Molecular Breeding for Improved Berry Quality in *Ribes*, *Rubus* and *Vaccinium*

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The James Hutton Institute

Vaccinium & Other Superfruits Symposium
Maastricht, June 2012

Fruit Breeding at JHI

- Commercially-funded breeding programmes
  - Raspberry, blackberry
  - Blackcurrant
- New techniques for selecting the plants we need
  - Marker–assisted breeding strategies
  - Faster and more specific cultivar development

Breeding techniques

- Expensive to run breeding programmes:
  - Lengthy timescales
    - Some traits take a long time to screen for, others are impossible to screen on a high-throughput basis
  - Field/glasshouse costs
- Timescales need to be reduced and efficiency needs to be increased
  - Time to cv. currently 12-15 years
- Extensive phenotyping in field, glasshouse and CE rooms
- Establish link between genotype and phenotype

Molecular Breeding

- Faster identification of genetically superior individuals in breeding populations
- Can be utilised in situations where:
  - Assessment in field takes a long time
    - Pest resistance (some)
  - Assessment can only be done on mature plants over time
    - Fruit quality
- Basic research development costs relatively high, deployment costs low
- No environmental effects
- **Must be associated with detailed phenotyping**

Existing markers in *Rubus* and *Ribes*

- Markers linked to pest/disease resistances
  - *Phytophthora* root rot (raspberry)
  - *Cecidophyopsis* gall mite (blackcurrant)

- SSR marker (root rot)
- PCR-based marker (gall mite)

- Time saved compared to field infestation plots (ca. 4 years) for screening of new lines from breeding programme

- **Markers now routinely deployed in JHI breeding programmes as a selection tool**

Brennan *et al*., 2009. TAG **118**: 205-212
Graham *et al*., 2011. TAG **123**: 585-601

Blackcurrant Breeding Objectives

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Fruit quality

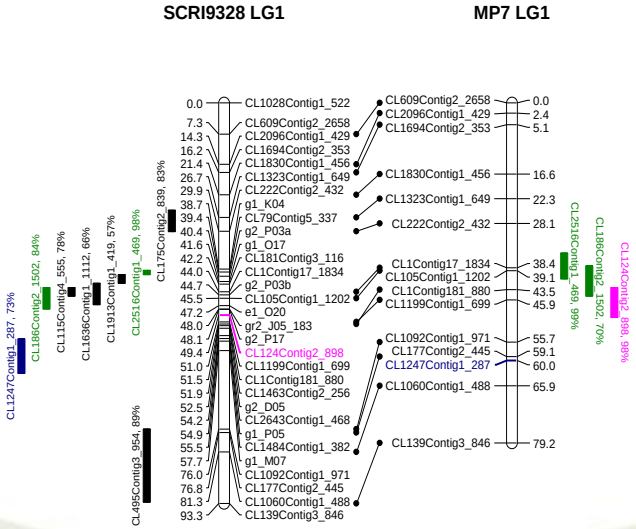
- High Brix/acid ratio
- Low total acidity
- Anthocyanins
  - Delphinidins preferentially selected
- Vitamin C (AsA)
  - > 140 mg/100 ml
- Sensory traits
- Berry size
  - 1 g minimum
  - Fresh market – 2 g minimum

Agronomic

- Environmental resilience
  - Winter chill levels
    - < 2000 h/7.2°C
- Pest resistance for low-input growing
- Acceptable crop yield
  - > 6 t/ha
  - Juice yield also quantified

Trait associations – *Ribes* fruit quality I

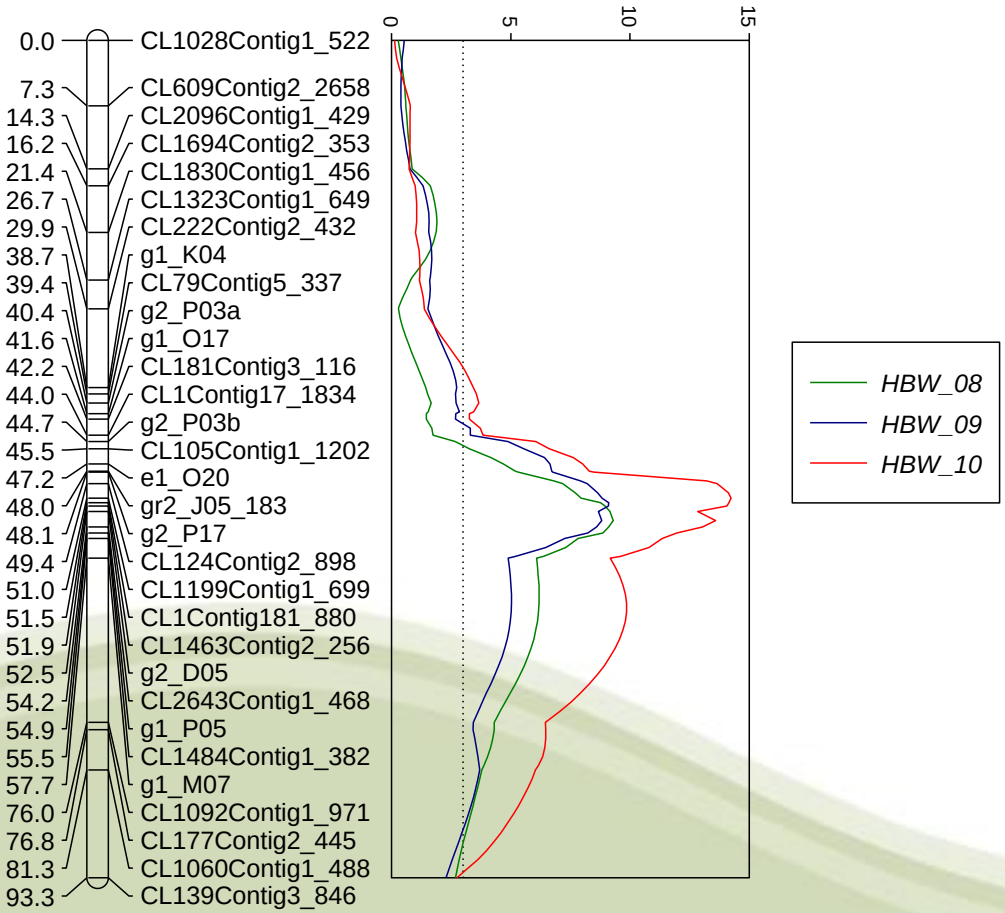
- Measurements across reference 9328 mapping population (ca. 300 plants) for 4 years at JHI
- SNP-based linkage map developed using transcriptome-based 2GS 454 sequencing
- Individual traits placed on genetic linkage map
  - Fruit size
  - Anthocyanins
- Associated molecular markers identified for validation in other germplasm



