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Fruit size QTL analysis of an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry

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Abstract Maximizing fruit size is critical for profitable sweet cherry (*Prunus avium* L.) production. Yet, despite its importance, little is known about the genetic control of fruit size. The objective of this study was to identify quantitative trait loci (QTLs) for fruit size and two essential components of fruit size, mesocarp cell number and size. This study utilized a double pseudo-testcross population derived from reciprocal crosses between a sweet cherry cultivar with ~8 g fruit, "Emperor Francis" (EF), and a wild forest sweet cherry selection with ~2 g fruit, "New York 54" (NY). A total of 190 F_1 progeny previously utilized for the construction of the linkage maps were evaluated in 2006 and 2007 for fruit weight, length, and diameter; mesocarp cell number and length; and pit length and diameter. In 2008, a subset of this population was again evaluated for fruit weight. Correlation analysis revealed that the three fruit size traits were highly correlated with each other, and mesocarp cell number, not cell length, was correlated with fruit size. Three QTLs were identified for each fruit size trait, and one QTL was identified for mesocarp cell number. Fruit size QTLs were found on linkage group 2 on the EF map (EF 2) and linkage groups 2 and 6 on the NY map (NY 2 and NY 6). On EF 2, the cell number QTL clustered with the fruit size QTL, suggesting that the underlying basis of the fruit size increase associated with this QTL was an increase in mesocarp cell number. On NY 6, pit length and diameter QTLs clustered with those for fruit size, suggesting that the underlying morphological basis of this fruit size QTL is the difference in pit size.

Keywords *Prunus avium* · Fruit size · Cell number · QTL mapping · Pseudo-testcross

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Introduction

Profitable sweet cherry production requires cultivars that achieve acceptable fruit size with large fruit size receiving a premium price in the market place (Whiting et al. 2006). For example, in many US markets, fruit size is the primary criterion by which fresh cherries are graded for sale with fruit averaging over 29 mm in diameter worth nearly twice as much as fruit less than 24 mm in diameter (Whiting et al. 2006). Despite the importance of large fruit size as a breeding goal, maximizing fruit size in sweet cherry breeding programs is difficult because the genetic control is not well understood. As a result, breeders are reluctant to use small-fruited germplasm to improve traits such as disease resistance as many years are required to regain the necessary fruit size and quality.

Sweet cherry fruit is composed of a thin protective exocarp, a fleshy mesocarp, and a stony endocarp (pit) containing the seed. The fleshy mesocarp can further be defined by its cellular components, cell number, and cell

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size. The relationship between fruit size and cell number and cell size has been investigated in apple (*Malus* × *domestica*; Harada et al. 2005), peach (*Prunus persica* L.; Scorza et al. 1991; Yamaguchi et al. 2002), pear (*Pyrus pyrifolia* L.; Zhang et al. 2006), and sweet cherry (Yamaguchi et al. 2004, Olmstead et al. 2007). In sweet cherry, Yamaguchi et al. (2004) concluded that cultivar differences in fruit size were due to both mesocarp cell number and cell size. Similarly, Olmstead et al. (2007) reported that varietal differences in fruit size were primarily due to differences in mesocarp cell number, not mesocarp cell size, although mesocarp cell size did differ among varieties. Most notably, however, unlike mesocarp cell size, mesocarp cell number was not found to be significantly influenced by environmental factors such as crop load or location, suggesting that mesocarp cell number would be a more useful target trait for genetic analysis (Olmstead et al. 2007).

Fruit size in sweet cherry has been considered a quantitative trait (Lamb 1953; Fogle 1961; Mathews, 1973) and therefore difficult to dissect using classical genetic methods. The advent of molecular markers has greatly facilitated genetic studies of quantitative traits by conducting quantitative trait locus (QTL) analysis to identify the genomic regions that control the trait of interest and quantify their genetic effects. The linked molecular markers identified in QTL analysis could then potentially be used in breeding practice via marker-assisted selection (MAS), where the selection is based on DNA sequence rather than the phenotype. MAS prior to field planting would greatly improve the efficiency of breeding for fruit size in cherry due to the large plant size and 3- to 5-year juvenility period.

In crop plants, QTLs for fleshy fruit size have been most thoroughly studied in tomato (*Lycopersicon esculentum* L.), resulting in the identification of 28 fruit size QTLs (see review by Grandillo et al. 1999; Prudent et al. 2009). In contrast, in rosaceous crops, the genetic dissection of fruit size has just begun and is limited to a small number of populations for which limited map coverage was available. These investigations include sour cherry (*Prunus cerasus* L.; Wang et al. 2000), peach (Yamamoto et al. 2002; Dirlewanger et al. 2002; Etienne et al. 2002; Quilot et al. 2004), and apple (King et al. 2001; Liebhard et al. 2003; Kenis et al. 2008). In sweet cherry, no QTL studies have previously been reported as comprehensive genetic maps have only recently been constructed (Olmstead et al. 2008; Clarke et al. 2009).

Our objective was to identify QTLs for fruit size and the components of fruit size (mesocarp cell size and cell number) in sweet cherry. Our approach was to utilize a cross between a large-fruited domesticated sweet cherry and a small-fruited wild forest "mazzard" sweet cherry to identify the genetic changes in fruit size that accompanied domestication. Identifying genetic changes associated with domestication has proven to be a valuable strategy to elucidate the underlying genetic changes associated with trait improvement (see review by Paterson 2002).

