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QTL analysis of flower and fruit traits in sour cherry

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Abstract The map locations and effects of quantitative trait loci (QTLs) were estimated for eight flower and fruit traits in sour cherry (*Prunus cerasus* L.) using a restriction fragment length polymorphism (RFLP) genetic linkage map constructed from a double pseudo-testcross. The mapping population consisted of 86 progeny from the cross between two sour cherry cultivars, Rheinische Schattenmorelle (RS)×Erdi Botermo (EB). The genetic linkage maps for RS and EB were 398.2 cM and 222.2 cM, respectively, with an average interval length of 9.8 cM. The RS/EB linkage map that was generated with shared segregating markers consisted of 17 linkage groups covering 272.9 cM with an average interval length of 4.8 cM. Eleven putatively significant QTLs (LOD >2.4) were detected for six characters (bloom time, ripening time, % pistil death, % pollen germination, fruit weight, and soluble solids concentration). The percentage of phenotypic variation explained by a single QTL ranged from 12.9% to 25.9%. Of the QTLs identified for the traits in which the two parents differed significantly, 50% had allelic effects opposite to those predicted from the parental phenotype. Three QTLs affecting flower traits (bloom time, % pistil death, and % pollen germination) mapped to a single linkage group, EB 1. The RFLP closest to the bloom time QTL on EB 1 was detected by a sweet cherry cDNA clone pS141 whose partial amino acid sequence was 81% identical to that of a Japanese pear stylar RNase.

Key words *Prunus cerasus* · QTL · Pseudo-testcross · Molecular markers · Polyploid

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Introduction

An important goal in sour cherry (*Prunus cerasus* L.) breeding is to develop cultivars with improved fruit quality, delayed bloom time to avoid spring freezes, and a range of ripening dates. Therefore, many flower and fruit traits such as bloom date, percentage pistil death, ripening date, fruit weight, and fruit soluble solids concentration are important for selection in a sour cherry breeding program. Unfortunately, direct selection for these traits can not be carried out until the seedlings flower and fruit after a minimum of 3–5 years of growth. If prior knowledge of linkage relationships between marker loci and important flower and fruit characteristics were available, undesirable individuals could be eliminated from progeny populations with marker-assisted selection as early as when the seedlings develop the first few leaves.

Linkage relationships between molecular markers and agronomically important quantitative traits have been extensively studied in many crop plants for over a decade (Edwards et al. 1987; Stuber et al. 1987, 1992; Paterson et al. 1988, 1990; Keim et al. 1990; Grandillo and Tanksley 1996). In tree fruit crops, quantitative trait locus (QTL) analyses have been reported for growth and development traits in apple (*Malus domestica*) (Conner et al. 1998) and fruit quality and disease resistance traits in peach (*P. persica*) (Dirlewanger et al. 1998; Quarta et al. 1998; Viruel et al. 1998). In contrast, no similar study has been reported in sour cherry. The delay has been due to the difficulties in the construction of a molecular linkage map for sour cherry because of the species' polyploid origin and mixed patterns of inheritance (disomic and tetrasomic)(Beaver and Iezzoni 1993; Wang et al. 1998). Recently, we constructed the first molecular linkage maps in sour cherry using restriction fragment length polymorphism (RFLP) markers (Wang et al. 1998) and in this report we describe the first QTL analysis in sour cherry. Our objectives were to estimate the locations and effects of QTLs affecting flower and fruit traits in sour cherry.

Materials and methods

Plant material

The mapping population utilized in this study is a double pseudo-testcross population (Lawson et al. 1995) which consisted of 86 progeny from the cross between two sour cherry cultivars, Rheinische Schattenmorelle (RS)×Erdi Botermo (EB). A double pseudo-testcross is a single cross between parents that are expected to have a high level of heterozygosity. Therefore, markers heterozygous in one parent and homozygous recessive in the other parent will segregate in the progeny as in a testcross. The parents, RS and EB, were chosen because they are from different geographic areas (Germany and Hungary, respectively) and differ with respect to important horticultural traits. The cross was made by hand pollination in 1990, and the resulting progeny trees were planted at the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, Michigan in the spring of 1991. The majority of the progeny flowered and fruited for the first time in 1993. A total of eight traits were evaluated for each progeny individual and the two parents. Five traits were evaluated over 3 years, and three traits were evaluated in 1 year. Details of trait evaluations are given below.

Traits measured

Bloom time

The bloom date of an individual was recorded as the day when approximately 50% of the flowers were open. Hourly temperature readings were available from an automated weather station at the Clarksville Horticultural Experiment Station. Time to bloom was expressed as degree days (DD) from January 1 with a base temperature of 4.4°C. Daily heat unit accumulation was calculated by summing the positive differences of hourly temperature readings minus 4.4°C and then dividing by 24. On the day of bloom, heat unit accumulation was calculated to hour 10, which was the approximate time the data were recorded. Bloom time was evaluated over 3 years (1995–1997).

Ripening time

The ripening time of an individual was recorded as the first day when the fruits could be easily pulled off the stems. Time to ripening was expressed as degree days (DD) following the same calculation as for bloom time except that the ripening date was used as the ending date. Time to ripening was evaluated for 3 years (1995–1997).

Flower bud death

Flower-bud death due to freeze damage is common in Michigan when the buds start swelling in the early spring. Flower buds in cherry contain from three to six flowers per bud that open to form a flower cluster. Following a spring freeze to –10°C on the night of April 5, 1995, flower-bud death was evaluated from the swelled buds. A flower bud was determined to be dead if the flowers within the bud were necrotic. About 20 flower buds from each individual were cut open to determine bud death, which was expressed as the percentage of dead buds. The data in percentage were angular-transformed (i.e., arcsin $\sqrt{Y}$ transformed) to normalize the distribution of the data for QTL analysis.

