This is to certify that the
dissertation entitled

SSR MAPPING AND A MODIFIED-BULK SEGREGANT
ANALYSIS FOR BLOOM TIME IN SOUR CHERRY

presented by

FATIH ALI CANLI

has been accepted towards fulfillment
of the requirements for the

PhD degree in Horticulture

Major Professor's Signature

Dec. 6, 2002

Date

SSR MAPPING AND A MODIFIED-BULK SEGREGANT ANALYSIS FOR BLOOM TIME IN SOUR CHERRY

By

Fatih Ali Canli

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

2002

ABSTRACT

SSR MAPPING AND A MODIFIED-BULK SEGREGANT ANALYSIS FOR BLOOM TIME IN SOUR CHERRY

By

Fatih Ali Canli

Two separate projects were carried out to aid breeding studies of sour cherry (*Prunus cerasus* L., 2n=4x=32). In the first project, 45 SSR markers from apple, peach, sour cherry and sweet cherry were screened and 10 informative SSRs yielding 17 markers that were added to previously developed sour cherry linkage map of two tetraploid sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB). The EB linkage map consisted of 118 markers in 19 linkage groups covering 337.8 cM. The average distance between two markers is 2.86 cM. The longest distance between two adjacent markers was 20.9 cM in linkage group 17. The RS linkage map consisted of 133 markers in 19 linkage groups covering 433.9 cM. The average distance between two adjacent markers is 19 cM in RS8. RS9 and RS12 from the previous map were combined into one linkage group with the addition of new markers. The EB and RS consensus map consisted of 161 markers covering 442.4 cM in 19 linkage groups. The average distance between two markers is 2.79 cM. The longest distance between two adjacent markers was 15 cM in linkage group 19. Several SSR markers were tightly linked to quantitative traits such as bloom time (*blm2*), fruit weight (*fw2*) and pistil death (*pd1*), which could facilitate marker assisted selection (MAS) for these traits.

In the second project, three different approaches were used to identify markers associated with bloom time in a sour cherry population from 'Balaton' x 'Surefire' cross. Initially a primer pair derived from pS141 sequence was employed. However, the primer amplified many bands between 140 bp and 500 bp and was not useful in determining any association. In a second approach, pchpgms3 SSR marker, which mapped to the EB1 and 8cM of pS141 probe, was tested for association with the bloom time. Bloom data was converted into degree-days and PCR amplification products of pchpgms3 SSR marker were tested for association. No significant relationship was detected between alleles of the pchpgms3 and bloom time. In a third approach, a modified bulk segregant analysis in combination with AFLP technique was used to screen two extreme phenotypic groups from bloom time. The average number of polymorphic bands was 10 per primer pair and the polymorphism rate ranged from 10% to 44% per primer pair. Screening of early and late extreme groups with 156 AFLP primer pairs resulted in the identification of three candidate bands in three different primer combinations (a 82 bp fragment in EGG/MCAC, a 78 bp fragment in ETT/MCCG, and a 94 bp fragment in EAA/MCGT) that were present in one extreme phenotypic group but not in the other. The establishment of an association of bloom time with markers assists a breeding program by allowing for selection early in the generation saving time and effort.

ACKNOWLEDGMENTS

I am very grateful to Dr. Ron Perry for his guidance, editorial assistance and financial support.

I would especially like to thank my committee members, Dr. James Kelly, Dr. David Douches and Dr. Rebecca Grumet for their guidance and helpful advise during the course of my study.

I appreciate Dr. Kenneth Sink, Dr. Amy Iezzoni, Dr. Dechan Wang, Dr. Eric J Hanson for giving me permission to use their facilities.

I also would like to thank Andre Loskutov, Veronica Vallego, Audrey Sebolt, Chris Owens, Nathanael Hauck, Pete Callow, Zafer Makaraci, Sedat Serce, Sergey Nesterenko, Steven Berkheimer and all the department of Horticulture community at MSU for their help with various aspects of my project during the course of my study.

I would like to take this opportunity to thank my wife and the children for their support, sacrifice and love over the years.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

Appendix

LIST OF FIGURES

Chapter 1

Chapter 2

LIST OF FIGURES (continued)

CHAPTER 1

DEVELOPMENT OF A SECOND GENERATION LINKAGE MAP FOR SOUR CHERRY USING SSR MARKERS

ABSTRACT

A second generation linkage map of two tetraploid sour cherry cultivars (*Prunus cerasus* L., 2n=4x=32), 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB) was constructed by addition of new SSR markers to a previously constructed map (Wang et al.1998).

Forty-five SSR primer pairs from apple, peach, sour cherry and sweet cherry were screened and 10 informative SSRs yielding 17 markers were added to the sour cherry linkage map. All apple primers showed amplification in sour cherry, but none of them amplified the expected size of bands and showed a complex banding pattern. Nine of 21 SSR from 'Redhaven' peach (*Prunus persica* (L) Batsch) amplified fragments with the expected size, and five were informative. The remaining SSR expressed a complex banding pattern. Of the eight SSR primers from sour cherry, six showed a complex banding pattern and two amplified fragments of the expected size. Only GA25 was informative and was incorporated into the map. Out of eight peach SSR primers reported by Sosinski et al. (2000), one (pchgms3) was mapped to the sour cherry map. Two out of four sweet cherry SSR markers were informative and placed into the map.

The EB linkage map consisted of 19 linkage groups covering 337.8 cM with 118 markers. The longest linkage group was EB1 covering 43 cM and the shortest was EB 6' where two markers were mapped to the same place. The average distance between two markers is 2.86 cM. The longest distance between two adjacent markers is 20.9 cM in linkage group 17. The RS linkage map consisted of 133 markers in 19 linkage groups covering 433.9 cM. The average distance between two markers is 3.26 cM. The longest

linkage group was RS8 covering 71.8 cM. The shortest linkage group was RS19 in which two markers were mapped to the same place. One group, which has only two markers mapped to the same place, was not assigned a group number. The longest distance between two adjacent markers was 19 cM in RS8. With the addition of new markers, the groups RS9 and RS12 from the previous map (Wang et al. 1998) were merged and combined into one linkage group named RS9. The EB and RS consensus map consisted of 161 markers covering 442.4 cM in 19 linkage groups. The largest linkage group is group 9 covering 44.5 cM. The average distance between two markers was 2.79 cM. The longest distance between two adjacent markers was 15 cM in linkage group19.

SSR markers tightly linked to important quantitative traits in sour cherry, such as bloom time, fruit weight and pistil death, were obtained. These markers could be utilized as a valuable tool for trait selection. The results from current study have shown that SSR primers are co-dominant, reproducible, highly polymorphic and have high utility for cross-species amplification within *Prunus*. SSR will be the marker of choice for comparative mapping and MAS studies in *Prunus*.

INTRODUCTION

Sour cherry is an allotetraploid species (*P. cerasus* L., 2n=4x=32) with sweet cherry (*P. avium* L., 2n=2x=16) and ground cherry (*P. fruticosa* Pall, 2n=4x=32) as the presumed ancestral species (Beaver et al. 1995; Iezzoni and Hancock 1996). Beaver and Iezzoni (1993) reported that sour cherry shows disomic inheritance which is characteristic of allopolyploids.

De Condolle (1884) stated that the sour cherry originated from the region surrounding the Caspian Sea, close to Istanbul, Turkey. Kolesnikova (1975) proposed that sour cherries could be divided into two ecological groups, a western European group and Middle Russian group. The former group is less winter hardy, but has better fruit quality than the latter. According to some authorities this is too restrictive, and they suggested that two areas of origin stretching from Switzerland to the Adriatic Sea and from the Caspian Sea to the far north existed (Webster 1996). Webster (1996) suggests that sour cherry thrives best in the areas with a Mediterranean type-climate.

Sour cherry is produced in significant quantities in about 40 countries. The majority of the world's sour cherries are produced in Russia (200,000 tons from commercial orchards and 180,000 tons from home gardens, 1986), Ukraine (2,400 tons from commercial gardens, 191,000 tons from home gardens, 1993), Yugoslavia (114,594 tons, 1990), Turkey (90,000 tons, 1990) and Hungary (78,000 tons,1990) (Webster and Looney 1996). Total production in US in 1999 was 115,804 tons with Michigan having 72.5% of the production (USDA/NASS 1999).

There is an increasing interest in constructing linkage maps of crop plants so that selection of DNA markers linked to a trait of interest can be used for trait selection at early stages of cultivar development (Scorza 1996). Molecular marker-based linkage maps have been useful for identifying and localizing important genes controlling both qualitatively and quantitatively inherited traits in tomato (Tanksley et al. 1989). DNA based markers can be used to identify related cultivars, to assess taxonomic relationships, and to indirectly select tagged loci affecting qualitative and quantitative traits. They also allow breeders to follow loci during the selection process, which helps reduce time spent in backcross programs (Scorza 1996). When compared to phenotypic markers, DNA based molecular markers have some advantages: they are developmentally stable, detectable in all tissues, not affected by environmental conditions and are insensitive to epistatic effects (Scorza 1996).

LITERATURE REVIEW

SSR's

Simple Sequence Repeats (SSRs, also known as SSR) are a class of DNA markers, consisting of tandem repeats of mono-, di-, tri-, tetra-, or penta-nucleotide units that are found throughout the genomes of most eukaryotic plants (Powell et al. 1991; Lit and Ludy 1989; Taramino and Tingey 1996). Due to the high rate of variation in the number of repeat units, the polymorphism level shown by SSRs is high (Taramino and Tingey 1996).

SSR are valuable markers due to their multiallelic nature, co-dominance, abundance and extensive genome coverage. They are easy to detect by PCR and require a small amount of template DNA. However, there are some disadvantages such as the time requirement for cloning and sequencing to identify useful SSR markers and high-resolution gels required to separate close alleles and score these markers (Powell et al. 1991).

Plant SSRs were first isolated and cloned from tropical species (Condit and Hubbell 1991). On average, there is a microsatellite in every 33-kb in plant nuclear genomes whereas they are found approximately every 6 kb in mammals (Wang et al. 1994). Copy number of these repeats varies among individuals and provides the basis for the polymorphism(s) used in selection studies (Condit and Hubbell 1991).

Broun and Tanksley (1996) screened tomato (*Lycopersicon esculentum* Mill.)genomic libraries with seventeen synthetic probes of 2 to 5 base pair tandem repeats. GAn and GTn sequences were most frequent in the tomato genome (estimated to be every 1.2 Mb). ATTn and GCCCn were estimated to be every 1.4 Mb and 1.5 Mb, respectively.

Condit and Hubbell (1991) studied the dinucleotide repeats in some tropical trees and reported that two-base repeats are abundant in plants providing a large number of polymorphic markers for studies of plant population genetics. They found that GT and AG repeat regions were abundant in six plant species genome studied and they estimated that there were $5x10^3$ to $3x10^5$ AC and AG repeats per genome.

Wang et al. (1994) reported that based on their survey in the Genebank, there was 1 SSR every 64.6 kb in DNA of monocotyledons versus 1 every 21.2 kb in DNA of dicotyledons. Mono-, di-, and tetranucleotide repeats were all located in non-coding regions. Fifty-seven percent of the trinucleotide SSRs containing GC base pairs were located in coding regions in algae. Ma et al. (1996) reported that there was approximately one (AC)n microstallite in every 292 kbp and one (AG)n microsatellite every 212 kbp in wheat (*Triticum aestivum* L.). The trinucleotide repeats (TCT)n and (TTG)n were 10 times less common than the two dinucleotide repeats tested and tetranucleotide tandem repeats were rare. Many of the SSRs had more than 10 repeats and maximum repeat number for (TCT)n was more than 50 in wheat (Ma et al. 1996).

SSRs have been assigned to the *Arabidopsis* linkage map, providing Sequence Tagged Sites (STSs) to relate physical and recombinational genetic maps (Powell et al. 1996).

Akkaya et al. (1995) reported that 40 SSR loci were mapped in a soybean (*Glycine max*) mapping population that consisted of 60 F2 plants from a cross between cultivars 'Clark' and 'Harasoy'. Although evidence of some clustering of SSR loci was also reported, a good overall coverage of the genome was obtained in soybean (Powell et al. 1996).

Guilford et al. (1997) demonstrated that (GT)15 and (GA)15 repeats are abundant in apple (*Malusxdomestica* Borkh.), occurring about every 190-kb and 120 kb, respectively. They have shown that SSRs isolated from a small insert library enriched for (GA) repeats contained numbers of repeats ranging from 7 to 39. Primers designed for SSR loci, which amplified these repeats in 21 different apple cultivars. The majority of the markers were highly polymorphic, diploid and showed single Mendelian inheritance. Twenty-five percent of the markers generated complex banding patterns agreeing with the amplification of more than one locus. They have also demonstrated that only three SSR markers were sufficient to differentiate between all 21 apple cultivars (Guilford et al. 1997).

Yamamoto et al. (2001) reported that SSRs derived from apple are conserved in pear and can be used to identify polymorphism in pear based on a study of 36 pear accessions. All SSRs derived from apple amplified fragments in all pear accessions tested (Yamamoto et al. 2001). All pear accessions were differentiated by using seven SSR loci that generated 79 alleles.

SSR's in *Prunus*

Cipriani et al. (1999) reported the sequence of 17 primer pairs of SSR loci cloned and sequenced from two genomic libraries of peach 'Redhaven' that were enriched for AC/GT and AG/CT repeats, respectively. Ten out of 17 loci showed Mendelian inheritance in a backcross population but the remaining seven SSRs did not segregate. The evaluation of these SSRs in ten peach genotypes has shown that 15 are polymorphic having 2-4 alleles each. The mean heterozygosity averaged on all loci was 0.32 which is significantly higher than that reported for isozymes and RFLPs and RADPs (Cipriani et al. 1999). In addition, 59% (10) of these SSRs demonstrated cross-species amplification with other *Prunus* species (Cipriani et al. 1999).

The isolation and sequencing of nine additional SSRs were reported from two genomic libraries of peach cultivar 'Redhaven' enriched for AC/GT and AG/CT repeats respectively. Seventeen of these SSRs showed Mendelian inheritance. An assay of polymorphism in 50 peach and nectarine cultivars showed that heterozygosity ranged from 0.04-0.74 with a mean of 0.47. SSR appeared for 2-8 alleles per locus (Testolin et al. 2000).

Sosinski et al. (2000) reported the identification of SSR loci in peach by screening a pUC8 genomic library and a λZAPII leaf cDNA library in addition to database searches. Their findings indicated that CT repeats occur every 100 kb, CA repeats every 420 kb and AGG repeats every 700kb in the peach genome. PCR primers were designed from SSR containing clones to amplify these regions (Sosinski et al. 2000). Sosinski et al. (2000) evaluated SSR polymorphism in 28 peach cultivars which showed one, two and four alleles per primer pair at the expected size for each locus. Five of these SSRs segregated in intraspecific peach-mapping crosses. Furthermore, Sosinski et al. (2000) tested SSRs for cross species amplification for use in comparative mapping both within the Rosaceae and with also unrelated species, *Arabidopsis thaliana* L. The SSR markers were found to be highly polymorphic, abundant and transportable between peach cultivars. Moreover, SSRs developed in Rosaceae species are useful for cross species amplification and may have utility in both intra and inter-family comparative mapping analysis. Heterozygosity values ranged from 21% to 56%, with an average value of 45%. These polymorphic SSR markers were used for DNA fingerprinting of 28 peach cultivars. All eight polymorphic markers were needed to discriminate between 28 cultivars.

Downey and Iezzoni (2000) studied the genetic diversity of black cherry germplasm (*Prunus serotina* Ehrh.) using SSR markers developed from sequences of other *Prunus* species; peach, sweet cherry and sour cherry. Four primer pairs were sufficient to identify 54 putative alleles for the 66 black cherry accessions assayed (Downey and Iezzoni 2000)

Cantini et al. (2001) used 10 SSR primer pairs to fingerprint 59 cherry accessions from Geneva, N.Y. The Geneva cherry accessions showed high levels of polymorphism with 4 to 16 putative alleles amplified per primer pair and heterozygosity values ranging from 67.9% to 100%. The 10 primer pairs differentiated between all 59 cherry accessions but two.

Abbot et al. (2000) reported that CT repeats are present in at least one in every 100 kb in peach, as compared to one in every 120 kb in apple (Guilford et al. 1997) and one in every 225 kb in rice (Wu and Tanksley 1993). CA repeats are less frequent (every 420 kb) in peach compared to apple (190 kb) and rice (480 kb). Markers generated from microstaellite sequences are highly polymorphic, transportable and abundant. SSR would be very useful for genetic mapping, map merging and cultivar identification in peach (Wu and Tanksley 1993).

Godoy and Jordano (2001) utilized SSRs for the exact identification of source trees that were produced by seed dispersed by animals. SSRs were used to identify the source tree (*Prunus mahaleb*) for 82.1% of the seeds collected. Remaining seeds came from other populations. Seed dispersal distances ranged from 0 to 316 m with about 62% of the seeds delivered within 15 m of the source trees.

Comparative mapping

Linkage maps generated in *Prunus* species can be compared using common markers that have been placed on all *Prunus* linkage maps. Comparative mapping offers important benefits for genome analysis. DNA probes can be used across-species in the same taxonomic family, increasing the number of genetic markers available. If the linkage maps are co-linear, the location of common single gene or Quantitative Trait Loci (QTL) in one species may predict results in other species (Paterson 1995). The use of the same SSR primers across species depends on conservation of primer sites flanking SSRs between related taxa. Cross-species amplification of SSR alleles with the same primers would increase value of these markers (Powell et al. 1996). Some studies have shown cross-species amplification indicating primer sequence conservation such as in rice (Wu and Tanksley 1993), grape (Thomas and Scott 1993) and Citrus spp (Kijas et al. 1995). Szewc-McFadden et al. (1996) reported that SSRs are abundant in *Brassica* spp. and these markers are conserved among the closely related species. Seventeen out of 21 SSR primer pairs amplified in the three *Brassica* species studied (Szewc-McFadden et al. 1996). Kijas et al. (1995) tested two sequence tagged SSR tagged sites (STSs) in citrus and related species and reported that they obtained amplification across species. Preliminary results in *Prunus* suggest that SSRs are frequently conserved among cherry, peach and almond (Abbott et al .2000).

With the increasing number of common loci identified in a series of *Prunus* species, the maps could be combined and homologous areas and regions of translocations, insertions and deletions detected. This would provide information on gene order conservation. Then studies of "synteny" in *Prunus* could potentially be extended to other species in the Rosaceae (Baird et al. 1996).

Assessment of genetic diversity in *Prunus* with molecular markers

Developing microsatellite markers requires sequencing and this hinders their broader application. An alternative approach was suggested by Wu et al. (1994). This approach, named random amplified microsatellite polymorphism (RAMP), includes the random amplification of microsatellites in combination with RAPD markers. The use of RAMP does not require sequencing and yields a larger number of bands per primer combination (Wu et al. 1994). Cheng et al. (2001) studied the genetic diversity of peach (*Prunus persica* (L.) Batsch) cultivars based on RAMP. Genetic relationships were assessed among 26 common peach cultivars (*P. persica* var. *vulgaris* Maxim.), 12 nectarine cultivars (*P. persica* var. *nectarina* Maxim.), and three flat peach cultivars (*P. persica* var. *platycarpa* Bailey) by using ten combinations of primers producing 82 polymorphic bands. Cluster analysis from RAMP data resulted in groupings which were consistent with the regions of origin of cultivars and classification of the cultivars.

Casas et al. (1999) employed 80 Random Amplified Polymorhic DNA (RAPD) primers to assess the genetic diversity of forty-one genotypes from both commercial *Prunus* rootstocks and clones from the breeding program at Aula Dei Experimental Station, Zaragoza, Spain. Seven RAPD primers produced a combined classification of the whole set of rootstock clones. Their analysis was successful in clustering rootstocks according to the classification based on morphological descriptors widely used to characterize *Prunus* clones. Manubens et al. (1999) clearly distinguished cultivars using AFLP fingerprinting for assessment of eight peach and six nectarine varieties. This technique was found to be more reliable than traditional assessment of agronomic traits of the adult plant. Goulao et al. (2001) employed seven AFLP and six inter-simple sequence (ISSR) primers for phenetic characterization of plum cultivars, resulting in amplification of 379 and 270 products, respectively. These markers are valuable in identification of specific genotypes and analysis of phenetics of plum (*Prunus domestica* L).

The use of AFLPs with its high polymorphism are useful for identification and genome analysis of sweet cherry cultivars (Struss et al. 2001). Ten out of 18 primer combinations were informative generating up to 80 bands for each primer pair. Seven to 33% of the amplified bands were polymorphic and all 38 cherry cultivars were clearly identified.

Bartolozzi et al. (1998) utilized 37 RAPD markers to study the genetic relatedness of 17 almond [*Prunus dulcis* (Mill.) D.A.Webb, syn. *P. amygdalus*, Batsch; *P. communis* (I.) Archangeli] cultivars from California and found that genetic diversity in almond is limited even if it is an obligate outcrossing cultivar. Three groups of cultivar origins can be distinguished with RAPD analysis: progeny derived from interbreeds of early California genotypes, bud-sport mutations, and progeny derived from crosses of California germplasm with genotypes originating from outside of California (Bartolozzi et al. 1998).