Materials and methods

Plant materials

The population used in this study consisted of 190 F_1 progeny individuals (~equal numbers from reciprocal crosses) that resulted from the cross between a landrace variety "Emperor Francis" (EF) and a wild "mazzard" sweet cherry "New York 54" (NY). EF is a large (~8 g) cultivated cherry, while NY is a wild forest cherry of northern European origin, termed "mazzard," that has very small fruits (~2 g). The crosses were carried out in the spring of 2001 (Olmstead et al. 2008), and seedling progenies were planted in the field in 2002 at Michigan State University's Clarksville Horticultural Experiment Station in Clarksville, MI, USA. The seedling trees together with their parents were planted at a spacing of 1.5 m within a row and 6.1 m between rows. In 2002, a subset of this population (94 progeny individuals) was grafted onto the Giesla® 6 (semidwarfing precocious) rootstock in a plot located at the same research station to provide a clonal replicate. Due to land and rootstock number limitations, the entire 190 progeny population was not replicated. The soil type for both orchards is Dryden sandy loam, and trees were provided supplemental irrigation by drip lines when needed May through July.

Crop load and harvesting

The majority of the progeny individuals flowered in 2006 when the trees were 4 years old. Large numbers of flowers were produced by the seedlings for the 3 years of this study, 2006 to 2008. However, in all 3 years, significant pistil freeze damage resulted in a reduction in flower numbers on the trees and even total flower loss for some seedlings. As a result, in 2006 and 2007, only 150 and 172 progeny individuals, respectively (78% and 89% of the total mapping population), had fruits. The most severe freeze occurred in spring 2008, which resulted in near 100% pistil death in the original own-rooted mapping population block. Fortunately, the plot with the grafted progeny subset did not experience significant pistil death, presumably due to better cold air drainage in this location. Therefore, the fruit data for 2008 was collected from the grafted subset (94 progeny individuals).

Due to raccoon and bird pressure, the orchards were surrounded by an electric fence to control raccoons, and

netting was used to control bird damage. In 2006 and 2007, regions of each tree containing fruits were netted beginning mid-June. In 2008, the entire grafted plot was enclosed in netting, which completely excluded bird predation. Since the exact harvest maturity date for each progeny individual was unknown, fruits were harvested multiple times from each tree at intervals of twice a week. These samples were pooled so that the largest fruit weight could be determined. In general, a total of ten to 25 fruits were harvested from each progeny individual with low values representing those trees for which insufficient fruits were available. The ranges of the harvest growing degree-day units (base 4.4°C) were 682–940, 715–1,025, and 760–1,200 for 2006, 2007, and 2008, respectively.

Trait measurements

In 2006 and 2007, three fruit size traits (fruit weight, length, and diameter), two cell traits (flesh mesocarp radial cell number and length), and two pit traits (pit diameter and length) were measured. Fruit weight was again evaluated in 2008. Fruit length and diameter were measured for each fruit at its longest and widest points using a digital caliper. The heaviest fruit among the samples was chosen to represent the genetic potential of each progeny individual, and its value was used in the analysis. Pits were removed from the largest fruits, and pit length and diameter were measured at its longest and widest points, respectively, using a digital caliper. Then, mesocarp length (ML) and diameter (MD) were calculated as ML=fruit length−pit length and MD=fruit diameter−pit diameter. In 2006 and 2007, mesocarp cell number and length were measured on the heaviest fruit sample according to Olmstead et al. (2007). Briefly, the fruit were cut from the skin to the endocarp wall along the diameter. After fixing and staining the tissue, a compound microscope was used to analyze the sample. Cell number was counted as the number of cells on the radial section, and the length of the radial section was measured. Cell length was calculated by dividing the radial section length by the number of cells. For parents EF and NY, mesocarp cell number and cell size were measured in 2006 and 2007 on five and one fruit per cultivar, respectively.

Statistical analysis for fruit size and cell measurements

The population mean, range, standard deviation, and the skewness of the distributions were calculated using the UNIVARIATE procedure of SAS (1999). Analysis of variance was performed using the data in a single year as one replication. Broad sense heritability was estimated with the formula $H^2 = \sigma_g^2/\left(\sigma_g^2 + \sigma_e^2/y\right)$, where $\sigma_g{}^2$ is the genetic variance of progeny lines, $\sigma_e{}^2$ is the error variance, and y is the number of years. The difference in fruit or cell measurements between two parents was compared using a t test ($P<0.05$) if multiple sample data was available for each parent. Pearson correlations between traits were calculated using CORR procedure of SAS (1999).

Linkage map and QTL analysis

The mapping population used in this study was a double pseudo-testcross population with heterozygous parents. In such a population, markers heterozygous in one parent and homozygous in the other parent will segregate as a test cross with a segregation ratio of 1:1, while dominant markers heterozygous in both parents will segregate as a F_2 cross with a segregation ratio of 3:1. In the previous study (Olmstead et al. 2008), a total of 197 markers, including 102 simple sequence repeat (SSR) markers, 61 amplified fragment-length polymorphism markers, 27 gene-derived markers, and seven sequence-related amplified polymorphism markers, were used to construct linkage maps with this mapping population. The previous study (Olmstead et al. 2008) generated eight linkage groups for each parent. In our QTL analysis, two SSRs on EF 2 (UCD-CH12 and pchgms1) were not included as their positions on the map were poorly supported. In addition, Ma007a that mapped on EF 2 and NY 2 was not included in the QTL analysis as genetic tests in other germplasm revealed the presence of null alleles, therefore complicating future utility of this marker (data not presented). Finally, the following three SSRs on NY 6 (CPSCT029, BPPCT009, and CPPCT023) were not included in the QTL analysis as an allele survey of these SSRs in a germplasm set called into question the robustness of these markers as we were unable to match allele genotypes with known pedigree relationships (data not presented). The deletion of these problematic markers resulted in only minor alterations in the cM distances between the remaining markers. The EF and NY maps in this QTL study spanned 676.0 and 561.7 cM with the average intervals of 4.9 and 6.5 cM, respectively.

QTL detection was carried out for the parental maps using the MapQTL 5.0 software package (Van Ooijen 2004). Kruskal–Wallis nonparametric test (KW test), interval mapping (IM), and multiple QTL mapping (MQM) were performed for each trait. In MQM, the marker cofactors were selected by the program and were used to control the genetic background for a better location of the QTLs. Only the results derived from MQM are presented. The limit of detection (LOD) thresholds were estimated with a 1,000-permutation test. The QTLs with LOD values higher than the genome-wide threshold at $P<0.05$ were considered significant. However, those QTLs with a LOD score greater than 3 and smaller than the threshold were also reported. The linkage maps and QTL positions were drawn using MapChart (Voorrips 2002).

