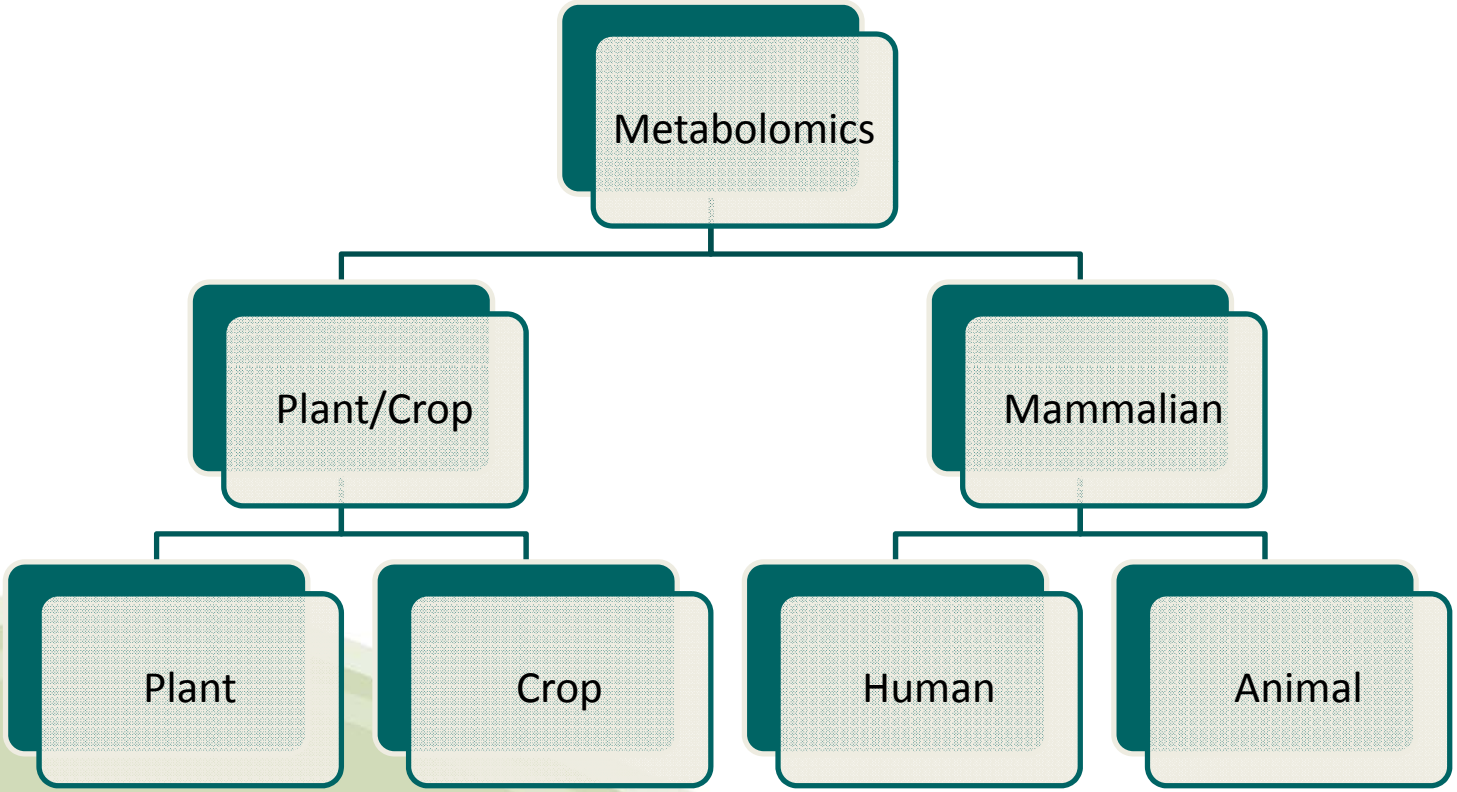
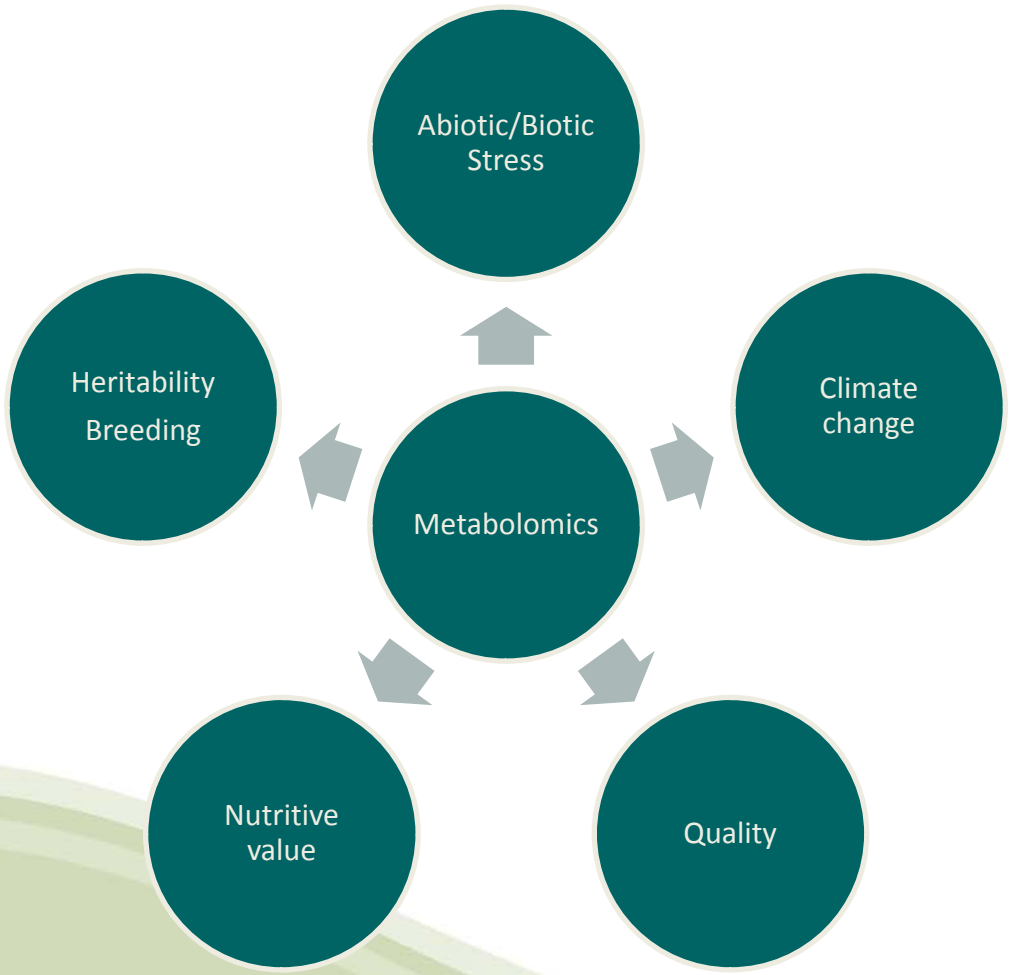
JHI Metabolomics Platform

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



Crossing, plant development,

storage, processing etc

Targeted analysis
Gravimetric, flavour & aroma taste
texture, disease resistance, bioactivity, etc etc.

Untargeted analysis: Metabolic profiling

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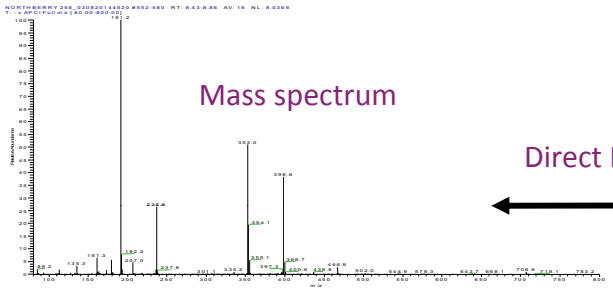
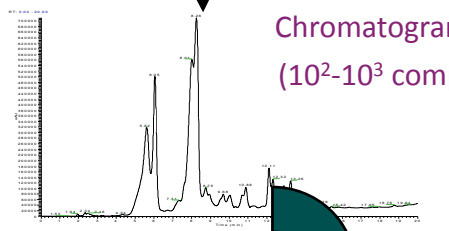
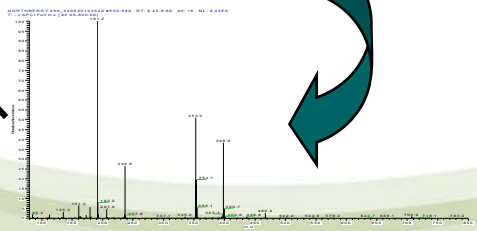
Direct Infusion MS

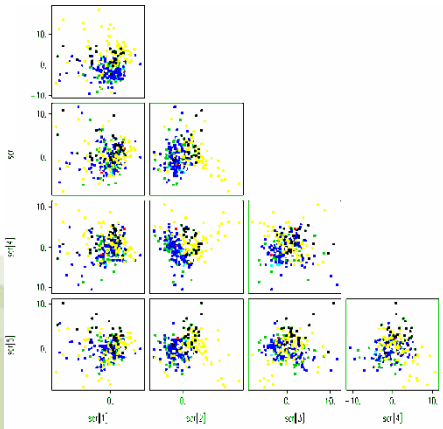


Mass spectrum

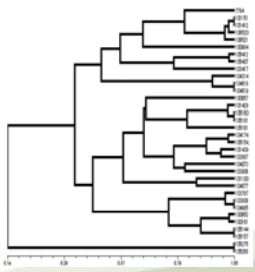


Chromatogram
(10^2-10^3 compounds)