Pistil death

Pistil death was evaluated during the bloom periods of 1995, 1996, and 1997 following natural freezing events. Ten open flowers were randomly selected for evaluation from each of the four sides (north, south, west, and east) of a tree. The dead pistils were counted to calculate the percentage of dead pistils in 40 flowers. The percentage data were angular-transformed in the same way as for flower-bud death data before QTL analysis.

Pollen germination

Percentage pollen germination was evaluated in 1996. Pollen was collected from flowers at anthesis, dried at room temperature overnight, and then germinated at room temperature in two separate experiments on Brewbaker and Kwack medium (1963) using 10% sucrose (Raptopoulos 1940). Pollen germination was determined under a light microscope after 3 h. The number of pollen grains germinated from a total of 100 pollen grains was recorded. The mean pollen germination percentage from the two experiments for each individual was used for QTL analysis. The data were angular-transformed in the same way as for flower bud-death before QTL analysis.

Fruit set

Fruit set, calculated as the percentage of flowers that set fruit, was measured in 1998 since the flowers had no apparent cold damage due to mild winter and spring temperatures. Two branches from opposite sides (east and west sides) of each tree were selected so that all branches had similar vigor, and each branch bore approximately 300 flowers.

Fruit weight and total soluble solids concentration

Fruit weight (g) and percentage soluble solids were evaluated for five ripe fruits from each parent and the progeny. Percentage soluble solids was measured with a refractometer as °Brix. The average of the five fruits was used for QTL analysis. These data were collected over 3 years (1995–1997).

Heritability estimates

For those traits that were evaluated in each of the 3 years, the broad-sense heritability (H) estimates were obtained from the analysis of variance based on the linear model:

$$Y_{ij}=\mu+y_i+g_j+\varepsilon_{ij}$$

where Y_{ij} is the phenotypic value of jth progeny (j=1, 2, 3, ..., 86) in ith year (i=1, 2, 3); μ is the mean value of the trait; y_i is the effect of the ith year on the trait; g_j is the genotypic effect of progeny j; and ε_{ij} is the year × genotype interaction. Broad-sense heritability (H) estimates were calculated using the following equations:

$$\sigma_g^2 = \frac{MS_g - MS_{residual}}{y}$$

$$H_{(broad\,sense)} = \frac{\sigma_g^2}{\sigma^2/y + \sigma_g^2}$$

where σ_g^2 is the genetic variance; MS_g is the estimated mean square of genotypes, and $MS_{residual}$ is the estimated mean square of residual error, σ^2, which is a measure of variability due to genotype × year interaction.

Molecular marker and QTL analysis

RFLP markers were used to construct linkage maps for each parent of the mapping population (Wang et al. 1998). All markers used were single-dose restriction fragments (SDRFs, Wu et al. 1992) which were either: (1) present in one but not both of the

Table 1 Mean phenotypic values and standard deviations (SD) for the progeny and parents (RS and EB), and the value range for the progeny

Trait	Mean±SD			Progeny range	
	RS	EB	Progeny	Minimum	Maximum
Bloom time (DD)	428.1±22.9	362.2±16.0	398.4±33.8	317.8	516.2
Ripening time (DD)	2474.9±262.7	1863.9±85.9	2084.8±233.3	1465.0	2712.0
Pistil death (%)	11.3±12.4	41.7±30.0	23.8±15.3	0.0	55.0
Fruit set (%)	16.0±0.2	13.4±1.3	6.8±6.7	0.0	34.4
Fruit weight (g)	5.5±0.5	7.4±0.8	4.7±1.2	2.3	8.8
Soluble solids (° Brix)	16.3±1.3	17.2±0.5	15.9±2.0	9.8	20.1
Pollen germination (%)	18.5±0.7	8.0±1.4	5.6±7.0	0.0	34.0
Flower-bud death (%)	0.0±0.0	55.0±17.7	33.4±26.3	0.0	100.0

parents and fit a 1:1 (presence:absence) segregation ratio, or (2) present in both parents and fit a 3:1 (presence:absence) segregation ratio. A total of 190 SDRF markers were used, of which 110 were present in one parent (67 and 43 markers in RS and EB, respectively) and 80 markers were present in both parents.

Our previous sour cherry linkage map (Wang et al. 1998) was generated by JOINMAP (Stam 1993), which is able to determine linkage relationships between markers segregating 1:1 and markers segregating 3:1 in a pseudo-testcross. Since QTL-CARTOGRAPHER (Basten et al. 1997) can not analyze data containing both 1:1 markers and 3:1 markers simultaneously from a pseudo-testcross mapping population, it was necessary to generate three linkage maps for QTL analysis. The three linkage maps constructed were the EB and RS maps using the 1:1 markers segregating in EB and RS, respectively, and a RS/EB map using the 3:1 markers. Linkage analyses were performed using MAPMAKER (Lander et al. 1987) and the Kosambi (1944) mapping function with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.30. Linkage group numbers assigned were the same as those previously used (Wang et al. 1998).

Means, standard deviations, and skewness of trait distribution were calculated for each trait. *t*-tests for significance of differences between means of parents and progeny were carried out for each trait, and correlations among traits were also calculated. All these analyses were accomplished using the analysis tools of MICROSOFT EXCEL 7.0.