QTL analysis and Marker Assisted Selection (MAS)

The genetic complexity of quantitative traits; ranging from an infinite number of genes with tiny effects to few genes with large effects has long been discussed. Current QTL mapping data suggests that few genes account for most of the variation with a greater number of genes responsible for smaller amount of the variance in many plant populations (Paterson 1995). High density genetic maps allow breeders to analyze the genome of an organism, indicating QTL locations affecting any characteristic that could be measured (Paterson 1995). Algorithms for QTL mapping in a wide range of experimental designs, including F_2, backcross, recombinant inbred and many other population designs were developed (Knapp et al. 1991; Carbonell et al. 1992). These algorithms all have been used to testing correlation between marker genotypes and quantitative phenotypes (Paterson 1995).

The effectiveness of molecular markers in marker assisted selection (MAS) depends on the linkage of the marker to the gene of interest. The closer the linkage between a marker and a gene, the more efficient the selection (Baird et al. 1996).

Bloom date, harvest date, fruit weight and pistil freeze tolerance and other quantitative traits were studied in a sour cherry mapping population (Wang 1998). Three markers, which mapped to linkage group 1, were found to be associated with harvest date and two unlinked markers were associated with fruit weight.

Osborn et al. (1987) identified RFLP markers linked to genes controlling the soluble solids (SS) content in tomato fruit by screening the F_2 population for the RFLP genotype for SS content. Analysis of variance of SS content for different RFLP genotypic classes indicated that RFLP alleles at one of the loci were significantly associated with variation for SS content. Tanksley and Hewitt (1988) found that SS content for tomato cultivars could be improved by indirect selection for the linked RFLP markers. In this case, due to the low heritability of SS, MAS based on molecular markers can maximize heritability and increase gain from selection for QTL (Knapp 1994).

Lu et al. (1999) stated that a codominant AFLP marker, EAA/MCAT10, co-segregates with the primary source of resistance to root-knot nematodes (*Meloidogyme incognita* and *hi. javanica*) in rootstock cultivars of peach. They cloned two allelic DNA fragments of this AFLP marker, then sequenced and converted to STSs. Four nucleotide differences (i.e. one addition and three substitutions) were observed between the two clones.

Hormaza (1999) developed an approach for early selection in sweet cherry combining RAPDs with embryo culture, which accelerates the breeding process. In their study, they used this approach to assess, with certainty, the paternity of embryos obtained after mixed pollinations with pollen of three sweet cherry cultivars.

Tao et al. (2000) searched for molecular markers for self-compatiblity by using the information about S-ribonucleases (S-RNases) of other *Prunus* species. By using oligonucleotide primers designed from conserved regions of *Prunus* S-RNases in PCR-amplification of five self-incompatible and six self-compatible cultivars, they found that self-compatible cultivars have a common band of approximate 1.5 kbp when genomic DNA is digested with *Hind*III and probed with the cDNA encoding S'-RNase of sweet cherry. They concluded that self-compatible cultivars possess a common S-RNase allele which can be utilized as a molecular marker for self-compatibility.

QTL analysis in *Prunus*

The genetics of late blooming in almond were studied using a DNA pooling technique to identify RAPD markers linked to a late blooming (*Lb*) gene (Ballester et al. 2001). The researchers were able to identify three RAPD markers associated with the *Lb* gene.

Dirlewanger et al. (1999) mapped QTL controlling fruit quality in peach using an F_2 population. The QTL for almost all qualitative components were on two linkage groups and the fraction of the total variation in each trait explained by the QTL was very high and accounted for up to 90 % of the variation of some characters. All the detected QTL displayed the same effect as the parental phenotypes for productivity, fresh weight, pH, quinic acid, sucrose and sorbitol content. On the contrary, some QTL for maturity date, titratable acidity, malic and citric acids and fructose, showed the same effect as parental phenotypes, but others displayed the opposite effect.

Wang et al. (2000) reported QTL analysis of flower and fruit traits in sour cherry using the RFLP map of EB and RS. The location and effects of QTL for eight traits and eleven putatively significant QTL (LOD > 2.4) were detected for six characters (bloom time, ripening date, % pistil death, % pollen germination, fruit weight, and soluble solid concentration). The percentage of phenotypic variation explained by a single QTL varied from 12.9 % to 25.9 %. The QTL for flower traits (bloom time, % pistil death and % pollen germination) were mapped to the same linkage group, EB 1.

Mapping in polyploids

Although a linkage map in sour cherry could provide broad potential advantages, linkage map construction in sour cherry is lagging compared to other *Prunus* species due to its polyploid origin. Construction of linkage maps in polyploids is difficult. There are large numbers of genotypes for each primer pair expected in a segregating population and these genotypes cannot always be identified by their banding patterns. Secondly, the genome constitution (allopolyploidy versus autopolyploidy) in many polyploids is not clearly understood (Wu et al. 1992). To overcome the difficulty of mapping in polyploids, Wu et al. (1992) proposed the use of Single Dose Restriction Fragments (SDRF). In the sour cherry mapping population, informative markers will be those that are Single Dose Restriction Fragments (SDRFs) in one or both parents [i.e., (+--- x ----), (---- x +---), or (+--- x +---), segregating 1:1, 1:1, or 3:1 respectively] (Wu et al. 1992; Hemmat et al. 1994; Sorrells 1992). To identify SDRFs with a confidence level of 98 % in the four ploidy levels, a population size of at least 75 is needed (Wu et al. 1992).

Software programs have been developed to aid with the mapping. JOINMAP was developed by Piet Stam at the center for Plant Breeding and Reproduction Research, Wagenigen, The Nederlands (Stam 1993). Like MAPMAKER (Lincoln et al. 1992), JOINMAP can construct maps of single crosses, but it also has advantages of merging maps obtained from distinct experiments and published recombination frequencies that are important in comparative mapping. Unlike MAPMAKER, JOINMAP can also be used with markers segregating in various ratios (3:1, 1:1) within the same cross (Baird et al. 1996).

Current status of mapping in *Prunus*

Linkage mapping was first initiated with diploid species due to the relative simplicity compared to polyploids. Linkage maps of peach (Chaparo et al. 1994; Rajapackse et al. 1995), peach X almond (Foolad et al. 1995), peach X *P. davidiana*

(Dirlewenger and Bodo 1994), almond (*P. dulcis*) and (Viruel et al. 1995), sweet cherry (Stockinger et al. 1996) were conducted.

Rajapakse et al. (1995) reported the construction of a genetic linkage map of peach. The map consisted of 47 markers covering 332 cM (RFLP, RAPD and morphological markers) based on 71 F_2 individuals derived from 'New Jersey Pillow' and KV77119. Fooled et al. (1995) constructed a linkage map of a dwarf peach selection (54P455) and an almond cultivar 'Padre' cross with 107 markers. Markers were assigned to nine different linkage groups covering 800 cM (11 markers remained unlinked). Viruel et al. (1995) constructed two linkage maps in almond using RFLP's. Eight linkage groups were constructed with the 93 heterozygous loci in 'Ferragnes' and eight linkage groups were constructed with 69 loci heterozygous in 'Tuano'. Dirlewanger and Bodo (1994) constructed a linkage map of peach with RAPD markers where eight linkage groups were identified. Lu et al. (1998) constructed a linkage map of peach rootstocks with (AFLP) markers in 55 F_2 individuals of the cross Lovell x Nemared. They have scored 169 AFLP markers from 21 different primer combinations and assigned 153 markers to 15 linkage groups covering 1297 cM with the average interval of 9.1 cM. Dirlewanger et al. (1998) constructed a linkage map of peach from an intraspecific F_2 population consisting of 249 markers including four agronomic characters (peach/nectarine, flat/round fruit, acid/non-acid fruit, and pollen sterility) and one isoenzyme, 92 RAPD, 50 RFLP, eight inter-microsatellite amplification (IMA), and 115 AFLP markers. The map will be useful in the detection of QTL's for controlling acid and sugar content, consists of 11 linkage groups covering 712 cM with the average density of 4.5 cM. The mapping population was generated from a flat non-acid peach, 'Fejalou

Jalousia(R)' and an acid round nectarine 'Fantasia' (Dirlewanger et al. 1998). Joobeur et al. (1998) constructed a saturated linkage map for *Prunus* using an almond x peach F_2 progeny with 246 markers (11 isozymes and 235 RFLPs) covering distance of 491 cM with the average map density of 2.0 cM/marker. The map had only four gaps of 10 cM. RFLPs come from 213 probes from the genomic and cDNA libraries of almond, peach, *P. ferganensis*, cherry, plum and apple, with an additional 16 almond probes of known genes. The order of locus on the map was almost identical and distances did not differ significantly among an intra specific almond map sharing 67 anchor loci.

Dettori et al. (2001) constructed a linkage map of a BC1 progeny (*Prunus persica* x (*P. persica* x *P. ferganensis*)) consisiting of 109 loci (74 RFLPs, 17 SSRs, 16 RAPDs, and two morphological traits) covering 521 cM on 10 linkage groups with an average distance between markers of 4.8 cM. JOINMAP 2.0 software was used to integrate loci segregating in five different ratios. Two monogenic traits, flesh adhesion (F/f) and leaf glands (E/e) were placed on the map. Homologies were found among the respective linkage groups. No relevant differences were observed in the linear order of the common loci (Dettori et al. 2001)

A second-generation linkage map was constructed for almond using RAPD and SSR markers (Joobeur et al. 2000). Fifty-four RAPD markers and SSRs were added to the molecular map previously constructed with 120 RFLPs and seven isozyme genes. Polymorphism was detected in six of the eight *Prunus* SSRs studied leading these to be mapped. All markers placed on the 8 linkage groups were previously identified resulting in a 5% increase to the previous map from 415 cM to 457 cM.

An RFLP genetic linkage map of two tetraploid sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB) was developed from the crosses of these two cultivars (Wang et al. 1998). The RS linkage map consists of 19 linkage groups covering 461.6 cM and EB linkage map consists of 16 linkage groups covering 279.2 cM. Fifty-three markers mapped in both parents allowed for the identification of 13 sets of homologous linkage groups. Homoeologous relations could not be determined since only 15 of the probes detected duplicate loci. Fifty-nine of the markers on the linkage maps were identified with probes, which are employed in other *Prunus* linkage maps.

Jauregui et al. (2001) reported developing a linkage map using an interspecific F_2 population between almond and peach with selected markers of eight linkage groups from previously developed *Prunus* maps. Contrary to expected eight linkage groups in *Prunus*, markers studied mapped to seven linkage groups and markers of groups 6 and 8 in previous maps formed a single group. By studying pollen fertility and chromosome behavior of meiosis in F1 generation, the presence of a reciprocal translocation between 'Garfi' almond and 'Nemared' peach was suggested (Jauregui et al. 2001).

Shimada et al. (2000) developed a genetic linkage map using 133 F_2 plants from an intraspecific cross among peach cultivars in Japan. The map of the rootstock cultivar, 'Akame', and the ornamental peach, 'Juseitou' contained 83 markers consisting of 41 RAPD, 30 AFLP, and Inter-SSR, PCR-RFLP markers and also three morphological trait loci, brachytic dwarf (*dw*), red leaf (*Gr*) and narrow leaf (*nl*). The map had ten linkage groups ranging in length from 17 to 244 cM and covered more than 960 cM. The morphological characteristic, *nl* co-segregated with the *dw* locus. DNA markers found to be linked to *Gr* and *dw* loci, could be utilized in peach breeding.

The expanded *Prunus* genetic linkage map constructed from peach and almond covers 1,144 cM (Bliss et al. 2002). Sour cherry linkage map, being tetraploid, should be two times the length of the peach map. However the published map covers only one fourth of the expected length due the difficulty of having informative markers in tetraploids compared to diploids (Wang et al. 1998). The objective of this study is to identify informative SSR markers and incorporate these markers onto the sour cherry map. Incorporation of informative SSR markers may lead to identification of homoeologous linkage groups in sour cherry that would be very valuable tools for comparative mapping studies in *Prunus*. Additionally, if mapped close to the QTL of important traits in sour cherry, these SSR markers would be valuable tools in MAS for these traits.

MATERIALS AND METHODS

Mapping population, plant material and DNA extraction

The sour cherry mapping population is a 'pseudotestcross' in which informative markers are those that are homozygous recessive in one parent and heterozygous in the other parent and segregate 1 : 1 (Hemmat et al. 1994). Eighty-four progeny from crosses of 'Rheinische Schattenmorelle' (RS) x 'Erdi Botermo' (EB) were used as a mapping population. EB and RS were chosen as parents because they differ from each other for important horticultural traits such as fruit firmness, fruit color, pistil freeze susceptibility, cold hardiness, bloom date and fertility. Additionally, these parents originated from different geographical regions (Germany and Hungary, respectively) (Wang et al. 1998). Young unfolded leaves were collected from trees of the mapping population located at the Clarksville Horticultural Experiment Station of Michigan State University. Leaves were frozen at $-80\ ^0$C overnight and lyophilized for 2-3 days. DNA isolation was conducted according to Stockinger et al. (1996).

SSR primers

The information on 45 SSR primer pairs from 'Redhaven' peach (Testolin et al. 2000), apple (Guilford et al. 1997) and sweet cherry (Sosinski et al. 2000) was used in this study. Sequences of peach primers (pchgms and pchcms series) and sweet cherry primers (PS08E08, PS12A02, PS01H03 and PS07A02) were provided by Sosinski et al. (2000). Sour cherry SSR primers were derived from a small-insert genomic DNA library (A. Iezzoni, Horticulture Dept., Michigan State University, East Lansing, Michigan).

Annealing temperature for each primer pairs.

To find the optimum and highest annealing temperature for each primer, a Stratagene Robocycler with a temperature gradient was used. EB was used as the template DNA in the PCR mixture in optimization since the genomic library was constructed from this parent. The reaction and a 123 bp ladder was run on a 0.9% agarose gel to determine the highest optimum annealing temperature to reduce the change of mismatching and confirm the size of the amplified fragment. The gel was stained with ethidium bromide (0.5 mg/μl) for 15 minutes and rinsed with double distilled water for one minute.

Screening primers for polymorphism using PCR

After determining the optimum annealing temperature for each primer pair, another DNA amplification reaction was set up with each primer pair and both parents and 12 progeny to find the primers that identify segregating fragments. Five μl of the PCR products was first run on a 0.9 % agorose gel to verify amplification. To identify the presence of polymorphism., 4 μl of each remaining reaction was run on a 4 % polyacrylamide gel and the bands was detected by using the DNA silver staining protocol of Promega (Promega Corporation, Madison , WI).

PCR with informative markers

After identifying SSR primers that were polymorphic, another DNA amplification reaction was conducted on the remaining progeny in the mapping population as follows; 1X PCR buffer, 0.2 mM of dNTP's, 2.5 mM of $MgCl_2$, 50 ng DNA, 0.6 unit TAQ DNA polymerase enzyme(Boehringer Mannheim Biochemicals) and ddH_2O was added to a volume of 25 μl. DNA amplification reactions were performed in a thermocycler (model

9600; Perkin Elmer Applied Biosystems, Inc., Foster City, California). The amplification products were separated by electrophoresis for 2.5 h at 80 W on a 6% polyacrylamide sequencing gel (Bio-Rad), then silver stained with sequence staining kit by Promega and sizes were estimated using a 10 bp ladder (Gibco BRL).

Scoring, X^2 analysis and map construction

The primers, which showed polymorphisms based on size in the polyarcylamide gel, were scored for the absence or presence of a band in the mapping population.

In the mapping population, informative markers are those that are SDRFs in one or both parents [i.e., (+---x----), (----x+---), or (+---x+---), segregating 1:1, 1:1, or 3:1, respectively] (Wu et al. 1992; Hemmat et al. 1994; Sorrells 1992). Fragments which differed between both parents were tested for fit to a 1:1 (presence:absence) ratio. Fragments which are present in both parents were tested for fit to a 3:1 (presence : absence) ratio. Those markers, which fitted the appropriate ratios at the 5% level, were used in linkage analysis. The SSR data of 84 progenies was added to the previously constructed RFLP data (Wang et al. 1998). A linkage map was generated from the RFLP, and SSR data with JOINMAP V2.0 (Stam 1993).

RESULTS AND DISCUSSION

Forty-five SSR primer pairs were tested to find informative markers in sour cherry (Table 1.1). Ten of these primer pairs were informative (Table 1.2), yielding 17 SDRF. A second generation linkage map of two tetraploid sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' was constructed (Fig. 1.1) by the addition of new SSR markers to a map previously constructed by Wang et al. (1998).

The revised EB linkage map consisted of 19 linkage groups covering 337.8 cM with 118 markers. Seventeen markers remained unlinked. The longest linkage group is EB1 covering 43 cM and the shortest is EB6' where two markers were mapped to the same place. The average distance between two markers is 2.9 cM. The longest distance between two adjacent markers is 20.9 cM in linkage group 17 (Fig. 1.1). The published EB linkage map published by Wang et al. (1998) consisted of 95 SDRF in 16 groups covering 279.2 cM. The incorporation of 23 new markers provided a 20% (58.6 cM) increase over the length of the previous map. Seventeen markers remained unlinked. Marker order was mostly conserved when compared to the earlier map. The average distance (cM/marker) between two loci decreased from 3.5 to 2.9 cM in the EB map.

The revised RS linkage map consisted of 133 markers in 17 linkage groups covering 433.9 cM. The average distance between two markers is 3.26 cM. The longest linkage group is RS8 covering 71.8 cM. The shortest linkage groups are RS19 where two markers were mapped to the same place. The longest distance between two adjacent markers is 19 cM in RS8. Twenty-six markers were unlinked. The addition of new markers to the linkage map would incorporate these unlinked markers to the linkage map.

Table 1.1. Summary of the 45 SSR primers derived from peach, sweet cherry, sour cherry and apple tested in current study.

Primer	Sequence	Source	Comments
01a6	(Guilford et al.1997)	Apple	Complex banding pattern
02b1	(Guilford et al.1997)	Apple	Complex banding pattern
23f1	(Guilford et al.1997)	Apple	Complex banding pattern
26c6	(Guilford et al.1997)	Apple	Complex banding pattern
Pchcms1	(Sosinski et al. 2000)	Peach	Complex banding pattern
Pchcms2	(Sosinski et al. 2000)	Peach	Not polymorphic
Pchcms3	(Sosinski et al. 2000)	Peach	Not polymorphic
Pchgms1	(Sosinski et al. 2000)	Peach	Many bands between 140 and 300bp with complex banding pattern
Pchgms5	(Sosinski et al. 2000)	Peach	Insufficient PCR product
Pchgms3	(Sosinski et al. 2000)	Peach	Informative (see Table 1.2)
Pchgms2	(Sosinski et al. 2000)	Peach	Insufficient PCR product
B4G3	(Appendix, Table 1)	Peach	Insufficient PCR product
PS01H03	(Sosinski et al. 2000)	Sweet cherry	Insufficient PCR product
PS07A02	(Sosinski et al. 2000)	Sweet cherry	Insufficient PCR product
PS08E08	(Sosinski et al. 2000)	Sweet cherry	Informative (see Table 1.2)
PS12A02	(Sosinski et al. 2000)	Sweet cherry	Informative (see Table 1.2)
PS09F08	(Joobeur et al. 2000)	Sweet cherry	Amplified complex bands between 250-140 bp.

Table 1.1. (cont'd).

Marker	Sequence Reference	Source	Comments
GA25(PceGA 25)	(Cantini et al. 2001)	Sour cherry	Informative (see Table 1.2)
GA65 (PceGA65)	(Appendix, Table 1.1)	Sour cherry	Amplified 6 bands ranging from 340bp to 247 bp, band patterning is not consistent, not workable
GA50 (PceGA50)	(Appendix, Table 1.1)	Sour cherry	Amplified bands between 178 to 143, not scorable
GA57 (PceGA57)	(Appendix, Table 1.1)	Sour cherry	Amplified many bands of not expected size between 500 and 130 bp
GA55 (PceGA55)	(Appendix, Table 1)	Sour cherry	Amplified many bands of not expected size between 500 and 130 bp
GA26 (PceGA26)	(Appendix, Table 1)	Sour cherry	Amplified many bands between 500 and 100bp
GA77 (PceGA77)	(Appendix, Table 1)	Sour cherry	Informative (see Table 1.2)
GA34(PceGA 34)	(Downey and Iezzoni 2000)	Sour cherry	Informative (see Table 1.2)
UDP98-24	(Testolin et al. 2000).	Peach	There are complex bands 73 to 64 bp, not expected size, very faint to be scored
UDP98-22	(Testolin et al. 2000).	Peach	Informative (see Table 1.2)
UDP98-410	(Testolin et al. 2000).	Peach	Informative (see Table 1.2)
UDP98-411	(Testolin et al. 2000).	Peach	Informative (see Table 1.2)
UDP98-412	(Testolin et al. 2000).	Peach	Amplified many bands of not expected size between 500 and 90 bp
UDP98-414	(Testolin et al. 2000).	Peach	Amplified many bands
UDP98-416	(Testolin et al. 2000).	Peach	Insufficient PCR product

Table 1.1. (cont'd).

