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Inheritance of Antioxidants in a New Zealand Blackcurrant (*Ribes nigrum* L.) population

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Abstract. A six parent modified half diallel of blackcurrants was planted in a randomised complete block design at Irwell, Canterbury, New Zealand. Variance components, heritability and breeding values were estimated for nine antioxidant traits from processed whole blackcurrant fruit harvested in 2004. Additive genetic effects were the largest with relatively small dominance and experimental design effects. Phenotypic and genetic correlations were moderately high ($r_p > 0.53$, $r_g > 0.46$) between all traits apart from correlations with relative antioxidant activity and bioavailability ratios and the correlation between delphinidin rutinoside and cyanidin-glucoside. Narrow-sense heritability estimates were moderate to high (0.46-0.80) except for the relative antioxidant activity and bioavailability ratios (0.28), indicating that phenotypic selection of parents may be successful. Implications are discussed for breeding blackcurrants with increased antioxidant levels.

Introduction

In recent times, there has been a growing interest in the medicinal properties of foods. Anthocyanins are phenolic compounds that cause the colour in blackcurrants and have a wide range of beneficial medicinal properties because of their antioxidant properties (Ghosh 2005). Fifteen anthocyanins have been identified in blackcurrants (Slimestad and Solheim 2002), of which the four main anthocyanins are the 3-O-glucosides and the 3-O-rutinosides of delphinidin and cyanidin: delphinidin glucoside (Dp-Glu), delphinidin rutinoside (Dp-Rut), cyanidin-glucoside (Cy-Glu) and cyanidin-rutinoside (Cy-Rut).

The New Zealand blackcurrant breeding programme aims to improve the antioxidant content of blackcurrants by using anthocyanin content as a selection criterion. Varying levels of anthocyanins have been found in blackcurrants (Moyer *et al.* 2002), and studies in other berryfruit show anthocyanins are moderately heritable (Connor *et al.* 2002a, 2002b; Connor *et al.* 2002c; Connor *et al.* 2005a; Connor *et al.* 2005b). This study reports on genetic parameter estimation for anthocyanin content and antioxidant activity in a blackcurrant population at Canterbury, New Zealand. In addition, implications for breeding are discussed.

Materials and methods

<u>Sampling</u>

A six parent modified half diallel (no reciprocals and no selfs) of blackcurrants was planted in a randomised complete block design at Irwell, Canterbury, New Zealand. There were four replicates and fifteen plots per replicate. Observations were taken from six individual seedlings per plot for the analysis. The berries were inspected weekly and a single sample of 200 g was harvested when the berries were fully black. Within three hours of harvest, the sample was frozen and kept frozen until processed.

Laboratory analysis

To prepare extract for analysis 20 g of fruit was homogenised in aqueous alcohol using a T25 Ultra Turrax. The blended fruit were sealed tightly and stored at 2°C for 48 hours in the dark to complete the extraction of polyphenolics. A portion of extract (approx. 5 ml) was centrifuged (3000 rpm, 10 minutes) and the supernatant stored at -20°C until analysis for phenolics, anthocyanins and antioxidant capacity.

Antioxidant capacity (ORAC) for the blackcurrant extracts was measured using the $ORAC_{FL}$ method (Ou *et al.* 2001). The total phenolic (TPH) content of the blackcurrant extracts was determined according to the Folin-Ciocalteu procedure using visible spectroscopy (Singleton *et al.* 1999). A subset of phenolics, total anthocyanin (ACY), and anthocyanin content of the four main anthocyanins: delphinidin-glucoside (Dp-Glu), delphinidin-rutinoside (Dp-Rut), cyanidin-glucoside (Cy-Glu), and cyanidin-rutinoside (Cy-Rut) in the extracts were determined by HPLC (Connor *et al.* 2005c).

The delphinidin / cyanidin ratio (Dp:Cy) and the glucoside / rutinoside ratio (Glu:Rut) were calculated to give an indication of relative antioxidant activity and of bioavailability, respectively, at similar levels of ACY. Higher Dp:Cy and Glu:Rut ratios may indicate higher antioxidant activity and bioavailability.

