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BULK SEGREGANT ANALYSIS FOR BLOOM TIME QTL IN SOUR CHERRY (*Prunus cerasus* L.)

presented by

Ann Marie Bond

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Plant Breeding and Genetics/Horticulture

Major Professor's Signature

May 11, 2004

Date

BULK SEGREGANT ANALYSIS FOR BLOOM TIME QTL IN SOUR CHERRY (*Prunus cerasus* L.)

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture and Genetics

2004

ABSTRACT

BULK SEGREGANT ANALYSIS FOR BLOOM TIME QTL IN SOUR CHERRY (*Prunus cerasus* L.)

By Ann Marie Bond

Spring freeze damage to sour cherry (*Prunus cerasus* L.) flower buds is the major limiting factor to sour cherry production in the U.S. Significant crop reductions from spring freezes occur approximately every three years. In 2002, the losses to spring freeze damage were particularly devastating with 95% of the sour cherry crop in Michigan destroyed. One breeding approach to minimize the potential for freeze injury to sour cherry flowers is to develop late-blooming cultivars that would have an increased chance of avoiding spring freeze damage. Fortunately there is extensive variation for bloom time in sour cherry with extremely late blooming germplasm available.

The goal of this project was to investigate the inheritance of bloom time in tetraploid sour cherry (2n = 4x = 32) using a QTL approach. The progeny population used was from a cross between 'Balaton' and the late blooming sour cherry cultivar 'Surefire'. QTL discovery was done using a bulk segregant approach comparing late and early blooming progeny individuals using SSR and AFLP markers. Using this approach, an AFLP marker was identified that was significantly linked to a putative QTL, termed *blm*3, controlling bloom time. The late allele, contributed by the late blooming parent 'Surefire', explained 16.5% of the phenotype variance and delayed bloom time by approximately 15 degree days. Future characterization of this QTL and other QTL controlling bloom time may suggest ways to delay bloom in sour cherry and other Rosaceous species, thereby reducing the probability of spring freeze damage and crop loss.

DEDICATION

I would like to dedicate my thesis to the men and women of the United States Armed Forces who unselfishly risk their lives, home and abroad, to preserve the way of life that I have the liberty and opportunity to enjoy.

ACKNOWLEDGEMENTS

I am very grateful to my advisor, Dr. Amy Iezzoni, for her guidance, financial support, and editorial assistance. I would like to thank my committee, Dr. Dechun Wang, Dr. Jim Hancock, and Dr Dave Douches, for their patience, understanding, and last minute advice. Also, I would like to extend countless thanks to members of both the Iezzoni lab and the Hancock lab – your wonderful sense of humor, words of advice, and constant guidance were of immeasurable value.

To my roommates Lee Ann Pramuk and Janelle Glady – without whom I'd never have gotten his far – thanks so much for strawberries and coconuts! I would also like to thank the 7th Michigan Volunteer Infantry Company B for their constant support and love – you have become my Michigan family and will forever have a special place in my heart.

And finally, Mom, Dad, Sarah, and Seth – Thanks for all the constant support, patience, and love. You have helped me attain the seemingly impossible by believing in me when I needed it the most! Bluebirds and Dandelions to you always!

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I. INTRODUCTION

Spring freeze damage to sour cherry (*Prunus cerasus* L., 2n = 4x) flower buds is the major limiting factor to sour cherry production in the United States. (Ricks, 1992). Significant crop reductions from spring freeze events occur approximately every three years. In 2002, the losses to spring freeze damage were particularly devastating with ~95% of the Michigan sour cherry crop destroyed (Kleweno and Matthews, 2003). The genetic uniformity of sour cherry production in the U.S., a monoculture of the 400 year old cultivar Montmorency, also set the stage for such a disaster.

Within the flower, the pistil is the most sensitive tissue, and in the vast majority of freeze events, the pistil tissue is killed while the other flower tissues remain un-injured (Dennis and Howell, 1974). For example, sour cherry leaves can cold acclimate to survive temperatures of -13.2°C, where as cherry pistils are killed at temperatures between -2 to -3°C (Owens et al., 2001). Initial investigations suggest that cherry pistils may be deficient in their ability to cold acclimate due to absence in *CBF1* activity (Owens et al., 2002). *CBF1* is a transcription factor that has been demonstrated to be required for cold acclimation in other plant species (Jaglo et al., 2001).

One breeding approach to minimize the potential for freeze injury to sour cherry flowers is to develop late-blooming cultivars. Since flowers become increasingly sensitive to freeze injury as they develop in the spring (Ballard et al., 1971), late blooming cultivars have an increased chance of avoiding spring freeze damage. Fortunately there is extensive variation in sour cherry for bloom time, as well as available

germplasm that blooms significantly later than Montmorency (Iezzoni and Hamilton, 1985; Iezzoni and Mulinix, 1992).

Previous studies in *Prunus* species identified Quantitative Trait Loci (QTL) for bloom time. In an inter-specific peach x almond cross, a major QTL for bloom time (called *Lb*) was identified on linkage group 4 (Arus et al., 1999; Ballester et al., 1998). In a peach cross, 6 QTL for flowering time were identified with a large QTL on Group 5 (Yamamoto et al., 2001). In the sour cherry cross Rheinische Schattenmorelle x Erdi Botermo, two QTL for bloom time were identified (Wang et al., 2000). However, the alleles identified at these loci, *blm*1 and *blm*2, conferred early bloom. A second cherry cross, Balaton x Surefire, was made to develop a pseudo-testcross population for the identification of late blooming QTL allele. Surefire was chosen as a parent because it is one of the latest blooming sour cherry selections.

Since bloom time in sour cherry has a high heritability (Wang et al., 2000) and QTL analyses have been successful in *Prunus*, we hypothesize that it should be possible to identify additional or orthologous QTL for bloom time in the Balaton x Surefire cross. Our objective was to identify QTL controlling bloom time and the alleles at these QTL that contribute to late bloom. QTL discovery was done using a bulk segregant approach (Mikas et al., 1996; Michelmore et al., 1991) comparing late and early blooming progeny individuals with Simple Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) marker analysis.

II. LITERATURE REVIEW

A. Origin and history of sour cherry

Cherries are members of the Rosaceae family, which as a whole, is economically important in the globe's temperate regions for both their fruits and ornamentals. Cherries have been valued since ancient times as one of the first tree fruits to ripen in temperate growing regions. The two main groups of cherries, the diploid sweet (*Prunus avium*, 2n = 2x = 16) and tetraploid sour cherries (syn. tart cherries, 2n = 4x = 32), are reported to have originated in an area that includes Asia minor, Iran, Iraq, and Syria (Watkins, 1976). The first diploid *Prunus* species arose in central Asia: sweet, sour, and ground cherry *Prunus fruticosa*, (a low growing bush cherry native to Russia) were early derivatives of this ancestral *Prunus*. Sour cherry is believed to have arisen through natural hybridizations between ground cherry and sweet cherry (Olden and Nybom, 1968).

B. Floral development and freeze susceptibility

Cherry production is limited to temperate regions that experience moderately cold winter temperatures. The cherry tree requires a dormancy period each year that begins with defoliation in the fall. Spring growth starts when the dormancy or chilling requirement has been satisfied and temperatures increase sufficiently in the spring. In colder regions, cherry production is limited by cold mid-winter temperatures. For example, temperatures below -30°C can result in wood or flower bud injury, particularly in the more cold-sensitive sweet cherries. However, in some cherry growing regions, low-temperature damage to flower buds is the most important factor limiting yields.

Vegetative bud burst and flowering occur in the spring in response to warm temperatures favorable for rapid plant growth (above 10°C). Individual cherry flowers are perfect, consisting of sepals, petals, stamens, and a pistil. The stamen is made up of a filament and a pollen-bearing anther where as the pistil is comprised of the stigmatic surface, the style and the ovary containing a pair of ovules. In *Prunus*, one ovule typically develops into the seed while the other ovule aborts very early. The ovary develops into the fleshy pericarp of the drupe fruit.

Since flowers become increasingly sensitive to freeze injury as they develop in the spring (Ballard et al., 1971; Dennis and Howell, 1974), late blooming cultivars have an increased chance of avoiding spring freeze damage. Therefore, late bloom is a common goal in *Prunus* breeding programs (Bailey and Hough, 1975).

Differences in bloom time among selections are frequently compared using heat accumulation above a given threshold to reach a designated pheonological state (Baskerville, 1968; Baskerville and Emin, 1969). The resulting calculated "degree-days" is therefore a more useful comparative measure for bloom time than calendar days. Since all the flower buds on a tree do not open at the same time, bloom time is sometimes evaluated when 50 percent of the blossoms are estimated to be open, i.e. 50% bloom (Wang et al., 2000).

C. Variation in bloom time

Sour cherries offers a unique opportunity to study late bloom in *Prunus*, as sour cherry and its parental species, *P. fruticosa*, are the latest blooming commercially important *Prunus* species (Iezzoni et al., 1990). Presumably *P. fruticosa* evolved

extremely late bloom time to avoid spring freeze damage in its native habitat in central Russia. Due to continued introgression between sour cherry and *P. fruticosa*, there is extensive variation in sour cherry for bloom time, including germplasm that blooms significantly later than Montmorency (Iezzoni and Hamilton, 1985; Iezzoni and Mulinix, 1992).

D. Genetic analysis of bloom time in *Prunus*

Various linkage maps have been created in *Prunus* species; however, most of the linkage maps have been constructed from diploid crosses among peach selections or between peach and almond (Arus et al., 2003; Ballester et al., 1998; Yamamoto et al., 2001). Recently, SSRs (syn. micro satellite markers) have been suggested to be a valuable marker resource for comparative mapping (Aranzana et al., 2001). For example, 109 SSRs that were originally developed for peach and cherry, are being mapped on an almond x peach F_2 population. They tested the marker variability between 25 cultivars (14 peach and 11 nectarine) and found 24 of the 35 SSRs to be polymorphic and consequently could distinguish between all of the individuals in the test (Aranzana et al., 2001). Testolin (2000) did similar research studying genetic origin and fingerprinting in peach, and all were polymorphic. Of the 26 SSR primer pairs they tested, 17 exhibited Mendelian inheritance. SSRs from peach and cherry were also found to be useful to distinguish among sour cherry germplasm accessions (Cantini et al., 2001). Additionally, AFLPs have been used to increase marker coverage and gene tagging.

Unfortunately publication of linkage maps from different researchers originally resulted in different numbers associated with corresponding linkage groups. For example,

numbered consecutively, the nomenclature used by European researchers for chromosomes 1, 2, 3, 4, 5, 6, 7, and 8 (Aranzana et al., 2003), are numbered 5, 2, 4, 6, 8, 3, 1, and 3, in a Japanese peach map [Groups 8 and 6 are shown as one linkage group (3)] (Yamamoto et al., 2001). This complicates the comparative QTL analysis. Fortunately, since 2002, the nomenclature of the European groups, as first published by Joobeur et al. (2000), has been adopted as the official nomenclature for *Prunus*.

Despite the preliminary stages of linkage mapping in diploid *Prunus*, QTL for bloom time have been reported independently by three groups (Ballester et al., 1998; Yamamoto et al., 2001; Wang et al., 2000). In an inter-specific peach x almond cross, a major QTL for bloom time (*Lb*) was identified on linkage group 4 (Arus et al., 1999; Ballester et al., 1998). In a peach cross, 6 QTL for flowering time were identified with a large QTL on the Japanese peach map, Group 5 (Yamamoto et al., 2001). In the sour cherry cross Rheinische Schattenmorelle x Erdi Botermo, two bloom time QTL were identified (Wang et al., 2000); however, the alleles *blm*1 and *blm*2 conferred early bloom. The objective of this study is to identify QTL controlling bloom time, but also to identify the alleles at these loci that may contribute the desired late bloom phenotype.

