Are polyphenols viable targets for soft fruit breeding?
A model study with blackcurrant.

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The increase of general and specific polyphenol level is a target for enhancement in crops, such as soft fruit, since accruing evidence suggests they exhibit beneficial effects against a number of degenerative diseases. Blackcurrant (Ribes nigrum) represents a rich source of putatively beneficial polyphenols, such as anthocyanins, flavonols and phenolic acids, and is a widely consumed fruit, mainly in juice form. Here we report some advances in our research into the inheritance of nutritional and beneficial health traits in soft fruit.

Conclusion
The cross yielded progeny exhibiting a diversity of polyphenolic structure and quantity. The state-of-the-art analytical approaches (‘omics) facilitated this screening in a timescale significantly quicker (~x10) than traditional analytical approaches.

Results
As part of a broad study looking at polyphenol inheritance we have analysed the progeny (200 lines) from a well established segregating cross derived from the blackcurrant parental lines S36/1/100 (high juice quality) and S10-2-27/28 (gall mite resistant). Metabolomic screening was undertaken for all progeny and the profiles of the parental lines are shown in Fig. 1.

Methods
LC/MS: Analysis was performed on single-stage LTQ Orbitrap mass spectrometer, coupled to LC and diode array.
LC conditions: Eluent: A-water (0.1% formic acid) and B-acetonitrile (50%) + 0.1% formic acid, Injection volume of 8 µl, and flow of 200 µl.min⁻¹ at 50°C. Gradient: 5-50% B to 22 min, 100%B to 32 min, 100%B at 34 min.
MS conditions- Resolution: 100,000; MS range: 80 – 2000 m/z; Full scan in positive mode
Processing data- Sieve V 1.2™
RT- 2.2 min; frame m/z- 0.02, threshold - 500,000
Statistical Analysis - Simca P 11™.

General and specific polyphenols across the progeny
Total anthocyanin levels (mg/ 100 ml of juice) were used to classify the segregating population into 4 groups (Fig. 2). The same classification was used for OPLS analysis of the LC/MS data; a valid model to discriminate the genotypes is shown in Fig. 3. The loading plots identified the outlier genotypes producing not only higher levels of anthocyanins but mainly conjugated anthocyanin-flavonols (Fig. 4). A similar strategy was used to analyse the general phenolics across the progeny, where OPLS analysis (Fig. 5) generated 3 groups (Fig.6), according to the total phenolics levels (mg/100 ml juice).

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Figure 1
Figure 2.
Figure 3. OPLS analysis (Y= 1, 2, 3 and 4)
Observations (N) = 383, Variables (K) = 2561, A= 1+21, R²X = 0.72, R²Y= 0.97, Q²(cum)= 0.79
Figure 4.
Figure 5. OPLS analysis (Y= 1, 2 and 3)
Observations (N) = 383, Variables (K) = 2561, A= 1+20, R²X = 0.701, R²Y= 0.98, Q²(cum)= 0.88

Anthocyanins: m/z 611, 595, 625, 449, 609, 579
Flavonols:
Myr-3-Rut m/z 627
Quercetin-3-O-glu m/z 465
Isorhamnetin-3-Glu m/z 479
Isorhamnetin-3-glu-malonate m/z 565
Gallocatechin-Delphinidin (DP)-3
Gallocatechin-cyanidin (CD)-3-glu
Gallocatechin-3-glucosylrutin (GR3G) m/z 817, 865, 823, 949, 939, 959
Gallocatechin-3-O-glucosylrutin (GR3G) m/z 785
Gallocatechin-3-O-glucosylrutin (GR3G) m/z 785

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