Results

Phenotypic analysis

The parents of the mapping population, EF and NY, differed significantly ($P<0.05$) for fruit weight, length, and diameter (Table 1). For example, the mean fruit weight for EF ranged from 8.8 to 10.4 g compared to 1.6 to 2.4 g for NY. In 2006, EF had significantly ($P<0.05$) more mesocarp cells and longer cells than NY. In 2007, cell number and cell length were only measured for one fruit from each parent; therefore, the values could not be compared statistically. However, the parental values for mesocarp cell number in 2007 were consistent with values in 2006, while cell length showed less of a separation. The significant differences in fruit size and mesocarp cell length and number are in agreement with previous fruit measurements from EF and NY taken in 2003 and 2004 (Olmstead et al. 2007).

For each fruit size and cell trait, the genetic variation among the progeny individuals was highly significant ($P<0.0001$). As the population distributions for each trait were similar between years, only year 2006 data were graphically presented (Fig. 1). All the fruit size and cell measurements showed continuous distributions, the characteristic of a quantitative trait. Fruit weight, length, and diameter also fit normal distributions; however, the means of these distributions were well below the midparent values. To determine if these small fruit size values for the progeny individuals could be due to the freeze damage limiting the number of available fruit and/or our concern to harvest prior to bird or raccoon predation, we conducted a third year of evaluation for fruit weight. The fruit evaluated in 2008 were harvested from trees that did not sustain any significant freeze damage and were completely enclosed by netting and thus protected from bird or raccoon predation. The fruit weights between years were highly correlated ($P<0.0001$), with coefficients of 0.65 between 2006 and 2007, 0.66 between 2006 and 2008, and 0.62 between 2007 and 2008. However, fruit size values from 2008 were higher than those recorded in the previous 2 years (Table 1), suggesting that the small fruit sizes in 2006 and 2007 might in part have resulted from our inability to sample fully mature fruit due to the bird and raccoon predation. Despite the increase in overall fruit size of the progeny, the distribution of 2008 values was still shifted towards the small-fruited parent, and no progeny had a fruit weight approaching the size of the large-fruited parent. In contrast to the whole fruit measurement, transgressive segregation was observed for increases in mesocarp cell number and cell length relative to the large-fruited parent (Fig. 1).

The broad sense heritabilities for the fruit size and cell traits ranged from 0.67 to 0.94 (Table 1). The heritability for cell number was the highest (0.94), indicating cell number was consistent over years and controlled mostly by genetic factors. Similarly, Olmstead et al. (2007) reported that mesocarp cell numbers in five sweet cherry cultivars, including EF and NY, did not vary significantly across locations and years and even between differed crop loads. Fruit weight had a higher heritability than either of the linear measurements of fruit size (e.g., fruit diameter or length). The fruit weight heritabilities calculated from maximum fruit weight (Table 1) were slightly higher than the heritabilities from mean fruit weight (0.73 for 2006 and

Table 1 Fruit size and mesocarp cell measurements for "Emperor Francis,", "New York 54," and their F_1 progenies investigated at Michigan State University's Clarksville Horticultural Experiment Station in Clarksville, MI in 2006, 2007, and 2008

Trait	Year	Parents[a]		F_1 population			
		EF	NY	Mean	Range	SD	H^{2b}
Fruit weight (g)	2006	10.4 a[c]	1.6 b	3.4	1.6–5.3	0.8	0.76
	2007	8.8 a	2.0 b	3.7	1.8–5.5	0.7	
	2008	9.9 a	2.4 b	4.8	3.1–7.5	1.0	
Fruit length (mm)	2006	23.9 a	12.3 b	15.9	12.6–19.1	1.3	0.68
	2007	21.7 a	13.4 b	15.9	13.1–18.3	1.1	
Fruit diameter (mm)	2006	27.9 a	13.9 b	17.7	13.4–21.2	1.7	0.69
	2007	25.1 a	14.9 b	17.9	14.3–20.9	1.4	
Cell number	2006	42.6 a	22.8 b	32.4	20.0–51.0	6.5	0.94
	2007	45.0	23.0	32.7	21.0–53.0	5.7	
Cell length (mm)	2006	0.19 a	0.15 b	0.18	0.13–0.26	0.03	0.67
	2007	0.22	0.20	0.19	0.06–0.29	0.03	

[a] The means were calculated from samples of five fruit except for mesocarp cell number and cell length in 2007

[b] Broad sense heritability. For comparison, the fruit weight heritability listed in the table was estimated using 2006 and 2007 data. The fruit weight heritability is 0.82 if based on 3 years' data

[c] Means followed by different letters within the same row are significantly different at $P<0.05$

