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RFLP MAPPING, QTL IDENTIFICATION, AND CYTOGENETIC ANALYSIS IN SOUR CHERRY

By

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ABSTRACT

RFLP MAPPING, QTL IDENTIFICATION, AND CYTOGENETIC ANALYSIS IN SOUR CHERRY

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Three separate but related projects were carried out to establish a foundation for the utilization of molecular markers and cytogenetic tools in the genetic study and breeding of tetraploid sour cherry (*Prunus cerasus* L., 2n=4x=32).

In the first project, restriction fragment length polymorphism (RFLP) linkage maps of two tetraploid sour cherry cultivars, Rheinische Schattenmorelle (RS) and Erdi Botermo (EB), were constructed from 86 progeny from the cross RS x EB. The RS linkage map consists of 126 single dose restriction fragment (SDRF, Wu et al. 1992) markers assigned to19 linkage groups covering 461.6 cM. The EB linkage map has 95 SDRF markers assigned to 16 linkage groups covering 279.2 cM. Fifty-three markers mapped in both parents were used as bridges between both maps and 13 sets of homologous linkage groups were identified. Fifty-nine of the markers on the linkage maps were detected with probes used in other *Prunus* genetic linkage maps. Six of the sour cherry linkage groups may be homologous with six of the eight genetic linkage groups identified in peach and almond.

In the second project, the map locations and effects of quantitative trait loci (QTLs) for eight flower and fruit traits in sour cherry were estimated using the RFLP genetic linkage maps constructed in the first project. Eleven putatively significant QTLs (LOD > 2.4) were detected for six characters (bloom time, ripening time, % pistil death, % pollen germination, fruit weight and soluble solids concentration). The percentage of

phenotypic variation explained by a single QTL ranged from 12.9 % to 25.9 %. Fifty percent of the QTLs identified for the traits in which the two parents differed significantly had allelic effects opposite to those predicted from the parental phenotype. Three QTLs affecting flower traits (bloom time, % pistil death, and % pollen germination) mapped to a single linkage group, EB1. The RFLP closest to the bloom time QTL on EB1 was detected by a sweet cherry (*P. avium* L.) cDNA clone pS141 whose partial amino acid sequence was 81% identical to that of a Japanese pear (Pyrus pyrifolia Nakai) stylar RNase.

In the final project, genomic *in situ* hybridization (GISH) was used to examine meiotic pairing behavior and parental genomic contributions in the allotetraploid sour cherry. Three sour cherry cultivars were studied: Montmorency, Rheinische Schattenmorelle (RS), and Erdi Botermo (EB). GISH analysis suggested that EB may have a higher genomic contribution from *P. avium* than *P. fruticosa* (the two putative progenitor species). In contrast, GISH analysis only identified a relatively few number of species-specific chromosomes and chromosome segments in RS, suggesting that significant intergenomic recombination had occurred. In the meiotic analyses, in addition to the normal bivalent pairing configuration, univalents, trivalents, and quadrivalents were frequently observed in the pollen mother cells of the three cultivars. RS had the most bivalents and the least number of quadrivalents. Montmorency and EB had approximately the same numbers of bivalents and quadrivalents. RS had a bivalents to non-bivalents ratio of 4.4:1 while EB and Montmorency had a ratio of 3.5:1. The ratio of bivalents to non-bivalents may be an important factor in determining the proportion of balanced and unbalanced meiotic products.

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CHAPTER 1

GENETIC LINKAGE MAP IN SOUR CHERRY USING RFLP MARKERS

ABSTRACT

Restriction fragment length polymorphism (RFLP) linkage maps of two tetraploid sour cherry (*Prunus cerasus* L., 2n=4x=32) cultivars, Rheinische Schattenmorelle (RS) and Erdi Botermo (EB), were constructed from 86 progeny from the cross RS x EB. The RS linkage map consists of 126 single dose restriction fragment (SDRF, Wu et al. 1992) markers assigned to 19 linkage groups covering 461.6 cM. The EB linkage map has 95 SDRF markers assigned to 16 linkage groups covering 279.2 cM. Fifty-three markers mapped in both parents were used as bridges between both maps and 13 sets of homologous linkage groups were identified. Homoeologous relationships among the sour cherry linkage groups could not be determined because only 15 probes identified duplicate loci. Fifty-nine of the markers on the linkage maps were detected with probes used in other *Prunus* genetic linkage maps. Six of the sour cherry linkage groups may be homologous with six of the eight genetic linkage groups identified in peach and almond. Twenty one fragments expected to segregate in a 1:1 ratio segregated in a 2:1 ratio. Three of these fragments were used in the final map construction because they all mapped to the same linkage group. Six fragments exhibited segregation consistent with the expectations of intergenomic pairing and/or recombination.

INTRODUCTION

The sour cherry (*Prunus cerasus* L.) industry in the United States desperately needs new improved cultivars to remain competitive in the world market and to reduce pesticide and fungicide use. The sour cherry industry in the United States is a monoculture of a 400 year-old cultivar from France, Montmorency. This cultivar must be sprayed approximately 10-15 times a year to control various insects and diseases to produce marketable fruit. It is also affected by numerous virus and mycoplasma diseases that cannot be completely controlled, and yields are frequently reduced because flower buds are killed by mid-winter cold temperatures or spring freezes.

A critical stage in the development of new cultivars is the selection of desired individuals from breeding populations. Selections are traditionally based on the phenotypic performance, which depends on the plant's genetic potential and the environment in which it grows. To distinguish the genetic component from the environmental component of a phenotypic trait such as the yield, a resource-intensive experiment has to be carried out. Moreover, in sour cherry breeding, selection for fruit traits can not begin until seedlings pass a 3 - 5 years of juvenile stage.

With the advancement of molecular technology, genetic markers can be used to start the selection as early as the seedling develops the first leaf (Beckman and Sollar 1983; Darvasi and Sollar 1994). Genetic markers are heritable entities, which can be associated with economically important traits. The ideal genetic markers to be used in marker-assisted selection (MAS) are those which are not influenced by the environment and are tightly linked to the trait under selection (Staub et al. 1996).

There are two general categories of genetic markers: phenotypic markers and genotypic markers. Phenotypic markers include morphological traits and isozymes. Morphological traits controlled by a single locus and reproducible over a range of environments can be used as genetic markers. Isozymes, which are differently charged protein molecules with the same activity, can also be used as genetic markers. Due to their phenotypic nature, morphological traits and isozymes are influenced by the environment (Staub et al. 1982) and the number of informative markers of both types is limited. These two factors often restrict their utility (Staub et al. 1996). On the other hand, genotypic markers are not limited in number and are not influenced by the environment in which the plant grows. Genotypic markers include all DNA markers.

Restriction fragment length polymorphisms (RFLPs) were the first commonly used DNA markers. RFLPs are revealed by cutting DNA with restriction enzymes and using labeled DNA fragments as probes. Restriction enzymes cut DNA molecules at specific nucleotide sequences (recognition sites), resulting in fragments of different sizes. Mutations within restriction sites, as well as insertions or deletions of DNA fragments between two restriction sites, result in variation in sizes of restriction fragments. This variation can be visualized with labeled probes by Southern blotting (Southern 1975). Probes are usually genomic or cDNA fragments of 500 to 3000 base pairs. Different species of the same genus often have sequence homology within the probe DNA fragment, allowing RFLP probes to be shared among species. This makes RFLPs ideal markers for comparative mapping. RFLPs are not subject to subtle changes in detection procedure, therefore they are highly reproducible. The disadvantage of RFLPs is that they require a large amount of sample DNA. Since the introduction of polymerase chain

reaction (PCR) (Mullis et al. 1986), RFLPs are gradually being replaced by PCR-based DNA markers, which require a very small amount of sample DNA.

Many types of PCR-based markers have been developed. Among the commonly used types are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs). RAPD markers are generated by PCR amplification of random genomic DNA segments with single primers (usually 10 nucleotides long) of arbitrary sequence (Williams et al. 1990). AFLPs are based on PCR amplification of restriction enzyme-digested DNA fragments with two selective primers (Zabeau and Vos 1993). SSRs are tandem arrays of two or more nucleotides. The polymorphisms result from variation in the number of repeats in a given motif. SSRs are detected by PCR amplification of the repeat motif with two primers designed from the sequenced regions flanking the repeat motifs.

Each of the three types of PCR-based markers has advantages and disadvantages. RAPDs are technically the easiest to use among the three types. But extensive primer testing is involved in generating RAPD markers. AFLPs can be generated in large numbers with minimum primer testing. However, they are technically the most complicated to use. SSRs are the most informative and reproducible. But extensive initial cloning and sequencing are required to generate SSR markers. In addition to the advantage of less sample DNA requirement, the analysis of PCR-based markers takes less time than that of RFLPs. However, because PCR is error prone and sensitive to contamination, PCR-based markers are less reproducible than RFLPs. The other disadvantage of PCR-based markers as compared to RFLPs is that they are generally not usable across species, not even across populations within species because the sequence

information revealed by a PCR-based marker is limited (Staub et al. 1996).

In order to use genetic markers in marker-assisted selection, the linkage relationships among genetic markers and economic traits such as fruit quality must be determined and presented in the form of genetic linkage maps. A genetic linkage map is constructed from a mapping population in which the genetic markers are segregating. Different types of mapping populations are typically used in map construction for diploid species. Among the commonly used are F_2, backcross (BC), recombinant inbred line (RI), and doubled haploid populations. A completely classified F_2 population provides the maximum genetic information for map construction (Mather 1951). BC and doubled haploid populations provide only half the genetic information of F_2 population when codominant markers are used (Mather 1951). When dominant markers are used, BC and doubled haploid populations are more informative than F_2 populations (Mather 1951). RI populations are less informative then backcross populations at low marker saturation (Staub et al. 1996).

Normally, mapping populations are developed from crosses between relatively homozygous inbred lines. In outcrossing species such as sour cherry, apple, and most fruit tree species, homozygous inbred lines are not available due to inbreeding depression. This precludes the use of regular backcross populations for genetic analysis in these species. Instead, a pseudotestcross design is typically used in which the variety of interest is crossed to a standard variety known to be homozygous recessive for the traits being investigated. The segregation ratio for a single gene traits is either 1:1 or 1:0 in such a population. A refinement of this approach is a double pseudotestcross in which genetic analysis are performed on both parents in a controlled cross by keeping track of which

loci are heterozygous in each parent. Hemmat et al. (1994) employed this approach to construct genetic maps for apple with each parent in such a double pseudotestcross.

Marker assisted selection would be especially advantageous for sour cherry breeding. Sour cherry seedlings require a minimum of 3-5 years of growth before they flower and fruit. If prior knowledge of linkage relationships between marker loci and important flower and fruit characteristics were available, undesirable individuals could be eliminated from progeny populations allowing more resources to be devoted to promising individuals. Additionally, in whole genome BC selection using RFLP markers, it is estimated that the recurrent parent genotype could be reconstructed and the introduced gene maintained in three BC generations as opposed to the six BC generations required without RFLP genotyping (Tanksley et al. 1989). Map-based BC selection is especially attractive in sour cherry where a reduction in three BC generations could mean a saving of 9-15 years.

Linkage map construction in sour cherry is complicated due to the species' polyploid origin. The presumed progenitor species of the tetraploid sour cherry are the diploid sweet cherry (*P. Avium L*, 2n=2x=16) and the tetraploid ground cherry (*P. fruticosa* Pall, 2n=4x=32)(Olden and Nybom 1968). Although sour cherry exhibits primary disomic inheritance, there is evidence from allozyme segregation data for occasional intergenomic pairing characteristic of a segmental allopolyploid (Beaver and Iezzoni 1993).

Construction of genetic linkage maps for polyploids is inherently more difficult than for diploids for the following reasons: (1) a large number of genotypes is expected for a single locus in a segregating population; (2) poorly characterized genome

constitution and/or chromosome pairing behavior are observed; (3) genome characterization is complicated due to multiple fragments (Wu et al. 1992; Sorrells 1992). To overcome these difficulty, several approaches have been employed, including: construction of linkage maps for diploid relatives, using aneuploid stocks, taking advantage of haploid or doubled haploid populations. However, one or more of these approaches may not be feasible for certain species, including sour cherry. Moreover, the gene order in the polyploid may have changed. One approach that is applicable to all polyploid species is the use of single-dose restriction fragment (SDRF, Wu et al. 1992). A SDRF is a fragment that is present in a single dose in a parent and that segregates in a ratio of 1:1 in the progeny.

Despite the potential utility of a genetic linkage map in the tetraploid sour cherry, no linkage relationships have been reported. In *Prunus*, linkage maps are most advanced in the diploid species. Genetic linkage maps have been constructed for: peach (*P. persica*) (Chaparro et al. 1994; Rajapakse et al. 1995), almond (*P. amygdalus*, syn. *P. dulcis*) (Viruel et al. 1995), sweet cherry (*P. avium*) (Stockinger et al. 1996), sweet cherry x *P. incisa*, sweet cherry x *P. nipponica* (Bošković et al. 1997), peach x almond (Foolad et al. 1995) and peach x *P. davidiana* interspecific hybrid populations (Dirlewanger and Bodo 1994). The markers used for these maps were predominantly RFLPs except for the sweet cherry map constructed by Stockinger et al. (1996), for which RAPDs were used.

As in apple (Hemmat et al. 1994), the linkage mapping population in sour cherry is a 'pseudotestcross' in which informative markers are those that are heterozygous in one parent and homozygous recessive in the other parent and segregate 1:1. However, in the tetraploid sour cherry, if a band is present in one of the parents, the parental genotype can

be ++++, +++-, ++--, +-+-, or +---. Approximately 75 progeny are required to conclusively identify the informative SDRF genotype (+---) based on 1:1 segregation (Wu et al. 1992).

The objective of this study was to construct low density RFLP linkage maps for two sour cherry cultivars and compare these maps to previously constructed *Prunus* RFLP maps. RFLP probes developed by other researchers were used to facilitate comparative mapping; specifically the alignment of sour cherry linkage groups with the 8 linkage groups identified in peach and almond (Arús, pers. comm.; Bliss, pers. comm.).

MATERIALS AND METHODS

Mapping population and DNA isolation

The mapping population consisted of 86 progeny from the cross between two sour cherry cultivars, Rheinische Schattenmorelle (RS) x Erdi Botermo (EB). RS and EB were chosen because they originated from different geographic areas (Germany and Hungary, respectively) and differed in important horticultural traits such as bloom date, cold hardiness, fruit quality and percent fruit set. The parents and progeny population are maintained at the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, MI.

Young unfolded leaves were collected from 7-year-old trees and transported to the laboratory in coolers filled with dry ice. The leaf samples were frozen at -80 C overnight and then lyophilized for 48 - 72 hours. DNA isolation for Southern analysis followed the procedure of Stockinger et al. (1996) with the following modifications: four hundred mg

of lyophilized leaves were placed in a 50-ml centrifuge tube together with five 4 mm glass beads (Fischer Scientific, Pittsburgh, PA) and shaken vigorously for 4 minutes with a paint shaker to grind the sample to a fine powder prior to the addition of extraction buffer.

Source of DNA probes

DNA clones from the following sources were used to identify informative RFLP markers (Table 1): (1) Plum genomic and peach cDNA clones (F. Bliss & S. Arulsekar; Univ. of CA, Davis, CA), (2) peach genomic clones (S. Rajapakse & A. Abbott; Clemson Univ., Clemson, SC), (3) peach cDNA clones (A. Callahan; USDA, Kearneysville, WV), (4) almond genomic and cDNA clones (P. Arús; IRTA, Barcelona, Spain), (5) *Pst*I genomic clones from the sweet cherry cultivar Emperor Francis, and (6) cDNA clones from a stylar cDNA library from the sweet cherry cultivar Emperor Francis.

Sweet cherry genomic clones: A genomic library was constructed using size fractionated *Prunus avium* cv. Emperor Francis DNA. Methylation sensitive *Pst*I (Boehringer Mannheim, Indianapolis, IN) was used to digest genomic DNA which was isolated as described (Stockinger et al. 1996) except that an additional CTAB-chloroform extraction was performed followed by ethanol precipitation. The plasmid vector, pUC19, was cut with *Pst*I and dephosphorylated with calf intestinal alkaline phosphatase (Gibco BRL, Gaithsburg, MD). Size selection of genomic DNA was achieved by fractionating the digested DNA on a 1 % TAE agarose gel (Sambrook et al. 1989). Fragments 500bp to 2000bp were isolated from the gel by placing a piece of DEAE NA45 membrane (Schleicher & Schuell, Keene, NH) into the gel and electrophoresing the appropriately sized DNA into the membrane. The membrane was prepared and the DNA was

Table 1. Probes from other *Prunus* research groups which were unlinked or mapped to one or more locations in sour cherry. RS and EB refer to the Rheinische Schattenmorelle and Erdi Botermo linkage groups, respectively.