Because bloom time in sour cherry has a high heritability (Wang et al., 2000) and QTL analyses have been successful in *Prunus,* we hypothesize it should be possible to identify additional or orthologous QTL for bloom time in a second sour cherry cross. This second cherry cross, Balaton x Surefire, was designed to assist in the identification of late blooming alleles as Surefire is one of the latest blooming sour cherry selections. Marker analyses of the progeny of this cross would initially consist of markers previously been demonstrated to be linked to published bloom time QTL (Arus, 2003; Bliss et al.,

2002; Wang et al., 1998; Yamamoto et al., 2001). Most of these markers are SSRs. Since these markers have been previously mapped in other *Prunus* species, it should be possible to determine if marker order and distance have been conserved between sour cherry and the other *Prunus* species. If necessary, genome coverage to look for additional QTL could be accomplished with AFLP analyses as was performed in peach to map a gene associated with nematode resistance (Lu et al., 1998).

QTL identification in a heterozygous polyploid crop such as sour cherry is more challenging than in a diploid. Only markers that meet the criteria of a single dose restriction fragment can be placed on the linkage map, and most loci are duplicated in a polyploid. This requires that only markers in simplex in one or both parents can be mapped. QTL alleles must also meet this criteria for linkage mapping.

Because identifying QTL in a polyploid species can be quite a challenge, the strategy is to use a bulk segregant analysis (Michelmore et al., 1991), whereby the initial goal is to identify marker polymorphisms that differ between a group of late blooming and early blooming selections. Bulk segregant analysis has previously been used to identify markers associated with QTL for quantitative traits (Mansur et al., 1993; Mikas et al., 1996). The association of marker(s) with a QTL for bloom time would subsequently be tested by scoring all progeny for the marker of interest.

III. MATERIALS AND METHODS

A. Plant material.

The two sour cherry cultivars used as parents to produce the population for QTL analysis were 'Balaton' and 'Surefire'. Balaton is a landrace Hungarian variety called Ujfehertoi Furtos. Surefire is a cultivar released from the New York State Agricultural Experiment Station, Cornell University, from a cross between Borchert Black Sour x NY 6935 (Richmorency x Schattenmorelle) (R. Andersen, pers. comm.). Surefire was chosen as the late blooming parent as it is one of the extremes for this trait. The Balaton x Surefire cross was made in 1996. One hundred ninety-seven (197) seedlings were planted at the Michigan State University Clarksville Horticultural Experimental Station (CHES), Clarksville, Michigan in the spring of 1998. The seedlings were planted in field number 27e and spaced within a row 1.5 meters apart, with the rows 6.1 meters apart. The following progeny individuals were not included in this study as they are suspected to have resulted from out-crossing or self-pollination [designated by row(tree)]: 1(65), 2(20), 2(34), 2(54), 3(06), 3(11), 3(35), 4(08), 4(23), 4(36), 4(39), 4(47) (A. Sebolt and N. Hauck, pers. comm.). With these individuals eliminated, the final population size was 185. The following progeny have not yet been genotyped for their *S*-alleles: 2(5), 2(10), 2(14), 2(21), 2(22), 2(28), 2(29), 2(30), 2(31), 2(46), 2(49), 2(60), 3(9), 3(10), 3(13), 3(23), 3(32), 3(43), 3(50), 3(51), 3(54), 3(56), 3(60), 4(1), 4(4), 4(5), 4(17), 4(18), 4(19), 4(20), 4(30), 4(33), 4(37), 4(50), 4(51), 4(52), 4(53), 4(54), 4(59), 4(60), 4(63), 4(64), 4(65), and 4(66).

B. Evaluation of bloom time.

Bloom time observations were made daily (at 10AM beginning mid April) to record the date when approximately 50% of the flowers had opened for each seedling tree. Time to bloom was expressed as degree days (DD) from January 1 with a base temperature of 4.4°C using hourly temperature readings collected at the Automated Weather Station located at CHES (Baskerville and Emin, 1969). Daily heat unit accumulation was calculated by summing the positive differences of hourly temperature readings minus 4.4°C, then dividing by 24. On the date of 50% bloom, heat unit accumulation was calculated to hour 10, the approximate time data was recorded. Bloom time was evaluated over five years (1999 - 2003).

C. Determination of early and late blooming bulk populations.

In order to divide the population into two extreme phenotypic groups to form the early and late bulks, the progeny were organized chronologically for each year using the date of 50% bloom for each individual. For example, in 2003, the earliest blooming progeny [individuals 3(24) and 3(25)] reached 50% bloom on April 28th or 177.2 DD. This day was recorded as day "a". April 29th was recorded as day "b" and so on until day "e". This was performed for each year of available bloom data. A table was made including a list of progeny with their bloom date for each year and the corresponding bloom day letter code. Progeny with consistent early blooming dates and late blooming dates were chosen to form the "early bulk" and "late bulk", respectively. The individuals in the early and late bulks were screened using the SSR and AFLP markers described

below. Any marker(s) that was found to be present in one bulk but absent in the other, was subsequently genotyped for the entire progeny population of 151.

D. DNA isolation:

Young leaves were collected from the parents and each progeny individual, placed immediately on dry ice, transported back to the laboratory, and placed in the -80°C freezer for 24 hours. The samples were then freeze-dried for at least 48 hours and then frozen at -20°C for storage. DNA was isolated using the CTAB method described by Stockinger et al. (1996).

E. SSR analysis:

Twenty-eight SSR primer pairs from both cherry and peach with known *Prunus* linkage map positions were used to screen the parents and bulk progeny individuals (Aranzana et al., 2001; Cantini et al., 2001; Cipriani et al., 1999; Dirlewanger et al., 2002; Sosinksi et al., 2000; Testolin et al., 2000; Wunsch et al., 2002) (Table 1). In an attempt to target those regions where bloom time QTL had previously been reported in *Prunus*, 10, 5, and 3 SSR primer pairs that map to *Prunus* linkage groups 1, 4, and 2, were tested (respectively). Primer pairs were selected by proximity (within 20 cM) to the published QTL. PCR amplifications were performed in a Perkin Elmer Cetus DNA Thermocycler 480 following procedures described in Cantini et al. (2001). To test the success of the PCR reaction, PCR products were separated by electrophoresis in 1% agarose gels with 0.1% ethidium bromide. For genotyping, the PCR products were mixed with 7.6 ul of formamide dye (10 ml 98% formamide, 200 ul 0.5mM EDTA, 10 mg

Table 1. *Prunus* linkage group(s) and reference information for the twenty-eight SSR primer pairs used in this study – Linkage groups are numbered as in Joobeur et al. (2000).

Primer Pair	Linkage Group (s)	Reference
BPPCT008	G6	Dirlewanger et al. (2002)
BPPCT015	G4	Dirlewanger et al. (2002)
BPPCT021	G1	Dirlewanger et al. (2002)
BPPCT023	G4	Dirlewanger et al. (2002)
BPPCT038	G5	Dirlewanger et al. (2002)
BPPCT040	G4	Dirlewanger et al. (2002)
CPPCT002	G3	Aranzana et al. (2001)
CPPCT003	G1 and G4	Aranzana et al. (2001)
CPPCT026	G1	Aranzana et al. (2001)
CPPCT027	G1	Aranzana et al. (2001)
CPPCT034	G1	Aranzana et al. (2001)
PceGA25	G5	Cantini et al. (2001)
PceGA59	G1	Cantini et al. (2001)
PceGA34	G2	A. Iezzoni (pers. comm..)
pchgms3	G1	Sosinski et al. (2000)[y]
pchgms5	G6	Sosinski et al. (2000)[y]
PMS2	G7	Cantini et al. (2001)
PMS3	G4	Cantini et al. (2001)
PMS67	G1	Cantini et al. (2001)
UDP96-008	G3	Cipriani et al. (1999)[z]
UDP96-018	G1	Cipriani (1999)[z]
UDP97-403	G3	Cipriani (1999)[z]
UDP98-022	G1	Testolin et al. (2000)[y]
UDP98-025	G2	Testolin et al. (2000)[y]
UDP98-405	G7	Cipriani (1999)[z]
UDP98-409	G8	Cipriani (1999)[z]
UDP98-411	G2	Testolin et al. (2000)[y]
UDP98-412	G6	Testolin et al. (2000)[y]

[z] Further used in *Prunus persica* and *P. avium* by Testolin et al. (2000), Wünsch et al. (2002).

[y] Further used in *P. persica* L. and *P. avium* L. by Wünsch et al. (2002).

bromophenol blue, 10 mg Xylene Cyanol), heated at 95°C for 5 minutes and immediately placed on ice. Five microliters of each sample were loaded on a denaturing 6% polyacylamide gel. The samples were then electrophoresed at 80 watts for 2.5 hours and stained using the Silver Staining Protocol from the Promega Technical Manual. (Promega Corporation; Madison, Wisconsin)

F. AFLP analysis:

DNA digestion, adaptor ligation, pre-selective amplification and selective amplifications were carried out according to standard procedures described by Vos et al. (1995). Primer combinations included three selective bases for one primer (*Mse* I) and both two and three selective bases for the other (*Eco* RI) (Table 2). PCR products were first checked for successful amplification by electrophoresis in 1% agarose gels stained with 0.1% ethidium bromide. The PCR products were then prepared for polyacrylamide gel electrophoresis by mixing the reaction products with 7.6 ul of formamide dye (10 ml 98% formamide, 200 ul 0.5mM EDTA, 10 mg bromophenol blue, 10 mg Xylene Cyanol), heated at 95°C for 5 minutes and immediately placed on ice. Five microliters of each sample were loaded on a denaturing 6% polyacrylamide gel. The samples were then electrophoresed at 80 watts for 2.5 hours and stained using the silver staining protocol according to Promega (Promega Corporation; Madison, Wisconsin).

G. QTL analysis:

A Nested Factor Design was used for a Single Marker QTL Analysis to determine if any marker is associated with bloom time. The linear additive model for the Analysis

of Variance is presented below. The two years represented the replications for this model. Computations were done using SAS (SAS Institute, Inc., 1999). Input files can be found in Appendix A.

$$y_{ijkl} = \mu + group_i + year_j + tree(group)_k + year*group_{ij} + e_{ijkl}$$

where y_{ijkl} = date of bloom in degree days

μ = grand mean

$group_i$ = marker present/absent by genotype

$year_j$ = replications, 2002 and 2003

$tree(group)_k$ = progeny nested within the group

$year*group_{ij}$ = interaction

e_{ijkl} = years

Table 2. Selective nucleotide sequences used for AFLP analyses with the restriction enzymes *Eco* RI and *Mse* I.