QTL mapping was performed using composite interval mapping (CIM) (Zeng 1994; Jansen and Stam 1994), which is an extension of interval mapping (Lander and Botstein 1989). Interval mapping calculates the likelihood score for a putative QTL placed in any position within an interval flanked by two adjacent markers. CIM extends this method by fitting the most significant markers outside the interval into the model, allowing more precise and efficient mapping of QTLs (Zeng 1994).

QTL analysis was carried out with the program QTL-CARTOGRAPHER (Basten et al. 1997). CIM was run with model 6 of the program and a window size of 10 cM for all analyses. The number of markers for the background control was set to 5, which means that the 5 most significant markers outside the interval under analysis were fitted to the model. The markers used for the background control were detected through forward and backward stepwise regression. The likelihood value of the presence of a QTL was expressed as LOD score $\log_{10}(L_1/L_0)$, where L_1 is the maximized likelihood of the model with the putative QTL and L_0 is the maximized likelihood of the model without the QTL. The threshold of the LOD score for declaring a putative QTL significant was chosen to be 2.4, which is approximately equivalent to applying a significance level of 0.001 for any single test. The estimate of the QTL position is the point where the maximum LOD score was found in the region under consideration. A one-LOD support interval was constructed for each QTL as described by Lander and Botstein (1989).

The phenotypic variance explained by a single QTL was estimated by the square of the partial correlation coefficient (R^2). Estimates of the R^2 value and the additive effect of a single QTL at its peak LOD position were obtained from the output of QTL analysis using the program QTL-CARTOGRAPHER (Basten et al. 1997).

For traits evaluated over 3 years, each year was considered as a different environment. Therefore, the data from each year were analyzed separately. When a putative QTL was detected in more than 1 year, the mean of the 3 years was analyzed and the results were reported as the generalized results for the QTL.

Results and discussion

Distribution of traits

All traits evaluated exhibited continuous variation, which is typical of quantitative or polygenic inheritance (Fig. 1). The two parents, RS and EB, differed significantly ($P<0.05$) for five traits, including bloom time, ripening time, fruit weight, percentage flower bud death, and percentage pollen germination (Table 1). There were no significant differences between the two parents for soluble solids concentration, percent pistil death, and percentage fruit set (Table 1). Transgressive segregation was observed for all traits analyzed (Table 1; Fig. 1).

The progeny distribution for bloom time was normal (Fig. 1), and the mean was similar to the mid-parent value of 395 (Table 1). The difference in bloom time for the two parents (66 degree days) was statistically significant ($P<0.05$). However, parental values were not the two extremes; 22% of the progeny bloomed later than the late parent and 13% of the progeny bloomed earlier than the early parent (Fig. 1).

The difference in ripening time between the two parents (611 degree days) was statistically significant ($P<0.05$) (Table 1; Fig. 1). The progeny mean was not statistically different from the average of the two parents, and the distribution was normal. Seventy-three percent of the progeny values fell into the range defined by the values of the two parents; 6% of the progeny ripened later than the late parent and 21% of the progeny ripened earlier than the early parent.

The two parents differed significantly ($P<0.001$) for percentage flower bud death, and EB had 55% more damage than RS (Table 1). The distribution of progeny values was significantly skewed towards a smaller percentage of death (Fig. 1); however, the progeny mean was not significantly different from the average of the two parents and only 48% of the progeny had a lower percentage flower-bud death than the mid-parent. Twenty-