*Russell et al., BMC Plant Biology 2011, **11:147***

Blackcurrant berry weight

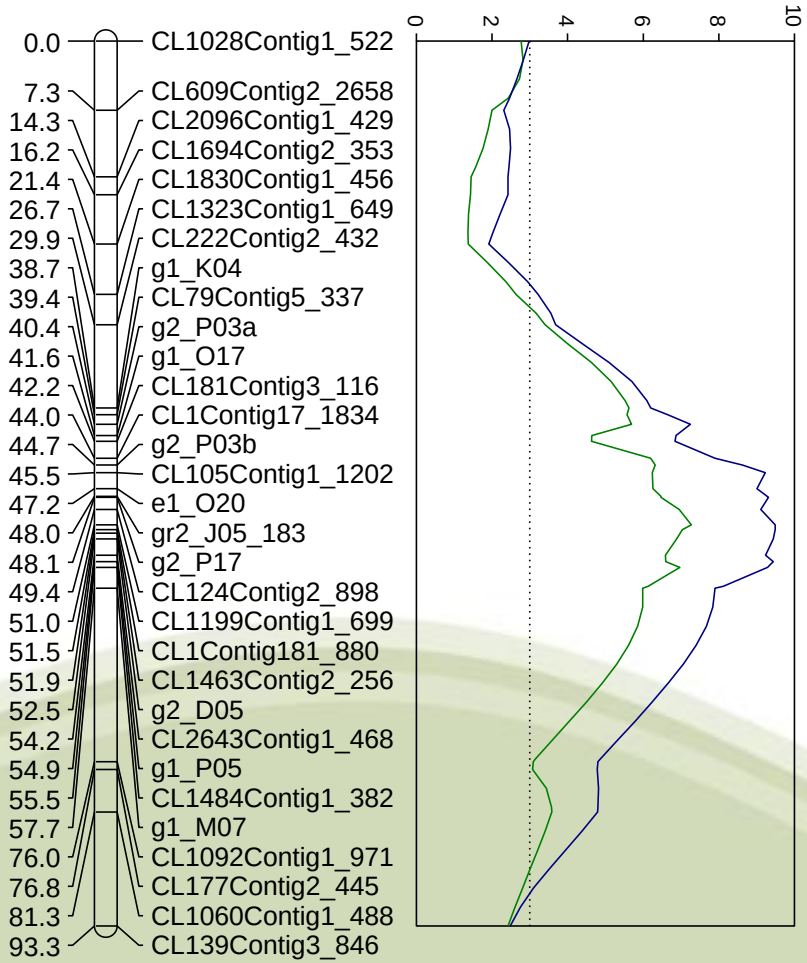
SCRI9328 LG1



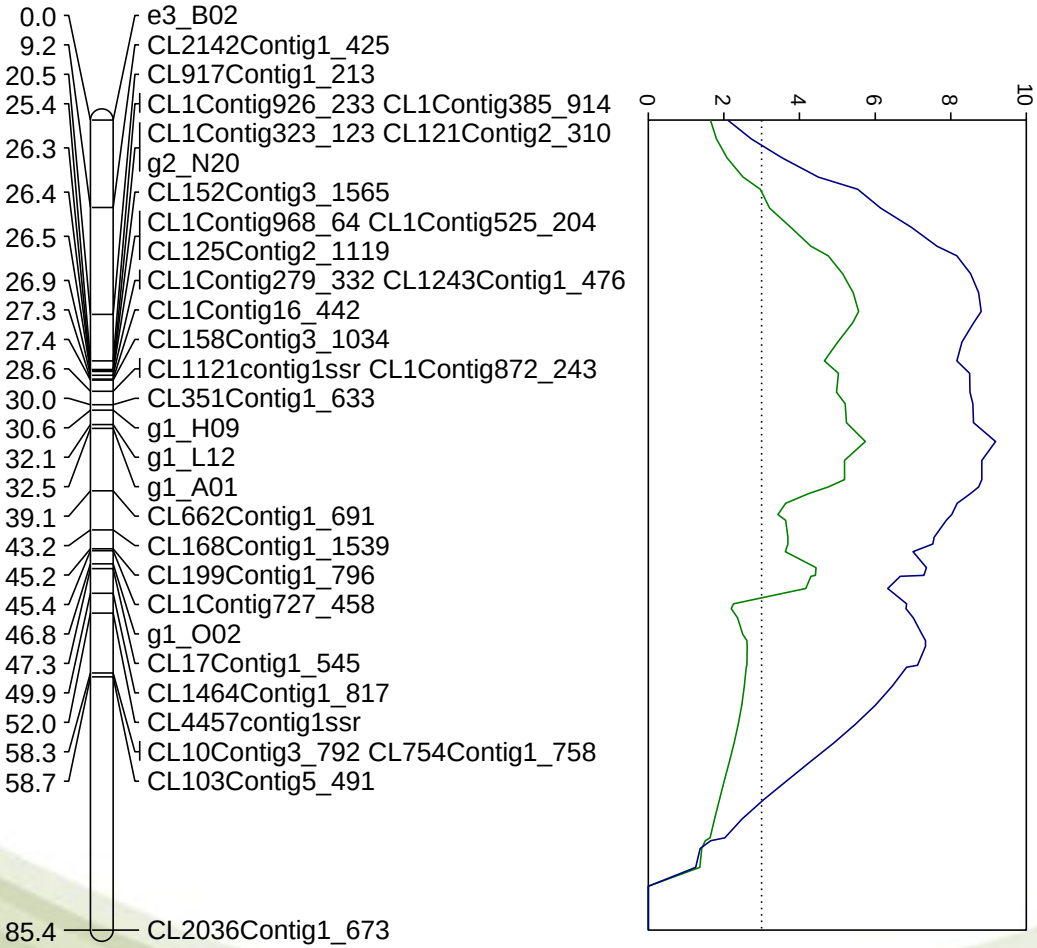
Smaller effects on LG4 and LG5, but less consistent

