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Genetic Variability in Apple Fruit Polyphenol Composition in *Malus* × *domestica* and *Malus sieversii* Germplasm Grown in New Zealand

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Supporting Information

ABSTRACT: Variations in the concentrations of flavan-3-ol, oligomeric procyanidin, chlorogenic acid, dihydrochalcone, flavonol, and anthocyanin polyphenol groups and total polyphenols were examined in the fruit peel and cortical flesh of 93 (80 *Malus* × *domestica* and 13 *Malus sieversii*) apple genotypes in at least 1 year between 2003 and 2005 grown at one site in New Zealand (NZ). Differences among genotypes accounted for 46−97% of the total variation in the concentrations of total polyphenols and each of the individual phenol groups in the flesh and peel in both species, whereas effects of year and genotype × year were minimal, except for peel flavonols in *M.* × *domestica* and flesh flavonols in both species. In these cases, differences among genotypes accounted for less than 30% of the total variation, which was less than the variation found for the interaction between genotype and year. Total polyphenol concentrations among genotypes were spread over a 7- and 9-fold range in the flesh and a 4- and 3-fold range in the peel of *M. sieversii* and *M.* × *domestica*, respectively, with the spread in concentrations of individual polyphenol groups in each tissue and within each species varying from a 2-fold to over a 500-fold range. Higher concentrations were generally found in *M. sieversii*. In *M.* × *domestica*, cultivars and breeding selections originating in NZ had lower average flesh and peel total polyphenols and chlorogenic acid than older cultivars previously imported into NZ from overseas countries.

KEYWORDS: Apple, *Malus*, polyphenol, repeatability, cultivar, HPLC

INTRODUCTION

Polyphenols are an important class of phytochemicals in fruits. They have a number of functions in the plant including resistance to disease and phytoprotection, are colorants and attractants to fruit-eating animals, and aid in identifying when fruits are edible. Plant polyphenols may also have positive effects on human health, although mechanisms by which they might exert such effects are unclear. A high human intake of foods rich in polyphenol compounds, such as fruits and vegetables, has been inversely associated with risks of coronary heart disease and mortality[1−3] and stroke.[4] Polyphenols have strong antioxidant activities, and the onset of these diseases is associated with the oxidation of low density lipoproteins in the vasculature. However, in vivo action of dietary antioxidants may also include the induction of protective enzymes and processes.[5]

Increasing the uptake of fruits and vegetables has received major encouragement in several countries as a means of reducing disease. The health of fruit and vegetable consumers might also be improved by enhancing health-related compounds in produce, such as polyphenols. However, in some foods and fruits, higher polyphenol concentrations are also associated with unfavorable bitter and/or astringent tastes.[6,7]

Within fruits, apples have high concentrations of polyphenols and, having a high rate of consumption, constitute a major source of polyphenols in the human diet.[8,9] Not only is the total polyphenol concentration high in apple fruit, but a complex range of polyphenols is present. These include hydroxycinnamic acids (mainly chlorogenic acid), flavan-3-ols (catechins and oligomeric procyanidins), hydrochalcones (phloridzin), flavonols (quercetin glycosides), and the red-colored anthocyanins.

Breeding new cultivars is one means by which polyphenols may be altered in apple fruit. To develop optimum strategies for changing concentrations, an understanding of the genetic variability within the breeding germplasm and its stability in different environments is essential. Total and individual polyphenols in dessert cultivars of *Malus* × *domestica* have been shown to vary by up to 10 times,[10−15] but little data are available on the variability outside material commonly cultivated. By analyzing the juice of over 300 noncommercial genotypes from 20 *Malus* species, a 400-fold variation in total fruit polyphenol concentration has been reported.[16] While most of the species considered were crab apples and extremely small-fruited,[17] the high polyphenol concentrations found in *Malus sieversii* fruit were interesting, as this species is also known to produce large-sized apples with desirable fruit and horticultural traits.[18] Accordingly, there is substantial interest in utilizing *M. sieversii* germplasm for apple cultivar improvement.

Many environmental factors, such as light,[19,20] fertilization practices, pesticide applications and pathogen attack,[21] can also influence polyphenol concentrations in apple. However, the importance of these environmental factors relative to genetic

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factors and their interactions are little known, yet are important to understand and quantify to design appropriate breeding and fruit sampling strategies. For most studies comparing cultivars, there has been little replication by year and/or site, and where this has occurred, only averages have been displayed, with little comment on the relative stability of genotypic effects. In several Polish studies, phenols were shown to vary from one year to another depending on apple genotype.[22,23]

Many studies comparing cultivars have extracted polyphenols from the fruit as a whole. Peel and flesh each provide a significant contribution to the total amount of consumed polyphenols in a whole apple; however, polyphenol composition in each tissue can be quite different.[23−25] This suggests that polyphenol accumulation in each tissue may be under different genetic control, and the responsiveness of polyphenolic accumulation to environmental factors may also be tissue dependent.

In this work, we determine the stability of different polyphenols across years and characterize its genetic variability in peel and flesh tissue separately in a subset of *M.* × *domestica* and *M. sieversii* grown at one site in New Zealand (NZ). We show that a wide range of concentrations for different polyphenols is present across different apple germplasm and that this genetic variation is stable across years, apart from that of the flavonols.

MATERIALS AND METHODS

Plant Material. Ninety-three apple genotypes (Table 1) planted in 1- or 2-tree plots at the Plant & Food Research (PFR) site in Hawke's Bay, New Zealand (39°39′S, 176°53′E) were assessed in the study. *M.* × *domestica* genotypes were planted in a germplasm repository in 1995−1998 on 'M. 9' and 'MM. 106' rootstock or as elite advanced selections from the PFR breeding program planted in advanced selection blocks, from 1995 to 2001, on 'MM. 106' rootstock. Twenty-eight of the 532 genotypes in the germplasm repository and 47 of the 212 advanced selections were randomly chosen for the study and were compared with five commercial cultivars ('Red Delicious', 'Royal Gala', 'Braeburn', 'Fuji', and 'Sciros'), also planted in the repository. *M. sieversii* seedlings planted in 2000 on their own roots had been originally collected as open-pollinated seed in Kazakhstan in 1995 and 1996[26] and imported into New Zealand in 1997. One seedling was chosen randomly from each of 13 families, which were chosen also at random from the 58 families available in the planting. All blocks were managed according to standard commercial procedures.

Experimental Section. Variability in polyphenol composition among all genotypes was determined in 2003−2005. Sixty-three *M.* × *domestica* genotypes were tested in only 1 year: two in 2003, 32 in 2004, and 29 in 2005 (Table 1). An additional 12 *M.* × *domestica* genotypes were analyzed over all 3 years, and five were analyzed over 2 years. Nine *M. sieversii* genotypes were analyzed over 2 years, with an additional four genotypes assessed in 1 year only. At harvest, nine fruits from each genotype were sampled randomly from the outside of the tree canopy when they were mature, based on a starch pattern index of 3 on a scale of 0 (100% starch = immature) to 6 (0% starch = over mature). Individual fruit weight varied from 150 to 350 g (*M.* × *domestica*) and 55 to 135 g (*M. sieversii*).

Polyphenol Analysis. To measure the concentration of polyphenolics by high-performance liquid chromatography (HPLC), the nine apples were sampled and extracted within 24 h, generally following the methods described in Schieber et al.[27] and McGhie et al.[24] The nine apples from each genotype were randomly assigned to three replicates each containing three fruit. Four plugs (10 mm diameter × 15 mm in length) were cut from the equator of each fruit at perpendicular locations, and the peel was carefully separated from the cortex. The 12 peel discs and 12 cortex plugs of each replicate were each combined to produce three peel and three cortex samples for each genotype. Peel and cortex samples were extracted with 5 and 50 mL of ethanol/water/formic acid (80:20:1, v/v/v), respectively. After homogenization using an IKA UltraTurrax (Global Science, Auckland, New Zealand), all samples were left to extract at 2 °C overnight. Extracts were centrifuged at 3000 rpm for 10 min, and the extracts were stored at −20 °C for a maximum of 4 months until analyzed by reversed-phase HPLC.