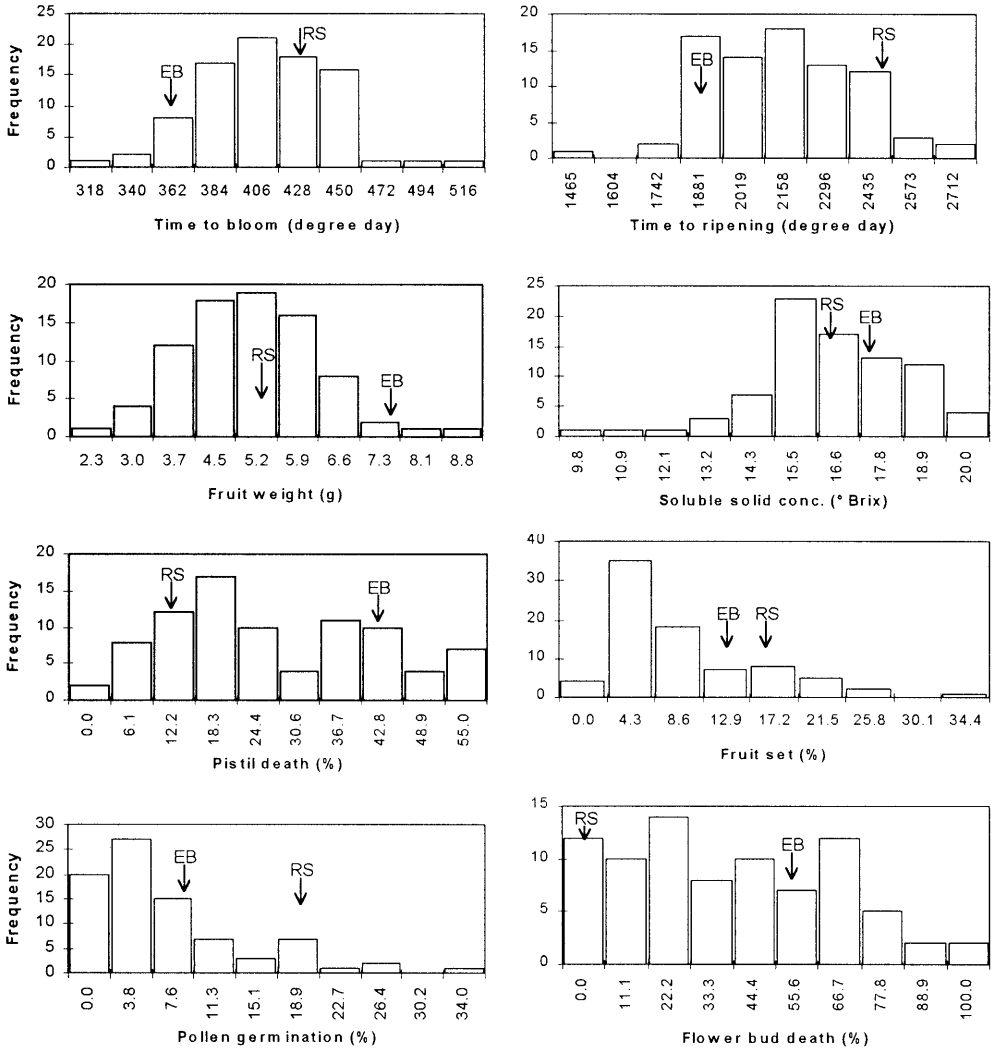

Fig. 1 Frequency distributions for each character in the mapping population. Means for the parents *RS* and *EB* are shown by *arrows*

three percentage of the progeny had a higher percentage flower-bud death than EB (Fig. 1).

RS had a lower percentage pistil death than EB; however, the difference was not significant (Fig. 1, Table 1). The progeny distribution was skewed toward the lower values; however, angular transformation of the percentage data reduced the skewness from 0.35 to 0.02.

RS and EB had similar values for percentage fruit set (Table 1). The distribution of progeny values was skewed toward lower values with 84% of the progeny having reduced percentage fruit set than the average of the two parents (Fig. 1). The skewness was reduced from 1.50 to 0.37 after angular transformation of the percentage data. One progeny plant had over a two times higher percentage fruit set than the mid parent.

EB had a significantly (P<0.05) lower pollen germination percentage than RS. Low percentage pollen germination was more prevalent among the progeny than high percentage pollen germination (Fig. 1). Of the progeny 75% had a lower percentage pollen germination than EB. The progeny mean was significantly (P<0.001) less than the mean of RS but not significantly different from the mean of EB (Table 1). Although low percentage pollen germination was more prevalent, 5% of the progeny had a higher percentage pollen germination than that of the RS parent.

Mean fruit weight of EB was significantly larger than that of RS (P<0.05). Mean fruit weight for the progeny was significantly (P<0.05) lower than the mean of RS, the small-fruited parent (Table 1; Fig. 1). Progeny fruit weight ranged from 2.3 to 8.8 g. Small fruit weight appeared to be dominant with 77% of the progeny having fruits smaller than those of the small-fruited parent. However, 1 progeny individual had fruits over 6 standard deviations larger than EB, the large-fruited parent.

Fruit from RS and EB had similar percentage soluble solids. The progeny distribution ranged from 9.8% to 20.1% soluble solids and was skewed towards the higher parental values (Fig. 1).

Heritability of traits

The broad-sense heritability estimates for bloom date, ripening date, pistil death, fruit weight and fruit soluble solids ranged from 0.91 to 0.50 (Table 2). The heritabili-

Table 2 Broad-sense heritability estimates for five traits evaluated over 3 years from the progeny in the sour cherry mapping population

Trait	H_{BS}
Bloom time	0.91
Ripening time	0.62
Pistil death	0.50
Fruit weight	0.88
Soluble solids	0.64

ty estimates from bloom time and fruit weight were relatively high, indicating that the values for the two traits were consistent over years. The lower heritability estimates for ripening time, soluble solids, and pistil death indicated a stronger genotype x year interaction for these three traits.

Correlation of traits

Three significant correlations were found among the traits analyzed. A significant (P<0.05) negative correlation was observed between bloom time and percentage pistil death (r=–0.25). Early flowering was also found associated with pistil freeze damage in almonds (Viti et al. 1994). Presumably, the earlier the flowers open, the more likely their pistils would be exposed to freezing temperatures. A significant (P<0.0001) negative correlation was found between bloom time and fruit weight (r=–0.45). This correlation may be associated with the polyploid origin of sour cherry. The two presumed progenitor species of the allotetraploid sour cherry are sweet cherry (*P. avium* L.) and ground cherry (*P. fruticosa* Pall.). Sweet cherry is early-blooming and large-fruited compared to ground cherry, which is late-blooming and small-fruited. Additionally, a significant (P<0.05) positive correlation was observed between percentage pistil death and fruit soluble solids concentration (r=0.24). The basis for this last correlation is unclear.

Genetic linkage maps

The RS and EB linkage maps identified 23 linkage groups. Fifteen linkage groups were a subset of the 19 linkage groups of the RS map, and the other 8 linkage groups were a subset of the 16 linkage groups of the EB map described previously (Wang et al. 1998). The RS and EB maps covered 398.2 cM and 222.2 cM, respectively, with an average interval length of 9.8 cM. The RS/EB map consisted of 17 linkage groups covering 272.9 cM with an average interval length of 4.8 cM.

QTL analysis

Eleven QTLs were identified for six traits: bloom time, % pistil death, % pollen germination, ripening time, fruit weight and soluble solids concentration (Table 3; Figs. 2–3). No QTLs were identified for flower bud death and % fruit set.