Blackcurrant – Ascorbic acid

SCRI9328 LG1

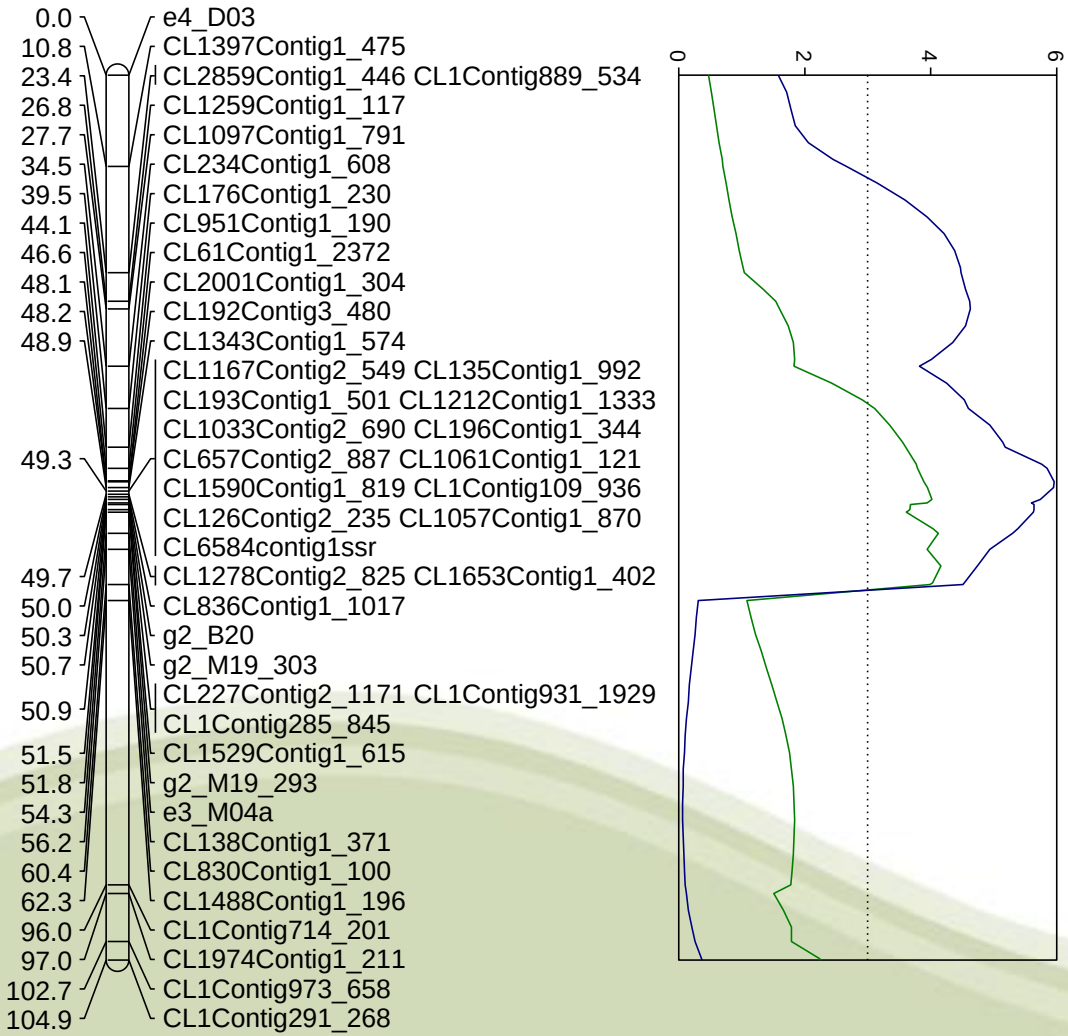


SCRI9328 LG5



Blackcurrant total anthocyanins

SCRI9328 LG3

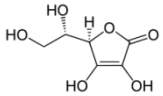


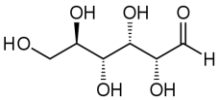
Also major QTLs for citrate in this area. Also anthocyanin (pomace) in both years, and Brix

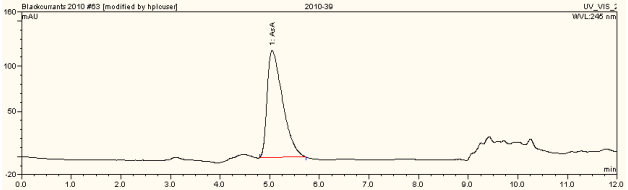
Trait associations – *Ribes* fruit quality II

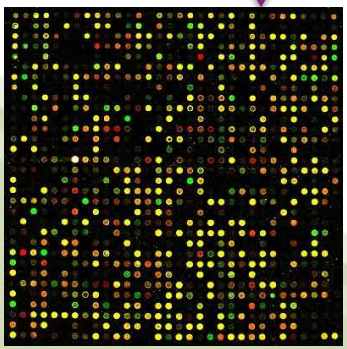
- **Use of gene expression data from ripening fruit linked to metabolomic analyses**

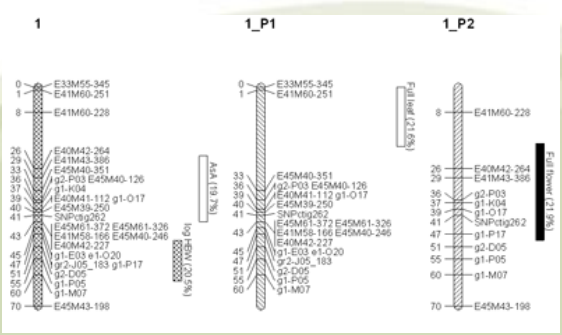
- Fruit quality analysed at various stages
- Gene expression monitored across stages using Agilent microarrays
- Key genes mapped, markers identified for the various quality and nutritional traits