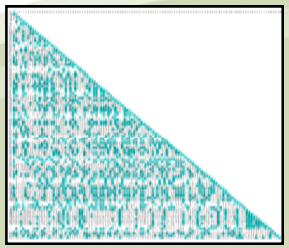




Principal component analysis of MS data

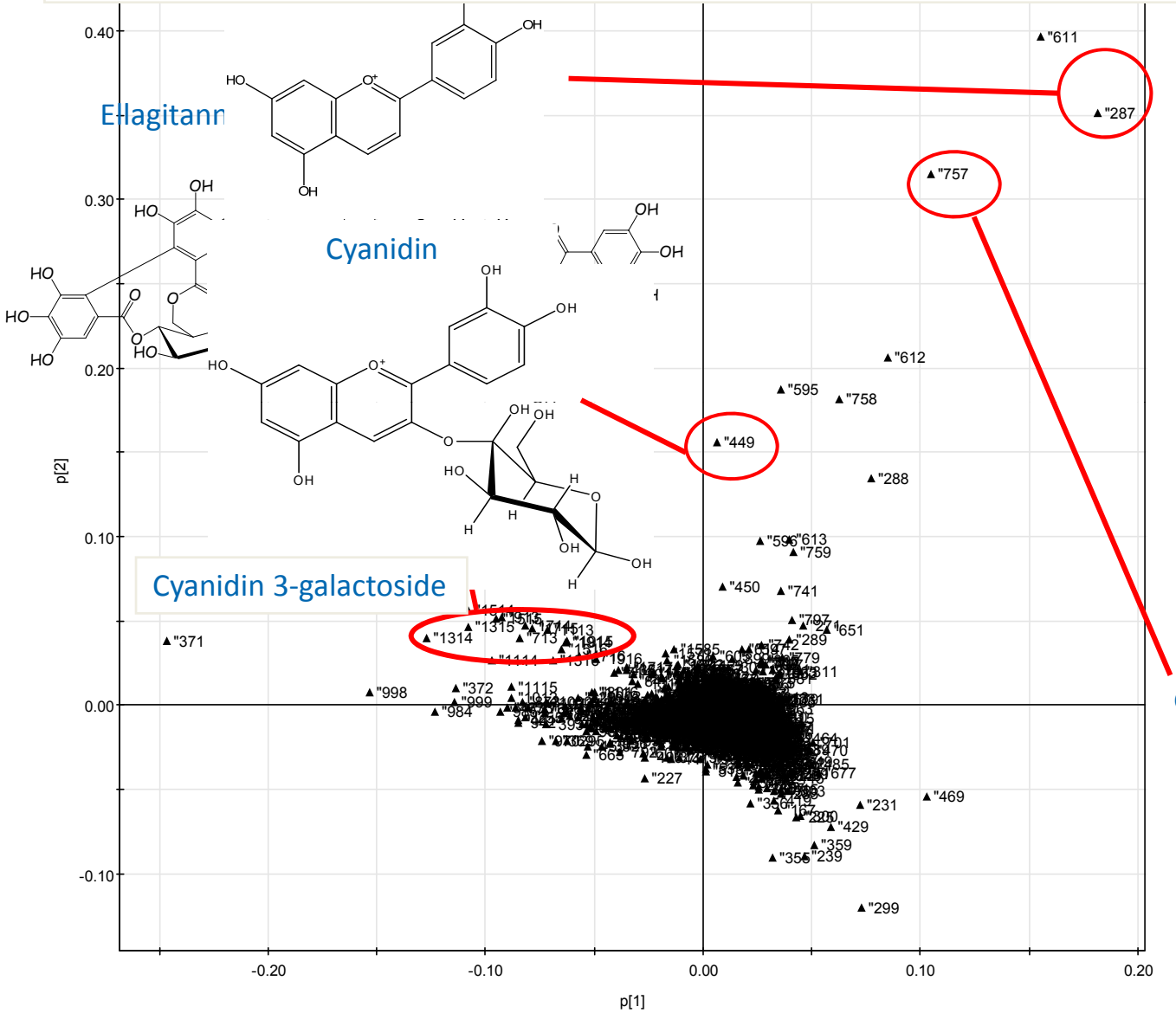


Hierarchical cluster analysis:
Measure of (phytochemical) biodiversity



Correlation Network:
Interrelate metabolite changes. Pathway cross talk

Raspberry segregating population: 2 environments, 3 harvest years



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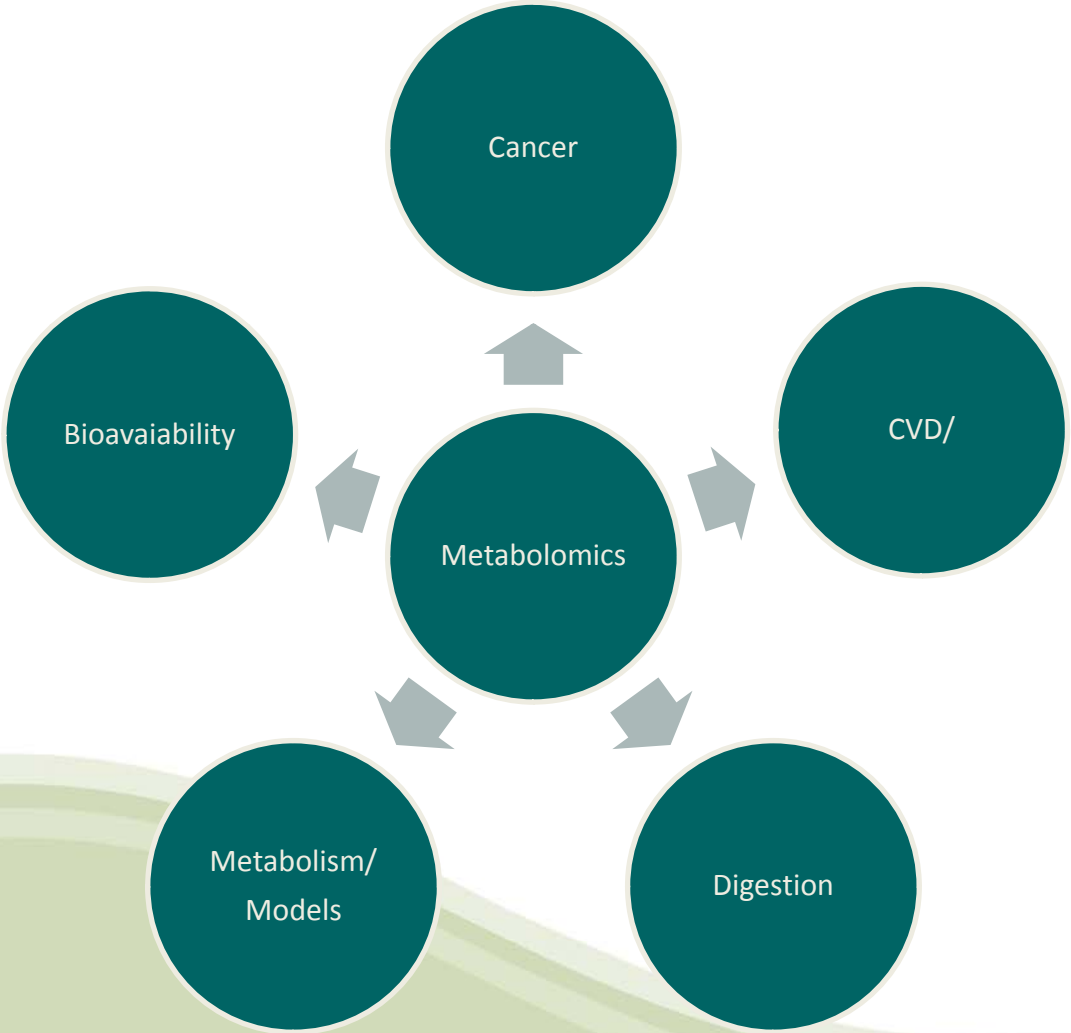
PCA plots can then be interrogated (mined) to see what masses (compounds) are causing the segregation

