Developing markers for breeding in *Rubus* and *Ribes*

EUBerry WP1 D1.3

Rex Brennan

The James
Hutton
Institute

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Outline

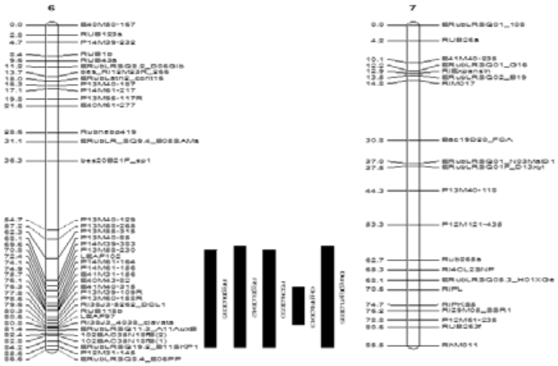
- Breeding programmes
- Traits of interest
- Marker development
- Mapping of markers and links to traits
- Validation and deployment
- Next steps and new opportunities

Breeding programmes within EUBerry

- *Rubus*
  - UK, France, Italy, Norway, Poland
- *Ribes*
  - UK, Poland
- Funding of programmes is generally based in the private (commercial) sector
  - Underpinning science, eg. marker development, is usually funded by government or EU
- Current use of molecular selection techniques is generally restricted to UK
  - Greater marker availability

Rubus – Phytophthora root rot

- QTL locations on raspberry linkage map
  - Two main QTL on LGs 3 & 6
  - Related in part to root vigour
- Marker identification
  - SSR linked with root rot resistance identified in cv. Latham
    - Rub 118b, 110 bp
  - Validated across wide range of germplasm
  - Marker used to identify clones in *Rubus* BAC library
    - Sequencing, genes in region identified



Graham et al. (2011) Towards an understanding of the nature of resistance to *Phytophthora* root rot in red raspberry. TAG 123: 585-601

Rubus – Phytophthora root rot

- Marker deployed in *Rubus* breeding programme since 2011
  - Identification of resistant germplasm
    - Parents for future crossing
  - Identification of resistant segregants in breeding populations
  - Confirmation of resistance status of existing trial seedlings
    - cv. Glen Ericht released to industry

Ribes – *Cecidophyopsis* gall mite I

- Most serious pest/disease problem facing blackcurrant producers
    - Difficult to control due withdrawal of chemicals, spray timing
    - Also vector of Blackcurrant Reversion Virus
- Resistance gene *Ce* derived from gooseberry (*R. grossularia*)
    - Introgressed into blackcurrant (*R. nigrum*) in 1970s at EMR, extensive backcrossing at JHI to restore key fruit quality traits
- Identification of resistant plants in breeding populations – field infestation plot for 4 years
- Reference 9328 mapping population replicated in infestation plot
    - Phenotype data collected for 4 years
    - Resistant/susceptible bulks identified

Ribes – Cecidophyopsis gall mite II

- AFLP marker identified linked to gall mite resistance
- PCR-based marker developed and high-throughput assay designed
- Accuracy in diverse *Ribes* germplasm *ca*. 95%
- Marker now routinely deployed in breeding programme at seedling (pre-field) stage
    - Saving time within programme
    - Reduction of field costs
- New resistant material released to commerce
    - Cv. Ben Finlay

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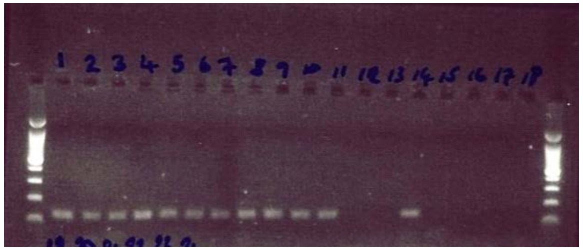
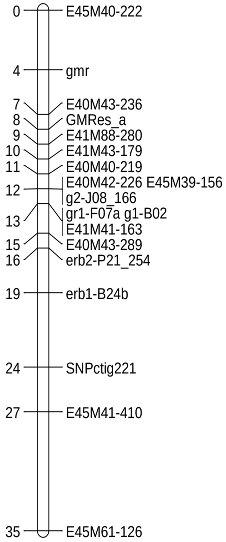




Brennan, Jorgensen, Gordon, Loades, Hackett and Russell (2009) *Theor. Appl. Genet.* **118**: 205 - 212