The HPLC system was a Waters Alliance 2690 with a Waters 996 photodiode array detector (Waters, Milford, MA). The analytical column used was a Synergi Hydro 4.6 mm × 250 mm, 4 μm (Phenomenex, Auckland, New Zealand) maintained at 35 °C. The injection volume was 5 μL. A gradient elution was performed with solvent A (5% formic acid in water) and solvent B (acetonitrile) at a flow rate of 1.0 mL min^{-1}. The elution was as follows: 0−10 min, 5% B isocratic; 10−30 min, linear gradient from 5 to 30% B; 30−35 min, 30% B isocratic; 35−40 min, linear gradient from 30 to 80% B; 40−45 min, 80% B isocratic; 45−50 min, linear gradient from 80 to 5% B to return to the initial conditions before injecting another sample at 54 min. Spectral data (260−550 nm) were collected for the entire run. Catechin, epicatechin, phloridzin, phloridzin 2-xyloside, and oligomeric procyanidins were quantified using chromatograms extracted at 280 nm; quercetin, quercetin glycosides, and chlorogenic acid at 370 nm; and cyanidin glycosides at 530 nm. Chromatographic data were collected and analyzed using the Waters Millennium Chromatography Manager 4.0. Chemical standards were prepared as individual stock solutions in methanol (100 μg mL^{-1}) and stored at −20 °C. Combined working calibrations solutions (0−50 μg mL^{-1}) were prepared in methanol. Other components were quantified using the standard curve of a related compound. Cyanidin glycosides were quantified as cyanidin 3-*O*-glucoside equivalents and unidentified oligomeric procyanidins as epicatechin equivalents.

Individual polyphenols were grouped into six categories: flavan-3-ols (= catechin + epicatechin); oligomeric procyanidins; flavonols (= quercetin 3-rutinoside, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, quercetin 3-arabino-furanoside, quercetin 3-arabino-pyranoside, and quercetin 3-rhamnoside); chlorogenic acid; dihydrochalcones (= phloridzin + phloridzin 2-xyloside); and anthocyanin [cyanidin 3-*O*-galactoside (Cy3 gal)]. Total polyphenol concentrations, which included identified and unidentified HPLC peaks, were determined by taking the sum of all of the peaks detected between 9 and 32 min and quantified as epicatechin equivalents.

Chemicals and Reagents. Liquid chromatography−mass spectrometry grade acetonitrile was purchased from Fischer Scientific (Auckland, New Zealand), methanol (ChromAR) was from Mallinckrodt Chemicals (Auckland, New Zealand), formic acid was from Merck Chemicals (Auckland, New Zealand), and ethanol (95%) was from LabServ (Auckland, New Zealand). Authentic standards of quercetin 3-rutinoside, quercetin 3-galactoside quercetin 3-glucoside, quercetin 3-rhamnoside, phloridzin, cyanidin 3-*O*-galactoside, and chlorogenic acid were purchased from Extrasynthese (Genay, France). Catechin and epicatechin were purchased from Sigma (Sydney, Australia).

Flesh Astringency and Bitterness. In 2005, two extra fruits were harvested on the same dates as those designated for polyphenol analysis from each of 23 *M.* × *domestica* and nine *M. sieversii* genotypes for a sensory assessment of flesh bitterness and astringency. Two thin wedges of cortical tissue were cut from each of the two apples (blush and opposite sides), the skin was removed, and the wedges were combined and chewed by one "expert" assessor trained in taste evaluation of large numbers of apple fruit. The presence or absence of bitterness and astringency was each recorded separately for each genotype. Solutions of caffeine (0.1%) and alum (0.2%) were used as reference standards for bitterness and astringency, respectively.

Statistical Analysis. *M.* × *domestica* genotypes on clonal rootstocks had been previously selected from seedling populations (based on fruit quality), while those of *M. sieversii* were from unselected material on

Table 1. Introduction Date, Country of Origin, Parents Where Known, and Year(s) of Fruit Polyphenolic Assessment for 93 *Malus* Genotypes[a]

species	genotype	date of selection/ introduction	country of origin	parents	assessment years
M. × *domestica*	'Biesterfelder Reinette'	1905[c]	Germany		2005
	'Boskoop'	1856[c]	Netherlands		2005
	'Braeburn'	1952[d]	New Zealand		2005
	'Cambridge Pippin'	1883[c]	United Kingdom		2005
	'Camoesa de Llobregat'	1600[c]	Spain		2005
	'Dayton'	1975[e]	United States	NJ123249 × PRI1235100	2005
	'Democrat'	1900[f]	United States		2005
	'Devonshire Quarrenden'	1678[f]	United Kingdom		2005
	'Egremont Russet'	1872[f]	United Kingdom		2005
	'Finkenwerder Prinz'	1860[c]	Germany		2005
	'Fuji'	1962[d]	Japan	'Rall's Janet' × 'Red Delicious'	2004, 2005
	'Geheimrat Oldenburg'	1904[c]	Germany	'Minister von Hammerstein' × 'Baumann's Reinette'	2005
	'Gewüürzluiken'	1885[g]	Germany		2005
	'Hetlina'	1800[h]	Czech Republic		2005
	'Holly'	1869[c]	United States		2005
	'Idagold'	1944[c]	United States	'Spitzenburg' × 'Wagener'	2005
	'Kent'	1974[f]	United Kingdom	'Cox's Orange Pippin' × 'Jonathan'	2005
	'Kidd's Orange Red'	1924[c]	New Zealand	'Cox's Orange Pippin' × 'Red Delicious'	2005
	'Laxton's Triumph'	1902[c]	United Kingdom	'King of the Pippins' × 'Cox's Orange Pippin'	2005
	'Liberty'	1974[i]	United States	'Macuon' × PRI 54-12	2005
	'Mayflower'	1850[j]	United States		2005
	'Orlean's Reinette'	1776[f]	France		2005
	'Priscilla'	1967[c]	United States	'Starking Delicious' × PRI 610-2	2005
	'Red Baron'	1926[c]	United States	'Golden Delicious' × 'Red van Buren'	2005
	'Red Delicious'[b]	1880[c]	United States		2003−2005
	'Roter Eiserapfel'	1700[c]	Germany		2005
	'Royal Gala'[b]	1960[d]	New Zealand	'Golden Delicious' × 'Kidd's Orange Red'	2003−2005
	'Salome'	1884[c]	United States		2005
	'Sciros'	1991	New Zealand	'Gala' × 'Splendour'	2003−2005
	'Spartan'	1936[c]	Canada	'McIntosh' × 'Yellow Newtown'	2005
	T009	1996	New Zealand	'Braeburn' × 'Royal Gala'	2004,2005
	T016	2001	New Zealand	('Gala' × 'Splendour') × ('Braeburn' × A180-390)	2003−2005
	T021	2001	New Zealand	'Red Delicious' × ('Gala' × 'Splendour')	2004
	T023	2000	New Zealand	'Golden Delicious' × A746-18	2004
	T025A	1996	New Zealand	'Braeburn' × 'Royal Gala'	2003−2005
	T025B	2001	New Zealand	('Gala' × 'Splendour') × 'Northern Spy'	2004
	T027	2001	New Zealand	'Red Delicious' × ('Gala' × 'Splendour')	2004
	T032	1996	New Zealand	'Braeburn' × 'Royal Gala'	2003−2005
	T037	1996	New Zealand	('Golden Delicious' × 'Red Dougherty') × 'Redfree'	2004
	T040	2002	New Zealand	'Cripp's Pink' × ('Gala' × 'Splendour')	2004
	T046	2000	New Zealand	'Golden Delicious' × A746-18	2004
	T054	1997	New Zealand	'Falstaff' × ('Cox's Orange Pippin' × 'Idared')	2004
	T055	1997	New Zealand	'Falstaff' × ('Cox's Orange Pippin' × 'Idared')	2004
	T056	2001	New Zealand	'Orin' × 'Baujade'	2004
	T058	2001	New Zealand	'Red Delicious' × ('Gala' × 'Splendour')	2004
	T071	2001	New Zealand	'Red Delicious' × A163-42	2004
	T074	1995	New Zealand	'Royal Gala' × ('Golden Delicious' × 'Red Dougherty')	2004
	T081A	1998	New Zealand	'Golden Delicious' × ?	2004
	T081B	2001	New Zealand	'Baujade' × ('Royal Gala' × 'Braeburn')	2004
	T082	2000	New Zealand	'Golden Delicious' × ('Cox's Orange Pippin' × 'Idared')	2004
	T084A	2001	New Zealand	('Gala' × 'Splendour') × ('Braeburn' × A180-390)	2004
	T084B	2001	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2004

