Tree Genetics & Genomes
DOI 10.1007/s11295-008-0161-1

ORIGINAL PAPER

Construction of an intra-specific sweet cherry (*Prunus avium* L.) genetic linkage map and synteny analysis with the *Prunus* reference map

James W. Olmstead · Audrey M. Sebolt · Antonio Cabrera · Suneth S. Sooriyapathirana · Sue Hammar · Gloria Iriarte · Dechun Wang · Charles Y. Chen · Esther van der Knaap · Amy F. Iezzoni

Received: 5 October 2007 / Revised: 24 March 2008 / Accepted: 14 April 2008

Abstract Linkage maps of the sweet cherry cultivar 'Emperor Francis' (EF) and the wild forest cherry 'New York 54' (NY) were constructed using primarily simple sequence repeat (SSR) markers and gene-derived markers with known positions on the *Prunus* reference map. The success rate for identifying SSR markers that could be placed on either the EF or NY maps was only 26% due to two factors: a reduced transferability of other *Prunus*-species-derived markers and a low level of polymorphism in the mapping parents. To increase marker density, we developed four cleaved amplified polymorphic sequence markers (CAPS), 19 derived CAPS markers, and four insertion–deletion markers for cherry based on 101 *Prunus* expressed sequence tags. In addition, four gene-derived markers representing orthologs of a tomato vacuolar invertase and fruit size gene and two sour cherry sorbitol transporters were developed. To complete the linkage analysis, 61 amplified fragment length polymorphism and seven sequence-related amplified polymorphism markers were also used for map construction. This analysis resulted in the expected eight linkage groups for both parents. The EF and NY maps were 711.1 cM and 565.8 cM, respectively, with the average distance between markers of 4.94 cM and 6.22 cM. A total of 82 shared markers between the EF and NY maps and the *Prunus* reference map showed that the majority of the marker orders were the same with the *Prunus* reference map suggesting that the cherry genome is colinear with that of the other diploid *Prunus* species.

Keywords *Prunus* · Genetic linkage map · Synteny analysis · Sweet cherry

Communicated by E. Dirlewanger

Electronic supplementary material The online version of this article (doi:10.1007/s11295-008-0161-1) contains supplementary material, which is available to authorized users.

J. W. Olmstead · A. M. Sebolt · S. S. Sooriyapathirana · S. Hammar · D. Wang · C. Y. Chen · A. F. Iezzoni
Michigan State University,
East Lansing, MI 48824, USA

A. Cabrera · G. Iriarte · E. van der Knaap
Ohio Agriculture Research and Development Center,
The Ohio State University,
Wooster, OH 44691, USA

A. F. Iezzoni (✉)
Department of Horticulture, Michigan State University,
East Lansing, MI 48824, USA
e-mail: iezzoni@msu.edu

Present address:
J. W. Olmstead
Washington State University,
Yakima County Extension,
Yakima, WA 98901, USA

Present address:
C. Y. Chen
USDA/ARS, National Peanut Research Laboratory,
Dawson, GA 39842, USA

Introduction

The genus *Prunus* contains many economically important tree fruit and nut crops including peach [*Prunus persica* (L.) Batsch], apricot (*Prunus armeniaca* L.), plum (*Prunus*

salicina Lindl., *Prunus domestica* L.), cherry (*Prunus avium* L., *Prunus cerasus*, L.), and almond [*Prunus dulcis* (Miller) D.A. Webb]. The construction of genetic linkage maps for these species has and continues to be an important research goal to facilitate quantitative trait locus (QTL) analysis and gene tagging for use in marker-assisted selection. *Prunus* linkage maps are especially well developed in peach (Chaparro et al. 1994; Rajapakse et al. 1995; Dirlewanger et al. 1998; Abbott et al. 1998; Lu et al. 1998; Yamamoto et al. 2001; Dirlewanger et al. 2006), almond (Viruel et al. 1995; Joobeur et al. 2000), and apricot (Hurtado et al. 2002; Vilanova et al. 2003; Lambert et al. 2004; Dondini et al. 2007). The most detailed map is the *Prunus* reference map that was constructed from an interspecific almond cv. 'Texas' × peach cv. 'Earlygold' (abbr. T × E) F_2 mapping population (Joobeur et al. 1998; Aranzana et al. 2003). This reference map consists of 562 markers, 264 of which are simple sequence repeat (SSR) markers arranged in the expected eight linkage groups (G1–G8), covering 519 cM, with an average marker density of 0.92 cM (Dirlewanger et al. 2004a; Howad et al. 2005).

