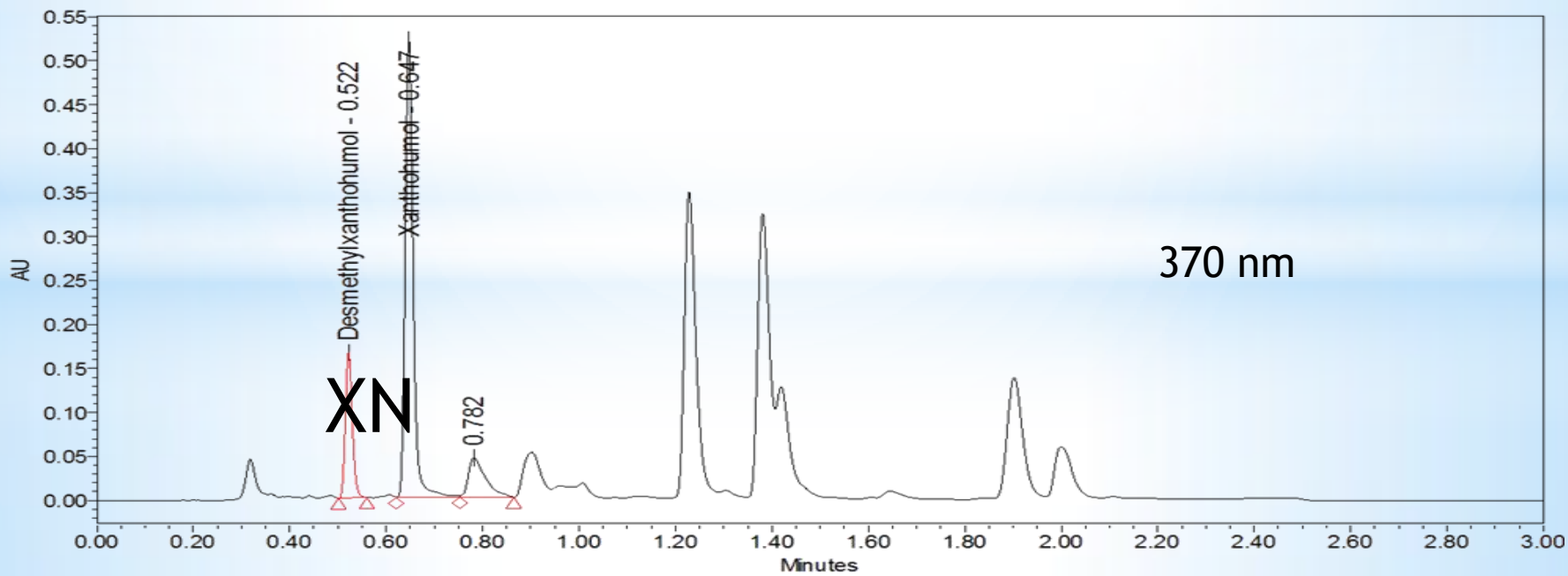
Classic Separation

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



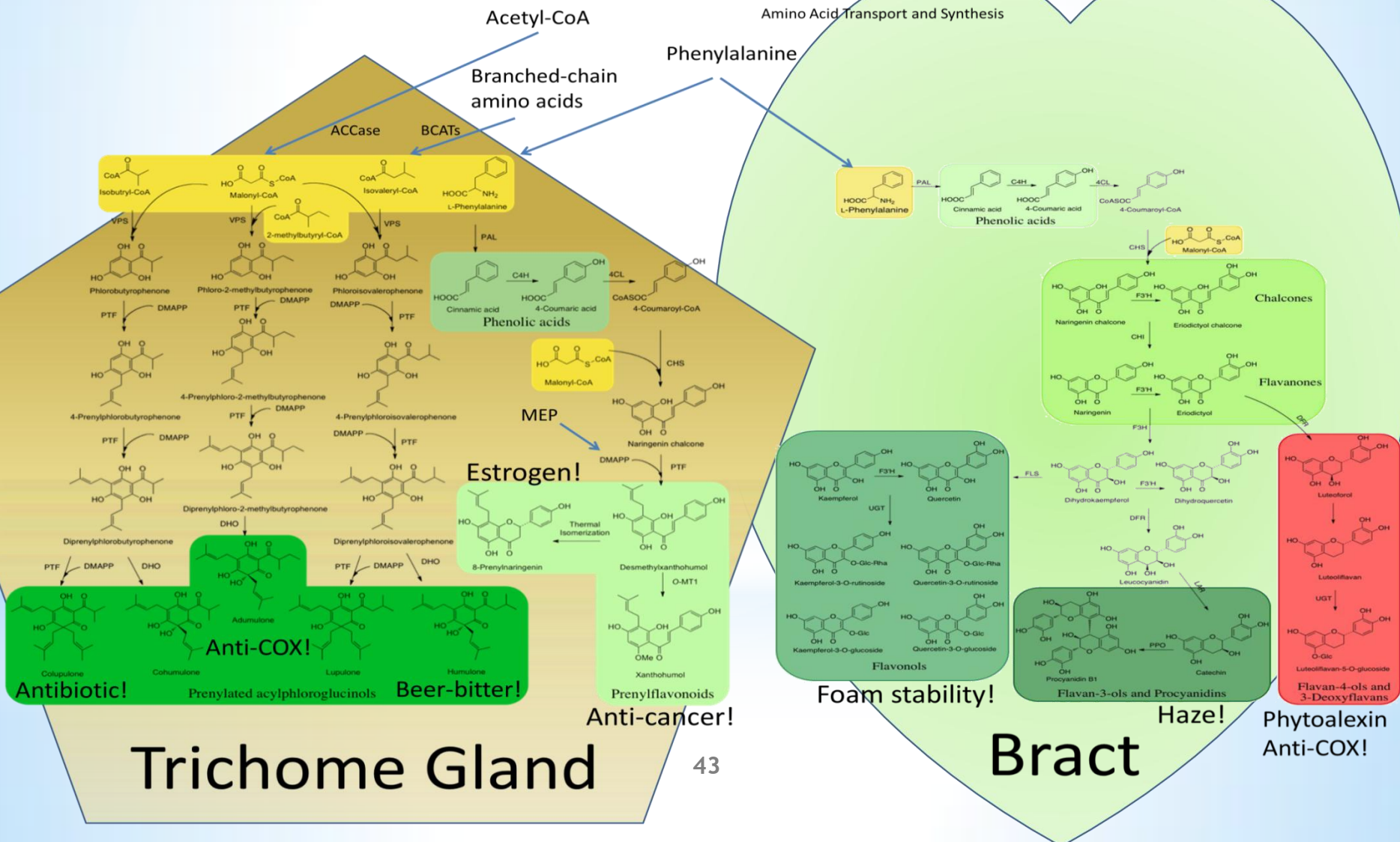


▲ 2.5 minutes

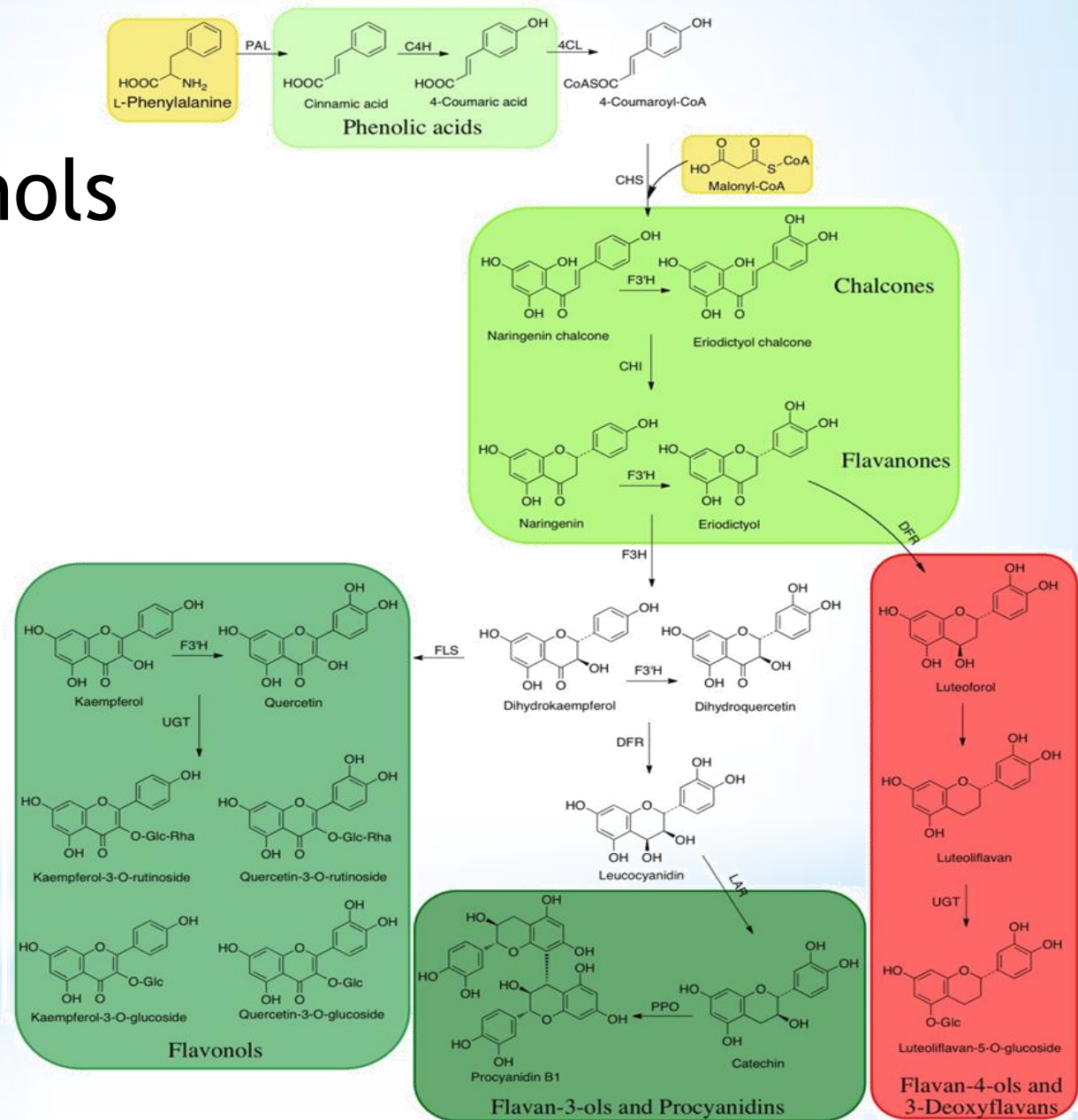


Bois du Giliou

Metabolic Precursors

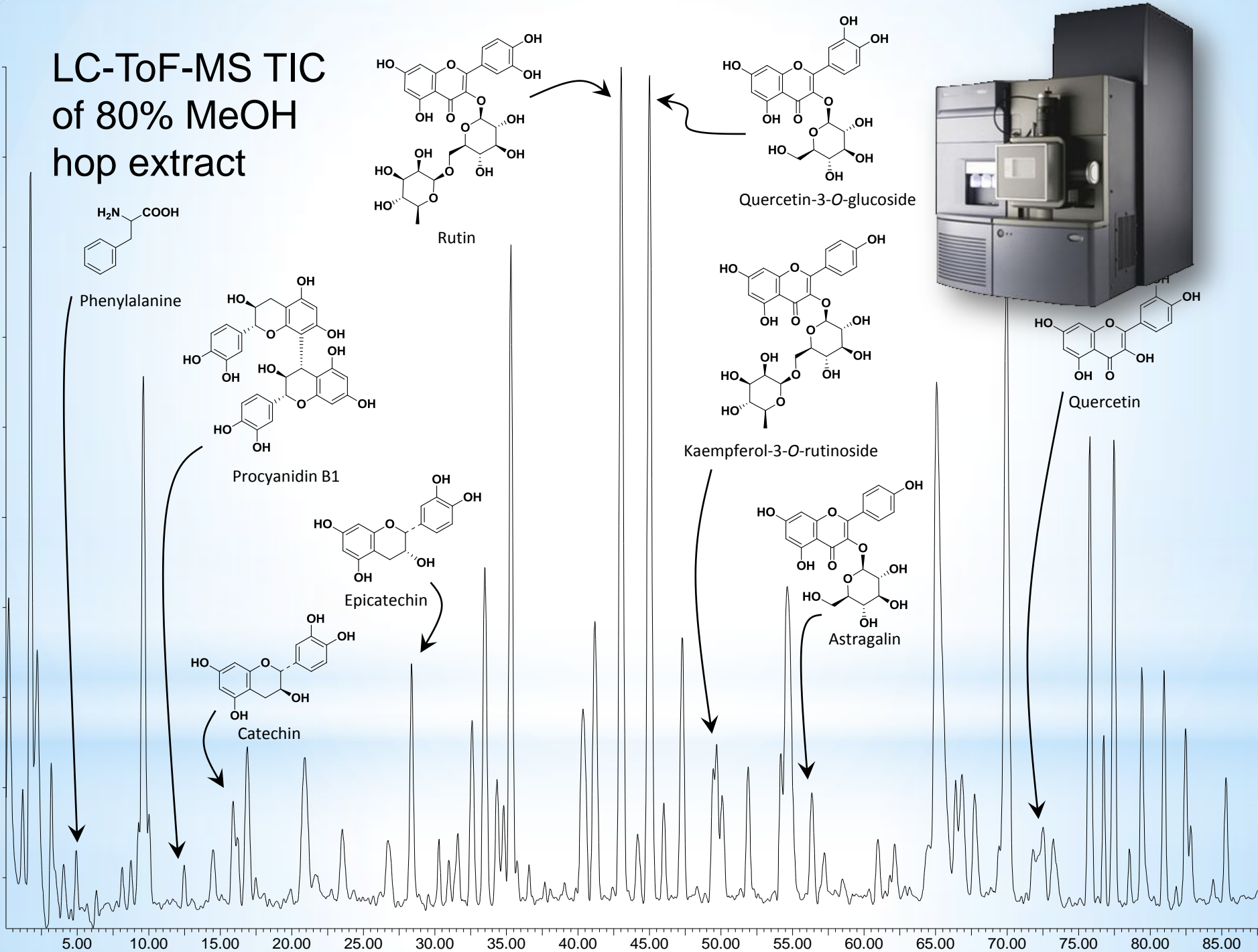


Polyphenols



Tannins

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



Scholar

12 results (0.05 sec)

Articles

Case law

My library

Any time

Since 2014