Two base pairs	Three base pairs
Eco RI + AA / *Mse* I + CAA	*Eco* RI + AAA / *Mse* I + CGT
Eco RI + AA / *Mse* I + CAC	*Eco* RI + AGG / *Mse* I + CAC
Eco RI + AC / *Mse* I + CAA	*Eco* RI + ATA / *Mse* I + CAC
Eco RI + AC / *Mse* I + CAC	*Eco* RI + ATA / *Mse* I + CCG
Eco RI + AC / *Mse* I + CAG	*Eco* RI + ATA / *Mse* I + CCT
Eco RI + AG / *Mse* I + CAA	
Eco RI + AT / *Mse* I + CAC	
Eco RI + AG / *Mse* I + CAC	
Eco RI + AT / *Mse* I + CAA	
Eco RI + AT / *Mse* I + CAC	

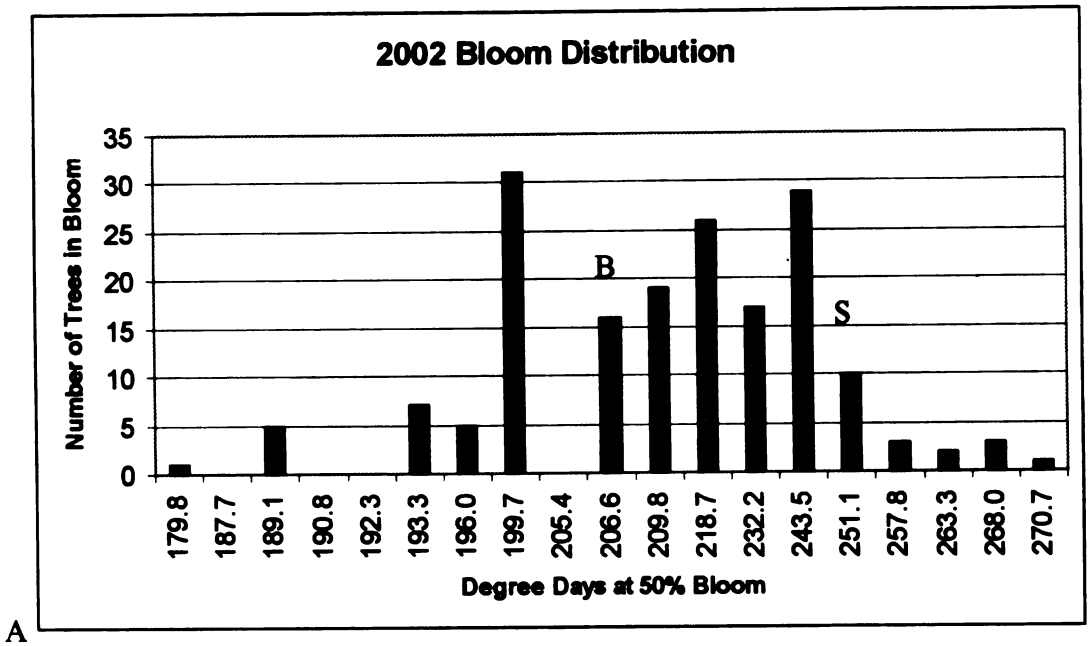
IV. RESULTS

A. Variation in bloom time

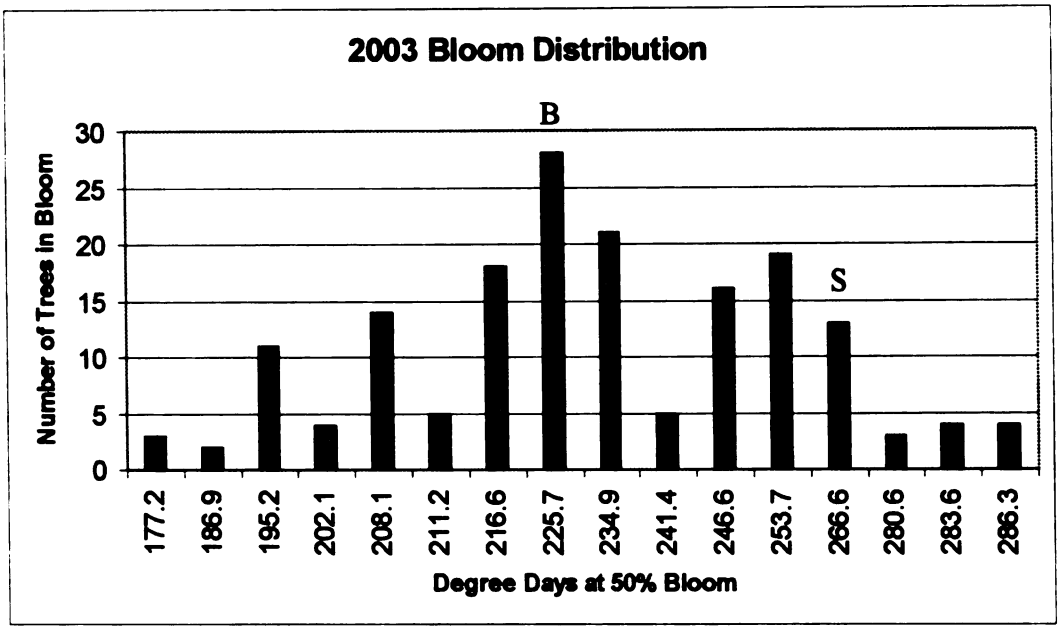
Bloom time was recorded for all blooming progeny in the Balaton x Surefire population over five years (A. Iezzoni, unpublished data) (Appendix E). The number of blooming progeny reached a maximum of 175 individuals in 2002. In 1999, only 33 of the 3 year old seedlings bloomed followed by 85 individuals in 2000, 143 individuals in 2001, 175 individuals in 2002 and finally, 170 individuals in 2003. This trend likely represents the differences in the length of juvenility period among the seedlings with all the seedlings flowering by age seven. To maximize the number of progeny evaluated only year 2002 and 2003 bloom data was used in the Single Marker Analysis (Appendix A for SAS input code).

Bloom data was converted from calendar days to degree days in order to compare bloom time over years on a heat unit basis. The progeny distribution for bloom time spanned 90.9 heat units in 2002 and 109.1 heat units in 2003 (Figure 1). The heat units accumulated for 50% bloom differed significantly between the two years; however, the year x progeny interaction was not significantly different over the two years. Balaton reached 50% bloom earlier than Surefire, exhibiting differences of 44.5 degree days in 2002 and 40.9 degree days in 2003.

The progeny exhibited transgressive segregation for both early and late bloom time (Figure 1). In 2002, 28% of the progeny bloomed earlier than the early blooming Balaton parent and 5% of the progeny bloomed later than the late blooming Surefire parent. In 2003, 34% of the progeny bloomed earlier than Balaton and 6% of the progeny



A



B

Figure 1. Frequency distributions of 50% bloom for the progeny population in 2002 (A) and 2003 (B). Bloom dates for the parents Balaton and Surefire are identified by B and S, respectively. Degree days were calculated in Celsius with a base temp of 4.4°C.

bloomed later than Surefire. These early and late blooming progeny individuals provided the opportunity to select individuals for bulk segregant analysis.

B. Selection of early and late blooming bulk populations

In order to divide the population into two extreme phenotypic groups to form the early and late bulks, the progeny were organized chronologically for each year using the date of 50% bloom for each individual. The population was divided into two extreme groups (denoted early and late) and the bloom data was organized chronologically for each year, the date of 50% bloom for each individual. Nine progeny with consistent early blooming dates were chosen to be a part of the "early bulk" for marker analysis: 2(6), 2(61), 3(2), 3(24), 3(25), 3(46), 3(54), 4(31), and 4(34) (Table 3). The range in heat units to reach 50% bloom of the individuals chosen for the early bulk was 177.2 to 216.6 degree days in 2003. The progeny population mean was 232.9 degree days. These selected progeny had noticeably earlier bloom time in comparison to the population mean over all years.

Table 3. Bloom data code and degree days (°C) to reach 50% bloom for the nine progeny selected for the early bulk (- = no data, letters refer to bloom code as described in Materials and Methods).

Early bulk	1999		2000		2001		2002		2003	
	rank	hours	Rank	hours	rank	hours	rank	hours	rank	hours
2(6)	f	226.7	c	221.8	b	188.7	-	199.7	-	211.2
2(61)	a	187.2	c	221.8	b	188.7	a	179.8	c	195.2
3(2)	-	-	c	221.8	b	188.7	f	193.3	-	216.6
3(24)	b	192.4	a	211.8	b	188.7	c	189.1	a	177.2
3(25)	-	-	-	-	-	-	f	193.3	a	177.2
3(46)	-	-	-	-	b	188.7	c	189.1	b	186.9
3(54)	-	-	-	-	c	205.3	c	189.1	e	208.1
4(31)	-	-	e	234.5	a	178.7	c	189.1	c	195.2
4(34)	-	-	d	228.1	b	188.7	f	193.3	a	177.2

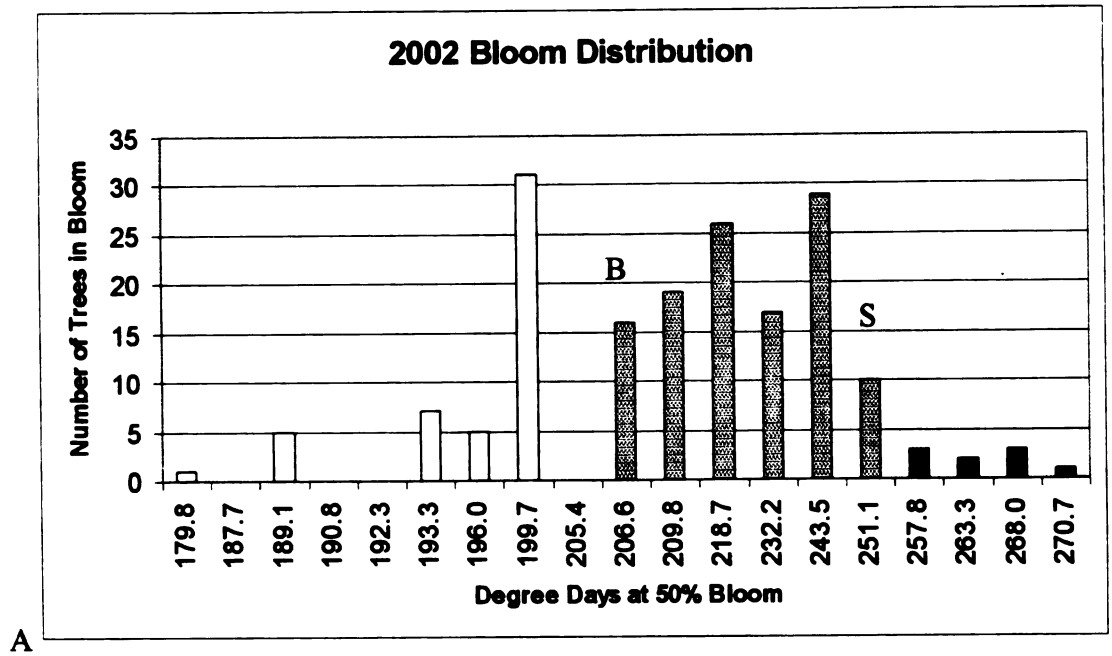
Similarly for late bloom, the last day of 50% bloom for the population in 2003 was May 13th or 286.3 DD. This day was recorded as day "z", May 12th was recorded as "y" and so on until day "v". Nine progeny with consistent late blooming dates were chosen to be part of the "late bulk" for marker analysis: 2(7), 2(39), 3(26), 3(51), 4(22), 4(24), 4(32), 4(45), and 4(66) (Table 4). The nine progeny individuals selected for each bulks represented 4.9% of the total population. The range in heat units to reach 50% bloom of the individuals chosen for the late bulk was 246.6 to 286.3 degree days in 2003. The progeny population mean was 232.9 degree days.

Table 4. Bloom data code and degree days (°C) to reach 50% bloom for the nine progeny selected for the late bulk (- = no data, letters refer to bloom code as described in Materials and Methods).