Probe*	Linkage group(s) in sour cherry map	References
AC1	unlinked	Viruel et al. 1995
AC6	unlinked	
Pru2	RS 8	
AC27	RS 2, EB 2	
AG6	RS 12	
AG8	EB 13	
AG10	RS 7, EB 7	
AG21	RS 2	
AG40	RS 17, EB 17, RS 18, EB 18	
Ext1	RS 8, EB 8	
Ole1	RS 2	
B4G10	EB 6, RS 17	Rajapakse et al. 1995
B6D1	unlinked	
B7H2	RS 16	
B8A3	RS 19	
CPM2	RS 5, EB 5	Bliss (pers comm);
CPM6	RS 12	Foolad et al. 1995
CPM12	RS 1, EB 1	
CPM20	RS 5, EB 5, RS 5', RS 6, EB 6	
CPM23	RS 6, EB 6, EB 14	
CPM30	RS 5	
CPM39	RS 6, EB 6, RS 17, EB 17, RS 18,	
CPM48	RS 7, EB 7, EB 7'	
CPM53	RS 4, EB 4	

Table 1. (cont'd)

Probe*	Linkage group(s) in sour cherry map	References
CPM57	RS 9, EB 9	Bliss (pers. comm.);
CPM58	RS 4, EB 4	Foolad et al. 1995
CPM59	RS 2	
CPM64	RS 7, EB 7	
CPM67	RS 7, EB 7	
CPM70	EB 5, RS 5', RS 19	
CPM90	RS 2	
CPM104	RS 6, RS 6'	
PLG10	unlinked	
PLG86	RS 2, EB 2	
Hsp4	RS 2, EB 2	Callahan (pers. comm.)
pch108	unlinked	
pch202	RS 5, EB 5	
pch205	RS 3	

*AC = almond cDNA clones, Pru2 = cDNA for the seed protein Prunin (P. Arús, personal comm.), AG = almond genomic clones, Ext1 = cDNA for Extensine, Ole1 = cDNA for Oleosine, B- = peach genomic clones, CPM = peach mesocarp cDNA clones, PLG = plum genomic clones, Hsp4 = peach cDNA for a heat shock protein, pch108 = peach cDNA for chlorophyll A/B binding protein, pch202 = peach cDNA for a thioredoxin, pch205 = peach cDNA for a water stress protein.

recovered according to the manufacturer's instructions. The size selected DNA and pUC19 DNA were concentrated in a Microcon concentrator (Amicon Inc., Beverly, MA), heated to 65 C for 5 min, then ligated in a 10 μl reaction with T4 DNA ligase (Boehringer Mannheim, Indianapolis, IN) as described by Sambrook et al. (1989). Recombinant plasmid DNA was then transformed by electroporation into *E. coli* DH5-α electrocompetent cells using the manufacturer's protocol (Bio-Rad Laboratories, Hercules, CA). White colonies were picked from LB plates containing ampicillin (125 μg/ml), X-gal (40 μg/ml), and IPTG (0.95 μg.ml), for further analyses. Inserts were amplified by PCR using primers which flank the multiple cloning site of pUC19 (Promega, Madison, WI). The size of amplified insert DNA was checked on a 1 % agarose gel. The approximate copy number of cloned fragments was determined by dot blotting. One hundred nanograms of insert DNA was blotted onto a Zeta-Probe GT membrane (Bio-Rad Laboratories, Hercules, CA) with control DNAs which were known to be low, medium, and high copy in the cherry genome. The dot blots were hybridized with sour cherry genomic DNA labeled with ^{32}P dCTP using a nick translation kit (Boehringer Mannheim, Indianapolis, IN). Prehybridization and hybridization conditions were as described by Stockinger et al. (1996). These genomic clones are identified by "EF" referring to Emperor Francis and the clone number.

Sweet cherry cDNAs: RNA was isolated from approximately 1 g of stylar tissue from the sweet cherry cultivar Emperor Francis by the method of Manning (1991) with the following modifications: four phenol chloroform isoamylalcohol (25:24:1) extractions were performed and the [Na+] in the first butoxyethanol precipitation was adjusted to 100 mM. Stylar cDNA was prepared using a cDNA synthesis kit (Boehringer Mannheim,

Indianapolis, IN) and a cDNA amplification protocol (Jepson et al. 1991). This stylar cDNA was subsequently used in a PCR amplification with two degenerate primers, ATNCA(T/C)GGN(C/T)TNTGGCC and (C/G)(A/T)(A/G)CANGTNCC(A/G)TG(T /C)TT, designed to amplify ribonuclease sequences. Primer design was based on conserved amino acids identified by aligning several S-allele and ribonuclease amino acid sequences (T-H. Kao, personal communication). Four major bands resulting from amplification with the degenerate primers were isolated from a 5 % native polyacrylamide gel (Sambrook et al. 1989). These fragments were then reamplified, cloned into pUC118, and copy number determined as described above for the sweet cherry genomic clones. These probes were identified by "PS" for *Prunus* stylar tissue and the clone number.

Southern analysis

DNA (6μg) of both parents and 12 progeny was digested with 20 - 30 units of one of six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Hind*III, *Pst*I, or *Xba*I; Boehringer Mannheim Biochemicals, Indianapolis, IN) and separated on a 0.9 % agarose gel for 30 h at 23V. Southern analysis was performed according to Stockinger et al. (1996) using Hybond-N+ membranes (Ambersham, Arlington Heights, IL).

Probe DNAs were prepared by PCR amplification of the inserts from pUC19 or Bluescript plasmids (Stratagene, La Jolla, CA) using a pair of primers flanking the cloning sites. Radiolabelling of probes with ^{32}P-dCTP (DuPont, Boston, MA) was done using the random priming method of Feinberg and Vogelstein (1983). Those enzyme and probe combinations that identified useful polymorphisms from the two parents and 12 progeny were used to genotype the additional 74 progeny in the mapping population.

X^2 and linkage analysis

Informative markers for a pseudotestcross mapping population are single-dose restriction fragment (SDRFs) that differ between parents and segregate in a 1:1 (presence:absence) ratio and SDRFs present in both parents that segregate in a 3:1 ratio (Wu et al. 1992). Therefore, markers which differed between parents were tested for fit to a 1:1 (presence:absence) ratio. Markers present in both parents were tested for fit to a 3:1 (presence:absence) ratio. Those markers which fit the appropriate ratios at the 5 % level were used in the linkage analysis.

Markers present in one parent that did not fit to a 1:1 ratio were tested for fit to a 5:1 or 2:1 ratio. A 5:1 ratio would be expected for tetrasomic inheritance of a double dose restriction fragment (DDRF, +-+- x ----; Wu et al. 1992). A ratio of 2:1 could probably represent either (1) a skewed 1:1 ratio due to possibly gametophytic selection or a lethal allele or (2) a skewed 3:1 ratio which would be expected for disomic inheritance of a DDRF (+-+- x ----). Markers which fit a 2:1 ratio at the 5 % level were included in an initial linkage analysis; however, only those 2:1 markers that exhibited linkage with another 2:1 marker were included in the final linkage analysis. These linked 2:1 markers may identify linkage groups which have been preferentially selected. The other 2:1 markers were not used because their genotype (DDRF or SDRF) could not be determined based on the 2:1 ratio.

Linkage analyses were performed with JoinMap V2.0 (Stam 1993) using a minimum LOD score of 3.0 and a maximum recombination fraction of 0.35. Distances were calculated by the Kosambi function and expressed in centi-Morgans . Multiple loci detected using the same probe were labeled with a letter after the probe designation.

Where possible, linkage groups were numbered based upon suspected homology with previously constructed peach and almond linkage maps (Bliss, personal comm.; Viruel et al. 1995).

RESULTS AND DISCUSSION

Two hundred sixty probes were tested to select informative probes. Ninety-nine probes were found to detect polymorphic markers. Eighty-two of these probes were able to identify SDRFs (Table 2).

Seventy-six percent of the polymorphic markers detected with the selected probes fit the expected ratios for SDRFs. A total of 190 SDRFs were identified, of which, 110 SDRF markers fit a 1:1 ratio. RS and EB were heterozygous for 67 and 43 of these 1:1 markers, respectively. A total of 80 SDRF markers were present in both parents and fit a 3:1 ratio (Table 2).

Twenty seven segregating fragments present in one parent and absent in the other parent did not fit a 1:1 ratio (Table 3). Of these fragments, 9 were present in RS and absent in EB, and 19 were present in EB and absent in RS. Of the 9 RS fragments, 8 fragments fit 1:2 or 2:1 ratios and one fit a 5:1 (+,-) ratio . Of the 19 EB fragments, 13 fragments fit 1:2 or 2:1 ratios, and 3 fragments fit a 5:1 (+,-) ratio. The other 3 fragments had distorted presence:absence ratios of 79:6, 81:2 and 84:2.

Sour cherry is an allotetraploid originated from two distinct species, *P. avium* and *P fruticosa*. Disomic inheritance is characteristic of an allotetraploid. In a cross between two strict allotetraploids, if a band is present in one parent and absent in the other, the

Table 2 RFLP genetic analysis of progeny from Rheinische Schattenmorelle (RS) x Erdi Botermo (EB).

Mapping population size	86
Number of probes tested	260
Number of polymorphic probes	99
Number of probes that identified SDRFs	82
Number of markers segregating 1:1 in RS	67
Number of markers segregating 1:1 in EB	43
Number of markers present in EB and RS segregating 3:1	80
Number of linkage groups in RS map	19
Number of linkage groups in EB map	16
Map units for RS map	461.6 cM
Map units for EB map	279.2 cM
Number of markers mapped in RS map	130
Number of markers mapped in EB map	100
Number of unlinked markers in RS	17
Number of unlinked markers in EB	23

Table 3 Polymorphic markers that were present in one parent and did not segregate 1:1 in progeny.

Marker name	Parent		No. of individual		tested ratio	x^2 value
	RS	EB	+	-		
EF146H1	+	-	27	57	1:2	0.08
EF176EV3	+	-	27	55	1:2	0.03
EF60EI	+	-	33	52	1:2	1.08
EF187EI4	+	-	52	33	2:1	1.08
EF158H4	+	-	51	30	2:1	0.46
B8A3X1	+	-	49	28	2:1	0.29
EF66EI1	+	-	54	30	2:1	0.19
AC27EV4	+	-	55	23	2:1	0.59
EF48EV1	+	-	66	16	5:1	0.36
PLG86EI1	-	+	28	52	1:2	0.09
EF66EI2	-	+	31	53	1:2	0.44
CPM6EV2	-	+	31	51	1:2	0.68
EF176H3	-	+	50	32	2:1	1.12
AG40H4	-	+	52	33	2:1	1.08
CPM53a	-	+	52	33	2:1	1.08
PS41EV2	-	+	52	32	2:1	0.80
PLG10H3	-	+	49	30	2:1	0.71
EF71EI2	-	+	53	32	2:1	0.66
EF182a	-	+	54	30	2:1	0.19
CPM70EI3	-	+	55	30	2:1	0.13
EF156a	-	+	58	25	2:1	0.45
EF173X1	-	+	55	22	2:1	0.87
EF187EV5	-	+	64	18	5:1	1.42
EF156H4	-	+	65	18	5:1	1.29
EF132X4	-	+	62	14	5:1	0.11
EF172EV4	-	+	79	6	-	-
PLG10H2	-	+	81	2	-	-
PLG10H1	-	+	84	2	-	-

band will theoretically either be present in all progeny or segregates at a 1:1 (SDRF) or a 3:1 (DDRF) ratio when there is no intergenomic recombination. The 1:2 ratios observed in this study could be a skewed 1:1 ratio resulting from gametophytic selection. The 2:1 ratio could be either a skewed 1:1 ratio or a skewed 3:1 ratio as discussed above. The 5:1 ratio observed for the 4 markers could only be explained as the results of tetrasomic inheritance from a cross +-+- x ----. The segregation ratios of 79:6, 81:2 and 84:2 could be explained as the results from loss of fixed heterozygosity. Skewed segregation ratios and loss of fixed heterozygosity in sour cherry were also observed in a genetic study using allozymes by Beaver and Iezzoni (1993). A 2:1 ratio was accepted and the expected 3:1 ratio was rejected for three out of nine inheritance ratios for three unlinked allozyme loci (Beaver and Iezzoni 1993). Fifteen out of 308 progeny exhibited a loss of fixed heterozygosity for *6-Pgd-2* (Beaver and Iezzoni 1993). The observations of a few markers showing tetrasomic inheritance and loss of fixed heterozygosity indicate that intergenomic chromosome pairings occur in a low frequence during meiosis of sour cherry. Cytogenetic studies support the theory that some of the segregation results are due to intergenomic recombination. Meiosis-I in sour cherry should result in the formation of 16 bivalents. However, quadrivalents were frequently observed for the mapping parents RS and EB (see Chapter 3).

Thirty-two fragments that were present in both parents and segregating in the progeny did not fit a 3:1 ratio which was expected from segregation of a SDRF in each parent (+--- x +---) (Table 4). Nine of these fragments fit a 2:1 ratio which could be a skewed 3:1 ratio resulted from gametophytic selection or zygotic lethal genes. The other fragments had segregation ratios ranging from 5:1 to 84:1. In these cases, it is possible

Table 4 Polymorphic markers that were present in both parents and did not segregate 3:1 in progeny.

Marker Name	No. of		Ratio of
	+	-	+/-
AG6EI2	47	35	1.3
EF127EV2	49	35	1.4
EF182EV1	50	34	1.5
EF61EV	51	34	1.5
AC1H	45	29	1.6
CPM12EI2	51	32	1.6
EF187H1	52	30	1.7
EF50D2	54	30	1.8
OleoEI1	52	28	1.9
AG6EI3	71	13	5.5
EF180X	66	12	5.5
EF132X1	65	11	5.9
AC27EV3	70	10	7.0
pch205EI1	73	10	7.3
EF87EV	75	10	7.5
EF191X1	69	9	7.7
EF187EV2	72	9	8.0
EF67EV2	76	9	8.4
EF77H3	77	8	9.6
CPM6EV1	75	6	12.5
EF187H6	77	6	12.8
EF187EI1	78	6	13.0
CPM104EI1	79	5	15.8
AG40H3	81	4	20.3
EF156H2	80	3	26.7
EF77H1	82	3	27.3
CPM20H6	80	2	40.0
EF48EV3	81	2	40.5
CPM43EI3	81	2	40.5
EF187EI5	83	2	41.5
CPM90H2	83	2	41.5
EF133H3	84	1	84.0

that one or both of the parents was double dose for the scored fragment (+-+- x +--- or +-+- x +-+-). However, the progeny size of 86 was too small to statistically distinguish between these various segregation hypotheses.

Linkage analysis of the 21 markers segregating 2:1 or 1:2 revealed that only three of these markers, EF156a, CPM53a, and EF182a, were linked. These three markers were added to the data containing the 190 SDRFs for map construction. Two maps were constructed separately, one for the parent RS and the other for the parent EB.

The RS linkage map consists of 130 markers assigned to 19 linkage groups covering 461.6 cM (Fig. 1). Seventeen markers remained unlinked. Four redundant markers were removed from the map because each of them was mapped to the same location with another marker detected by the same probe. The longest linkage group in RS map, RS8, is 71.8 cM while the shortest linkage group, RS7', is 5.8 cM. The average length of all linkage groups is 24.3 cM. The longest distance between two adjacent markers is 20.5 cM (RS3). The average distance between two adjacent markers is 4.3 cM.

The EB linkage map possesses 100 markers assigned to 16 linkage groups covering 279.2 cM (Fig. 1). Twenty-three markers were unlinked. Five redundant markers were removed from the map. The longest linkage group in EB map, EB7, is 35.5 cM while the shortest linkage group, EB6', is 0 cM. The average length of all linkage groups is 17.5 cM. The longest distance between two adjacent markers is 20.9 cM (EB17). The average distance between two adjacent markers is 3.5 cM.

Thirteen EB linkage groups homologous to the RS linkage groups were identified using 53 bridging markers heterozygous in both parents. EB counterparts of RS linkage

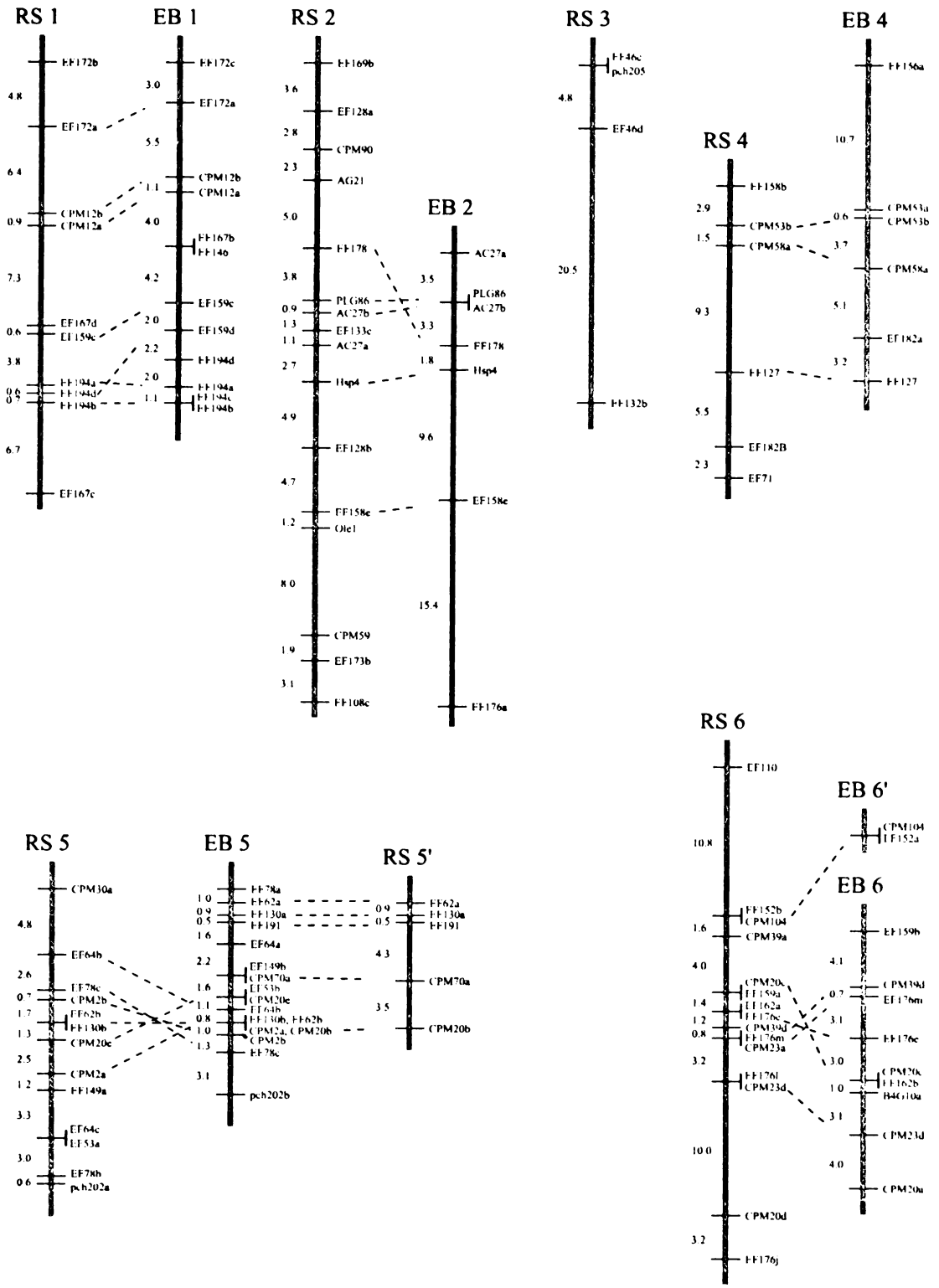

Figure 1. RFLP maps for Rheinische Schattenmorelle (RS) and Erdi Botermo (EB). Markers shown on the right are identified by the probe followed by a letter when more than one marker is generated from a single probe. Correspondences between anchor loci of RS and EB linkage groups are shown with dashed lines.