Elevation or reduction in known (or unknown) compounds

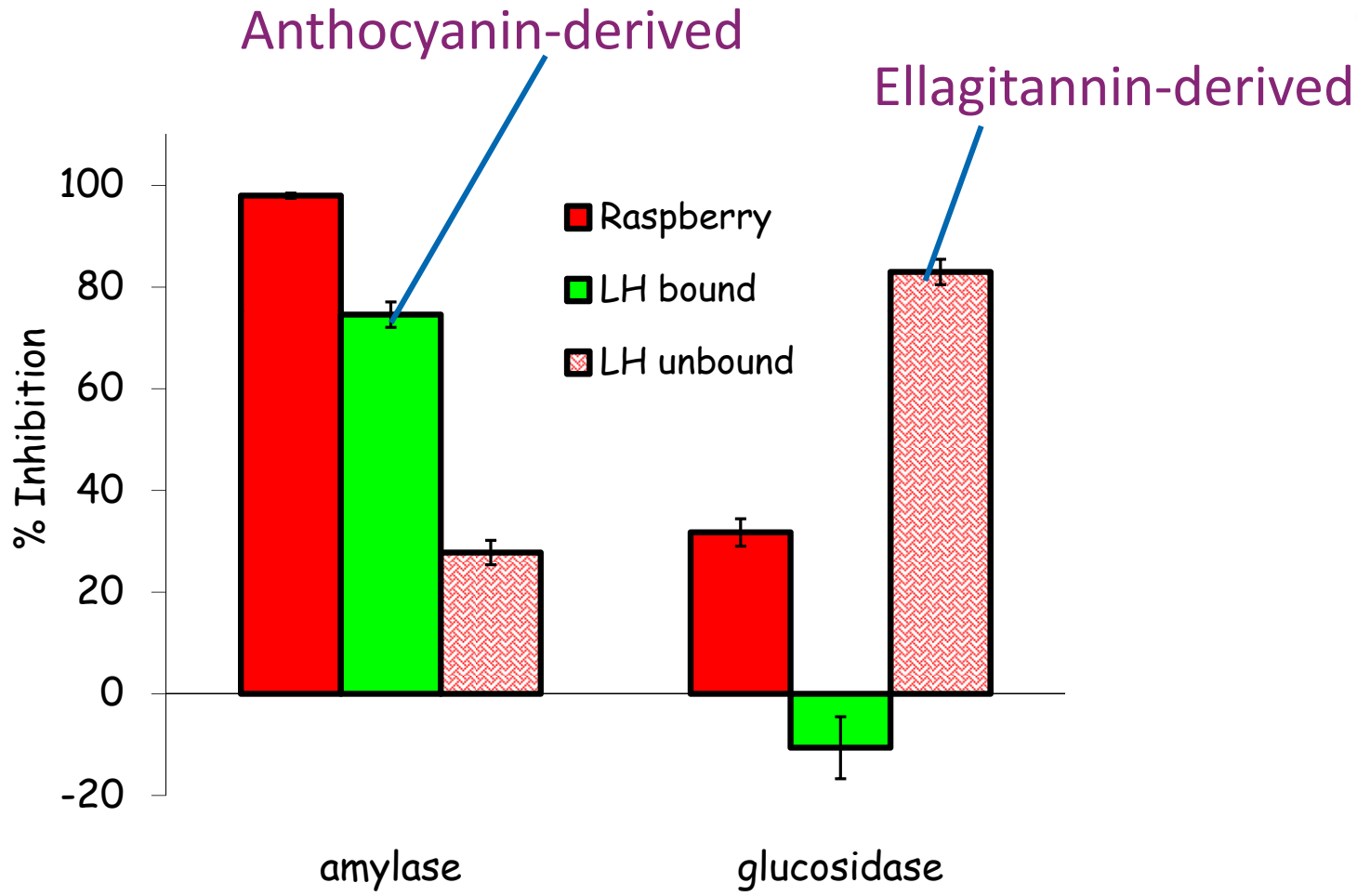
Cy-3-(2G-glucosylrutinoside)

scri living technology

JHI Mammalian Metabolomics

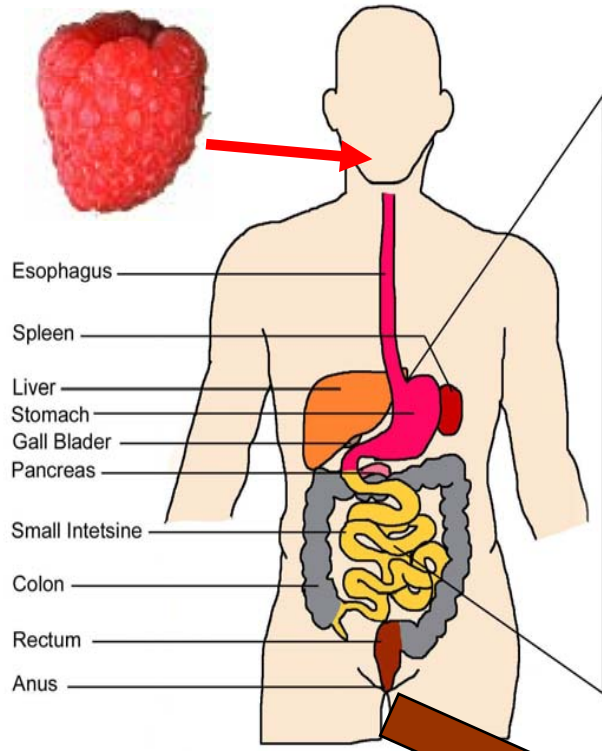


Impact of soft fruit consumption on diabetes; Inhibition of starch digestion



S07.021 (Wed. 1:30)
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

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But not the same subjects!

Phenylacetic acid increased in 7/10 subjects

4-Hydroxy phenylacetic acid increased in 6/10 subjects

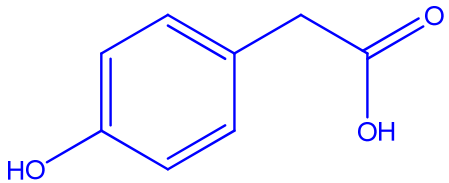
3-Hydroxy phenylacetic acid increased in 5/10 subjects

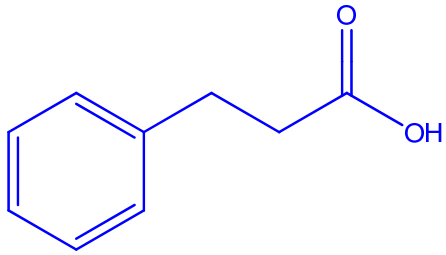
3-Phenylpropionic acid increased in 6/10 subjects

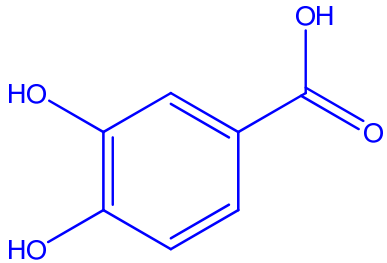
3-(4-Hydroxy)-phenylpropionic acid increased in 5/10 subjects

3,4-Dihydroxy benzoic acid increased in 7/10 subjects

4-Hydroxy benzoic acid increased in 2/10 subjects

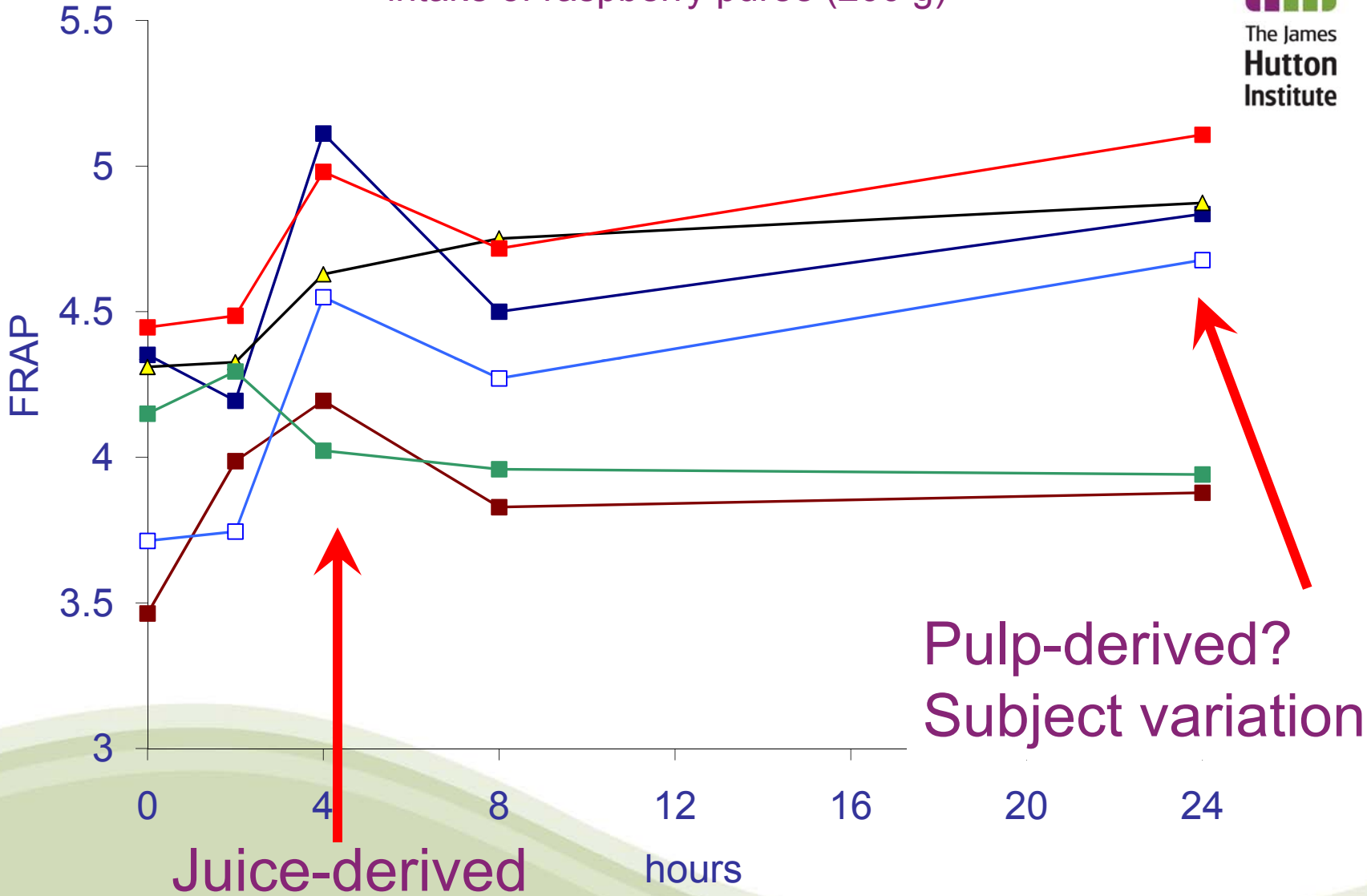






- Predominantly anthocyanin derived
- Fits evidence from model studies with faecal inocula but shows large inter-individual variation.
- Due to differences in diet or microflora?
- A proper confined study is required: defined diet, labelled fruit/anthos?