Table 1. Continued

species	genotype	date of selection/ introduction	country of origin	parents	assessment years
	T092	1999	New Zealand	'Fuji' × 'Sciros'	2003−2005
	T096	1999	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2003
	T099	1997	New Zealand	'Royal Gala' × 'Braeburn'	2003, 2004
	T105	2000	New Zealand	'Akane' × 'Sciearly'	2003−2005
	T112	2001	New Zealand	('Gala' × 'Splendour') × ('Braeburn' × A180-390)	2004
	T118	2000	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2004
	T135	2000	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2004
	T145	1997	New Zealand	'Royal Gala' × 'Braeburn'	2003−2005
	T152A	1997	New Zealand	'Royal Gala' × 'Braeburn'	2003, 2005
	T152B	1999	New Zealand	'Fuji' × 'Sciros'	2004
	T157	1998	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2004
	T161	2001	New Zealand	'Red Delicious' × A163-42	2004
	T167	2000	New Zealand	'Akane' × 'Sciearly'	2003
	T169	2001	New Zealand	('Gala' × 'Splendour') × A172-2	2004
	T190A	1996	New Zealand	'Braeburn' × 'Royal Gala'	2003−2005
	T190B	2001	New Zealand	'Red Delicious' × A163-42	2004
	T191	2000	New Zealand	'Royal Gala' × ('Gala' × 'Splendour')	2004
	T193	1997	New Zealand	'Braeburn' × 'Royal Gala'	2003−2005
	T207	2001	New Zealand	('Gala' × 'Splendour') × A172-2	2004
	T221	2000	New Zealand	'Golden Delicious' × ('Cox's Orange Pippin' × 'Idared')	2004
	T252	2001	New Zealand	('Gala' × 'Splendour') × A92-23	2004
	T260	2001	New Zealand	'Red Delicious' × 'Priscilla'	2004
	T271	2000	New Zealand	'Akane' × 'Sciearly'	2003−2005
	T272	1996	New Zealand	'Braeburn' × 'Royal Gala'	2004
	T281	1997	New Zealand	'Braeburn' × 'Royal Gala'	2003, 2005
	'Willie Sharp'	1920[k]	New Zealand		2005
	'Worcester Pearmain'	1874[f]	United Kingdom		2005
	'Yellow Bellflower'	1817[c]	United States		2005
M. sieversii	GMAL3596.4.116	1995	Kazakhstan	GMAL3596 open pollinated	2004, 2005
	GMAL3609.2.150	1995	Kazakhstan	GMAL3609 open pollinated	2004, 2005
	GMAL3634.2.30	1995	Kazakhstan	GMAL3634 open pollinated	2004, 2005
	GMAL3677.1.105	1995	Kazakhstan	GMAL3677 open pollinated	2004, 2005
	GMAL3683.7.134	1995	Kazakhstan	GMAL3683 open pollinated	2004, 2005
	GMAL3688.1.114	1995	Kazakhstan	GMAL3688 open pollinated	2004
	GMAL3691.2.183	1996	Kazakhstan	GMAL3691 open pollinated	2004, 2005
	GMAL4026.7.061	1996	Kazakhstan	GMAL4026 open pollinated	2004, 2005
	GMAL4040.7.013	1996	Kazakhstan	GMAL4040 open pollinated	2004, 2005
	GMAL4042.7.044	1996	Kazakhstan	GMAL4043 open pollinated	2004
	GMAL4045.2.001	1996	Kazakhstan	GMAL4045 open pollinated	2004, 2005
	GMAL4263.5.154	1996	Kazakhstan	GMAL4263 open pollinated	2004
	GMAL4302.8.171	1996	Kazakhstan	GMAL4303 open pollinated	2004

[a] Genotypes beginning with "T" indicate advanced selections from Plant & Food Research's apple breeding program. [b] 'Hawke's Red Delicious', sport of 'Delicious' discovered in New Zealand ~1955; 'Royal Gala', sport of 'Gala' discovered in New Zealand ~1970. [c] Ref 38. [d] Ref 39. [e] Ref 40. [f] Ref 41. [g] Ref 42. [h] Frantisek Paprstein, personal communication. [i] Ref 43. [j] Ref 44. [k] Ref 45.

their own roots. Therefore, the two species could not be directly compared, and we focused our attention on variation within each species. A mixed modeling approach was taken to determine the importance of genotype (G) relative to year (Y) and the genotype × year (G × Y) interactive effects. Covariance estimates were calculated for each effect for each species separately, using the restricted maximum likelihood (REML) method, assuming all of these effects to be random. The mixed model used was

$$y_{ijk} = \mu + g_i + y_j + gy_{ij} + e_{ijk} \quad (1)$$

where y_{ijk} is the measurement on the k-th sample of the g-th genotype in the y-th year, μ is the overall mean, g_i is the random effect of the i-th genotype, y_j is the random effect of the j-th year, gy_{ij} is the interactive random effect of the i-th genotype with the j-th year, and e_{ijk} is the residual. We assume $g_i \sim N(0, \sigma_G^2)$, $y_i \sim N(0, \sigma_Y^2)$, $gy_{ij} \sim N(0, \sigma_{GY}^2)$, and $e_{ijk} \sim N(0, \sigma^2)$, where σ^2_G = genotypic variance, σ^2_Y = year variance, σ^2_{GY} is the genotype × year variance, and σ^2 is the residual error. Polyphenol data were log transformed to stabilize the variances before all analyses.