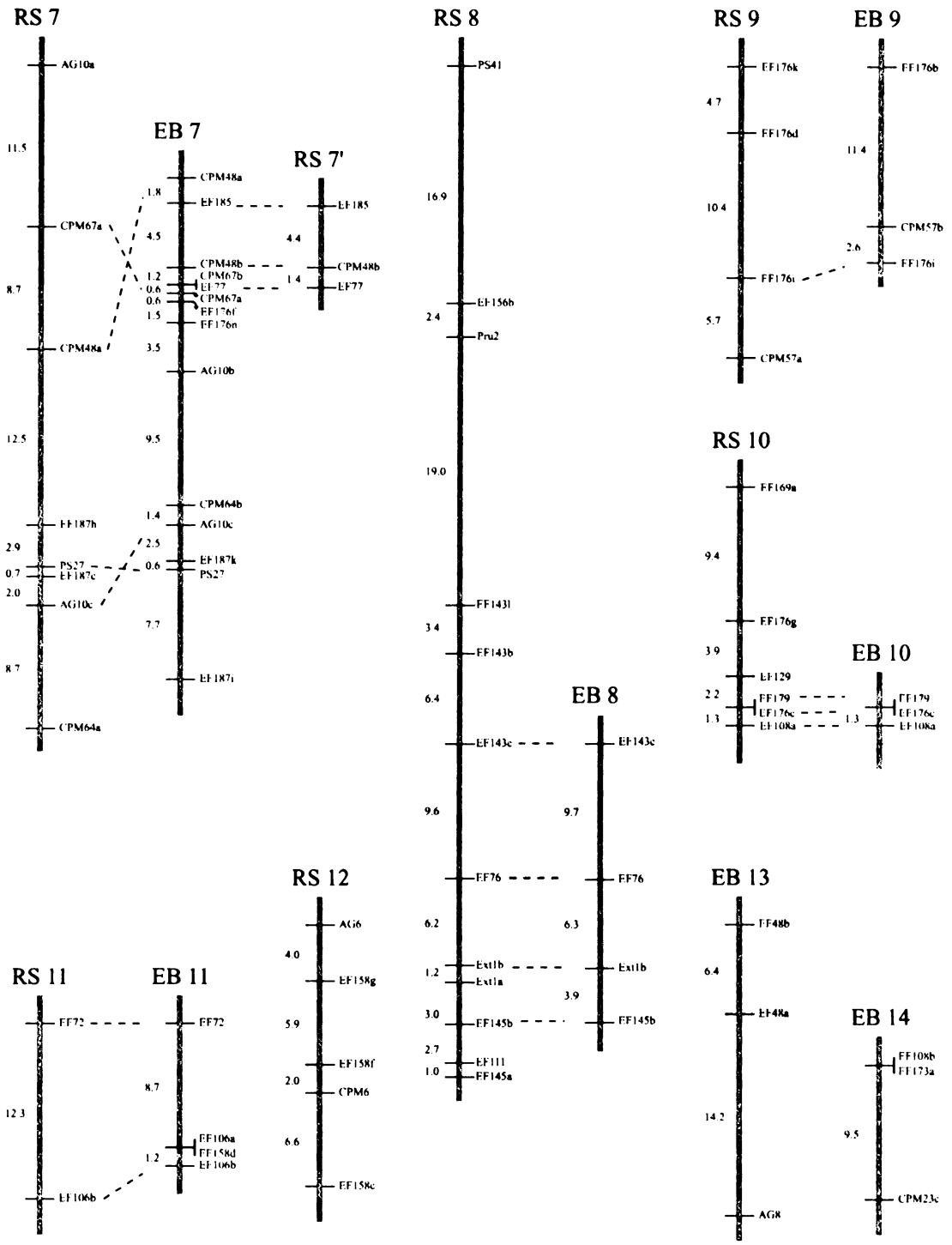

Figure 1 (cont'd)

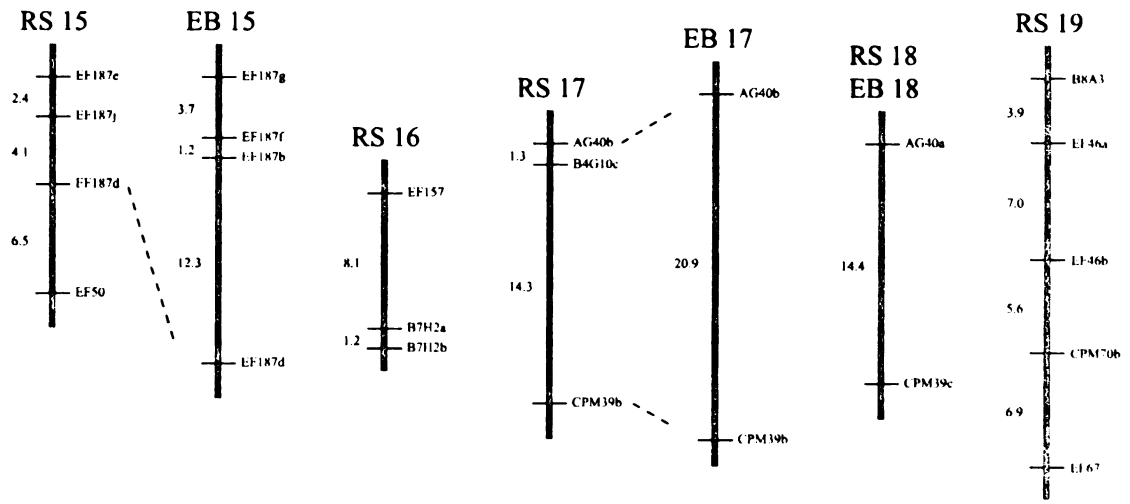

Figure 1 (cont'd)

groups 3, 12, 16, and 19, were not identified. Conversely, RS counterparts of EB linkage groups 13 and 14 were also not identified. Two EB linkage groups were homologous to RS linkage group 6. The longer of the two was named EB6 and the shorter was named EB6'. Two homologous RS linkage groups were identified for each of the two EB linkage groups, 5 and 7. As for EB linkage group 6, RS5 and RS7 were used to name the longer linkage groups, and RS5' and RS7' were used to name the shorter linkage groups of RS linkage group 5 and 7, respectively. In all these cases, the two linkage groups homologous to the same linkage group of the other parent may actually be two segments of a single linkage group. When more markers are added to the map, the two linkage groups may eventually become one linkage group.

The three markers that fit a 2:1 ratio were mapped to EB Group 4. All three markers had an overabundance of the allele unique to EB, suggesting that the region containing these alleles may have been preferentially selected.

Since sour cherry is a tetraploid with x=8, the ultimate goal is to identify 16

linkage groups and the homoeologous relationships among these linkage groups. For example, Groups 17 and 18 may be homoeologous groups because markers identified with probes AG40 and CPM39 mapped an average of 18.2 and 14.4 cM apart in both linkage groups, respectively (Fig. 1). However, no other homoeologous segments could be identified with the set of probes used in this analysis. The ideal probe for identifying homoeologous linkage groups in a tetraploid is a probe that identifies 2 segregating bands which map to different linkage groups. Of the 82 probes that identified mapped fragments, only 15 probes met this criterion. Forty-six probes identified only one mapped fragment, and 21 probes identified two or more fragments which were mapped to the same linkage group.

Fifty-nine markers on the linkage maps were detected with probes placed on other *Prunus* linkage maps. Based on these common probes (Table 5), linkage groups were numbered according to suspected homology to the previously constructed almond x peach map (Arús, personal comm.) and the peach x almond map (Bliss, personal comm.). Six of the sour cherry linkage groups share 2 or more probes with the corresponding linkage groups in the almond x peach and the peach x almond maps (Table 5), suggesting that they may be homologous to the corresponding linkage groups. The map distances between markers detected by shared probes are generally consistent with those in the almond x peach and peach x almond maps (Table 6). For example, group 2 markers identified with the probes AG21 and Ole1 mapped 25.6 cM apart in RS (Fig. 1) and 24 cM apart in almond x peach (Arús, personal comm.). Another example is that the map distance between group 2 markers identified with the probes CPM90 and PLG86 is 11.1 cM in RS (Fig. 1) and 13.2 cM in peach x almond (Bliss, personal comm.). However,

Table 5 Shared probes on which the assignment of linkage group number of sour cherry maps were based.

Group number	Probes common with the corresponding linkage group in the almond x peach and peach x almond linkage maps
1	CPM12
2	AC27, AG21, CPM59, CPM90, PLG86, Ole1
4	CPM53, CPM58
5	CPM2, CPM20
6	CPM20, CPM23, CPM39
7	AG10, CPM48, CPM64, CPM67
8	Pru2, Ext1

Table 6 Distances of common pairs of markers which were mapped in sour cherry and other *Prunus* species.

Probe pair in common	Linkage group number	Distance in sour cherry map (cM)		Distance in other *Prunus* map (cM)	
		RS	EB	almond x peach	peach x almond
AG21 - Ole1	2	25.6	-	24	-
AC27 - AG21	2	9.7, 12.1	-	13	-
AC27 - Ole1	2	13.5, 15.9	-	11	-
CPM90 - PLG86	2	11.1	-	-	13.2
PLG86 - CPM59	2	24.8	-	-	48.2
CPM53 - CPM58	4	1.5	3.7	-	27.9
Pru2 - Ext1	8	44.6	-	52	-

inconsistency in map distances between markers detected by shared probes was also found (Table 6). For example, group 4 markers identified with the probes CPM53 and CPM58 mapped 27.9 cM apart in peach x almond (Bliss, personal comm.) but just 1.5 cM and 3.7 cM apart in RS and EB, respectively (Fig. 1). The general consistency in map distances of common markers between sour cherry linkage groups and the corresponding linkage groups in the almond x peach and peach x almond maps provide further support for the likelihood of homologous relationship between the corresponding linkage groups. These associations, however, are preliminary until more alignment comparisons can be made.

Sweet cherry, a diploid *Prunus*, is suspected to be an ancestral progenitor of sour cherry. Unfortunately, it is not possible to compare the sour cherry map with the previously published maps from sweet cherry, sweet cherry x *P. incisa*, and sweet cherry x *P. nipponica*, because these diploid maps consist exclusively of RAPD and isozyme markers (Bošković et al. 1997; Stockinger et al. 1996)

The longest *Prunus* linkage map published is a peach x almond map consisting of approximately 800 cM (Foolad et al. 1995). Given that sour cherry is a tetraploid, a map of comparable coverage should be 1500 cM. Our current maps cover only one third of this expected total length. The requirements for informative marker state in a tetraploid make it more challenging to add more markers to the map than that for a diploid. A project to develop and map simple sequence repeat (SSR) loci is currently underway in our laboratory to determine if potentially higher levels of heterozygosity at SSR loci will increase the likelihood of identifying informative markers and identifying homoeologous linkage groups in sour cherry. Additionally, if SSRs are conserved among *Prunus*

species, they would be excellent markers for comparative mapping.

The maps constructed in this study are the first genetic linkage maps for sour cherry. They form the base for further genetic studies of important traits such as fruit quality and stress tolerance in sour cherry using molecular markers. The following chapter will describe the first application of these maps to identify quantitative trait loci (QTL) controlling flower and fruit traits in sour cherry.

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CHAPTER 2

QTL ANALYSIS OF FLOWER AND FRUIT TRAITS IN SOUR CHERRY

ABSTRACT

The map locations and effects of quantitative trait loci (QTLs) were estimated for eight flower and fruit traits in sour cherry (*Prunus cerasus* L.) using a restriction fragment length polymorphism (RFLP) genetic linkage map constructed from a double pseudo-testcross. The mapping population consisted of 86 progeny from the cross between two sour cherry cultivars, Rheinische Schattenmorelle (RS) x Erdi Botermo (EB). The genetic linkage maps for RS and EB were 398.2 cM and 222.2 cM, respectively, with an average interval length of 9.8 cM. The RS/EB linkage map that was generated with shared segregating markers consisted of 17 linkage groups covering 272.9 cM with an average interval length of 4.8 cM. Eleven putatively significant QTLs (LOD > 2.4) were detected for 6 characters (bloom time, ripening time, % pistil death due to freeze damage, % pollen germination, fruit weight and soluble solids concentration). The percentage of phenotypic variation explained by a single QTL ranged from 12.9 % to 25.9 %. Fifty percent of the QTLs identified for the traits in which the two parents differed significantly had allelic effects opposite to those predicted from the parental phenotype. Three QTLs affecting flower traits (bloom time, % pistil death due to freeze damage, and % pollen germination) mapped to a single linkage group, EB1. The RFLP closest to the bloom time QTL on EB1 was detected by a sweet cherry cDNA clone pS141 whose partial amino acid sequence was 81% identical to that of a Japanese pear stylar RNase.

INTRODUCTION

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

Linkage relationships between molecular markers and agronomically important traits have been extensively studied in many crop plants for over a decade (Edwards et al. 1987; Stuber et al. 1987, 1992; Paterson et al. 1988, 1990; Keim et al. 1990; Heyes et al. 1993; Wang et al. 1994; Toroser et al. 1995; Grandillo and Tanksley 1996; Rebai et al. 1997; Pilet et al. 1998). In tree fruit crops, a QTL analyses was reported for growth and development traits in apple (Conner et al. 1998) and QTL analyses for fruit size and fruit sugar content are underway in peach (A. Abbott, pers. comm). In contrast, no QTL analyses have been reported in sour cherry. The delay has been due to the difficulties in the construction of a molecular linkage map for sour cherry because of the species' polyploid origin and mixed patterns of inheritance (disomic and tetrasomic) (Beaver and Iezzoni 1993; Wang et al. 1998) . Recently, we constructed the first molecular linkage

maps in sour cherry using RFLP markers (Wang et al. 1998) and in this report we describe the first QTL analysis in sour cherry. Our objectives were to estimate the locations and effects of QTLs affecting flower and fruit traits in sour cherry.

MATERIAL AND METHODS

Plant material

The mapping population utilized in this study is a double pseudo-testcross population (Lawson et al. 1995) which consisted of 86 progeny from the cross between two sour cherry cultivars, Rheinische Schattenmorelle (RS) x Erdi Botermo.(EB). RS and EB were chosen because they are from different geographic areas (Germany and Hungary, respectively) and differ for important horticultural traits. The trees are planted at the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, MI. A total of 8 traits were evaluated for each progeny individual and the two parents. Five traits were evaluated over 3 years and three traits were evaluated in one year. Details of trait evaluations are given below.

Traits measured

Bloom time

The bloom date of an individual was recorded as the day when approximately 50% of the flowers were open. Hourly temperature readings were available from an automated weather station at the Clarksville Horticultural Experiment Station. Time to bloom was expressed as degree days (DD) from January 1 with a base temperature of 4.4

°C. Daily heat unit accumulation was calculated by summing the positive differences of hourly temperature readings minus 4.4 °C and then dividing by 24. On the day of bloom, heat unit accumulation was calculated to hour 10, which was the approximate time the data were recorded. Bloom time was evaluated over 3 years (1995 - 1997).

Ripening time

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

Flower bud death

Flower bud death due to freeze damage is common in Michigan when the buds start swelling in the early spring. Following a spring freeze to -10 °C on the night of April 5, 1995, flower bud death was evaluated from the swelled buds. About twenty flower buds from each individual were cut open to determine bud death, which was expressed as the percentage of dead buds. The data in percentage were angular transformed (i.e. $\arcsin\sqrt{Y}$ transformed) to normalize the distribution of the data for QTL analysis.

Pistil death

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

Pollen germination

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

Fruit set

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

Fruit weight and total soluble solids concentration

Fruit weight (g) and percent soluble solids were evaluated for five ripe fruits from each parent and the progeny. Percent soluble solids was measured with a refractometer as ^{o} Brix. The average of the five fruits was used for QTL analysis. These data were collected over 3 years (1995-1997).

Molecular marker and QTL analysis

RFLP markers were used to construct linkage maps for each parent of the

mapping population (Wang et al. 1998). All markers used were single dose restriction fragments (SDRFs, Wu et al. 1992) which were either: (1) present in one but not both of the parents and fit a 1:1 (presence:absence) segregation ratio, or (2) present in both parents and fit a 3:1 (presence:absence) segregation ratio. A total of 190 SDRF markers were used, of which 110 were present in one parent (67 and 43 markers in RS and EB, respectively) and 80 markers were present in both parents.

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

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

QTL mapping was performed using composite interval mapping (CIM) (Zeng 1994; Jansen and Stam 1994) which is an extension of interval mapping (Lander and

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

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

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

For traits evaluated over three years, each year was considered as a different environment. Therefore, the data from each year were analyzed separately. When a

putative QTL was detected in more than one year, the mean of the three years was analyzed and the results were reported as the generalized results for the QTL.

RESULTS AND DISCUSSION

Distribution of traits

All traits evaluated exhibited continuous variation which is typical of quantitative or polygenic inheritance (Fig. 1). The two parents, RS and EB, differed significantly ($P < 0.05$) for 5 traits, including bloom time, ripening time, fruit weight, percent flower bud death, and percent pollen germination (Table 1). There were no significant differences between the two parents for soluble solids concentration, percent pistil death, and percent fruit set (Table 1). Transgressive segregation was observed for all traits analyzed (Table 1; Fig. 1).

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

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

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

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

the progeny ripened earlier than the early parent.