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

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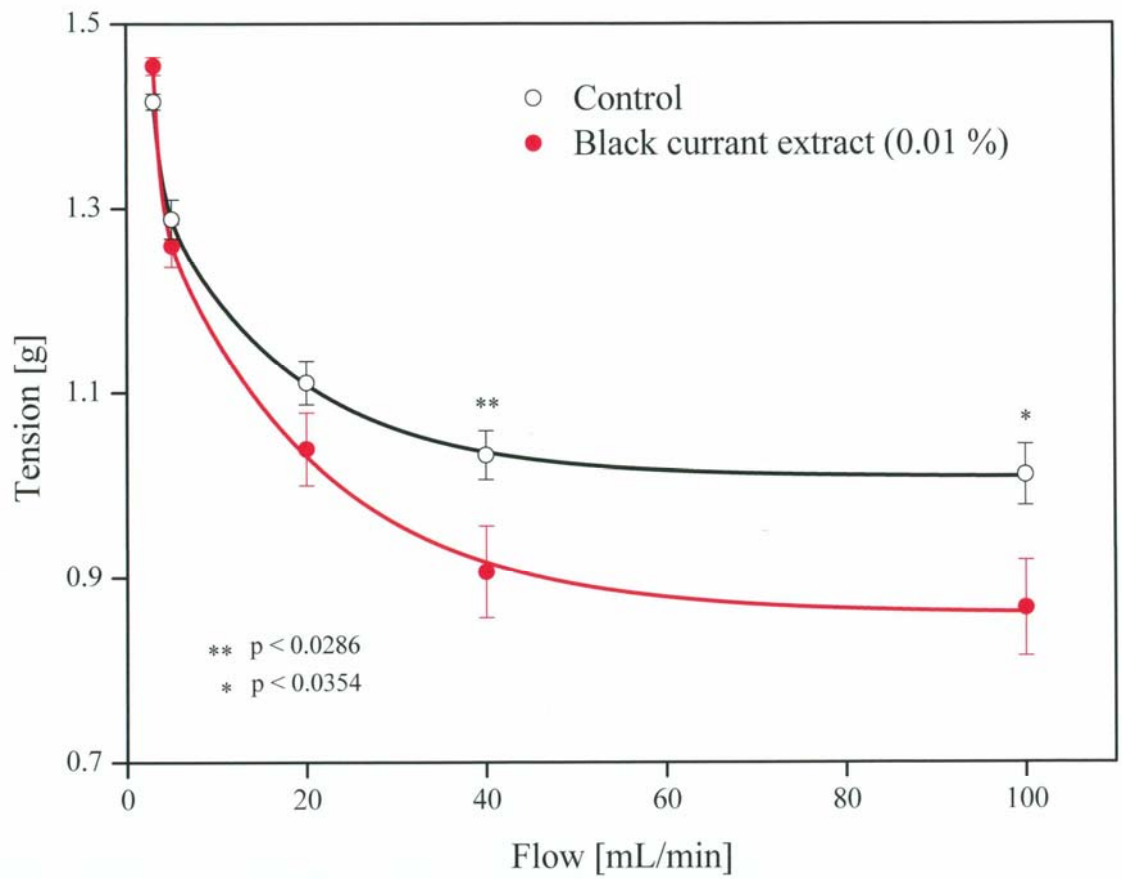
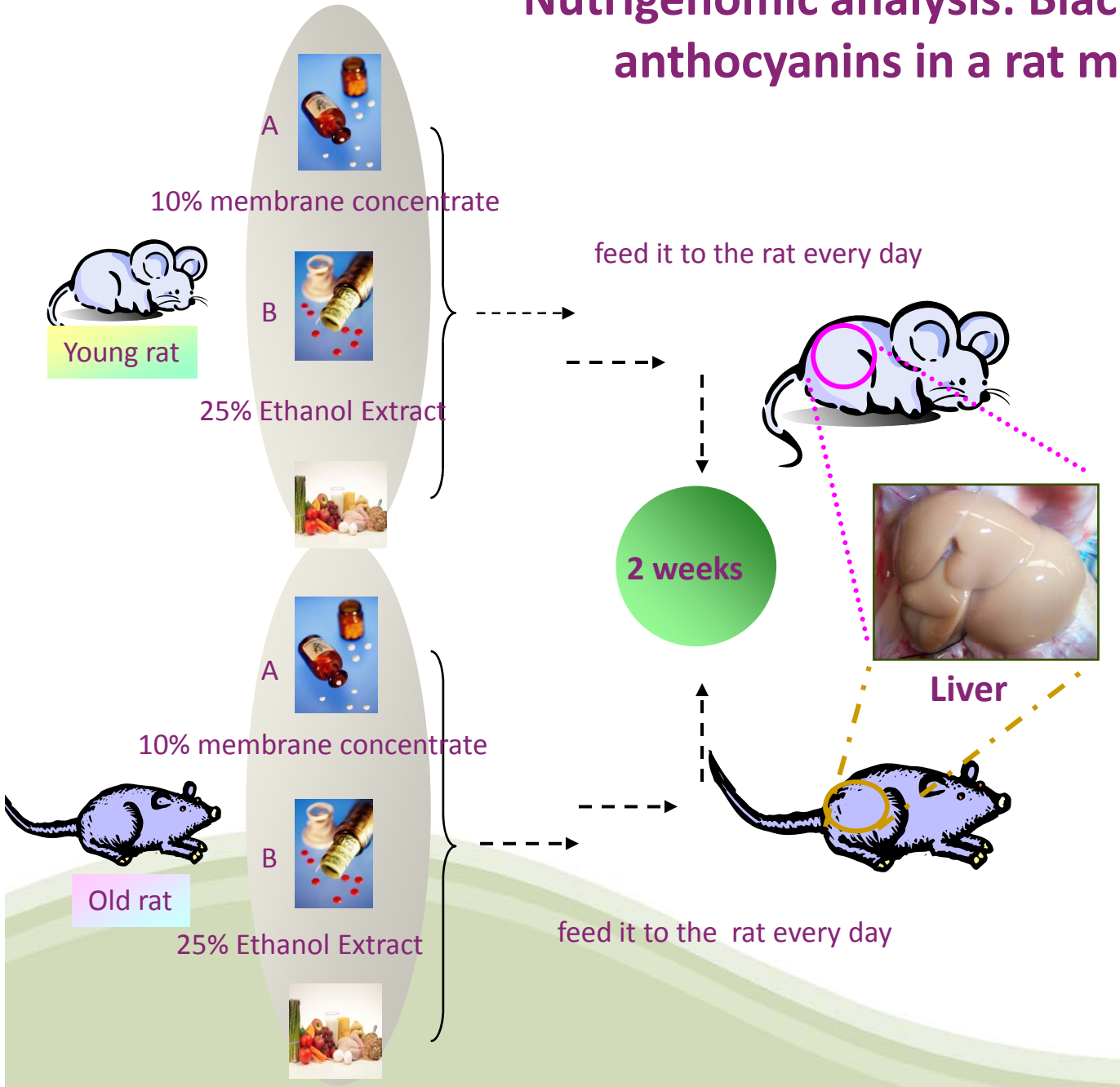
- Flow-dependent isometric tension was measured in segments of isolated human intracerebral arteries from consciousness areas: derived from brain surgery.
- The anthocyanin driven vasodilatation may have a beneficial effect on the cognitive functions in dementia of the Alzheimer type, in the prevention of TIA and stroke
- Flow-dependent relaxation is almost identical to fluvistatin.



Nutrigenomic analysis: Blackcurrants anthocyanins in a rat model.



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The Extract Company BerryPharma ARTEMIS NUTRITIONALS EUROPE

Nutrigenomic analysis: Blackcurrants anthocyanins in a rat.