Comprehensive alignments of available *Prunus* linkage maps in the diploid peach, almond, and apricot ($2n=2x=16$) reveal a strong colinearity among the genomes (Dirlewanger et al. 2004a, b; Lambert et al. 2004; Dondini et al. 2007). In contrast, the synteny of a sweet cherry ($2n=2x=16$) linkage map with the T × E reference map remains to be thoroughly examined as comparative mapping with cherry has lagged behind other *Prunus* species due to the low number of mapped markers in cherry that are also on other *Prunus* linkage maps (Stockinger et al. 1996; Bošković and Tobutt 1998; Wang et al. 1998). More recently, partial linkage maps were constructed of two elite sweet cherry cultivars from 133 F_1 progeny from a 'Regina' × 'Lapins' population in the Institut National de la Recherche Agronomique (INRA) of Bordeaux, France (Dirlewanger et al. 2004a). The 'Regina' and 'Lapins' maps have 30 and 28 SSRs in common with the T × E map and only one SSR was detected in a nonhomologous linkage group. These results are in agreement with the high level of synteny reported in *Prunus* (Dirlewanger et al. 2004a, b; Lambert et al. 2004; Dondini et al. 2007). However, more complete genome coverage in cherry is necessary to rigorously examine synteny across all eight linkage groups.

Reciprocal F_1 intra-specific sweet cherry mapping populations were generated at Michigan State University to complement the other international efforts and provide a population suitable for QTL analysis of fruit traits that would have been improved through domestication, namely, fruit size and quality. Reciprocal crosses were made between the domesticated parent 'Emperor Francis' (EF) and the wild forest tree cherry 'NY 54' (NY). EF is an old landrace variety originating from Northern Europe that has fruit of approximately 6–8 g and has a yellow flesh with pink blush on the skin. Our primary reason for choosing EF as the domesticated parent was because it is a founding clone that is in the pedigree of almost every newly released sweet cherry cultivar (Choi and Kappel 2004). For example, EF is a great-grandparent of 'Lapins,' a parent of the INRA sweet cherry mapping population. Therefore, QTL validation could be greatly facilitated when using pedigree genotyping or association mapping approaches. NY is a wild "mazzard" clone originally obtained from a German forest and introduced at the New York State Agricultural Experiment Station, Cornell University (R.L. Andersen, personal communication). NY is typical of wild forest cherries, having small fruit (~2 g) with black skin, purple flesh, and unpalatable taste. This particular clone was selected because its self-incompatibility alleles are S_2S_6, and it is therefore fully compatible with EF (S_3S_4) providing the capability to conduct a genetic analysis of those traits linked to the *S*-locus (Ikeda et al. 2005). *S*-allele genotyping of 511 progeny from the EF and NY populations previously determined that all four *S*-locus progeny classes (S_2S_3, S_2S_4, S_3S_6, S_4S_6) are equally represented. This is in contrast to many other *Prunus* mapping populations where only one pollen *S*-allele type is compatible in the style resulting in just two *S*-locus progeny classes (Foolad et al. 1995; Joobeur et al. 1998; Bliss et al. 2002; Foulongne et al. 2003; Vilanova et al. 2003; Lambert et al. 2004). However, it is more difficult to map other markers using our sweet cherry mapping population compared to the T × E mapping population as: (1) our cherry population is from an intra-specific cross and the T × E population is from an inter-specific cross and (2) our cherry population is an F_1 and therefore a marker must be heterozygous in at least one parent to be mapped, while the T × E population is an F_2 and therefore markers monomorphic in the peach and/or almond parents can also be placed on the linkage map.

SSR markers derived from either cDNA or genomic sequences have thus far been the markers of choice for *Prunus* linkage mapping. However, with the increase in available *Prunus* expressed sequence tag (EST) sequences, many of which have been assigned locations on the peach transcript map (Horn et al. 2005), we sought to increase the marker density of our cherry maps with the addition of gene-derived markers. Markers that are based on expressed gene sequences are particularly useful as they represent nonrepetitive regions of the genome and are sufficiently conserved across the Rosaceae family to be useful in synteny analysis. Moreover, gene-derived markers also permit future association studies that relate gene function to phenotypes of interest. The majority of the markers designed were cleaved amplified polymorphic sequence

markers (CAPS; Konieczny and Ausubel 1993) and derived CAPS (dCAPS) markers that can detect single-nucleotide polymorphisms (SNP) that differentially affect the presence or absence of a restriction enzyme cut site (Michaels and Amasino 1998; Neff et al. 1998). Contrary to SSR markers that require high-resolution acrylamide-based systems to separate the fragments, CAPS and dCAPS can be visualized on lower-resolution agarose gel systems which are available in many laboratories.

The objectives of this work were (1) to construct two new sweet cherry genetic linkage maps from progenies generated from reciprocal crosses that would be useful for future QTL detection, (2) develop gene-derived markers that could be visualized on agarose gels to further increase marker density, and (3) compare marker orders and positions between the sweet cherry maps generated and the *Prunus* T × E reference map.