Repeatability estimates (r) for each group were then calculated as follows:[28]

$$r = \frac{\sigma_G^2}{(\sigma_G^2 + \sigma_{GY}^2 + \sigma^2)} \quad (2)$$

Best linear unbiased predictors (BLUPs) of total and individual polyphenol concentrations were estimated for each genotype over years

Table 2. Covariance Parameter Estimates and Genotype Repeatabilities (r) for Flesh and Peel Polyphenol Concentrations in *M.* × *domestica*[a]

tissue	source	total	flavan-3-ols	procyanidin oligomers	dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
flesh	genotype (G)	0.182 ± 0.033	0.70 ± 0.14	0.221 ± 0.045	0.138 ± 0.025	2.58 ± 0.42	3.1 ± 2.3	
	year (Y)	0.007 ± 0.008	ND[b]	0.017 ± 0.020	0.006 ± 0.007	0.037 ± 0.040	1.00 ± 1.2	
	G × Y	0.010 ± 0.005	ND	0.029 ± 0.014	0.010 ± 0.005	0.033 ± 0.010	4.9 ± 1.9	
	residual	0.020 ± 0.002	0.520 ± 0.046	0.059 ± 0.006	0.019 ± 0.001	0.011 ± 0.001	2.19 ± 0.21	
	total variation explained by G (%)	83	57	68	80	97	27	
	r	0.86	0.57	0.72	0.83	0.98	0.31	
peel	genotype (G)	0.062 ± 0.013	0.145 ± 0.028	0.077 ± 0.016	0.162 ± 0.034	14.8 ± 2.6	0.059 ± 0.034	4.91 ± 0.93
	year (Y)	0.008 ± 0.009	0.028 ± 0.036	0.055 ± 0.056	0.094 ± 0.097	0.041 ± 0.120	0.004 ± 0.010	0.026 ± 0.071
	G × Y	0.010 ± 0.004	0.026 ± 0.008	0.016 ± 0.006	0.036 ± 0.011	0.57 ± 0.33	0.085 ± 0.030	0.66 ± 0.23
	residual	0.016 ± 0.001	0.009 ± 0.001	0.018 ± 0.002	0.014 ± 0.001	1.65 ± 0.158	0.056 ± 0.005	0.590 ± 0.056
	total variation explained by G (%)	65	70	46	53	87	29	79
	r	0.70	0.81	0.69	0.76	0.87	0.30	0.90

[a] Data log transformed before analyses. [b] ND, not detected.

Table 3. Covariance Parameter Estimates and Genotype Repeatabilities (r) for Flesh and Peel Polyphenol Concentrations in *M. sieversii*[a]

tissue	source	total	flavan-3-ols	procyanidin oligomers	dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
flesh	genotype (G)	0.26 ± 0.11	0.29 ± 0.12	0.41 ± 0.17	0.26 ± 0.11	0.36 ± 0.16	0.65 ± 3.23	
	year (Y)	ND[b]	0.003 ± 0.006	0.003 ± 0.007	ND	0.001 ± 0.006	2.0 ± 3.4	
	G × Y	0.006 ± 0.004	0.007 ± 0.005	0.009 ± 0.008	0.011 ± 0.010	0.025 ± 0.014	4.7 ± 3.3	
	residual	0.009 ± 0.002	0.009 ± 0.002	0.020 ± 0.004	0.026 ± 0.006	0.009 ± 0.002	0.93 ± 0.20	
	total variation explained by G (%)	95	94	93	88	91	8	
	r	0.95	0.95	0.93	0.88	0.91	0.10	
peel	genotype (G)	0.157 ± 0.067	0.28 ± 0.12	0.27 ± 0.12	0.224 ± 0.097	1.28 ± 0.53	0.56 ± 0.28	11.2 ± 4.7
	year (Y)	0.005 ± 0.009	0.054 ± 0.081	0.059 ± 0.087	0.003 ± 0.006	0.010 ± 0.019	ND	0.027 ± 0.118
	G × Y	0.007 ± 0.005	0.030 ± 0.016	0.022 ± 0.013	0.010 ± 0.008	ND	0.147 ± 0.081	0.11 ± 0.26
	residual	0.011 ± 0.002	0.008 ± 0.002	0.015 ± 0.003	0.015 ± 0.003	0.099 ± 0.019	0.079 ± 0.017	1.13 ± 0.24
	total variation explained by G (%)	87	75	74	89	92	71	90
	r	0.90	0.88	0.88	0.90	0.93	0.71	0.90

[a] Data log transformed before analyses. [b] ND, not detected.

for each species. Pearson correlation coefficients were determined on these data, and principal component analysis (PCA) was then carried out on the correlation matrix to evaluate relationships among individual polyphenols in the apple genotypes. All analyses were carried out using PROC MIXED and PROC PRINCOMP in SAS (SAS 9.2, SAS Institute).

RESULTS AND DISCUSSION

Stability in Polyphenol Composition. Genotype (G) accounted for most of the variation in total polyphenol concentration within each species, with more variation being explained in the flesh (83−95%) than in the peel (65−88%) for each species (Tables 2 and 3). Year or G × Y explained no more than 10% of the total variation in each tissue for both species. The ranking of individual genotypes for total flesh polyphenol concentration showed relatively little change from one year to another. Of the 12 *M.* × *domestica* genotypes that were assessed in all 3 years, eight genotypes changed within-year ranking by no more than two places, and none changed rank by more than four places (Table 4). The rank change for total peel polyphenol concentrations was only slightly greater, with seven genotypes changing rank by two places or fewer and three genotypes changing rank by five or six places.

These among versus within genotype differences can also be quantified by the repeatability estimate.[28] Genotype repeatabilities were higher for total flesh (0.86−0.95) than total peel polyphenols (0.70−0.90) within each species (Tables 2 and 3). More importantly, they were all sufficiently high such that there would be little gain in accuracy by carrying out more than 1 year's measurements to ascertain the total polyphenolic concentrations in peel and flesh for an apple genotype relative to that of another,[29] at least at this particular site.

Table 4. Total Flesh Polyphenol (μg g^{-1} FW), Total Peel Polyphenol (μg cm^{-2}), and Peel Flavonol (μg cm^{-2}) Concentrations for 12 *M.* × *domestica* Genotypes Assessed in Each of 3 Years[a]

		year		
polyphenol	genotype	2003	2004	2005
total flesh	'Red Delicious'	1212 a	953 e	889 e
	'Royal Gala'	871 h	692 j	754 i
	'Sciros'	1141 c	1108 c	898 d
	T016	978 f	779 g	764 h
	T025A	866 i	797 f	767 g
	T032	859 j	729 i	628 k
	T092	692 l	637 k	641 j
	T105	1180 b	1154 b	1015 a
	T145	1068 d	1044 d	994 b
	T190A	760 k	574 l	593 l
	T193	921 g	774 h	801 f
	T271	1033 e	1274 a	908 c
total peel	'Red Delicious'	884 a	800 a	871 a
	'Royal Gala'	611 f	531 g	480 f
	'Sciros'	588 g	693 b	640 b
	T016	628 c	542 f	497 e
	T025A	291 l	237 l	224 l
	T032	494 k	462 j	362 k
	T092	612 e	546 e	467 i
	T105	509 j	450 k	399 j
	T145	619 d	559 d	554 c
	T190A	566 h	523 i	469 h
	T193	688 b	525 h	473 g
	T271	562 i	570 c	503 d
peel flavonols	'Red Delicious'	110 j	94 k	124 e
	'Royal Gala'	133 e	107 j	110 h
	'Sciros'	120 h	196 a	167 b
	T016	127 f	109 i	117 f
	T025A	105 k	57 l	79 l
	T032	160 d	159 e	91 k
	T092	202 b	166 d	139 c
	T105	112 i	110 g	99 j
	T145	170 c	179 c	169 a
	T190A	127 f	127 f	129 d
	T193	223 a	187 b	114 g
	T271	95 l	110 g	106 i

[a] Letters refer to ranking order within each year from highest (a) to lowest (l).

G × environment interactions on total polyphenol concentrations in apple fruit, whether by year or by geographical region suggested in previous investigations,[22,24,30] have not been consistent. Comparing our findings with studies also involving a large number of genotypes, a high G with minimal G × Y interaction for total peel polyphenols was indicated by Nybom et al.[30] in Sweden. However, considerable cultivar rank changes for total peel polyphenols between two consective years was shown in Polish-grown germplasm.[22] In an analysis of 10 commercial cultivars in three regions of New Zealand, some changes in genotype rank for both total peel and flesh polyphenol concentrations occurred among regions.[24] However, our reanalysis of data presented in McGhie et al.[24] still showed that G was substantially higher than the G × region interaction by over 3- (peel) and 4-fold (flesh) with high repeatabilities (Supporting Information, Table 1). These different polyphenolic responses to the environment may be because different genotypes were used in each study and/or the environmental factors that influence tissue polyphenol accumulation varied much more in some studies than others.