Since 2013

Since 2010

Custom range...

Sort by relevance

Sort by date

☒ include patents☒ include citations☐ Create alert

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

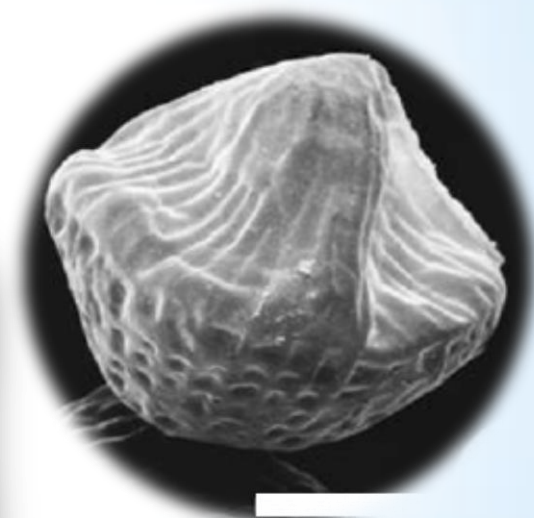
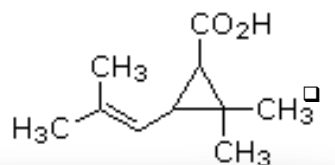
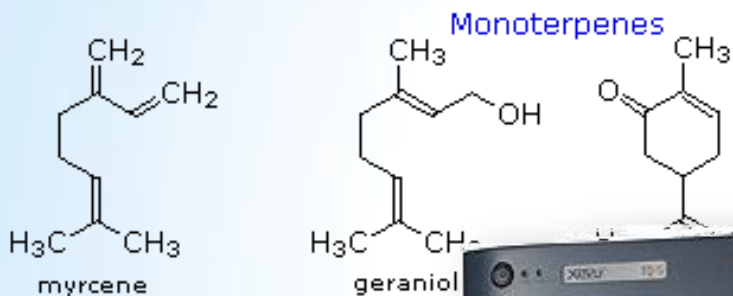
..., C Ma, M Figueroa, D Kincaid, [PD Matthews](#)... - **Food chemistry**, 2014 - Elsevier

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 ...