Marker	Sequence	Source	Comments
UDP98-409	(Testolin et al. 2000).	Peach	A 160 bp band, not polymorphic 163 bp not polymorphic
UDP96-018	(Testolin et al. 2000).	Peach	Amplified bands of not expected size about 500 bp
UDP96-008	(Testolin et al. 2000).	Peach	Informative (see Table 1.2)
UDP96-003	(Testolin et al. 2000).	Peach	Amplified very close 3-4 bands at 100bp of not scorable nature
UDP96-001	(Testolin et al. 2000).	Peach	A 130bp band, not polymorphic A 118bp band segregating 10:1 A 105bp band segregating 2:1
UDP96-005	(Testolin et al. 2000).	Peach	Amplified many bands between 250 and 500 bp
UDP96-019	(Testolin et al. 2000).	Peach	Amplified many bands between 250 and 500 bp
UDP97-403	(Testolin et al. 2000).	Peach	150bp can not be separated form 149b band, not scorable 100bp not polymorphic
UDP98-405	(Testolin et al. 2000).	Peach	Informative (see Table 1.2)
UDP98-406	(Testolin et al. 2000).	Peach	A 99bp band segregating 2:1 A 97bp band, not polymorphic
UDP98-407	(Testolin et al. 2000).	Peach	Amplified many bands larger than 500 bp
pS141	(Appendix, Table A.1)	Sweet cherry	Amplified many bands between 500 and 140bp.

Table 1.2 Segregation ratios and product sizes of informative SSR primers in sour cherry mapping population from 'Rheinische Schattenmorelle' (RS) x 'Erdi Botermo' (EB) .

Primer	Plant source	Reference	Product size	RS	EB	Ratio
GA34	Sour cherry	(Downey and	184	-	+	
(PceGA34)		Iezzoni 2000)	175	+	-	1:1
			170	+	-	1:1
			161	+	-	1:1
			143	+	+	
PS12A02	Sweet cherry	(Sosinski et al.	178	-	+	1:1
		2000)	167	-	+	1:1
			162	+	-	1:1
			160	+	+	
			148	-	+	1:1
PS08E08	Sweet cherry	(Sosinski et al.	188	-	+	1:1
		2000)	184	+	+	
			175	+	+	
Pchgms3	Peach	(Sosinski et al.	189	-	+	1:1
		2000)	182	+	+	
			178	+	+	3:1
			174	+	-	1:1
GA25	Sour cherry	(Cantini et al.	199	-	+	1:1
(PceGA25)		2001)	187	-	+	1:1
			174	+	+	3:1
			162	+	+	
UDP96-008	Peach	(Testolin et al.	158	+	-	
		2000)	155	+	+	
			148	-	+	
			139	-	+	1:1
			135	+	+	
			128	+	-	
UDP98-405	Peach	(Testolin et al.	112	+	-	1:1
		2000)	105	+	+	
			103	-	+	
			100	+	+	
			97	-	+	
UDP98-22	Peach	(Testolin et al.	104	+	-	1:1
		2000)	98	+	-	1:1
			90	+	+	
UDP98-410	Peach	(Testolin et al.	139	+	-	1:1
		2000)	134	+	-	2:1
			131	-	+	
UDP98-411	Peach	(Testolin et al.	164	-	+	1:2
		2000)	154	+	+	3:1
			150	+	+	
			131	+	+	3:1

- = absence of a band, + = presence of a band.

Figure 1.1., pages 31, 32 and 33. The second-generation linkage maps of two sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB), generated by addition of SSR markers to the previously constructed RFLP map (Wang et al. 1998). SSR markers are underlined. Lines represent anchor loci correspondences between RS and EB linkage groups. When two linkage groups in one cultivar are homologous to a linkage group in the other cultivar, the shorter of the two is marked, i.e., RS5'.

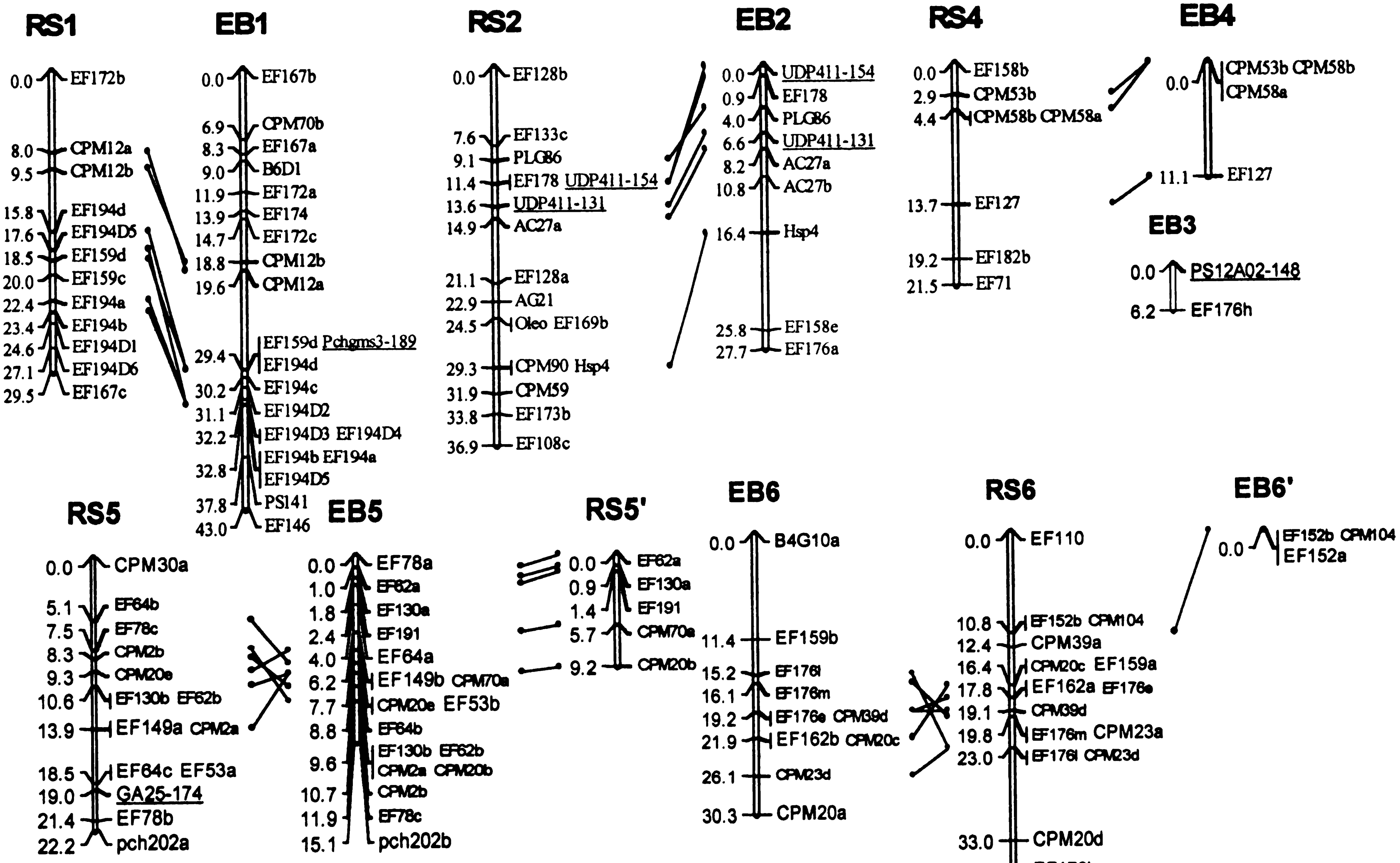
RS1
0.0 EF172b
8.0 CPM12a
9.5 CPM12b
15.8 EF194d
17.6 EF194D5
18.5 EF159d
20.0 EF159c
22.4 EF194a
23.4 EF194b
24.6 EF194D1
27.1 EF194D6
29.5 EF167c
EB1
0.0 EF167b
6.9 CPM70b
8.3 EF167a
9.0 B6D1
11.9 EF172a
13.9 EF174
14.7 EF172c
18.8 CPM12b
19.6 CPM12a
29.4 EF159d Pchgms3-189
EF194d
30.2 EF194c
31.1 EF194D2
32.2 EF194D3 EF194D4
EF194b EF194a
32.8 EF194D5
37.8 PS141
43.0 EF146
RS2
0.0 EF128b
7.6 EF133c
9.1 PLG86
11.4 EF178 UDP411-154
13.6 UDP411-131
14.9 AC27a
21.1 EF128a
22.9 AG21
24.5 Oleo EF169b
29.3 CPM90 Hsp4
31.9 CPM59
33.8 EF173b
36.9 EF108c
EB2
0.0 UDP411-154
0.9 EF178
4.0 PLG86
6.6 UDP411-131
8.2 AC27a
10.8 AC27b
16.4 Hsp4
25.8 EF158e
27.7 EF176a
RS4
0.0 EF158b
2.9 CPM53b
4.4 CPM58b CPM58a
13.7 EF127
19.2 EF182b
21.5 EF71
EB4
0.0 CPM53b CPM58b
CPM58a
11.1 EF127
EB3
0.0 PS12A02-148
6.2 EF176h
RS5
0.0 CPM30a
5.1 EF64b
7.5 EF78c
8.3 CPM2b
9.3 CPM20e
10.6 EF130b EF62b
13.9 EF149a CPM2a
18.5 EF64c EF53a
19.0 GA25-174
21.4 EF78b
22.2 pch202a
EB5
0.0 EF78a
1.0 EF62a
1.8 EF130a
2.4 EF191
4.0 EF64a
6.2 EF149b CPM70a
7.7 CPM20e EF53b
8.8 EF64b
9.6 EF130b EF62b
CPM2a CPM20b
10.7 CPM2b
11.9 EF78c
15.1 pch202b
RS5'
0.0 EF62a
0.9 EF130a
1.4 EF191
5.7 CPM70a
9.2 CPM20b
EB6
0.0 B4G10a
11.4 EF159b
15.2 EF176l
16.1 EF176m
19.2 EF176e CPM39d
21.9 EF162b CPM20c
26.1 CPM23d
30.3 CPM20a
RS6
0.0 EF110
10.8 EF152b CPM104
12.4 CPM39a
16.4 CPM20c EF159a
17.8 EF162a EF176e
19.1 CPM39d
19.8 EF176m CPM23a
23.0 EF176l CPM23d
33.0 CPM20d
36.2 EF176j
EB6'
0.0 EF152b CPM104
EF152a

Fig. 1.1

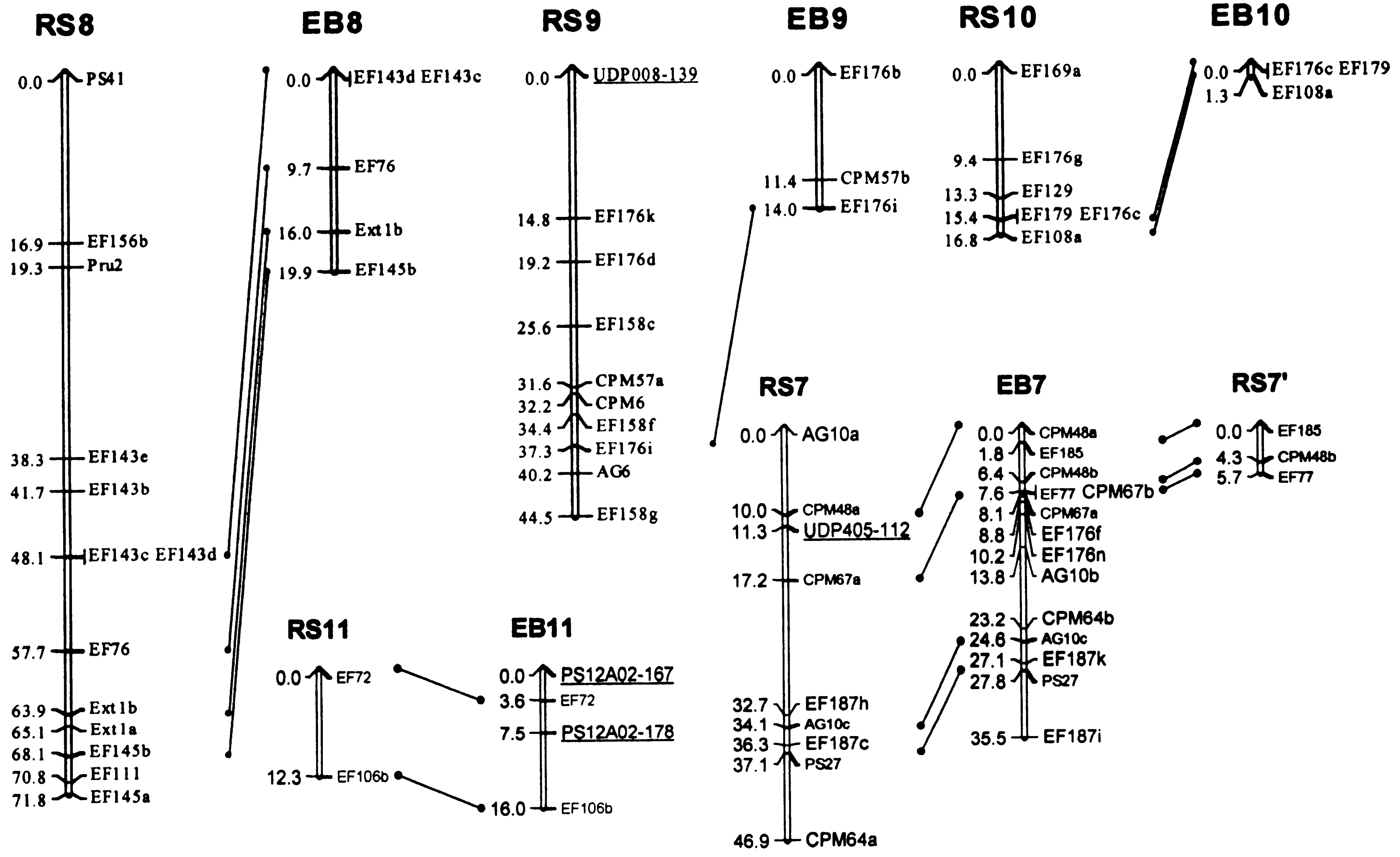
RS8
0.0 PS41
16.9 EF156b
19.3 Pru2
38.3 EF143e
41.7 EF143b
48.1 EF143c EF143d
57.7 EF76
63.9 Ext1b
65.1 Ext1a
68.1 EF145b
70.8 EF111
71.8 EF145a
EB8
0.0 EF143d EF143c
9.7 EF76
16.0 Ext1b
19.9 EF145b
RS9
0.0 UDP008-139
14.8 EF176k
19.2 EF176d
25.6 EF158c
31.6 CPM57a
32.2 CPM6
34.4 EF158f
37.3 EF176i
40.2 AG6
44.5 EF158g
EB9
0.0 EF176b
11.4 CPM57b
14.0 EF176i
RS10
0.0 EF169a
9.4 EF176g
13.3 EF129
15.4 EF179 EF176c
16.8 EF108a
EB10
0.0 EF176c EF179
1.3 EF108a
RS11
0.0 EF72
12.3 EF106b
EB11
0.0 PS12A02-167
3.6 EF72
7.5 PS12A02-178
16.0 EF106b
RS7
0.0 AG10a
10.0 CPM48a
11.3 UDP405-112
17.2 CPM67a
32.7 EF187h
34.1 AG10c
36.3 EF187c
37.1 PS27
46.9 CPM64a
EB7
0.0 CPM48a
1.8 EF185
6.4 CPM48b
7.6 EF77 CPM67b
8.1 CPM67a
8.8 EF176f
10.2 EF176n
13.8 AG10b
23.2 CPM64b
24.6 AG10c
27.1 EF187k
27.8 PS27
35.5 EF187i
RS7'
0.0 EF185
4.3 CPM48b
5.7 EF77

Fig. 1.1 (cont'd)

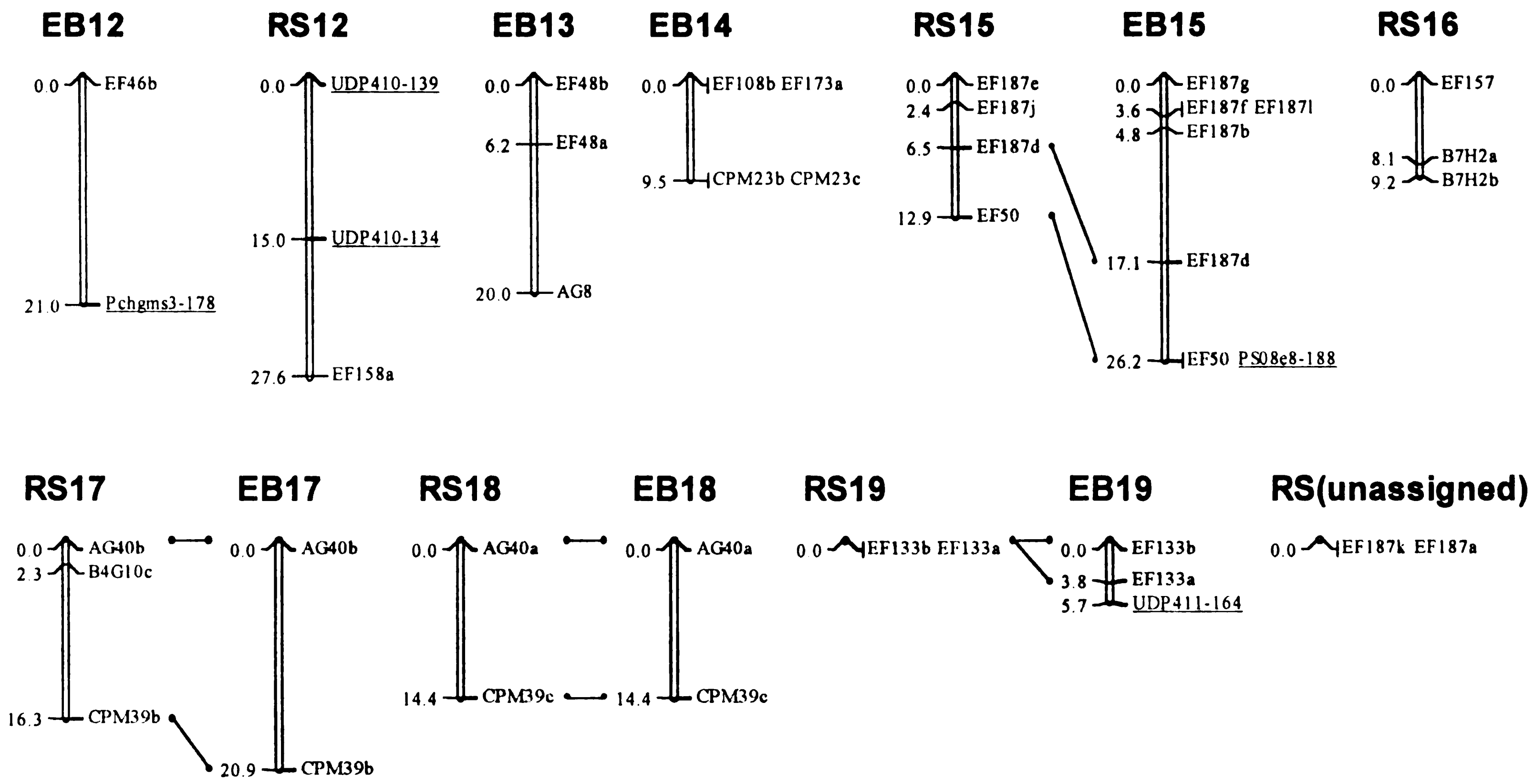
EB12
0.0 EF46b
21.0 Pchgms3-178
RS12
0.0 UDP410-139
15.0 UDP410-134
27.6 EF158a
EB13
0.0 EF48b
6.2 EF48a
20.0 AG8
EB14
0.0 EF108b EF173a
9.5 CPM23b CPM23c
RS15
0.0 EF187e
2.4 EF187j
6.5 EF187d
12.9 EF50
EB15
0.0 EF187g
3.6 EF187f EF187l
4.8 EF187b
17.1 EF187d
26.2 EF50 PS08e8-188
RS16
0.0 EF157
8.1 B7H2a
9.2 B7H2b
RS17
0.0 AG40b
2.3 B4G10c
16.3 CPM39b
EB17
0.0 AG40b
20.9 CPM39b
RS18
0.0 AG40a
14.4 CPM39c
EB18
0.0 AG40a
14.4 CPM39c
RS19
0.0 EF133b EF133a
EB19
0.0 EF133b
3.8 EF133a
5.7 UDP411-164
RS(unassigned)
0.0 EF187k EF187a

Fig. 1.1 (cont'd)

With the addition of new markers, RS9 and RS12 from the previous map combined into one linkage group as RS9 (Fig. 1.1). The previous RS linkage map (Wang et al. 1998) possesses 126 SDRF assigned to 19 linkage groups covering 461.6 cM. A six percent decrease (27.7 cM) in the map distance was observed. This is caused by the fact that linkage group 9 and 12 from the earlier map combined into one named group 9. Twenty-six markers remained unlinked. Marker order was generally conserved. The average distance (cM/marker) between two loci decreased from 4.3 to 3.3 cM in the RS map. Counterparts of fourteen RS linkage groups homologous to the EB linkage groups were detected (Fig. 1.1).

The revised EB and RS consensus map (Fig. 1.2) consisted of 161 markers covering 442.4 cM in 19 linkage groups. Forty-nine markers remained unlinked. The largest linkage group is group 9 covering 44.5 cM. The shortest linkage group is group19 covering 5.7 cM. The average distance between two markers is 2.79 cM. The longest distance between two adjacent markers is 16.9 cM in linkage group 8 (Fig. 1.1). The previous EB and RS consensus map (Wang et al. 1998) consisted of 144 SDRF in 16 groups covering 443.1 cM. The incorporation of 17 new markers did not change the length of the previous map significantly. The average distance (cM/marker) between two loci decreased from 3.07 to 2.8 cM in the revised map. Marker order in the new map was mostly conserved when compared to the previous EB and RS consensus map.