- Environmental influences on gene expression











Reduction of seedling numbers using marker-assisted breeding - *Ribes*

Marker for berry size
Est. 2013

Marker for gall mite resistance
2012

Markers for anthocyanins, sugars, vitamin C
Est. 2015

Reduced seedling numbers – but increased relevance to industry needs

Faster field selections and cv. releases

Raspberry quality traits

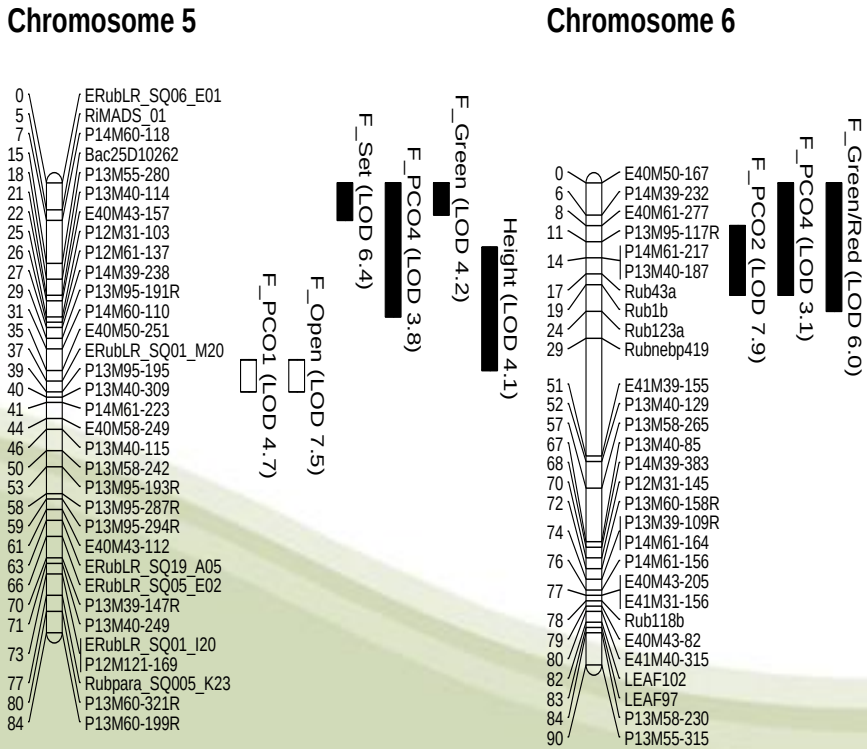
- **Commercial traits assessed across seasons and environments:**
  - **Ripening**
  - **<u>Cropping season</u>**
  - **Colour**
  - **pH**
  - **<u>Anthocyanins</u>**
  - **Berry size**
  - **Sensory traits**
  - **Brix**
  - **<u>Volatiles</u>**
  - **Raspberry ketone**