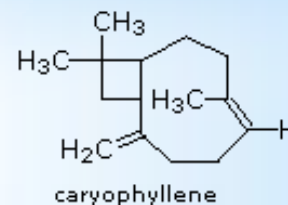
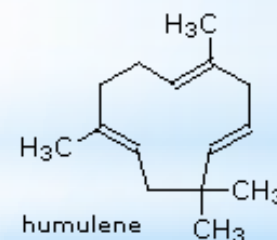
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Terpenoids

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



Besquiterpenes



ngaione

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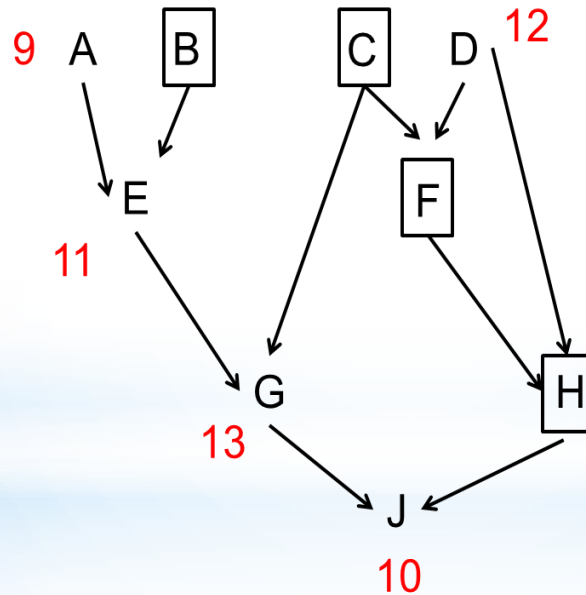
Evolution of Breeding Systems Technology

Single Cross
Select



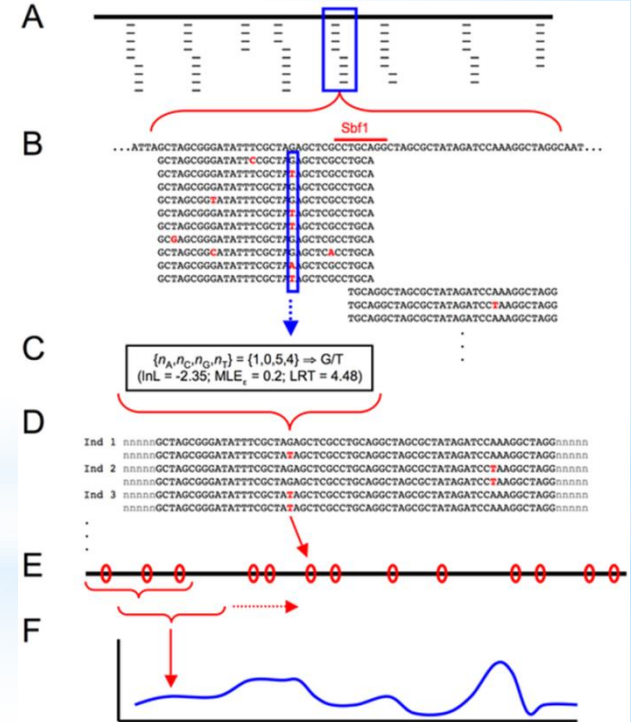
Quantitative
Genetics

Calculate EBVs for Male Alpha



Alpha Acid $h^2 = 0.5$

Molecular
Quantitative
Genetics



‘Omics>>>

Motivation for molecular marker development

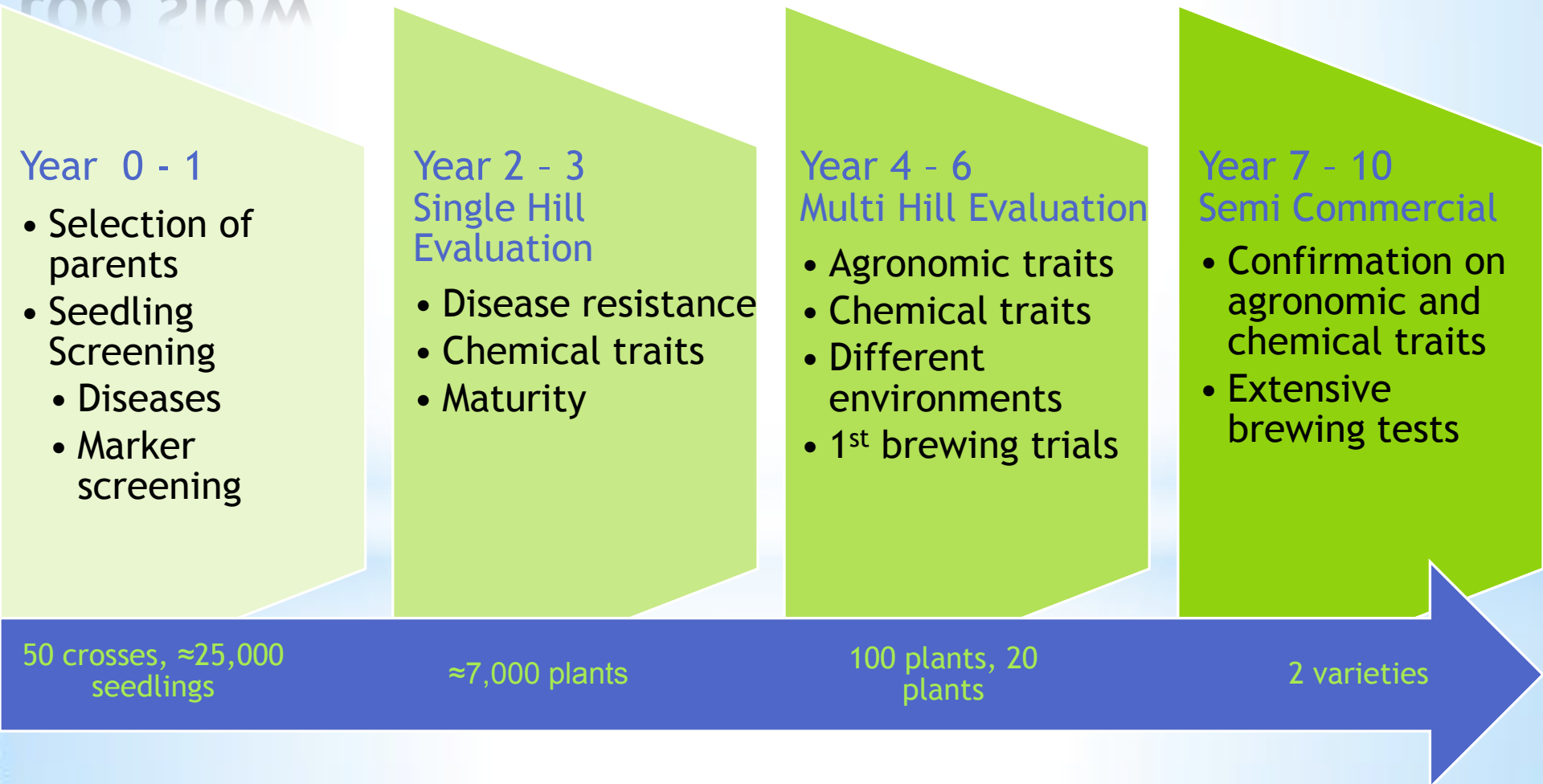
➤ What are markers good for?

- ✓ 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



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

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

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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