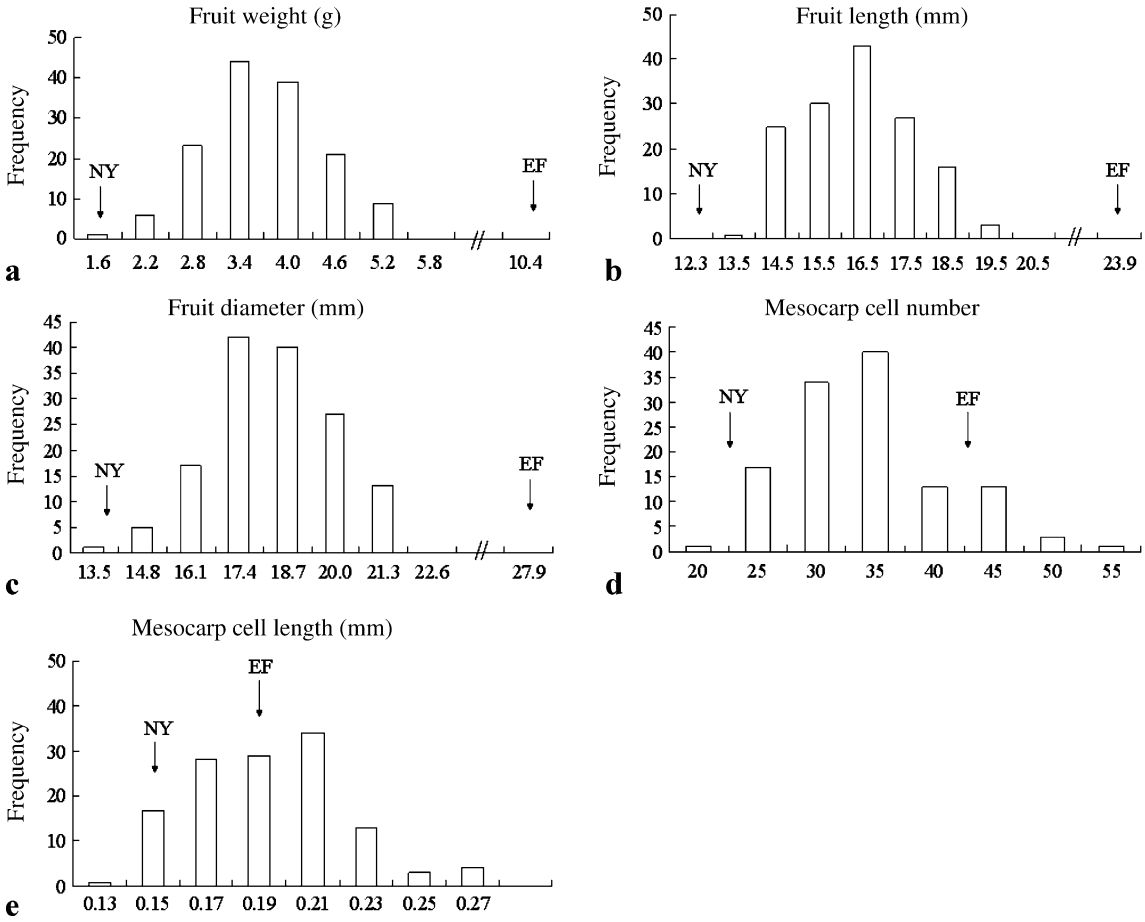

Fig. 1 **a**–**e** Frequency distributions of fruit size and mesocarp cell measurements for a pseudo-testcross population derived from "Emperor Francis" (*EF*)×"New York 54" (*NY*) in 2006. Means for the parents EF and NY are shown by *arrows*. **a** Fruit weight. **b** Fruit length. **c** Fruit diameter. **d** Mesocarp cell number. **e** Mesocarp cell length

2007 and 0.79 for 2006–2008). Therefore, maximum fruit weight was used in the analyses.

The correlation coefficients among the traits were similar between 2006 and 2007 (Table 2). Highly significant correlations were found among fruit weight, length, and diameter ($r>0.80$ for all the pairwise combinations, $P<0.0001$), suggesting that any of these three measures can be used to represent fruit size. This result is expected since fruit weight will increase as fruit dimensions increase. The high correlations ($r>0.90$) between these fruit size measurements have also been observed in a tomato F_2 population (Lippman and Tanksley 2001). Mesocarp cell number was positively correlated with fruit weight, length, and diameter with correlation coefficients ranging from 0.30 ($P<0.001$) to 0.49 ($P<0.0001$), indicating that increased mesocarp cell number contributed significantly to a larger fruit. In contrast, mesocarp cell length did not significantly ($P<0.05$) correlate with any of the three fruit size measurements. In addition, mesocarp cell number negatively correlated with cell length, which is consistent with the absence of any progeny individual exhibiting a fruit weight equal to or above the large-fruited parent. Pit length and diameter were also highly correlated with each other and all three fruit size measurements (Table 2). However, pit size was not correlated with cell measurements except pit diameter and cell number ($P<0.05$).

QTL analyses

The results from the three QTL detection methods, KW test, IM, and MQM, were consistent. Fruit weight QTLs were detected on two of the eight *Prunus* linkage groups (Table 3 and Fig. 2). Three fruit weight QTLs were identified that were located on linkage group 2 of the EF map (EF 2), and linkage groups 2 and 6 of the NY map (NY 2 and NY 6). The QTL on EF 2 was significant in all 3 years and explained 27.8%, 15.9%, and 54.0% of the phenotypic variation in 2006, 2007, and 2008, respectively. Similarly, the fruit weight QTL on NY 2 was detected in all 3 years and explained 17.8%, 27.5%, and 44.7% of the phenotypic variation, respectively. The third fruit weight QTL on NY 6 was significant in 2007 and 2008 and explained 32.4% and 30.3% of the phenotypic variation,

Table 2 Pearson's correlation coefficients among fruit size, cell, and pit size traits measured in 2006 and 2007 on an F_1 population derived from the cross "Emperor Francis" × "New York 54"

Trait	Year	Fruit length	Fruit diameter	Cell number	Cell length	Pit length	Pit diameter
Fruit weight	2006	0.83***	0.94***	0.49***	−0.09	0.46***	0.64***
	2007	0.82***	0.91***	0.41***	0.02	0.38***	0.50***
Fruit length	2006		0.80***	0.39***	−0.08	0.56***	0.56***
	2007		0.83***	0.30**	0.05	0.52***	0.46***
Fruit diameter	2006			0.42***	−0.04	0.41***	0.62***
	2007			0.37***	0.06	0.38***	0.47***
Cell number	2006				−0.74***	0.11	0.19*
	2007				−0.68***	0.11	0.28*
Cell length	2006					0.00	−0.01
	2007					0.07	−0.11
Pit length	2006						0.59***
	2007						0.64***

*$P<0.05$; **$P<0.001$; ***$P<0.0001$; others are not significant at $P<0.05$

respectively. In 2006, this QTL was not significant, but it had a LOD score of 3.4.