Key traits - *Rubus*

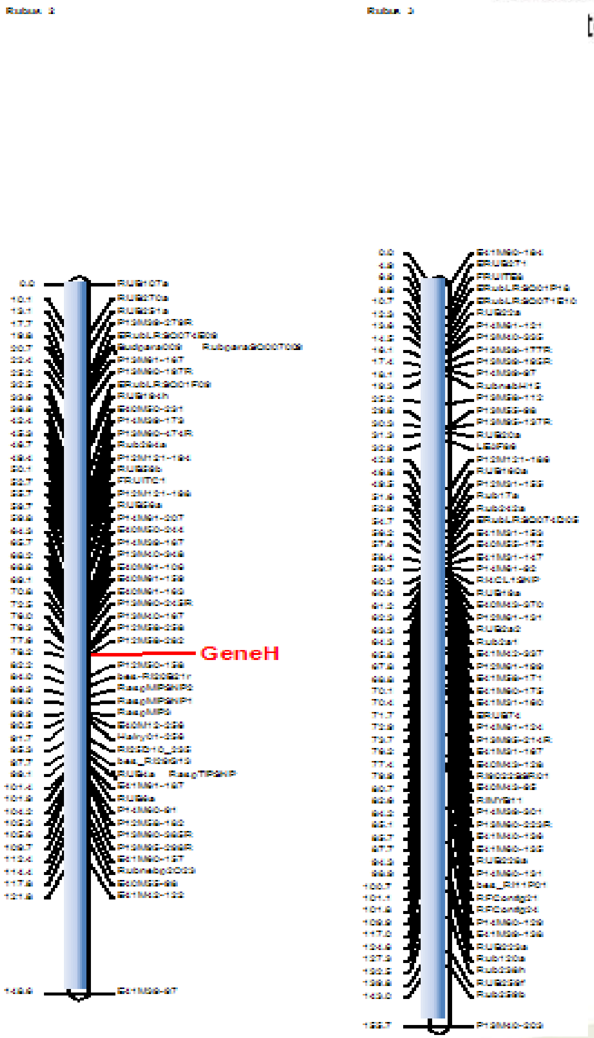
- Developmental traits
  - Ripening season
  - Late (eg. Octavia) vs. early (eg. Glen Moy)
- Fruit quality traits
  - Berry size
  - Key variable with both consumer and economic impact
- Compositional traits
  - Anthocyanins
    - Total and individual

Rubus marker development

- Initial plan was to use an association mapping approach to identify key regulatory genes and associated markers across diverse germplasm
    - Collections at JHI
- Attempts to identify associations (including already known) were broadly unsuccessful
        - Population sizes too small
            - Usually < 200
        - Highly heterozygous material
- Other mapping populations were therefore developed
    - Latham x Glen Moy - reference population at JHI
    - Autumn Treasure x Glen Fyne – flowering habit etc.

Rubus

- Glen Moy x Latham mapping population
- QTL for traits mapped
- Glen Moy genome sequence is now available, and has been used to investigate the sequences underlying QTL to identify genes for further evaluation
- Gene-based approach for markers



Rubus – ripening traits

Trait	Marker/nearest searchable marker	LG	Glen Moy Scaffolds	Key genes on scaffold
Ripening	Gene H - ERubLR_SQ12.1_B12CAF (20D22)	2	Sc1147	Very large no genes as have bacs also eg. CCR4 UPF Ubc enz
Ripening	Gene H - ERubLR_SQ5.3_E0360S (11G23)	2	Sc1131	KH domain LLR Cy B 60S
Ripening	P13M55_98 closest – ERubLRSQ13.2E09	3	Sc11986	expansin
Ripening	E41M31_153 closest RiM015	3	Sc2901	mRNA decapping Nectarin 2
Ripening	233a	3	Sc3423	b-glucanase umecyanin NBP

Rubus – other traits

Trait	Marker type	Marker details	Availability/source	Deployment
softening	EST	JHIRi20954	James Hutton Institute	Under validation
	SSR	Rub119a	James Hutton Institute	Under validation
	SSR	Rub120a	James Hutton Institute	Under validation
	EST	JHIRi_33046	James Hutton Institute	Under validation
	SSR	Rub22	James Hutton Institute	Under validation
	SSR	Rub242	James Hutton Institute	Under validation

Rubus

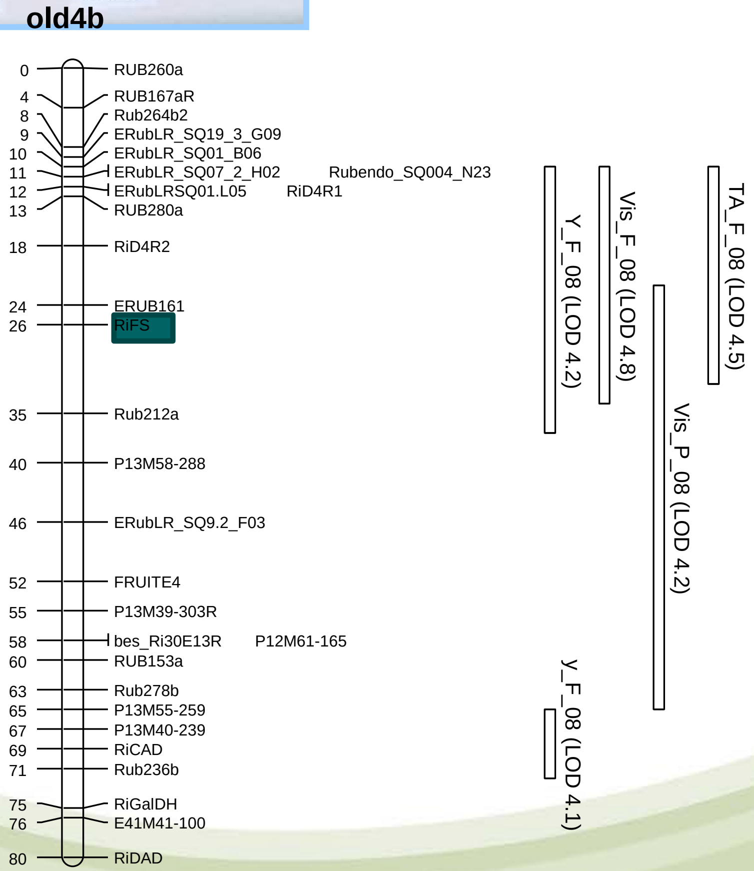
Berry size	BAC sequence	Ri31G22	James Hutton Institute	Under validation
	SSR	Rub137	James Hutton Institute	Under validation
	EST	ERubLRSQ10.2	James Hutton Institute	Under validation
	EST	ERubLRSQ5.3E03	James Hutton Institute	Under validation
	EST	ERubEndoSQ07_P15	James Hutton Institute	Under validation