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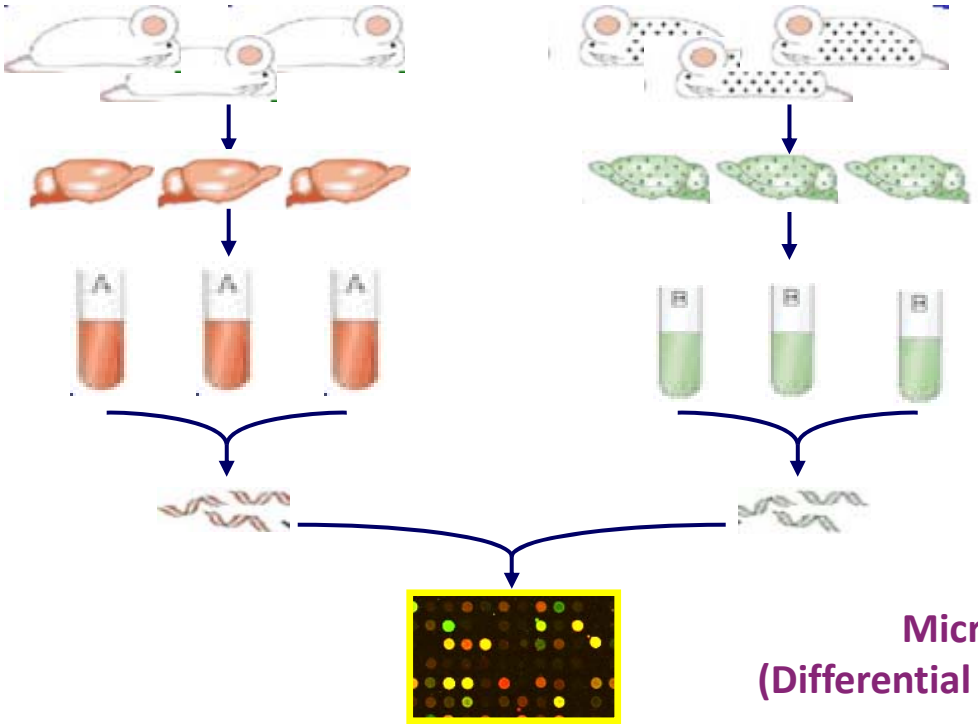
Control (No feed) **Experiment (feeded)**



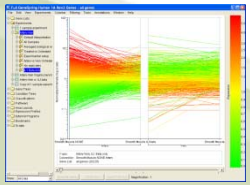
Specimen collection & storage

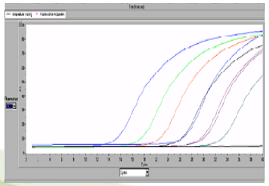
RNA isolation

Microarray experiment (Differential Gene Expression Profiling)

Rat Nutrigenomics array

Bioinfomatics analysis





Targeted genes Validation by Q-PCR

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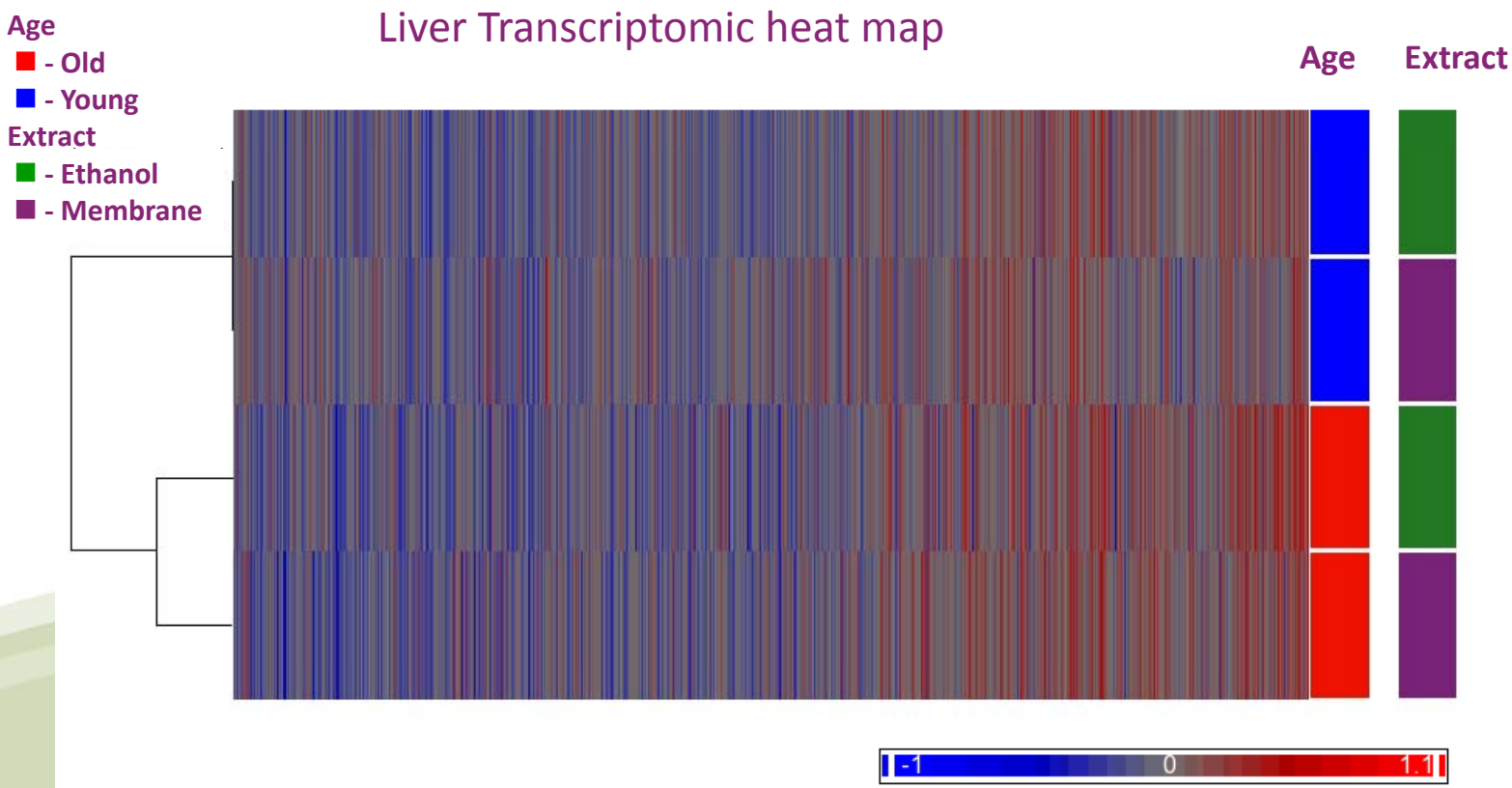
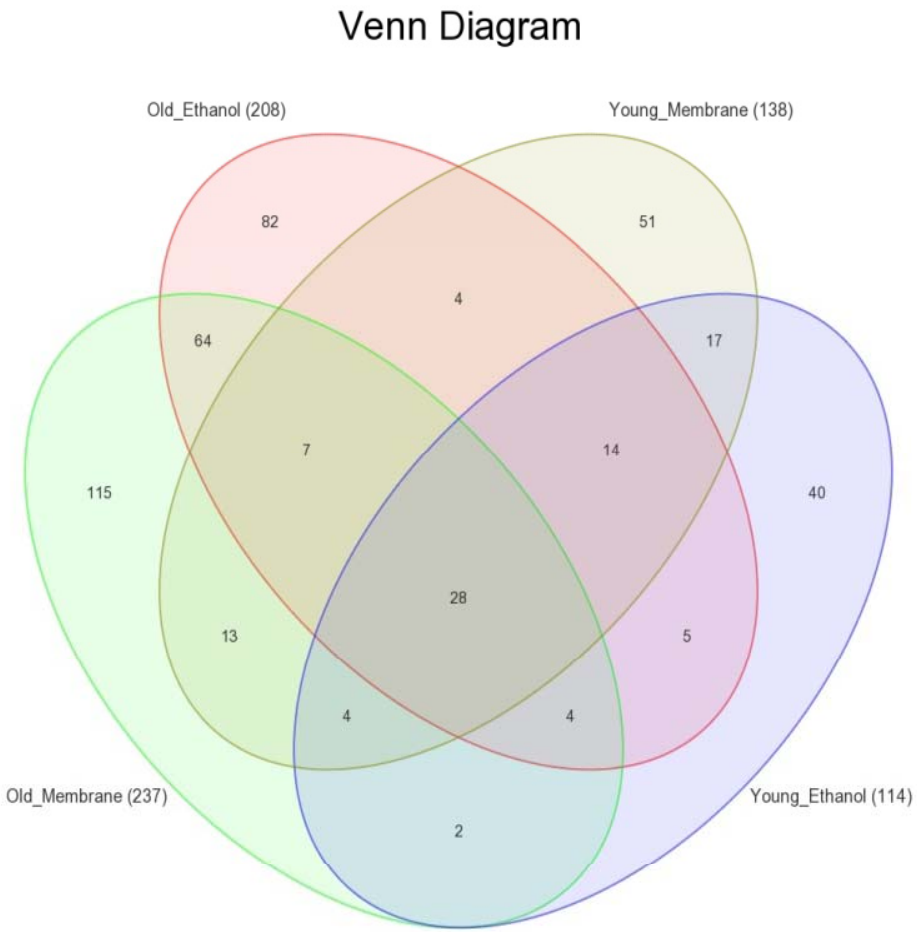
Blackcurrant anthocyanin intervention
Rat transcriptomic analysis