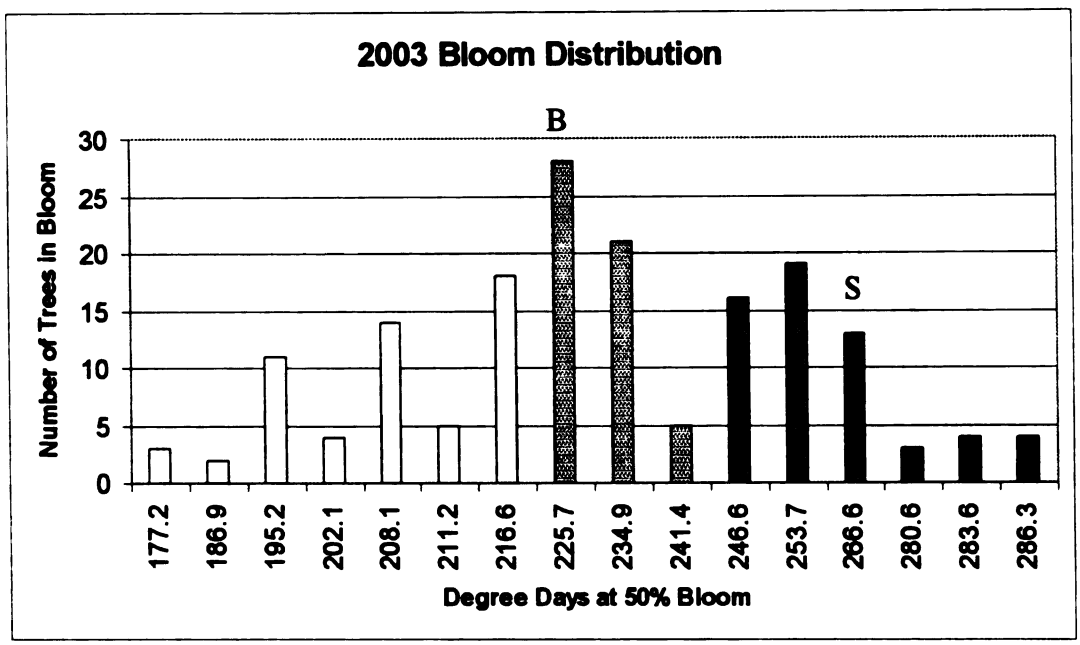
Late bulk	1999		2000		2001		2002		2003	
	rank	hours	Rank	hours	rank	hours	rank	hours	rank	hours
2(7)	v	284.5	w	289.7	-	239.3	x	263.3	w	266.6
2(39)	-	-	z	343.4	z	274.8	y	268.0	y	283.6
3(26)	-	-	-	-	-	-	-	-	z	286.3
3(51)	-	-	-	-	x	255.9	y	268.0	-	246.6
4(22)	z	318.8	z	343.4	x	255.9	w	257.8	z	286.3
4(24)	-	-	y	324.1	z	274.8	z	270.7	z	286.3
4(32)	-	-	-	-	-	221.8	w	257.8	w	266.6
4(45)	-	-	x	306.6	x	255.9	v	251.1	x	280.6
4(66)	-	-	x	306.6	-	-	y	268.0	z	286.3

C. SSR analyses:

The SSR genotypes for 28 primer pairs were determined for the two parents and, individually, for the 17 progeny in the early and late bulks. No fragments were amplified with five of the primer pairs (Table 5). Considering the remaining primer pairs tested, the number of fragments amplified from these sour cherry individuals ranged from 0 to 9



A



B

Figure 2. Frequency distributions of the degree days needed for the progeny individuals to reach 50% bloom in 2002 (A) and 2003 (B). The degree days for the parents Balaton (B) and Surefire (S) are shown as inserted letters respectively. White bars indicate degree days for progeny within the early bulk and dark grey bars for the respective late bulk.

Table 5. SSR primer pairs, fragments observed, and percent polymorphic fragments for Balaton, Surefire, and the 18 individuals in the early and late bloom bulk populations.* (see Appendix F for SSR genotyping data).

Primer combination	No. of fragments	Observed monomorphic fragments (size in base pairs)	Observed polymorphic fragments (size in base pairs)	Percent polymorphism
BPPCT0008	9	96, 100, 102, 109, 120, and 127	94, 98, and 115	3/9 = 0.33
BPPCT015	4	160	132, 158, and 167	3/4 = 0.75
BPPCT021	2	230 and 234		-
BPPCT038	7	107, 110, 113, 118, 120, 130, 135		-
CPPCT003	6	248, 250, 260	230, 240, 330	3/6 = 0.50
CPPCT026	2	157	155	1/2 = 0.50
CPPCT027	4	68, 70, 84, 86		-
CPPCT034	6	178, 180	154, 156, 190, 192	4/6 = 0.60
PceGA25	2	168, 170		-
PceGA59	7	182, 184, 190, 195	178, 228, 230	3/7 = 0.43
pchgms3	6	178, 186	176, 180, 184, 190	4/6 = 0.60
pchgms5	2	168, 170		-
PMS2	6	135, 138	130, 132, 141, 145	4/6 = 0.60
PMS3	5		158, 160, 186, 188, 190	5/5 = 1.00
UDP96-008	4	128, 130, 132, 135		-
UDP96-018	6	202, 204, 254, 256, 262, 264		-
UDP97-403	4	138, 140, 142	100	1/4 = 0.25
UDP98-022	7	92, 100, 102, 104	94, 96, 98	3/7 = 0.43
UDP98-405	5	105	102, 104, 109, 115	4/5 = 0.80
UDP98-409	3	122	118, 120	2/3 = 0.66
UDP98-411	4	132, 133, 140, 162		-
UDP98-412	4	98, 100, 120, 122, 124		-

*no amplification was observed for primer pairs BPPCT023, BPPCT040, CPPCT002, PceGA34, and UDP98-025

(Table 5). Nine SSR primer pairs produced only monomorphic fragments, while the other 14 primer pairs amplified fragments were polymorphic among the parents and progeny tested (Appendix F). None of the polymorphic SSR fragments were consistently present in individuals from one bulk and absent from individuals in the other bulk. Therefore, this SSR analysis did not reveal a marker that could be tested as a candidate for linkage to a bloom time QTL.

D. AFLP analyses:

Fifteen AFLP primer combinations were used to screen the parents and individuals from both the early and late bulks. All fragment sizes were scored for all the individuals tested. The monomorphic and polymorphic fragments were summarized to evaluate the feasibility of future AFLP linkage mapping in this population (Table 6 and 7). Initially, primer combinations with three selective nucleotides for both *Eco* RI and *Mse* I were used, which exhibited an average of 21 bands per gel (averaging 30% polymorphism). Based upon Vilanova et al. (2003), two selective nucleotides were used with the *Eco* RI primer producing on average of 39 bands per gel (averaging 14% polymorphism).

Only one fragment was found that was present in one parent, absent in the other parent and differentially present in the bulk progeny. The candidate marker of ~265 bp was identified with AFLP primers *Eco* RI + AAA and *Mse* I + CGT, and therefore the marker name assigned to this fragment was E-AAA/M-CGT$_{265}$ (Figure 3). This primer pair was initially screened with both parents, eight early bulk individuals, and six late

Table 6. AFLP analysis of Balaton, Surefire, and the bulk progeny individuals utilizing primers *Eco* RI +ANN and *Mse* I + CNN.

Primer combination	No. of fragments	Observed monomorphic fragments (size in base pairs)	Observed polymorphic fragments (size in base pairs)	Percent polymorphism
Eco RI + AAA/ *Mse* I + CGT	19	170, 180, 205, 222, 240, 250, 272, 285, 289, 290, 330 , and 3 bands > 330	155, 218, 220, 265, and 1 band > 330	5/19 = 0.26
Eco RI + AGG/ *Mse* I + CAC	19	135, 154, 156, 166, 168, 208, 210, 218, 245, 250, 290, and 1 bands > 330	128, 140, 152, 180, 275, 330, and 1 band > 330	7/19 = 0.37
Eco RI + ATA/ *Mse* I + CAC	33	103, 105, 140, 142, 145, 155, 157, 160, 178, 186, 188, 190, 192, 198, 200, 218, 230, 250, 266, 268, 280, 282, 320, 330 and 1 bands > 330	108, 114, 146, 148, 205, 223, 224, and 1 band > 330	8/33 = 0.24
Eco RI + ATA/ *Mse* I + CCG	8	270, 290, 300, 320	248, 250, 310, 330	4/8 = 0.50
Eco RI + ATA/ *Mse* I + CCT	28	110, 130, 163, 186, 188, 198, 200, 210, 238, 240, 260, 273, 275, 290, 310, and 10 bands > 330	265, 330, and 1 band > 330	3/28 = 0.11

Table 7. AFLP analysis of Balaton, Surefire, and the bulk progeny individuals utilizing primers *Eco* RI +AN and *Mse* I + CNN.

Primer combination	No. of fragments	Observed monomorphic fragments (size in base pairs)	Observed polymorphic fragments (size in base pairs)	Percent polymorphism
Eco RI + AA/ *Mse* I + CAA	49	116, 118, 120, 122, 125, 130, 150, 158, 165, 173, 175, 186, 189, 194, 11 bands > 200 and < 250, 12 bands > 250 and < 330, and 8 bands > 330	163, 192, 1 band > 200 and < 250, 1 band > 250, and < 330	4/49 = 0.08
Eco RI + AA/ *Mse* I + CAC	29	142, 150, 165, 202, 212, 220, 230, 247, 250, 258, 260, 262, 286, 288, 300, 310, 314, and 10 bands > 330	2 bands > 330	2/29 = 0.07
Eco RI + AC/ *Mse* I + CAA	31	105, 110, 114, 116, 118, 120, 128, 130, 143, 145, 165, 181, 183, 186, 188, 2 bands > 200 and < 250, 5 bands > 250 and < 330, and 2 bands > 330	135, 175, 3 bands > 200 and < 250, 1 bands > 250 and < 330 and 3 bands > 330	9/31 = 0.29
Eco RI + AC/ *Mse* I + CAC	36	102, 105, 112, 114, 120, 122, 130, 136, 138, 150, 186, 188, 190, 192, 198, 212, 220, 222, 275, 290, 307, 310, 325, and 7 bands > 330	172, 218, 230, 257, 295, 330	6/36 = 0.17
Eco RI + AG/ *Mse* I + CAA	46	120, 122, 125, 132, 133, 134, 138, 140, 150, 152, 160, 172, 174, 176, 180, 186, 188, 11 bands > 200 and < 250, 7 bands > 250 and < 330, and 8 bands > 330	2 bands > 200 and < 250, 1 band > 250 and < 330	3/46 = 0.07

Primer combination	No. of fragments	Observed monomorphic fragments (size in base pairs)	Observed polymorphic fragments (size in base pairs)	Percent polymorphism
Eco RI + AG/ *Mse* I + CAC	28	107, 110, 117, 119, 121, 140, 152, 156, 162, 168, 190, 205, 210, 235, 268, 280, 310, and 1 band > 330	117, 132, 135, 137, 180, 265, 270, 272, 300, 330	10/28 = 0.36
Eco RI + AT/ *Mse* I + CAA	71	112, 113, 132, 140, 143, 147, 150, 166, 168, 170, 172, 176, 178, 185, 188, 192, 196, 200, 202, 208, 210, 220, 224, 228, 234, 236, 238, 240, 245, 250, 252, 259, 260, 265, 270, 288, 294, 296, 310, 312, 320, 322, 325, 330, and 23 bands > 330	148, 215, 230, and 1 band > 330	4/71 = 0.06
Eco RI + AT/ *Mse* I + CAC	23	116, 125, 127, 158, 160, 180, 181, 188, 190, 198, 200, 212, 258, 290, 320, and 7 bands > 330	260	1/23 = 0.04

Figure 3. Segregation for E-AAA/M-CGT$_{265}$ among the parents and bulk progeny in this particular PCR reaction. B= Balation, S=Surefire, Early bulk = 2(6), 2(61), 3(2), 3(24), 3(25), 3(46), 4(31), 4(34), Late bulk = 2(7), 3(26), 3(51), 4(22), 4(24), 4(45).