Figure 1.2., pages 36 and 37. The consensus map of two sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB), constructed from combined data of AFLP and SSR markers using JoinMap with a minimum LOD of 3.0 and a maximum recombination frequency of 0.35. SSR markers are underlined.

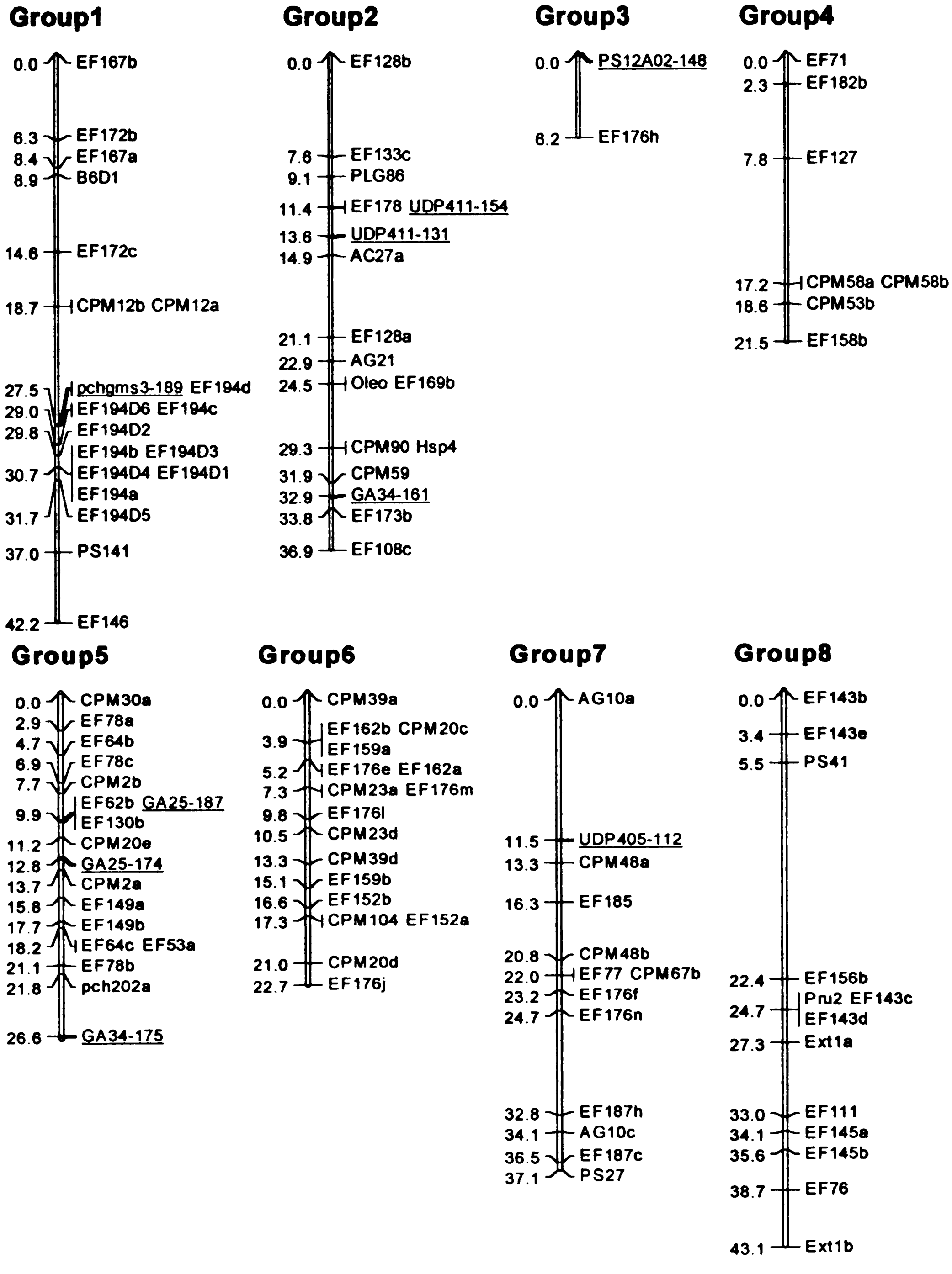
Group1
0.0 EF167b
6.3 EF172b
8.4 EF167a
8.9 B6D1
14.6 EF172c
18.7 CPM12b CPM12a
27.5 pchgms3-189 EF194d
29.0 EF194D6 EF194c
29.8 EF194D2
EF194b EF194D3
30.7 EF194D4 EF194D1
EF194a
31.7 EF194D5
37.0 PS141
42.2 EF146
Group2
0.0 EF128b
7.6 EF133c
9.1 PLG86
11.4 EF178 UDP411-154
13.6 UDP411-131
14.9 AC27a
21.1 EF128a
22.9 AG21
24.5 Oleo EF169b
29.3 CPM90 Hsp4
31.9 CPM59
32.9 GA34-161
33.8 EF173b
36.9 EF108c
Group3
0.0 PS12A02-148
6.2 EF176h
Group4
0.0 EF71
2.3 EF182b
7.8 EF127
17.2 CPM58a CPM58b
18.6 CPM53b
21.5 EF158b
Group5
0.0 CPM30a
2.9 EF78a
4.7 EF64b
6.9 EF78c
7.7 CPM2b
EF62b GA25-187
9.9 EF130b
11.2 CPM20e
12.8 GA25-174
13.7 CPM2a
15.8 EF149a
17.7 EF149b
18.2 EF64c EF53a
21.1 EF78b
21.8 pch202a
26.6 GA34-175
Group6
0.0 CPM39a
EF162b CPM20c
3.9 EF159a
5.2 EF176e EF162a
7.3 CPM23a EF176m
9.8 EF176l
10.5 CPM23d
13.3 CPM39d
15.1 EF159b
16.6 EF152b
17.3 CPM104 EF152a
21.0 CPM20d
22.7 EF176j
Group7
0.0 AG10a
11.5 UDP405-112
13.3 CPM48a
16.3 EF185
20.8 CPM48b
22.0 EF77 CPM67b
23.2 EF176f
24.7 EF176n
32.8 EF187h
34.1 AG10c
36.5 EF187c
37.1 PS27
Group8
0.0 EF143b
3.4 EF143e
5.5 PS41
22.4 EF156b
Pru2 EF143c
24.7 EF143d
27.3 Ext1a
33.0 EF111
34.1 EF145a
35.6 EF145b
38.7 EF76
43.1 Ext1b

Fig. 1.2.

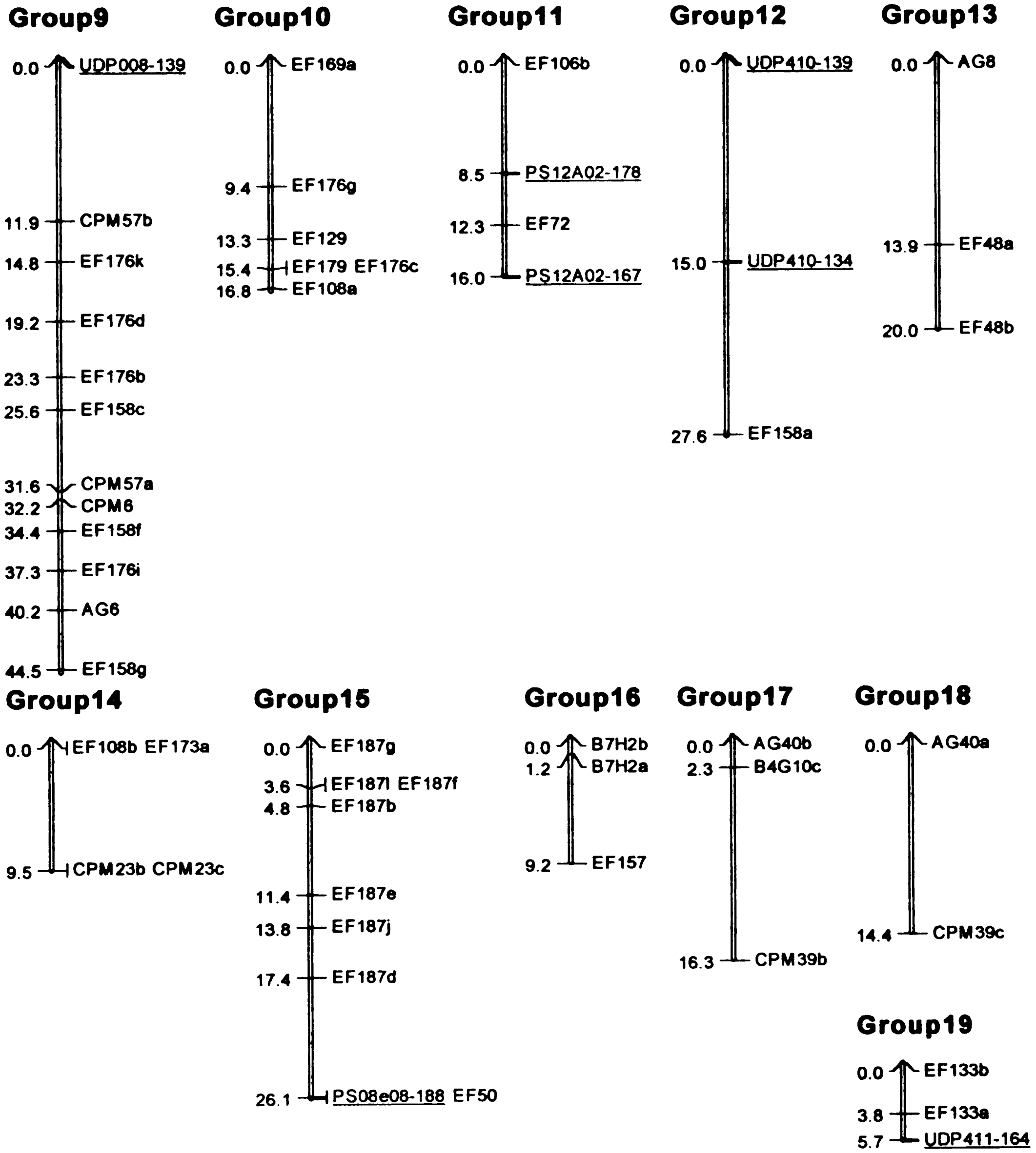
Group9
0.0 UDP008-139
11.9 CPM57b
14.8 EF176k
19.2 EF176d
23.3 EF176b
25.6 EF158c
31.6 CPM57a
32.2 CPM6
34.4 EF158f
37.3 EF176i
40.2 AG6
44.5 EF158g
Group10
0.0 EF169a
9.4 EF176g
13.3 EF129
15.4 EF179 EF176c
16.8 EF108a
Group11
0.0 EF106b
8.5 PS12A02-178
12.3 EF72
16.0 PS12A02-167
Group12
0.0 UDP410-139
15.0 UDP410-134
27.6 EF158a
Group13
0.0 AG8
13.9 EF48a
20.0 EF48b
Group14
0.0 EF108b EF173a
9.5 CPM23b CPM23c
Group15
0.0 EF187g
3.6 EF187l EF187f
4.8 EF187b
11.4 EF187e
13.8 EF187j
17.4 EF187d
26.1 PS08e08-188 EF50
Group16
0.0 B7H2b
1.2 B7H2a
9.2 EF157
Group17
0.0 AG40b
2.3 B4G10c
16.3 CPM39b
Group18
0.0 AG40a
14.4 CPM39c
Group19
0.0 EF133b
3.8 EF133a
5.7 UDP411-164

Fig. 1.2 (cont'd)

Homologous relations for linkage groups were identified using 60 bridging markers heterozygous in both parents (Fig. 1.1). Fifteen EB linkage groups homologous to the RS linkage groups were identified. RS counterparts of EB linkage groups 3, 12, 13 and 14 were not identified. EB counterpart of RS linkage group 16 was also not identified (Fig. 1.1).

Two EB linkage groups were homologous to RS6. The longer of two was named EB6 and the shorter was named EB6'. Two RS linkage groups were homologous to EB5. The longer one was named RS5 and the shorter was named RS5'. Similarly, two RS linkage groups were homologous to EB7. The longer one was named RS7 and the shorter was named RS7' (Fig. 1.1). In all three cases, the two linkage groups homologous to the same linkage group of the other parent may become one linkage group when the map is saturated as stated by Wang et. al. (1998).

Four of the apple primers published by Guilford et al. (1997) were tested in sour cherry. All of the apple primers tested (01a6, 02b1, 23fl and 26c6) showed PCR amplification in sour cherry (Table 1.1). SSRs isolated from apple did amplify alleles of expected size in pear (Yamamato et al. 2001) and in peach (60%) (Sosinski et al. 2000). In our study, none of the primers amplified the expected size of bands in sour cherry and they showed a complex banding pattern amplifying many bands in sour cherry (Table 1.1). Apple SSR primers gave similar results in apricot as (Sosinski et al. 2000) in sour cherry. Although 80% of the apple primers amplified in apricot, they all showed a complex banding pattern.

Twenty-one out of the 26 SSR primers developed from 'Redhaven ' peach cultivar (Testolin et al. 2000) tested in this study showed PCR amplification in sour cherry (Table 1.1). UDP96-010, UDP96-013, UDP96-015, UDP97-401 and UDP98-408 SSR primers were not studied since they were reported to show no amplification in sour cherry (Cipriani et al. 1999). Of the 21 SSR primers tested, only nine (UDP98-024, UDP98-406, UDP98-411, UDP-96-001, UDP-97-403, UDP98-405, UDP-98-406, UDP98-410 and UDP98-411) amplified bands of the expected size in sour cherry. Five of these nine markers also resulted in useful SDRF and four of them were incorporated into the sour cherry linkage map. Although UDP98-22 SSR primers amplified polymorphic bands and segregated 1:1 (Table 1.2), it did not map on any of the linkage groups. The sour cherry linkage map is not saturated. The lack of a saturated map in sour cherry limited the mapping of potentially useful marker such as UDP98-22. Similar limitations were concluded by Sosinski et al. (2000) for a peach map. They reported that five out of ten SSR they studied were informative, however only one of them (pchgms1) was incorporated into the peach map due to lack of saturation. The remaining 18 peach SSR primers showed a complex banding pattern with amplification of many bands per primer pair and were not useful for genetic and mapping studies in our sour cherry mapping population (Table 1.1).

All peach SSR primers reported by Sosinski et al. (2000) demonstrated PCR amplification in sour cherry. The pchgms1 presented a complex banding pattern between 140-300bp. Pchcms2 and pchcms3 were not polymorphic. Pchgms2, pchgms5 and B4G3 showed weak amplification and were not suitable as primers. The pchcms1 amplification product displayed a complex banding pattern resulting in many bands of unexpected size. Pchgms3 was informative, exhibiting SDRF and mapped to the sour cherry linkage map (Table 1.1). Since pchcms4 and pchcms5 were reported to show a complex banding pattern in sour cherry (Sosinski et al. 2000), these two markers were excluded in this study.

Sweet cherry SSR primers were also tested (Table 1.1) in sour cherry. Two of them were informative (PS08e08 and PS12A02) and were incorporated into the sour cherry map (Table 1.3).

The primer designed from pS141 probe was determined not to be useful as it amplified many bands between 140 bp and 500 bp. This probe had mapped to a region in the sour cherry linkage map group1 and that was associated with bloom time (Table 1.1).

Five of the SSR primers (GA26, GA65, GA50, GA57 and M55) developed from sour cherry (Amy Iezzoni, Department of Horticulture, Michigan State University, East Lansing, MI) showed a complex banding pattern. GA34 amplified 8 bands between 143 and 184 bp, but was not reproducible (Table 1.1). GA77 produced three bands, one being polymorphic, however it did not segregate in 1:1 or 3:1 ratio (Table 1.1). Of the sour primers tested, GA25 produced an expected ratio that was applicable to the sour cherry linkage map (Table 1.3).

Bliss et al. (2002) reported mapping four SSR loci in a *Prunus* map based on an interspecific cross between almond and peach. They mapped pchgms1 and GA34 to group 2, GA77 to group 4 and PS12A02 to group 8. Since GA77 did not produce a band segregating at a ratio of 1:1 and 3:1, this loci also was not mapped in the sour cherry map. PS12A02 (GK12A02) was mapped to group EB11 (Fig. 1.1) and EB and RS3 (Fig. 1.2) of sour cherry map.

Table 1.3 SSR markers incorporated into linkage groups of sour cherry map from cultivars 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB). Numbers refer to the linkage groups.

SSR primer	Sequence reference	Linkage groups in EB and RS consensus map	Linkage groups in RS map	Linkage groups in EB map
UDP98-410	(Testolin et al. 2000)	12	12	
UDP98-410	(Testolin et al. 2000)	12	12	
UDP98-411	(Testolin et al. 2000)	19		2
UDP98-411	(Testolin et al. 2000)	2	2	2
UDP98-411	(Testolin et al. 2000)	2	2	2
pchpgms3	(Sosinski et al. 2000)			12
pchpgms3	(Sosinski et al. 2000)	1		1
PS12A02	(Sosinski et al. 2000)	11		11
PS12A02	(Sosinski et al. 2000)	11		11
PS12A02	(Sosinski et al. 2000)	3		3
UDP98-405	(Testolin et all. 2000)	7	7	
UDP96-008	(Testolin et all. 2000)	9	9	
GA25 (PceGA25)	(Cantini et al. 2001)	5		
GA25 (PceGA25)	(Cantini et al. 2001)	5	5	
PS08E08	(Sosinski et al. 2000)	15	15	15

Joobeur et al. (2000) included six SSRs in the almond map. They mapped pchgms3 and PS9f8 into group 1, pchgms1 into group 2, PS12e2 to group4, PS7a2 into group 6 and PS8e8 into group 7 of the almond map (Table 1.4). In sour cherry, pchgms3 was placed on EB1, PS12e2 to EB11, and PS8e8 into EB15 (Table 1.4). The pchgms3 locus mapped into group 1 in both almond and sour cherry maps. PS7e2 could not be mapped in sour cherry map due to low DNA amplification and pchgms1 was also not mapped due to lack of DNA amplification. PS8e8 mapped to group 15 of sour cherry map, where it mapped group 7 of almond map. PS12e2 mapped to EB11 and EB3 and RS3 of the sour cherry map, where it mapped to group 4 of almond map and to group 8 of almond x peach map.

GA34 amplification products were very hard to score due to closeness of the alleles to each other and not always reproducible. These markers were mapped into group2 and group5 of EB and RS consensus map. The map location of the GA34 marker in sour cherry is in good agreement with the almond and peach map (Bliss et al. 2002), where it mapped between CPM59 and CPM90 markers in both maps. The map location of peach pchgms3 marker in sour cherry agrees with the results of Joobeur et al. (2000), where it mapped into the middle section of linkage group 1 on both maps.

The linkage groups in the previous sour cherry map (Wang et al. 1998) and in the revised map were numbered according to suspected homology to the almond x peach map (Bliss et al. 2002) and the almond map (Joobeur et al. 2000). Six linkage groups in sour cherry share two or more common markers (Table 1.4) with the corresponding linkage groups in the almond x peach map and in the almond map. These results suggest that these six linkage groups of sour cherry might be homologous to the corresponding linkage groups in the almond x peach map and the almond map. The distances between common markers shared between these maps are generally consistent. For example, markers Pru2 and Ext1 were mapped 44.6 cM apart in sour cherry (Fig. 1.2) and 41 cM apart in the almond x peach map. CPM39 and CPM20 were mapped 21 cM apart in sour cherry (Fig. 1.2) and 25.2 cM apart in the almond x peach map. However, inconsistencies in map distances between shared markers also exist. For example, markers PLG86 and CPM59 were mapped 50.4 cM apart in the almond x peach map, however, only 22.8 cM apart in sour cherry (Fig. 1.2). These conclusions about the homology relations are preliminary until more common markers are incorporated to these maps.

Table 1.4. Common markers which were used in the assignment of sour cherry linkage groups.

Linkage group	Shared markers of the sour cherry map with the corresponding linkage groups in the almond x peach map (Bliss et al. 2002) and the almond map (Joobeur et al. 2000)
1	pchgms3, CPM12
2	GA34, AC27, AG21, Ole1, PLG86, CPM59, CPM90
4	CPM58, CPM53
5	CPM20, CPM2
6	CPM20, CPM39, CPM23
7	CPM67, CPM48, AG10, CPM64
8	Ext1, Pru2

Eight out of 26 SRR markers developed by Testolin et al. (2000) were assigned into a peach map constructed with F_2 progenies from cultivars 'Akame' and ' Jeseitou' (Yamamoto et al. 2001). When compared to our results, six of the eight SSR markers (UDP96-01, UDP96-03, UDP96-05. UDP9619, UDP98406, and Udp-409) also amplified in sour cherry, however none of them were informative which prevented mapping (Table 1).

Table 1.5. Linkage group locations of SSR loci in maps of several *Prunus* species. Numbers refer to linkage groups.