Two QTLs were identified for bloom time on two different linkage groups, EB 1 (*blm*1) and Group 2 (*blm*2) (Fig.2A, B). The QTL, *blm*1, explained 19.9% of the phenotypic variation. This QTL had the effect predicted by the parental phenotype, with an allele from the early-blooming parent, EB, reducing bloom time by 27.8 degree days. This QTL was the only QTL identified in this study that was consistently detected in each of the 3 years analyzed. The QTL *blm*2 explained 22.3% of the phenotypic variance and was detected in 2 of the 3 years and in all three years when the data were combined. The stabilities of the bloom time QTLs are likely due to the ease of scoring for this trait plus the conversion of the calendar day data to a heat accumulation value which reduces the variation among years. As a result, the bloom time data for all 3 years had the lowest average coefficient of variation (3.0%) of all the quantitative traits analyzed.

Table 3 QTLs detected for each trait. QTLs are named according to trait abbreviations, and a number is used to distinguish QTLs affecting the same trait. Data were based on the analysis of trait means over 3 years except for the trait percentage pollen germination and the QTL *pd*1

Trait	QTL	Linkage group[a]	Interval length (cM)	LOD peak position (cM)	Nearest marker	Maximum LOD	R^2 (%)	Genetic effect: a[b]
Bloom (degree day)	*blm*1	EB 1	>21.5	81.1	pS141	3.6	19.9	–27.8
	*blm*2	Group 2	>20.1	32.1	PLG86	3.3	22.3	–10.1
Pistil death (%)	*pd*1	EB 1	28.8	14.8	EF194c	2.6	12.9	– 2.1
	*pd*2	RS 8	>14.7	0.0	EF156b	2.7	14.3	1.5
Pollen germination (%)	*pgr*	EB 1	>14.0	4.0	EF146	3.0	17.0	1.4
Ripe (degree day)	*rp*1	RS 4	>10.0	0.0	EF158b	4.1	21.5	197.5
	*rp*2	Group 6	> 8.7	4.5	CPM20e	3.7	25.9	156.2
Fruit weight (g)	*fw*1	EB 4	26.5	10.01	EF182a	2.3	13.7	0.9
	*fw*2	Group 2	>20.1	32.1	PLG86	2.5	15.5	0.6
Soluble solids	*ssc*1	EB 7	> 6.0	0.0	AG10b	3.2	16.5	1.9
Concentration (° Brix)	*ssc*2	RS 6	25.8	23.1	EF159a	2.5	13.1	– 1.5

[a] Linkage groups as assigned in Wang et al. (1998)
[b] a, Additive value of the QTL