Blackcurrant anthocyanin intervention
Rat transcriptomic analysis

The James Hutton Institute



Pathway enrichment/regulation and ontology as a consequence of blackcurrant anthocyanin intervention

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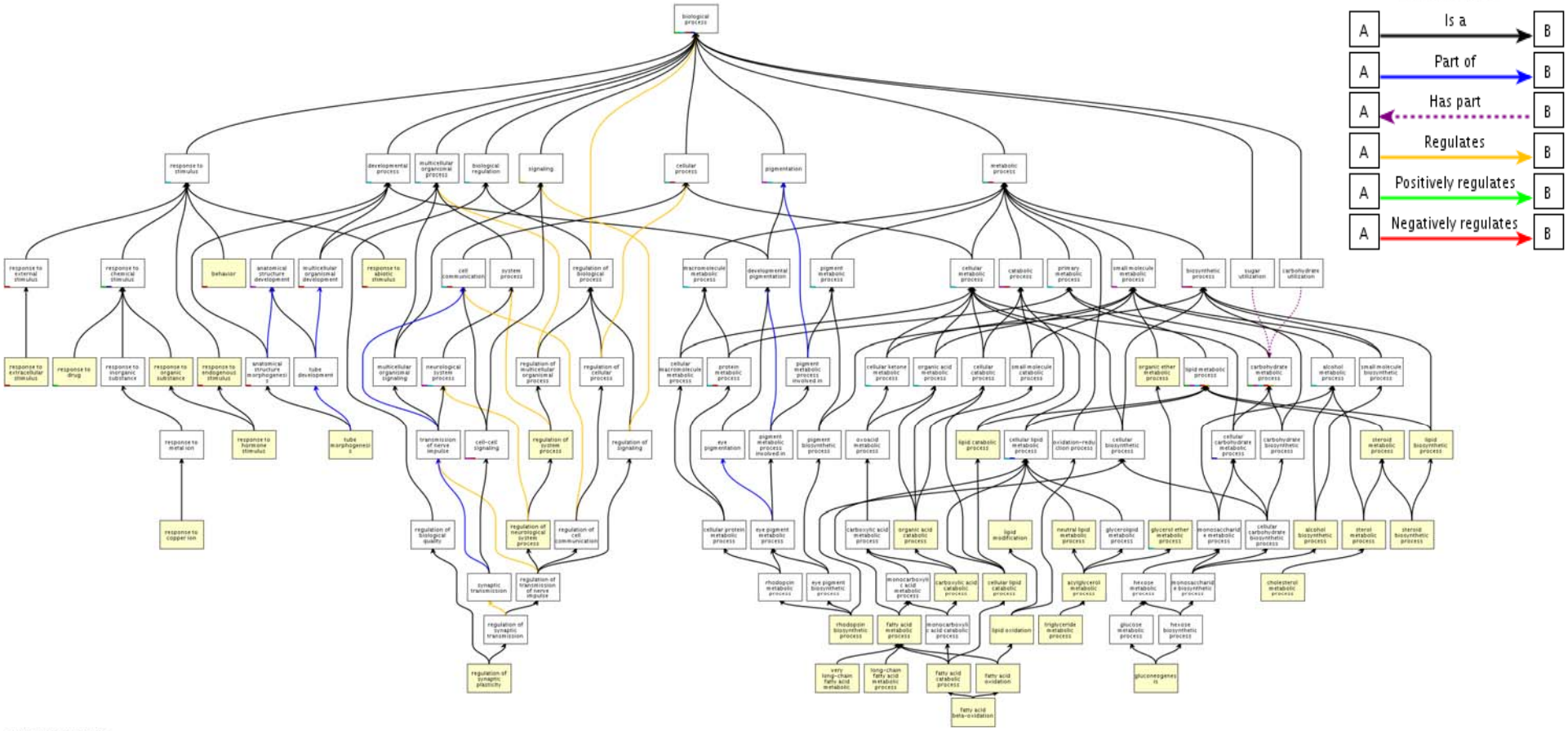
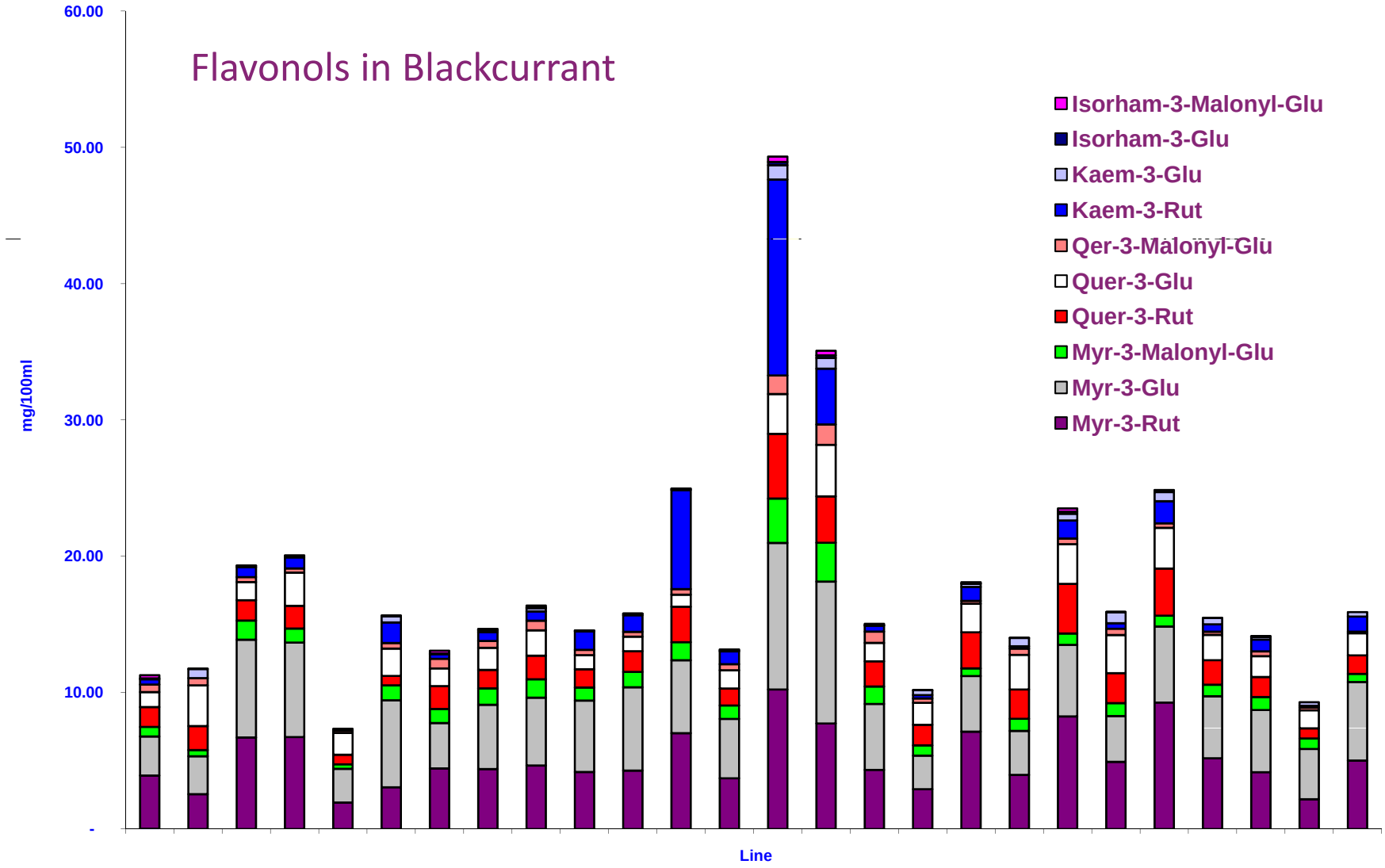
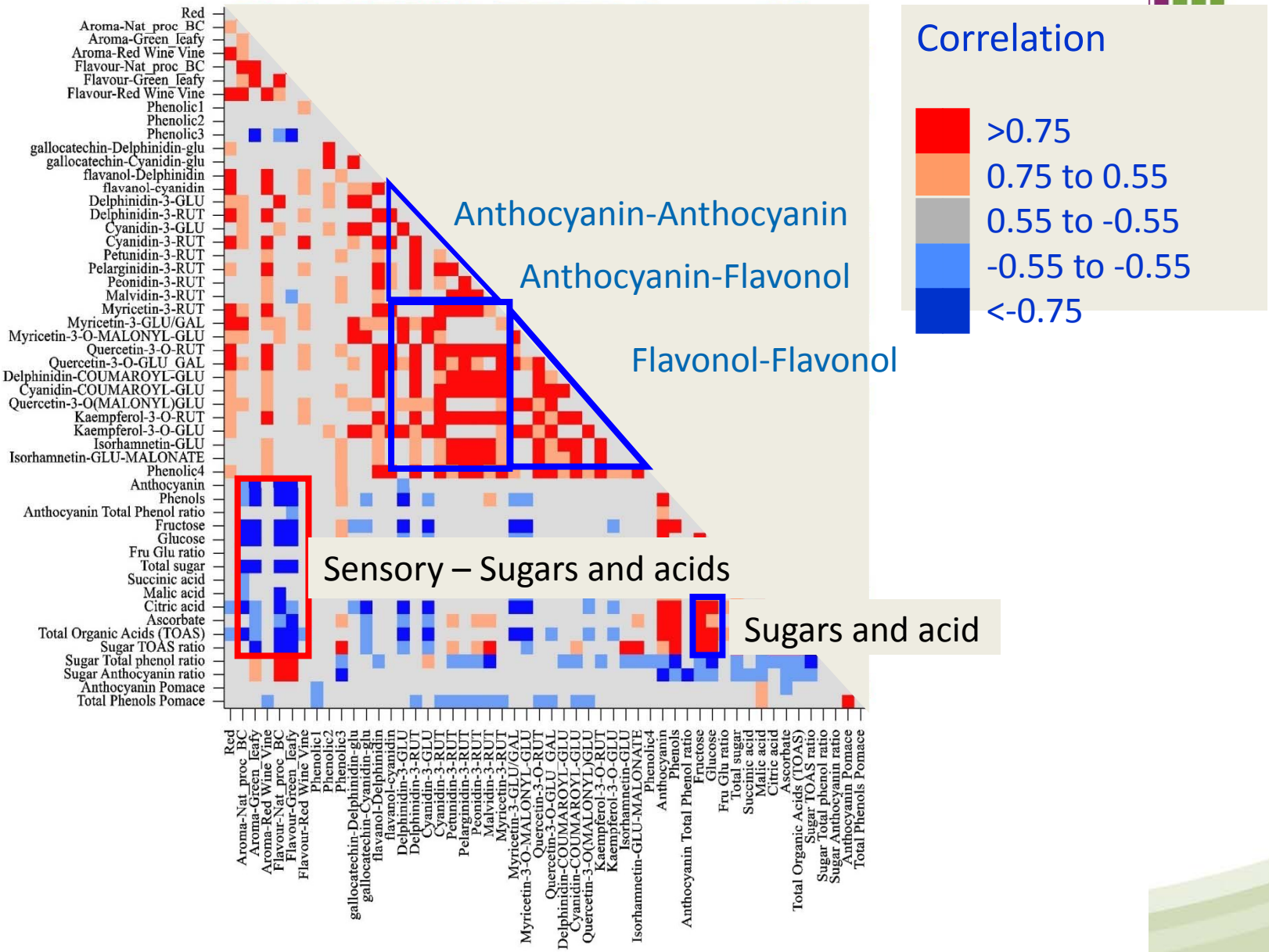