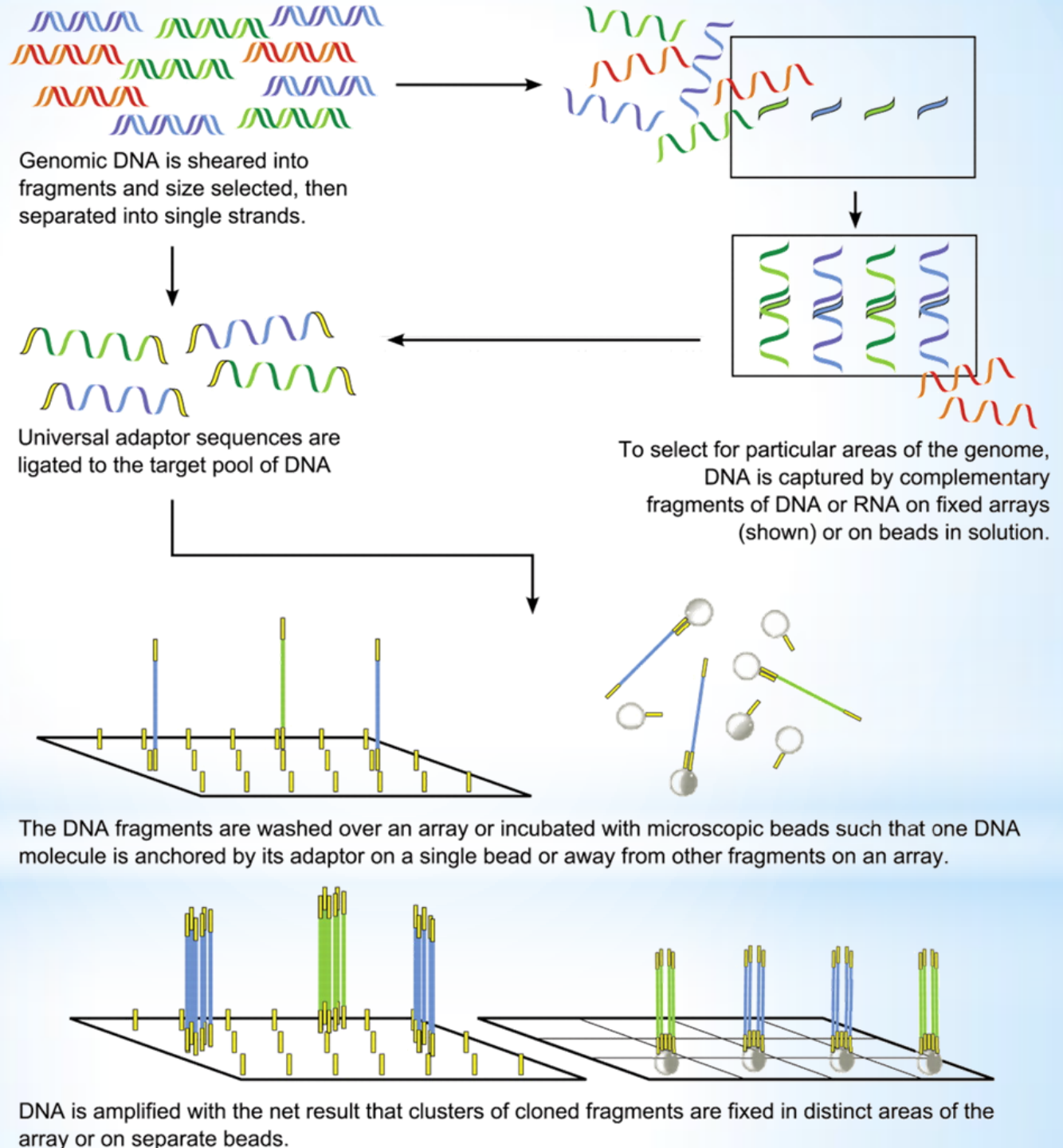
demonstration of utility, a cluster analysis showed substantial congruence to previous studies. The cultivar's genetic distances. The large number of demonstrated SSRs have the potential to

Molecular Marker Development

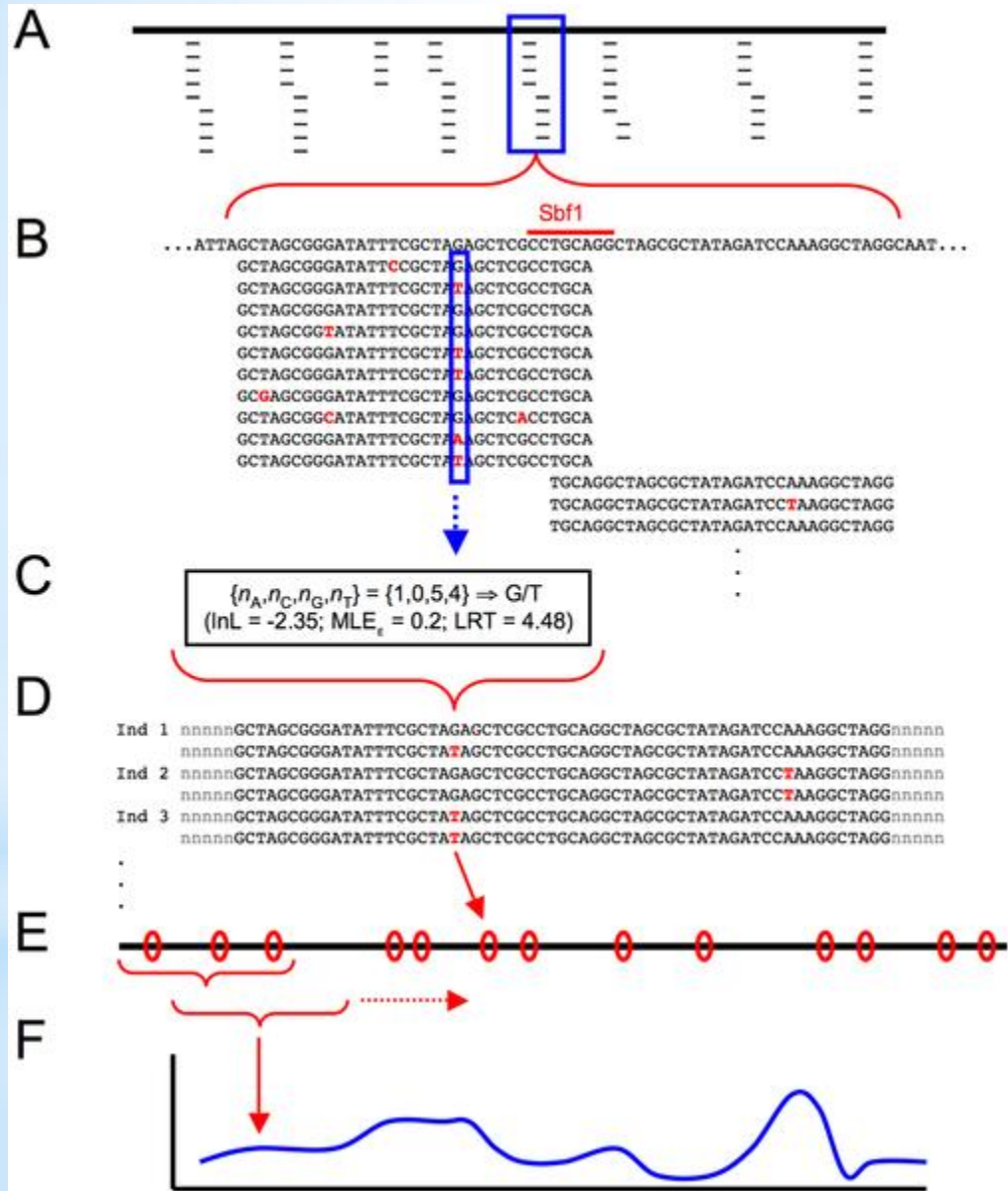
- ❑ 600 Diversity Array Technology markers (2010)
 - DNA hybridization micro array (give citation)
- ❑ 1000 Genic Simple Sequence Repeat markers (2011)
 - Transcriptome mining (give citation)
- ❑ 300, 000 Single Nucleotide Polymorphisms (2013)
 - Genotyping-by-sequencing (give example >)

Next Generation Sequencing provides Massive Molecular Marker Data

3-5 million
reads of 64
nucleotides
per plant



7-6001A0100-DA-2601160C100



GBS Revolution:

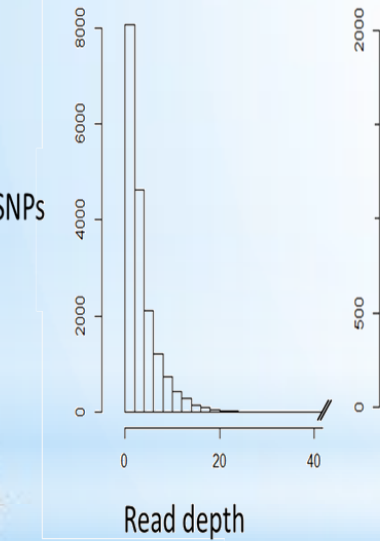
- 2-5 million makers per analysis & plant
- >1000 analyses completed
- 3 billion markers scored
- 64 bp gene tags around each marker