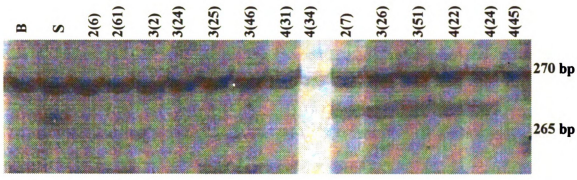


Table 8. Presence or absence of E-AAA/M-CGT$_{265}$ in Balaton, Surefire and the bulk progeny individuals. (na = no amplification in this particular PCR reaction).

Balaton	absent	Surefire	present

(early bulk)		(late bulk)	
2(6)	absent	2(7)	present
2(61)	absent	3(26)	present
3(2)	absent	3(51)	present
3(24)	absent	4(22)	present
3(25)	absent	4(24)	present
3(46)	absent	4(32)	na
4(31)	absent	4(45)	absent
4(34)	absent	4(66)	na

bulk individuals. E-AAA/M-CGT_{265} was present in Surefire, absent in Balaton and present in all but one of the progeny in the late bulk and absent in all of the progeny in the early bulk (Figure 3, Table 8). The one late bulk progeny individual that did not have this marker, 4(45), was early in bloom time relative to the other individuals in the late bulk. (Although in 2003, individual 4(45) bloomed 280.6 DD designated day "x" for that year – Appendix C).

The differential presence of E-AAA/M-CGT_{265} between the parents and bulks suggested that it might be linked to a genomic region important in the genetic control of bloom time. Therefore this marker was further screened on a larger number of progeny individuals to test this association.

E. QTL analysis of marker E-AAA/M-CGT_{265} with bloom time.

An additional 137 progeny were genotyped for marker E-AAA/M-CGT_{265} (Appendix F). The marker was present in Surefire, absent in Balaton and present and absent in 45 and 106 progeny individuals, respectively (Appendix G). The marker was tested for a 1:1 (+/-) ratio, the ratio expected from segregation of a single dose restriction fragment. The marker segregation deviated significantly for this expected ratio, therefore a 1:2 (+/-) ratio was tested. The segregation data fit a 1:2 (+/-) ratio. This suggested that the segregation exhibited by marker E-AAA/M-CGT_{265} is due to significant skewing in favor of the absence of the marker. It appears to show selection against the presence of

E-AAA/M-CGT$_{265}$, which would mean the allele inherited from Surefire is linked to something being selected against.

Table 9. Chi Square Analysis of the segregation of marker E-AAA/M-CGT$_{265}$ in the Balaton and Surefire population to a 1:1 and 1:2. (*Significance at $P < 0.05$ for deviation from expected ratio, n.s. = not significant).

Expected ratio (+ : -)	Observed ratio (+ : -)	x^2
1 : 1	45 : 106	23.4*
1 : 2	45 : 106	0.6 n.s.

The association of marker state for E-AAA/M-CGT$_{265}$ and bloom time was tested using Single Marker Analysis of Variance. Three analyses were performed, one for year 2002, one for year 2003, and then a third analysis with both years combined. In all three analyses, marker E-AAA/M-CGT$_{265}$ was significantly associated with bloom time indicating linkage to a QTL controlling bloom time (Table 11). Specifically, the data indicates the marker E-AAA/M-CGT$_{265}$ is linked in coupling with a QTL allele in Surefire that confers late bloom time. This putative QTL is named *blm*3 following the nomenclature of Wang et al. (2000) in which two QTL for bloom time (*blm*1 and *blm* 2) were identified.

When looking at the significance of growing degree days, both 2002 and 2003 show a significant difference between the two marker 'groups'. In both cases, the present fragment group, or *Blm*3 group is significantly later. When the degree day data was analyzed over both years, again the two groups were significantly different which prevents the possibility of combining the data. The allele *Blm*3 was again found to be significantly associated with late bloom (Appendix H).

Table 10. Analysis of variance for the association of marker state for E-AAA/M-CGT_{265} with heat unit accumulation to 50% bloom time among 151 progeny evaluated in 2002 and 2003.

Source	df	Mean Square	F value	P value
group	1	14277.00	18.88	<.0001
year	1	7860.37	69.19	<.0001
group*year	1	135.30	1.19	0.2769
tree(group)	150	787.79	6.93	<.0001
error	145	113.60		

The mean heat unit accumulation to 50% bloom for those progeny that had the late blooming *Blm*3 allele was significantly greater than the heat unit accumulation to 50% bloom for those progeny not exhibiting *Blm*3 and therefore by default, having the *blm*3 allele (Table 9). This QTL was associated with a delay in bloom time of 16.7 heat units in 2002, 14.1 heat units in 2003, and 15 heat units in both years combined (Table 11).

Table 11. Mean heat unit accumulation for 50% bloom for progeny separated by the presence or or absence of *blm*3. (a and b denote significant difference within columns at $P < 0.05$).

	Marker	2002	2003	2002 & 2003
*Blm*3	present	230.25 a	240.64 a	235.45 a
*blm*3	absent	213.52 b	226.54 b	220.03 b

Using the mean squares calculated in the Single Marker Analysis (Table 10), the broad sense heritability for bloom time, and therefore the percent phenotypic variance explained by this marker, was 16.5 %. This was calculated using the procedure outlined below:

Source		
group	M1	$\sigma^2_e + 2\sigma^2_{tree(group)} + 151\ \sigma^2_{group}$
tree(group)	M2	$\sigma^2_e + 2\sigma^2_{tree(group)}$
residual	M3	σ^2_e

$\sigma^2_{group} = (M1 - M2) / 151 = (14277.00 - 787.79) / 151 = 89.33$
$\sigma^2_{tree} = (M2 - M3) / 2 = (787.79 - 113.60) / 2 = 337.10$

$$r^2 = \frac{\sigma^2_{group}}{\sigma^2_{group} + \sigma^2_{tree(group)} + \sigma^2_e} = \frac{89.33}{89.33 + 337.10 + 113.60} = 0.1654$$

$$h^2 = \frac{\sigma^2_{tree(group)}}{\sigma^2_{tree(group)} + \sigma^2_e} = \frac{337.10}{337.10 + 113.60} = 0.7479$$

V. DISCUSSION

The major outcome of this research was the identification of a marker significantly linked to a putative QTL, termed *blm*3, controlling bloom time. The late allele identified (which is contributed by the late blooming parent, Surefire) explained about 16.5% of the phenotypic variance and delayed bloom time by approximately 15 degree days. Because of the importance of late bloom as a potential escape mechanism for spring freeze damage, the goal was to identify late blooming QTL alleles. This allele, *blm*3, had the opposite effect of two previously identified bloom time alleles identified in the sour cherry cross Erdi Botermo x Schattenmorelle, *blm*1 and *blm*2 (Wang et al., 2000). The genetic effect of both *blm*1 and *blm*2 was to promote early bloom time. For example, *blm*1 explained 19.9% of the phenotypic variance and reduced bloom time by 27.8 degree days. *Blm*2 explained 22.3% of the variation for bloom time and was detected in two of the three years evaluated (Wang et al., 2000). The presence of early blooming alleles in the Erdi Botermo x Schattenmorelle cross was due to the use of the early blooming Erdi Botermo as a parent as the early allele, *blm*1, was present in Erdi Botermo. In contrast, using a different cross with the late blooming Surefire parent resulted in the identification of a late blooming QTL.

The lower percent of phenotypic variance exhibited by *blm3* as compared to *blm1* and *blm2* could be due to several factors. Because trees are permanent plantings, replications were accomplished for this study in the form of years – which themselves were significantly different from each other. Also variation could be explained by lack of

tight linkage between E-AAA/M-CGT$_{265}$ and the *blm3* allele. Further replications across years would reduce this potential for error.

It is not known whether *blm*3 is found at the same loci as *blm*1, *blm*2, or the other bloom time QTL previously identified in *Prunus*. This is because the linkage map position for *blm*3 has not been identified. For example, *blm*1 and *blm*2 were mapped to *Prunus* linkage groups EB1 and Group2 (Wang et al., 2000), while the major QTL identified as *Lb* is located on linkage group 4 (Aruset al., 2003; Ballester, 1998). In an attempt to target these regions where bloom time QTL had previously been reported in *Prunus*, 10, 5, and 3 SSR primer pairs that map to *Prunus* linkage groups 1, 4, and 2, were tested (respectively). Primer pairs were selected by proximity to the published QTL (within 20cM on either side of the estimated QTL). However, none of these SSR primer pairs identified fragments that were differentially present between the late and early bulks. Therefore, AFLP markers were used to try to increase the efficiency of tagging a QTL location.

AFLP analysis was done using the two standard restriction enzymes, *Eco* RI and *Mse* I, with three selective nucleotides. Subsequently, *Eco* RI was changed to use two selective nucleotides (instead of three), as done in apricot, to identify higher numbers of polymorphic bands (Vilanova et al., 2003). This change produced comparatively more bands (as E+AN is 1/4 less selective than the use of E+ANN) although the percent polymorphic bands was lower. While this proves to be a faster method for screening primer combinations (16 as opposed to 64), it may not reveal as many polymorphisms due to co-migration of same sized bands amplified from different regions of the genome. Given the percent polymorphism evident using three selective nucleotides with the

restriction enzyme *Eco* RI, this population exhibits enough polymorphism to construct a AFLP linkage map.

Since *blm3* is present in Surefire but absent from approximately a third of the progeny, this marker must be in single dose. However, the lack of fit to a 1:1 ratio suggests that this marker is on a chromosome region exhibiting extremely skewed segregation in favor of fragment absence. The skewed ratio is not due to linkage with the duplicate self-incompatibility loci as a single factor analysis using bloom time and *S*-allele marker data was not significant.

Since *blm3* explains approximately 16.5% of the phenotypic variance for bloom time, and confers late bloom, future goals for this project will include the cloning, sequencing, as well as physical and linkage mapping of this late bloom QTL. Additionally AFLP analysis could be continued with the goal of identifying additional markers linked to late bloom QTL.

Identifying markers for bloom time that can be used for marker assisted selection is not the major long term goal of this study. Although it might be possible to use markers to eliminate early blooming individuals prior to field planting, and therefore conserve time and resources, phenotyping for bloom time is very simple and easier than a genotyping screen. Instead the long term goal is to understand the inheritance and genetic control of late bloom time in sour cherry. This is because sour cherry exhibits significantly later bloom time than any other horticulturally important *Prunus* species, presumably due to its introgression with the late blooming *P. fruticosa*. Therefore sour cherry potentially contains genes/alleles that maximize the bloom delay exhibited in *Prunus*. For example, compared to peach, sweet cherry, and apricot, the late blooming

sour cherry selections are significantly later blooming. The transgressive segregation exhibited by the progeny in the Balaton x Surefire cross further suggests the loci controlling bloom time in this allotetraploid species are heterozygous and therefore could be identified using a QTL approach. Future characterization of the loci that control bloom time in sour cherry may suggest ways to delay bloom in other early blooming Rosaceous species, thereby reducing the probability of spring freeze damage and crop loss.