SSR marker	Sour cherry	Almond (Joobeur et al. 2000)	Peach and almond (Bliss et al. 2002)	Peach (Sosinski et al. 2000)	Peach (Dettori et al. 2001)	Peach (Yamamoto et al. 2001)	Apricot (Hurtado et al. 2002)
Pchgms1	c.b	2	2	1	-	-	7
Pchgms3	1	1	-	+	-	-	-
pchgms4	-	-	-	-	-	-	4
pchgms5	-	-	-	-	-	-	1
pchcms5	-	-	-	-	-	-	3
GA34*^{a}	5, 2	-	2	-	-	-	-
GA25*^{b}	5	-	-	-	-	-	-
GA77*^{c}	p.n.s	-	4	-	-	-	-
PS12A02	3, 11	4	8		-	-	-
PS07A02	w.a	6	-	-	-	-	-
PS08E08	15	7	-	-	-	-	-
PS09F08	c.b	1	-	-	-	-	-
UDP-405	7	-	-	-	-	-	-
UDP-008	9	-	-	-	3	-	
UDP-410	12	-	-	-	2	-	4
UDP-411	2 , 19	-	-	-	2	-	4
UDP-022	I.f	-	-	-	1	-	-
UDP-408	n.a	-	-	-	-	1	-
UDP-005	c.b	-	-	-	-	5	2
UDP-004	-	-	-	-	-	6	-
UDP-019	c.b	-	-	-	-	3	-
UDP-001	p.n.s	-	-	-	6	3	-
UDP-015	n.a	-	-	-	8	3	-
UDP-406	p.n.s	-	-	-	2	7	4
UDP-010	-				6	-	3
UDP-409	n.p	-	-	-	8	3	2
UDP-018	c.b	-	-	-	1	-	2
UDP-013	n.a	-	-	-	2	-	4
UDP-401	n.a	-	-	-	5	-	-
UDP-412	c.b	-	-	-	6	-	-
UDP-024	c.b	-	-	-	4	-	-
UDP-415	n.a	-	-	-	7	-	-
UDP-003	c.b	-	-	-	4	6	-
UDP-416	w.a	-	-	-	6	-	-

n.a = no amplification, n.p = not polymorphic, c.b = complex banding pattern, p.n.s = polymorphic but no SDRF, w.a = weak amplification, I.f = informative. *a,b, and c are also called PceGA34, PceGA25 and PceGA77, respectively

Dettori et al. (2001) reported that 17 out of 26 peach microstellites tested were polymorphic in peach and incorporated into a peach genetic linkage map in a backcross progeny (*Prunus persica* x (*P. persica x P. ferganensis*) (Table 1.5). UDP-411 mapped into linkage group 2 in both sour cherry and peach map. UDP-008 mapped into linkage group 9 of the sour cherry map and linkage group 3 of the peach map. UDP-410 mapped into linkage group 12 of sour cherry map and into linkage group 2 of the peach map.

Out of 45 SSR primer pairs, 22 % (10 loci) were found to be informative yielding 17 informative SDRF that were incorporated in the map EB and RS consensus map in current study. This is comparable to the results in apricot where out of 45 SSR screened, 13 (28%) loci were mapped (Hurtado et al. 2002).

QTL locations for six fruit and flower traits were detected by Wang et al. (1998). In current study, SSR markers were mapped to the locations of QTL detected earlier (Table 1.6). Two peach SSR markers UDP411-154 and UDP411-131 (Fig. 1.3) were linked to the bloom time (*blm2*) location, at the distances of 4.5 cM and 2.3 cM, respectively (Table 1.6). The same peach markers are also tightly linked to fruit weight QTL (*fw2*), at the distances of 4.5 cM and 2.3cM respectively (Table 1.6). The pchgms3-189 marker was mapped to 8.4 cM of the PS141 which located in bloom time (*blm1*) area. The same marker is also tightly linked to EF194c marker at a distance of 0.8 cM which is located in pistil death (*pd1*) area (Table 1.6). UDP405-112 marker mapped 11.1 cM distance of the AG10b marker which is the closest marker to soluble solids concentration (*ssc2*) QTL location (Table 1.6). SSR markers obtained are horticulturally very important. Being tightly linked to important traits and highly polymorphic, these SSR markers will be utilized for breeding for these traits saving considerable time and resources. A negative correlation was found between bloom time and percent pistil death (r = - 0.25) and also a negative correlations exits between bloom time and fruit weight (r = - 0.45) (Wang et al. 1998). The existence of correlation between these traits further increases the value of these markers enabling breeder to select more than one trait at the same time.

Table 1.6. QTL detected for flower and fruit traits in sour cherry cultivars 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB) by Wang et al. (1998) and SSR markers incorporated to these QTL locations in current study.

Trait	QTL	Linkage group	R^2	Nearest RFLP maker(s)	Nearest SSR marker	SSR distance to RFLP marker
Bloom time	Blm1	EB1	19.9	PS141	pchgms3-189	8.4 cM
	Blm2	EB and RS 2	22.3	PLG86	UDP411-154 UDP411-131	4.5 cM 2.3 cM
Pistil death (%)	Pd1	EB1	12.9	EF194c	pchgms3-189	0.8 cM
	Pd2	RS8	14.3	EF156b		
Pollen germination(%)	Pgr	EB1	17.0	EF146		
Ripening time	Rp1	RS4	21.5	EF158b		
	Rp2	EB and RS 6	25.9	CPM20e		
Fruit weight (g)	Fw1	EB4	13.7	EF182a		
	Fw2	EB and RS 2	15.5	PLG86	UDP411-154 UDP411- 131	4.5 cM 2.3cM
Soluble solids concentration	Ssc1	EB7	16.5	AG10b	UDP405-112	11.1cM
	Ssc2	RS6	13.1	EF159a		

R^2 = amount of phenotypic variance explained by QTL (Coefficient of determination).

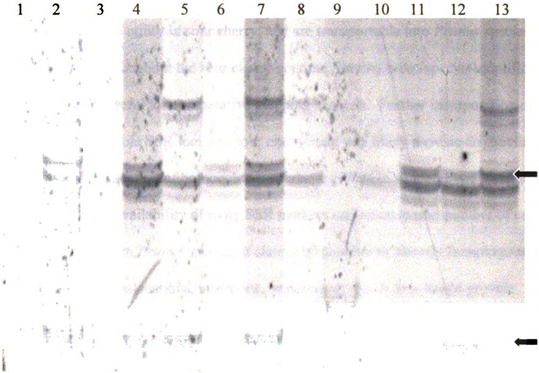

Fig. 1.3. DNA fragment patterns of the SSR primer UDP411. Arrows indicates two segregating fragments which were mapped to bloom time 2 (*blm2*) location. The upper arrow indicates a 154 bp fragment named UDP411-154 and the lower arrow indicates a 131 bp fragment named UDP411-131. Lanes 1-13 are progenies from 'Rheinische Schattenmorelle' (RS) x 'Erdi Botermo' (EB) population.

The expanded *Prunus* genetic linkage map constructed from peach and almond covers 1144 cM (Bliss et al. 2002). The sour cherry linkage map, being tetraploid (2n = 4x = 32), should have 16 linkage groups covering two times of the length of the peach map. Mapping has a drawback in sour cherry due to the requirement for SDRF in a tetraploid state, which limits the availability of informative markers. However, new SSR primer pairs were recently published by Aranzana et al. (2002), Dirlewanger et al. (2002) and Wang et al. (2002). Incorporation of new markers should extend the current sour cherry map and bring the linkage group number down to 16 groups.

The results in this study clearly indicate that SSR developed in other *Prunus* species have good utility in sour cherry, and are transportable into *Prunus* species. SSR are distributed throughout the sour cherry genome. Having cross-species amplification, they are highly useful for comparative mapping analysis. Further incorporation of currently unavailable SSR loci into sour cherry map will likely provide an excellent source for identification of homoeologous linkage groups in sour cherry (Wang et al. 1998). With the availability of more SSR markers and an increased number of common SSR loci mapped in *Prunus* species, it should be possible to identify homologous areas, and regions of translocations, insertions, or deletions. Such data would provide information on gene order conservation in *Prunus* and the family Rosaceae.

LITERATURE CITED

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan P (1994) Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci. 35:1439-1445

Aranzana MJ, Garcia-Mas J, Carbo J, Arus P (2002) Development and variability analysis of microsatellite markers in peach. Plant Breeding 121: 87-92

Arus P. Messeguer R. Viruel M, Tobutt K, Dirlewanger E, Santi F. Quarta R. and Ritte' E (1994) The European *Prunus* mapping project. Euphytica 77: 97-100

Baird WV, Ballard RE, Rajapakse S, Abbott AG (1996) Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. HortScience 31: 1099-1106

Ballester J, Company RSI, Arus P, de Vicente MC (2001) Genetic mapping of a major gene delaying blooming time in almond. Plant Breeding 120:268-270

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-83

Bartolozzi F, Warburton ML, Arulsekar S, Gradziel TM (1998) Genetic characterization and relatedness among California almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. J. Am. Soc. Hortic. Sci. 123:381-387

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137-144

Beaver JA, Iezzoni AF, Ramn C (1995) Isozyme diversity in sour, sweet and ground cherry. Theor. Appl. Genet. 90:847-852

Bidel LPR, Renault P, Pages L, Riviere LM (2000) Mapping meristem respiration of *Prunus persica* (L.) Batsch seedlings: potential respiration of the meristems, O-2 diffusional constraints and combined effects on root growth. Journal of Experimental Botany 51:755-768

Bliss FA, Arulsekar S, Foolad MR, Beccerra V, Gillen AM, Warburton AM, Dandekar AM, Kocsisne GM, Mydin KK (2002) An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach. Genome 45:520-529

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996) Isolation and characterization of new polymorphic SSR loci in grape. Genome 39:628-633

Broun P, Tankley S (1996) Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol. Gen. Genet. 250:39-49

Cantini C, Iezzoni AF, Lamboy WF, Boritzki M, Struss D (2001) DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats J. Am. Soc. Hortic. Sci. 126:205-209

Carbonell EA, Gerig TM, Balansard E, Asins MJ (1992) Interval mapping in the analysis of nonadditive quantitative trait loci. Biometrics 48:305-315

Casas AM, Igartua E, Balaguer G, Moreno MA (1999) Genetic diversity of *Prunus* rootstocks analyzed by RAPD markers. Euphytica 110:139-149

Cervera MT, Remington D, Frigerio JM, Storme V, Ivens B, Boerjan W, Plomion C (2000) Improved AFLP analysis of tree species. Canadian Journal of Forest Research Revue Canadienne de Recherche Forestiere 30:1608-1616

Chaparro JS, Werner DJ, O'Malley D, Sederoff RR (1994) Targeted mapping and linkage analysis of morphological, isozvme, and RAPD markers in peach. Theor. Appl. Genet. 87:805-815

Cheng HY, Yang WC, Hsiao JY (2001) Genetic diversity and relationship among peach cultivars based on Random Amplified Microsatellite Polymorphism (RAMP). Botanical Bulletin of Academia Sinica 42:201 -206

Cipriani G, Lot G, Huang WG, Marrazzo T, Peterlunger E, Testolin R. (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99:65-72

Company RSI, Felipe AJ, Aparasi JG (1999) A major gene for flowering time in almond. Plant Breeding 118:443-448

Condit R, Hubbell SP (1991) Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome 34:66-71

Dettori MT, Quarta R, Verde I (2001) A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. Genome 44:783-790

De Candolle A (1884) Origin of cultivated plants. Kegan Paul, London.

Dirlewanger E, Bodo C (1994) Molecular qanetic map of peach. Euphytica 77:101-103

Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTL controlling fruit quality in peach (*Prunus persica* (L.) Batsch). Theor. Appl. Genet. 98:18-31

Dirlewanger E, Pronier V, Parvery C, Rothan C, Guye A, Monet R (1998) Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. Theor. Appl. Genet. 97:888-895

Dirlewanger E, Cosson P, Tavaud M (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105:127-138

Downey SL, Iezzoni AF (2000) Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. J. Am. Soc. Hort. Sci. 125:76-80

Edwards MD. Helentjaris T. Wright S. Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. Theor. Appl. Genet. 83:765-774

Etienne C, Rothan C, Moing A, Plomion C, Bodenes C, Svanella-Dumas L, Cosson P, Pronier V, Monet R, Dirlewanger E (2002) Candidate genes and QTLs for sugar and organic acid content in peach *[Prunus persica* (L.) Batsch]. Theor. Appl. Genet. 105:145-159

Foolad MR, Arulsekar S. Becerra V, Bliss FA (1995) A genetic map of Prunus based on an interspesific cross between peach and almond. Theor. Appl. Genet. 91:262-269

Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA SSR. Molecular Ecology 10:2275-2283

Goulao L, Monte-Corvo L, Oliveira CM (2001) Phenetic characterization of plum cultivars by high multiplex ratio markers: Amplified fragment length polymorphisms and inter-simple sequence repeats. J. Am. Soc. Hortic. Sci. 126: 72-77

Guilford P, Prakash S, Zhu JM (1997) Microsatellites in *Malus domestica* (apple): Abundance, polymorphism and cultivar identification. Theor. Appl. Genet. 94: 249-254

Hemmat M, Weeden NF, Manganaris AG, Lawson NM, (1994) Molecular marker linkage map of apple. J. Hered. 85:4-11

Hormaza JI (1999) Early selection in cherry combining RAPDs with embryo culture. Scientia Horticulturae. 79:121-1261

Hormaza JI (2002) Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor. Appl. Genet. 104:321-328

Hurtado MA, Romero C, Vilanova S, Abbott AG, Llacer G, Badanes ML (2002) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.), and mapping of PPV (sharka) resistance. Theor. Appl. Genet. 105:182-191

Iezzoni AF, Hancock AM (1996) Chloroplast DNA variation in sour cherry. Proc. Intl. Cherry Sym. (Ed. C. Hampson, R. Anderson, R. Perry and A. Webster) Acta Hort. 410

Jauregui B, de Vicente MC, Messeguer R, Felipe A, Bonnet A, Salesses G, Arus P (2001) A reciprocal translocation between 'Garfi' almond and 'Nemared' peach. Theor. Appl. Genet. 102:1169-1176

Joobeur T, Viruel MA, de Vicente MC, Jauregui B, Ballester J, Dettori MT, Verde I, Truco MJ, Messeguer R, Batlle I, Quarta R, Dirlewanger E, Arus P (1998) Construction of a saturated linkage map for Prunus using an almond x peach F-2 progeny. Theor. Appl. Genet. 97:1034-1041

Joobeur T, Periam N, Vicente MC, King GJ, Arus P (2000) Development of a Second generation linkage map for almond using RAPD and SSR markers. Genome 43:649-655

Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1995) An evaluation of sequence tagged microsatellite site markers for genetic analysis within Ci *trus* and related species. Genome 38:349-355

Kimura T, Shi YZ, Shoda M (2002) Identification of Asian pear varieties by SSR analysis. Breeding Sci. 52:115-121

Knapp SJ (1994) Selection using molecular markers indexes, p.1-11. In: Proceeding of the second symposium of the American Society for Horticultural Science and Crop science sociey of America: Analysis of molecular marker data (Corvallis, Ore.). Amer. Soc. Hort. Sci., Alexandria, VA.

Kolesnikova AF (1975) Breeding and some biological characteristics of sour cherry in central Russia. USSR Priokstock Izdatel,stvo, Orel

Kowalski SP, Lan TH, Feldmann KA, Paterson AH (1994) Comparative mapping of *Arabidopsis thaliana* and *Brassica oleraceae* chromosomes reveals islands of conserved organization. Genetics 138:499-510

Lincoln SM, Daly M, Lander E (1992) Constructing genetic maps with MAPMAKER EXP 3.0. 3rd ed. Whitehead Inst. Tech. Rpt., Cambridge, Mass.

Litt M, Ludy JA (1989) A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 44:397-401

Lu ZX, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998) Construction of A genetic linkage map and identification of AFLP markers for resistance to root-knotnematodes in peach rootstocks. Genome 41:199-207

Lu ZX, Sossey-Alaoui K, Reighard GL, Baird WV, Abbott AG (1999) Development and characterization of a co-dominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding. Theor. Appl. Genet. 99:115-122

Manubens A, Lobos S, Jadue Y, Toro M, Messina R, Lladser M, Seelenfreund D (1999) DNA isolation and AFLP fingerprinting of nectarine and peach varieties (Prunus persica). Plant Molecular Biology Reporter 17:255-267

Ma ZQ, Roder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123-130

McCouch SR, Chen X, Panud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T, Blair M (1997) Microstellite marker development, mapping and applications in rice genetic and breeding. Plant Mol. Biol. 35:238-242

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88:9828-9832

Murray HO, Thompson WF (1980) Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 8:4321-4325

Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA, King RA (1995) Linkage analysis with multiplexed short tandem repeat polymorphism using infrared fluorescence and M13 tailed primers. Genomics 30:450-458

Osborn TC. Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor. Appl. Genet. 73:350-356

Paterson AH, Lin YR, Li Z. Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714-1718

Paterson AH (1995) Molecular dissection of quantitative traits: progress and prospects. Genome Research 5:321-333

Powell W. Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends in Plant Sci. 1:215-222

Rajapakse S. Belthoff LE, He G. Estager AK, Scorza R. Verde I Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995) Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor. Appl. Genet. 90:503-510

Sambrook J. Fritsche EF, Maniatis T (1989) Molecular cloning. A laboratory manual, 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Scorza R (1996) Genome mapping of Horticultural crops: Introduction to the colloquium. HortScience. 31(7)

Shimada T, Yamamoto T, Hayama H, Yamaguchi M, Hayashi T (2000) A genetic linkage map constructed by using an intra-specific cross between peach cultivars grown in Japan. Jpn. Soc. Hortic. Sci. 69:536542

Sorrells ME (1992) Development and application of RFLPs in polyploids. Crop Sci 32:1086-1091

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite Markers in peach [Prunus persica (L.) Batsch]. Theor. Appl. Genet. 101 (3):421-428

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J. 3:739-744

Stockinger EJ, Mulinix CA, Long CM, Brettin TS, Iezzoni AF (1996) A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J. Hered. 87: 214-218

Struss D, Boritzki M, Glozer K, Southwick SM (2001) Detection of genetic diversity among populations of sweet cherry (*Prunus avium* L.) by AFLPs. Journal of Horticultural Science & Biotechnology 76:362-367

Szewc-McFadden AK, Lamboy WF, McFerson JR (1996) Utilization of identified simple sequence repeats in *Malus domestica* (L.) Borkh. (Apple) for germplasm characterization. HortScience. 31:619 (Abst.)

Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble solids content in tomato-a re-examination. Theor. Appl. Genet. 75:811-823

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: New tools for an old science. Bio/Technology 7:257-264

Taramino G, Tingey S (1996) Simple sequence repeats fort germplasm analysis and mapping in maize. Genome 39:277-287

Tao R, Habu T, Yamane H, Sugiura A, Iwamoto K (2000) Molecular markers for self-compatibility in Japanese apricot (Prunus mume). HortScience 35:1121-1123

Testolin R, Marrazzo T, Cipriani G, Quarta R, Verde I, Dettori MT, Pancaldi M, Sansavini S (2000) Microsatellite DNA in peach (Prunus persica L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. Genome 43:512-520

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86:985-990

Viruel MA, Messeguer R. de Vicente MC, Garcia-Mas J. Puigdomenech P, Vargas F, Arus P (1995) A linkage map with RFLP and isozyme markers for almond. Theor. Appl. Genet. 91:964-971

Wang A, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 88:1-6

Wang D, Karle R, Brettin TS, Iezzoni AF (1998) Genetic linkage map in sour cherry using RFPL markers. Theor. Appl. Genet. 97:1217-1224

Wang D, Karle R, Iezzoni AF (2000) QTL analysis of flower and fruit traits in sour cherry. Theor. Appl. Genet. 100:535-544

Wang Y, Georgi LL, Zhebentyayeva TN, Reighard GL, Scorza R, Abbott AG (2002) High-throughput targeted SSR marker development in peach (*Prunus persica*). Genome 45:319-328

Weir B (1990) Genetic Data Analysis, Sinauer Assoc., Sunderland MA.

Webster AD (1996) The taxonomic classification of sweet and sour cherries and a brief history of their cultivation. In: Cherries; Crop physiology, production and uses. (Ed. Webster A.D. and Looney N.E.) Cab international. Wallinford, Oxon, UK.

Webster AD, Looney NE (1996) World distribution of sweet and sour cherry production. National statistics. In: Cherries; Crop physiology, production and uses. (Ed. Webster A.D. and Looney N.E.) Cab international. Wallinford, Oxon, UK.

Weber JL (1990) Informativeness of human (dC-dA)n. (dG-dT)n polymorphisms. Genomics 7:524-530

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. 83:294-300

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic-mapping of microsatellites in rice. Mol. Gen. Genet. 241:225-235

Wu KS, Jones R, Danneberger L, Scolnik PA (1994) Detection of microsatellite polymorphisms without cloning. Nucleic Acid Res. 22:3257-3258

Yamamoto T, Kimura T, Sawamura Y, Kotobuki K, Ban Y, Hayashi T, Matsuta N (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor. Appl. Genet. 102:865-870

Yamamoto T, Shimada T, Imai T (2001) Characterization of morphological traits based on genetic linkage map in peach. Breeding Sci. 51:271-278

CHAPTER 2

A MODIFIED-BULK SEGREGANT ANALYSIS FOR BLOOM TIME IN SOUR CHERRY

ABSTRACT

Developing genetic markers linked to bloom time in sour cherry (*Prunus cerasus* L., 2n=4x=32) is very important, because utilization of markers will help the indirect selection of varieties for desirable bloom time in early generations, saving time and effort. Three different approaches were used to identify markers associated with bloom time in a sour cherry population derived from crosses between two sour cherry cultivars, 'Balaton' and 'Surefire'.

