JHI Metabolomics Platform

GC-MS: 2 x GC-MS-(Thermo) & 2 x GC-MS ATD
LC-MS: 4 x iontrap LC-MS^n, (2 standard 2 state of the art)
1 Orbitrap (accurate mass), 1 iontrap MS^n
JHI Plant Metabolomics

- Abiotic/Biotic Stress
- Heritability Breeding
- Climate change
- Nutritive value
- Quality
Targeted analysis
Gravimetric, flavour & aroma taste
 texture, disease resistance, bioactivity, etc etc.

Crossing, plant development,
storage, processing etc

Untargeted analysis: Metabolic profiling

Mass spectrum

Hierarchical cluster analysis:
Measure of (phytochemical) biodiversity

Correlation Network:
Interrelate metabolite changes.
Pathway cross talk

Principal component analysis
of MS data

Compounds 1-500

Chromatogram
(10^2-10^3 compounds)
Raspberry segregating population: 2 environments, 3 harvest years

PCA plots can then be interrogated (mined) to see what masses (compounds) are causing the segregation.

Elevation or reduction in known (or unknown) compounds

Cy-3-(2G-glucosylrutinoside)
JHI Mammalian Metabolomics

- Cancer
- Bioavailability
- CVD/
- Metabolism/Models
- Digestion
Impact of soft fruit consumption on diabetes; Inhibition of starch digestion

Modulation of Digestive Enzymes by Berry Polyphenols: Potential Health Benefits
Mcdougall et al.
Faecal metabolism of berry polyphenols

- Metabolomic Profiling of faecal water metabolites in 10 free-living students after intake of raspberry puree (200 g/d for 14 d) by gas-chromatography mass spectrometry (GC-MS [and LC-MS]): Metabolomics
- Substantial ingestion of anthocyanins, ellagitannins etc.
- Focus on major phenolic metabolites.
- Some common metabolic patterns noted.
Faecal metabolism of berry polyphenols

Phenylacetic acid increased in 7/10 subjects
4-Hydroxy phenylacetic acid increased in 6/10 subjects
3-Hydroxy phenylacetic acid increased in 5/10 subjects
3-Phenylpropionic acid increased in 6/10 subjects
3-(4-Hydroxy)-phenylpropionic acid increased in 5/10 subjects
3,4-Dihydroxy benzoic acid increased in 7/10 subjects
4-Hydroxy benzoic acid increased in 2/10 subjects

- Predominantly anthocyanin derived
- Fits evidence from model studies with faecal inocula but shows large inter-individual variation.
- Due to differences in diet or microflora?
- A proper confined study is required: defined diet, labelled fruit/anthos?
Measure serum antioxidant capacity in free-living students after intake of raspberry puree (200 g)
Blackcurrant anthocyanins cause a flow-dependent increase in blood perfusion in isolated human intracerebral arteries

• Flow-dependent isometric tension was measured in segments of isolated human intracerebral arteries from consciousness areas: derived from brain surgery.

• The anthocyanin driven vasodilatation may have a beneficial effect on the cognitive functions in dementia of the Alzheimer type, in the prevention of TIA and stroke.

• Flow-dependent relaxation is almost identical to fluvistatin.
Nutrigenomic analysis: Blackcurrants anthocyanins in a rat model.

10% membrane concentrate feed it to the rat every day

25% Ethanol Extract

Young rat

2 weeks

Old rat

Liver

feed it to the rat every day
Nutrigenomic analysis: Blackcurrants anthocyanins in a rat.