Experimental outline

- Glen Moy x Latham reference mapping population
- 3 seasons and 3 environments
- Assessments of trait of interest
- QTL mapping
- Candidate gene analysis
- Microarray to examine changes in gene expression during fruit development

Ripening

- QTLs for the ripening stages were identified across four chromosomes 2, 3, 5 and 6.
- Each of the groups had markers that had a significant effect at various ripening stages.
- The work identified genetic markers associated with early or late bud break and short or long ripening periods.



Anthocyanins in Raspberry

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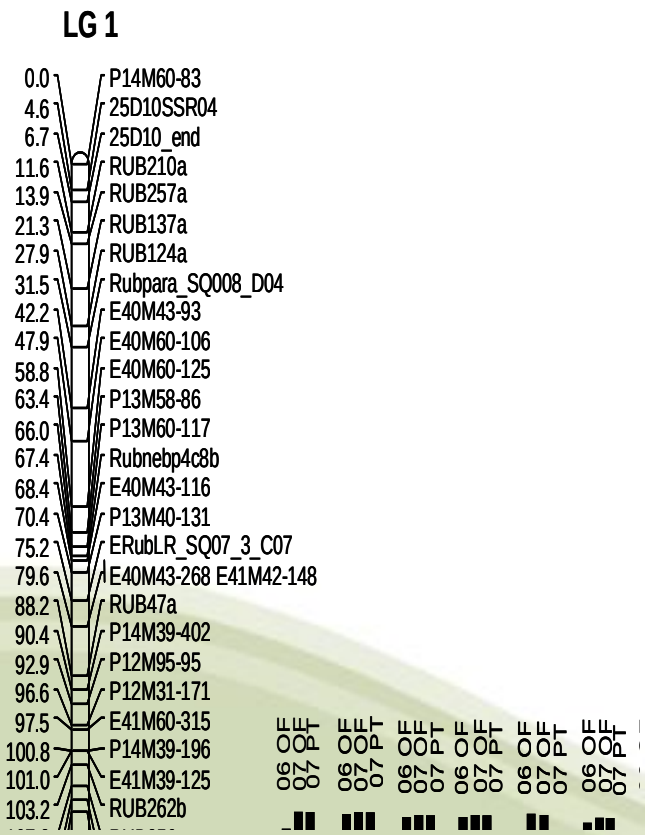
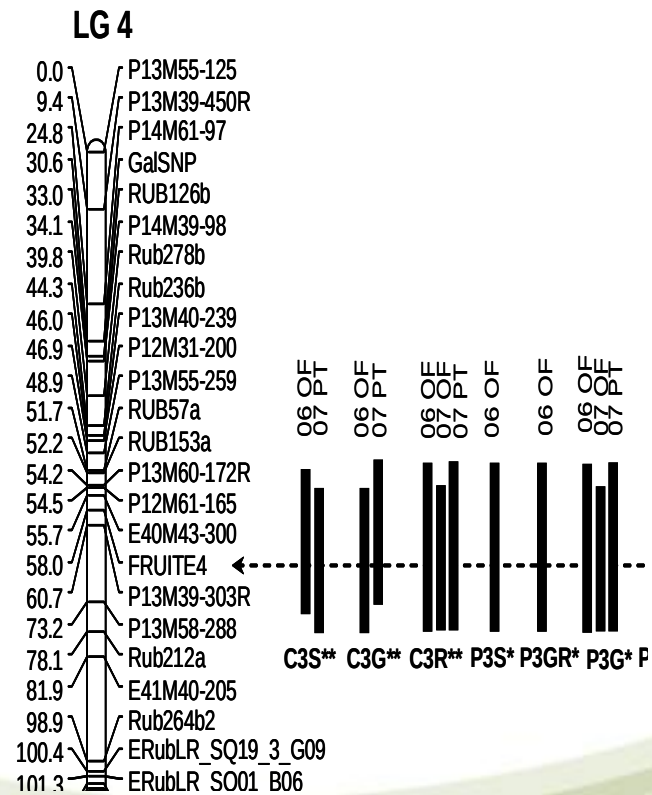
- Anthocyanins in mapping progeny and parents:
  - cyanidin-3-sophoroside (C3S)
  - cyanidin-3-glucoside (C3G)
  - cyanidin-3-glucosylrutinoside (C3GR)
  - cyanidin-3-rutinoside (C3R)
  - pelargonidin-3-sophoroside (P3S)
  - pelargonidin-3-glucoside (P3G)
  - pelargonidin-3-glucosylrutinoside (P3GR)
  - pelargonidin-3-rutinoside (P3R)