All polyphenols are synthesized in the flavonoid pathway, but with each polyphenol group having some specific structural and regulatory genes in the pathway that are unique to them,[31] each group may each respond differently to a set of environmental stimuli. A better understanding of the interactions between genetic and environmental factors on apple polyphenols might therefore be gained from assessing individual rather than the sum of polyphenols within each tissue. In our study, the amount of total variation accounted for by genotype in both species indeed did depend on polyphenol group. In *M.* × *domestica*, genetic variation accounted for nearly all (87−97%) of the total variation found for chlorogenic acid in each tissue, and accordingly, repeatabilities were also very high (Table 2). Flavan-3-ols, procyanidin oligomers, dihydrochalcones, and anthocyanins were intermediate with genetic variation accounting for 46−80% of the total variation found for each group with repeatabilities varing from 0.57 to 0.90.

In contrast, a much smaller amount of total variation in flavonol concentrations (27−29%) was accounted for by genotype. More important was the G × Y interaction explaining 42−44% of the total flavonol variation in each tissues. This was also demonstrated by the large changes in ranking that occurred for some *M.* × *domestica* genotypes but not others when assessed in different years (Table 4). Of the 12 common genotypes assessed in all 3 years, only four genotypes changed rank by fewer than three places, while six genotypes changed ranked by five or six places. T193 and 'Sciros' were ranked first and eighth, respectively, in order of peel flavonol concentration in 2003, second and first, respectively, in 2004, then seventh and second, respectively, in 2005. Repeatability estimates were much lower (r = 0.30, 0.31 in both peel and flesh, respectively) than found for the other polyphenol groups. A substantial increase in accuracy would be gained by repeating flavonol assessments for a genotype over at least 3 years.[29] Genetic variation explained at least 70% of the total variation for each polyphenol group in *M. siervesii*, except for the flesh flavonols where only 8% of the total was explained by genetic variation.

That the environment should so strongly influence flavonol accumulation in apple tissue for some *M.* × *domestica* genotypes and not for others, as compared with having a more consistent effect on the concentrations of other peel and flesh polyphenols, has been previously indicated by cultivar rank changes between years in Poland.[22] Our reanalysis of data extracted from the 10 cultivar × three region study in New Zealand[24] confirmed the instability and low repeatability of peel flavonols as compared with all other individual flesh and peel polyphenols (Supporting Information, Table 1). In contrast, Nybom et al.[30] found reasonably high correlations from one year to another for several quercetin glycosides in the peel across 99 apple cultivars in Sweden, although the quercetin concentrations measured in that study were considerably lower than reported in our work.

Concentrations of flavonols in apple fruit of a *M.* × *domestica* genotype may depend upon the exposure of the fruit to specific light and temperature conditions during fruit development, and

its sensitivity to those conditions. Flavonols in plants are particularly sensitive to the light and temperature environment as compared with other polyphenol compounds. This is not suprising given they have a specific functional role in plants of protection against ultraviolet (UV) radiation,[32] and thermal stresses can inhibit or promote their metabolism.[33] In apple, shading was associated with a large reduction in fruit peel flavonol concentrations across cultivars, but concentrations of phloridzin, catechin, and chlorogenic acid were much less affected.[19,20] Quercetin glycosides in the peel of mature apple fruit accumulated much more in response to UV-B radiation than did procyanidin compounds, with the magnitude of this response being cultivar- and temperature-dependent.[34]

Genetic Variability in Polyphenol Composition. As a high proportion of the genotypes in each species were tested only once and in different years of our study, a mixed model analysis adjusted genotypes for any possible year effects based on the 17 (*M.* × *domestica*) or 9 (*M. sieversii*) genotypes that were tested in more than 1 year. That there was negligible Y effects, and only small G × Y effects for all polyphenols (except flavonols) give confidence in the BLUPs generated for the different genotypes across years. However, genotype BLUPs for flavonols should be treated with caution because of the large G × Y effects. In addition, there is an assumption in this analysis that the Y and G × Y effects generated from the multiyear genotypes apply to those single year-tested genotypes. This may not be the case, and further assessments would be required to verify this assumption.

Total flesh polyphenol concentrations in *M.* × *domestica* genotypes were spread over a 9-fold range, with the PFR selection T055 having the lowest of 273 μg g^{-1} fresh weight (FW) and 'Devonshire Quarrenden' the highest flesh polyphenol concentration at 2326 μg g^{-1} FW (Table 5). Similarly in *M. sieversii*, total flesh polyphenol concentrations were spread over a 6-fold range, with a minimum concentration within the bounds of the *M.* × *domestica* genotypes of 1038 μg g^{-1} FW and a maximum concentration of over 7000 μg g^{-1} FW (Table 6), in broad agreement with an earlier report that compared apple juice samples.[16] Polyphenol concentrations in the peel varied less than in the flesh in both species, spread only over a 3.2 times range in *M.* × *domestica* and a 4-fold range in *M. sieversii.* Again, the minimum concentration for the latter species was within the bounds of *M.* × *domestica*, while the maximum was double that of the highest *M.* × *domestica* genotype. The spread in concentrations within individual polyphenol groups varied from a 2-fold range (peel flavonols in *M.* × *domestica*) to over a 500-fold range (flesh chlorogenic acid in *M.* × *domestica*).

Apart from the flavonols, variations of total and most individual polyphenols in the peel and flesh of dessert apples of *M.* × *domestica* in our study are broadly similar to those previously reported of commercial cultivars.[11,24] Concentrations of approximately 2500 μg g^{-1} FW and 1000 μg cm^{-2} in flesh and peel, respectively, appear to be generally the maximum found for dessert apples across a range of germplasm. These thresholds may exist because fruits with higher concentrations of polyphenols may elicit bitter or astringent tastes; hence, genotypes with such fruit may have been selected against in the past. In cider apples, fruits from bitter cultivars had higher contents of flavan-3-ols and/or dihydrochalcones than nonbitter cultivars.[35] In 2005, the only *M. sieversii* seedling whose flesh did not taste bitter or astringent in our study (GMAL4045.2.001) had a flesh polyphenol concentration of 909 μg g^{-1} FW, wheras all other *M. sieversii* genotypes had concentrations greater than 2845 μg g^{-1} (Supporting Information, Table 2). The relationships between flesh polyphenol concentrations and flesh bitterness or astringency in *M.* × *domestica* were not so clear, and more extensive studies are required to understand and quantify the influence of polyphenol composition on fruit taste in apple.

It is noteworthy, and is further suggestive of a strong environmental influence on flavonol accumulation in apple, that concentrations of peel flavonols in some other reports assessing apple cultivars, including several genotypes in common with the present work[22,23] and germplasm,[30] were substantially lower than those found in the present study.

To gain a global view of genotype differences in polyphenol composition within each species, PCA was performed on the combined data set of total polyphenol plus individual polyphenol groups (seven peel and six flesh), over the 80 *M.* × *domestica* genotypes and separately over the 13 *M. sieversii* genotypes. For *M.* × *domestica*, the first two principal components (PCs) accounted for 51% of the total variance, with PC1 (35%) explaining twice as much variation as PC2 (16%; Figure 1A). PC3 explained another 14% of polyphenol variation, while other PCs each explained ≤10% of total variance (data not shown). Total polyphenols, oligomeric procyanidins, flavan-3-ols, and dihydrochalcones in both tissues were highly positively correlated with PC1 (Figure 1A). In contrast, the flavonols and chlorogenic acid in both tissues and Cy3 gal had relatively low correlations with PC1. Polyphenol groups tended to diverge in the PC2 dimension mainly based on tissue type. Cy3 gal, flavonols, flavan-3-ols, oligomeric procyanidins, and total polyphenols, all in the peel, had positive correlations with PC2. In contrast, all flesh polyphenols (except flavan-3-ols) were negatively correlated with PC2, with flesh dihydrochalcones and chlorogenic acid in both tissues showing stronger correlations than the rest.