Database for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (**DAVID**) - http://david.abcc.ncifcrf.gov/
QuickGO (http://www.ebi.ac.uk/QuickGO/)

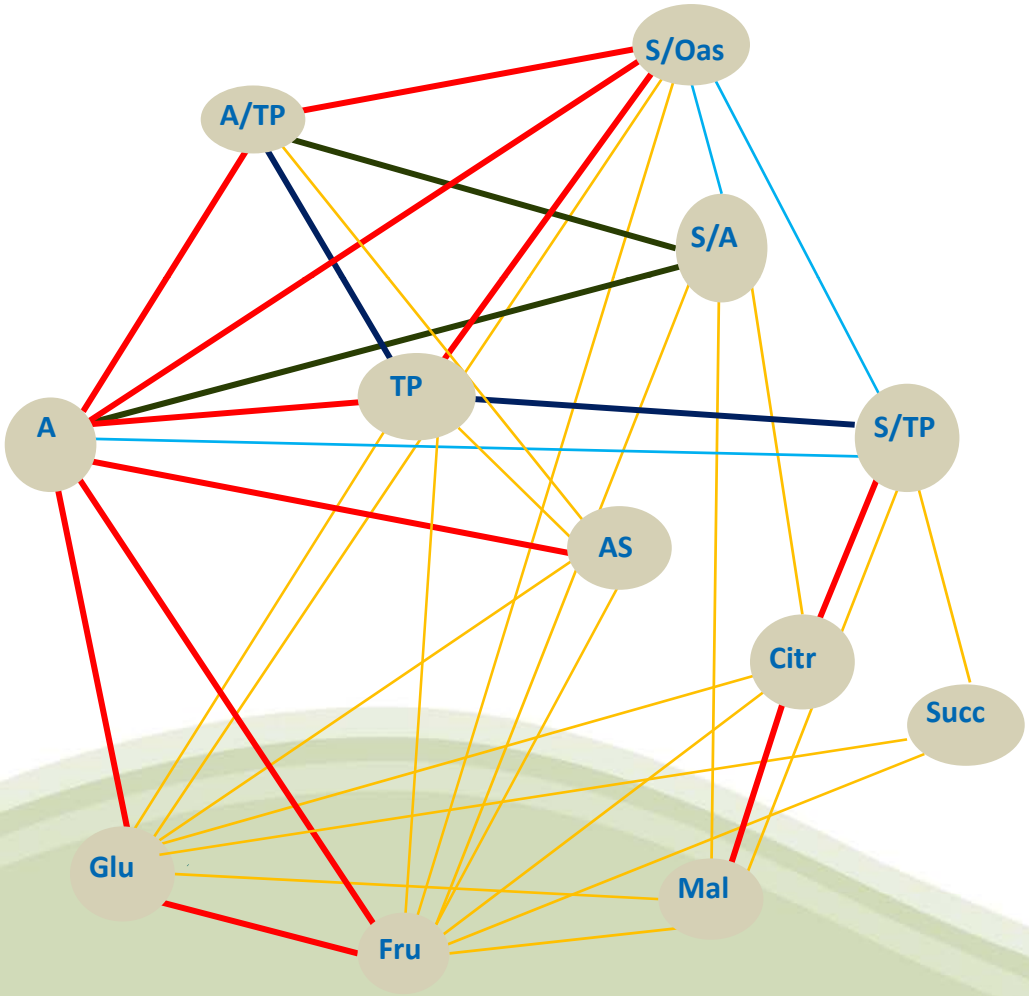
Polyphenolic Diversity



Soft fruit: Phytochemical Correlation Matrix



Metabolite Correlation Network



A and TP $\xrightarrow{+}$ S/Oas

↑Oas → ↓S/Oas → ↓A and TP

Citr and Mal $\xrightarrow{+}$ S/TP and A

↑ A or TP → ↓S/TP or A → ↓ Oas

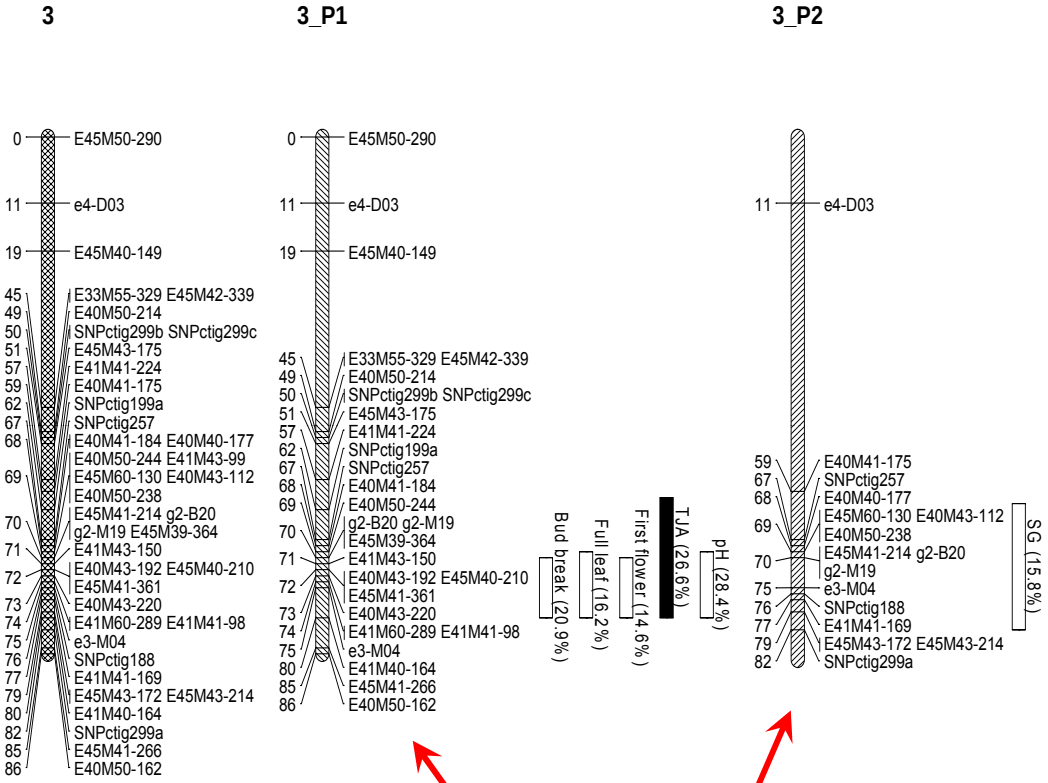
≥ 0.75

0.549 ≤ ≥0.749

-0.749≤ ≥ -0.549

≤ -0.75

Soft Fruit: Integration of ‘Omics, Genetics and Breeding

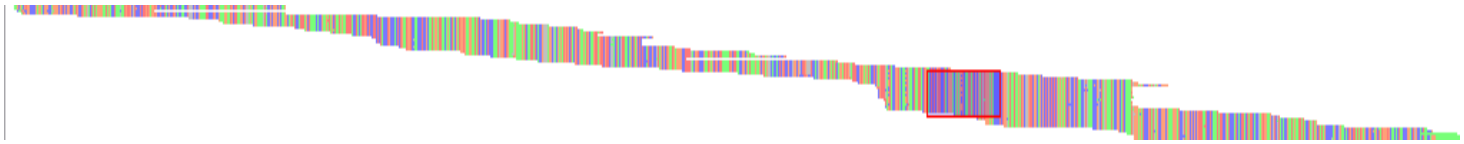


- Linkage maps being completed
- Extension of mapping population for confirmation of QTLs
- Mapping of key genes from ascorbate biosynthetic pathway and polyphenolic biosynthetic pathways.
- Correlate the genetic and metabolomic data
- Dissection of genetic and environment on food and health quality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