- Major QTL identified for all anthocyanins across seasons and sites
- Several transcription factors underlying QTL:
  - bHLH, FruitE4 encoding a basic leucine zipper (bZIP) transcription factor
  - Rub119 encoding a NAM (no apical meristem)-like transcription factor
- Markers linked to QTL under validation

QTL for anthocyanin production

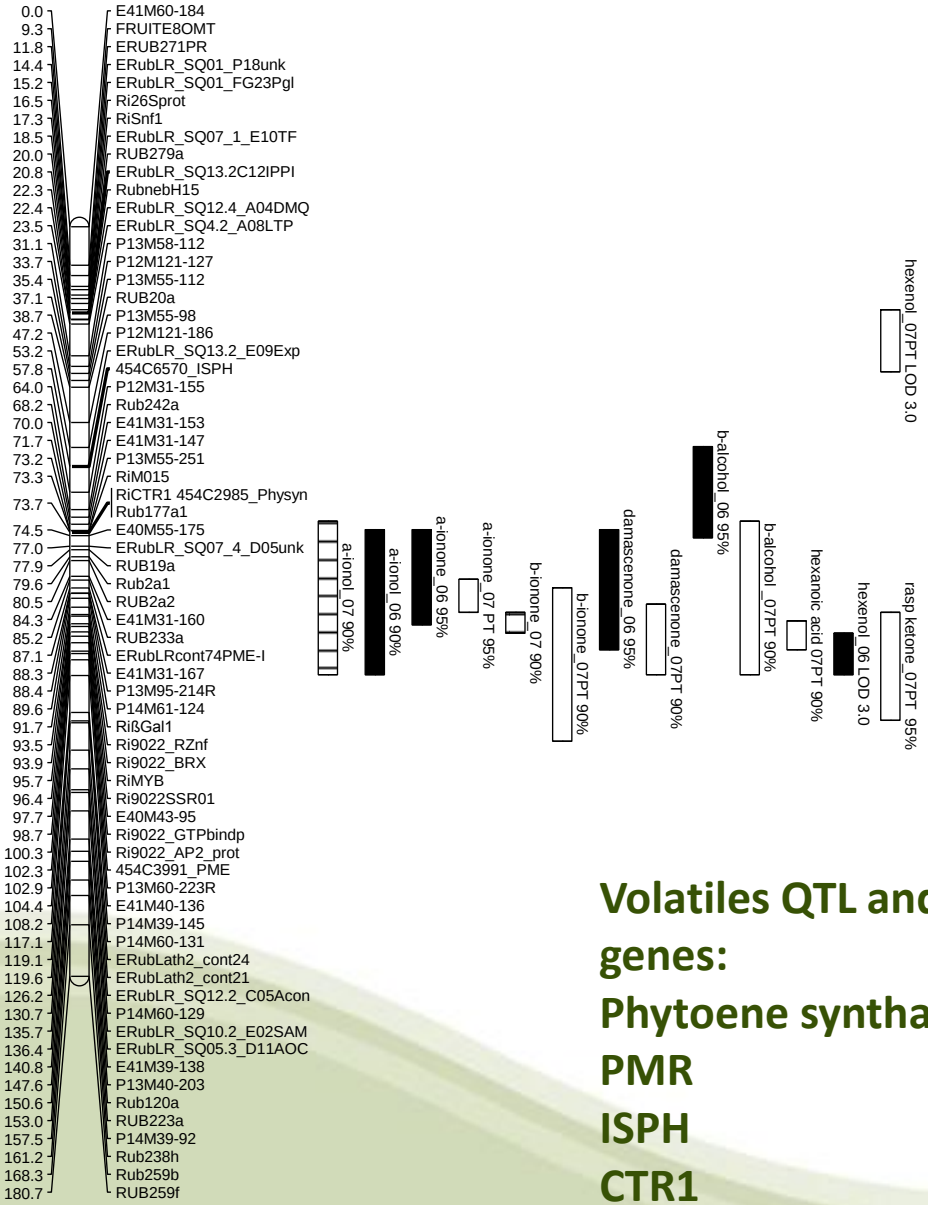




Kassim et al., 2009

Raspberry Volatiles Analysis

Season	Field 2006		Field 2007		Polytunnel 2007	
Sample	Progeny		Progeny		Progeny	
Volatile	Mean± SEM	Min-Max	Mean ± SEM	Min-Max	Mean ± SEM	Min-Max
b-damascenone	72.29 ± 4.02	0 – 463.8	87.10 ± 6.82	0 – 872.10	64.88 ± 4.71	3.17 - 368.8
b-ionone	13.65 ± 0.53	0.46 – 47.88	9.83 ± 0.44	0.61 – 44.33	6.43 ± 0.36	0.25 - 33.02
a-ionone	7.31 ± 0.19	1.65 – 17.16	3.86 ± 0.14	0.79 – 13.82	5.16 ± 0.24	0.22 - 21.61
a-ionol	2.24 ± 0.09	0.16 – 7.28	1.83 ± 0.09	0.24 – 11.68	5.96 ± 0.30	0.16 - 29.97
Linalool	4.72 ± 0.32	0.67 – 22.26	2.90 ± 0.17	0.33 – 14.52	4.39 ± 0.20	0.90 - 29.28