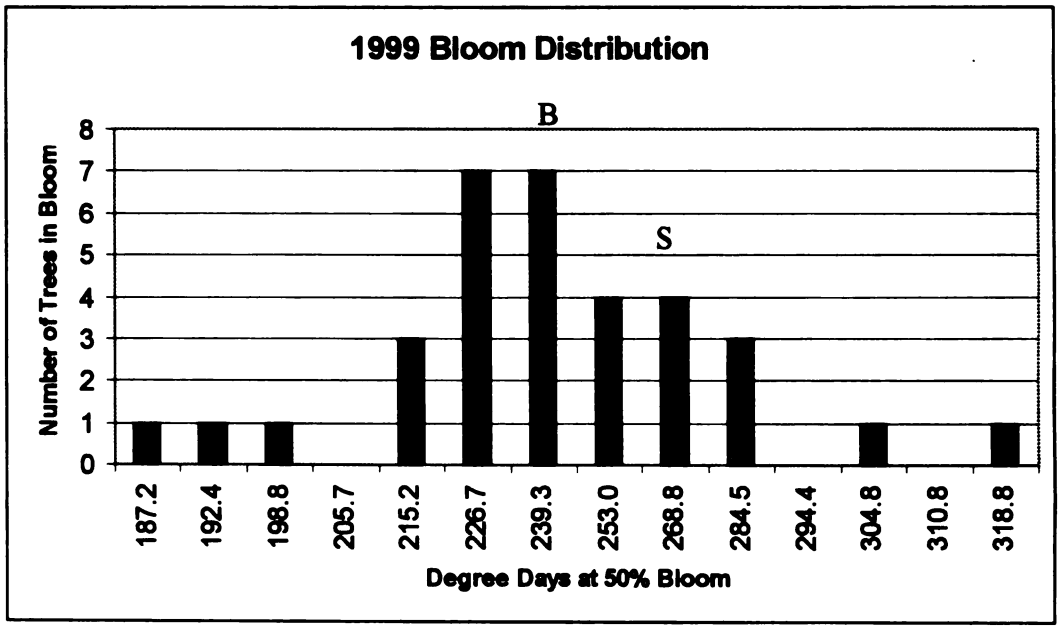
APPENDIX A

Abbreviated SAS input files:

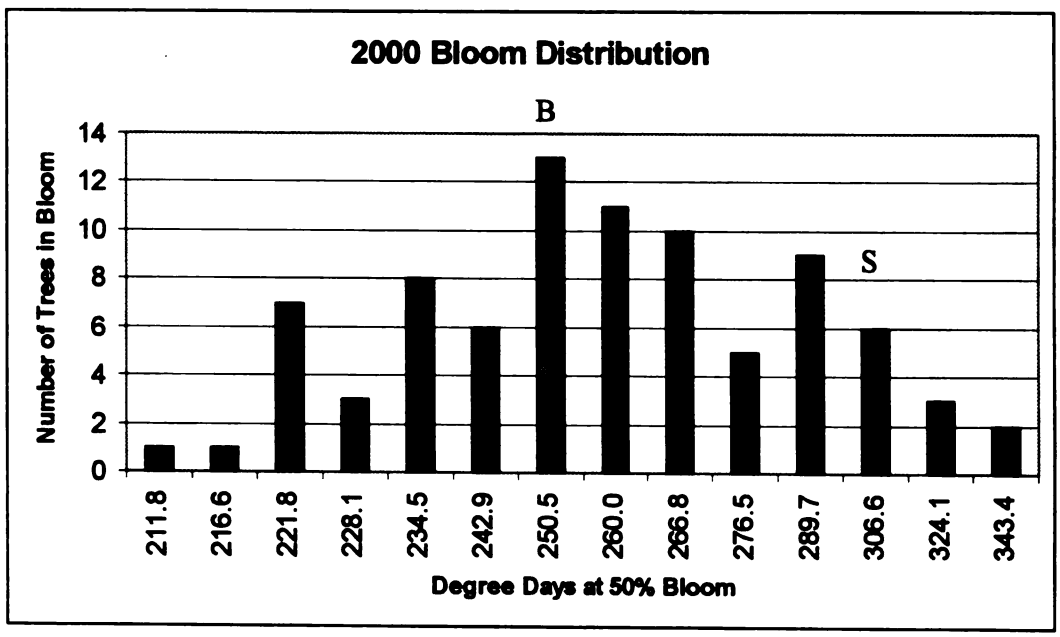
```
data cherryfinal;
input tree$ year group$ degdays;
cards;
1(66)  4       a       209.8
... deleted data ...
4(61)  5       a       266.6
;
run; quit;

proc sort; by group; run;

proc mixed data=cherryfinal;
class group tree;
model degdays = group/outpred=new11 ddfm=satterth;
random tree(group);
lsmeans group/pdiff adjust=tukey;
repeated /group=group;
run;

proc univariate plot normal;
var resid;
run;

proc plot data=new11;
plot resid*group/VPOS=19 HPOS=50;
plot resid*pred/VPOS=19 HPOS=50;
run;

proc mixed data=cherryfinal method=type3;
class group tree;
model degdays = group tree(group);
lsmeans group/pdiff adjust=tukey;
run;

proc mixed data=cherryfinal;
class group tree year;
model degdays = group year group*year;
random tree(group);
run;
```

APPENDIX B

Figure 1. Frequency distributions of 50% Bloom for the Balaton x Surefire progeny population over the first 3 years of bloom: 1999 (A), 2000 (B), and 2001 (C). Bloom date for the parents Balaton (B) and Surefire (S) are shown as inserted letters correspondingly.

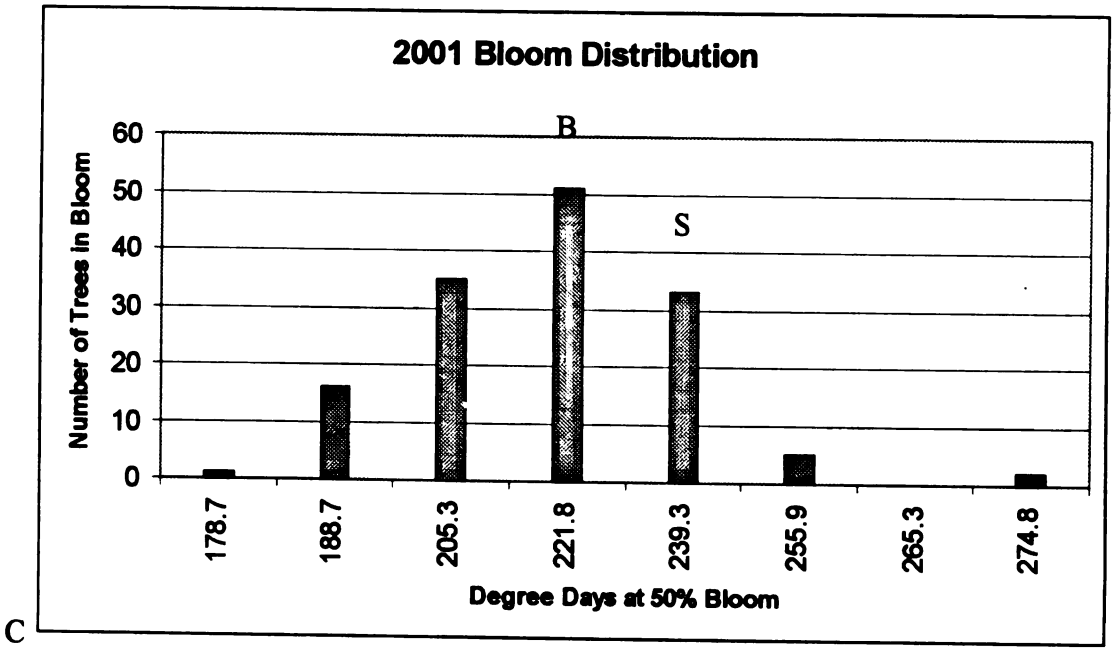


A



B

B. Figure 1. (continued)



C

APPENDIX C

Table 1. Day and degree day comparisons for 50% bloom to identify early and late blooming individuals for the early and late bulks (- = no assignment).

	1999		2000		2001		2002		2003	
letter	date	hours	date	hours	date	hours	date	hours	date	hours
a	4/27	187.2	4/24	211.8	4/29	178.7	4/24	179.8	4/28	177.2
b	4/28	192.4	4/25	216.6	4/30	188.7	4/25	187.7	4/29	186.9
c	4/29	198.8	4/26	221.8	5/1	205.3	4/26	189.1	4/30	195.2
d	4/30	205.7	4/27	228.1	-	-	4/27	190.8	5/1	202.1
e	5/1	215.2	4/28	234.5	-	-	4/28	192.3	5/2	208.1
f	5/2	226.7	4/29	242.9	-	-	4/29	193.3	5/3	211.2
v	5/6	284.5	5/3	276.5	-	-	5/8	251.1	5/9	253.7
w	5/7	294.4	5/4	289.7	-	-	5/9	257.8	5/10	266.6
x	5/8	304.8	5/5	306.6	5/4	255.9	5/10	263.3	5/11	280.6
y	5/9	310.8	5/6	324.1	5/5	265.3	5/11	268.0	5/12	283.6
z	5/10	318.8	5/7	343.4	5/6	274.8	5/12	270.7	5/13	286.3

Table 1. Growing degree days data (°C) for all Balaton x Surefire progeny individuals.

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
Balaton		05/03/99	239.3	04/30/00	250.5	05/02/01	221.8	05/03/02	206.6	05/05/03	225.7
Surefire		05/05/99	268.8	05/05/00	306.6	05/03/01	239.3	05/08/02	251.1	05/10/03	266.6
1	66	05/02/99	226.7	04/30/00	250.5	05/01/01	205.3	05/04/02	209.8	05/05/03	225.7
2	4					05/01/01	205.3	05/04/02	209.8	05/02/03	208.1
2	5	05/01/99	215.2	04/26/00	221.8	04/30/01	188.7	05/03/02	206.6	05/05/03	255.7
2	6	05/02/99	226.7	04/26/00	221.8	04/30/01	188.7	05/01/02	199.7	05/03/03	211.2
2	7	05/06/99	284.5	05/04/00	289.7	05/03/01	239.3	05/10/02	263.3	05/10/03	266.6
2	8			05/01/00	260.0	05/02/01	221.8	05/07/02	243.5	05/09/03	253.7
2	9			05/05/00	306.6	05/03/01	239.3	05/08/02	251.1	05/12/03	283.6
2	10					05/02/01	221.8	05/06/02	232.2	05/09/03	253.7
2	11					05/03/01	239.3	05/05/02	218.7	05/06/03	234.9
2	12					05/03/01	239.3	05/07/02	243.5	05/05/03	225.7
2	13					05/01/01	205.3	05/05/02	218.7	05/06/03	234.9
2	14	05/06/99	284.5	05/04/00	289.7	05/02/01	221.8	05/08/02	251.1	dead	
2	15					05/02/01	221.8	05/05/02	218.7	05/09/03	253.7
2	16					04/30/01	188.7	05/01/02	199.7	05/03/03	221.2
2	17			04/26/00	221.8	05/02/01	221.8	05/01/02	199.7	05/02/03	208.1
2	18							05/03/02	206.6	04/30/03	195.2
2	19			05/06/00	324.1	05/03/01	239.3	05/09/02	257.8	05/08/03	246.6
2	21							dead		dead	
2	22					04/30/01	188.7	05/01/02	199.7	05/01/03	202.1
2	23					05/01/01	221.8	05/01/02	199.7	05/06/03	234.9
2	24					04/30/01	188.7	05/01/02	199.7	05/02/03	208.1
2	25							05/03/02	206.6	05/02/03	208.1
2	26					no flowers		no flowers		no flowers	
2	27					no flowers		05/07/02	243.5	no flowers	