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

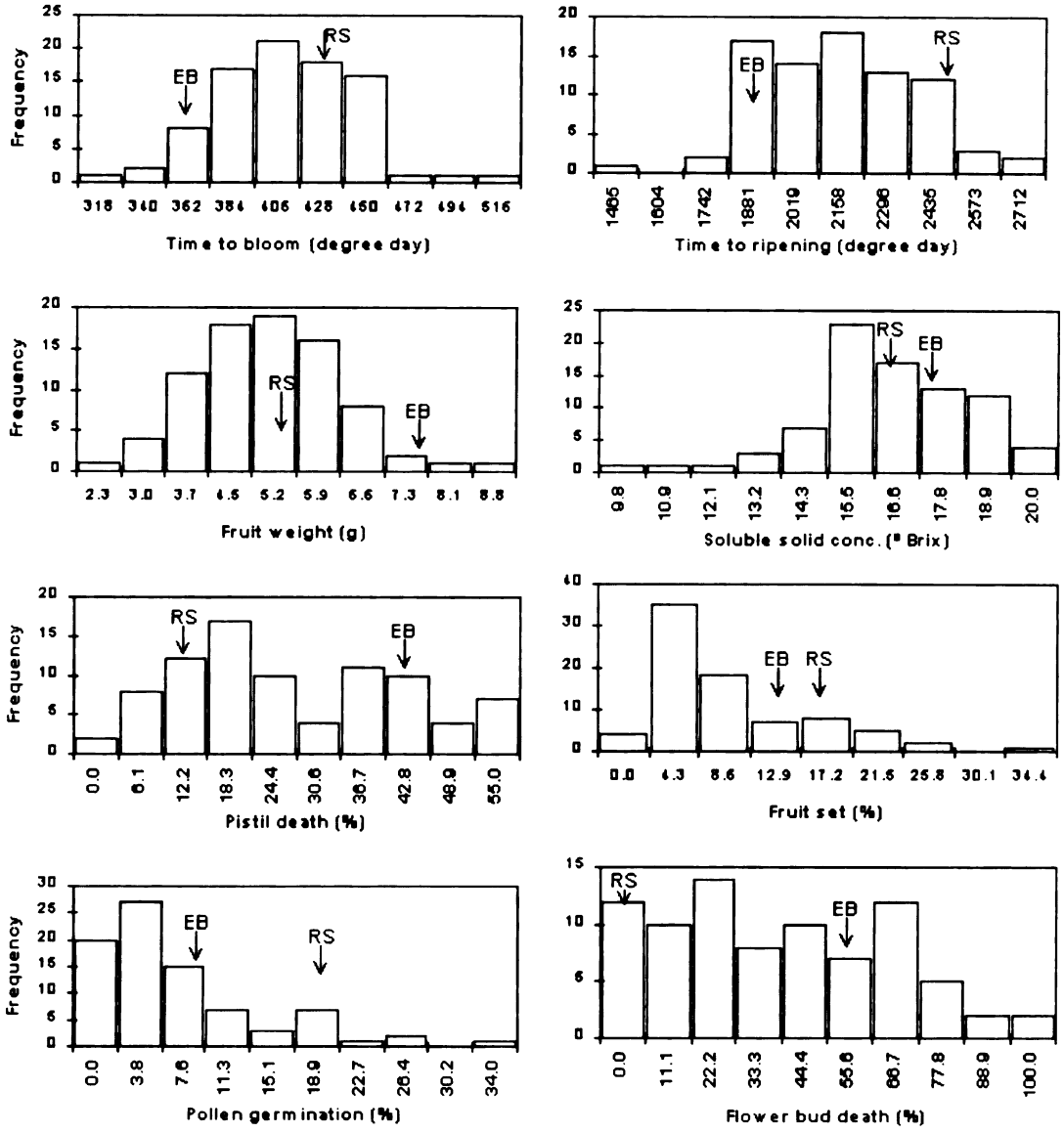

Figure 1. Frequency distributions for each character in the mapping population. Means for the parents RS and EB are shown by arrows.

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

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

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

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

Fruit from RS and EB had similar percent soluble solids. The progeny distribution

ranged from 9.8 to 20.1 % soluble solids and was skewed towards the higher parental values (Fig. 1).

Correlation of traits

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

Genetic linkage maps

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

272.9 cM with an average interval length of 4.8 cM.

QTL analysis

Eleven QTLs were identified for 6 traits: bloom time, % pistil death, % pollen germination, ripening time, fruit weight and soluble solids concentration (Table 2, Fig. 2 and Fig. 3). No QTLs were identified for flower bud death and % fruit set.

Two QTLs were identified for bloom time on two different linkage groups, EB1 (*blm*1) and Group 2 (*blm*2) (Fig. 2, A and B). The QTL, *blm*1, explained 19.9 % of the phenotypic variation. This QTL had the effect predicted by the parental phenotype, with an allele from the early blooming parent, EB, reducing bloom time by 27.8 degree days. This QTL was the only QTL identified in this study that was consistently detected in each of the three years analyzed. The QTL *blm*2 explained 22.3% of the phenotypic variance and was detected in 2 of the 3 years and in all three years when the data were combined. The stabilities of the bloom time QTLs are likely due to the ease of scoring for this trait plus the conversion of the calendar day data to a heat accumulation value which reduces the variation among years. As a result, the bloom time data for all three years had the lowest average coefficient of variation (3.0 %) of all the quantitative traits analyzed.

Two QTLs were detected for percent pistil death on linkage groups EB1 (*pd*1) and RS8 (*pd*2) (Fig. 2, C and D). The QTLs *pd*1 and *pd*2 explained 12.9 % and 14.3 % of the phenotypic variance, respectively. Both QTLs had effects in the direction opposite to those predicted by the phenotype of the parents. An EB allele of *pd*1 reduced the percent pistil death by 2.1 % while a RS allele of *pd*2 increased percent pistil death by 1.5 %. The QTLs *pd*1 and *pd*2 were both detected with the threshold LOD score in only one of

Table 2 QTLs detected for each trait. QTLs are named according to trait abbreviations and a number is used to distinguish QTLs affecting the same trait. Data were based on the analysis of trait means over three years except for the trait percent pollen germination and the QTL *pd*1.

Trait	QTL	Linkage group [a]	Interval length (cM)	LOD peak position (cM)	Nearest marker	Max. LOD	R^2	Genetic effect: a[b]
Bloom (degree day)	*blm*1	EB1	> 21.5	81.1	pS141	3.6	19.9	-27.8
	blm2	RS/EB2	> 20.1	32.1	PLG86	3.3	22.3	-10.1
Pistil death (%)	*pd*1	EB1	28.8	14.8	EF194c	2.6	12.9	-2.1
	*pd*2	RS8	>14.7	0.0	EF156b	2.7	14.3	1.5
Pollen germination (%)	*pgr*	EB1	> 14.0	4.0	EF146	3.0	17.0	1.4
Ripe (degree day)	*rp*1	RS4	> 10.0	0.0	EF158b	4.1	21.5	197.5
	rp2	RS/EB6	> 8.7	4.5	CPM20e	3.7	25.9	156.2
Fruit weight (g)	*fw*1	EB4	26.5	10.01	EF182a	2.3	13.7	0.9
	fw2	RS/EB2	> 20.1	32.1	PLG86	2.5	15.5	0.6
Soluble solids concentration (° Brix)	*ssc*1	EB7	> 6.0	0.0	AG10b	3.2	16.5	1.9
	*ssc*2	RS6	25.8	23.1	EF159a	2.5	13.1	-1.5

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

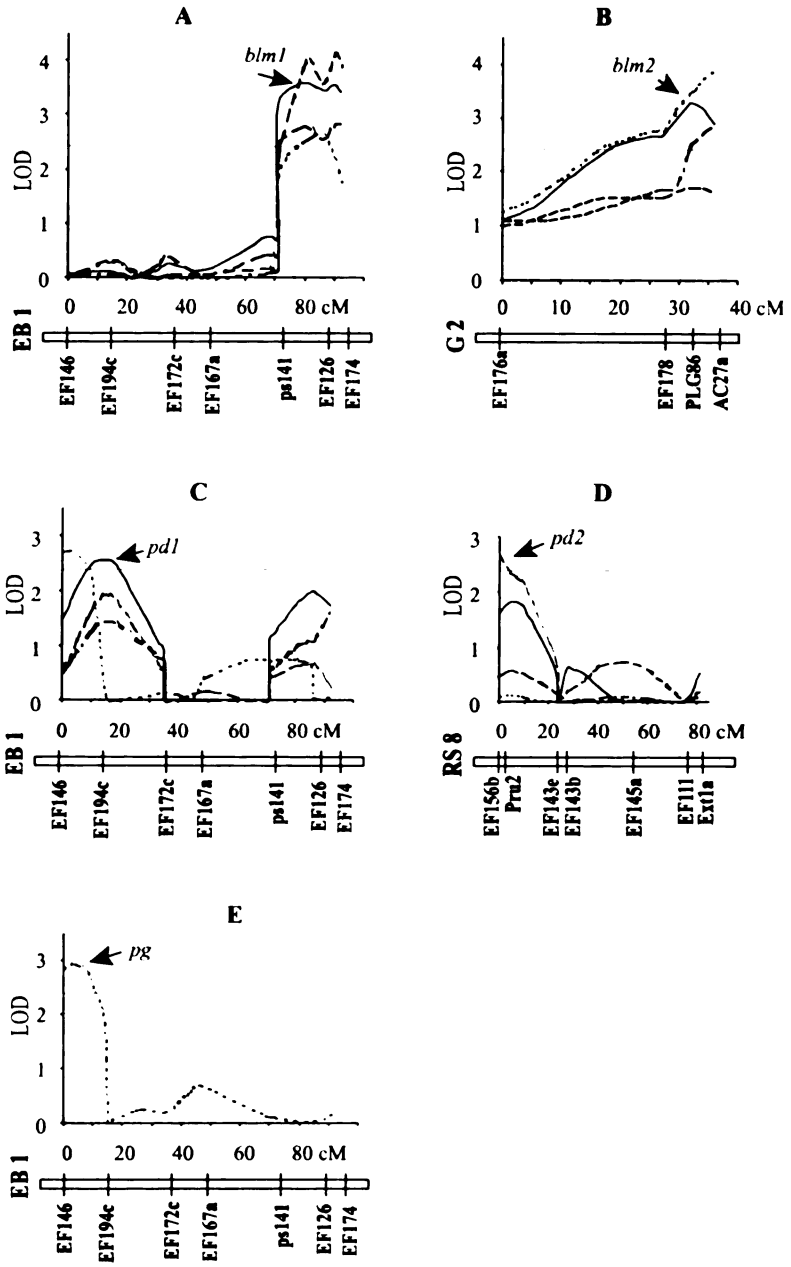

Fig. 2. LOD scores for bloom date on linkage groups EB 1 (*blm*1) (**A**); and Group 2 (*blm*2) (**B**); pistil death (*pd*) on linkage groups EB 1 (**C**); RS 6 (**D**) and pollen germination % (*pg*) on linkage group EB1 (**E**). Peak LOD scores for each trait are indicated by arrows. Linkage groups are shown below the x-axes. The horizontal line indicates the level of significance at LOD = 2.4. Curves represent results from individual years of 1995 (---), 1996 (·····), 1997 (— · —), and over years (———).

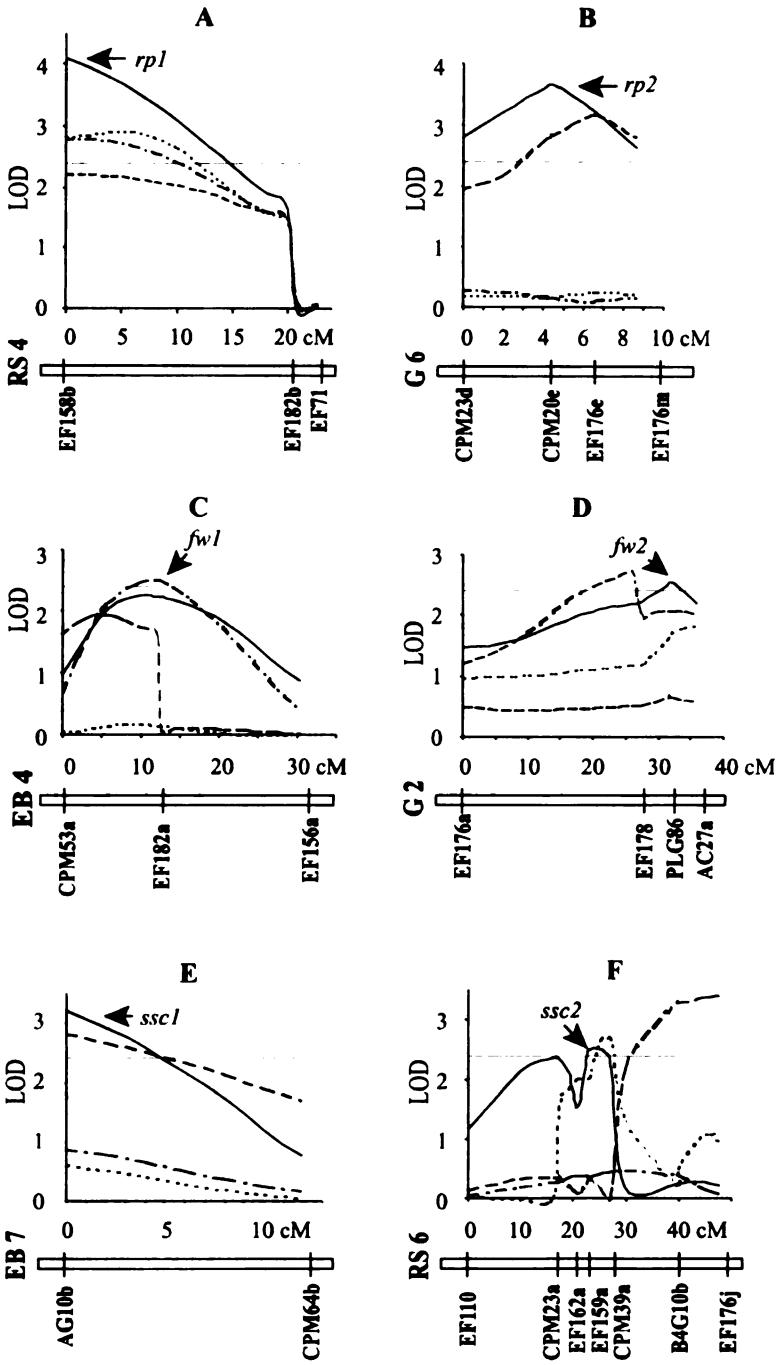

Figure. 3. LOD scores for ripening date on linkage groups RS4 (*rp*1) (**A**) and Group 6 (*rp*2) (**B**); soluble solids concentration on linkage groups EB7 (*ssc*1) (**E**) and RS6 (*ssc*2) (**F**), and fruit weight on linkage groups EB4 (*fw*1) (**C**) and Groups 2 (*fw*2) (**D**). Peak LOD scores for each trait are indicated by arrows. Linkage groups are shown below the x-axes. The horizontal line indicates the level of significance at LOD = 2.4. Curves represent results from individual years of 1995, 1996, 1997, and over years (See the legend for Fig. 2 for details).

the three years analyzed and were identified in different years, 1995 and 1996, respectively.

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

One QTL, *pg*, was found for percent pollen germination on linkage group EB1 (Fig. 2E). This QTL explained 17.0 % of the phenotypic variance. It had an effect opposite to that predicted by the phenotype of the parent, with an EB allele increasing the pollen germination rate by 1.4 %.

Two QTLs were identified for ripening time on two different linkage groups, RS4 (*rp*1) and Group 6 (*rp*2)(Fig. 3, A and B). The QTL *rp*1 was detected in two of the three years analyzed and was responsible for 21.5% of the phenotypic variance. This QTL had the effect predicted by the parental phenotype, with an allele from the late ripening parent, RS, increasing ripening time by 197.5 degree days. The QTL *rp*2 was detected in one of three years and was responsible for 25.9% of the phenotypic variance.

Two QTLs were identified for fruit weight on two different linkage groups, EB4

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

Two QTLs were identified for soluble solids concentration on two different linkage groups, EB7 (*ssc*) and RS6 (*ssc*2) (Fig. 3, E and F). The QTL *ssc*1 was detected with the threshold LOD score of 2.4 using 1995 data and the average data of 1995, 1996, and 1997. The QTL *ssc*2 was detected with the critical LOD score of 2.4 using the data of 1995, 1996, and the average data of 1995, 1996, and 1997. The QTLs *ssc*1 and *ssc*2 explained 16.5 % and 13.1 % of the phenotypic variance, respectively. The two QTLs were from different parents and had opposite effects. An EB allele of *ssc*1 increased percent soluble solids by 1.9 ° Brix while a RS allele of *ssc*2 decreased percent soluble solids by 1.5 ° Brix.

Previous QTL studies on other plant species have identified regions of the genome that seem to contain clusters of QTLs (Edwards et al. 1987, Fulton et al. 1997). In tomato for example, a 25-cM region of linkage group 1 contained QTLs for many fruit quality traits (Fulton et al. 1997). In our study, QTLs affecting three flower traits, bloom time, pollen germination percent, and pistil death in 1996, mapped to linkage group EB1 (Fig. 2; Table 2). Two QTLs, *pg* and *pd*1, mapped at the lower end of the linkage group. The positions of the peak LOD scores for QTLs *pg* and *pd*1 were 10.8 cM apart; however, the intervals for the two QTLs overlapped. The third QTL, *blm*1, mapped to the other end of

the linkage group closest to the RFLP marker pS141. Since pS141 is a clone derived from sweet cherry stylar cDNA (Iezzoni and Brettin 1998), partial sequence was obtained to determine if this RFLP identified a putative gene. Following a BLAST search (Altschul et al. 1990) using 185 nucleotides, the closest nucleotide and amino acid similarity to pS141 was a non-S-allele RNase identified from pear stylar cDNA (Norioka et al. 1996). The pear RNase and pS141 have 81% amino acid homology, suggesting that pS141 also identifies a non-S-allele stylar RNase (Fig. 4). With the putative identification of pS141 as identifying a stylar RNase, 4 genes affecting floral traits mapped to EB1.