Three fruit length QTLs and three fruit diameter QTLs were identified at similar genomic regions as the fruit weight QTLs (Table 3 and Fig. 2). All these QTLs were significant in the years analyzed (2006 and 2007) except for the fruit diameter QTL on EF 2, which was only significant in 2006. However, the fruit diameter data in 2007 did suggest the presence of a possible QTL on EF 2 with a LOD score of 3.5. On EF 2, NY 2, and NY 6, the 2-LOD support intervals of the QTLs for fruit weight, length, and diameter all overlapped.

A QTL for mesocarp cell number was detected on EF 2 (Table 3 and Fig. 2). This QTL was only significant in 2006 and accounted for 31.9% of the phenotypic variation. However, in 2007, mesocarp cell number exhibited a peak with a LOD score of 3 at the similar genomic region as in 2006. The 2-LOD support intervals for the mesocarp cell number QTL overlapped with the fruit size QTLs on EF 2. Therefore, it is possible that these QTLs are under similar genetic control where the primary effect is to increase mesocarp cell number and ultimately fruit size. If this is the case, the fruit size/cell number QTL identified on EF 2 may contribute to the increase in fruit size that accompanied the domestication of sweet cherry. In contrast to mesocarp cell number, not a single QTL was identified for mesocarp cell length.

Since no mesocarp cell size or cell number QTL mapped to the fruit size QTL on NY 6, we investigated the possibility that the morphological basis of the fruit size QTL on NY 6 was due to differences in pit size, a theory put forth in peach by Quilot et al. (2004). In the analyses, we used linear pit measurements (length and diameter) instead of pit weight as pit weight can vary greatly depending on seed development (Olmstead et al. 2007). The QTL analyses using data from 2 years (2006 and 2007) identified significant pit length and diameter QTLs that colocated with the fruit size QTL on NY 6 (Table 3 and Fig. 2). Furthermore, QTL analyses of mesocarp size showed that QTLs for mesocarp length and diameter colocated with fruit size QTLs on EF 2 and NY 2 in both years (2006 and 2007). However, a QTL for mesocarp length on NY 6 was only significant in 2007. These suggest that differences in pit size might be the major underlying morphological factor resulting in the increases in fruit size associated with the QTL on NY 6.

Parental haplotypes for QTLs

A QTL can segregate for a maximum of four different alleles in a diploid F_1 pseudo-test population ($Q_1Q_2 \times Q_3Q_4$); therefore, it is possible that one QTL can be detected in both parental maps. Therefore, for linkage group 2, we considered whether the QTLs on EF 2 and NY 2 likely represented the same QTL. On EF 2, the QTL peak position and the largest allele effect was associated with PR96 (Table 3, Fig. 2). On NY 2, the QTL peak position and the largest allele effect was associated with the shared dominant marker EAA-MCCC-370, which is only ~6 cM away from PR96 on the EF map. In addition, the 2-LOD support intervals for the QTLs on EF 2 and NY 2 partially overlapped based on the positions of the shared codominant SSR markers CPSCT038 and BPPCT034. Therefore, it is possible that the fruit weight QTLs on EF 2 and NY 2 belong to the same QTL, and this QTL is segregating for more than two alleles.

If one QTL has more than two alleles, three types of allelic effects are possible, including two parental effects

Table 3 Summary of QTLs for fruit weight, fruit length, fruit diameter, mesocarp cell number, pit length, and pit diameter identified in an F_1 population derived from the cross "Emperor Francis" × "New York 54" using the multiple QTL mapping method of MapQTL

LG	QTL (peak position[a])	2006		2007		2008[b]	
		LOD	R^{2c}	LOD	R^2	LOD	R^2
Fruit weight							
Threshold[c]		3.7; 4.0		3.8; 3.8		3.9; 4.7	
EF 2	21.4 (PR96)	6.6	27.8	3.8	15.9	10.2	54.0
NY 2	48.9 (EAA-MCCC-370)	6.4	17.8	7.4	27.5	14.3	44.7
NY 6	56.8 (EPPCU3090)	3.4[d]	7.6	7.9	32.4	7.7	30.3
Fruit length							
Threshold		3.9; 4.0		3.7; 3.7			
EF 2	28.9 (UDAp-461)	6.0	26.0	5.1	21.9		
NY 2	41.4 (BPPCT034)	7.7	19.4	7.4	21.2		
NY 6	56.8 (EPPCU3090)	7.6	23.2	11.2	37.8		
Fruit diameter							
Threshold		3.9; 3.7		3.8; 4.1			
EF 2	30.9 (MA005c)	7.4	24.5	3.5[d]	14.5		
NY 2	44.4 (BPPCT034)	7.3	26.1	6.5	39.1		
NY 6	56.8 (EPPCU3090)	4.0	4.2	7.7	39.4		
Mesocarp cell number							
Threshold		4.5		3.8			
EF 2	19.9 (UDA-059)	5.1	31.9	3.0[d]	17.0		
Pit length							
Threshold		3.6		4.0			
NY 6	58.7 (EAA-MCAC-420)	9.0	35.8	8.7	33.8		
Pit diameter							
Threshold		3.5		3.8			
NY 6	57.4 (PR86)	4.8	20.5	6.4	39.4		
Mesocarp length (fruit length−pit length)							
Threshold		4.8; 4.9		3.8; 3.8			
EF 2	30.9 (MA005C)	6.6	24.5	4.4	31.6		
NY 2	38.4 (EAT-MCCC-150)	9.9	58.1	7.2	32.0		
NY 6	56.8 (EPPCU3090)			8.2	67.1		
Mescocarp diameter (fruit diameter−pit diameter)							
Threshold		3.8; 3.8		3.9; 3.7			
EF 2	28.9 (UDAp-461)	4.9	21.2	4.4	24.6		
NY 2	42.4 (BPPCT034)	6.0	34.6	4.3	15.8		

LG linkage group, R^2 percentage of phenotypic variation explained by a QTL

[a] QTL peak position in the most significant environment is expressed in cM, and the closest markers are indicated in bracket

[b] Traits not measured in 2008 are blank

[c] Thresholds for each trait in each year and each map. When two numbers are presented, the first and second numbers are for the thresholds on the EF and NY maps, respectively

[d] LOD score is smaller than the threshold

(difference in effect of the alleles inherited from each parent) and one interaction effect (genetic effect coming from both parents; Sewell et al. 2002). Therefore, the interpretation of the desirable QTL alleles in those regions is more complicated than in other crops where QTL analyses are done with homozygous recombinant inbred lines or F_2 populations that include both homozygous marker classes. Yet, determining the favorable QTL alleles, identified by their flanking markers, is critical for further QTL validation and eventual utilization in MAS.