Fruit colour

Marker RiFLS LG 4

Flavanol synthase impact of alleles

	aa	ab	bb	SED	Sig
Y	15.5	15.0	14.6	0.20	**<.001**
Visual	2.92	3.25	3.66	0.149	**<.001**
TA	-0.39	0.06	0.28	0.157	**<.001**
C3S	200.2	237.5	256.6	22.32	**.019**
C3GR	**62.5**	**78.1**	**89.8**	**10.58**	**.043**



Rubus - anthocyanins

Total Anthocyanin	BAC sequence	Ri29G13	James Hutton Institute	Validation stage	McCallum et al 2010
	AFLP	P13M40-85	James Hutton Institute	Validation stage	
	Gene	RiFLS	James Hutton Institute	Validation stage	
	SSR	Rub2a	James Hutton Institute	Validation stage	
Individual anthocyanins	Gene	bHLH	James Hutton Institute	Validation stage	McCallum et al 2010
	Gene	bZIP	James Hutton Institute	Validation stage	

McCallum, S., Woodhead, M., Hackett, C.A., Kassim, A., Paterson, A. & Graham, J. 2010. Genetic and environmental effects influencing fruit colour. *Theoretical and Applied Genetics* 121:611-627

Key traits - *Ribes*

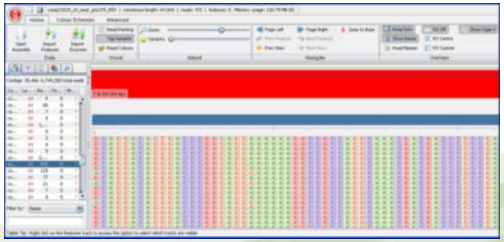
- Fruit quality traits
  - Physical traits, eg. berry size
  - Key attribute in the selection of fresh-market types
  - Wide range of variation within blackcurrant germplasm
    - Big Ben berry weight ca. 2 g
    - Breeding selections – < 0.6g

- Nutritional traits
  - Total anthocyanins
  - Wide range of variation based on spectrophotometric readings
    - High levels, eg. Ben Alder ($E_{515} > 1.4$)
    - Very low levels, eg. Ben Connan ($E_{515} < 0.8$)

Ribes marker development

- Linkage map of blackcurrant 9328 reference mapping population
  - Key phenotypic traits added, QTL/marker associations identified

- Genotyping by sequencing (GBS)
  - Reads mapped against reference transcriptome assembly
  - 1.58k high-confidence SNPs identified
  - Map length increased 33% to 780.7 cM
  - Model for mapping and identifying SNPs in crop species lacking reference genome

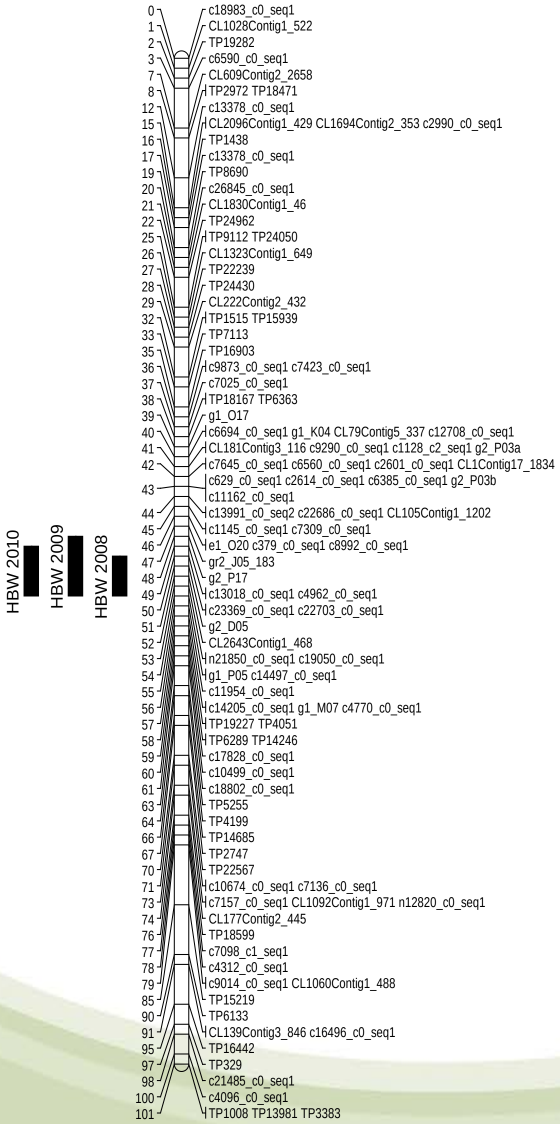


Russell *et al*. (2013) Molecular Breeding **33**: 835-849

Berry size

Trait associations – berry size

- Measurements across reference 9328 mapping population for 4 years at JHI
- Individual traits placed on new linkage map
  - **Fruit size**
  - **Brix**
  - Anthocyanins
  - Ascorbic acid
- Associated SNPs identified for evaluation in other germplasm