Geraniol	2.64 ± 0.08	0.68 – 8.87	1.82 ± 0.07	0.44 – 10.74	3.68 ± 0.14	0.11 - 15.46
(Z)-3-hexenol	22.35 ± 0.34	0.71 – 28.15	9.06 ± 0.29	0.63 – 28.18	16.70 ± 0.67	0.07 - 54.03
Acetic acid	1.39 ± 0.06	0.06 – 8.26	0.64 ± 0.05	0.01 – 4.05	0.72 ± 0.03	0.01 - 3.54
Hexanoic acid	6.54 ± 0.35	0.89 – 41.68	7.97 ± 0.30	1.71 – 22.88	7.04 ± 0.34	0.28 - 30.84
Acetoin	1.02 ± 0.05	0.09 – 4.74	1.03 ± 0.04	0.10 – 4.79	0.80 ± 0.05	0.02 – 4.95
Benzyl alcohol	0.59 ± 0.03	0.15 – 2.18	1.07 ± 0.04	0.09 – 3.16	2.67 ± 0.11	0 - 9.94



Volatiles QTL and some of the underlying genes:

Phytoene synthase

PMR

ISPH

CTR1

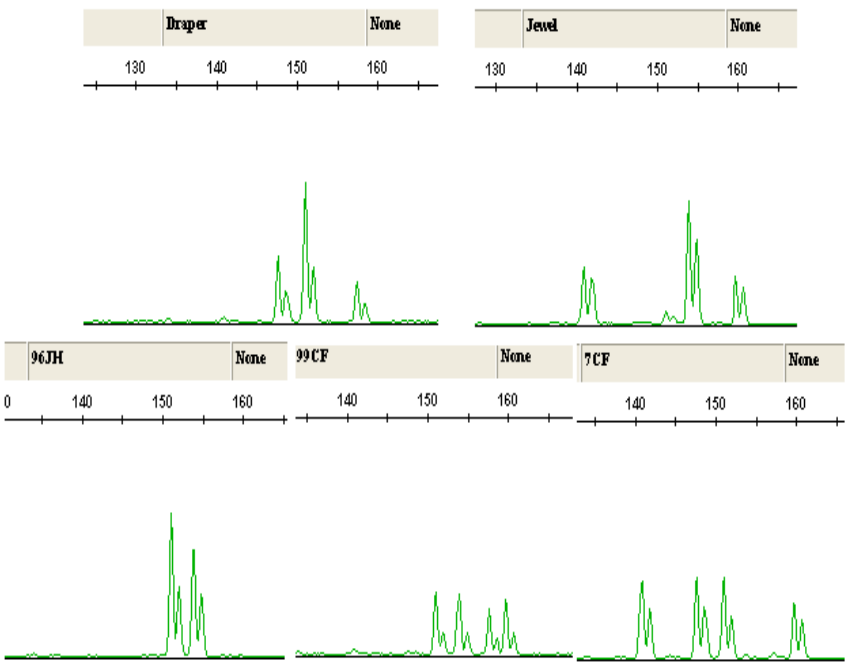
IPPI

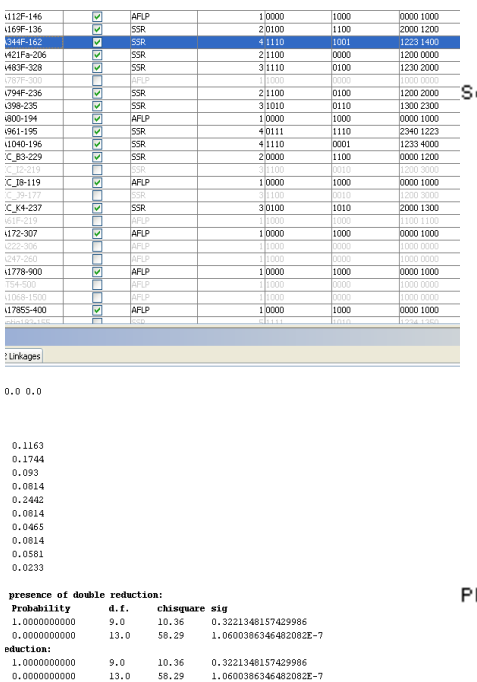
Vaccinium

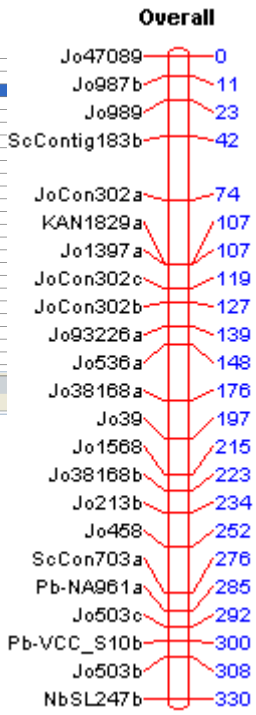
- Mapping of key traits
- Mapping population developed (Draper x Jewel), segregating for:
  - Fruit size
  - Firmness
  - Flavour
    - Sugar/acid ratios
- Tetraploid mapping developed at JHI for potato used to map traits