To examine the allele effects of the fruit weight QTLs identified in this study, we defined the parental haplotypes with markers in the QTL regions, only considering those haplotypes that did not have a recombination between the parental markers. The recombinant individuals were not included in the haplotype table because it was not known where the recombination had occurred relative to the QTL. For the QTLs on EF 2 and NY 2, we defined the parental haplotypes using CPSCT038 and BPPCT034. Marker CPSCT038 has unique fragments of 192 bp in NY and 204 bp in EF and one common fragment of 190 bp in both parents. Marker BPPCT034 has unique fragments of 225 bp in NY and 235 bp in EF and one common fragment of 255 bp in both parents. Since both parents have QTLs in this region, four haplotypes, termed "a" and "b" in NY and "c" and "d" in EF, were defined as in Table 4. Progeny individuals with the "ac" haplotypes had the smallest mean fruit weight in all 3 years analyzed (Table 5). Among the

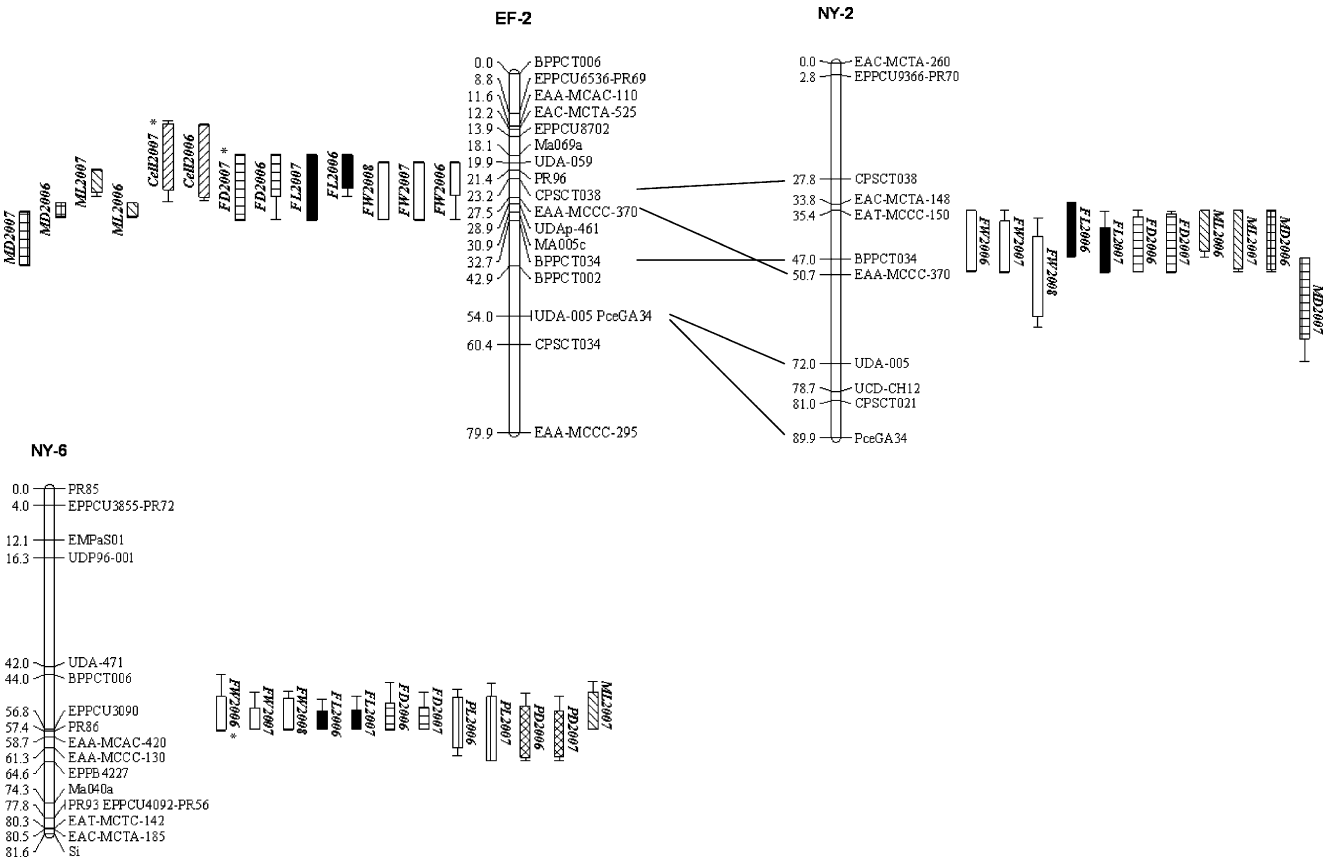

Fig. 2 Locations of QTLs for fruit weight (*FW*), fruit length (*FL*), fruit diameter (*FD*), mesocarp cell number (*Cell*), pit length (*PL*), and pit diameter (*PD*), mesocarp length (*ML*), and mesocarp diameter (*MD*) in 2006, 2007, and 2008 using the multiple QTL mapping method. *Blank bars* represent QTLs for fruit weight. *Black bars* represent QTLs for fruit length. *Bars filled with parallel lines* represent QTLs for fruit diameter. *Bars filled with slash lines* represent QTLs for cell number. *Bars filled with vertical lines* represent QTLs for pit length. *Bars filled with cross lines* represent QTLs for pit diameter. *Bars filled with back slash lines* represent QTLs for mesocarp length. *Bars filled with both parallel and vertical lines* represent QTLs for mesocarp diameter. 1-LOD and 2-LOD support intervals of each QTL are marked by *thick* and *thin bars*, respectively. Only linkage groups including the QTLs are presented. The QTL positions are listed on the *left side* of linkage group EF 2 and *right side* of linkage groups NY 2 and NY 6. The QTLs with *asterisk* indicate they are not significant but have a LOD score of over 3