In first method, a primer pair designed from the sequence of an AFLP probe pS141 that mapped to the linkage group 1 of 'Erdi Botermo' (EB) (Chapter 1) was used to find markers associated with the bloom time. The primer amplified too many bands between 140 bp and 500 bp, and therefore did not distinguish any specific association with the trait.

In a second approach, the pchpgms3 SSR marker, which mapped to the EB1 at 8.4 cM distance from the pS141 probe, was tested for association with bloom time. Bloom data was converted into degree-days and tested for association with PCR amplification products of the pchpgms3 SSR marker, (189bp, 176bp and 174bp) which were polymorphic in the progeny (the progeny set is shown in table 2.2). There was no significant relationship between alleles amplified by pchpgms3 primer and bloom time data in sour cherry.

In a third strategy, a modified bulk segregant analysis in combination with AFLP technique was used to screen progenies from two extreme phenotypic classes for bloom time. The average number of polymorphic bands was 10.7 per primer pair and the

percentage of polymorphism ranged from 10% to 44% for primer pair combinations. Screening of early and late extreme groups with 156 AFLP primer pairs resulted in the identification of three candidate bands in three different primer combinations (a 82 bp fragment in EGG/MCAC primer pair combination, a 78 bp fragment in ETT/MCCG primer pair combination, and a 94 bp fragment in EAA/MCGT primer pair combination). These candidate bands were present in an early bloom time group but not in late group or versa visa.

INTRODUCTION

Consistent yield is one of main objectives of sour cherry breeding programs (Iezzoni 1996). In some cherry growing regions, such as Michigan, where 72.5 % of the sour cherries in US are produced (USDA/NASS 1999), low temperature damage to flower buds and flowers is the most common factor reducing yield (Thompson 1996). Therefore, cold hardiness of sour cherry flower buds is one of the most important breeding objectives for these cold production regions (Iezzoni 1996). A delay in the spring floral bud development could decrease crop loss from a spring freeze (Iezzoni 1996). Therefore, the development of new later blooming varieties would avoid some of the loss due to spring freeze injury.

Bloom time in cherry is a quantitative trait, but has high broad sense heritability (0.91) (Wang et al. 2000) probably due to low number of genes controlling the trait. Identification of markers linked to Quantitative Trait Loci (QTL) controlling bloom time in sour cherry could expedite the development of new cultivars or improvement of current cultivars with late blooming characteristics using marker assisted selection (MAS).

c

c

s

T

sp

ge

LITERATURE REVIEW

QTL identification strategies

DNA markers are essential tools in plant genetics with particular value in gene mapping and marker assisted selection. Genetic markers linked with QTL may enable indirect selection of complex traits. Molecular markers have been successfully used to map individual genetic factors or QTL controlling complex traits (Hurtado et al. 2002; Ballester et al. 2001; Dirlewanger et al. 1999). While such experiments are useful, they require large populations, and are labor-intensive (Wang and Paterson 1994). Construction of separate linkage maps to identify QTL for each complex trait in many different populations is frequently not feasible (Miklas et al. 1996).

More efficient alternatives to the construction of saturated linkage maps for identifying QTL have been developed. Bulked segregant analysis (BSA) (Michelmore et al. 1991) and selective genotyping (Lander and Botstein 1989) have been used to identify markers linked to targeted QTL. In these approaches, polymorphic markers are evaluated across two DNA pools (BSA method) or groups of lines (selective genotype method). One DNA pool or group of lines consists of the most resistant (one-extreme) and the other the most susceptible (other extreme) lines within the population. Markers that co-segregate within groups with the trait of interest are mapped across the entire population. Thus, only a few select markers are mapped and analyzed for association with the specific quantitative trait (Miklas et al. 1996). Chen et al. (1994) used the selective genotyping approach with the objective of rapidly locating putative resistance loci.

DNA pooling based only upon phenotypic information, or BSA has been employed mostly in the analysis of simply inherited traits. Wang and Paterson (1994) assessed the utility of phenotype-based DNA pools for tagging QTL in F_2, backcross (BC), recombinant inbred (RI), and doubled haploid (DH) populations. The effects of population size, portion of population selected, magnitude of the phenotypic effect of individual QTL alleles (QTL allele effect), and effects of both dominance and deviations from Mendelian segregation ratios, are taken into consideration. It was suggested that BC populations are better than F_2 populations, but less efficient than RI or DH populations, for "tagging" QTL using phenotype-based DNA pools. The use of phenotype-based DNA pools might be successful in tagging QTL of very large effect, but is unlikely to permit comprehensive identification of the majority of QTL affecting a complex trait (Wang and Paterson 1994). To tag QTL using phenotype-based DNA pools, Wang and Paterson (1994) recommended several considerations, including (1) the use of crosses having a wide variation; (2) the use of large populations (3) the use of homozygous populations, i.e., RI or DH lines; and (4) the replication of phenotypic evaluations, facilitated by the use of homozygous populations, but also possible by using F_2 or BC –derived lines for phenotype evaluations (Wang and Paterson 1994).

Koester et al. (1993) used F_2 and F_3 progeny derived from crosses between the near isogenic lines (NIL) and the recurrent parent of maize to identify QTL controlling days to flowering and plant height. Mansur et al. (1993) used recombinant inbred lines (RIL) exhibiting an extreme phenotype for each trait (eg. early and late plants maturity) by having two bulked DNA samples prepared for each trait. When an RFLP marker was linked to a QTL, one parental allele predominated in the bulked DNA from a particular phenotype; while the other allele was associated with the opposite phenotype. Chalmers et al. (1993) successfully used DH populations of barley in combination with RAPDs to identify molecular markers linked to genes controlling the milling energy (ME) requirement. Their work involved the construction of bulks by combining DNA from DH families representing the extreme members of the distribution for ME. Miklas et al. (1996) investigated the use of selective mapping to expedite identification of RAPDs associated with QTL conditioning bean golden mosaic virus (BGMV) or common bacterial blight (CBB) resistance using RIL populations of common bean (*Phaseolus vulgaris* L.). They used a BSA of as few as three individuals and tested 101 RAPDs identified as polymorphic between the parents tested across resistant vs. susceptible bulks for BGMV reaction. Fourteen of 22 RAPDs which were selectively mapped because they co-segregated among lines within bulks, and were linked with seven of the nine QTL. BSA and selective genotyping was equally effective and less costly than completely classifying the entire population with each marker.

Use of AFLP'S in *Prunus*:

Amplified fragment length polymorphism (AFLP) is used increasingly in a variety of genetic analyses due to its suitability for high-throughput analyses (Cervera et al. 2000). Cervera et al. (2000) evaluated an AFLP system and found it to be a powerful tool in forest tree genetics for genetic variability studies, genome mapping purposes and fingerprinting of different tree species such as *Prunus* spp., *Eucalyptus* spp., *Quercus* spp., *Populus* spp., and *Pinus* spp, .

Campalans et al. (2001) used the cDNA-AFLP (amplified restriction fragment polymorphism derived technique for RNA fingerprinting) method to find differentially expressed genes during dehydration of almond.

AFLPs are useful for identification and genome analysis of sweet cherry cultivars (Struss et al. 2001). Struss et al. (2001) found that ten out of 18 primer combinations were informative. Seven to thirty-three of the amplified bands were polymorphic and all 38 sweet cherry cultivars were clearly identified. Goulao et al. (2001) stated that AFLP and ISSR techniques are useful for identification of genotypes and analysis of phenetic relationships in plum (*Prunus domestica* L.). They used six ISSR and seven AFLP primers resulting in the amplification of 270 and 379 fragments, respectively. Several cultivars fall the same group with both AFLP and ISSR analysis. The phenetic classification from the two methods were similar ($r = 0.73$, for the diploid group) but, ISSR had better reproducibility and a higher percentage of polymorphisms (87.4% vs. 62.8%) (Goulao et al. 2001). Manubens et al. (1999) employed AFLP fingerprinting for assessment of peach and nectarine (*Prunus persica* ssp *nucipersia*) varieties and distinguished eight peach and six nectarine varieties more consistently when compared to traditional identification based on assessment of agronomic traits of the adult plant.

Shimada et al. (1999) studied the AFLP system for usefulness in obtaining information on reproducibility, efficiency and frequency of polymorphisms, in the peach using fluorescein isothiocyanate (FITC) and biotin detection systems. An almost identical band pattern was obtained from different methods of DNA extraction and between replications. AFLP analysis resulted in 2.5 polymorphic bands between 'Akame' and Juseitou' per primer, which is 20 times more than those obtained by RAPD analysis leading to discrimination of closely related cultivars. They concluded that AFLP analysis is a useful system for cultivar identification, the parentage, and mapping work in peach.

Lu et al. (1999) found that a co-dominant AFLP marker, EAA/MCAT10, co-segregates with the primary source of resistance to root-knot nematodes (*Meloidogyme incognita* and *hi. javanica*) in rootstock cultivars of peach. Two allelic DNA fragments of this AFLP marker were cloned, then sequenced and converted to sequence tagged sites (STS). Four nucleotide differences (i.e. one addition and three substitutions) were observed between the two clones. Then they evaluated the STS marker system for peach germplasm improvement by PCR-amplifying germplasm with the Mij3F/Mij1R primer pair and then digesting with Sau3 AI. The banding patterns of the EAA/MCAT10 STS markers were able to distinguish among the three genotypes - homozygous resistant, heterozygous resistant and homozygous susceptible - in the 'Lovell' x 'Nemared' cross. Moreover, the results of the rootstock survey were consistent with nematode infection response of each rootstock.

Bulk segregant analysis

The conventional method of locating and comparing QTL requires a segregating population of plants where each one is genotyped with molecular markers. Another approach is to group plants according to the phenotype of the trait of interest and test for differences in allele frequency between the population bulks: bulk-segregant analysis (BSA) (Michelmore et al. 1991). A marker that is polymorphic between the parents of the population and closely-linked to a major QTL regulating a trait of interest will co-segregate with that QTL. For example a marker will co-segregate with the phenotype of a trait if the QTL has a major effect. If two extreme groups are analyzed with the polymorphic marker, the frequency of the two marker alleles present within each of the two bulks will deviate significantly from the 1:1 ratio expected for most populations. Since, in many species, chromosomal locations of many markers was determined, the location of linked QTL could be deduced without genotyping each individual in a segregating population. This method was used in composite populations of maize to locate QTL effecting yield under drought conditions (Quarrie et al. 1999).

Bentolila and Hanson (2001) used BSA to identify markers closely linked to the restorer of fertility (*Rf*) locus in petunia in a large BC1 population produced from two different parental lines carrying *Rf*. They were able to identify an amplified fragment length polymorphism (AFLP) marker that co-segregates with Rf.

Decousset et al. (2000) employed BSA in a BC2 population segregating for the Ppd-HI photoperiod response gene and were able to identify six AFLP markers closely linked to the Ppd-H1 gene.

Smiech et al. (2000) used BSA with RAPD's to identify markers to distinguish between resistant and susceptible forms tomato (*Lycopersicon esculentum* Mill.). They stated that 28 out of 271 primers produced polymorphism which were tested for linkage to the resistance phenotype. They were able to identify 5 primers enabling them to distinguish between resistant and susceptible forms in a F_2 segregating progeny developed from resistant x susceptible parents for tomato spotted wilt virus. They concluded that the selection of TSWV resistant individuals can be facilitated by MAS.

Badenes et al. (2000) used BSA with RAPD markers to identify markers linked to male sterility and self-compatibility in apricot. Their screening of 228 primers yielded a marker linked to male-fertility (M4-950) but none to S alleles. With a second approach of the screening of primers in a subset of seedlings, they were able to identify two markers linked to the Sc allele and three markers linked to male-fertility.

Dong et al. (2000) used BSA with the AFLP technique to identify molecular markers linked to the thermosensitive genic male sterility (TGMS) gene in a F_2 population of a cross between a TGMS indica mutant, TGMS-VN1, and a fertile indica line, CH1 of rice (*Oryza sativa*) . Out of 200 AFLP primer combinations surveyed, they identified four AFLP markers (E2/M5-600, E3/M16-400, E5/M12-600, and E5/M12-200) linked to the TGMS gene in the coupling phase.

Wise et al. (1999) employed BSA with AFLP analysis to identify DNA markers closely linked to the *Rf*8 locus, which mediates partial fertility restoration of T-cytoplasm maize. They stated that these findings would help a better understanding of mechanisms of nuclear-directed mitochondrial RNA processing and fertility restoration.

Yu and Wise (2000) identified three markers linked to the Pea crown-rust resistance cluster using AFLP-based BSA in pea (*Lathyrus sativus*).

The Beta (*B*) locus effects fruit beta-carotene content in tomato (*Lycopersicon esculentum* Mill.) (Zhang and Stommel 2000). Zhang and Stommel (2000) employed BSA with 1018 random primers for RAPD analysis and 64 primer pairs for AFLP analysis in an F_2 population segregating for B, and identified polymorphic bands which distinguished two bulked DNA samples. One single 100 bp AFLP amplified band distinguished the NILs and co-segregated with Beta modifier (MOB) and was shown to be closely linked to the locus.

Lecouls et al. (1999) used BSA with RAPD analysis to identify markers linked to the *Mal* gene (controls a high and wide-spectrum resistance to root-knot nematode) using segregating progenies crossed by host parents. They were able to identfy four dominant coupling-phase markers from a total of 660 10-base primers tested.

A single recessive gene, *ana*, produces the anasazi pattern of partly colored seedcoats in common bean (Bassett et al. 2000). Bassett et al. (2000) identified molecular markers linked in coupling to the *Ana* (OM9 (200), 5.4 cM) gene using BSA.

Bloom time studies in *Prunus*

Flowering time is generally considered to be inherited quantitatively, however, a single gene controlling late flowering in a qualitative manner was identified in progenies tracing back to a single mutant in almond (Company et al. 1999). The effect of this allele in almond progenies is modified by quantitatively inherited minor genes.

Wang et al. (2000) reported QTL analysis of flower and fruit traits in sour cherry using the RFLP map of EB and RS. They estimated the location and effects of QTL for eight traits. They reported that they detected eleven putatively significant QTL (LOD > 2.4) for six characters (bloom time, ripening date, % pistil death, % pollen germination, fruit weight, and soluble solid concentration) and the percentage of phenotypic variation explained by a single QTL varied from 12.9 % to 25.9 %. QTL for flower traits (bloom time, % pistil death and % pollen germination) were mapped to the same linkage group, EB1. A negative correlation was found between bloom time and percent pistil death (r = - 0.25) (Wang et al. 2000).

Ballester et al. (2001) studied the genetics of late bloom in almond. BSA was used to in an F_1 population to identify RAPD markers linked to the *Lb* gene, which is located on the linkage group 4. They were able to identify three RAPD markers associated with the *Lb* gene. One of them ($OKP10_{1350}$) placed at 5.4 cM from *Lb* and possibly can be used as a selective marker for flowering time. Plants with *Lb* allele bloomed about two weeks later and this allele had dominant gene action.

Bloom time QTL were also identified in the peach map (Yamamato et al. 2001). Six QTL were located in 4 different linkage groups. One of the QTL mapped in group 6 and another 3 QTL into group 3. The last bloom time QTL located was at the end of the group 1.

Low temperature damage to floral organs in spring is the most common factor in reducing the yield in some regions (Thompson 1996). Therefore, the development of new late blooming varieties would avoid some of the loss due to spring freeze injury. Identification of markers linked to bloom time QTL in sour cherry could expedite the development new cultivars with late blooming characteristics using MAS. The objective of this study is to search for such candidate markers associated with bloom time in sour cherry using different approaches, which includes testing of the genetic markers that are mapped close to the bloom time QTL for association with bloom time (Chapter 1) and BSA.

MATERIALS AND METHODS

Plant material and bloom time

Bloom time was scored on approximately 200 progenies from a cross between two sour cherry cultivars, ‘Balaton’ x ‘Surefire’, for three consecutive (1999-2001) years. Bloom time was recorded as the time when approximately 50% of the flowers were open. ‘Balaton’ x ‘Surefire’ population was chosen because this cross displayed considerable variation in bloom time (Figures 2.2, 2.3 and 2.4). Temperature readings were obtained from the Clarksville Horticultural Experiment Station. Bloom date data was converted into degree days (DD) from January 1 with a base temperature of 4.4 °C (Table 2.2). The positive differences of hourly temperature readings from 4.4 °C were summed to calculate daily heat unit accumulations.

Selection of bulks

Two groups of three plants were selected from each extreme of the bloom time distribution of ‘Balaton’ x ‘Surefire’ population for selective genotyping. Three progenies, 3-24, 4-47 and 2-61, were selected as early group because they were the earliest flowering individuals over three years (Fig. 2.2, 2.3, and 2.4). The other three progenies, 4-22, 2-19 and 2-39, were selected as the late group since they were the latest flowering progenies for three years (Fig. 2.2, 2.3, and 2.4). These selected progenies carry enough flowers (about 10 flowers) to assess the bloom time accurately. Two groups were screened with a total of 156 AFLP primer pair combinations (Table 2.1).

DNA extraction

Young unfolded leaves were obtained from trees of the ‘Balaton’ x ‘Surefire’ population located at Clarksville Horticultural Experiment Station of Michigan State

University and were brought to the laboratory in a cooler and frozen at –80 ^{0}C overnight and lyophilized for 2-3 days. DNA isolation was conducted according to Stockinger et al. (1996).

Primers and PCR conditions for approaches 1 and 2

A primer pair (5'-GGCTCCTACCCATCTAACTGTGA-3', 5'GTCCCGTGCT TTTCCCATTC-3') was designed from the sequence of a RFLP probe pS141, which is a clone, derived from sweet cherry stylar cDNA (Iezzoni and Brettin 1998). The primer sequence for pchpgms3 SSR primer was given by Sosinski et al. (2000). These two primer pairs were PCR-amplified as follows: 1X PCR buffer, 0.2 mM of dNTP's, 2.5 mM of $MgCl_2$, 50 ng DNA, 0.6 unit Tag DNA polymerase enzyme (Boehringer Mannheim Biochemicals) and ddH_2O to a volume of 25 µl. PCR reactions were performed in a thermocycler (model 9600; Perkin Elmer Applied Biosystems, Inc., Foster City, California). The PCR products were electrophoresed for 2.5 hrs at 80W on a 6%polyacrylamide gel with a 38 × 50 cm Sequi-Gen GT sequencing cell (BioRad Laboratories Inc., Hercules, CA, USA). Silver staining was conducted with a commercial kit (Promega # Q4132) according to instructions. Fragment sizes were estimated using a 10 bp ladder (Gibco BRL).

Modified –BSA and AFLP procedure for third aproach

The DNA pooling technique proposed by Michelmore et al. (1991) with a modification was used to find candidate markers that are present in one group but not in the other. The modification was made by not mixing the DNA of plants from the same group and keeping them separate.

AFLP markers were used because they do not have a very high development cost, genotyping cost is moderate, and produces more bands (up to 100 per gel) per gel than any other markers.

Digestion, adapter ligation, preamplification, and selective amplification were done as described (Vos et al. 1995; Barrett and Kidwell 1998), except with the following modifications described by Hazen et al. (2002); 2 μl of restriction ligation product was combined with 25 ng of *Mse*I and *EcoR*I, 0.5 mM dNTPs, 1X PCR buffer (10 mM Tris-HCl, pH 7.2 50 mM KCl, and 0.1% Triton X-100), 0.5 U Taq polymerase, 1.5 mM MgCl2, total volume 20 μl. Preamplification was done with the following thermocycler profile [94 .C 2 min – 26 cycles (94 .C 1 min, 56 .C 1 min, 72 .C 1 min) – 72 .C 5 min]. The PCR product from preamlification was diluted six times with sterile water. One microliter of the dilute preamplification product was added to 19 μl of the following cocktail (25 ng *EcoR*I primer, 30 ng *Mse*I primer, 0.4 mM dNTPs, 1X PCR buffer, 0.4 U Taq polymerase, 1.5 mM MgCl2) and selective amplification was carried out with the following profile [94 .C 2 min – 12 cycles with annealing temperatures decreasing by 0.7 .C each step (94 .C 30 sec, 65 .C 30 sec, 72 .C 1 min) – 23 cycles (94 .C 30 sec, 56 .C 30 sec, 72 .C1min) – 72 .C 2 min]. The screening of early and late bulks was done by 156 AFLP primer combinations (Table 2.1).

Electrophoresis

The selective amplification products were separated by electrophoresis for 2.5 hrs at 80 W on a 6% polyacrylamide sequencing gel on a 38 × 50 cm Sequi-Gen GT sequencing cell (BioRad, Hercules, CA), then silver stained with sequence staining kit by

Promega (# Q4132) and sizes were estimated using a 10 bp ladder (Gibco BRL #10821-015).

RESULTS AND DISCUSSION

Three different approaches were used to identify markers linked to bloom time in sour cherry population from the 'Balaton' x 'Surefire' cross.

In the first approach, a primer pair designed from pS141 sequence was used to find a marker associated with the bloom time. The pS141 probe mapped in a region of (*blm1*) the linkage group 1 of EB, which explained the 19.9 % of the phenotypic variation (Wang et al. 2000). Therefore, a primer designed from the sequence of pS141 probe could have been a useful marker in selecting for bloom time in early generations. However, the primer designed from the sequence amplified many bands between 140 bp and 500 bp and no specific band was available to test for association with bloom time.