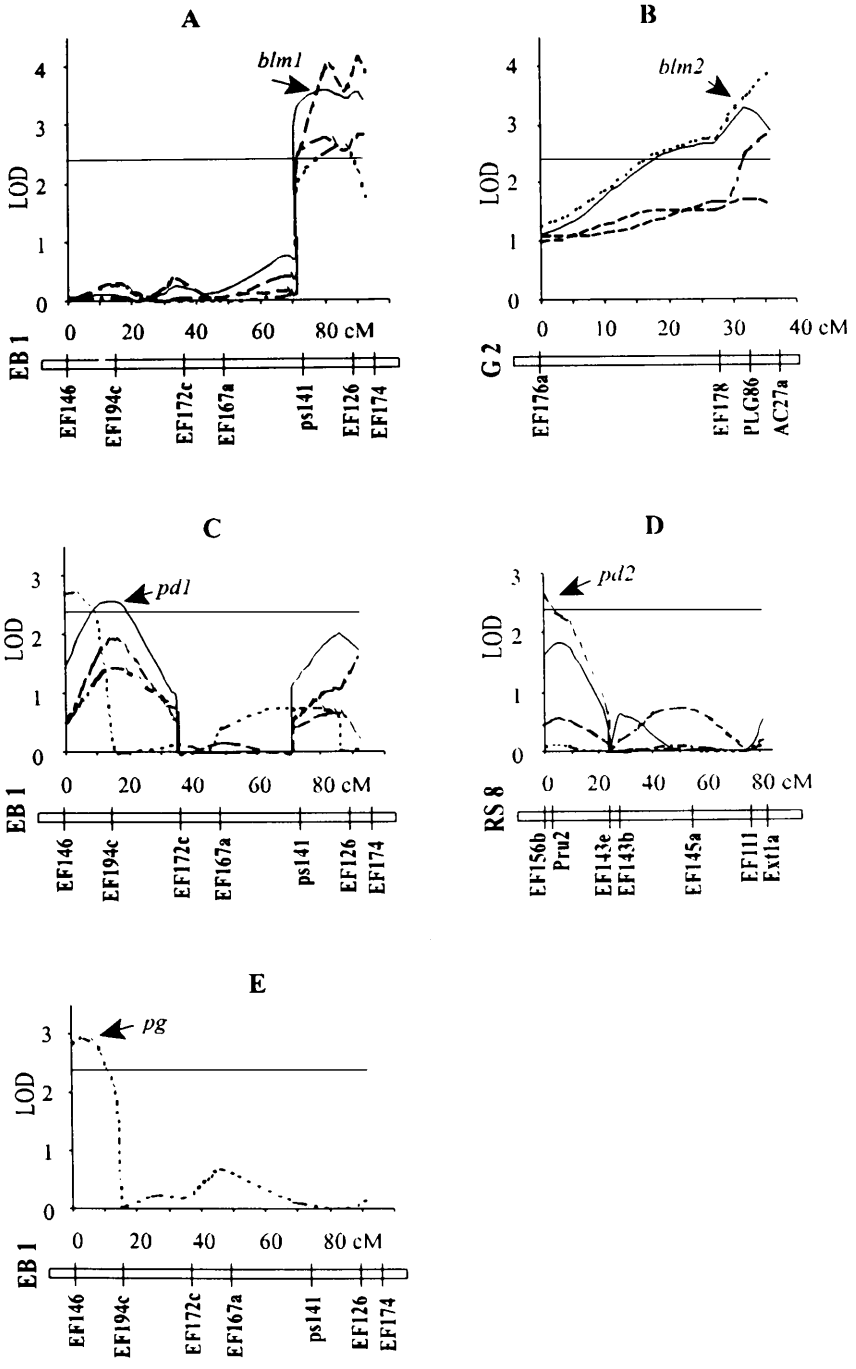

Fig. 2A–E LOD scores for bloom date on linkage groups EB 1 (*blm*1) **(**A**)** and Group 2 (*blm*2) **(B)**; pistil death (*pd*) on linkage groups EB 1 **(C)** and RS 8 **(D);** pollen germination percentage (*pg*) on linkage group EB 1 **(E)**. Peak LOD scores for each trait are indicated by *arrows*. Linkage groups are shown *below* the *x-axes*. The *horizontal line* indicates the level of significance at LOD=2.4. *Curves* represent results from individual years of 1995 (– – –), 1996 (-----), 1997 (–––), and over years (——)

Two QTLs were detected for percentage pistil death on linkage groups EB 1 (*pd*1) and RS 8 (*pd*2) (Fig. 2D). The QTLs *pd*1 and *pd*2 explained 12.9% and 14.3% of the phenotypic variance, respectively. Both QTLs had effects in the direction opposite to those predicted by the phenotype of the parents. An EB allele of *pd*1 reduced the percent pistil death by 2.1%, while a RS allele of *pd*2 increased percentage pistil death by 1.5%. The QTLs *pd*1 and *pd*2 were both detected with the threshold LOD score in only 1 of the 3 years analyzed and were identified in different years, 1995 and 1996, respectively.

Since pistil death in 1995 and 1996 was caused by freezing events that occurred at different stages of flower development, it is not surprising that different QTLs were identified for the different years. In 1995, the only damaging freezing event after bud break was –10°C which occurred 21 days before bloom. In contrast, there were two damaging freezing events in 1996. The first freezing event occurred 12 days before the population started blooming, when the temperature declined to –2.6°C for 11 h. The second freezing event was 4 days after the population started blooming, when the air temperature was below –1.5°C for 3 h. Consequently, the average percentage pistil death of the progeny population was larger in 1996 than in 1995, 40.9% and 8.7%, respectively.