LG1

QTLs associated with 100wt berry and E515- map location and SNP marker development

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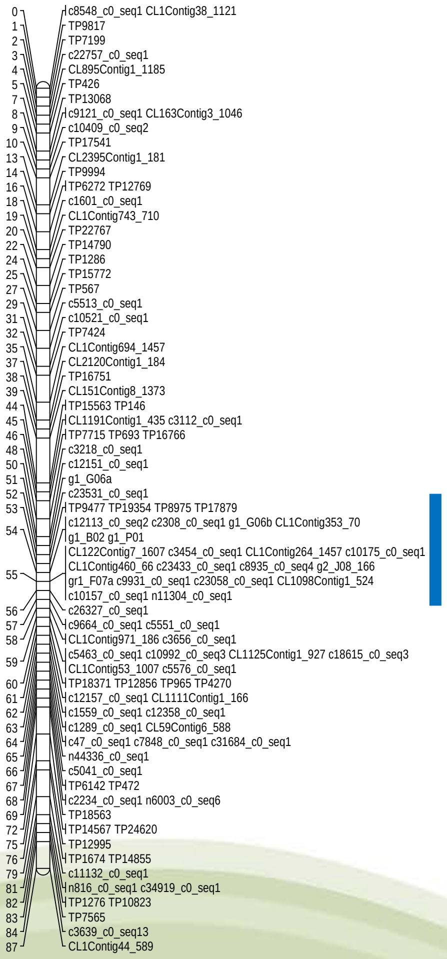
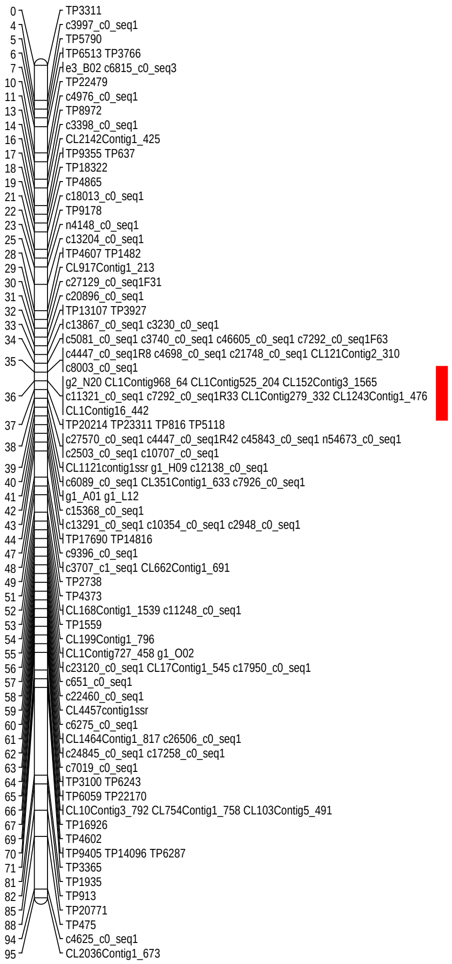


Total Anthocyanin

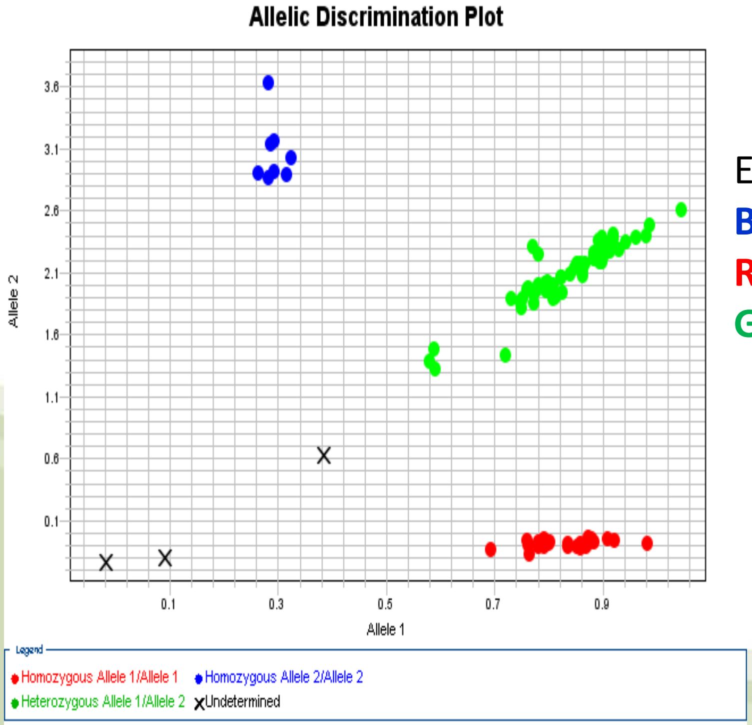


100wt QTL

- 1578 GbS markers mapped to 8 linkage groups
- A region on LG 5 at 36cM was identified for total anthocyanin
- A region on LG2 at 55cM was identified for 100 berry weight