In a second approach, the pchpgms3 SSR marker, which mapped to the EB1 linkage group at 8.4 cM from the pS141 probe (Chapter 1, Fig.1.1) was tested for association with bloom time. Bloom data was converted into degree days (Table 2.1) and tested for association with PCR amplification products of the pchpgms3 SSR marker (189bp, 176bp and 174bp) which was polymorphic between the parents and in the progeny. The significance of differences between marker levels (0 versus 1) of pchgms3 SSR were tested (Table 2.2) using three years of bloom data expressed in degree days (DD) (Table 2.1). The model ($Y_{ijkm} = \text{Mean} + M1_i + M2_j + M3_k + \text{Year}_m + E_{ijkm}$) also included year to eliminate the effect of missing data (Table 2.1) and a total of 74 observations were used (Table 2.1) . Degree of freedom is 68 (74 – 6 = 68). There were no significant differences between marker levels of alleles amplified by pchpgms3 primer and bloom time (Table 2.2).

Table 2.1. Degree days (DD) for bloom time and PCR amplification by pchgms3 primer pair for each progeny in 'Balaton' x 'Surefire' population.

Plant	Marker			DD			
	pchgms3 -189bp	pchgms3 -176bp	pchgms3 -174bp	1999	2000	2001	Average
4-47	0	1	0	389.0	212.2	178.9	260.0
3-24	0	1	1	418.0	212.2	163.6	264.6
2-61	1	1	0	406.5	223.1	173.0	267.5
3-20	0	1	0	430.5	218.1	173.0	273.8
2-31	1	1	0	457.5	235.5	201.2	298.0
2-29	0	1	0	474.0	235.5	187.4	298.9
2-44	0	1	0	474.0	235.5	201.2	303.5
1-66	1	0	1	474.0	251.3	187.4	304.2
2-32	0	1	0	474.0	259.3	201.2	311.5
2-54	0	1	0	474.0	243.5	217.5	311.6
4-56	0	1	0	517.0	223.1	201.2	313.7
2-56	0	1	1	494.5	251.3	201.2	315.6
4-14	0	1	1	494.5	251.3	201.2	315.6
3-37	1	1	0	474.0	287.6	201.2	320.9
3-59	0	1	1	494.5	267.4	201.2	321.0
3-42	0	1	1	494.5	251.3	217.5	321.1
2-45	1	0	1	517.0	267.4	217.5	333.9
4-35	0	1	1	544.5	235.5	234.9	338.3
3-50	1	1	0	517.0	267.4	234.9	339.7
2-33	0	1	0	494.5		201.2	347.8
4-46	0	1	1	544.5	287.6	217.5	349.8
4-54	0	1	0	574.5	287.6	234.9	365.6
Balaton	1	1	0	494.5	251.3		372.9
Surefire	0	1	1	544.5	287.6		416.0
1-25	1	0	1	457.5			457.5
1-26	0	0	1	474.0			474.0
1-42	0	1	1	517.0			517.0
1-27	1	1	0	574.5			574.5
3-66	0	1	0	616.5			616.5

1 = presence of the marker; 0 = absence of the marker, DD = degree days from January 1 with a base temperature of 4.4 °C.

Table 2.2. Differences of least squares means for pchgms3 marker levels and years for bloom data in ‘Balaton’ x ‘Surefire’ population. The significance of differences between marker levels (0 versus 1) of pchgms3 SSR were tested using three years of bloom data expressed in degree days (DD).

Effect	M1	M2	M3	Year	M1	M2	M3	Year	Estimate	Error	df	t value	Pr>[t]
M1	0				1				-4.5	11.2	68	-0.40	0.6897
M2		0				1			-15.1	17.9	68	-0.85	0.4000
M3			0				1		-12.2	10.1	68	-1.20	0.2340
Year				1999				2000	244.3	10.2	68	23.88	<.0001
Year				2000				2001	291.3	10.3	68	28.14	<.0001
Year				2000				2001	46.9	10.9	68	4.30	<.0001

M1 = pchgms3-189, M2 = pchgms3-176, M3 = pchgms3-174, 0 = absence of the band, 1 = presence of the band
The model (Y_{ijkm} = Mean + $M1_i$ + $M2_j$ + $M3_k$ + $Year_m$ + E_{ijkm}) also included year to eliminate the effect of missing data and a total of 74 observations were used. Degrees of freedom is 68 (74 – 6 = 68).

Ballester et al. (2001) reported a QTL for bloom time on linkage group 4 in the almond map that explained 79% of the phenotypic variation. Three RAPD markers were identified in almond in association with bloom time using BSA, with one at a 5.4 cM distance from the late blooming gene (*Lb*) which could be used in MAS. Incorporation of more common co-dominant markers, such as SSRs, between a sour cherry and an almond map might have potential for evaluation of association of these markers with bloom time assuming the gene order is conserved between the two species. Currently the sour cherry and peach x almond hybrids map share two common RFLP markers in this linkage group namely; CPM58 and CPM53 (Chapter 1). Recently Dettori et al. (2001) incorporated two SSR markers to the linkage 4 of peach; UDP96-003 and UDP98-024. The UDP-003 is only 7 cM away from the FG3 marker which is located also next to the bloom time QTL of the almond map. Unfortunately UDP96-003 marker could not be incorporated into sour cherry linkage map due to the complex banding pattern. Although UDP98-024 marker was polymorphic and informative, it did not map due to the low saturation in the sour cherry map (Chapter 1).

Yamamato et al. (2001) reported that they have identified six bloom time QTL in peach map locating in four different linkage groups. One of the QTL mapped in group 6 and another three into group 3. Linkage group 3 contains four SSR loci recently incorporated, however, none of these markers were informative in sour cherry (Chapter 1). The last bloom time QTL located at the end of the group 1 in their study as in the case of sour cherry (Wang et al. 1998) where a microsatelite marker, pchgms3, was incorporated (Chapter 1). However no association was found in our study between this marker and bloom time (Chapter 1).

In the third method, a modified BSA in combination with AFLP technique was used to identify candidate markers associated with bloom time. The selected individuals used for bulk analysis are indicated on Figures 2.2-2.4. There were 156 AFLP primer pair combinations, which were used to screen early and late bulks of the population to find candidate markers present in one bulk but not in the other. AFLP primer combinations resulted in 1 to 34 polymorphic bands for each primer pair (Table 2.2). The average number of polymorphic bands was 10.65 per primer pair and the polymorphism rate ranged from 10% (ECA/MCCT and EGG/MCAT) to 44% (EAA/MCGA) per primer pair combination.

Screening of early and late bulks with 156 AFLP primer pair combinations resulted in the identification of three candidate bands in three different primer combinations that were present in early bulk but absent in late bulk or visa versa. The EGG/MCAC primer combination amplified a band at 82 bp that was present in the early group but not in the late group (Fig. 2.1-c). The Balaton parent also had the band of the early group; 'Surefire' did not possess this band.

ETT/MCCG primer combination resulted in an amplified band of 78 bp, which is present in the late group, but not in the early group (Fig. 2.1-b). The parent, 'Balaton', also had the band as the late group; 'Surefire' did not have this band.

EAA/MCGT primer combination amplified a band at 94 bp, which is present in the late group, but not in the early group (Fig. 2.1-a).

Table 2.3 Number of polymorphic bands produced by AFLP primer pair combinations in 'Balaton' x 'Surefire' population.

Primer combination		No. of polymor. bands	Primer combination		No. of polymor. bands	Primer combination		No. of polymor. bands
ECA	MCGC	7	ECC	MCGG	na	EGG	MCCC	3
	MCTC	2		MCGC	10		MCCG	na
	MCCG	na		MCAA	7		MCGC	11
	MCAA	5		MCGG	10		MCTC	12
	MCGT	na		MCGA	22		MCAA	12
	MCGA	26		MCAT	na		MCGT	6
	MCAT	14		MCTC	28		MCGG	2
	MCAG	na		MCCG	15		MCGA	5
	MCCT	7		MCCC	10		MCAT	3
	MCTA	16		MCCT	na		MCAG	na
	MCCA	na		MCAG	na		MCCT	na
	MCCG	10		MCTA	6		MCTA	na
	MCCC	10		MCCA	4		MCCA	7
	MCAC	6		MCAC	5		MCAC	10
	MCTT	11		MCTT	9		MCTT	2
				MCGT	24		MCAG	7
ETT	MCAC	8	ECT	MCGC	na	EAT	MCTC	3
	MCTT	na		MCTC	4		MCCC	na
	MCTC	12		MCAA	13		MCTA	3
	MCCC	11		MCCC	21		MCTC	13
	MCTA	9		MCCG	15		MCAA	na
	MCTC	9		MCGG	12		MCGT	5
	MCAT	14		MCGA	na		MCGA	na
	MCAA	na		MCAT	na		MCGG	na
	MCGT	6		MCAG	na		MCCA	na
	MCAT	14		MCCT	2		MCAG	14
	MCGG	11		MCTA	7		MCCG	9
	MCCA	19		MCCA	7		MCGC	na
	MCAG	13		MCGT	na		MCAT	na
	MCCG	15		MCAC	26		MCGA	na
	MCGC	10		MCTT	18		MCCT	11
	MCGA	24					MCAC	14
							MCGG	9

Table 2.2. (cont'd)

Primer combination		No. of polymor. bands	Primer combination		No. of polymor. bands	Primer combination		No. of polymor. bands
EAG	MCGA	5	EGC	MCTC	3	EAA	MCCG	22
	MCGG	23		MCGA	6		MCCC	9
	MCTC	12		MCAT	4		MCAA	12
	MCAG	6		MCAA	5		MCTC	8
	MCCT	1		MCTC	8		MCAT	8
	MCCA	2		MCAG	5		MCGC	18
	MCTA	na		MCCT	3		MCTA	6
	MCGT	17		MCTA	14		MCCA	na
	MCAT	18		MCCA	7		MCAG	na
	MCGC	2		MCGT	na		MCCT	na
	MCCG	10		MCGC	na		MCGT	18
	MCAA	8		MCCC	na		MCGG	21
	MCCC	16		MCGG	2		MCGA	34
	MCAC	9		MCAC	7		MCAC	na
	MCTT	na		MCTT	14		MCTT	6
				MCCG	12			
EAC	MCAT	7	EAC	MCTC	na	EAC	MCAC	26
	MCCC	na		MCGT	na		MCTT	19
	MCAG	na		MCCG	na		MCTC	na
	MCCT	4		MCGC	na			
	MCTA	na		MCGA	na			
	MCCA	10		MCCG	na			
	MCAA	na		MCGA	na			

na = data is not available, polymor. = polymorphic.

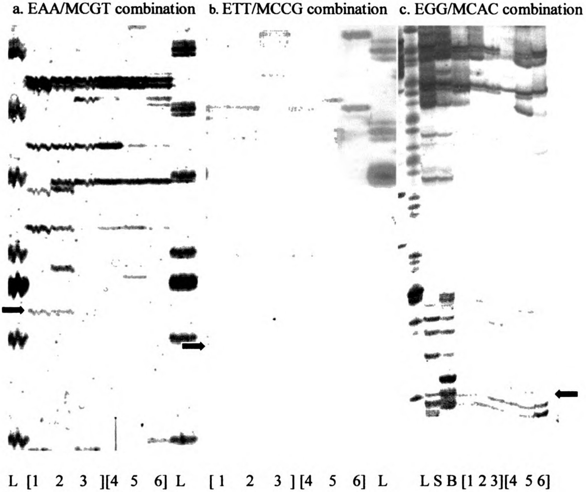

Figure 2.1 a-c. Three candidate AFLP bands that are present in one group, but not in another; a 94 bp band pointed by an arrow in EAA/MCGT primer combination is present in late group, but not in early group. A 78 bp band pointed by an arrow in ETT/MCCG primer combination is present in late group, but not in early group. EGG /MCAC primer combination has a band pointed by an arrow at 82 bp that present in early group, but not in late group. Lane L is 10 bp ladder; lanes 1, 2 and 3 are late group (2-19, 4-22, and 2-39, respectively); lanes 4, 5, and 6 are early group (4-47, 2-61, and 3-24, respectively); lane S is Surefire and lane B is Balaton.

The significant relationship between these AFLP markers and the bloom time could be confirmed by genotyping the whole 'Balaton' and 'Surefire' with these markers and testing for a statistically significant relationship. Then, informative AFLP markers could be converted into STS as explained in Chapter 3 and be utilized in MAS for late blooming.

For a better understanding of the genetics of bloom time, the AFLP markers obtained here could be incorporated into the existing sour cherry linkage map in EB and RS population as explained in Chapter 3. The incorporation of these markers into the map will provide useful linkage information between these AFLP markers. If these markers are not closely linked and if each one maps independent of each other or into different linkage groups, then this information may allow us to have better understanding of the number of genes controlling bloom time. The amount of variation explained by the locations of the AFLP markers could also be calculated using QTL CARTOGRAPHER (Basten et al. 1997). If these candidate markers are closely linked or map in to the same location, this might indicate that they are part of the same QTL.

The bloom time data for 'Balaton' x 'Surefire' population exhibited continuous variation in all three years, which is typical of quantitative inheritance. Distributions of flowering time of progenies in all three years were normal (Fig. 2.2, 2.3 and 2.4) and the means (Table 2.4) were similar to the mid-parent values. The absence of a bimodal distribution suggests that there is no major dominant gene for bloom time in 'Balaton' x 'Surefire' population. In contrary, the bloom time distribution in almond showed a bimodal distribution due to the presence of a major dominant *Lb* gene (Company et al. 1999). The difference in bloom time for 'Balaton', and 'Surefire' (43.10 DD) was not significant ($P < 0.05$). Parental values were not the extremes (Tables 2.1 and 2.4) and transgressive segregation was observed for bloom time distribution of the progenies (Fig. 2.2, 2.3 and 2.4).

Table 2.4. Mean phenotypic values, standard deviations and value range for the bloom time distribution of the progenies of 'Balaton' x 'Surefire'. All data expressed as degree days.

Year	Mean	SD	Max.	Min.
1999	470.3	125.6	616.0	389.0
2000	261.8	28.4	339.0	212.2
2001	200.5	15.3	273.6	163.6

DD = degree days from January 1 with a base temperature of 4.4 °C.
SD = standard deviation.

From a breeding standpoint, availability of informative markers associated with bloom time is very important, because utilization of markers will help selection of varieties for bloom time early in the generation, saving time and effort. These candidate markers may also be incorporated into existing *Prunus* maps and lead to isolation of genes for bloom time.

Figure 2.2. Bloom dates of progenies of 'Balaton' x 'Surefire' in 1999. Selected progenies for bulk segregant analysis are shown in bold.

Apr 26	Apr 27	Apr 28	Apr 29	May 1	May 2	May 3	May 4	May 5	May 6	May 8	May 10
					4-44						
					3-37						
					2-54	4-25					
					2-44	4-14					
					2-32	3-59		4-46			
				3-29	2-29	3-42		4-35	4-54		
				2-34	2-5	2-56	4-56	3-50	2-14		
				2-31	1-66	2-33	4-2	3-44	2-7		
4-47	2-61	3-21	3-20	2-4	1-65	Balaton	2-45	Surefire	2-6	3-66	4-22

Figure 2.3. Bloom dates of progenies of 'Balaton' x 'Surefire' in 2000. Selected progenies for bulk segregant analysis are shown in bold.

Apr 24	Apr 25	Apr 26	Apr 27	Apr 28	Apr 29	Apr 30	May 1	May 2	May 3	May 4	May 5	May 6	May 7
						4-64							
						4-44							
						4-38							
						4-26	4-61	4-7		4-54			
						4-25	4-60	4-6		4-46			
				4-31		4-15	4-43	3-62		4-39			
				4-29		4-14	4-42	3-59		4-37			
				4-21	4-2	3-63	4-11	3-50		3-44			
		3-16		3-49	3-28	3-42	4-8	3-35	4-1	3-38			
		3-2		3-27	3-18	2-56	3-5	2-58	3-17	3-37	4-66		
		2-61		2-44	2-54	2-46	2-62	2-52	3-10	2-55	4-58	4-33	
		4-56	4-34	2-37	2-42	1-66	2-36	2-45	4-55	2-14	4-45	4-24	
3-24		2-6	4-28	2-31	2-34	1-65	2-32	2-43	4-23	2-7	3-22	4-22	3-57
4-47	3-20	2-5	3-29	4-35	2-30	Balaton	2-8	3-21	4-4	Surefire	2-9	2-19	2-39

Figure 2.4. Bloom dates of progenies of 'Balaton' x 'Surefire' in 2001. Selected progenies for bulk segregant analysis are shown in bold.

4/27	4/28	4/29	4/30	5/1	5/1	5/2	5/2	5/3	5/3	5/4	5/5	5/6
						4-61	3-47	3-52				
						4-59	3-43	3-52				
						4-55	3-42	3-50				
						4-52	3-36	3-45				
						4-46	3-33	3-44				
						4-41	3-27	3-38				
				3-63	3-54	4-40	3-19	3-34				
			4-21	3-55	3-51	4-32	3-12	3-22	4--65			
			4-8	3-59	3-41	4-30	3-11	3-21	4-64			
			3-62	3-56	3-37	4-28	3-8	3-10	4-62			
			3-49	4-60	3-33	4-26	2-64	3-5	4-57			
			3-35	4-56	3-31	4-25	2-62	2-63	4-54			
			3-29	4-49	2-60	4-23	2-55	2-59	4-45	4-66		
			3-18	4-44	2-57	4-12	2-54	2-58	4-39	4-58		
		4-47	1-65	4-42	2-56	4-7	2-51	2-53	4-38	4-53		
		4-43	1-66	4-29	2-48	4-6	2-45	2-46	4-37	4-22		
	4-36	3-46	2-43	4-19	2-44	4-4	2-35	2-40	4-35	4-20		
	4-34	3-28	2-37	4-18	2-42	54-1	2-17	2-38	4-33	3-51		
	4-31	3-16	2-34	4-15	2-33	3-64	2-15	2-28	4-27	3-17		
	3-20	2-24	2-29	4-14	2-32	3-61	2-14	2-11	4-16	2-52		
	3-2	2-22	2-25	4-10	2-31	3-58	2-13	2-10	4-11	2-47		
	3-1	2-16	2-23	4-3	2-30	3-48	2-12	2-9	3-65	2-36		
3-24	2-61	2-6	2-5	4-2	2-20	2-4	2-8	2-7	3-57	2-19	4-24	2-39

LITERATURE CITED

Apostol J, Iezzoni AF (1992) Sour cherry breeding and production in Hungary. Fruit Variety Journal 16:11-15

Badenes ML, Hurtado MA, Sanz F, Archelos DM, Burgos L, Egea J, Llacer G (2000) Searching for molecular markers linked to male sterility and self-compatibility in apricot. Plant Breeding 119:57-160

Ballester J, Company RSI, Arus P, de Vicente MC (2001) Genetic mapping of a major gene delaying blooming time in almond. Plant Breeding 120:268-270

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 38:1261–1271

Basten, CJ, Weir BS, Zeng ZB (1997) QTL CARTOGRAPHER, reference manual and tutorial for QTL mapping. North Caroliana State University

Bassett MJ, Hartel K, McClean P (2000) Inheritance of the Anasazi pattern of partly colored seedcoats in common bean. J. Am. Soc. Hort. Sci.125:340-343

Bentolila S, Hanson MR (2001) Identification of a BIBAC clone that co-segregates with the petunia Restorer of fertility (Rf) gene. Molecular Genetics and Genomics 266:223-230

Campalans A, Pages M, Messeguer R (2001) Identification of differentially expressed genes by the cDNA-AFLP technique during dehydration of almond (*Prunus amygdalus*). Tree Physiology 21:633-643

Cervera MT, Remington D, Frigerio JM, Storme V, Ivens B, Boerjan W, Plomion C (2000) Improved AFLP analysis of tree species. Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere 30:1608-1616

Chalmers, K.J, Barua, U.M, Hackett, C.A, Thomas, W.T.B, Waugh, R, Powell, W (1993) Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theor. Appl. Genet. 87:314-320.