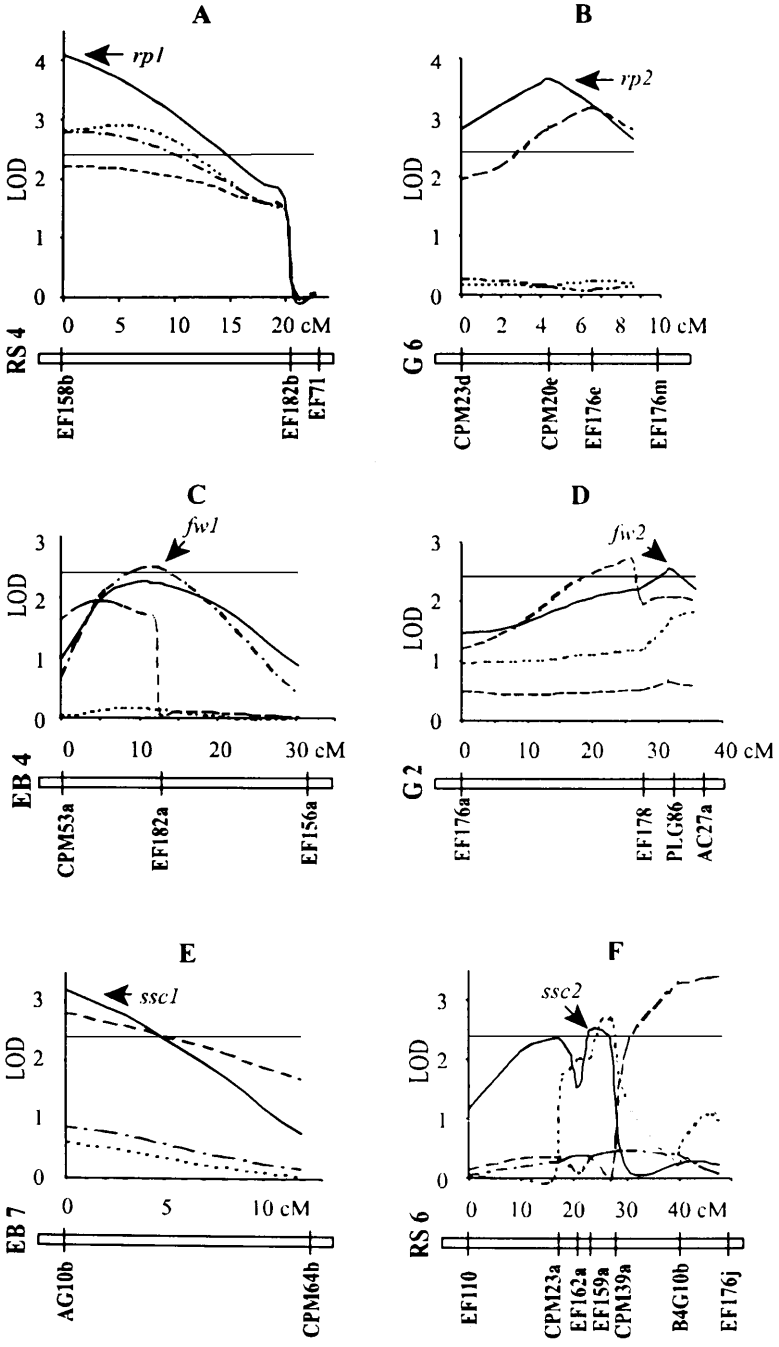

Fig. 3A–F LOD scores for ripening date on linkage groups RS 4 (*rp*1) **(A)** and Group 6 (*rp*2) **(B)**; soluble solids concentration on linkage groups EB 7 (*ssc*1) **(E)** and RS 6 (*ssc*2) **(F)**; fruit weight on linkage groups EB 4 (*fw*1) **(C)** and Group 2 (*fw*2) **(D)**. Peak LOD scores for each trait are indicated by *arrows*. Linkage groups are shown *below* the *x-axes*. The *horizontal line* indicates the level of significance at LOD=2.4. *Curves* represent results from individual years of 1995, 1996, 1997, and over years (see the legend for Fig. 2 for details)

One QTL, *pg*, was found for percentage pollen germination on linkage group EB 1 (Fig. 2E). This QTL explained 17.0% of the phenotypic variance. It had an effect opposite to that predicted by the phenotype of the parent, with an EB allele increasing the pollen germination rate by 1.4%.

Two QTLs were identified for ripening time on two different linkage groups, RS 4 (*rp*1) and Group 6 (*rp*2)(Fig.3A, B). The QTL *rp*1 was detected in 2 of the 3 years analyzed and was responsible for 21.5% of the phenotypic variance. This QTL had the effect predicted by the parental phenotype, with an allele from the late-ripening parent, RS, increasing ripening time by 197.5 degree days. The QTL *rp*2 was detected in 1 of 3 years and was responsible for 25.9% of the phenotypic variance.

Two QTLs were identified for fruit weight on two different linkage groups, EB 4 (*fw*1) and Group 2 (*fw*2) (Fig. 3C, D). The 2 QTLs were both detected with the threshold LOD score in only 1 of the 3 years analyzed and were identified in the same year, 1997. The QTLs *fw*1 and *fw*2 were responsible for 13.7% and 15.5% of the phenotypic variance, respectively. The effect of the QTL *fw*1 was in the direction predicted by the phenotype, with an allele from the large-fruited parent, EB, increasing fruit weight by 0.9 g.

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Pa    LGFRP NYKDG SYPSN CDPDS VFDKS EISEL MSNLE KNWPS LxCPS xNGFR
Pp    HGLWP NYKDG GYPSN CDPDS VFDKS QISEL LTSLN KNWPS LSCPS SNGYR
       *  * *****  **** ***** *****  ****    *  ***** * **   ** *

Pa    FWSHE WEKHG TC
Pp    FWSHE WEKHG TC
      ***** ***** **
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Fig. 4 Alignment of the amino acid sequences of the sweet cherry cv. Emperor Francis RNase (pS141) with the pear (*Pyrus pyrifolia*) non-S-RNase (D49529)(Norioka et al. 1996). The alignment maximizes homology at the nucleotide and amino acid sequence levels. The conserved amino acids are indicated by *asterisks*

Two QTLs were identified for soluble solids concentration on two different linkage groups, EB 7 (*ssc*1) and RS 6 (*ssc*2) (Fig. 3E, F). The QTL *ssc*1 was detected with the threshold LOD score of 2.4 using 1995 data and the average data of 1995, 1996, and 1997. The QTL *ssc*2 was detected with the critical LOD score of 2.4 using the data of 1995, 1996, and the average data of 1995, 1996, and 1997. The QTLs *ssc*1 and *ssc*2 explained 16.5% and 13.1% of the phenotypic variance, respectively. The 2 QTLs were from different parents and had opposite effects. An EB allele of *ssc*1 increased percentage soluble solids by 1.9° Brix, while a RS allele of *ssc*2 decreased percentage soluble solids by 1.5° Brix.

Previous QTL studies on other plant species have identified regions of the genome that seem to contain clusters of QTLs (Edwards et al. 1987; Fulton et al. 1997). In tomato, for example, a 25-cM region of linkage group 1 contained QTLs for many fruit quality traits (Fulton et al. 1997). In our study, QTLs affecting three flower traits, bloom time, pollen germination percentage, and pistil death in 1996, mapped to linkage group EB 1 (Fig. 2; Table 3). Two QTLs, *pg* and *pd*1, mapped at the lower end of the linkage group. The positions of the peak LOD scores for QTLs *pg* and *pd*1 were 10.8 cM apart; however, the intervals for the 2 QTLs overlapped. The third QTL, *blm*1, mapped to the other end of the linkage group closest to the RFLP marker pS141. Since pS141 is a clone derived from sweet cherry stylar cDNA (Iezzoni and Brettin 1998), a partial sequence was obtained to determine if this RFLP identified a putative gene. Following a BLAST search (Altschul et al. 1990) using 185 nucleotides, the closest nucleotide and amino acid similarity to pS141 was a non-S-allele RNase identified from pear stylar cDNA (Norioka et al. 1996). The pear RNase and pS141 have 81% amino acid homology, suggesting that pS141 also identifies a non-S-allele stylar RNase (Fig. 4). With the putative identification of pS141 as identifying a stylar RNase, 4 genes affecting floral traits mapped to EB 1.