Mark Coles
Nicholi Pitra

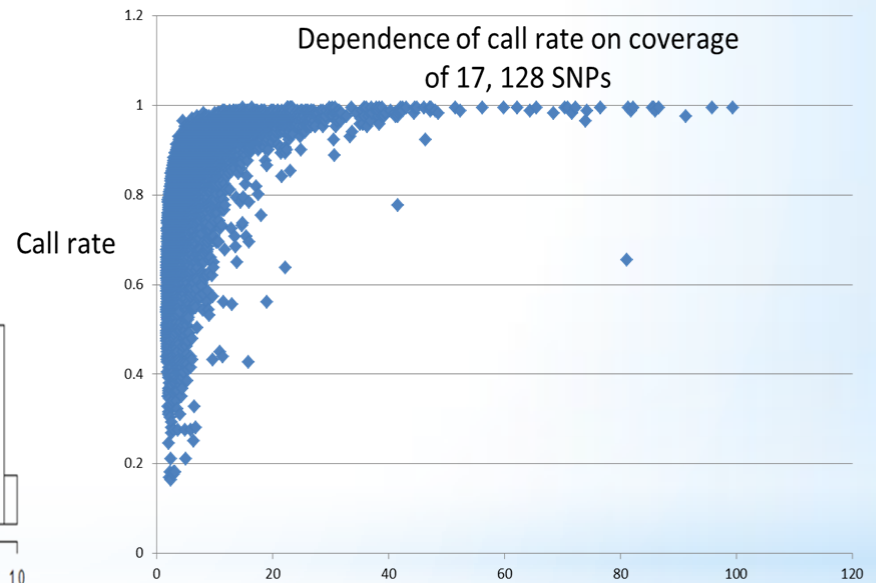
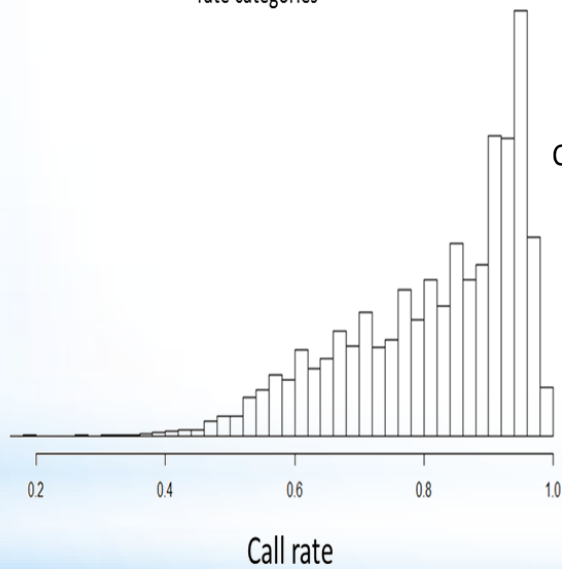
Genotyping-by-sequencing

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

Distribution of 17, 128 SNPs among read depth categories



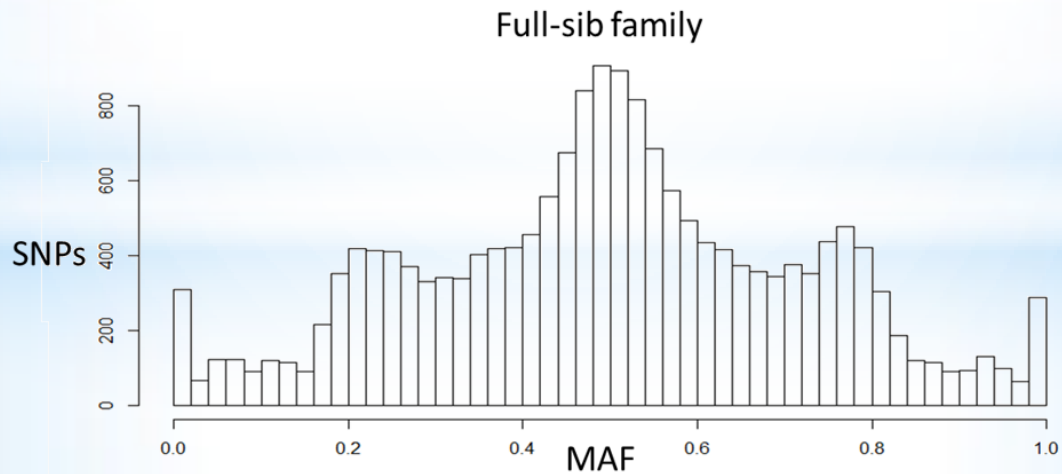
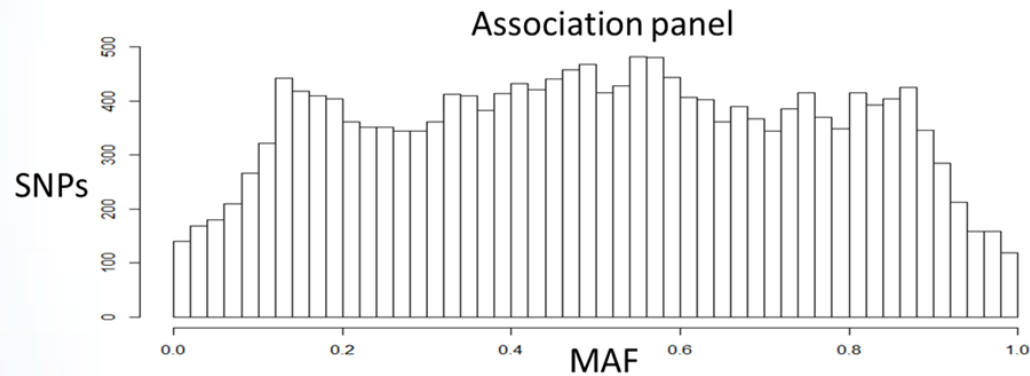
Distribution of 17, 128 SNPs among call rate categories



Genotyping-by-sequencing

GBS UNEAK TASSEL markers behave well genetically

Distribution of SNPs among minor allele frequency categories



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:

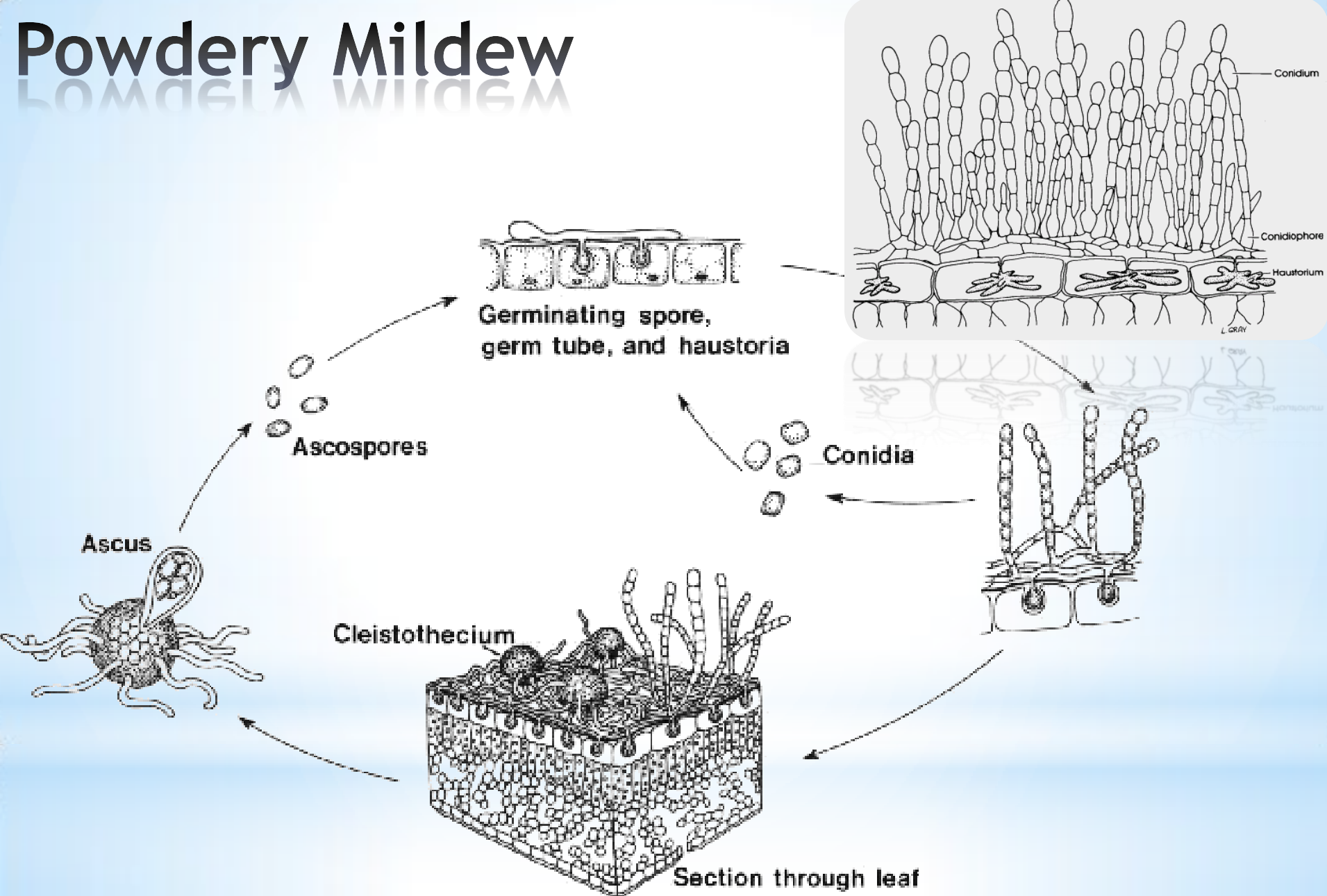
80% of breeding efforts are Breeding for disease resistance