For *M. sieversii*, the first two PCs accounted for 69% of the total variance, with PC1 (54%) explaining over three times as much variation as PC2 (15%). Other PCs each explained ≤9% of total variance (data not shown). All of the polyphenols (except flesh flavonols) were highly correlated with PC1 for this species. As in *M.* × *domestica*, total polyphenols, flavan-3-ols, oligomeric procyanidins, and dihydrochalones but also chlorogenic acid in both flesh and peel had strong positive correlations with PC1 (Figure 1B). Cy3 gal and peel flavonols were negatively correlated with PC1. These latter two groups were highly positively correlated with PC2, whereas chlorogenic acid and peel dihydrochalcone had moderate negative correlations with PC2. These results indicate that within the selected *M.* × *domestica* and unselected *M. sieversii* material, there was some commonality in the pattern of polyphenol distribution among genotypes. For both species, most fruit polyphenol variation among genotypes could be accounted for by concentrations of most polyphenols in both flesh and peel in one dimension and, somewhat independently, concentrations of peel Cy3 gal and flavonols and peel and flesh chlorogenic acid in a second dimension.

The pairwise component scores for each of the 80 genotypes in *M.* × *domestica* plotted for the first two PCs showed a good distribution throughout the plot, with no obvious area where genotypes were lacking (Figure 1C). Three genotypes were identified as outliers outside the 95% prediction ellipse. Concentrations of all of polyphenolic groups in both peel and flesh were all low for T046; peel polyphenols were low for T025A, but flesh chlorogenic acid was high, while the old English cultivar 'Egremont Russet' had high concentrations of flesh polyphenols, particularly chlorogenic acid in the peel and flesh and low Cy3 gal

Table 5. Flesh and Peel Polyphenol Composition for 80 *M.* × *domestica* Genotypes[a]

	flesh (μg g^{-1} FW)						peel (μg cm^{-2})						
genotype	total	flavan-3-ols	procyanidin oligomers	chlorogenic dihydrochalcones	chlorogenic acid	flavonols	total	flavan-3-ols	procyanidin oligomers	chlorogenic dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
'Biesterfelder Reinette'	1365	116	360	31.5	335	0.59	663	42	193	48.2	19.0	139	6.7
'Boskoop'	1374	107	508	33.8	249	0.69	611	37	249	24.7	7.0	128	6.8
'Braeburn'	527	81	239	12.1	61	0.52	505	34	179	11.6	0.1	148	12.0
'Cambridge Pippin'	751	61	312	12.9	121	0.04	439	23	142	9.4	7.4	142	1.3
'Camoesa de Llobregat'	663	89	254	15.2	100	0.41	373	34	152	8.9	1.2	110	1.3
'Dayton'	1719	186	515	17.9	373	0.75	778	59	247	10.2	26.4	165	12.2
'Democrat'	1032	129	548	9.5	52	0.67	799	61	328	10.2	ND[b]	150	29.5
'Devonshire Quarrenden'	2326	116	723	43.3	567	0.66	635	38	197	21.2	24.2	149	13.2
'Egremont Russet'	1545	118	514	29.7	337	0.79	667	42	245	30.3	43.5	115	ND
'Finkenwerder Prinz'	935	77	359	13.1	167	0.04	362	20	118	9.2	7.7	121	2.9
'Fuji'	669	90	204	14.6	125	0.13	521	35	162	8.5	7.7	169	12.8
'Geheimrat Oldenburg'	1096	93	470	11.8	153	0.72	595	47	244	8.8	7.0	134	11.4
'Gewürzluiken'	1880	135	822	28.8	247	0.82	550	42	234	15.7	0.4	114	1.2
'Hetlina'	1692	87	512	30.3	439	0.72	688	51	267	14.2	22.3	135	17.2
'Holly'	789	120	333	18.1	90	0.04	705	59	242	25.3	ND	146	24.4
'Idagold'	876	117	472	28.3	13	0.08	406	29	180	15.1	ND	103	ND
'Kent'	773	95	424	8.4	36	0.72	642	61	264	8.1	0.1	135	17.7
'Kidd's Orange Red'	572	105	291	15.2	26	0.44	620	40	240	15.6	ND	142	15.8
'Laxton's Triumph'	967	108	535	14.5	51	0.06	463	34	183	10.2	ND	129	5.5
'Liberty'	873	72	202	15.5	215	0.50	507	23	150	8.9	6.4	124	30.3
'Mayflower'	959	88	272	7.0	226	0.49	548	39	224	6.2	10.0	134	1.7
'Orlean's Reinette'	1103	85	384	18.4	218	0.77	508	32	178	15.9	25.8	122	3.2
'Priscilla'	1028	93	392	31.7	160	0.70	582	43	165	20.6	3.4	150	15.7
'Red Baron'	1180	122	373	16.7	245	0.82	431	54	154	10.1	9.3	118	5.4
'Red Delicious'	1006	147	458	27.9	95	0.29	834	77	347	26.1	1.7	115	34.6
'Roter Eiserapfel'	1221	71	401	15.1	312	0.69	697	37	231	11.0	36.7	160	7.1
'Royal Gala'	769	91	331	14.1	123	0.54	537	45	202	11.0	6.5	120	22.4
'Salome'	988	52	271	21.3	259	0.82	558	22	168	11.3	18.8	156	9.7
'Sciros'	1037	117	373	14.4	188	1.16	636	54	232	10.0	16.8	149	18.7
'Spartan'	648	74	211	10.7	112	0.45	489	27	159	10.0	5.9	128	24.7
T009	667	61	230	13.7	142	1.19	399	29	129	11.4	6.5	119	9.4
T016	836	170	307	17.6	101	0.48	551	55	180	20.8	2.8	121	17.4
T021	418	1	67	13.5	155	0.07	407	21	153	15.1	8.8	127	7.5
T023	1393	176	599	17.0	195	0.99	381	34	190	11.7	5.8	94	0.2
T025A	809	73	234	17.5	188	0.86	261	19	88	6.8	8.5	92	0.1