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

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

In this study, 50% of the QTLs identified for the traits in which the two parents differed significantly had allelic effects opposite to those expected from the parental phenotype. Such a high percentage of QTLs with allelic effects opposite to those predicted from the parent may explain the common transgressive segregation observed for all traits analyzed. Each parent was likely to possess both favorable and unfavorable alleles of different QTLs affecting the same trait. Recombinations of favorable alleles as well as unfavorable alleles from both parents would most likely generate transgressive

phenotypes. QTLs with effects opposite to those expected from parental phenotypes have been reported to be responsible for transgressive segregation in an interspecific tomato cross, where 36% of the QTLs had effects opposite to those predicted by the parental phenotypes and these QTLs were directly related to the appearance of transgressive individuals in the F_2 (de Vicente et al. 1993).

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

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

Additionally, identification of major QTL alleles is theoretically more difficult in a polyploid mapping population because in order to detect a QTL allele it would have to meet the same segregation requirement as a molecular marker, i.e. segregate as a single dose restriction fragment (Wang et al. 1998). The simplest case meeting this requirement could be diagramed as $Q_1Q_2Q_2Q_2$ x $Q_2Q_2Q_2Q_2$. Given this requirement

which favors the detection of a unique QTL allele (i.e. Q_1), it is not unexpected that half of the QTL alleles identified in sour cherry contrasted to the parental phenotype. This requirement also makes it theoretically more difficult to identify the QTL allele contributing to the parental phenotype if this allele is present in at least 2 copies (i.e. Q_2). There is some speculation in allotetraploid cotton, that this may be the case. In cotton, major QTL alleles donated from the high value parent were not detected presumable because they are present in more than one dose (Jiang et al. 1998). It is important to note however, that a QTL locus can still be identified by mapping the allele that is present in a single dose.

Improved map coverage should increase our ability to identify QTLs and estimate their location. The RS and EB linkage maps used in the QTL analysis represent only approximately one third of the estimated total sour cherry linkage map distance (Wang et al. 1998). In addition, the marker density in certain regions of the linkage maps was relatively low. For traits that exhibited little variation among years such as bloom and ripening time, additional QTLs might have been identified if a more complete linkage map were available. Additionally, the One-LOD support interval lengths could not be determined for five of the QTLs (*rp*1, *blm*1, *ssc1*, *pd*2, and *pg*), because these QTLs mapped to the ends of the linkage groups (Table 1, Figs. 2 and 3). Despite the limitations discussed above, the results confirm that significant QTLs can be identified for important flower and fruit traits in sour cherry.

It has been demonstrated in other plants that QTLs can be conserved among species and even across genera (Paterson et al. 1995). If QTLs were conserved within *Prunus* and then between *Prunus* and *Malus*, it might be possible to predict regions in

other species that might be homologous to QTL regions in sour cherry. The sour cherry linkage Groups 2, 4 and 7 which contain QTLs for boom date, ripening date, fruit weight, and soluble solids, are suspected to be homologous to the peach and almond linkage Groups 2, 4 and 7 based on shared RFLP markers (Wang et al. 1998). Ongoing QTL analyses in peach for fruit size and soluble solids (A. Abbott, per comm.) should provide data for QTL comparison between sour cherry and peach.

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

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CHAPTER 3

CYTOGENETIC ANALYSIS OF SOUR CHERRY USING GENOMIC *IN SITU* HYBRIDIZATION

ABSTRACT

Genomic *in situ* hybridization (GISH) was used to examine meiotic pairing behavior and parental genomic contributions in the allotetraploid sour cherry (*P. cerasus*). Three sour cherry cultivars were studied: Montmorency, Rheinische Schattenmorelle (RS), and Erdi Botermo (EB). GISH analysis suggested that EB might have a higher genomic contribution from *P. avium* than *P. fruticosa*. However, GISH analysis only identified a relatively few number of species specific chromosomes and chromosome segments in RS suggesting that significant intergenomic recombination has occurred. In the meiotic analyses, in addition to the normal bivalent pairing configuration, univalents, trivalents, and quadrivalents were frequently observed in the pollen mother cells of the three cultivars. RS had the most bivalents and the lowest number of quadrivalents. Montmorency and EB had approximately the same numbers of bivalents and quadrivalents. RS had a bivalents to non-bivalents ratio of 4.4:1 while EB and Montmorency had a bivalents to non-bivalents ratio of 3.5:1. The ratio of bivalents to non-bivalents may be an important factor in determining the proportion of balanced and unbalanced meiotic products.

INTRODUCTION

The MSU sour cherry (*Prunus cerasus* L.) germplasm collection is one of the largest in the world with material collected thoughout the species range. Individuals in this germplasm collection possess many important fruit quality and disease resistance traits that are important for future gains in cultivar breeding. However, use of superior individuals in the breeding program is severely limited because approximately 95% of the individuals in the germplasm collection are highly infertile with fruit set frequently between 0.1 and 10%; approximately 30% fruit set is needed to produce a commercial crop.

Even commercial cultivars and progeny resulting from commercial cultivars exhibit a high level of sterility. In the sour cherry cultivar Montmorency, 25 % to 40 % of the embryo sacs were non-functional (Furukawa and Bukovac 1989). In the progeny from the cross Rheinische Schattenmorelle x Erdi Botermo, pollen germination rate ranged from 34 % to 0 % (Table 1 in Chapter 2).

Sour cherry is a polyploid (2n=4x=32) presumed to be derived from sexual polyploidization between sweet cherry (*P. avium* L.; 2n=2x=16) and ground cherry (P. *fruticosa* Pall.; 2n=4x=32) (Olden and Nybom 1968). Low fertility is hypothesized to result from lack of complete bivalent pairing between homologues. Evidence for this is derived from several sources. Isozyme segregation consistent with occasional intergenomic pairing has been observed (Beaver and Iezzoni 1993). Segregation of seven restriction fragment length polymorphism (RFLP) markers in sour cherry revealed intergenomic pairing and recombination (Wang *et al.* 1998). Pollen mother cell meiosis

in sour cherry resulted in the frequent formation of aneuploid gametes (Murawski and Endlick 1962; Kotoman and Krylova 1977). Megasporogenesis exhibited abnormalities similar to microsporogenesis which resulted in the degeneration of the embryo sac (Leach and Tylus 1983; Murawski and Endlick 1962; Potemkina 1973; Manesu et al. 1980). Sour cherry, like other *Prunus*, has just two ovules per flower, one of which degenerates. Therefore, the degeneration of the other ovule due to aneuploidy will translate into reduced fruit set.

Lack of complete bivalent pairing between homologues in sour cherry could be explained by two theories: (1) homology between chromosomes of the progenitor species resulting in pairing of homoeologous chromosomes, and/or (2) unbalanced genomes due to introgression with progenitor species resulting in meiotic pairing irregularity. Since the generation of a viable allopolyploid requires some level of phylogenetic relatedness between the parental species, it is expected that some homology will exist between the two parental genomes. The resulting irregularities in pairing or segregation in the allopolyploids can lead to unbalance gametes and infertility (Heiser 1973). In this first case, one would suspect that there would be selection for a mechanism which would restrict bivalent pairing to homologues. In support of the second theory, sour cherry is not reproductively isolated from sweet and ground cherry, its presumed progenitor species (Olden and Nybom 1968), and crossing with its progenitor parent is prevalent (Hruby 1962). Unbalanced genomes can result from repeated introgression by one of the progenitor species and retard any move towards diploidization (Stebbins 1947).

Pairing between homoeologous chromosomes and genome balance can be investigated using the genomic *in situ* hybridization (GISH) technique (Schwarzacher et

al. 1994). GISH has been successfully used to distinguish chromosomes from different progenitor species in allopolyploid species (reviewed by Jiang and Gill 1994). To distinguish between two species, genomic DNA from one species was labeled and used as the probe, while unlabeled DNA from the other species was applied at a much higher concentration as a block (Anamthawat-Jónsson et al. 1990).

The objective of this research was to study the meiotic pairing behavior and determine the parental genome contributions for three sour cherry cultivars.

MATERIAL AND METHODS

Plant material

Three sour cherry cultivars, Montmorency, Rheinische Schattenmorelle (RS), and Erdi Botermo (EB), were chosen for the meiotic analysis. Montmorency is the only commercially grown cultivar in the United States. RS and EB were the two mapping parents for which genetic linkage maps have been constructed (Chapter 1). The three cultivars originated from different geographic regions. Montmorency originated from France and RS and EB originated from Germany and Hungary, respectively.

The two progenitor species of sour cherry, *P. avium* (sweet cherry) and *P. fruticosa* (ground cherry), were used in the GISH analysis of the meiotic chromosomes of the three sour cherry cultivars. The sweet cherry used in the GISH analysis was cultivar Emperor Francis. Two genotypes of ground cherry, PF-HortFarm and PF26e1(36), were used. Since ground cherry is distinct from sweet cherry and sour cherry in tree size, PF-HortFarm and PF26e1(36) were chosen based on their tree sizes. PF-HortFarm has the

largest tree size (about 2 m tall) while PF26e1(36) has the smallest tree size (about 0.5 m tall) in the *P. fruticosa* collection at Michigan State University.

Chromosome preparation

Meiotic chromosomes of pollen mother cells (PMC) were prepared from anthers of Montmorency, RS, and EB. Branches bearing flower buds were collected in February, 1998 when the plants were still in dormancy and had received enough chilling. The branches were kept at room temperature and the progress of meiosis was monitored. Flower bud samples were collected twice a day (in the morning and in the afternoon) until pollen grains were observed. Flower buds were fixed in ice-cold 3:1 methanol : acetic acid immediately after removal from branches and stored in the fixative at 4 ° C until use. The fixed anthers at first metaphase of meiosis were soaked in 45% acetic acid for three hours before chromosome preparation. Slides were prepared by placing the fixed anthers in a drop of 45% acetic acid on a pre-cleaned slide; the pollen mother cells were squeezed from the anthers and the anthers discarded. A cover-glass was added and the preparation was gently squashed beneath filter paper. The slides were observed using phase-contrast microscopy and the selected slides were frozen and stored at - 80 ° C until use.

Probe preparation and *in situ* hybridization

Total genomic DNAs of *P. fruticosa* PF-HortFarm and PF26e1(36) and *P. avium* cv. Emperor Francis were extracted as described by Stockinger et al. (1996) with the following modifications: 400 mg of lyophilized leaves were placed in a 50-ml centrifuge

tube together with five 4 mm glass beads (Fischer Scientific, Pittsburgh, PA) and shaken vigorously for 4 minutes with a paint shaker to grind the sample to a fine powder prior to the addition of extraction buffer. Subsequent procedures were the same as those described by Stockinger et al. (1996).

Total genomic DNAs of *P. fruticosa* and *P. avium* were separately labeled with either digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN) or biotin-14-dATP (GibcoBRL, Gaithersburg, MD) by nick translation for use as the *in situ* probes. Unlabeled total genomic DNA from *P. fruticosa* or *P. avium* was fragmented to pieces of 100 - 600 bp long by autoclaving for 5 min and then used as blocking DNA. When labeled DNA from *P. fruticosa* was used as the *in situ* probe, the unlabeled DNA from *P. avium* was used as blocking DNA . Conversely, when labeled DNA from *P. avium* was used as the probe, the unlabeled DNA from PF26e1(36), the small-sized genotype of *P. fruticosa* was used as blocking DNA .

The protocols followed for pretreatment of slide preparations, *in situ* hybridization, and detection of digoxigenin or biotin labeled probe were essentially those described by Schwarzacher et al. (1994). Chromosome preparations were treated with RNase A, pepsin and paraformaldehyde (Sigma, St. Louis, MO) as described in the protocols. The slide preparations were denatured in preheated solution of 70 % formamide, 2x SSC at 75 °C for 3 minutes and dehydrated in a cold (-20 °C) ethanol series (70 %, 95 %, and 100 %, 2 minutes each). Twenty µl of hybridization mixture containing 100 ng of labeled probe DNA, 1.5 to 10 µg of unlabeled blocking DNA, and 10 µg of sheared fish sperm DNA in 50 % formamide, 10 % dextran sulfate was applied to each slide. The hybridization mixture was denatured at 75 °C for 5 minutes, chilled on

ice for 5 minutes, and then pre-annealed at 37 °C for 5 minutes before application to the slide. The hybridization was allowed to occur at 37 °C for 16 hours. After hybridization, slides were washed in three changes of 50 % formamide, 2x SSC at 45 °C for 5 minutes each. Both the digoxigenin and biotin hybridization sites were visualized with the appropriate fluorescence conjugates. All preparations were counterstained with propidium iodide and/or DAPI (Sigma, St. Louis, MO). The preparations were mounted in Vectashield antifade (Vector Laboratories, Burlingame, CA), examined using a Olympus BX60F fluorescence microscope, and photographed using Kodak Ektachrome 400HC slide film.

RESULTS

Genomic *in situ* hybridization

All Montmorency chromosomes were labeled when the preparations were hybridized with labeled total DNA from *P. fruticosa* PF-HortFarm and blocked with unlabeled total DNA from *P. avium* at a blocking ratio of 50:1 (block:label) or less (Fig. 1). When the blocking ratio was above 50:1, the hybridization signal was either not present or very weak.

The EB chromosomes showed differential signals of hybridization when labeled total DNA from either *P. avium* or *P. fruticosa* PF26e1(36) was used as the probe and unlabeled total DNA from the other progenitor species was used as the blocking DNA with a blocking ratio of 50:1 (Fig. 2). When *P. avium* was used as the probe DNA, about 11 pairs of EB chromosomes showed a strong hybridization signal while the other 5 pairs

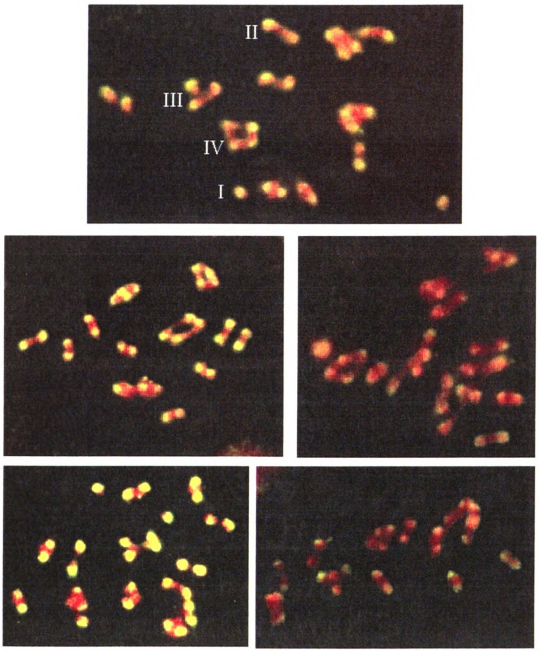

Figure 1. Fluorescent micrographs of metaphase PMC chromosomes from the sour cherry cultivar Montmorency after genomic in situ hybridization using total genomic DNA from *P. fruticosa* PF-HortFarm as the probe labeled with biotin and detected with fluorescein. The hybridization signal appears as yellow-green fluorescence while the unhybridized regions appear as orange-red with the counterstain propidium iodide. Examples of different meiotic pairing configurations are identified by roman numerals in the top micrograph as: I - univalent, II - bivalent, III - trivalent, and IV - quadrivalent.

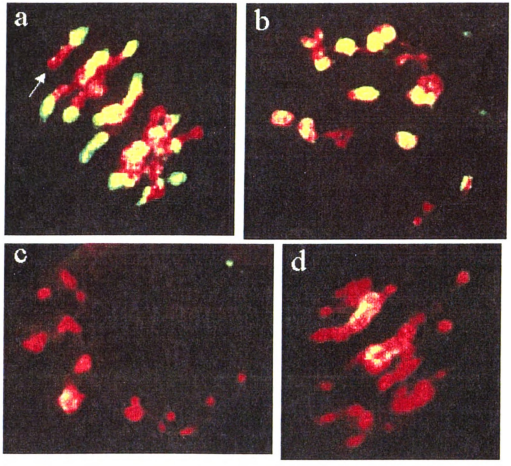

Figure 2 a-d. Fluorescent micrographs of metaphase PMC chromosomes from the sour cherry cultivar Erdi Botermo after genomic in situ hybridization using total genomic DNA from either *P. avium* (**a**, **b**), or *P. fruticosa* PF26e136 (**c**, **d**) as the probe. The probes were labeled with digoxigenin and detected with fluorescein. The hybridization signal appears as yellow-green fluorescence while the unhybridized regions appear as orange-red with the counterstain propidium iodide. The arrow in micrograph **a** points to a bivalent formed by homoeologous chromosomes.

showed a very weak or no hybridization signal (Fig. 2 a and b). In contrast, when *P. fruticosa* was used as the probe DNA, more than two thirds of the EB chromosomes showed no or very weak hybridization signal while a few chromosomes showed strong hybridization signals (Fig. 2 c and d).

Most RS chromosomes displayed hybridization when labeled and probed in both directions; i.e. total DNA from either *P. avium* or *P. fruticosa* PF26e1(36) was used as the probe and unlabeled total DNA from the other progenitor species was used as the blocking DNA with a blocking ratio of 50:1 (Fig. 3). However, the strength of the signal varied among chromosomes (Fig. 3).

When EB and RS chromosomes were hybridized with labeled total DNA from *P. fruticosa* PF-HortFarm and blocked with unlabeled total DNA from *P. avium* at a blocking ratio of 50:1 (block:label) or less, all chromosomes showed a signal of hybridization.