➤ 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

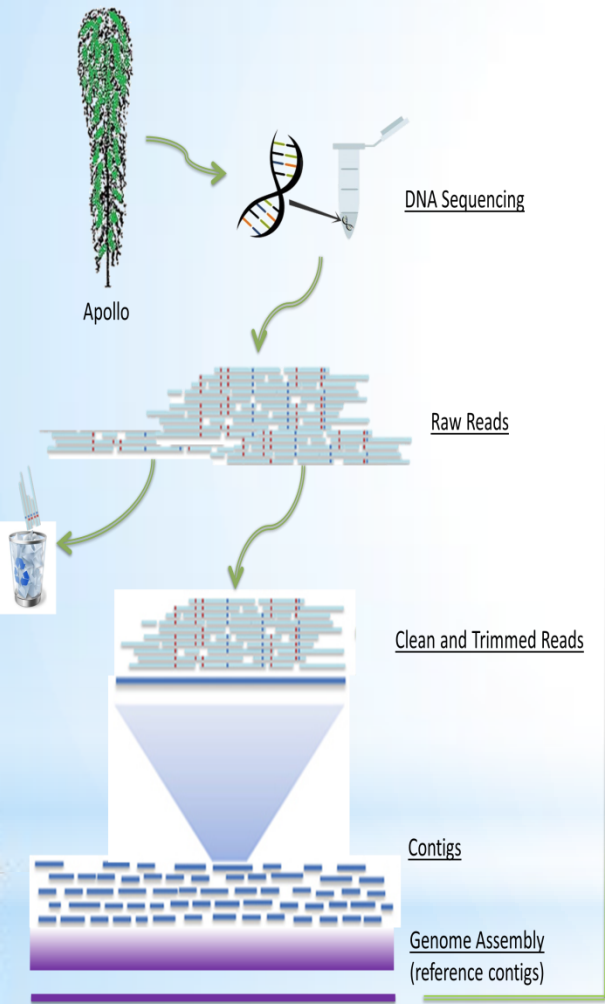
Gene	Source	Status in USA
R1, R3, Rb	Zenith	Tolerance
R2	Wye Target	Resistance
R4	Early Choice	Tolerance
R5	Cascade	Tolerance
R6	Nugget	Broken
19058mR6		Broken
Kazak 2000R	Kazak 2000	Resistant, HSR

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

CTCCGTAGCTAGCTTCGTGAGCGACTTCGGCGATTGGGATCGATCGTACGTACGTGACGGTAGCGTGCATCGATGGCTGGATCGGCTATCATCTCGGGCGATTAAATATATCTCTTTTCATATA
 CTCGGCGATTGGGATCGATCGTACGTACGTGACGGTAGCGTGC
 CTCGGCGATTGGGATCGATCGTACGTACGTGACGGTAGCGTGC
 CTCGGCGATTGGGATCGATCGTACGTACGTGACGGTAGCGTGC

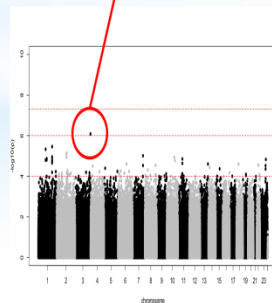
Mixed Liner Modeling

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

	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
.....