Development of markers

- Mapped genes were sequenced and SNP identified from parental lines (CL1contig-442 on LG5 and comp23058 on LG2)
- Simple easily scored KASpar markers were developed



Each dot represents an individual

Blue is homozygous i.e. AA

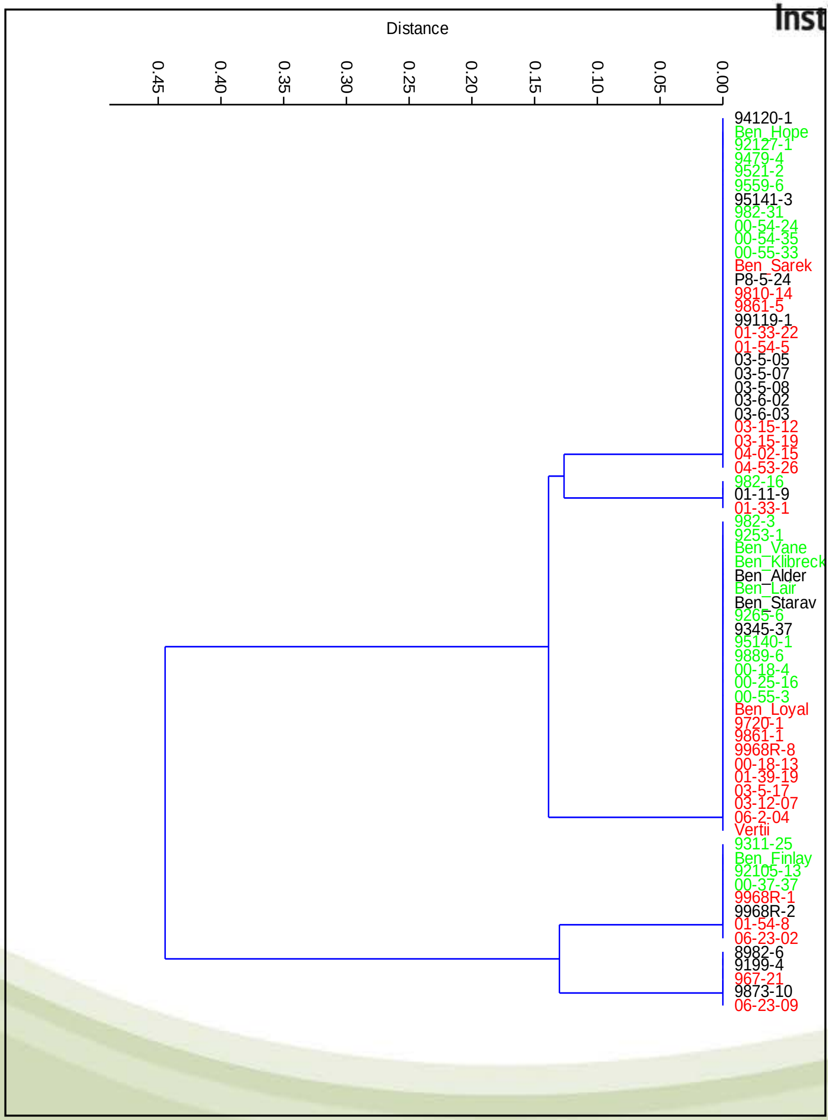
Red is homozygous i.e. GG

Green are heterozygotes i.e. AG

LG2 Anthocyanins

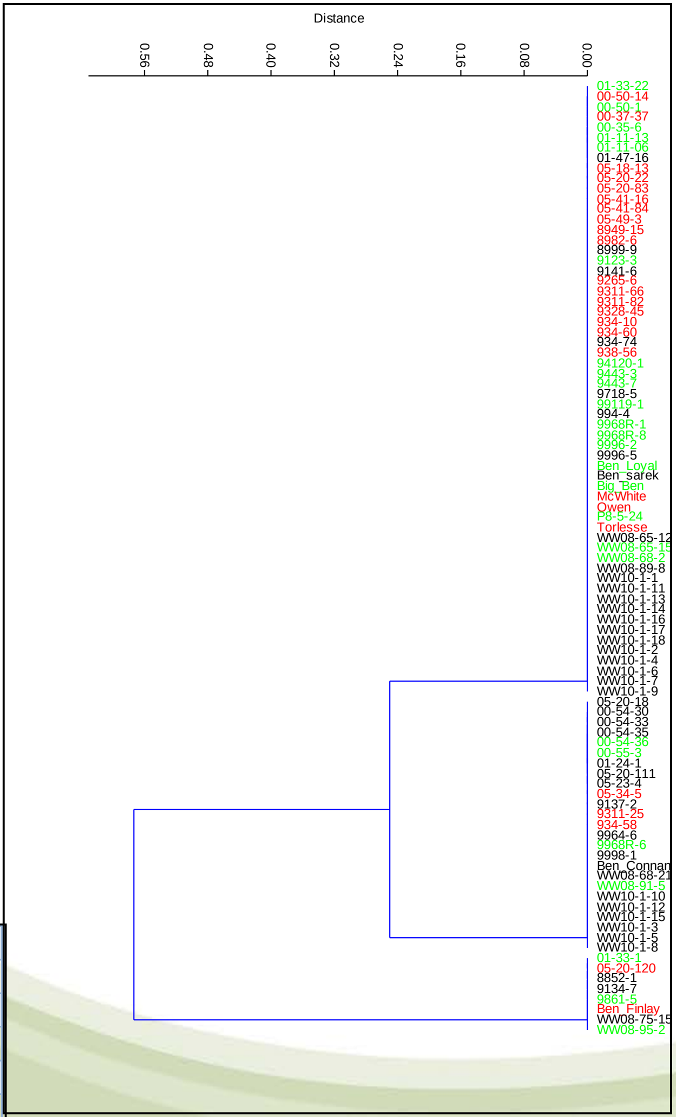
- 96 breeding lines and commercial cultivars
- E515 ranges from 0.48 to 1.63
- Two contrasting groups of 25 accessions
  - 0.48 – 0.71
  - 1.07 – 1.63
- 4 SNP (55cM to 59cM)
- 5 haplotypes but no clear association with E515