Company RSI, Felipe AJ, Aparasi JG (1999) A major gene for flowering time in almond. Plant Breeding 118:443-448

Chen, FQ, Prehn D, Hayes PM (1994) Mapping genes for resistance to barley stripe rust. Theor. Appl. Genet. 88:215-219

Decousset L, Griffiths S, Dunford RP, Pratchett N, Laurie DA (2000) Development of STS markers closely linked to the Ppd-H1 photoperiod response gene of barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 101:1202-1206

Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTL controlling fruit quality in peach (Prunus persica (L.) Batsch). Theor. Appl. Genet. 98:18-31

Dong NV, Subudhi PK, Luong PN, Quang VD, Quy TD, Zheng HG,Wang B and Nguyen HT (2000) Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. Theor. Appl. Genet. 100:727-734

Goulao L, Monte-Corvo L, Oliveira CM (2001) Phenetic characterization of plum cultivars by high multiplex ratio markers: Amplified fragment length polymorphisms and inter-simple sequence repeats. HortScience 126:72-77

Hazen SP, Leroy P, Ward RW (2002) AFLP in *Triticum aestivum* L.: patterns of genetic diversity and genome distribution. Euphytica 125:89-102

Hurtado MA, Romero C, Vilanova S, Abbott AG, Llacer G Badanes ML (2002) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.), and mapping of PPV (sharka) resistance. Theor. Appl. Genet. 105:182-191

Iezzoni AF, Hamilton RL (1985) Difference in spring floral bud development among sour cherry cultivars. HortScience 20:915-916

Iezzoni AF (1996) Sour cherry cultivars: objectives and methods of fruit breeding and characteristics of principal commercial cultivars: in Cherries: crop physiology, production and uses. (eds. By Webster A.D. and Looney N.E.) University press, Cambridge, UK 223-241

Iezzoni AF, Brettin TS (1998) Utilization of molecular genetics in cherry. Acta Horticulturae 468:55-62

Koester RP, Sisco PH, Stuber CH (1993) Identification of quantitative trait loci controlling days to flowering and plant height in two near isogenic lines of *Maize*. Crop. Sci. 33:1209-1216

Lu ZX, Sossey-Alaoui K, Reighard GL, Baird WV, Abbott AG (1999) Development and characterization of a codominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding. Theor. Appl. Genet. 99:115-122

Lander, ES Botstein D (1989) Mapping mendelian factors underlying quantitaive traits using RFLP linkage maps. Genetics 121:185-199

Lecouls AC, Rubio-Cabetas MJ, Minot JC, Voisin R, Bonnet A, Salesses G, Dirlewanger E, Esmenjaud D (1999) RAPD and SCAR markers linked to the Ma1 root-knot nematode resistance gene in Myrobalan plum (*Prunus cerasifera* Ehr.). Theor. Appl. Genet. 99:328-335

Manubens A, Lobos S, Jadue Y, Toro M, Messina R, Lladser M, Seelenfreund D (1999) DNA isolation and AFLP fingerprinting of nectarine and peach varieties (*Prunus persica*). Plant Molecular Biology Reporter 17:255-267

Mansur LM, Orf J, Lark KG (1993) Determining the linkage of quantitaive trait loci to RFLP markers using extreme phenotypes of recombinant inbreds of soybean (*Glycine max* L. Merr.). Theor. Appl. Genet. 86:914-918

Michelmore RW, Paran, I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA. 88:9828-9832

Miklas PN, Johnson E, Stone V, Beaver JS, Montoya C, Zapata M (1996) Selective mapping of QTL conditioning disease resistance in common bean. Crop. Sci. 36:1344-1351

Quarrie SA, Lazic-Jancic V, Kovacevic D, Steed A, Pekic S (1999) Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. Journal of Experimental Botany 50:1299-1306

Shimada T, Yamamoto T, Yaegaki H, Yamaguchi M, Yoshida M, Hayashi T (1999) Application of AFLP to molecular genetic analysis in peach. J. Japan. Soc. Hort. Sci. 68:67-69

Shimada T, Yamamoto T, Hayama H, Yamaguchi M, Hayashi T (2000) A genetic linkage map constructed by using an intraspecific cross between peach cultivars grown in Japan. J. Japan. Soc. Hort. Sci. 69:536542

Simoviki, K (1960) Relation between flowering time of fruit trees and frost period in the MacedonianPeople's Republic. Godisen zb. Zemjod-sum. Fak. Univ. Skopie 13:5-57

Smiech M, Rusinowski Z, Malepszy S, Niemirowicz-Szczytt K (2000) New RAPD markers of tomato spotted wilt virus (TSWV) resistance in Lycopersicon esculentum Mill. Acta Physiologiae Plantarum 22:299-303

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite Markers in peach [Prunus persica (L.) Batsch] Theor. Appl. Genet. 101:421-428

Stockinger EJ, Mulinix CA, Long CM, Brettin TS, Iezzoni AF (1996) A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J. Hered. 87:214-218

Struss D, Boritzki M, Glozer K, Southwick SM (2001) Detection of genetic diversity among populations of sweet cherry (*Prunus avium* L.) by AFLPs. J. Hort. Sci. 76:362-367

Thompson M (1996) Flowering, Pollination and fruit set: in Cherries: crop physiology, production and uses. (ed. By Webster A.D. and Looney N.E.) University press, Cambridge, UK 223-241

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res. 23:4407-4414

Wang D, Karle R, Brettin TS, Iezzoni AF (1998) Genetic linkage map in sour cherry using RFLP markers. Theor. Appl. Genet. 97:1217-1224

Wang D, Karle R, Iezzoni AF (2000) QTL analysis of flower and fruit traits in sour cherry. Theor. Appl. Genet. 100:535-544

Wang GL, Paterson AH (1994) Assessment of DNA pooling strategies for mapping of QTL. Theor. Appl. Genet. 88:355-361

Wise RP, Gobelman-Werner K, Pei D, Dill C, Schnable PS (1999) Mitochondrial transcript processing and restoration of male fertility in T-cytoplasm maize. J. Hered. 90:380-385

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. 83:294-300

Yamamoto T, Shimada T, Imai T (2001) Characterization of morphological traits based on genetic linkage map in peach. Breeding Sci. 51:271-278

Yu GX, Wise RP (2000) An anchored AFLP- and retrotransposon-based map of diploid Avena. Genome 43:736-749

Zhang Y, Stommel JR (2000) RAPD and AFLP tagging and mapping of Beta (B) and Beta modifier (Mo-B), two genes which influence beta-carotene accumulation in fruit of tomato (Lycopersicon esculentum Mill.). Theor. Appl. Genet. 100:368-375

CHAPTER 3

SUMMARY AND DISCUSSIONS

SUMMARY AND DISCUSSIONS

Two projects were carried out to aid breeding studies of sour cherry (*Prunus cerasus* L., 2n=4x=32). In the first project, 45 primer pairs developed from the sequences of *Prunus* microsatellites (simple sequence repeats, SSR) were screened and 10 informative SSR yielding 17 markers were added to a previously developed sour cherry linkage map of two tetraploid sour cherry cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB).

The previously published EB linkage map (Wang et al. 1998) consisted of 95 SDRF in 16 linkage groups covering 279.2 cM. With the addition of new markers the current linkage map consists of 118 SDRF in 18 linkage groups covering 337.8 cM. Seventeen markers remained unlinked. Therefore the addition of new markers provided a 20% (58.6 cM) increase over the length of the previous map. The previous RS linkage map (Wang et al. 1998) consisted of 126 SDRF assigned to 19 linkage groups covering 461.6 cM. The current linkage map now consists of 133 SDRF assigned to 19 linkage groups covering 433.9 cM. With the addition of new markers, a 27.7 cM decrease in the map distance was observed. This is caused by the fact that with the addition of the new markers, linkage groups 9 and 12 from the old map were combined into one linkage group named group 9 in the current map. Twenty-six markers were unlinked. The expanded *Prunus* genetic linkage map constructed from peach and almond covers 1144 cM (Bliss et al. 2002). The sour cherry linkage map, being tetraploid, should be twise the length of the peach map.

Homologous relations between EB and RS linkage groups were identified using 60 bridging markers heterozygous in both parents. Fifteen EB linkage groups homologous to the RS linkage groups were identified. RS counterparts of EB linkage groups 3, 12, 13 and 14 were not identified. An EB counterpart of RS linkage group 16 was also not identified (Fig. 1.1, Chapter 1).

Ideal markers are those which have two segregating bands mapping to two different linkage groups (Wang et al. 1998). In the current map, groups 2 and 19 could be homoeologous, since the markers amplified by the UDP11 and F133 mapped into different linkage groups at 6 cM and 5.7 cM apart in linkage groups 2 and 19, respectively (Fig. 1.1). The other suspected homeologous relation between group 17 and 18 detected by AG40 and CPM39 RFLP markers (Fig. 1.1) has already been reported by Wang et al. (1998). However, more data is needed to make more precise conclusions.

In the current study, SSR markers were incorporated to the locations QTL for six traits that were detected earlier by Wang et al. (1998) (Table 1.5, Chapter1). Two SSR markers, UDP411-154 and UDP411-131, were incorporated into linkage group 2 (Fig. 1.1, Chapter 1) and were tightly linked to bloom time location (*blm2*) at the distances of 4.5 cM and 2.3 cM, respectively (Table 1.5, Chapter 1). The same peach markers were also linked to fruit weight (*fw2*) location with the same distances as above (Table 1.5, Chapter 1). A significant negative correlation exists between bloom time and fruit weight ($r = -0.45$) (Wang et al. 1998). The pchgms3-189 marker was mapped 8.4 cM from the pS141, which is located in bloom time location (*blm1*) (Table 1.5, Chapter 1). The pchgms3-189 marker was also tightly linked to pistil death (*pd1*) location at a distance of 0.8 cM (Table 1.5, Chapter 1). A negative correlation was detected between bloom time and pistil death (Wang et al. 1998). The UDP405-112 marker was mapped 11.1 cM from the AG10b marker, which is located in soluble solids (*ssc2*) area (Table 1.5, Chapter 1). SSR markers, which were mapped into the QTL areas, are very valuable. The correlations mentioned above further increase the value of these SSR markers in sour cherry breeding by allowing breeders to select for different traits at the same time. The use of these SSR markers in MAS would make very important contributions to breeding of these traits.

Although SSRs are highly polymorphic, co-dominant and reproducible, very limited numbers are available for *Prunus* mapping due to the expensive and time consuming SSR development procedure. Recently, 41 SSR primer pair sequences from peach were published by Dirlewanger et al. (2002b). However, the number of SSRs available in *Prunus* falls considerably short of the goal to cover the complete genome for mapping purposes, which limits the use of these markers in MAS. A new methodology developed by Wang et al. (2002) provides a better alternative for *Prunus* mapping, because their approach combines high-throughput AFLP mapping, which allows quick map development, followed by targeted SSR development in AFLP marked region of interest. First, the region of interest is mapped with marker-dense AFLP mapping (or bulk-segregant analysis), then SSR linked to trait was developed by using these AFLP markers as a probe in a bacterial artificial chromosome (BAC) library. This methodology allows rapid identification of SSR loci that are tightly linked to the traits of interest.

Recently, 93 new SSR primer pairs were published by Aranzana et al. (2002), Dirlewanger et al. (2002b) and Wang et al. (2002) (Table 3.1). Furthermore, a presentation made at the 2002 Plant and Animal Genome conference indicates that 40 more SSR primer sequences are expected to be published in near future by Kimura et al. (2002) (Table 3.1).

Table 3.1. Recent progress in SSR mapping in *Prunus*.

Species	SSR information	Reference
Peach	41 SSR primer pair sequences available	(Dirlewanger et al. 2002)
Peach	17 SSR primer pair sequences available	(Wang et al. 2002)
Peach	35 SSR primer pair sequences available	(Aranzana et al. 2002)
Peach	58 SSR markers placed in a peach map	(Aranzana et al. 2001)
Peach	40 SSR were developed and half of them mapped in peach map	(Kimura1 et al. 2002)
Sweet cherry	SSR incorporated into sweet cherry map	(Dirlewanger et al. 2002a)

Cross species amplification of SSR primers in the current study and in other studies mentioned in Chapter 2 indicates that SSRs are highly polymorphic, transportable and frequently conserved in *Prunus* species and in the Rosaceae family. These provide an excellent tool for intra and inter family comparative mapping analysis and markers can be used for cross species amplification increasing the number of SSR markers available. SSRs in sour cherry are dispersed throughout the genome and not clustered in specific areas of the genome. The increased availability of SSR, their co-dominant nature and transportability makes SSR choice of markers and powerful tools for future comparative mapping, breeding and MAS studies in *Prunus*. Due to its diploid genome and small genome size, peach is a good candidate to be a model plant in Rosaceae for comparative mapping and for positional cloning of important genes (Sosinski et al. 2000). Including SSR markers into *Prunus* maps will provide a framework of anchor points, leading to map alignment and establishment of positions of QTLs and known genes (Aranzana et al.

2002). With the increased number of SSRs mapped in *Prunus* species, these anchor loci will enable a comparison of genome organization in the genus and will lead to establishment of a consensus map for the genus.

The objective of the second project was to identify candidate markers associated with bloom time in sour cherry using the 'Balaton' x 'Surefire' population. Several approaches were used such as testing the association between bloom time and markers that were mapped into bloom time locations in sour cherry or BSA. Low temperature damage to flowers is the most common factor in reducing the yield in some cherry growing regions (Thompson 1996), such as Michigan. Therefore, the development of new late blooming varieties to avoid the loss due to spring freeze injury is one of the most important breeding objectives for these cold regions (Iezzoni 1996)

Bloom time in cherry is a quantitative trait, but is highly heritabile (0.91) (Wang et al. 2000). Identification of markers linked to bloom time QTL in sour cherry could expedite the development of new cultivars or improvement of current cultivars with late blooming characteristics using MAS.

Bloom time data was collected from 'Balaton' and 'Surefire' population as explained (Chapter 2). The high heritability of the bloom time and the normal distribution of this trait in 'Balaton' and 'Surefire' population over three years suggest that bloom time is controlled by few number of genes with additive affect. If a dominant gene with major effect was involved, it would show bimodal distribution as in the case of almond (Ballester et al. 2001). As expected from the high heritability value, the bloom times of the progeny were consistent over three years and not significantly affected by the environmental conditions.

In the first approach, a primer pair designed from the sequence of a RFLP marker pS141 was employed to find a fragment, which is associated with the bloom time in 'Balaton' and 'Surefire' population. The pS141 is a clone derived from sweet cherry stylar cDNA (Iezzoni and Brettin 1998). The pS141 probe mapped in the region of bloom time QTL (*blm1*) in the linkage group 1 of EB, which explained the 19.9 % of the phenotypic variation (Wang et al. 2000). However the primer pair amplified a complex banding pattern and no specific band was available to test possible association with the bloom time and PCR amplification products of the primer pair.

In a second approach, the pchpgms3 SSR marker, which was mapped at a distance of 8.4 cM from the pS141 probe (10 cM in EB-RS consensus map) in EB1 (Chapter 1, Figure1.1), was tested for association with the bloom time. However, no significant relationship between alleles amplified by the pchpgms3 primer and bloom time was observed (Table 2.4, Chapter2). The large distance between marker location and the trait may invalidate its use as a diagnostic marker of QTL for bloom time in sour cherry. Another reason could be the low number of observations used in this experiment. Ballester et al. (2001) stated that the RAPD marker $OKP10_{1350}$ located 5.4 cM from the late blooming gene (*Lb*) could be a valuable diagnostic marker for late blooming, since the band was present in almost all late blooming plants and absent in most of the earlier-blooming plants in almond. Therefore, 10 cM distance from the trait of interest may not be useful whereas 5 cM may be a more practical distance to work with (Rajapakse et al. 1995).

In the third approach, screening of early and late extreme groups for bloom time with 156 AFLP primer pairs resulted in the identification of three candidate bands in three different primer combinations (a 82 bp band in EGG/MCAC, a 78 band in ETT/MCCG, and a 94 bp band in EAA/MCGT, Fig. 2.1, Chapter 2) that were present in the early bulk but not in the late or present in the late group but not in the early. After significant relationship between one or more of this candidate bands and bloom time is confirmed in the whole population, then these markers could be used in MAS.

Although AFLP markers are highly polymorphic and reliable in sour cherry, they are very expensive and time consuming to be used in MAS. Therefore, as a next step, candidate markers developed here could be converted into sequence tagged sites (STS) by excising the bands from gels, cloning and sequencing. An alternative approach also could be targeted SSR development (Wang et al. 2002) from the region(s) of interest after the candidate AFLP markers developed in this study are incorporated into the sour cherry map.

The candidate AFLP markers developed in the current study could be incorporated into the sour cherry map after the association with bloom time is confirmed in the entire population by genotyping all progenies of 'Balaton' and 'Surefire' with the candidate markers. Provided that candidate markers yield SDRF segregating 1:1 or 3:1 in EB and RS population and show Mendelian inheritance, these markers could be incorporated into the sour cherry map.

In conclusion, the AFLP markers obtained in chapter 2 and the SSR markers mapped to bloom time location (Chapter 1) are very important for MAS in sour cherry breeding and utilization of these markers will allow selection of new late blooming varieties earlier in generation resulting in saving of time, effort and resources. Incorporation of these markers into EB and RS map allow QTL analysis for bloom time to be performed. Therefore, the amount of variation explained by each location can be calculated. This information will give a better understanding of genetic basis of bloom time and will help estimating the number of genes that control bloom time in sour cherry.

LITERATURE CITED

Aranzana M, Ascasibar J, Garcia J (2001) Development and mapping of a set of SSR markers covering most of the *Prunus* genome. Plant and animal genome conferences IX, San Diego, CA, January 13-17 (Abst.)

Aranzana MJ, Garcia-Mas J, Carbo J, Arus P (2002) Development and variability analysis of microsatellite markers in peach. Plant Breeding 121:87-92

Bliss FA, Arulsekar S, Foolad MR, Beccerra V, Gillen AM, Warburton AM, Dandekar AM, Kocsisne GM, Mydin KK (2002) An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach. Genome 45:520-529

Dettori MT, Quarta R, Verde I (2001) A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. Genome 44:783-790

Dirlewanger E, Garbowski C , Claverie J , Renaud C, Zanetto A, Moing A, Arus P, Laigret F (2002a) Microsatellite markers for the construction of a linkage map in sweet cherry (Prunus avium l.) and map comparison between sweet cherry and other prunus species. Plant and animal genome conferences X. San Diego, CA, January 12-16 (Abst.)

Dirlewanger E, Cosson P, Tavaud M (2002b) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105:127-138

Etienne C, Rothan C, Moing A, Plomion C, Bodenes C, Svanella-Dumas L, Cosson P, Pronier V, Monet R, Dirlewanger E (2002) Candidate genes and QTLs for sugar and organic acid content in peach *[Prunus persica* (L.) Batsch]. Theor. Appl. Genet. 105:145-159

Hormaza JI (2002) Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor. Appl. Genet. 104:321-328

Hurtado MA, Romero C, Vilanova S, Abbott AG, Llacer G, Badanes ML (2002) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.), and mapping of PPV (sharka) resistance. Theor. Appl. Genet. 105:182-191

Iezzoni AF, Brettin TS (1998) Utilization of molecular genetics in cherry. Acta Horticulturae 468:55-62.

Jauregui B, de Vicente MC, Messeguer R, Felipe A, Bonnet A, Salesses G, Arus P (2001) A reciprocal translocation between 'Garfi' almond and 'Nemared' peach. Theor. Appl. Genet. 102:1169-1176

Kimura T, Shi YZ, Shoda M (2002) Identification of Asian pear varieties by SSR analysis. Breeding Sci. 52:115-121

Kimura T, Yamamoto T, Mochida K, Imai T, Matsuta N, Ban Y, Hayashi T (2002) Development and mapping of SSR markers in peach and pear. Plant and animal genome conferences X. San Diego, CA, January 12-16 (Abst.)

Shimada T, Yamamoto T, Hayama H (2000) A genetic linkage map constructed by using an intraspecific cross between peach cultivars grown in Japan. J. Jpn. Soc. Hortic. Sci. 69:536-542

Wang D, Karle R, Brettin TS, Iezzoni AF (1998) Genetic linkage map in sour cherry using RFLP markers. Theor. Appl. Genet. 97:1217-1224

Wang Y, Georgi LL, Zhebentyayeva TN, Reighard GL, Scorza R, Abbott AG (2002) High-throughput targeted SSR marker development in peach (*Prunus persica*). Genome 45:319-328

Yamamoto T, Shimada T, Imai T (2001) Characterization of morphological traits based on genetic linkage map in peach. Breeding Sci. 51:271-278

Yamamoto T, Kimura T, Sawamura Y, Kotobuki K, Ban Y, Hayashi T, Matsuta N (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor. Appl. Genet. 102:865-870

APPENDIX

Table A.1. Primer sequences and lab sources.

Primer pair	Orientation[a]	Sequence (5' to 3')	Lab source
pS141	F R	GGCTCCTACCCATCTAACTGTGA GTCCCGTGCTTTTCCCATTC	A. Iezzoni[c]
pchpgms3	F R	ACGC[b]TATGTCCGTACACTCTCCATG CAACCTGTGATTGCTCCTATTAAAC	Sosinski et al. 2000
GA65 (PceGA65)	F R	GAGAAGCATCCAATGGCAAAGTT CGGGGGCATCAATATACCTCAA	A. Iezzoni
GA50 (PceGA50)	F R	TTCCGTCCGAAGAAATGATTCA TAACTAATGCAGCAGAGCACA	A. Iezzoni
GA57 (PceGA57)	F R	CTTTCAGAACACGAGGCATAGTC GTGTGGAATTGTGAGCGGATAA	A. Iezzoni
GA55 (PceGA55)	F R	GGTACCGGGGGCATCACAC GTGTGGATTTGTGAGCGGATAA	A. Iezzoni
GA26 (PceGA26)	F R	CTTGCAGCTAGCTAGAGTGGTTTT GTGTGGAATTGTGAGCGGATAA	A. Iezzoni
GA77 (PceGA77)	F R	CCTTACCACTGGCATCATCA CAGCTGAGCAGGCAACAAAA	A. Iezzoni
B4G3	F R	CATTGTTCATGGGAGGAATT AGAACATCCCTAAAGGAGCA	A.G. Abbott[d]

[a] F = forward, R = reverse.

[b] This fourth nucleotide, a C in this study, is a G in the original pchpgms3 (Sosinski et a., 2000).

[c] A. Iezzoni, Horticulture Dept., Michigan State University, East Lansing, MI, USA.

[d] A.G. Abbott, Department of Biological Science, Clemson University, Clemson, SC,USA.