Pairing configuration at meiosis

Univalents, bivalents, trivalents, and quadrivalents were observed in PMCs at metaphase-I of the three sour cherry cultivars (Figs. 1 and 4a). Table 1 shows the average number of pairing configurations per PMC at metaphase I. RS had the most bivalents (12.9) and univalents (1.7) and the least number of quadrivalents (0.9). During anaphase I to telephase I, the univalents may not be included in the telophase nuclei (Fig. 4b). Montmorency and EB had approximately the same numbers of bivalents and quadrivalents. However, the quadrivalent configurations of Montmorency and EB differed. Most quadrivalents observed in the Montmorency PMCs were in ring or other

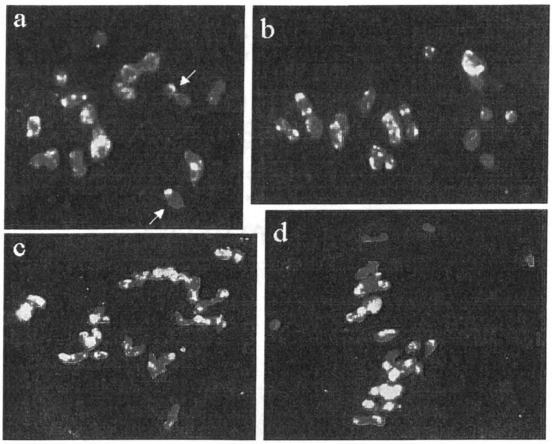

Figure 3 a-d. Fluorescent micrographs of metaphase PMC chromosomes from the sour cherry cultivar Rheinische Schattenmorelle after genomic in situ hybridization using total genomic DNA from either *P. fruticosa* PF26e136 (**a**, **b**), or *P. avium* (**c**, **d**), as the probe. The probes were labeled with digoxigenin and detected with fluorescein. The hybridization signal appears as yellow-green fluorescence while the unhybridized regions appear as orange-red with the counterstain propidium iodide. The arrows in micrograph **a** point to bivalents formed by homoeologous chromosomes.

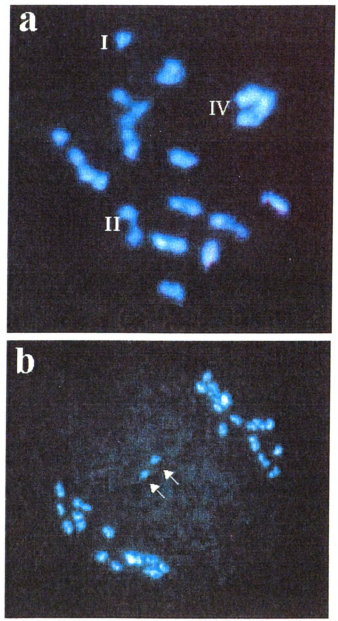

Figure 4 a, b Fluorescent micrographs of PMC chromosomes from sour cherry cultivar Rheinische Schattenmorelle. The chromosomes were stained with the DNA-specific dye DAPI and were shown by blue fluorescence. **a** Metaphase-I chromosomes showing examples of different meiotic pairing configurations identified by roman numerals as: I - univalent, II - bivalent, and IV - quadrivalent. **b** Telophase-I chromosomes showing two univalents (arrows) remain stationary at the equatorial plate when other chromosomes have reached the poles.

parallel configurations (Fig. 1) as diagrammed by Kuspira et al. (1985), whereas most quadrivalents observed in EB PMCs were in a linear chain configuration (Fig. 5). The quadrivalents observed in RS PMCs were in ring or open-ring configurations (Fig. 4a).

Table 1 Mean number of chromosome pairing configurations per PMC at metaphase I.

Cultivar	No. of PMCs analyzed	Uni-valent	Bi-valent	Tri-valent	Quadri-valent	Bivalent: nonbivalent
RS	36	1.7	12.9	0.3	0.9	4.4 : 1
EB	20	0.6	10.9	0.0	2.5	3.5 : 1
Montmorency	13	0.9	11.2	0.2	2.1	3.5 : 1

Non-bivalent configurations were found in most of the PMCs analyzed. Non-bivalent configurations were observed in 92.3 %, 80.6 % and 100 % of the PMCs of Montmorency, RS, and EB, respectively.

Bivalents formed by homoeologous chromosomes were observed with the GISH labeling. In some bivalent configurations, only one of the two chromosomes was labeled by the *P. avium* probe DNA (Fig. 2a) or by the *P. fruticosa* probe DNA (Fig. 3a).

DISCUSSION

One of our objectives was to use GISH analysis to discriminate the ancestral parental chromosomes and/or chromosome segments in three sour cherry cultivars. For GISH analysis to be effective, the blocking DNA from one progenitor species must presumably hybridize to sequences in common between the blocking DNA and the labelled probe. Then mainly species specific sequences would remain as sites for probe

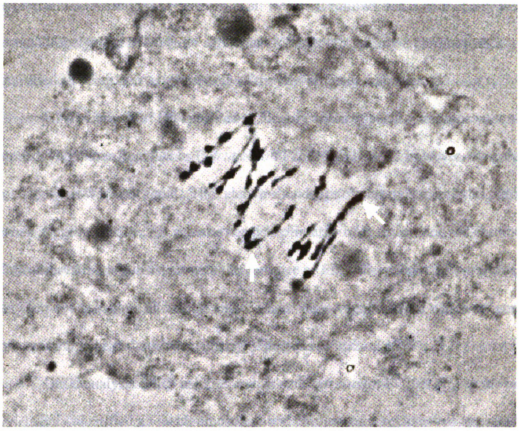

Figure 5 Phase-contrast micrograph of metaphase PMC chromosomes from sour cherry cultivar Erdi Botermo showing linear chain quadrivalents (arrows).

hybridization (Anamthawat-Jonsson et al. 1990). It follows that if sour cherry were a recent allopolyploid between *P. avium* and *P. fruticosa*, ideally 8 chromosomes would be identified as being derived from each of the two progenitor species.

GISH analysis of EB appeared to identify species specific chromosomes when probed with either *P. avium* or *P. fruticosa* PF26e1(36) DNAs; however, the parental contributions were not equal. The relative abundance of chromosomes hybridizing to *P. avium* suggests that the EB genome consists primarily of *P. avium* derived chromosomes. This observation is consistent with the pedigree of EB. EB is derived from a cross between two tetraploid cherries, Pandy 38 and Nagy Angol, both of which are considered to be natural hybrids with sweet cherry (Apostol and Iezzoni 1992).

GISH analysis of RS using *P. avium* and *P. fruticosa* PF26e1(36) as the probe DNAs only identified a few chromosomes and chromosome regions that appeared to be derived solely from *P. avium* or *P fruticosa*. Both *P. avium* and *P. fruticosa* PF26e1(36) DNAs hybridized to most of the chromosomes. Since the GISH technique was able to discriminate species specific chromosomes in EB, we felt that the GISH protocol was reliable. RS is an old landrace sour cherry variety from Germany and it is possible that continual recombination between the chromosomes from the two ancestral genomes has resulted in our inability to identify more species specific chromosomes. Our identification in RS of bivalents between presumably homoeologous chromosomes supports this theory (Fig. 3a).

When Montmorency chromosomes were probed with *P. fruticosa* PF-HortFarm and blocked with *P. avium* DNA, all the chromosomes exhibited hybridization signal. However, the GISH results with Montmorency are difficult to interpret since the converse

experiment was not done, i.e. *P. avium* was not used as the probe. Additionally, only *P. fruticosa* PF-HortFarm, and not *P. fruticosa* PF26e1(36) was used as the probe. For EB and RS, only PF26e1(36) was able to distinguish the two progenitor genomes of sour cherry. The failure of PF-HortFarm to distinguish the two progenitor genomes could be due to possible introgression of *P. avium* into its genome. *P. fruticosa* and *P. avium* are not reproductively isolated and the two species coexist in the wild (Olden and Nybom 1968). Trees of *P. avium* are typically tall and trees of *P. fruticosa* are typically very short. PF-HortFarm has the largest tree size in our *P. fruticosa* collection, indicating possible introgression by *P. avium*.

The meiotic analyses support our hypothesis that sour cherry is not completely diploidized with the expected16 bivalents at meiosis. Instead the three cultivars analyzed all exhibited meiotic irregularities. RS had the highest number of bivalents and the least number of quadrivalents at metaphase I, suggesting that RS may be the most diploidized among the three cultivars. GISH analyses revealed that most RS chromosomes hybridized to DNA probes from both progenitor species, indicating that the two genomes in RS have undergone significant intergenomic exchange. However, the presence of quadrivalents and trivalents at metaphase I indicates that the process of diploidization in RS is not completed. In contrast to RS, EB had the least number of bivalents and the most number of quadrivalents at metaphase I, suggesting that EB was the least diploidized among the three cultivars. GISH analyses revealed that EB had unbalanced genomes of the two progenitor species. Over two thirds of the EB chromosomes hybridized to the *P. avium* DNA probe and only less than one third of chromosomes hybridized to the *P. fruticosa* DNA probe.

The homology between the two progenitor species of sour cherry was significant enough to cause pairing between homoeologous chromosomes. Trivalents and quadrivalents were frequently observed in this study. Bivalents formed by homoeologous chromosomes were also found in this study.

The commonly observed non-bivalent pairing configurations in this study may explain the high level of sterility in sour cherry. Pollen mother cells with univalents, trivalents, and quadrivalents are the source of aneuploid gametes (Kuspira et al. 1985). During the disjunction of chromosomes at anaphase I to telophase I, univalents may remain more or less stationary at the equatorial plate (Fig. 4b) and fail to be included in either telophase nuclei (Singh 1993). The disjunction of chromosomes in a trivalent generally result in a 2:1 split of the three chromosomes to the opposite poles (Singh 1993). The disjunction of chromosomes in a quadrivalent may result in unequal split of the four chromosomes to the telophase nuclei (Kuspira et al. 1985). Most PMCs of the three sour cultivars analyzed in this study contained at least one of the three non-bivalent configurations. This is consistent with the reports that meiosis in PMCs of sour cherry resulted in the frequent formation of aneuploid gametes (Murawski and Endlick 1962; Kotoman and Krylova. 1977). Aneuploid gametes could be responsible for the low average pollen germination rate of 5.6 % for the 86 progeny from the cross RS x EB (Table 1 in Chapter 2).

The ratio of bivalents to non-bivalents may be an important factor in determining the proportion of balanced and unbalanced meiotic products and ultimately in determining the proportion of fertile and sterile gametes. RS had a higher ratio of bivalents to non-bivalents (4.4 : 1) than that of EB (3.5 : 1) (Table 1). As expected, RS

had a higher pollen germination rate (18.5 %) than that of EB (8.0 %) (Table 1 in Chapter 2).

In conclusion, GISH analysis failed to identify balanced parental genomic contributions in the sour cherry cultivars. Instead, the GISH evidence suggests that higher fertility levels may be associated with ancestral chromosomes that have undergone significant intergenomic recombination. Additionally, the relatively high number of PMCs exhibiting meiotic irregularities suggest that these meiotic disturbances may be contributing to low fertility in sour cherry.

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APPENDIX

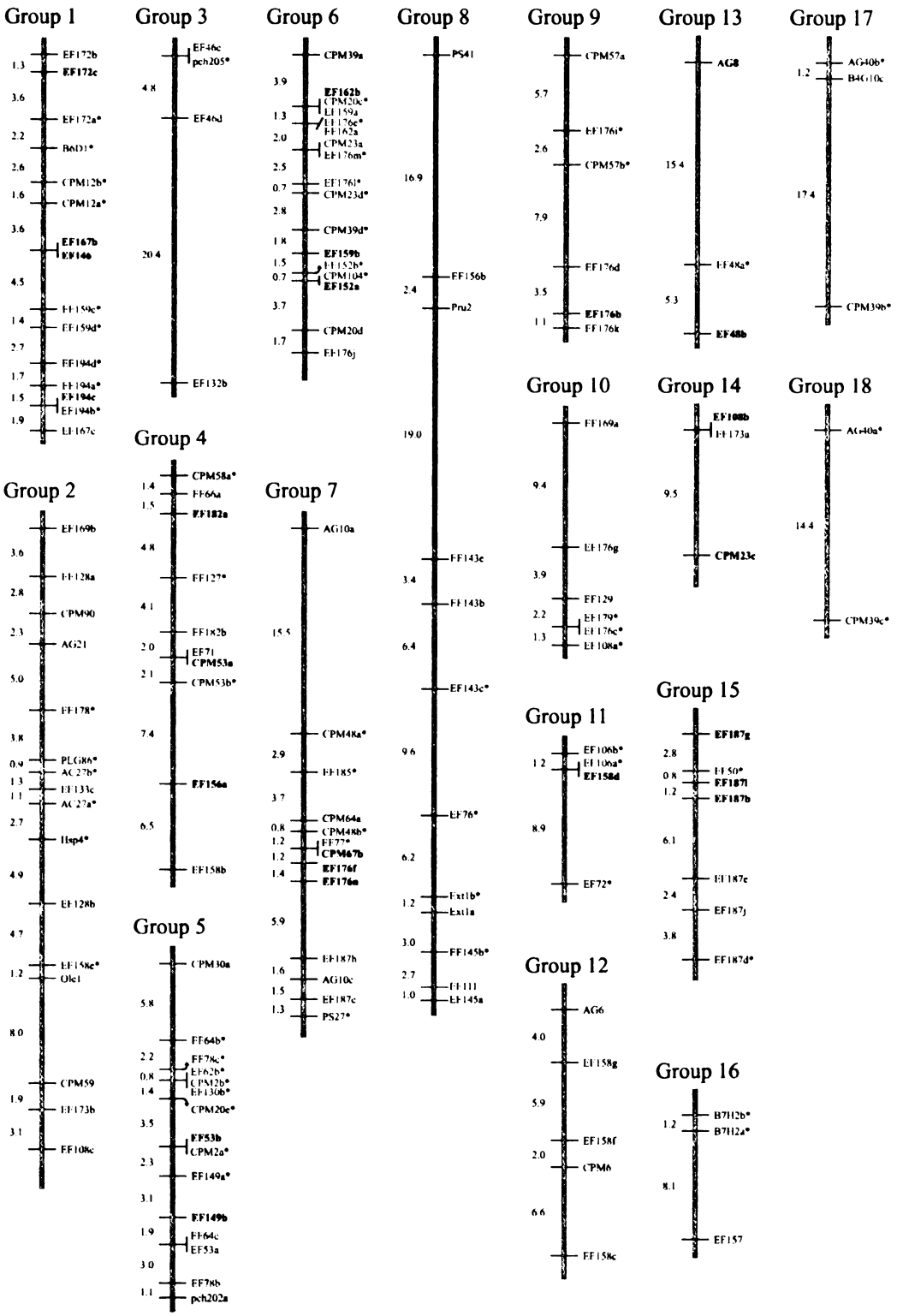

Figure 1. The consensus map of RS and EB maps (Chapter 1) constructed from the combined data using JoinMap with a minimum LOD of 3.0 and a maximum recombination frequency of 0.35. Markers in bold were present in EB only. Markers indicated by asterisks were present in both RS and EB. All other markers were present in RS only.

Table 1. Information about all single dose restriction fragment (SDRF) markers that fit the expected ratios

Marker name	Probe	Restriction Enzyme	Parent genotype* RS	EB	Expected ratio	Observed ratio	X^2
Pru2	AC12	*Xba* I	H	A	1 : 1	35 : 43	0.83
AC27a	AC27	Eco RV	H	H	3 : 1	57 : 26	1.62
AC27b	AC27	Eco RV	H	H	3 : 1	64 : 19	0.27
AG10a	AG10	Eco RI	H	A	1 : 1	41 : 42	0.02
AG10b	AG10	Eco RI	A	H	1 : 1	42 : 41	0.02
AG10c	AG10	Eco RI	H	H	3 : 1	63 : 21	0.02
AG21	AG21	Eco RV	H	A	1 : 1	46 : 39	0.59
AG40a	AG40	Hind III	H	H	3 : 1	69 : 16	1.91
AG40b	AG40	Hind III	H	H	3 : 1	64 : 21	0.03
AG6	AG6	Eco RI	H	A	1 : 1	36 : 48	1.73
AG8	AG8	Hind III	A	H	1 : 1	41 : 43	0.06
B4G10a	B4G10	Xba I	H	H	3 : 1	47 : 16	0.02
B4G10b	B4G10	Xba I	H	A	1 : 1	35 : 29	0.58
B4G10c	B4G10	Xba I	H	A	1 : 1	36 : 42	0.47
B6D1	B6D1	Hind III	H	H	3 : 1	51 : 18	0.03
B7H2a	B7H2	Eco RV	H	H	3 : 1	59 : 24	0.59
B7H2b	B7H2	Eco RV	H	H	3 : 1	66 : 16	1.48
B8A3	B8A3	Xba I	H	A	1 : 1	34 : 40	0.50
CPM104	PC104	Eco RI	H	H	3 : 1	59 : 25	0.90
CPM12a	PC12	Eco RI	H	H	3 : 1	56 : 27	2.33
CPM12b	PC12	Eco RI	H	H	3 : 1	61 : 22	0.08
CPM20a	PC20	Hind III	A	H	1 : 1	43 : 29	2.74
CPM20b	PC20	Hind III	H	H	3 : 1	61 : 21	0.02
CPM20c	PC20	Hind III	H	H	3 : 1	56 : 25	1.35
CPM20d	PC20	Hind III	H	A	1 : 1	50 : 33	3.49
CPM20e	PC20	Hind III	H	H	3 : 1	67 : 15	2.16
CPM23a	PC23	Eco RI	H	A	1 : 1	36 : 48	1.73
CPM23b	PC23	Eco RI	A	H	1 : 1	49 : 35	2.35
CPM23c	PC23	Hind III	A	H	1 : 1	49 : 35	2.35
CPM23d	PC23	Hind III	H	H	3 : 1	57 : 27	2.11
CPM2a	PC2	Eco RI	H	H	3 : 1	59 : 24	0.59
CPM2b	PC2	Eco RI	H	H	3 : 1	62 : 18	0.35
CPM30a	PC30	Hind III	H	A	1 : 1	47 : 38	0.96
CPM30b	PC30	Hind III	A	H	1 : 1	39 : 46	0.59
CPM39a	PC39	Eco RV	H	A	1 : 1	38 : 46	0.77
CPM39b	PC39	Eco RV	H	H	3 : 1	61 : 23	0.21
CPM39c	PC39	Eco RV	H	H	3 : 1	66 : 18	0.68
CPM39d	PC39	Eco RV	H	H	3 : 1	67 : 17	1.16
CPM43	PC43	Eco RI	H	A	1 : 1	44 : 39	0.31
CPM45a	PC45	Eco RI	A	H	1 : 1	36 : 49	2.00
CPM45b	PC45	Eco RI	A	H	1 : 1	41 : 44	0.12
CPM48a	PC48	Hind III	H	H	3 : 1	63 : 22	0.03
CPM48b	PC48	Hind III	H	H	3 : 1	69 : 16	1.91
CPM53b	PC53	Eco RI	H	H	3 : 1	68 : 17	1.28
CPM57a	PC57	Xba I	H	A	1 : 1	40 : 36	0.22
CPM57b	PC57	Xba I	H	H	3 : 1	62 : 15	1.42