other three possible progeny haplotypes, those with "bd" had the largest mean fruit size in 2006 and 2008 but not in 2007. However, progeny with the "bd" haplotypes had the highest mean mesocarp cell number, supporting our prediction that the "bd" haplotypes might result in the largest fruit size.

The four haplotype classes were not equally distributed; instead, there was an excess of progenies with the "ac" and "bc" haplotypes. The distorted genotype classes in this study might be due to alleles on haplotype "d" that negatively affected gametogenesis, fertilization, or embryogenesis. Therefore, to confirm that the haplotype combination "bd" results in the largest fruit size, a larger population will be necessary to avoid small sample sizes for the classes "ad" and "bd." However, our data do suggest that progenies with the haplotyes "ac" should be selected against. In fact, the genotyping of 30 large-fruited sweet cherry cultivars with these two markers only revealed a few cultivars with the "ad" or "bc" haplotypes but none with the "ac" or "bd" haplotypes (data not shown). This suggests that the large-fruited genotype in this QTL region has not been fixed in all our modern cultivars and would be a potential target QTL(s) for MAS. Future efforts will involve experiments to validate the presence and direction of this QTL region in different genetic backgrounds using closely linked codominant markers.

The QTL on linkage group 6 was only found for the NY map, indicating that there is no segregation for this QTL in EF. Therefore, we only defined the parental haplotypes "a" and "b" for NY using two closely linked markers, EPPCU3090 and PR86 (Table 4). For all 3 years, those progeny individuals that received the "b" haplotype consistently had larger fruit and larger pits than those that received the "a" haplotype (Table 6). Interestingly, the 180 bp fragment for EPPCU3090, potentially associated with the large-fruited QTL, was not found in any of the 30 large-fruited sweet cherry cultivars evaluated (data not

Table 4 Definitions of parental haplotypes for QTL regions on linkage groups 2 and 6

Linkage group	Parent	Haplotype	Molecular marker			
			CPSCT038	BPPCT034	EPPCU3090	PR86
2	NY	a	192[a]	225		
		b	190	255		
	EF	c	204	235		
		d	190	255		
6	NY	a			172	180
		b			180	210

[a] Allele fragment size in bp

presented). This suggests that haplotype "b" may have been selected against during domestication and subsequent breeding.

Discussion

The uniform distribution of fruit size and the prevalence of small-fruited progenies identified in this study are in agreement with previous findings in sweet cherry (Lamb 1953; Fogle 1961; Mathews 1973). However, the very low fruit size values for the progeny individuals, the majority of which were below the midparent value, suggest semi-dominance of small-fruited alleles. In addition, it is possible that NY is homozygous for one or more fruit size QTLs, and the presence of these dominant small fruit size alleles contributes to the small fruit size in all the progeny individuals. Our fruit size segregation results are similar to those of Quilot et al. (2004), who evaluated fruit size in a BC_2 peach population where the small-fruited parent was the wild *Prunus* species *Prunus davidiana*. Similar fruit size distributions were also found in crosses not involving small-fruited wild relatives. For example, in a cross between two *P. persica* parents, the mean fruit weight of the F_1 progeny was less than the parental midpoint (Dirlewanger et al. 1999). The same trend was reported in sweet cherry (Lamb 1953) and sour cherry (Wang et al. 2000). However, in sour cherry, the mean fruit weight of the F_1 progeny was less than the mean fruit weight of the small-fruited parent; yet, two progeny individuals had mean fruit weights higher than the large-fruited parent (Wang et al. 2000). The one exception to this apparent dominance of small-fruited alleles is in the cross between a rootstock peach and an ornamental peach cultivar (Yamamoto et al. 2001). In this case, the mean fruit weight of the F_1 progeny was higher than that of the large-fruited parent. However, both parents had small fruit, and the mean progeny fruit weight was far below that of commercial cultivars. Interestingly, semi-dominance of small fruit size alleles has been documented in tomato, a model for fleshy fruit development (Grandillo et al. 1999; Lippman and Tanksley, 2001). Collectively, these studies indicate that progeny with large fruit size may only rarely occur, suggesting that the ability to identify and aggregate positive fruit size alleles through MAS will be critical for continued cultivar improvement.

The fruit size QTL regions identified in our study were also identified in at least two other studies on *Prunus*. A fruit weight QTL was also reported on linkage group 2 in sour cherry (Wang et al. 2000) and peach (Quilot et al. 2004). Additionally, fruit size QTLs on the lower half of linkage group 6 were reported in peach multiple times (Dirlewanger et al. 1999, Etienne et al. 2002, Quilot et al. 2004). In contrast, previously reported fruit size QTLs not identified in our study include QTLs on linkage group 4 identified in two different *Prunus* populations [sour cherry, Wang et al. (2000), and peach, Quilot et al. (2004)] and QTLs on linkage group 1 in the peach BC_2 population that had the wild species *P. davidiana* as parent (Quilot et al. 2004). Clearly, more studies using shared markers and populations with different genetic backgrounds are needed to define the complement of QTLs controlling fruit size in *Prunus* species.