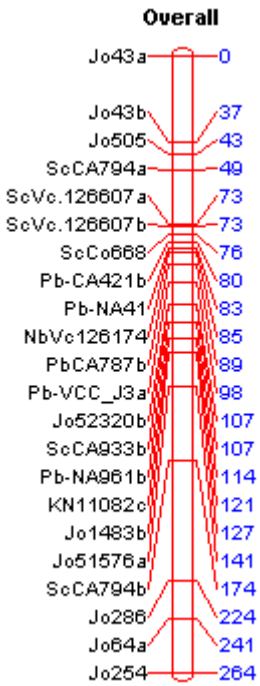
Linkage Analysis and QTL Mapping

- Separate markers into linkage groups, based on independent segregation
- Order markers to obtain linkage maps
- Relate marker data to phenotypic traits to identify regions containing QTLs

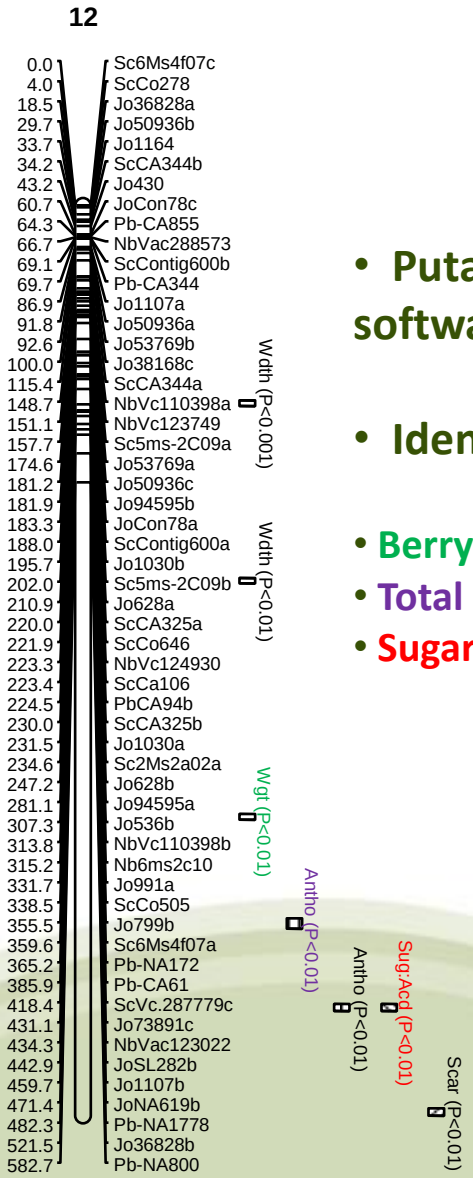








Vaccinium Fruit Quality QTL



- **Putative quality-related QTL mapped using TetraploidMap software**
- **Identification of linked markers in progress**
- **Berry Weight**
- **Total Anthocyanins (Cyanidin-3-glucoside equivalents mg/L)**
- **Sugar:Acid ratio**

Summary

- Marker-assisted breeding in berry fruit offers potential for faster cultivar development and reduction in both time and costs of breeding programmes
- Exploitation of genomics resources now possible even for minor crops
- Focus must be on quality traits important to industry and end-users
- Combining markers for both quality and agronomic traits is the long-term aim
- Improved phenotyping methods are essential

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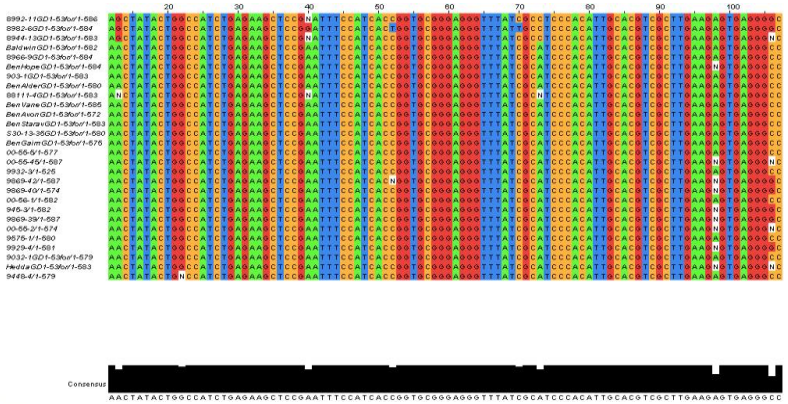

Acknowledgements

James Hutton Institute
Christine Hackett
Rob Hancock
Derek Stewart
Pete Hedley

Ribes
Sandra Gordon
Dorota Jarret
Joanne Russell
Linzi Jorgensen

Rubus
Julie Graham
Kay Smith
Mary Woodhead
Sandie Williamson
Alastair Patterson *(University of Strathclyde)*

Vaccinium
Susan McCallum
Julie Graham
Nahla Bassil (USDA, OR)
Jim Hancock (Michigan State U)