SNP Comp23058		
Genotype	High (%)	Low (%)
AG	36	44
GG	60	52
AA	4	4



LG5 Berry size (100wt)

- 96 breeding lines and commercial cultivars
- 100wt ranges from 45g to 182g
- Two contrasting groups of 25 accessions
  - 45 –78g
  - 127.5 – 182g
- 2 SNP (36.4cM co-located)
- 3 haplotypes but no clear association with berry size
- Other QTL and associated SNPs under investigation

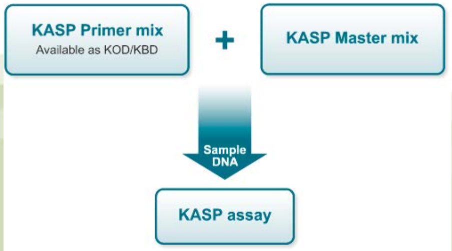


CL1Contig16-442		
Genotype	Small (%)	Large (%)
TC	80	72
CC	12	16
TT	8	12

Ribes – links to other EUBerry programmes

P2 (InHort, Poland)

- Visiting worker (B. Badek) developed expertise from JHI on use of KASP assay, SSR analysis etc.
- DNA from Polish populations analysed at JHI using putative berry size markers
- Data developed and retained for alignment with phenotype data from Poland (due in 2015)