In this study, 50% of the QTLs identified for the traits in which the two parents differed significantly had allelic effects opposite to those expected from the parental phenotype. Such a high percentage of QTLs with allelic effects opposite to those predicted from the parent may explain the common transgressive segregation observed for all traits analyzed. Each parent was likely to possess both favorable and unfavorable alleles of different QTLs affecting the same trait. Recombinations of favorable alleles as well as unfavorable alleles from both parents would most likely generate transgressive phenotypes. QTLs with effects opposite to those expected from parental phenotypes have been reported to be responsible for transgressive segregation in an interspecific tomato cross, where 36% of the QTLs had effects opposite to those predicted by the parental phenotypes and these QTLs were directly related to the appearance of transgressive individuals in the F_2 (de Vicente et al. 1993).

The QTLs detected for each individual trait explained from 17% to 47.4% of the phenotypic variance, with an average of 32.1%. These values are comparable to those from a QTL analysis of horticultural traits in tomato, where the cumulative action of all QTLs detected for each trait accounted for 12–59% of the phenotypic variation (Grandillo and Tanksley 1996). The extent of the phenotypic variance explained in our analysis is encouraging given the theoretical limitations of QTL mapping in a pseudo-testcross and a polyploid crop plus the present limited length of the sour cherry map.

For example, both sour cherry analyses were done with pseudo-testcross mapping populations. Since both parents in a pseudo-testcross can be heterozygous ($Q_1Q_2Q_3Q_4$+$Q_5Q_6Q_7Q_8$ for sour cherry), QTL identification in a pseudo-testcross population would theoretically be less likely than in a backcross-inbred population used in tomato since the effect of an individual allelic substitution would have to be sufficiently large to be identified in a segregating heterozygous background (Conner et al. 1998).

Additionally, identification of major QTL alleles is theoretically more difficult in a polyploid mapping population because in order to detect a QTL allele it would have to meet the same segregation requirement as a molecular marker; i.e., segregate as a single-dose restriction fragment (Wang et al. 1998). The simplest case meeting this requirement could be diagramed as $Q_1Q_2Q_2Q_2\times Q_2Q_2Q_2Q_2$. Given this requirement, which favors the detection of a unique QTL allele (i.e., Q_1), it is not unexpected that half of the QTL alleles identified in sour cherry contrasted to the parental phenotype. This requirement also makes it theoretically more difficult to identify the QTL allele contributing to the parental phenotype if this allele is present in at least two copies (i.e., Q_2). There is some speculation in allotetraploid cotton that this may be the case. In cotton, major QTL alleles

donated from the high value parent were not detected, presumably because they are present in more than one dose (Jiang et al. 1998). It is important to note, however, that a QTL locus can still be identified by mapping the allele that is present in a single dose.

Improved map coverage should increase our ability to identify QTLs and estimate their location. The RS and EB linkage maps used in the QTL analysis represent only approximately one-third of the estimated total sour cherry linkage map distance (Wang et al. 1998). In addition, the marker density in certain regions of the linkage maps was relatively low. For traits that exhibited little variation among years, such as bloom and ripening time, additional QTLs might have been identified if a more complete linkage map were available. Additionally, the one-LOD support interval lengths could not be determined for 5 of the QTLs (*rp*1, *blm*1, *ssc*1, *pd*2, and *pg*), because these QTLs mapped to the ends of the linkage groups (Table 1, Figs. 2, 3). Despite the limitations discussed above, the results confirm that significant QTLs can be identified for important flower and fruit traits in sour cherry.

It has been demonstrated in other plants that QTLs can be conserved among species and even across genera (Paterson et al. 1995). If QTLs were conserved within *Prunus* and then between *Prunus* and *Malus*, it might be possible to predict regions in other species that might be homologous to QTL regions in sour cherry. Sour cherry Linkage Groups 2, 4, 6, and 7, which contain QTLs for boom date, ripening date, fruit weight, and soluble solids, are suspected to be homologous to the peach and almond Linkage Groups 2, 4, 6 and 7 based on shared RFLP markers (Wang 1998). However, to date, fruit soluble solids is the only quantitative trait analyzed in this sour cherry study that has also been evaluated in peach (Dirlewanger et al. 1998). In both peach and sour cherry, a QTL for soluble solids was located on Linkage Group 6. This observation suggests that there might be a conserved QTL between these two *Prunus* species; however this possibility is still speculative since the density of shared markers was insufficient to resolve the comparative position of the peach and sour cherry soluble solids QTLs. If QTLs were conserved between sour cherry and peach it would greatly accelerate the pace of discovery of loci controlling important horticultural traits in both species since QTL locations in one species could be tested in the other species. This would be especially important for traits such as soluble solids which have a low heritability and, therefore, gain from selection may be improved by utilizing marker-assisted selection.

Unfortunately, the peach-almond homologue for sour cherry Linkage Group 1, which appears to have bloom-related traits, has not been identified. Due to the year to year stability in bloom time measurements and the universal importance of this trait in Rosaceous crops, bloom time would be an appropriate quantitative trait for QTL comparison among *Prunus* species and between *Prunus* and *Malus*.

The QTLs identified for bloom time are also the most promising QTLs for marker-assisted selection. These QTLs were consistently identified over years and the heritability for this trait is high, suggesting that there may be a limited number of loci controlling this trait. A major sour cherry breeding objective is to identify late-blooming selections that would have a greater likelihood of avoiding spring freeze events. In this study, both QTLs identified for bloom time had an early-blooming genetic effect. Currently these 2 QTLs are being tested in a second mapping population from a cross between a mid-season blooming variety and a very late-blooming variety to determine (1) whether these 2 QTLs can be validated in a second population, and (2) if late-blooming alleles of these 2 QTLs can be identified. If 1 or both of the QTLs identified for bloom time can be validated in the second mapping population and a QTL allele with a late-blooming effect can be identified, these QTLs would be useful for marker-assisted selection in the breeding program.

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