* H = presence of the marker; A = absence of the marker

Table 1. (cont'd)

Marker name	Probe	Restriction Enzyme	Parent genotype RS	EB	Expected ratio	Observed ratio	X^2
CPM58a	PC58	Hind III	H	H	3 : 1	65 : 17	0.93
CPM58b	PC58	Hind III	H	H	3 : 1	55 : 27	2.55
CPM59	PC59	Hind III	H	A	1 : 1	46 : 39	0.59
CPM64a	PC64	Eco RI	H	A	1 : 1	48 : 37	1.44
CPM64b	PC64	Eco RI	A	H	1 : 1	43 : 42	0.02
CPM67a	PC67	Eco RV	H	H	3 : 1	52 : 27	3.32
CPM67b	PC67	Eco RV	A	H	1 : 1	41 : 36	0.34
CPM6	PC6	Eco RV	H	A	1 : 1	36 : 46	1.23
CPM70a	PC70	Eco RI	H	H	3 : 1	66 : 18	0.68
CPM70b	PC70	Eco RI	H	H	3 : 1	63 : 22	0.03
CPM90	PC90	Hind III	H	A	1 : 1	43 : 42	0.02
EF106a	EF106	Dra I	H	H	3 : 1	68 : 14	2.98
EF106b	EF106	Dra I	H	H	3 : 1	62 : 20	0.05
EF108a	EF108	Xba I	H	H	3 : 1	52 : 21	0.47
EF108b	EF108	Xba I	A	H	1 : 1	42 : 31	1.67
EF108c	EF108	Xba I	H	A	1 : 1	44 : 31	2.27
EF110	EF110	Eco RV	H	A	1 : 1	36 : 43	0.63
EF111	EF111	Eco RV	H	A	1 : 1	45 : 38	0.60
EF126	EF126	Eco RV	A	H	1 : 1	38 : 45	0.60
EF127	EF127	Eco RV	H	H	3 : 1	65 : 20	0.15
EF128a	EF128	Xba I	H	A	1 : 1	40 : 33	0.68
EF128b	EF128	Xba I	H	A	1 : 1	40 : 34	0.50
EF128c	EF128	Xba I	A	H	1 : 1	38 : 37	0.03
EF129	EF129	Hind III	H	A	1 : 1	51 : 34	3.41
EF130a	EF130	Hind III	H	H	3 : 1	64 : 22	0.02
EF130b	EF130	Hind III	H	H	3 : 1	68 : 18	0.88
EF132a	EF132	Xba I	H	H	3 : 1	52 : 23	1.15
EF132b	EF132	Xba I	H	A	1 : 1	43 : 33	1.33
EF133a	EF133	Hind III	H	H	3 : 1	57 : 28	2.66
EF133b	EF133	Hind III	H	H	3 : 1	68 : 17	1.28
EF133c	EF133	Hind III	H	A	1 : 1	45 : 40	0.31
EF143a	EF143	Eco RV	H	A	1 : 1	41 : 43	0.06
EF143b	EF143	Eco RV	H	A	1 : 1	46 : 38	0.77
EF143c	EF143	Eco RV	H	H	3 : 1	69 : 15	2.49
EF143d	EF143	Xba I	H	H	3 : 1	67 : 13	3.52
EF143e	EF143	Xba I	H	A	1 : 1	41 : 39	0.06
EF145a	EF145	Eco RI	H	A	1 : 1	46 : 35	1.51
EF145b	EF145	Eco RI	H	H	3 : 1	67 : 14	2.79
EF146	EF146	Hind III	A	H	1 : 1	43 : 41	0.06
EF149a	EF149	Eco RV	H	H	3 : 1	58 : 24	0.70
EF149b	EF149	Eco RV	A	H	1 : 1	38 : 46	0.77
EF152a	EF152	Xba I	A	H	1 : 1	41 : 41	0.01
EF152b	EF152	Xba I	H	H	3 : 1	67 : 15	2.16
EF156b	EF156	Hind III	H	A	1 : 1	41 : 43	0.06
EF157	EF157	Eco RI	H	A	1 : 1	50 : 33	3.49
EF158a	EF158	Eco RI	H	A	1 : 1	48 : 34	2.40
EF158b	EF158	Eco RI	H	A	1 : 1	44 : 37	0.62
EF158c	EF158	Eco RI	H	A	1 : 1	39 : 44	0.31

Table 1. (cont'd)

Marker name	Probe	Restriction Enzyme	Parent genotype RS	EB	Expected ratio	Observed ratio	X^2
EF158d	EF158	Eco RI	A	H	1 : 1	42 : 41	0.02
EF158e	EF158	Hind III	H	H	3 : 1	55 : 22	0.45
EF158f	EF158	Hind III	H	A	1 : 1	35 : 43	0.83
EF158g	EF158	Hind III	H	A	1 : 1	36 : 42	0.47
EF159a	EF159	Eco RV	H	A	1 : 1	36 : 49	2.00
EF159b	EF159	Eco RV	A	H	1 : 1	41 : 44	0.12
EF159c	EF159	Eco RV	H	H	3 : 1	60 : 25	0.78
EF159d	EF159	Eco RV	H	H	3 : 1	64 : 21	0.03
EF162a	EF162	Dra I	H	A	1 : 1	36 : 48	1.73
EF162b	EF162	Dra I	A	H	1 : 1	37 : 47	1.20
EF167a	EF167	Xba I	A	H	1 : 1	39 : 40	0.03
EF167b	EF167	Xba I	A	H	1 : 1	33 : 46	2.15
EF167c	EF167	Xba I	H	A	1 : 1	43 : 37	0.46
EF167d	EF167	Xba I	H	A	1 : 1	40 : 40	0.01
EF169a	EF169	Eco RI	H	A	1 : 1	48 : 35	2.05
EF169b	EF169	Eco RI	H	A	1 : 1	45 : 38	0.60
EF172a	EF172	Eco RV	H	H	3 : 1	61 : 24	0.40
EF172b	EF172	Eco RV	H	A	1 : 1	41 : 44	0.12
EF172c	EF172	Eco RV	A	H	1 : 1	45 : 40	0.31
EF173a	EF173	Xba I	A	H	1 : 1	44 : 33	1.58
EF173b	EF173	Xba I	H	A	1 : 1	44 : 34	1.29
EF174	EF174	Xba I	A	H	1 : 1	35 : 42	0.65
EF176a	EF176	Eco RV	H	H	3 : 1	55 : 26	2.00
EF176b	EF176	Eco RV	A	H	1 : 1	45 : 37	0.79
EF176c	EF176	Eco RV	H	H	3 : 1	59 : 24	0.59
EF176d	EF176	Eco RV	H	A	1 : 1	35 : 46	1.51
EF176e	EF176	Eco RV	H	H	3 : 1	54 : 27	2.79
EF176f	EF176	Eco RV	A	H	1 : 1	40 : 41	0.02
EF176g	EF176	Eco RV	H	A	1 : 1	44 : 37	0.62
EF176h	EF176	Hind III	A	H	1 : 1	48 : 35	2.05
EF176i	EF176	Hind III	H	H	3 : 1	65 : 19	0.33
EF176j	EF176	Hind III	H	A	1 : 1	43 : 40	0.12
EF176k	EF176	Hind III	H	A	1 : 1	42 : 41	0.02
EF176l	EF176	Hind III	H	H	3 : 1	65 : 17	0.93
EF176m	EF176	Hind III	H	H	3 : 1	57 : 26	1.62
EF176n	EF176	Hind III	A	H	1 : 1	40 : 42	0.06
EF178	EF178	Eco RV	H	H	3 : 1	57 : 26	1.62
EF179	EF179	Eco RI	H	H	3 : 1	63 : 20	0.08
EF182b	EF182	Eco RV	H	A	1 : 1	45 : 37	0.79
EF185	EF185	Xba I	H	H	3 : 1	62 : 15	1.42
EF187a	EF187	Eco RI	H	H	3 : 1	69 : 16	1.91
EF187b	EF187	Eco RI	A	H	1 : 1	47 : 37	1.20
EF187c	EF187	Eco RV	H	A	1 : 1	43 : 36	0.63
EF187d	EF187	Eco RV	H	H	3 : 1	57 : 26	1.62
EF187e	EF187	Eco RV	H	A	1 : 1	49 : 33	3.13
EF187f	EF187	Eco RV	A	H	1 : 1	46 : 36	1.23
EF187g	EF187	Hind III	A	H	1 : 1	46 : 37	0.99
EF187h	EF187	Hind III	H	A	1 : 1	44 : 39	0.31

Table 1. (cont'd)

Marker name	Probe	Restriction Enzyme	Parent genotype RS	EB	Expected ratio	Observed ratio	X^2
EF187i	EF187	Hind III	A	H	1 : 1	39 : 44	0.31
EF187j	EF187	Hind III	H	A	1 : 1	50 : 33	3.49
EF187k	EF187	Hind III	H	H	3 : 1	67 : 16	1.62
EF187l	EF187	Hind III	A	H	1 : 1	47 : 36	1.47
EF191	EF191	Xba I	H	H	3 : 1	58 : 20	0.02
EF194D1	EF194	Dra I	H	A	1 : 1	46 : 38	0.77
EF194D2	EF194	Dra I	A	H	1 : 1	42 : 42	0.01
EF194D3	EF194	Dra I	A	H	1 : 1	41 : 43	0.06
EF194D4	EF194	Dra I	A	H	1 : 1	41 : 43	0.06
EF194D5	EF194	Dra I	H	H	3 : 1	56 : 27	2.33
EF194D6	EF194	Dra I	H	A	1 : 1	47 : 36	1.47
EF194a	EF194	Hind III	H	H	3 : 1	63 : 21	0.02
EF194b	EF194	Hind III	H	H	3 : 1	64 : 20	0.11
EF194c	EF194	Hind III	A	H	1 : 1	42 : 42	0.01
EF194d	EF194	Hind III	H	H	3 : 1	57 : 28	2.66
EF46a	EF46	Xba I	H	A	1 : 1	37 : 41	0.22
EF46b	EF46	Xba I	H	H	3 : 1	63 : 15	1.56
EF46c	EF46	Xba I	H	A	1 : 1	41 : 36	0.34
EF46d	EF46	Xba I	H	A	1 : 1	39 : 39	0.01
EF48a	EF48	Eco RV	H	H	3 : 1	62 : 20	0.05
EF48b	EF48	Eco RV	A	H	1 : 1	40 : 42	0.06
EF50	EF50	Dra I	H	H	3 : 1	70 : 14	3.35
EF53a	EF53	Xba I	H	A	1 : 1	41 : 39	0.06
EF53b	EF53	Xba I	A	H	1 : 1	36 : 44	0.81
EF62a	EF62	Xba I	H	H	3 : 1	57 : 20	0.03
EF62b	EF62	Xba I	H	H	3 : 1	62 : 16	0.97
EF64a	EF64	Hind III	A	H	1 : 1	38 : 46	0.77
EF64b	EF64	Hind III	H	H	3 : 1	65 : 20	0.15
EF64c	EF64	Hind III	H	A	1 : 1	41 : 43	0.06
EF67	EF67	Eco RV	H	A	1 : 1	36 : 49	2.00
EF71	EF71	Eco RI	H	A	1 : 1	44 : 41	0.12
EF72	EF72	Eco RV	H	H	3 : 1	63 : 20	0.08
EF76	EF76	Eco RV	H	H	3 : 1	66 : 17	1.04
EF77	EF77	Hind III	H	H	3 : 1	70 : 15	2.66
EF78a	EF78	Dra I	A	H	1 : 1	35 : 42	0.65
EF78b	EF78	Dra I	H	A	1 : 1	44 : 36	0.81
EF78c	EF78	Dra I	H	H	3 : 1	63 : 22	0.03
Ext1a	Extensine	Eco RV	H	A	1 : 1	50 : 35	2.66
Ext1b	Extensine	Eco RV	H	H	3 : 1	68 : 17	1.28
Hsp4	Hsp4	Eco RV	H	H	3 : 1	61 : 21	0.02
Oleo	Oleosine	Eco RI	H	A	1 : 1	48 : 34	2.40
pch202a	pch202	Hind III	H	A	1 : 1	46 : 38	0.77
pch202b	pch202	Hind III	A	H	1 : 1	40 : 44	0.20
pch205	pch205	Eco RI	H	H	3 : 1	57 : 26	1.62
PLG86	PLG86	Eco RI	H	H	3 : 1	57 : 26	1.62
PS141	PS141	Eco RI	A	H	1 : 1	37 : 43	0.46
PS27	PS27	Eco RV	H	H	3 : 1	62 : 22	0.05
PS41	PS41	Eco RV	H	A	1 : 1	42 : 40	0.06

Table 2. Degree days (DD) for bloom and ripening for each progeny in the mapping population for the years 1995, 1996, 1997 and the average.

Progeny	Bloom (degree days)				Ripe (degree days)			
	1995	1996	1997	average	1995	1996	1997	average
2(02)	-	516.2	-	516.2	-	-	-	-
2(03)	438.6	441.7	411.5	430.6	2149.5	1957.7	2107.8	2071.7
2(04)	343.7	410.9	369.3	374.6	1443.4	-	1486.5	1465.0
2(05)	354.0	393.1	396.3	381.1	2437	2109.8	2164.0	2236.9
2(06)	343.7	371.4	371.4	362.2	2605.6	2109.8	2311.1	2342.2
2(07)	354.0	410.9	396.3	387.1	2149.5	1900.8	1844.9	1965.1
2(08)	328.5	361.1	335.1	341.6	1789	1900.8	1880.4	1856.7
2(09)	393.6	393.1	371.4	386.0	1789	2109.8	1844.9	1914.6
2(10)	354.0	371.4	369.3	364.9	2711.9	2192.4	2395.8	2433.4
2(11)	343.7	364.9	357.4	355.3	2711.9	2351.1	2395.8	2486.3
2(12)	393.6	410.9	408.6	404.4	1628.2	2109.8	1844.9	1861.0
2(13)	366.5	393.1	369.3	376.3	-	-	2395.8	2395.8
2(14)	381.8	393.1	402.8	392.6	1789	2109.8	1844.9	1914.6
2(15)	414.2	410.9	402.8	409.3	2711.9	-	2520.4	2616.1
2(16)	354.0	393.1	369.3	372.1	1789	1900.8	1844.9	1844.9
2(17)	414.2	410.9	402.8	409.3	2711.9	2109.8	1955.8	2259.2
2(18)	393.6	410.9	371.4	392.0	1987.7	1957.7	2107.8	2017.7
2(19)	414.2	410.9	402.8	409.3	1859.4	-	-	1859.4
2(20)	414.2	410.9	396.3	407.1	2149.5	2109.8	2164.0	2141.1
2(22)	354.0	410.9	398.9	387.9	1815.6	2109.8	1844.9	1923.4
2(23)	438.6	441.7	408.6	429.6	1987.7	2109.8	2164.0	2087.2
2(24)	414.2	410.9	396.3	407.1	1987.7	2109.8	2164.0	2087.2
2(25)	438.6	441.7	466.5	448.9	-	2109.8	2164.0	2136.9
2(27)	328.5	361.1	357.4	349.0	1568.8	2109.8	1955.8	1878.1
2(28)	454.1	379	466.5	433.2	-	-	1955.8	1955.8
2(29)	313.8	361.1	335.1	336.7	1723.9	1900.8	1714.4	1779.7
2(30)	-	-	408.6	408.6	-	-	-	-
2(32)	354.0	393.1	369.3	372.1	2210.3	2192.4	2164.0	2188.9
2(33)	393.6	393.1	402.8	396.5	-	-	-	-
2(34)	414.2	410.9	398.9	408.0	1859.4	-	-	1859.4
2(35)	438.6	410.9	411.5	420.3	1628.2	2109.8	1844.9	1861.0
2(36)	438.6	441.7	411.5	430.6	-	2109.8	2107.8	2108.8
2(37)	438.6	441.7	408.6	429.6	2210.3	2109.8	2107.8	2142.6
2(38)	438.6	441.7	411.5	430.6	-	2109.8	2107.8	2108.8
2(39)	393.6	410.9	402.8	402.4	-	2109.8	2311.1	2210.5
2(40)	438.6	441.7	411.5	430.6	-	-	2164.0	2164.0
2(41)	393.6	410.9	398.9	401.1	2711.9	2109.8	1955.8	2259.2
2(42)	414.2	441.7	419.3	425.1	2210.3	-	2107.8	2159.1
2(43)	414.2	441.7	419.3	425.1	2149.5	2109.8	1844.9	2034.7
2(44)	393.6	410.9	396.3	400.3	-	-	2107.8	2107.8
2(45)	414.2	379	455.7	416.3	-	2109.8	1844.9	1977.4
2(46)	-	-	455.7	455.7	-	-	-	-
2(47)	414.2	441.7	411.5	422.5	1915.6	-	1844.9	1880.3
2(48)	366.5	393.1	381.5	380.4	2711.9	2109.8	-	2410.9
2(49)	438.6	441.7	455.7	445.3	2711.9	-	1844.9	2278.4

Table 2. (cont'd)