Table 5 Fruit weight (FW) in grams and cell number (CN) of haplotype combinations for the QTL region on linkage group 2

Haplotype combination[a]	Year					
	2006		2007		2008	
	FW	CN	FW	CN	FW	CN
ac (44)	3.0 (34)	30.1 (30)	3.6 (44)	30.1 (34)	4.0 (25)	–
ad (8)	3.4 (6)	31.0 (4)	4.3 (8)	30.9 (7)	5.2 (4)	–
bc (32)	3.5 (25)	31.3 (19)	4.4 (29)	31.8 (22)	5.3 (14)	–
bd (13)	4.1 (9)	37.6 (7)	4.2 (12)	35.9 (10)	5.4 (3)	–

Numbers in brackets are the number of progeny individuals

– N/A

[a] a, b, c, and d are the haplotypes as defined in Table 4

Table 6 Fruit weight (FW) in grams, pit diameter (PD), and length (PL) in millimeter of haplotypes for the QTL region on linkage group 6 as defined in Table 4

Haplotype	Year						
	2006			2007			2008[a]
	FW	PD	PL	FW	PD	PL	FW
a (82)	2.9 (50)	7.9 (50)	9.2 (50)	3.6 (53)	8.2 (53)	9.5 (53)	4.4 (43)
b (72)	3.5 (55)	8.4 (55)	9.9 (55)	4.3 (55)	8.5 (55)	10.2 (55)	5.4 (38)

Numbers in brackets are the number of progeny individuals

[a] Pit size was not available in 2008

Previous studies of diverse fruit species have determined that mesocarp cell number and/or size played a major role in the overall increase in fruit size (Frary et al. 2000; Yamaguchi et al. 2002, 2004; Harada et al. 2005; Olmstead et al. 2007). In our study, cell number, not cell size, made contributions to large fruit. This result is consistent with that of Quilot and Génard (2008), who compared four peach groups with different percentages of a wild, small-fruited peach genome. They found that the percentage of the wild peach genome affected maximal mesocarp cell number but not the maximal mesocarp cell size suggesting that increased mesocarp cell numbers resulted in the large fruit size in cultivated peach genotypes. This similar result has also been revealed in other fruit crops such as avocado (*Persea americana* Mill.; Cowan et al. 1997); melon (*Cucumis melo* L. *reticulatus*; Higashi et al. 1999), olive (*Olea europaea* L.; Rapoport et al. 2004), peach (Scorza et al. 1991), and pear (Zhang et al. 2006) by the comparisons among genotypes. However, our analysis failed to identify any QTL for mesocarp cell length despite the significant progeny phenotypic variation and the relatively high broad sense heritability. The reasons for this are unknown. We speculate that this may be due to regions of poor map coverage or a larger number of genes with small effects controlling cell length. Besides, epistatic effects or QTLs at a poorly polymorphic state in parents could also contribute to the lack of detected QTLs. Alternatively, the environmental variation influencing cell length may have been sufficient to prevent us from detecting any significant QTL. For example, our prior work that included an analysis of EF and NY indicated that mesocarp cell length is strongly influenced by the environment compared to mesocarp cell number (Olmstead et al. 2007). The higher heritability of mesocarp cell number relative to cell size was also supported by our results, i.e., 0.94 versus 0.67.

The clustering of QTLs for the highly correlated fruit size traits (weight, diameter, and length) implies the presence of multiple closely linked genes or a common genetic mechanism that results in an increase in mass and both linear dimensions. Most likely, the clustered QTLs in this study fit the latter explanation since it is logical that fruit weight increases according to the fruit dimensions. Colocated QTLs for these traits have been found in tomato (Lippman and Tanksley 2001) and peach (Quilot et al. 2004). The colocation of the mesocarp cell number QTL with the fruit size QTL on EF 2 suggests that the increase in fruit size manifest by this QTL is due to increases in mesocarp cell number. Increased mesocarp cell number has been proposed to be the morphological change that contributed to the increased fruit size of domesticated varieties. Therefore, this QTL may represent an important genetic change that distinguishes wild cherry from its domesticated counterparts. Increases in organ size as a result of increases in cell number have previously been associated with domestication QTLs for tomato fruit size (*fw*2.2, Frary et al. 2000), rice kernel weight (*GW*2, Liu et al. 2003; Song et al. 2007), and the size of the outer glume in the rice flower (*qSW*5, Shomura et al. 2008). Interestingly, *ORFX*, the gene that underlies the fruit weight QTL in tomato (*fw2.2*), has been proposed to be a negative regulator of cell division, and the wild-type gene behaves in a dominant manner to reduce carpel cell number and thus fruit weight (Frary et al. 2000).

In our analyses, neither mesocarp cell number nor cell size QTLs colocated with the fruit weight, length, and diameter QTLs identified on NY 6. However, QTLs identified for linear measurements (length and width) of pit size did cluster with the fruit size QTLs on NY 6. As in Quilot et al. (2004), it is possible that the fruit size QTL identified herein on NY 6 may really represent increased pit size as a component of total fruit size. This would be analogous to the contribution of increased locule number in tomato to increased fruit size (Lippman and Tanksley 2001). If pit size were under different genetic control than flesh size, this would represent an alternative avenue to increased fruit size; i.e., fruit size is increased through a larger pit without concomitant decrease in flesh area. The idea of differential genetic control of pit and flesh of cherry is supported by Tukey and Young (1939), who concluded that mesocarp and pit cells arise from distinct cell

populations that are distinguishable prior to anthesis in sour cherry. Additionally, Olmstead et al. (2007) suggested that genetic increases in fruit size could occur without an associated increase in pit diameter when comparing five sweet cherry cultivars.

In conclusion, the repeated examples of the prevalence of small-fruited individuals in *Prunus* populations and the rarity of large-fruited individuals indicates that the ability to preselect those seedlings predicted to have acceptable fruit size prior to field planting will dramatically increase the efficiency of *Prunus* breeding programs. The parental haplotypes in the QTL regions defined in this analysis will serve as a starting point for QTL validation in other populations and the narrowing of the target QTL regions. The analyses of other populations will also contribute to the discovery of new QTLs and to the study of synteny between different *Prunus* species at the QTL level.

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