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

$$y = X\beta + Zu + e \quad h^2 = \frac{Var(A)}{Var(P)}$$



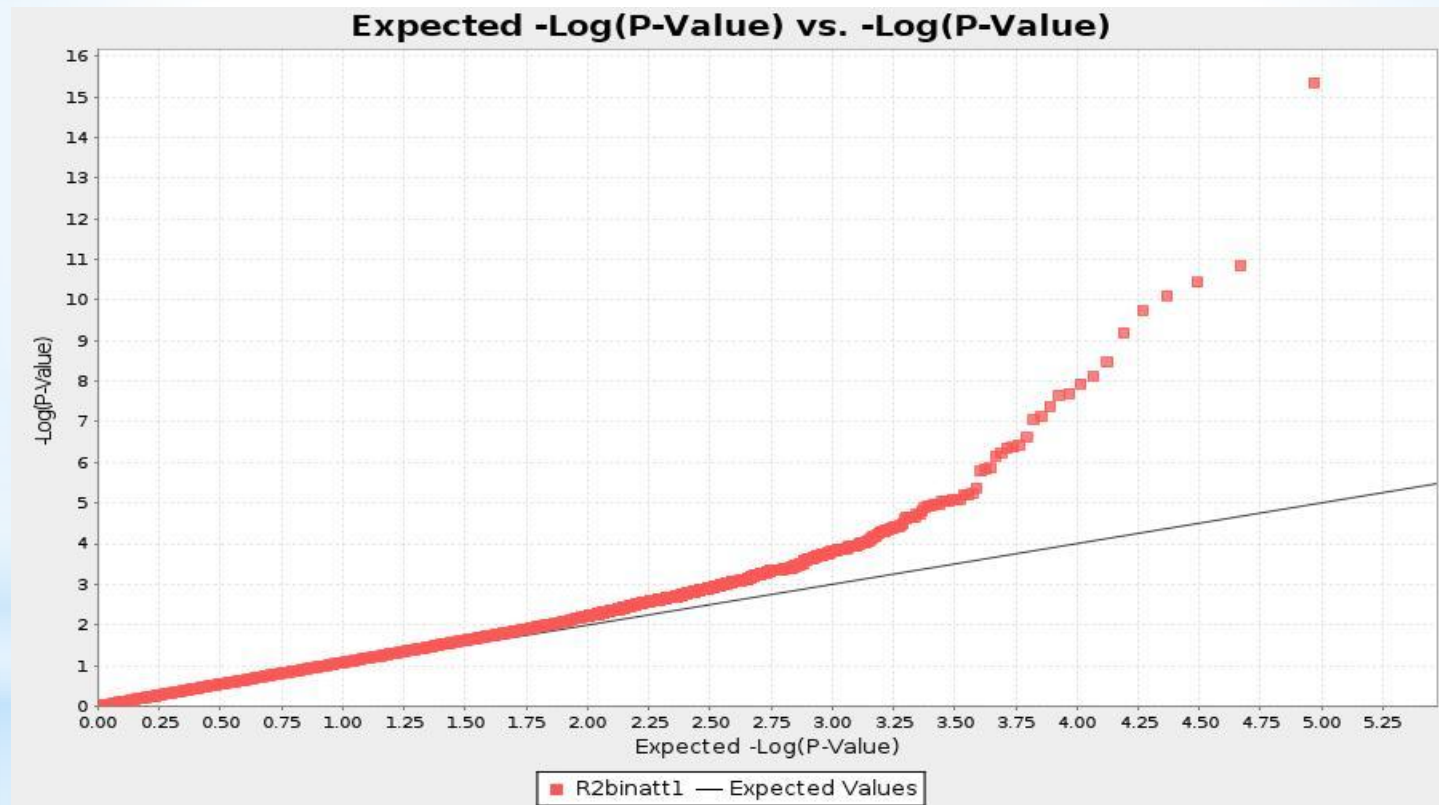
Apply the Marker



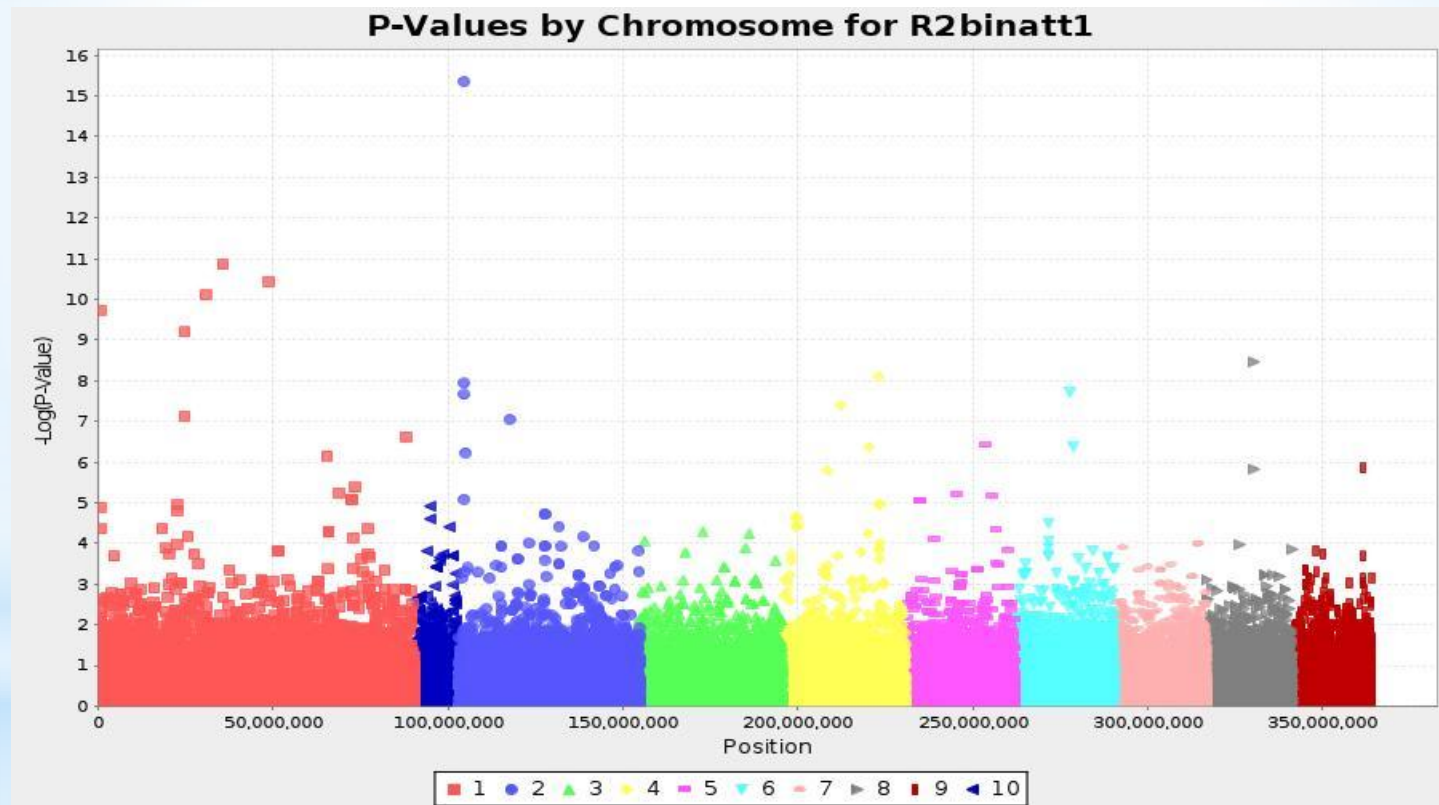
Analyze Results and Advance Breeding Program

	rs11	rs12	rs13	rs14	rs15	rs16	rs17	rs18	rs19	rs20	rs21	rs22	rs23	rs24	rs25	rs26	rs27	rs28	rs29	rs30	rs31	rs32	rs33	rs34	rs35	rs36	rs37	rs38	rs39	rs40	rs41	rs42	rs43	rs44	rs45	rs46	rs47	rs48	rs49	rs50	rs51	rs52	rs53	rs54	rs55	rs56	rs57	rs58	rs59	rs60	rs61	rs62	rs63	rs64	rs65	rs66	rs67	rs68	rs69	rs70	rs71	rs72	rs73	rs74	rs75	rs76	rs77	rs78	rs79	rs80	rs81	rs82	rs83	rs84	rs85	rs86	rs87	rs88	rs89	rs90	rs91	rs92	rs93	rs94	rs95	rs96	rs97	rs98	rs99	rs100	rs101	rs102	rs103	rs104	rs105	rs106	rs107	rs108	rs109	rs110	rs111	rs112	rs113	rs114	rs115	rs116	rs117	rs118	rs119	rs120	rs121	rs122	rs123	rs124	rs125	rs126	rs127	rs128	rs129	rs130	rs131	rs132	rs133	rs134	rs135	rs136	rs137	rs138	rs139	rs140	rs141	rs142	rs143	rs144	rs145	rs146	rs147	rs148	rs149	rs150	rs151	rs152	rs153	rs154	rs155	rs156	rs157	rs158	rs159	rs160	rs161	rs162	rs163	rs164	rs165	rs166	rs167	rs168	rs169	rs170	rs171	rs172	rs173	rs174	rs175	rs176	rs177	rs178	rs179	rs180	rs181	rs182	rs183	rs184	rs185	rs186	rs187	rs188	rs189	rs190	rs191	rs192	rs193	rs194	rs195	rs196	rs197	rs198	rs199	rs200	rs201	rs202	rs203	rs204	rs205	rs206	rs207	rs208	rs209	rs210	rs211	rs212	rs213	rs214	rs215	rs216	rs217	rs218	rs219	rs220	rs221	rs222	rs223	rs224	rs225	rs226	rs227	rs228	rs229	rs230	rs231	rs232	rs233	rs234	rs235	rs236	rs237	rs238	rs239	rs240	rs241	rs242	rs243	rs244	rs245	rs246	rs247	rs248	rs249	rs250	rs251	rs252	rs253	rs254	rs255	rs256	rs257	rs258	rs259	rs260	rs261	rs262	rs263	rs264	rs265	rs266	rs267	rs268	rs269	rs270	rs271	rs272	rs273	rs274	rs275	rs276	rs277	rs278	rs279	rs280	rs281	rs282	rs283	rs284	rs285	rs286	rs287	rs288	rs289	rs290	rs291	rs292	rs293	rs294	rs295	rs296	rs297	rs298	rs299	rs300	rs301	rs302	rs303	rs304	rs305	rs306	rs307	rs308	rs309	rs310	rs311	rs312	rs313	rs314	rs315	rs316	rs317	rs318	rs319	rs320	rs321	rs322	rs323	rs324	rs325	rs326	rs327	rs328	rs329	rs330	rs331	rs332	rs333	rs334	rs335	rs336	rs337	rs338	rs339	rs340	rs341	rs342	rs343	rs344	rs345	rs346	rs347	rs348	rs349	rs350	rs351	rs352	rs353	rs354	rs355	rs356	rs357	rs358	rs359	rs360	rs361	rs362	rs363	rs364	rs365	rs366	rs367	rs368	rs369	rs370	rs371	rs372	rs373	rs374	rs375	rs376	rs377	rs378	rs379	rs380	rs381	rs382	rs383	rs384	rs385	rs386	rs387	rs388	rs389	rs390	rs391	rs392	rs393	rs394	rs395	rs396	rs397	rs398	rs399	rs400	rs401	rs402	rs403	rs404	rs405	rs406	rs407	rs408	rs409	rs410	rs411	rs412	rs413	rs414	rs415	rs416	rs417	rs418	rs419	rs420	rs421	rs422	rs423	rs424	rs425	rs426	rs427	rs428	rs429	rs430	rs431	rs432	rs433	rs434	rs435	rs436	rs437	rs438	rs439	rs440	rs441	rs442	rs443	rs444	rs445	rs446	rs447	rs448	rs449	rs450	rs451	rs452	rs453	rs454	rs455	rs456	rs457	rs458	rs459	rs460	rs461	rs462	rs463	rs464	rs465	rs466	rs467	rs468	rs469	rs470	rs471	rs472	rs473	rs474	rs475	rs476	rs477	rs478	rs479	rs480	rs481	rs482	rs483	rs484	rs485	rs486	rs487	rs488	rs489	rs490	rs491	rs492	rs493	rs494	rs495	rs496	rs497	rs498	rs499	rs500	rs501	rs502	rs503	rs504	rs505	rs506	rs507	rs508	rs509	rs510	rs511	rs512	rs513	rs514	rs515	rs516	rs517	rs518	rs519	rs520	rs521	rs522	rs523	rs524	rs525	rs526	rs527	rs528	rs529	rs530	rs531	rs532	rs533	rs534	rs535	rs536	rs537	rs538	rs539	rs540	rs541	rs542	rs543	rs544	rs545	rs546	rs547	rs548	rs549	rs550	rs551	rs552	rs553	rs554	rs555	rs556	rs557	rs558	rs559	rs560	rs561	rs562	rs563	rs564	rs565	rs566	rs567	rs568	rs569	rs570	rs571	rs572	rs573	rs574	rs575	rs576	rs577	rs578	rs579	rs580	rs581	rs582	rs583	rs584	rs585	rs586	rs587	rs588	rs589	rs590	rs591	rs592	rs593	rs594	rs595	rs596	rs597	rs598	rs599	rs600	rs601	rs602	rs603	rs604	rs605	rs606	rs607	rs608	rs609	rs610	rs611	rs612	rs613	rs614	rs615	rs616	rs617	rs618	rs619	rs620	rs621	rs622	rs623	rs624	rs625	rs626	rs627	rs628	rs629	rs630	rs631	rs632	rs633	rs634	rs635	rs636	rs637	rs638	rs639	rs640	rs641	rs642	rs643	rs644	rs645	rs646	rs647	rs648	rs649	rs650	rs651	rs652	rs653	rs654	rs655	rs656	rs657	rs658	rs659	rs660	rs661	rs662	rs663	rs664	rs665	rs666	rs667	rs668	rs669	rs670	rs671	rs672	rs673	rs674	rs675	rs676	rs677	rs678	rs679	rs680	rs681	rs682	rs683	rs684	rs685	rs686	rs687	rs688	rs689	rs690	rs691	rs692	rs693	rs694	rs695	rs696	rs697	rs698	rs699	rs700	rs701	rs702	rs703	rs704	rs705	rs706	rs707	rs708	rs709	rs710	rs711	rs712	rs713	rs714	rs715	rs716	rs717	rs718	rs719	rs720	rs721	rs722	rs723	rs724	rs725	rs726	rs727	rs728	rs729	rs730	rs731	rs732	rs733	rs734	rs735	rs736	rs737	rs738	rs739	rs740	rs741	rs742	rs743	rs744	rs745	rs746	rs747	rs748	rs749	rs750	rs751	rs752	rs753	rs754	rs755	rs756	rs757	rs758	rs759	rs760	rs761	rs762	rs763	rs764	rs765	rs766	rs767	rs768	rs769	rs770	rs771	rs772	rs773	rs774	rs775	rs776	rs777	rs778	rs779	rs780	rs781	rs782	rs783	rs784	rs785	rs786	rs787	rs788	rs789	rs790	rs791	rs792	rs793	rs794	rs795	rs796	rs797	rs798	rs799	rs800	rs801	rs802	rs803	rs804	rs805	rs806	rs807	rs808	rs809	rs810	rs811	rs812	rs813	rs814	rs815	rs816	rs817	rs818	rs819	rs820	rs821	rs822	rs823	rs824	rs825	rs826	rs827	rs828	rs829	rs830	rs831	rs832	rs833	rs834	rs835	rs836	rs837	rs838	rs839	rs840	rs841	rs842	rs843	rs844	rs845	rs846	rs847	rs848	rs849	rs850	rs851	rs852	rs853	rs854	rs855	rs856	rs857	rs858	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Q-Q plot of MLM associations