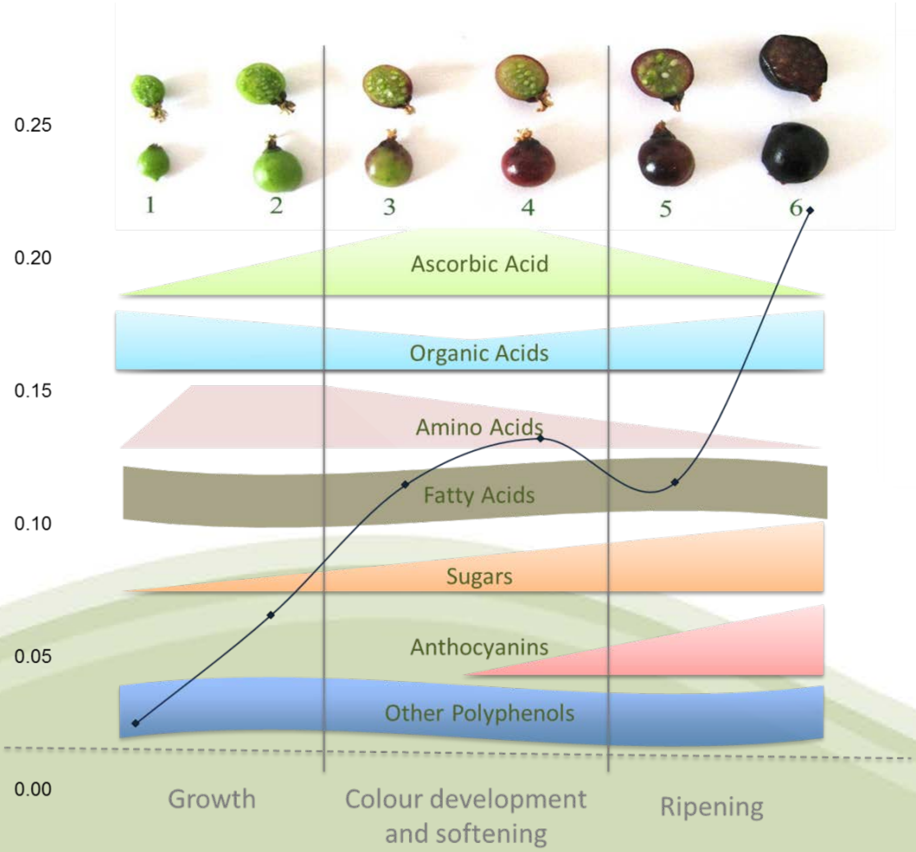


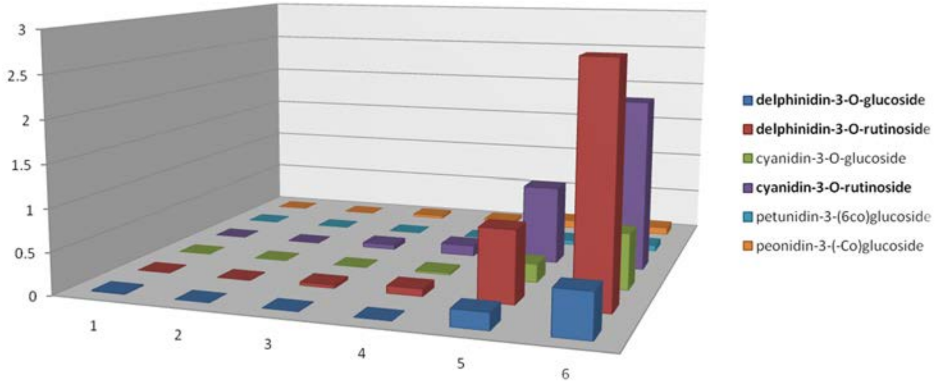
Plant material from Poland

- **Parental genotypes:**
  - ’Polares’, ‘Foxendown’,‘Titania’,
  - ‘Big Ben’, ‘Bona’, ‘Sejaniec Goł.’
- **Populations:**
- ‘Foxendown’ x ‘Polares’ (29 individuals)
- ‘Bona’ x ‘Sejaniec Goł’ (50 individuals)
- ‘Titania’ x ‘Big Ben’ (85 individuals)
- ‘Polares’ x ‘Big Ben’ (44 individuals)

Exploiting Developmental Changes to Identify Genes Underlying Fruit Quality in Blackcurrant

- **Fruit of blackcurrant cv. Ben Finlay assessed throughout season**
- **Profound changes during development**
- **Reflected in phytochemical profiles**





Phytochemical changes used to identify candidate genes controlling key quality traits

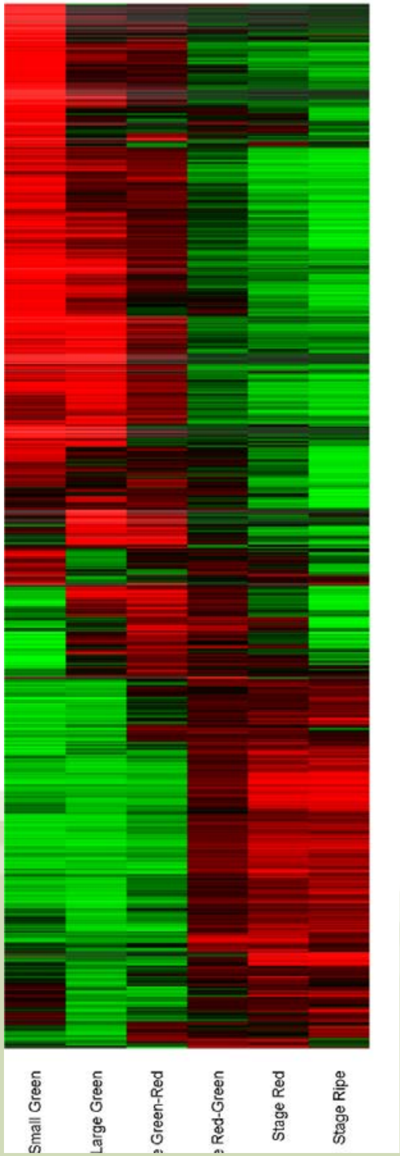
Gene expression profiles at different stages of ripeness from blackcurrant microarrays