Progeny	Bloom (degree days)				Ripe (degree days)			
	1995	1996	1997	average	1995	1996	1997	average
2(50)	438.6	441.7	411.5	430.6	-	2109.8	1844.9	1977.4
2(51)	393.6	410.9	402.8	402.4	2149.5	2109.8	2395.8	2218.4
2(52)	381.8	393.1	381.5	385.5	-	2109.8	2164.0	2136.9
2(53)	343.7	393.1	369.3	368.7	-	-	1844.9	1844.9
2(54)	381.8	410.9	381.5	391.4	2711.9	-	-	2711.9
2(55)	454.1	441.7	440.4	445.4	1987.7	-	1844.9	1916.3
2(56)	381.8	410.9	396.3	396.3	1628.2	1957.7	1844.9	1810.3
2(58)	366.5	393.1	369.3	376.3	1723.9	1900.8	1844.9	1823.2
2(59)	381.8	410.9	396.3	396.3	2711.9	2192.4	2107.8	2337.4
2(60)	354.0	393.1	381.5	376.2	-	-	2520.4	2520.4
2(62)	366.5	371.4	357.4	365.1	-	2109.8	2164.0	2136.9
2(63)	393.6	441.7	411.5	415.6	1987.7	-	-	1987.7
2(64)	328.5	371.4	357.4	352.4	-	-	2311.1	2311.1
2(65)	354.0	393.1	369.3	372.1	1568.8	2109.8	1844.9	1841.2
2(66)	438.6	410.9	411.5	420.3	1885.8	2109.8	2107.8	2034.5
3(02)	-	379	455.7	417.3	-	2351.1	-	2351.1
3(03)	343.7	382.3	369.3	365.1	1885.8	1957.7	1844.9	1896.1
3(04)	454.1	441.7	419.3	438.4	2437	1957.7	2107.8	2167.5
3(05)	414.2	441.7	440.4	432.1	2711.9	1957.7	2395.8	2355.1
3(06)	454.1	441.7	440.4	445.4	2210.3	2192.4	1844.9	2082.5
3(07)	354.0	410.9	396.3	387.1	2679	-	2107.8	2393.4
3(08)	393.6	393.1	371.4	386.0	2776.3	2109.8	1844.9	2243.7
3(09)	354.0	382.3	369.3	368.5	-	2109.8	1955.8	2032.8
3(10)	381.8	393.1	369.3	381.4	-	-	1844.9	1844.9
3(13)	343.7	371.4	357.4	357.5	2711.9	2192.4	2107.8	2337.4
3(14)	381.8	410.9	371.4	388.0	2711.9	2109.8	2273.0	2364.9
3(16)	393.6	410.9	408.6	404.4	1628.2	1601.2	1642.6	1624.0
3(18)	393.6	410.9	396.3	400.3	2711.9	2109.8	1844.9	2222.2
3(20)	438.6	441.7	411.5	430.6	1628.2	-	1844.9	1736.6
3(21)	414.2	410.9	396.3	407.1	1885.8	1957.7	1844.9	1896.1
3(22)	438.6	441.7	411.5	430.6	2645.2	-	2311.1	2478.2
3(24)	303.3	326.3	323.8	317.8	1654.7	2109.8	2311.1	2025.2
3(25)	414.2	410.9	408.6	411.2	2744.3	2109.8	2107.8	2320.6
3(27)	474.8	-	-	474.8	2210.3	-	-	2210.3
3(28)	381.8	393.1	381.5	385.5	2210.3	1957.7	-	2084.0
3(29)	328.5	361.1	335.1	341.6	1789	1900.8	1844.9	1844.9
3(31)	343.7	361.1	357.4	354.1	2210.3	2109.8	1844.9	2055.0
3(32)	328.5	345.6	335.1	336.4	-	1957.7	1844.9	1901.3
3(34)	328.5	364.9	369.3	354.2	1885.8	-	1844.9	1865.4
3(35)	343.7	393.1	369.3	368.7	1885.8	1900.8	1880.4	1889.0
3(37)	414.2	441.7	407.7	421.2	-	-	1844.9	1844.9

Table 3. Fruit weight, soluble solids concentration for each progeny in the mapping population for the years 1995, 1996, 1997 and the average.

Progeny	Fruit weight (g)				soluble solids concentration (°Brix)			
	1995	1996	1997	average	1995	1996	1997	average
2(02)	-	-	-	-	-	-	-	-
2(03)	3.43	3.53	4.06	3.67	18.63	16.86	18.04	17.84
2(04)	4.25	-	4.86	4.56	17.50	-	12.34	14.92
2(05)	5.17	4.60	6.56	5.44	19.23	15.76	17.48	17.49
2(06)	6.46	5.10	6.46	6.00	13.57	13.86	14.6	14.01
2(07)	4.59	4.05	4.60	4.41	17.45	13.28	16.52	15.75
2(08)	5.78	6.25	5.40	5.81	16.95	17.8	18.24	17.66
2(09)	5.13	-	5.02	5.08	9.50	20	22.72	17.41
2(10)	4.54	3.79	5.44	4.59	15.50	15.9	18.88	16.76
2(11)	6.66	7.37	5.56	6.53	20.27	18.52	16.92	18.57
2(12)	3.75	4.78	4.36	4.30	15.20	20.9	18.84	18.31
2(13)	-	-	4.56	4.56	-	-	9.76	9.76
2(14)	4.88	6.18	5.68	5.58	15.85	13.6	15.52	14.99
2(15)	7.46	-	8.38	7.92	14.67	-	15.52	15.09
2(16)	4.90	5.22	4.52	4.88	15.90	15.8	17.44	16.38
2(17)	5.48	5.90	6.04	5.81	14.28	12.6	17	14.63
2(18)	4.40	4.55	4.18	4.38	17.40	18.24	18.88	18.17
2(19)	3.89	-	-	3.89	13.38	-	-	13.38
2(20)	6.37	6.74	6.38	6.49	15.04	14.3	15.28	14.87
2(22)	3.80	3.27	3.78	3.62	15.03	14.2	17.56	15.60
2(23)	6.09	4.12	6.02	5.41	15.40	16.3	19.68	17.13
2(24)	5.79	6.09	7.36	6.41	13.85	14.9	15.6	14.78
2(25)	-	3.00	3.60	3.30	-	11.5	13.5	12.50
2(27)	3.56	4.45	4.78	4.26	13.93	14.6	14.74	14.42
2(28)	-	-	5.50	5.50	-	-	17.48	17.48
2(29)	4.69	5.40	4.28	4.79	16.60	14	15.72	15.44
2(30)	-	-	-	-	-	-	-	-
2(32)	4.95	4.45	5.14	4.85	16.27	14.28	16.44	15.66
2(33)	-	-	-	-	-	-	-	-
2(34)	4.53	-	-	4.53	15.08	-	-	15.08
2(35)	4.13	5.08	4.64	4.61	14.50	18.5	18.56	17.19
2(36)	-	-	2.90	2.90	-	14.5	19.36	16.93
2(37)	2.72	2.67	3.70	3.03	20.82	15.6	19.8	18.74
2(38)	-	4.48	5.22	4.85	-	19.9	15.56	17.73
2(39)	-	4.53	5.74	5.14	-	15	14.48	14.74
2(40)	-	-	5.98	5.98	-	-	16.68	16.68
2(41)	4.21	3.32	4.12	3.88	12.70	18	16.84	15.85
2(42)	2.52	-	2.14	2.33	14.37	-	14.52	14.44
2(43)	5.25	5.26	5.52	5.34	17.90	15	16.36	16.42
2(44)	-	-	5.58	5.58	-	-	18.52	18.52
2(45)	-	3.25	2.92	3.09	-	14.64	16.72	15.68
2(46)	-	-	-	-	-	-	-	-
2(47)	3.54	-	3.38	3.46	15.43	-	15.44	15.44
2(48)	6.13	4.77	-	5.45	21.30	17.56	-	19.43
2(49)	4.97	-	4.00	4.49	-	-	17.92	17.92
2(50)	-	3.73	2.80	3.27	-	13.52	13.16	13.34
2(51)	6.52	4.82	6.08	5.80	14.30	15.08	14.88	14.75

Table 3. (cont'd)

Progeny	Fruit weight (g)				soluble solids concentration (°Brix)			
	1995	1996	1997	average	1995	1996	1997	average
2(52)	-	3.54	5.10	4.32	-	14.14	14.96	14.55
2(53)	-	-	3.14	3.14	-	-	16.16	16.16
2(54)	3.81	-	-	3.81	11.65	-	-	11.65
2(55)	3.99	-	3.54	3.76	14.90	-	14.52	14.71
2(56)	3.60	4.36	4.46	4.14	15.00	15.72	17.08	15.93
2(58)	5.61	6.30	4.86	5.59	15.37	15.2	16.64	15.74
2(59)	3.72	3.44	3.54	3.57	11.33	12.9	14.8	13.01
2(60)	-	-	4.00	4.00	-	-	9.96	9.96
2(62)	-	7.04	7.24	7.14	-	15.96	16.92	16.44
2(63)	3.25	-	-	3.25	14.97	-	-	14.97
2(64)	-	-	4.00	4.00	-	-	16.6	16.60
2(65)	3.25	4.96	5.40	4.54	14.67	15.04	14.24	14.65
2(66)	6.45	5.28	6.28	6.00	14.57	15.52	16.88	15.66
3(02)	-	3.23	-	3.23	-	13.68	-	13.68
3(03)	6.78	5.27	6.06	6.04	14.87	14.84	15.52	15.08
3(04)	4.83	4.52	4.00	4.45	20.00	18.6	16.76	18.45
3(05)	4.31	5.29	4.44	4.68	16.15	18.84	15.68	16.89
3(06)	2.86	2.30	3.06	2.74	19.25	21	18.12	19.46
3(07)	7.05	-	6.38	6.71	16.87	-	16.96	16.91
3(08)	5.89	5.51	4.78	5.39	18.00	16.84	17	17.28
3(09)	-	5.38	4.38	4.88	-	19.5	20.56	20.03
3(10)	-	-	2.30	2.30	-	-	15.72	15.72
3(13)	5.84	5.32	6.36	5.84	12.43	13.44	14.96	13.61
3(14)	3.26	5.77	4.18	4.40	16.00	15.52	16.4	15.97
3(16)	4.92	4.34	5.66	4.97	15.12	13.2	17	15.11
3(18)	4.56	4.46	4.92	4.65	12.80	13.88	15.12	13.93
3(20)	3.06	-	3.72	3.39	14.04	-	16.52	15.28
3(21)	5.08	5.23	4.12	4.81	15.20	15.94	15.76	15.63
3(22)	4.09	-	4.48	4.29	14.27	-	14.76	14.51
3(24)	7.49	9.00	9.86	8.78	14.30	17.2	14.34	15.28
3(25)	5.31	4.67	5.66	5.21	12.20	13.27	15.72	13.73
3(27)	2.93	-	-	2.93	17.96	-	-	17.96
3(28)	5.39	4.98	-	5.18	19.08	18.58	-	18.83
3(29)	5.56	5.31	5.76	5.54	16.23	14.2	14.56	15.00
3(31)	4.14	3.75	5.32	4.40	19.05	19	17.72	18.59
3(32)	-	7.05	6.16	6.60	-	19.86	17.64	18.75
3(34)	3.57	-	4.44	4.01	11.97	-	14.04	13.00
3(35)	5.30	5.16	5.00	5.15	15.94	16	16	15.98
3(37)	-	-	3.80	3.80	-	-	20.08	20.08

Table 4. Pistil death, fruit set, pollen germination, flower bud death for each progeny in the mapping population.

Progeny	Pistil death (%)				Fruit	Pollen	Flower bud
	1995	1996	1997	average	set (%)	germnation (%)	death (%)
2(02)	-	15.0	-	15.0	0.0	0.0	0.0
2(03)	5.0	30.0	25.0	20.0	3.7	4.5	6.7
2(04)	15.0	90.0	45.0	50.0	2.4	6.5	0.0
2(05)	0.0	17.5	32.5	16.7	15.2	1.0	7.1
2(06)	12.5	25.0	30.0	22.5	2.3	4.0	44.4
2(07)	2.5	0.0	17.5	6.7	0.4	8.5	12.5
2(08)	7.5	85.0	20.0	37.5	9.6	0.5	40.0
2(09)	0.0	22.5	50.0	24.2	3.4	5.5	56.3
2(10)	7.5	52.5	40.0	33.3	17.6	11.0	76.9
2(11)	17.5	97.5	20.0	45.0	14.7	2.5	50.0
2(12)	5.0	95.0	55.0	51.7	9.9	0.0	23.1
2(13)	10.0	30.0	2.5	14.2	1.8	3.5	72.2
2(14)	25.0	75.0	57.5	52.5	5.4	0.0	66.7
2(15)	0.0	82.5	12.5	31.7	4.9	3.5	7.1
2(16)	5.0	30.0	45.0	26.7	6.6	1.5	35.7
2(17)	0.0	20.0	40.0	20.0	2.0	4.0	30.0
2(18)	2.5	10.0	37.5	16.7	16.0	18.0	21.4
2(19)	10.0	90.0	27.5	42.5	2.1	1.0	40.0
2(20)	0.0	12.5	12.5	8.3	-	7.0	12.0
2(22)	12.5	70.0	57.5	46.7	5.2	1.0	81.3
2(23)	5.0	92.5	0.0	32.5	8.7	3.0	14.3
2(24)	17.5	12.5	12.5	14.2	0.4	2.0	19.0
2(25)	0.0	2.5	-	1.3	17.6	10.0	5.3
2(27)	10.0	60.0	2.5	24.2	8.1	0.0	0.0
2(28)	-	20.0	-	20.0	-	0.0	25.0
2(29)	15.0	75.0	27.5	39.2	34.4	6.0	18.8
2(30)	-	-	0.0	0.0	-	-	-
2(32)	10.0	65.0	20.0	31.7	13.7	18.0	10.0
2(33)	7.5	20.0	-	13.8	-	3.5	40.0
2(34)	12.5	12.5	22.5	15.8	0.4	10.5	64.3
2(35)	0.0	90.0	12.5	34.2	0.8	0.0	0.0
2(36)	0.0	17.5	27.5	15.0	0.5	3.0	0.0
2(37)	0.0	45.0	2.5	15.8	0.6	1.5	20.0
2(38)	15.0	5.0	47.5	22.5	1.2	0.0	68.0
2(39)	2.5	2.5	12.5	5.8	1.9	0.0	0.0
2(40)	2.5	12.5	5.0	6.7	4.1	0.0	34.8
2(41)	0.0	15.0	12.5	9.2	3.0	4.0	0.0
2(42)	0.0	40.0	7.5	15.8	0.5	0.0	20.0
2(43)	15.0	95.0	30.0	46.7	2.8	1.5	42.1
2(44)	15.0	10.0	12.5	12.5	2.0	0.0	66.7
2(45)	12.5	5.0	0.0	5.8	4.2	17.5	20.0
2(46)	-	-	0.0	0.0	-	-	-
2(47)	12.5	60.0	55.0	42.5	0.0	0.0	13.3
2(48)	25.0	10.0	57.5	30.8	3.7	17.0	63.2
2(49)	10.0	42.5	42.5	31.7	15.2	25.0	35.7
2(50)	5.0	17.5	7.5	10.0	11.1	12.0	46.2

Table 4. (cont'd)

Progeny	Pistil death (%)				Fruit	Pollen	Flower bud
	1995	1996	1997	average	set (%)	germnation (%)	death (%)
2(51)	12.5	5.0	50.0	22.5	6.9	0.0	28.6
2(52)	5.0	0.0	0.0	1.7	2.6	1.5	57.9
2(53)	5.0	12.5	20.0	12.5	4.2	2.5	25.0
2(54)	7.5	30.0	5.0	14.2	5.8	2.5	47.1
2(55)	0.0	0.0	2.5	0.8	1.7	17.0	5.0
2(56)	17.5	25.0	70.0	37.5	23.3	0.0	58.8
2(58)	5.0	62.5	50.0	39.2	19.3	0.0	
2(59)	0.0	5.0	2.5	2.5	10.1	5.0	6.3
2(60)	20.0	17.5	22.5	20.0	7.5	5.5	100.0
2(62)	2.5	2.5	27.5	10.8	3.8	1.0	72.0
2(63)	15.0	100.0	7.5	40.8	5.4	4.0	33.3
2(64)	32.5	90.0	37.5	53.3	7.3	1.5	20.0
2(65)	0.0	10.0	7.5	5.8	11.0	10.5	6.3
2(66)	0.0	5.0	30.0	11.7	18.6	15.5	20.0
3(02)	-	10.0	2.5	6.3	1.4	0.0	0.0
3(03)	0.0	0.0	27.5	9.2	21.8	4.5	20.0
3(04)	0.0	37.5	10.0	15.8	13.7	2.5	0.0
3(05)	2.5	20.0	27.5	16.7	13.2	20.0	16.7
3(06)	-	42.5	32.5	37.5	2.2	0.0	0.0
3(07)	2.5	15.0	27.5	15.0	0.0	1.5	0.0
3(08)	2.5	5.0	10.0	5.8	2.2	15.0	10.0
3(09)	7.5	47.5	45.0	33.3	16.1	3.5	66.7
3(10)	22.5	52.5	42.5	39.2	0.3	0.0	92.9
3(13)	2.5	45.0	2.5	16.7	7.2	8.5	63.2
3(14)	7.5	17.5	60.0	28.3	6.6	13.5	-
3(16)	17.5	100.0	32.5	50.0	8.1	16.5	10.0
3(18)	0.0	15.0	10.0	8.3	3.0	0.0	41.2
3(20)	0.0	57.5	17.5	25.0	2.3	3.5	45.0
3(21)	2.5	22.5	7.5	10.8	0.0	8.5	56.5
3(22)	0.0	90.0	57.5	49.2	7.2	3.5	45.5
3(24)	17.5	75.0	50.0	47.5	0.3	3.5	47.1
3(25)	0.0	20.0	0.0	6.7	0.6	0.0	38.5
3(27)	-	-	-	-	-	-	30.8
3(28)	22.5	7.5	30.0	20.0	19.3	24.5	70.6
3(29)	12.5	50.0	42.5	35.0	4.4	0.5	81.3
3(31)	12.5	72.5	22.5	35.8	6.3	5.0	33.3
3(32)	27.5	65.0	72.5	55.0	5.3	2.0	52.9
3(34)	32.5	25.0	60.0	39.2	2.5	4.5	64.7
3(35)	15.0	60.0	32.5	35.8	10.1	34.0	60.0
3(37)	0.0	57.5	20.0	25.8	5.4	6.0	0.0