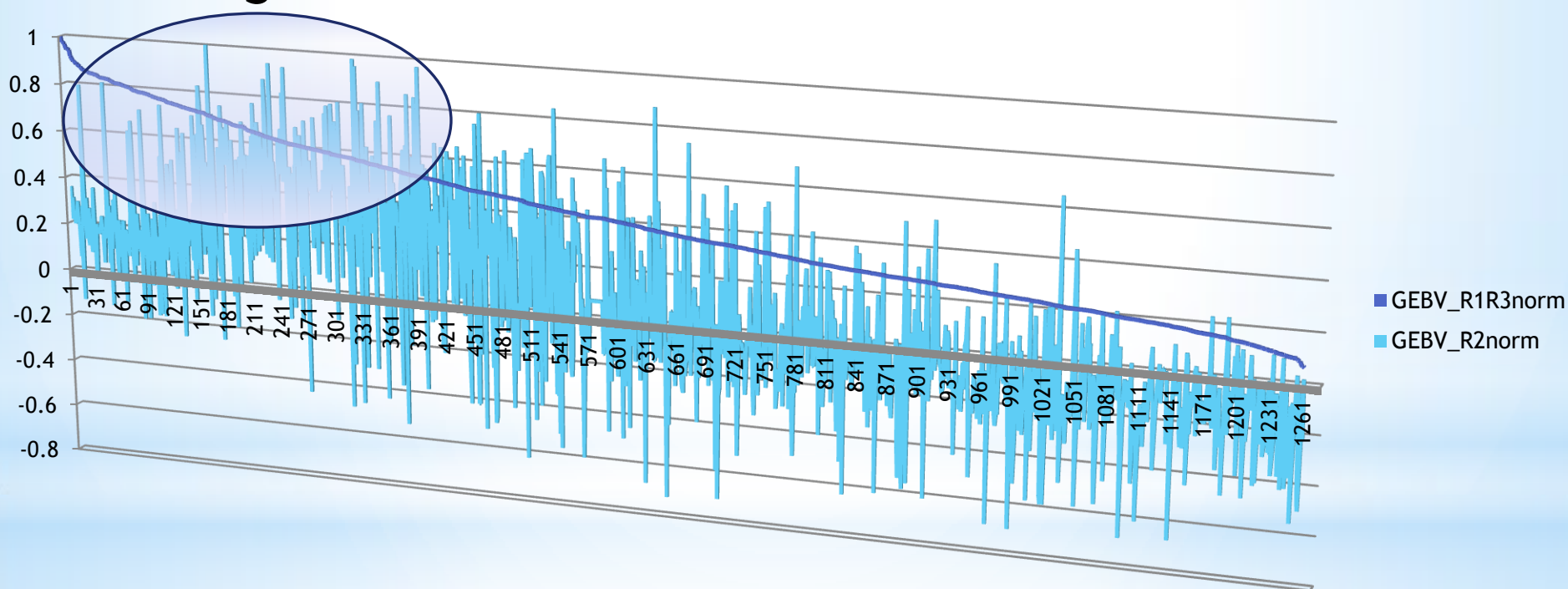


Manhattan Plot of MLM associations



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

High GEBV for both sets of markers



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

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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 ApeK1 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 $p < 1 \times 10^{-4}$. 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.

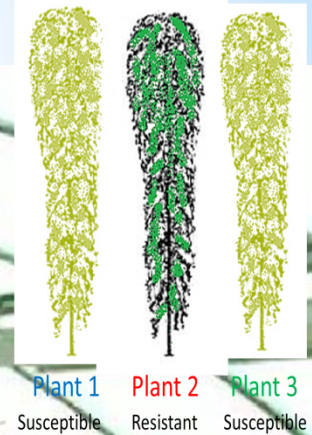
Abbreviations: WGAS=whole genome association study; SNP=single nucleotide polymorphism, NGS=next generation sequencing

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

2. Generating GBS Markers



Phenotype

Extract DNA

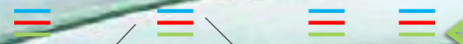


Cut and Sequence
Genomic DNA

Specific Cut Sites

3. Genome Based Selection

Mapping Reads to Reference



Single Nucleotide
Polymorphism Detection

.....CTCCGTAGCTAGCTTCGTGAGCGATTCGCGATTCCGGATCGATGACGTACGTACGCTGATCGATCGATGGATCGGCTATCATCTGCGGCGATTAATATATATTCTCTTTTCATATA.....
CTTCGGCGATTTCGGGATCGATGACGTACGTACGTGACGGTAGCGTG
CTTCGGCGATTTCGGGATCGATGACGTACGTGACGGTAGCGTG
CTTCGGCGATTTCGGGATCGATGACGTACGTGACGGTAGCGTG

Mixed Linear Modeling

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

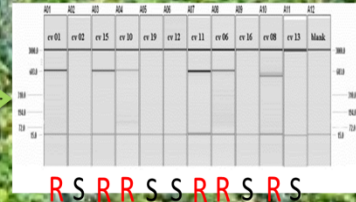
	Plant_1	Plant_2	Plant_3
Plant_1	1.0	0.825	0.356
Plant_2	0.825	1.0	0.074
Plant_3	0.356	0.074	1.0

	Resistant
Plant_1	yes
Plant_2	no
Plant_3	yes

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

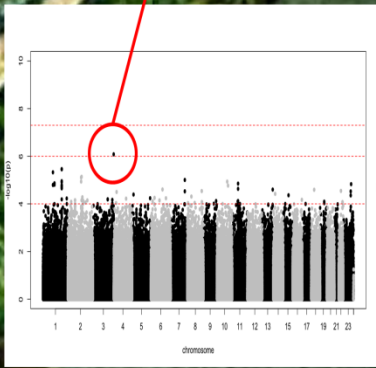
$$h^2 = \frac{Var(A)}{Var(P)}$$

Analyze Results and Advance
Breeding Program



R = resistant
S = susceptible

Apply
the
Marker



Summary

- ✓ Hops exhibit great morphological, genetic and chemical 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



Thank you very much.

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Steiner Asia Limited