Statistical analysis

The data were analysed using the following individual plant ('animal model') mixed model:

$$y = \mu + Block_i + Family_j + Plot_{ij} + Plant_{ijk} + error_{ijk}$$

Where *μ* is the population mean, *Block* is a fixed effect, and *Family* (specific combining effect), *Plot* and *Plant* (additive genetic effects) are the random effects. Plots of residuals were examined for deviations from normality and homogeneity of variance using R (R Development Core Team, 2005). Data were transformed if necessary, and thus reported genetic parameters are for the transformed, rather than the raw data. The expected values and variances of the model are:

$E(y_{ijk}) = \mu + Block_i$

$Var(Family_j) = \sigma_f^2$, $Var(Plot_{jk}) = \sigma_p^2$, $Var(Plant_{ijk}) = \sigma_a^2$, $Var(error_{ijk}) = \sigma_e^2$ where σ_f^2, σ_p^2, σ_a^2 and σ_e^2 correspond to the family (estimates ¼ dominance), plot, additive and residual variances respectively. All covariances between random effects were assumed to be zero. Finally, the relationship between individuals was taken into account using a numerator relationship matrix.

The *Plot* and *Family* factors were not statistically significant and so were removed and a simplified model was fitted:

$$y = mu + Block_i + Plant_{ij} + Error_{ij}$$

The additive genetic and residual variance components were estimated by restricted maximum likelihood techniques using ASReml software (Gilmour *et al.* 1998).Narrow-sense heritability and the phenotypic and genetic correlations were calculated from the variance components and the standard error was calculated with Pearson's approximation of the variance of a ratio (Gilmour *et al.* 1998).

Results and discussion

The range of data for ACY, TPH and ORAC was similar to the range in values found by Moyer et al. (2002) for blackcurrants (Table 1). The four main anthocyanins make up 99% of the ACY (Table 1). Slimestad and Solheim (2002) also found these four main anthocyanins made up more than 97% of the total anthocyanin content in blackcurrants. Rutinosides were more abundant than the glucosides, which is also, what Slimestad and Solhiem observed.

Correlation - ACY **v**. TPH

Although the ACY was approximately half of the TPH and values ranged widely, ACY and TPH were closely correlated (r_p= 1.00, r_p = 0.98 Table 2). This suggests that the expression of non-anthocyanin phenolics was linked to expression of anthocyanins. Similar high correlations were found by Moyer et al. (2002) for blueberries (r_p = 0.93) and some hybrid berries (r_p = 0.57 to 0.83) but a lower correlation was found for blackcurrants (r_p = 0.63).

Correlation - ACY and TPH v. ORAC

In this study both ACY and TPH had similar, large, positive phenotypic and genetic correlations with antioxidant activity measured by ORAC (r_p = 0.77, r_g = 1.0) (Table 2). Connor et al. also found high phenotypic correlations (r_p = 0.9 to 0.97) between antioxidant activity and TPH and moderately high phenotypic correlations (r_p =0.5-0.73) between antioxidant activity and ACY for blueberry (Connor *et al.* 2002a; Connor *et al.* 2002c), blackberry and hybridberry (Connor *et al.* 2005a; Connor *et al.* 2005b), and red raspberry (Connor *et al.* 2005c). Moyer *et al.* (2002) found a lower correlation between TPH and ORAC in blackcurrants (r_p = 0.44) than in blueberries (0.79) or *Rubus* sp. (r_p = 0.92), and speculated that this may be due to the higher levels of ascorbic acid (a non-phenolic antioxidant) in blackcurrants. Ascorbic acid was not measured in this study but, because of the high correlation between TPH and ORAC, ascorbic acid must have been highly correlated to TPH in this population.

Both Moyer *et al.* (2002) and Connor *et al.* (2002a; 2002c) reported that ACY had lower correlations than TPH with antioxidant activity. In this study ACY and TPH had similar correlations to ORAC because of the close relationship between the expression of ACY and TPH.