Control (No feed) → Specimen collection & storage → RNA isolation → Microarray experiment (Differential Gene Expression Profiling) → Rat Nutrigenomics array → Bioinformatics analysis → Targeted genes Validation by Q-PCR

Experiment (fed)
Blackcurrant anthocyanin intervention
Rat transcriptomic analysis
Blackcurrant anthocyanin intervention
Rat transcriptomic analysis
Pathway enrichment/regulation and ontology as a consequence of blackcurrant anthocyananin intervention

Database for Annotation, Visualization and Integrated Discovery (DAVID) - http://david.abcc.ncifcrf.gov/
QuickGO (http://www.ebi.ac.uk/QuickGO/)
Polyphenolic Diversity

Flavonols in Blackcurrant

Graph showing the levels of different flavonols in blackcurrant, with various lines representing different compounds.
Soft fruit: Phytochemical Correlation Matrix

Correlation
- >0.75
- 0.75 to 0.55
- 0.55 to -0.55
- -0.55 to -0.75
- < -0.75

Anthocyanin-Anthocyanin
Anthocyanin-Flavonol
Flavonol-Flavonol
Sensory – Sugars and acids
Sugars and acid
Metabolite Correlation Network

- S/Oas
- A
- TP
- S/A
- Citr
- Mal
- S/TP
- Glu
- Succ
- Fru
- AS
- A/TP

- A and TP to S/Oas
- S/Oas to A and TP
- Citr and Mal to S/TP and A
- A or TP to S/TP or A to Oas

Correlation strengths:
- Red: $\geq 0.75$
- Orange: $0.549 \leq \geq 0.749$
- Teal: $-0.749 \leq \geq -0.549$
- Black: $\leq -0.75$
Soft Fruit: Integration of ‘Omics, Genetics and Breeding

- Linkage maps being completed
- Extension of mapping population for confirmation of QTLs
- Mapping of key genes from ascorbate biosynthetic pathway and polyphenolic biosynthetic pathways.
- Correlate the genetic and metabolomic data
- Dissection of genetic and environment on food and health quality parameters

Genetic (AFLP, SSR, Next gen sequencing) ↔ Metabolomics
Next Generation Sequencing in *Ribes*

- Large scale 454 transcriptomic sequencing of two *Ribes* genotypes (9328 reference mapping parents)
- > 700k reads (117.9 Mbp of blackcurrant transcriptome)
- Reads assembled into 46411 contigs
- 7245 SNPs and 3179 SSRs discovered
- Set of 384 SNPs selected using `Tablet` programme, range of germplasm assessed on Illumina BeadXpress platform
- New 384-SNP under development, also Genotyping By Sequencing is being investigated.
- Trait associations in development through field phenotyping
Maps and markers

- New SNP-based linkage map of blackcurrant produced for 9328 reference mapping population and also for new MP7 mapping population (Ben Finlay x Hedda)
- Shared QTLs and markers between maps

Russell et al. (2011) Identification, utilisation and mapping of novel transcriptome-based markers from blackcurrant (Ribes nigrum) BMC Plant Biology 11: 147
Trait associations

- **Fruit quality traits**
  
  Metabolomic analysis for sugars, organic acids and phenolics
  
  Putative QTL developed on SNP map, validation in progress
  
  Putative markers for berry size

- **Dormancy-related traits**
  
  Differentially-regulated genes identified using microarrays mapped to LG3 on original linkage map in area where QTL for budbreak and flowering is located
  
  Ongoing analysis using SNP map and diverse germplasm including population segregating for chilling requirement with Plant and Food (NZ)
  
  Significance for future sustainability of blackcurrant production
Genetic control of processing quality traits in blackcurrant (*Ribes nigrum* L.)

**Dorota Jarret**

**Aims of the project**

- Understand genetic control of the biosynthesis of nutritional and sensory compounds
- Identify map locations for the genes of interest
- Identify and deploy markers linked to key traits
- Assess environmental effects on important fruit quality traits

**Approaches**

- Mapping population of 150 individuals
- Quality compounds analysis
- Measurement of gene activity and genotyping

**Important outcomes for future breeding**

- Use of marker-assisted selection of new cultivars with elevated levels of nutritionally important and sensory compounds
- Understanding expression and inheritance of quality traits
- Enrichment of the blackcurrant genetic map
Development of an Agilent Rubus microarray

Microarray principles

2-colour microarrays: fluorescent detection
pseudo-colouring

level of activity = spot intensity
relative gene activity (A:B) = colour

Red: active in A
Green: active in B
Orange: active in both

8x 60k format
Probes (55k)

Targets
RNA: Stages of fruit development
fluorescent dye

Latham
Moy

RNA:

hybridisation