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Table 5. Continued

	flesh (μg g^{-1} FW)						peel (μg cm^{-2})						
genotype	total	flavan-3-ols	procyanidin oligomers	chlorogenic dihydrochalcones	chlorogenic acid	flavonols	total	flavan-3-ols	procyanidin oligomers	chlorogenic dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
T025B	733	121	429	14.4	ND	0.07	409	42	164	12.0	ND	115	3.5
T027	1111	138	436	17.7	192	0.93	421	40	192	15.9	8.5	115	ND
T032	729	98	432	16.9	38	0.29	441	31	175	13.5	ND	131	0.5
T037	589	144	274	10.0	11	1.22	460	39	176	11.3	6.9	115	7.1
T040	569	136	236	11.3	25	1.03	441	40	165	8.9	ND	112	18.2
T046	279	34	174	14.6	11	0.34	282	15	106	9.1	ND	117	0.3
T054	961	100	347	14.5	206	14.5	654	36	196	11.1	14.9	170	25.9
T055	273	2	134	15.9	32	15.9	471	24	151	10.9	ND	124	27.8
T056	1055	137	468	12.8	137	1.09	643	41	260	12.1	9.2	172	0.2
T058	989	132	311	14.8	241	0.07	519	47	223	15.8	15.2	110	13.4
T071	995	162	487	16.9	44	0.98	446	55	176	11.3	ND	99	11.6
T074	337	25	124	10.4	75	0.07	386	15	126	9.7	5.2	120	22.5
T081A	923	139	298	18.3	172	18.3	540	38	213	13.3	14.5	146	0.5
T081B	682	117	222	10.2	123	0.91	569	36	188	7.0	13.8	176	2.2
T082	744	107	331	12.8	86	0.96	340	37	162	7.7	3.6	88	ND
T084A	481	119	179	10.7	51	1.24	468	50	162	10.2	0.8	116	18.0
T084B	956	140	544	14.9	41	0.16	511	46	213	9.0	ND	129	11.4
T092	653	84	209	12.9	120	1.19	537	47	214	9.7	6.7	154	7.1
T096	585	102	294	10.4	33	0.86	601	52	224	9.7	ND	153	19.7
T099	444	70	271	14.2	16	0.07	363	20	121	10.5	ND	124	7.9
T105	1120	118	499	19.4	163	1.55	454	32	177	15.9	8.9	113	6.0
T112	938	171	393	11.9	82	0.07	487	56	203	13.3	1.6	119	8.0
T118	364	46	222	18.5	17	0.82	423	17	155	15.7	ND	128	12.8
T135	554	118	298	11.9	9	0.07	503	53	208	7.3	ND	123	13.4
T145	920	170	341	14.0	129	2.02	572	62	197	11.9	2.0	157	10.4
T152A	1050	93	361	13.3	215	0.79	445	39	164	12.9	13.8	98	ND
T152B	890	114	261	19.9	197	0.07	608	48	232	13.2	14.5	154	8.3
T157	1341	165	573	19.8	207	0.07	536	52	241	12.7	15.3	119	8.3
T161	1462	221	815	27.5	47	1.25	741	68	323	26.6	0.3	138	6.7
T167	697	116	343	12.3	41	0.05	462	45	189	12.1	0.1	113	12.6
T169	1038	142	582	17.9	55	0.15	520	40	196	12.9	4.5	138	9.6
T190A	634	103	369	15.5	20	0.11	517	48	214	9.7	ND	128	12.0
T190B	1046	121	562	30.0	63	0.92	623	54	230	16.8	1.4	134	22.0
T191	735	164	392	11.1	9	0.07	414	50	174	7.6	ND	98	11.8
T193	830	103	335	15.7	138	1.35	553	50	180	9.9	10.8	155	12.0
T207	1342	188	534	26.7	203	1.02	695	64	306	12.4	13.2	136	13.7
T221	1219	127	504	18.0	208	1.07	480	30	178	16.4	13.4	122	10.4

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Table 5. Continued

	flesh (μg g^{-1} FW)						peel (μg cm^{-2})					
genotype	total	flavan-3-ols	procyanidin oligomers	dihydrochalcones	chlorogenic acid	flavonols	total	flavan-3-ols	procyanidin oligomers	dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
T252	722	126	382	14.4	24	0.15	634	50	208	9.2	ND	116	48.4
T260	1080	165	550	26.1	55	0.38	656	52	238	16.2	ND	143	25.9
T271	1067	156	574	15.4	63	0.59	543	52	246	12.1	ND	111	8.9
T272	675	109	309	11.9	268	0.39	675	40	245	9.1	19.2	167	14.3
T281	773	128	262	13.6	128	0.76	568	51	211	10.3	2.5	158	6.0
'Willie Sharp'	1312	120	567	20.8	190	0.52	472	44	176	16.9	10.3	123	0.6
'Worcester Pearmain'	1489	140	480	14.4	356	0.50	480	43	165	7.6	19.8	125	11.1
'Yellow Bellflower'	1225	71	420	25.9	277	0.71	527	40	213	13.4	18.3	123	0.8
minimum	273	1	37	7	ND	ND	260	14	87	6	ND	87	ND
maximum	2326	221	822	43	567	2.0	834	77	347	48	44	176	48
average	943	112	383	17	141	0.6	530	31	199	13.2	7.7	130	11
coefficient of variation	39	36	38	40	79	69	22	42	25	47	116	15	84
proportion of total		12	41	2	15	0.1		6	38	2	1	25	2

[a] Values are back-transformed (e^x) BLUPs that have been estimated on log transformed data. [b] ND, not detected.

concentrations. The same plot for *M. sieversii* revealed one extreme seedling (GMAL4045.2.001) having a high negative PC1 relatively to the other seedlings, but all genotypes were within the 95% prediction ellipse (Figure 1D).

Sources of Variation within *M.* × *domestica*. We then explored factors that might influence these variations within *M.* × *domestica*. Date of genotype introduction varied over 400 years, from 1600 to 2001 (Table 1). The more advanced the introduction date (and therefore more modern the genotype), the lower ($P < 0.04$) were the first PCA component ($r = -0.23$), the concentrations of total polyphenols in the flesh ($r = -0.35$), chlorogenic acid in flesh ($r = -0.30$) and peel ($r = -0.24$), and flesh dihydrochalcones ($r = -0.30$), and higher the second PCA component ($P = 0.05$, $r = 0.22$). Concentrations of other polyphenols were not associated ($P > 0.10$) with date of genotype origin. However, when New Zealand-originated genotypes were removed from this data set, all correlations became nonsignificant ($P > 0.09$; $n = 28$), despite year of origin ranging from 1600 to 1975.

All genotypes tested originating after 1990 were developed in New Zealand, whereas all but two cultivars ('Willie Sharp' and 'Kidd's Orange Red') originating before 1950 were developed from countries outside New Zealand (Table 1). Examination of the influence of country of origin further showed that New Zealand genotypes ($n = 52$) had a lower first PCA component ($P = 0.01$) than genotypes originating outside New Zealand, but the second PCA component average was not different ($P = 0.41$; Figure 1C). Total flesh ($P < 0.001$) and peel ($P = 0.005$) polyphenol concentrations were lower for genotypes originating in New Zealand (768 μg g^{-1} FW for flesh and 487 μg cm^{-2} for peel) than for those originating outside New Zealand (1038 μg g^{-1} FW for flesh and 562 μg cm^{-2} for peel). New Zealand cultivars and selections also had lower flesh ($P = 0.004$) and peel ($P = 0.03$) chlorogenic acid than those from outside New Zealand. However, no other differences were found for the other polyphenols (data not shown). Concentrations of total or individual flesh or peel polyphenols in cultivars originating from the United States ($n = 12$), United Kingdom ($n = 5$), and Germany ($n = 6$) were similar ($P > 0.13$).

In a survey of 67 apple cultivars grown in Poland, most of which had been bred or selected in Europe, Wojdylo et al.[36] concluded that new cultivars had similar or higher concentrations of a range of individual polyphenols than old cultivars. In contrast, in Germany, Keller et al.[37] observed that dessert apple cultivars released after 1950 had generally lower polyphenol concentrations as compared with older cultivars used mainly for juice and cider. Much of the New Zealand-bred material in our study is derived from two relatively low flesh polyphenolic cultivars of 'Gala' (or its red mutant 'Royal Gala') and 'Braeburn' (Table 1) and has been selected for high eating quality and against bitter or astringent tastes. In contrast, old cultivars imported into New Zealand were placed in the cultivar collection irrespective of fruit taste.