The high genetic correlations between ACY, TPH and ORAC suggests that any one of these measures will co-select for the other traits in this population and that only one of these measures need be taken.

Correlation - ORAC, ACY, TPH v. individual anthocyanins

ORAC, ACY and TPH had moderately large positive phenotypic (r_p > 0.53) and genetic (r_g > 0.59) correlations with each of the individual major anthocyanin compounds (Table 2This suggests that selection based on any of these traits would result in genetic progress for the others.

Correlations between individual anthocyanins

The individual anthocyanin compounds had positive genetic correlations with each other apart from a correlation close to zero between cyanidin-glucoside and delphinidin-rutinoside (Table 2), which may be genetically independent. Individual anthocyanins did not have higher correlations with ORAC than ACY. Based on similar results, Connor et al. (2005b) concluded that there is little value in using any of the individual anthocyanins to select blackcurrants with high antioxidants. However, as discussed in the next section, a higher heritability for Dp-Rut combined with large positive correlations with ORAC suggest that selection based on Dp-Rut may result in a faster response to selection.

Correlations between individual anthocyanins and ratios

An expected positive relationship between the Dp:Cy and the Glu:Rut ratios with the anthocyanins in the numerator and the expected negative correlation between the ratios and anthocyanins in the denominator of the ratio provide a valuable indicator of the reliability of the calculated correlations.

The Dp:Cy ratio did not show the expected phenotypic correlations between the ratio and the numerator and denominator components and the only non-zero correlation was within one standard error of zero (Table 2). The Glu:Rut ratio did show the expected positive correlation with numerator terms and negative correlation with denominator terms. The Glu:Rut ratio was independent from the Dp:Cy ratio and although it showed low negative correlation with ACY, TPH or ORAC, this was within one standard error of zero, suggesting that selection for high Glu:Rut ratio may not have an impact on overall ACY, TPH or ORAC. The poor performance of these ratios in discriminating differences and the high standard errors associated with the ratios casts some doubt on the usefulness of these measures.

Future correlation work

Connor et al. (2005a) found a reasonable amount of genotype by environment interaction (GXE) for ACY, TPH and antioxidant activity in *Rubus* sp., suggesting that selection based on these traits needed to be measured over several seasons or locations. It needs to be confirmed whether similar GXE effects occur with blackcurrants.

Heritability

The heritability of the Dp:Cy and glucoside: rutinoside ratios was low (0.28) (Table 3), reflecting the variability in the data. The heritabilities for the individual anthocyanin components ranged from moderate heritability (0.46) to high heritability (0.80) (Table 3). The high standard errors (range 0.25 to 0.31) mean that the true heritabilities could be considerably different from these values. Increasing the number of genetic entries in trials would contribute to reducing the size of the standard errors of the genetic parameters. A similar pattern of heritability emerges from the literature on the same traits (Connor *et al.* 2002b; Connor *et al.* 2005c).

The highest heritability was for Dp-Rut (h^2 = 0.80), which was also highly correlated to ORAC, ACY and TPH, and so selection on this trait may result in rapid gains in antioxidant and anthocyanin levels in blackcurrants.

Table 1. Blackcurrant anthocyanin trait data summary

Trait (µg/g fresh weight)	Minimum	Mean	Maximum
Total anthocyanins (ACY)	1,253	3,357	6,166
Delphinidin glucoside (Dp-Glu)	128	489	1,032
Delphinidin rutinoside (Dp-Rut)	378	1,380	2,739
Cyanidin-glucoside (Cy-Glu)	56	189	647
log Cyanidin-glucoside	1.75	2.24	2.81
Cyanidin-rutinoside (Cy-Rut)	513	1,252	2,838
Total phenolics (TPH)	2,365	6,341	12,264
Delphinidin : cyanidin ratio (Dp:Cy)	0.60	1.32	2.22
Glucoside : Rutinoside ratio (Glu:Rut)	0.12	0.26	0.58
log Glucoside : rutinoside ratio	-0.91	-0.60	-0.24
Oxygen radical absorbance capacity (ORAC)	4.23	10.45	16.05

Table 2. Anthocyanin and antioxidant traits from blackcurrant fruit. Phenotypic correlations (upper triangle) and genotypic correlations (lower triangle) with standard errors in brackets.