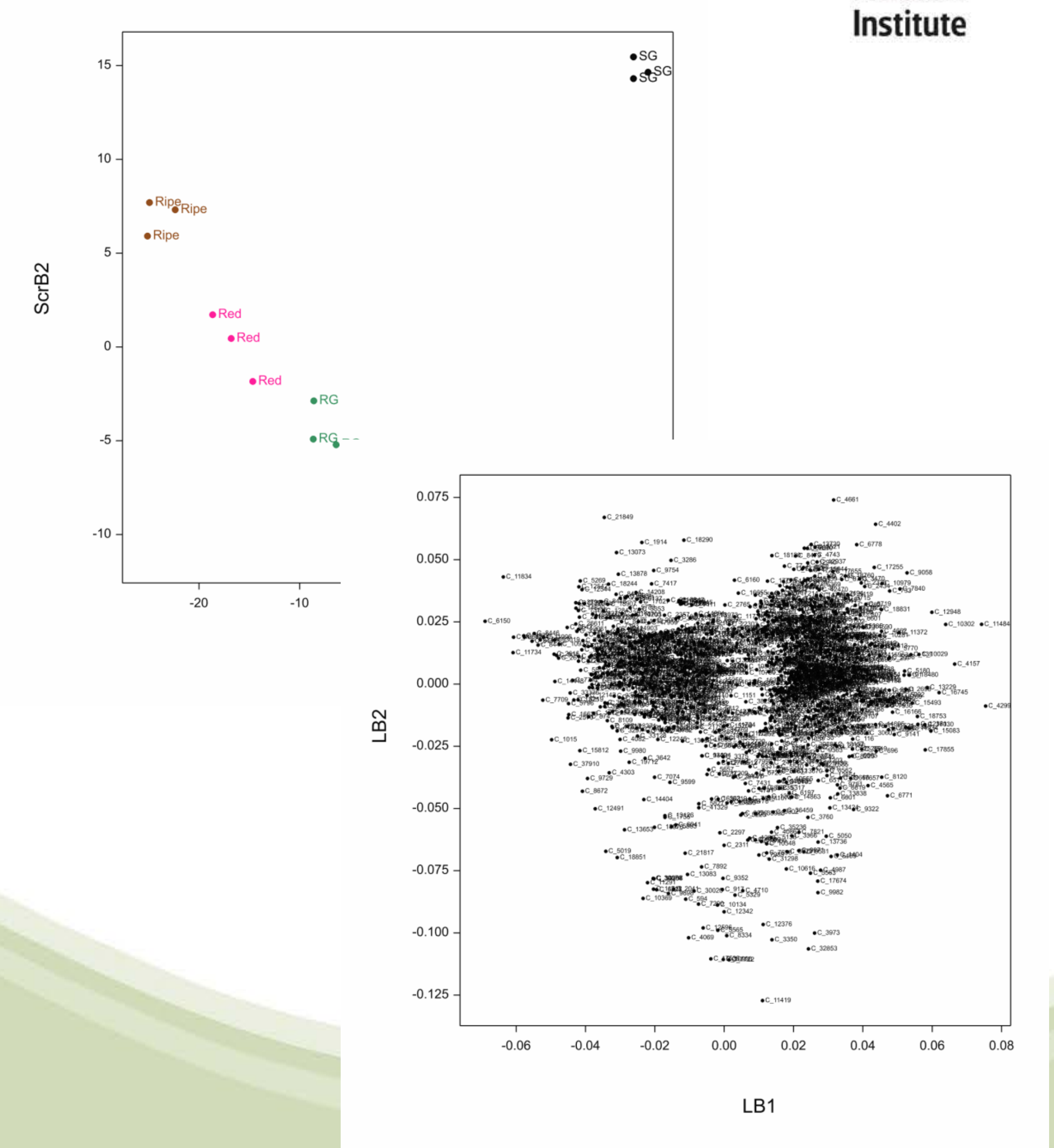
Correlation of gene expression with phytochemical profiles in progress

ANOVA
0.5%
(1433)

859
Arabidopsis
homologues

940 Grape
homologues





Ribes – Genome assembly

- Genome sequencing of *Ribes nigrum* cv. Ben Finlay
  - Shotgun whole genome sequencing (WGS)
    - 50x coverage on Illumina HiSeq (2 x 100 bp reads)
  - Mate-pair (MP) scaffolding sequencing
    - 1x coverage on Illumina MiSeq (2 x 150 bp reads), reduced to 0.6 x coverage after trimming and de-duplication

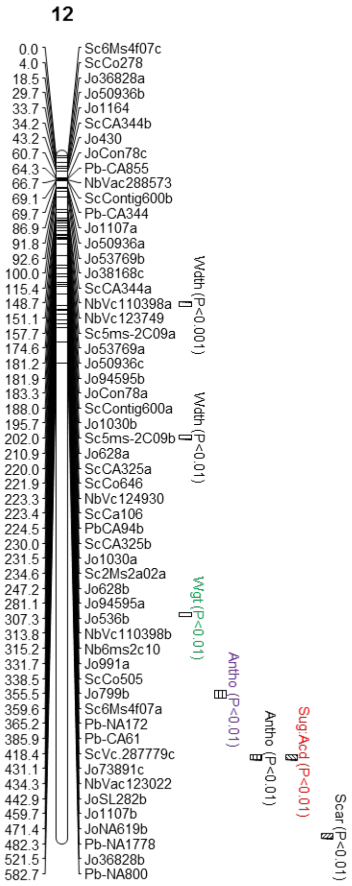
- Optimisation of assembly and scaffolding software parameters
  - 520k sequence scaffolds
  - Covering 735 Mbp of the estimated 2 Gbp genome

- Will serve as reference for Genotyping by Sequencing (GbS) and RNAseq transcript analysis

cv. Ben Finlay

Marker availability

- Essential that all robust markers are available to breeders after validation
- Work will continue on the traits identified as important for breeding of raspberry and blackcurrant
  - *Ribes* berry size and anthocyanins – other QTL and associated SNPs to be tested, genome sequence
- Sequences etc. will be published and can then be used by other groups
- Further collaborations welcomed in molecular breeding development for *Rubus* and *Ribes* (and *Vaccinium*)



The result, therefore, of our present enquiry is, that we find no vestige of a beginning, no prospect of an end. *James Hutton, 1788*

James Hutton Institute

Ribes
Sandra Gordon
Linzi Jorgensen
Joanne Russell
Pete Hedley

Rubus
Julie Graham
Kay Smith
Sandie Williamson

Christine Hackett, BioSS
Linda Milne
Micha Bayer

InHort, Poland
Bogusia Badek
Stan Pluta
Malgorzata Korbin

InHort
SKIERNIEWICE

UK Raspberry Breeding Consortium

LUCOZADE RIBENA SUNTORY

EUBerry

The Scottish Government