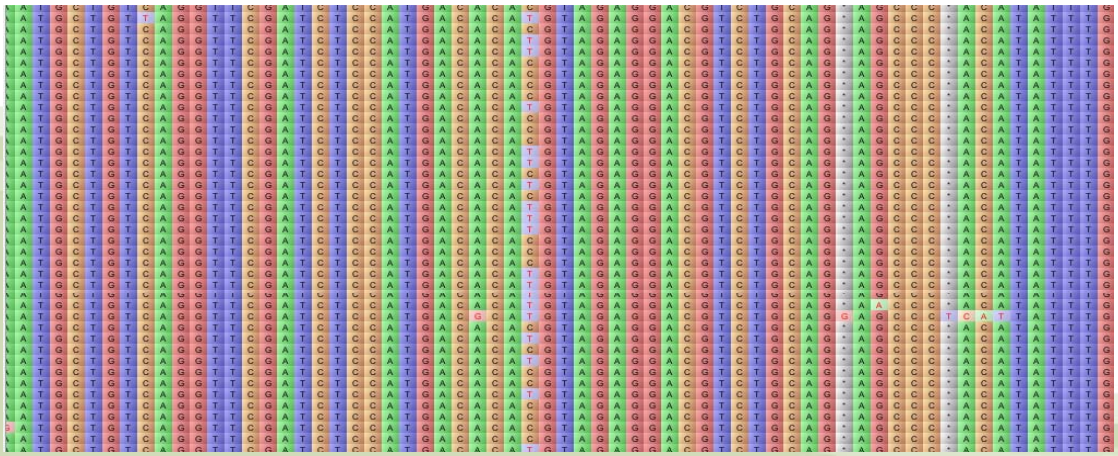
Genetic (AFLP, SSR, Next gen sequencing) ↔ Metabolomics

Next Generation Sequencing in *Ribes*

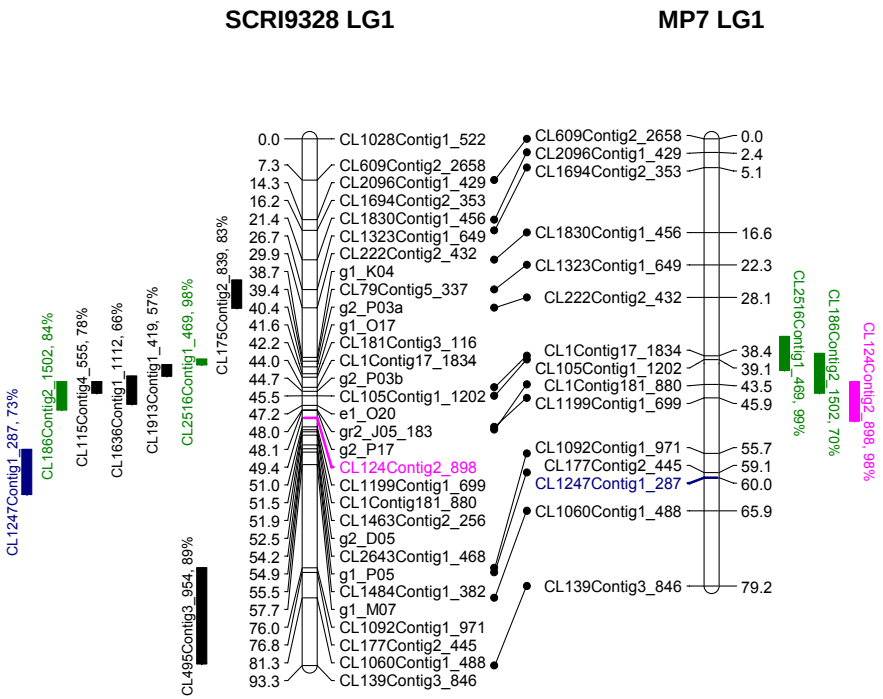
- Large scale 454 transcriptomic sequencing of two *Ribes* genotypes (9328 reference mapping parents)
- > 700k reads (117.9 Mbp of blackcurrant transcriptome)
- Reads assembled into 46411 contigs
- 7245 SNPs and 3179 SSRs discovered
- Set of 384 SNPs selected using `Tablet' programme, range of germplasm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



SNP



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

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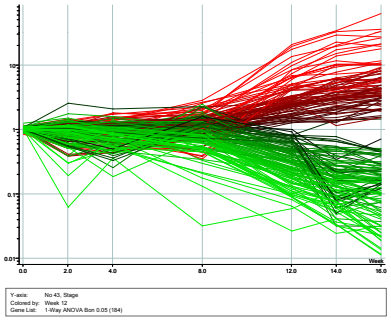
- **Fruit quality traits**

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)

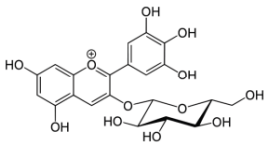
Significance for future sustainability of blackcurrant production

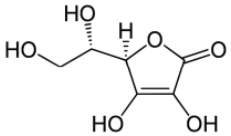
Genetic control of processing quality traits in blackcurrant (*Ribes nigrum* L.)

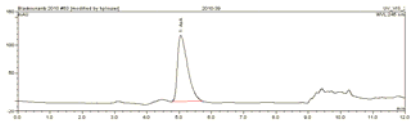
Dorota Jarret

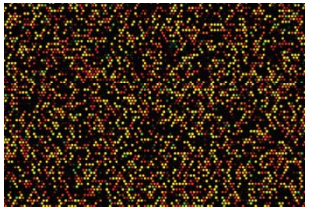
Aims of the project

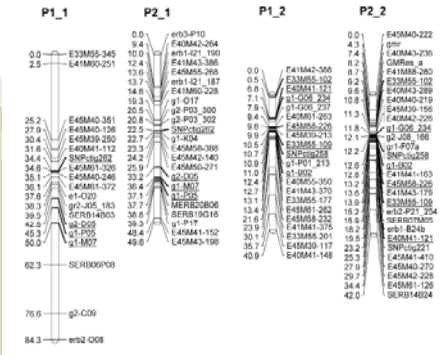
- Understand genetic control of the biosynthesis of nutritional and sensory compounds
- Identify map locations for the genes of interest
- Identify and deploy markers linked to key traits
- Assess environmental effects on important fruit quality traits











Approaches

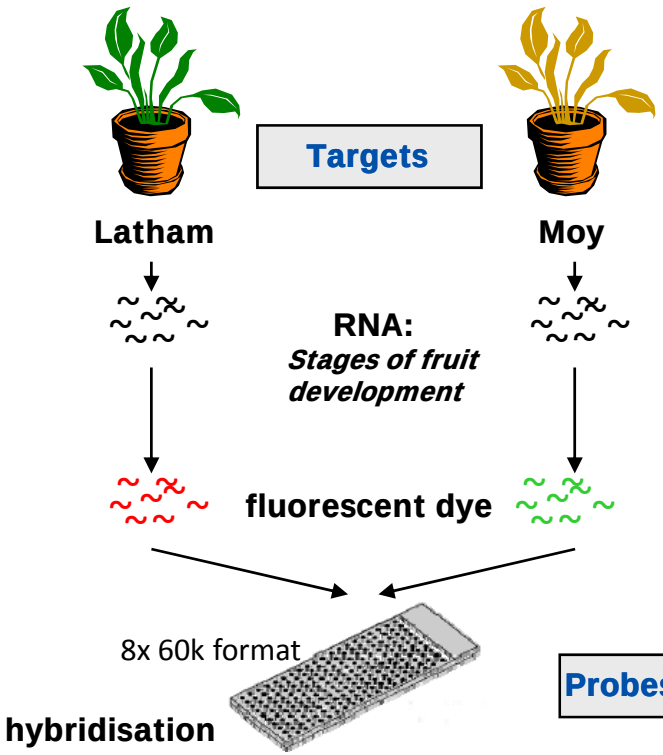
- Mapping population of 150 individuals
- Quality compounds analysis
- Measurement of gene activity and genotyping

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