Despite these differences in average polyphenolic profiles between the two groups, heterogeneity within the New Zealand as compared with the non-New Zealand group was similar (Figure 1C). Several New Zealand genotypes with very high positive PC1 scores were evident. The high concentrations of flesh polyphenols, generally better eating quality and storing ability of these genotypes as compared with older imported cultivars and a reasonable genetic diversity (Table 1), make this

Table 6. Flesh and Peel Polyphenol Composition for 13 *M. sieversii* Genotypes[a]

	flesh (μg g^{-1} FW)						peel (μg cm^{-2})						
genotype	total	flavanols	procyanidins	dihydrochalcones	chlorogenic acid	flavonols	total	flavanols	procyanidins	dihydrochalcones	chlorogenic acid	flavonols	cyanidin 3-galactoside
GMAL3596.4.116	6682	984	3683	127	382	2.75	1049	112	488	39.5	24.9	59	ND[b]
GMAL3609.2.150	7069	1203	2345	166	866	2.15	887	146	350	37.5	35.5	15	ND
GMAL3634.2.030	4352	709	2173	112	227	2.29	623	69	290	27.4	5.2	41	ND
GMAL3677.1.105	4114	770	1843	179	208	0.98	923	119	353	55.3	8.3	65	ND
GMAL3683.7.134	4458	853	1646	153	302	1.66	740	91	327	27.5	3.8	81	1.0
GMAL3688.1.114	5854	1354	2395	58	267	2.24	1232	171	527	23.6	10.8	93	ND
GMAL3691.2.183	3072	308	1618	85	197	1.94	620	47	277	34.0	2.6	39	1.7
GMAL4026.7.061	5250	624	2431	168	364	2.31	941	94	371	47.0	9.3	135	2.8
GMAL4040.7.013	3937	412	1315	153	575	2.02	606	57	227	51.9	31.8	22	ND
GMAL4042.7.044	5735	1326	2274	187	300	2.19	1320	201	480	88.1	6.2	23	0.2
GMAL4045.2.001	1038	289	270	50	90	2.16	325	37	75	16.1	1.5	108	1.7
GMAL4263.5.154	5657	624	2386	223	635	2.37	1174	98	457	69.2	65.1	94	0.3
GMAL4302.8.171	2864	373	1132	67	441	0.89	609	60	242	47.8	22.1	54	ND
minimum	1037	289	270	50	90	0.9	325	37	75	16	1	14	ND
maximum	7069	1354	3684	224	866	2.7	1319	200	526	88	65	135	2.8
average	4621	756	1963	136	373	2.0	850	100	344	43	17.5	64	0.6
coefficient of variation	36	49	42	42	57	26	34	49	37	45	104	58	156
proportion of total (%)		10	42	3	8	0.04		12	40	5	2	8	0.07

[a] Values are back-transformed (e^x) BLUPs that have been estimated on log transformed data. [b] ND, not detected.

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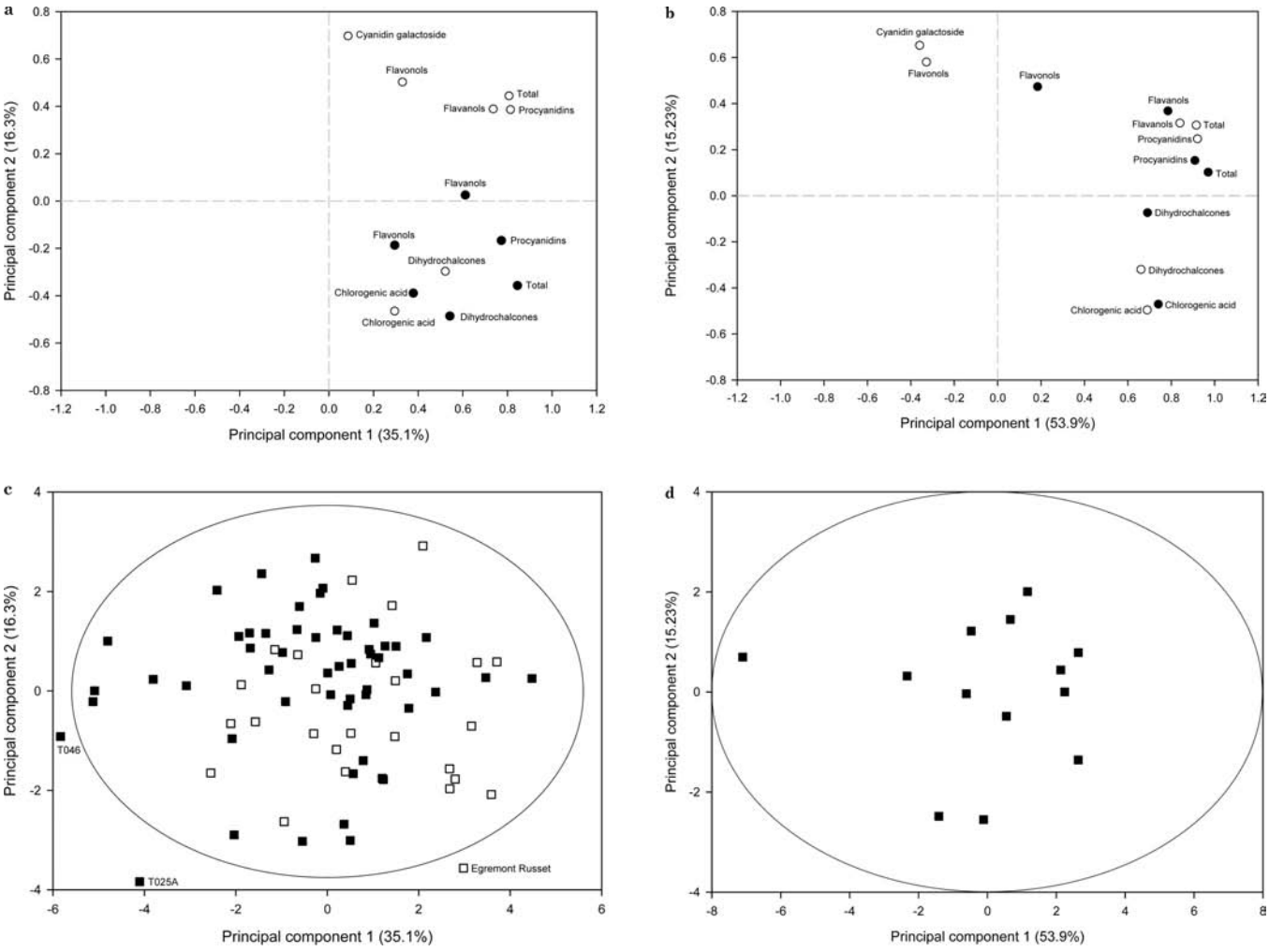

Figure 1. PCA plots between PCs 1 and 2 for apple fruit polyphenol concentrations indicating polyphenol group (a and b) and genotypes (c and d) for *M.* × *domestica* (a and c) and *M. sieversii* (b and d). For panels c and d, ellipse = 95% prediction ellipse. For panels a and b, cortical flesh tissue = solid circle and peel tissue = open circle. For panel c, genotypes of New Zealand origin = solid square, and genotypes of non-New Zealand origin = open square.

germplasm well-suited to improving the concentrations of polyphenols in future breeding activities.

Conclusions. This study clearly confirms considerable variation in polyphenol composition in the fruit peel and flesh among current commercial cultivars of *M.* × *domestica* and extends this to some of the elite selections within the PFR breeding program and old cultivars imported into New Zealand from other countries, as well as some large-fruited *M. sieversii* germplasm. While the New Zealand-bred material had lower average flesh polyphenol concentrations than non-New Zealand germplasm, sufficient variability for breeding exists in the concentrations of total as well as individual polyphenol groups within the New Zealand-bred material. Apart from the flavonols in *M.* × *domestica*, this variation is relatively stable from year to year, at least at the Hawke's Bay site in New Zealand, where only 1 year of assessment would be required to ascertain a genotype's fruit polyphenol concentration.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Table of covariance parameter estimates and genotype repeatabilities for flesh and peel polyphenol concentrations across 10 apple cultivars assessed in three regions of New Zealand (data extracted from ref 24). Table of presence or absence of bitter or astringent tastes and total polyphenol concentration in the cortical flesh of fruit from 25 *M.* × *domestica* and nine *M. sieversii* genotypes (2005). This material is available free of charge via the Internet at http://pubs.acs.org.

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