D. Table 1. (continued)

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
2	28							05/06/02	232.2	05/05/03	225.7
2	29	05/02/99	226.7			05/01/01	205.3	05/01/02	199.7	05/02/03	208.1
2	30			04/29/00	242.9	05/01/01	205.3	05/01/02	199.7	05/06/03	234.9
2	31	05/01/99	215.2	04/28/00	234.5	05/01/01	205.3	05/03/02	206.6	05/05/03	225.7
2	32	05/02/99	226.7	05/01/00	260.0	05/02/01	221.8	05/01/02	199.7	05/05/03	225.7
2	33	05/03/99	239.3	05/01/00	260.0	05/02/01	221.8	05/03/02	206.6	05/04/03	216.6
2	35					05/03/01	239.3	05/07/02	243.5	05/06/03	234.9
2	36			05/01/00	260.0	sick		05/07/02	243.5	05/07/03	241.4
2	37			04/28/00	234.5	04/30/01	188.7	05/01/02	199.7	05/02/03	208.1
2	38					05/03/01	239.3	05/07/02	243.5	05/08/03	246.6
2	39			05/07/00	343.4	05/06/01	274.8	05/11/02	268.0	05/12/03	283.6
2	40					05/03/01	239.3	05/07/02	243.5	05/05/03	225.7
2	41					no flowers		05/06/02	232.2	05/08/03	246.6
2	42			04/29/00	242.9	05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
2	43			05/02/00	266.8	05/01/01	205.3	05/01/02	199.7	05/02/03	208.1
2	44	05/02/99	226.7	04/28/00	234.5	05/01/01	205.3	05/04/02	209.8	05/05/03	225.7
2	45	05/04/99	253.0	05/02/00	266.8	05/02/01	221.8	05/05/02	218.7	05/05/03	225.7
2	46			04/30/00	250.5	05/03/01	239.3	05/05/02	218.7	05/07/03	241.4
2	47					05/03/01	239.3	05/07/02	243.5	05/10/03	266.6
2	48					05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
2	49			dead		dead		dead		dead	
2	50							05/05/02	218.7	05/06/03	234.9
2	51					05/02/01	221.8	05/04/02	209.8	05/03/03	221.2
2	52			05/02/00	266.8	05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
2	53					05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
2	55			05/04/00	289.7	05/02/01	221.8	05/01/02	199.7	05/04/03	216.6
2	56	05/03/99	239.3	04/30/00	250.5	05/02/01	221.8	05/04/02	209.8	05/05/03	225.7
2	57					05/01/01	205.3	05/03/02	206.6	05/02/03	208.1

D. Table 1. (continued)

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
2	58			05/02/00	266.8	05/03/01	239.3	05/05/02	218.7	05/07/03	241.4
2	59					05/03/01	239.3	05/05/02	218.7	05/08/03	246.6
2	60					05/01/01	205.3	05/01/02	199.7	05/05/03	225.7
2	61	04/27/99	187.2	04/26/00	221.8	04/30/01	188.7	04/24/02	179.8	04/30/03	195.2
2	62			05/01/00	260.0	05/02/01	221.8	05/08/02	251.1	05/06/03	234.9
2	63					05/02/01	221.8	05/01/02	199.7	05/05/03	225.7
2	64					05/02/01	221.8	05/05/02	218.7	05/05/03	225.7
2	65							04/26/02	189.1	05/02/03	208.1
2	66							05/06/02	232.2	05/02/03	208.1
3	1					04/30/01	188.7	05/01/02	199.7	04/30/03	195.2
3	2			04/26/00	221.8	04/30/01	188.7	04/29/02	193.3	05/04/03	216.6
3	3							05/06/02	232.2	05/06/03	234.9
3	4							no flowers		dead	
3	5			05/01/00	260.0	05/03/01	239.3	05/07/02	243.5	05/08/03	246.6
3	7							05/04/02	209.8	05/08/03	246.6
3	8					05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
3	9							no flowers		dead	
3	10			05/03/00	276.5	05/03/01	239.3	05/05/02	218.7	05/08/03	246.6
3	12					05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
3	13			dead		dead		dead		dead	
3	14					no flowers		05/01/02	199.7	05/05/03	225.7
3	15					no flowers		05/04/02	209.8	05/05/03	225.7
3	16			04/26/00	221.8	04/30/01	188.7	05/01/02	199.7	04/30/03	195.2
3	17			05/03/00	276.5	05/03/01	239.3	05/07/02	243.5	05/10/03	266.6
3	18			04/29/00	242.9	05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
3	19					05/02/01	221.8	05/01/02	199.7	05/04/03	216.6
3	20	04/29/99	198.8	04/25/00	216.6	04/30/01	188.7	04/29/02	193.3	04/30/03	195.2
3	21			05/02/00	266.8	05/02/01	221.8	05/06/02	232.2	05/08/03	246.6

D. Table 1. (continued)

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
3	22			05/05/00	306.6	05/03/01	239.3	05/06/02	232.2	05/10/03	266.6
3	23			dead		dead		dead		dead	
3	24	04/28/99	192.4	04/24/00	211.8	04/30/01	188.7	04/26/02	189.1	04/28/03	177.2
3	25							04/29/02	193.3	04/28/03	177.2
3	26							no flowers		05/13/03	286.3
3	27			04/29/00	242.9	05/02/01	221.8	05/04/02	209.8	05/07/03	241.4
3	28			04/29/00	242.9	04/30/01	188.7	04/29/02	193.3	05/01/03	202.1
3	29	05/01/99	215.2	04/27/00	228.1	05/01/01	205.3	04/30/02	196.0	05/04/03	216.6
3	30					no flowers		05/05/02	218.7	05/08/03	246.6
3	31					05/02/01	221.8	05/04/02	209.8	05/05/03	225.7
3	32					05/02/01	221.8	05/04/02	209.8	05/09/03	253.7
3	33					05/01/01	205.3	05/04/02	209.8	05/06/03	234.9
3	34					05/03/01	239.3	05/07/02	243.5	sick	
3	36					05/02/01	221.8	05/05/02	218.7	05/06/03	234.9
3	37	05/02/99	226.7	05/04/00	289.7	05/01/01	205.3	05/05/02	218.7	05/02/03	208.1
3	38			05/04/03	289.7	05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
3	39					no flowers		05/07/02	243.5	05/08/03	246.6
3	40					no flowers		05/07/02	243.5	05/09/03	253.7
3	41					05/02/01	221.8	05/04/02	209.8	05/04/03	216.6
3	42	05/03/99	239.3	04/30/00	250.5	05/02/01	221.8	05/05/02	218.7	05/09/03	253.7
3	43					05/02/01	221.8	04/30/02	196.0	05/05/03	225.7
3	44	05/05/99	268.8	05/04/00	289.7	05/03/01	239.3	05/08/02	251.1	05/08/03	246.6
3	45					05/03/01	239.3	05/08/02	251.1	05/08/03	246.6
3	46					04/30/01	188.7	04/26/02	189.1	04/29/03	186.9
3	47					05/02/01	221.8	05/05/02	218.7	05/05/03	225.7
3	48					05/02/01	221.8	05/06/02	232.2	05/08/03	246.6
3	49			04/28/00	234.5	05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
3	50	05/04/99	253.0	05/02/00	266.8	05/03/01	239.3	05/06/02	232.2	05/12/03	283.6

D. Table 1. (continued)

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% BI	GDD	50% BI	GDD	50% BI	GDD
3	51					05/04/01	255.9	05/11/02	268.0	05/08/03	246.6
3	52					05/03/01	239.3	05/07/02	243.5	05/06/03	234.9
3	53					no flowers		05/03/02	206.6	05/04/03	216.6
3	54					05/01/01	205.3	04/26/02	189.1	05/02/03	208.1
3	55					05/01/01	205.3	05/01/02	199.7	04/29/03	186.9
3	56					sick		05/01/02	199.7	05/06/03	234.9
3	57					05/02/01	221.8	05/05/02	218.7	05/04/03	216.6
3	58					05/02/01	221.8	05/07/02	243.5	05/06/03	234.9
3	59	05/03/99	239.3	05/02/00	266.8	05/01/01	205.3	05/04/02	209.8	05/05/03	225.7
3	60					no flowers		no flowers		few flowers	
3	61					05/02/01	221.8	05/03/02	206.6	05/04/03	216.6
3	62			05/02/00	266.8	05/01/01	205.3	04/29/02	193.3	04/30/03	195.2
3	63			04/30/00	250.5	05/01/01	205.3	04/29/02	193.3	05/01/03	202.1
3	64					05/03/01	239.3	05/05/02	218.7	05/06/03	234.9
3	65							05/03/02	206.6	05/09/03	253.7
3	66	05/08/99	304.8			no flowers		no flowers		05/12/03	283.6
4	1			05/03/00	276.5	05/02/01	221.8	05/05/02	218.7	05/05/03	225.7
4	2	05/04/99	253.0	04/29/00	242.9	05/01/01	205.3	05/05/02	218.7	05/07/03	241.4
4	3					05/02/01	221.8	05/03/02	206.6	05/05/03	225.7
4	4			05/03/00	276.5	few flowers		05/07/02	243.5	05/10/03	266.6
4	5					no flowers		no flowers		dead	
4	6			05/02/00	266.8	05/02/01	221.8	05/05/02	218.7	05/09/03	253.7
4	7			05/02/00	266.8	05/02/01	221.8	05/03/02	206.6	05/06/03	234.9
4	9					no flowers		05/06/02	232.2	05/09/03	253.7
4	10					05/01/01	205.3	05/03/02	206.6	05/02/03	208.1
4	11			05/01/00	260.0	05/02/01	221.8	05/07/02	243.5	05/09/03	253.7
4	12					05/02/01	221.8	05/08/02	251.1	05/10/03	266.6
4	13							05/07/02	243.5	05/06/03	234.9

D. Table 1. (continued)

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
4	14	05/03/99	239.3	04/30/00	250.5	05/01/01	205.3	05/04/02	209.8	05/04/03	216.6
4	15			04/30/00	250.5	05/01/01	205.3	05/04/02	209.8	05/05/03	225.7
4	16					05/04/01	255.9	05/08/02	251.1	05/10/03	266.6
4	17					no flowers		no flowers		no flowers	
4	18					05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
4	19					05/01/01	205.3	05/04/02	209.8	05/05/03	225.7
4	20					05/03/01	239.3	05/10/02	263.3	dead	
4	21			04/28/00	234.5	05/01/01	205.3	04/30/02	196.0	05/03/03	211.2
4	22	05/10/99	318.8	05/07/00	343.4	05/04/01	255.9	05/09/02	257.8	05/13/03	286.3
4	24			05/06/00	324.1	05/06/01	274.8	05/12/02	270.7	05/13/03	286.3
4	25	05/03/99	239.3	04/30/00	250.5	05/02/01	221.8	05/04/02	209.8	05/05/03	225.7
4	26			04/30/00	250.5	05/02/01	221.8	05/03/02	206.6	05/06/03	234.9
4	27					05/02/01	221.8	05/07/02	243.5	05/06/03	234.9
4	28			04/27/00	228.1	05/02/01	221.8	05/01/02	199.7	05/04/03	216.6
4	29			04/28/00	234.5	05/01/01	205.3	05/01/02	199.7	05/05/03	225.7
4	30					05/02/01	221.8	05/05/02	218.7	05/04/03	216.6
4	31			04/28/00	234.5	04/29/01	178.7	04/26/02	189.1	04/30/03	195.2
4	32					05/02/01	221.8	05/09/02	257.8	05/10/03	266.6
4	33			05/06/00	324.1	05/02/01	221.8	05/07/02	243.5	05/06/03	234.9
4	34			04/27/00	228.1	04/30/01	188.7	04/29/02	193.3	04/28/03	177.2
4	35	05/05/99	268.8	04/28/00	234.5	05/03/01	239.3	05/07/02	243.5	05/10/03	266.6
4	37			05/04/00	289.7	05/02/01	221.8	05/07/02	243.5	05/10/03	266.6
4	38			04/30/00	250.5	05/02/01	221.8	05/06/02	232.2	05/10/03	266.6
4	40					05/02/01	221.8	05/06/02	232.2	05/09/03	253.7
4	41					05/02/01	221.8	05/01/02	199.7	05/06/03	234.9
4	42			05/01/00	260.0	05/01/01	205.3	05/04/02	209.8	05/02/03	208.1
4	43			05/01/00	260.0	04/30/01	188.7	04/30/02	196.0	04/30/03	195.2
4	44	05/02/99	226.7	04/30/00	250.5	05/01/01	205.3	05/04/02	209.8	05/05/03	225.7