	ACY	Dp-Glu	Dp-Rut	Log Cy-Glu	Cy-Rut	TPH	Dp:Cy	Log Glu:Rut	ORAC
ACY	1.00	0.79 (0.05)	0.88 (0.04)	0.60 (0.09)	0.89 (0.04)	0.98 (0.00)	0.00 (0.00)	-0.09 (0.11)	0.77 (0.05)
Dp-Glu	0.88 (0.11)	1.00	0.56 (0.10)	0.78 (0.05)	0.62 (0.08)	0.78 (0.05)	0.00 (0.22)	0.49 (0.10)	0.64 (0.07)
Dp-Rut	0.86 (0.12)	0.66 (0.27)	1.00	0.20 (0.15)	0.62 (0.12)	0.83 (0.05)	0.00 (0.29)	-0.36 (0.10)	0.64 (0.08)
log Cy-Glu	0.59 (0.32)	0.70 (0.25)	0.09 (0.48)	1.00	0.66 (0.07)	0.62 (0.08)	0.00 (0.23)	0.62 (0.08)	0.53 (0.09)
Cy-Rut	0.83 (0.14)	0.73 (0.23)	0.46 (0.37)	0.87 (0.12)	1.00	0.89 (0.04)	0.00 (0.25)	-0.12 (0.11)	0.69 (0.07)
TPH	1.00 (0.00)	0.88 (0.12)	0.84 (0.14)	0.61 (0.30)	0.85 (0.13)	1.00	0.00 (0.24)	-0.06 (0.11)	0.77 (0.05)
Dp:Cy	0.32 (0.42)	0.00 (0.64)	0.00 (0.65)	0.00 (0.65)	0.00 (0.63)	0.00 (0.64)	1.00	0.00 (0.18)	0.28 (0.21)
log Glu:Rut	-0.35 (0.45)	0.11 (0.50)	-0.64 (0.31)	0.39 (0.43)	-0.11 (0.50)	-0.34 (0.46)	0.00 (0.67)	1.00	0.00 (0.11)
ORAC	1.02 (0.02)	0.89 (0.12)	0.79 (0.18)	0.69 (0.26)	0.91 (0.09)	1.04 (0.04)	0.37 (0.53)	-0.29 (0.47)	1.00

Table 3. Blackcurrant antioxidant trait narrow sense heritability and standard errors for anthocyanin expression in blackcurrant fruit.

Trait	**Narrow sense heritability**	**s.e.**
ACY	0.64	0.29
Cp-Glu	0.47	0.25
Dp-Rut	0.80	0.31
Cy-Glu	0.46	0.25
Cy-Rut	0.62	0.29
TPH	0.55	0.28
Dp:Cy ratio	0.28	0.18
Glu:Rut ratio	0.28	0.18
ORAC	0.55	0.27

Conclusion

The high correlations between ORAC and ACY show that the blackcurrants in this population are good candidates for improving the antioxidant content by selection based on ACY.

High positive correlations between ACY and the four main individual anthocyanins mean that selection based on ACY should also increase the amounts of each of the four main individual anthocyanins.

The high heritability of Dp-Rut and high genetic correlation with ACY and ORAC suggests greater gains can be made in ACY and ORAC by selecting on Dp-Rut. However, Dp-Rut was independent of Cy-Glu and so selection would need to be made based on Cy-Glu as well, if improvement in this trait is also needed.

Future work on GXE and correlations with agronomic traits would be valuable to determine whether selection can be made in one year at one site, and how selection for antioxidant content will affect other agronomic traits.

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