		1999		2000		2001		2002		2003	
Row	Tree	Bloom	GDD	Bloom	GDD	50% Bl	GDD	50% Bl	GDD	50% Bl	GDD
4	45			05/05/00	306.6	05/04/01	255.9	05/08/02	251.1	05/11/03	280.6
4	46	05/05/99	268.8	05/04/00	289.7	05/02/01	221.8	05/06/02	232.2	05/09/03	253.7
4	48					no flowers		05/05/02	218.7	05/03/03	211.2
4	49					05/01/01	205.3	04/30/02	196.0	05/01/03	202.1
4	50					no flowers		05/03/02	206.6	04/30/03	195.2
4	51					no flowers		05/01/02	199.7	04/30/03	195.2
4	52					no flowers		05/06/02	232.2	05/08/03	246.6
4	53					no flowers		05/06/02	232.2	no flowers	
4	54	05/06/99	284.5	05/04/00	289.7	sick		05/06/02	232.2	sick	
4	55			05/03/00	276.5	05/02/01	221.8	05/05/02	218.7	05/05/03	225.7
4	56	05/04/99	253.0	04/26/00	221.8	05/01/01	205.3	05/01/02	199.7	05/04/03	216.6
4	57					05/03/01	239.3	05/06/02	232.2	05/11/03	280.6
4	58			05/05/00	306.6	05/04/01	255.9	05/08/02	251.1	05/11/03	280.6
4	59					no flowers		05/01/02	199.7	04/30/03	195.2
4	60			05/01/00	260.0	05/02/01	221.8	05/03/02	206.6	sick	
4	61			05/01/00	260.0	05/02/01	221.8	05/05/02	218.7	05/10/03	266.6
4	62					05/03/01	239.3	05/07/02	243.5	05/09/03	253.7
4	63					few flowers		05/07/02	243.5	05/09/03	253.7
4	64			04/30/00	250.5	05/02/01	221.8	05/05/02	218.7	05/06/03	234.9
4	65					05/03/01	239.3	05/05/02	218.7	05/08/03	246.6
4	66			05/05/00	306.6			05/11/02	268.0	05/13/03	286.3
earliest		04/27/99	187.2	04/24/00	211.8	04/29/01	178.7	04/24/02	179.8	04/28/03	177.2
(-)st dev		04/30/99	213.5	04/27/00	232.3	04/30/01	201.1	05/01/02	199.0	05/02/03	208.0
average		05/03/99	243.9	05/01/00	262.0	05/01/01	219.8	05/05/02	220.1	05/06/03	232.9
(+)st dev		05/05/99	274.3	05/04/00	291.7	05/03/01	238.5	05/07/02	241.2	05/09/03	257.8
latest		05/10/99	318.8	05/07/00	343.4	05/06/01	274.8	05/12/02	270.7	05/13/03	286.3
st dev			30.4		29.7		18.7		21.1		24.9

D. Table 1. (continued)

Table 1. Presence or absence data for tested SSR pairs including parents and progeny evaluated.

Primer Pair	Image name	Peach	Balaton	Surefire	2(61)	2(65)	4(22)	4(24)
BPPCT008	03-03-03b, 03-03-04b	112, 114, 116	94, 96, 98, 100, 109, 120, 122, 126	94, 96, 98, 100, 109, 115, 120	94, 96, 98, 100, 120, 122, 126	94, 96, 98, 100, 109, 115, 120, 122, 126	94, 96, 98, 100, 109, 115, 120, 122, 126	94, 96, 98, 100, 109, 115, 120
BPPCT015	03-01-28	132, 140, 150, 155, 167, 168, 169, 170	-	-	160	158, 160	132, 158, 160, 167	132, 158, 160, 167
BPPCT021	03-02-14b	245, 247	-	-	230, 234	na	230, 234	230, 234
BPPCT023	03-02-14b	na	-	-	na	na	na	na
BPPCT038	03-04-13	na	107, 110, 113, 118, 120, 130, 135	na	-	-	-	-
BPPCT040	03-02-14b	na	-	-	na	na	na	na
CPPCT002	03-04-13	na	95	na	-	-	-	-
CPPCT003	03-02-14a	136, 155, 164	-	-	230, 248, 250, 260, 330	230, 248, 250, 260, 330	248, 250, 260	240, 248, 250, 260
CPPCT026	03-02-14b	177, 179	-	-	157	155, 157	155, 157	155, 157
CPPCT027	03-01-28	na	-	-	78, 70, 84, 86	78, 70, 84, 86	78, 70, 84, 86	78, 70, 84, 86
CPPCT034	03-02-14a		-	-	154, 156, 178, 180, 190, 192	154, 156, 178, 180	154, 156, 178, 180	178, 180, 190, 192
PceGA25	03-03-03a	na	na	160, 162	-	-	-	-

Primer Pair	Image name	Peach	Balaton	Surefire	2(61)	2(65)	4(22)	4(24)
PceGA59	02-11-26	180, 182	178, 182, 184, 190, 195, 228, 230	182, 184, 190, 195	178, 182, 184, 190, 195	182, 184, 190, 195	182, 184, 190, 195	178, 182, 184, 190, 195, 228, 230
PceGA34								
pchgms3	03-01-28	178	-	-	178, 180, 184, 186, 190	176, 178, 180, 184, 186	178, 180, 184, 186	178, 186
pchgms5	03-03-03a	na	168, 170	168, 170	-	-	-	-
PMS2	03-03-25b, 03-04-13	na	135, 138, 141, 145	130, 132, 135, 138,	-	-	-	-
PMS3	03-02-14b	135, 136, 138	-	-	158, 160	188, 190	186, 188, 190	158, 160, 186, 188, 190
PMS67								
UDP96-008	03-03-04a, 03-03-25a	na	128, 130, 132, 135	na	-	-	-	-
UDP96-018	03-03-25a	195, 242, 259	202, 204, 254, 256, 262, 264	202, 204, 254, 256, 262, 264	-	-	-	-
UDP97-403	03-04-13	150, 152	100, 138, 140, 142	138, 140, 142	-	-	-	-
UDP98-022	03-03-25b, 03-04-13	100, 110, 112, 120, 122, 124	92, 94, 96, 98, 100, 102, 104	92, 100, 102, 104	-	-	-	-
UDP98-025	03-03-04a	142, 143, 145	na	na	-	-	-	-

E. Table 1. (continued)

E. Table 1. (continued)

Primer Pair	Image name	Peach	Balaton	Surefire	2(61)	2(65)	4(22)	4(24)
UDP98-405	03-03-03b, 03-03-04b	107, 109	102, 104, 105, 109, 115	104, 105, 109, 115	102, 104, 105	102, 104, 105, 109	105, 109	102, 104,
UDP98-409	03-03-04a	120, 122, 125	122	118, 120, 122	-	-	-	-
UDP98-411	03-04-13	148, 150, 155, 157	132, 133, 140, 162	132, 133, 140, 162	-	-	-	-
UDP98-412	03-03-03b	120, 122, 124	98, 100, 120, 122, 124	98, 100, 120, 122, 124	-	-	-	-

APPENDIX F

Table 1. Presence or absence for E-AAA/M-CGT$_{265}$ from all the parents and progeny evaluated.

	Balaton	absent	Surefire	present

1(66)	absent	2(55)	absent	3(34)	present	4(13)	absent
2(04)	absent	2(56)	present	3(36)	present	4(14)	absent
2(05)	absent	2(57)	present	3(37)	absent	4(15)	present
2(06)	absent	2(58)	present	3(38)	present	4(16)	present
2(07)	present	2(59)	absent	3(39)	absent	4(18)	absent
2(09)	present	2(60)	absent	3(40)	absent	4(19)	absent
2(10)	absent	2(61)	absent	3(41)	present	4(21)	absent
2(11)	absent	2(62)	absent	3(42)	absent	4(22)	present
2(12)	present	2(63)	absent	3(43)	absent	4(24)	present
2(13)	absent	2(64)	absent	3(45)	present	4(25)	absent
2(15)	absent	2(65)	absent	3(46)	absent	4(26)	absent
2(16)	absent	2(66)	absent	3(47)	present	4(27)	present
2(17)	absent	3(01)	absent	3(48)	absent	4(28)	present
2(18)	present	3(02)	absent	3(49)	absent	4(29)	absent
2(19)	present	3(03)	absent	3(50)	absent	4(30)	absent
2(22)	absent	3(05)	present	3(51)	present	4(31)	absent
2(24)	absent	3(07)	present	3(52)	absent	4(33)	present
2(28)	absent	3(08)	present	3(53)	absent	4(34)	absent
2(29)	absent	3(10)	absent	3(54)	absent	4(35)	absent
2(30)	absent	3(12)	absent	3(55)	absent	4(37)	absent
2(31)	absent	3(14)	absent	3(57)	absent	4(40)	absent
2(32)	present	3(15)	present	3(58)	present	4(41)	absent
2(35)	present	3(16)	absent	3(59)	present	4(42)	absent
2(36)	present	3(17)	absent	3(60)	absent	4(43)	absent
2(37)	present	3(19)	absent	3(61)	absent	4(44)	absent
2(38)	absent	3(20)	present	3(62)	absent	4(45)	absent
2(40)	absent	3(21)	present	3(63)	absent	4(46)	absent
2(41)	absent	3(22)	absent	3(64)	absent	4(49)	present
2(42)	absent	3(24)	absent	3(65)	present	4(50)	absent
2(43)	absent	3(25)	absent	4(01)	absent	4(51)	absent
2(45)	absent	3(26)	present	4(02)	absent	4(52)	absent
2(46)	present	3(27)	present	4(03)	absent	4(54)	present
2(47)	present	3(28)	present	4(04)	absent	4(55)	absent
2(48)	absent	3(29)	absent	4(07)	absent	4(56)	absent
2(50)	absent	3(30)	absent	4(09)	absent	4(58)	present
2(51)	absent	3(31)	absent	4(10)	absent	4(59)	absent
2(52)	present	3(32)	absent	4(11)	present	4(61)	absent
2(53)	absent	3(33)	absent	4(12)	absent		

Balaton = absent Surefire = present Progeny = 106 absent and 45 present (Total = 151)

APPENDIX G

Table 1. SAS output for "group" comparison where group a is absent for E-AAA/M-CGT_{265} and b is present (therefore representing *blm*3 and *Blm*3 respectively, includes 2002 and 2003).

Least Squares Means						
Effect	Group	Estimate	Std Error	DF	t Value	Pr > | t |
group	a	220.08	1.9483	141	112.96	< .0001
group	b	235.21	3.0052	137	78.27	< .0001

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