Materials and methods

Plant material

In 2001, pollen was collected from NY and EF trees in the National Research Support Project 5 (NRSP5) planting in Prosser, WA, USA. NY was used as a maternal parent in Washington State, and pollen was transported to Michigan for use in reciprocal crosses with EF as the maternal parent. From the crosses, 617 F_1 individuals were planted at Michigan State University's Clarksville Horticultural Experiment Station in Clarksville, MI, USA in the spring of 2002. The seedlings were planted at 1.5 and 6.1 m within and between row spacing, respectively. From the total population, a linkage mapping subset of 190 individuals was selected. This subset consisted of 86 individuals from the NY × EF cross, 103 individuals from the EF × NY cross, and one individual with no reciprocal cross information. Approximately equal numbers of progeny from each of the four *S*-allele groups (48, S_2S_3; 49, S_2S_4; 47, S_3S_6; 46, S_4S_6) were included in the mapping population. These four *S*-allele groups were shown previously to segregate according to the expected 1:1:1:1 Mendelian ratio (Ikeda et al. 2005).

DNA isolation

For DNA extraction, young unfolded leaves from the parents and each progeny individual were collected, placed immediately on dry ice, transported to the laboratory, and placed at −80°C for at least 24 h. Leaves were lyophilized for 48 h and stored long term at −20°C after which DNA was isolated using the cetyl trimethylammonium bromide method described by Stockinger et al. (1996).

DNA markers

A total of 433 SSR markers used for linkage map construction were derived from cDNA and genomic libraries of six *Prunus* species: *P. armeniaca* (apricot), *P. dulcis* (almond), *P. persica* (peach), *P. salicina* (plum), *P. avium* (sweet cherry), and *P. cerasus* (sour cherry; Table 1). SSR markers were screened using the two parents and six progeny individuals. For the majority of the SSR markers, the PCR conditions were constant except that the recommended annealing temperature for each individual primer pair was used: 94°C for 5 min, 35 cycles of 94°C (45 s), *X*°C (45 s), 72°C (90 s), and a final extension step of 72°C for 5 min, where *X*=the published optimum annealing temperature for each primer. For the EMPA and EMPaS primers, a touchdown polymerase chain reaction (PCR) temperature profile was used as described by Clarke and Tobutt (2003). The reaction mixture contained 1× PCR buffer, 2.5 mM $MgCl_2$, 120 µM of each deoxynucleotide triphosphate, 2.5 pmol of each primer, 50 ng of genomic DNA, and 0.3 U *Taq* polymerase (Invitrogen Corporation, Carlsbad, CA, USA) in a 12.5-µl reaction.

Amplified fragment length polymorphism (AFLP) analysis consisting of genomic DNA digestion with *Eco*RI and *Mse*I restriction enzymes, adapter ligation, preamplification, and selective amplification using *Eco*RI plus two and *Mse*I plus three selective nucleotide primers were similar to those from Vos et al. (1995) with modifications described by Hazen et al. (2002).

Two sequence-related amplified polymorphism (SRAP) forward and reverse primer combinations me1–em1 and me1–em2 were used as reported by Li and Quiros (2001); me1=5′-TGAGTCCAAACCGGATA, em1=5′-GACTGCGTACGAATTAAT, em2=5′-GACTGCGTACGAATTTGC. DNA amplification was performed as in Li and Quiros (2001).

Gene-derived markers

The *S*-locus was scored using the *S-RNase* primer pair Pru-C2 and PCE-R (Tao et al. 1999; Yamane et al. 2001) under previously described conditions (Ikeda et al. 2005). The S_2-*RNase*-specific PCR primer pair, PaS2-F and PaS2-R, was used for confirmation of S_2 presence (Sonneveld et al. 2001). Primer sequences for *Prp*FT, a peach homolog to a *Malus* × *domestica* T-like protein, were also utilized (Silva et al. 2005). In addition, the following three candidate genes were developed into markers: *INV5*, corresponding to a vacuolar invertase (Fridman et al. 2000) and *SorT1* and *SorT2*, corresponding to sorbitol transporters (Gao et al. 2003).

EST sequences were selected from the bin map as well as the *Prunus* physical map which were both anchored to

Table 1 Origins and proportion of amplified, heterozygous, and mapped simple sequence repeat markers used in the development of the 'NY 54' × 'Emperor Francis' sweet cherry genetic linkage maps

SSR name	*Prunus* species	Number of SSRs					Reference
		Origin	Tested	Amplified[a]	Heterozygous[b]	Number Mapped (%)[c]	
AMPA	*P. armeniaca*	Genomic	4	4	2	2 (50)	Hagen et al. 2004
BPPCT	*P. persica*	Genomic	35	31	14	13 (37)	Dirlewanger et al. 2002
CPDCT	*P. dulcis*	Genomic	22	16	5	3 (14)	Mnejja et al. 2005
CPPCT	*P. persica*	Genomic	27	25	8	8 (30)	Aranzana et al. 2002
CPSCT	*P. salicina*	Genomic	24	18	5	5 (21)	Mnejja et al. 2004
EMPA	*P. avium*	Genomic	11	11	5	4 (36)	Clarke and Tobutt 2003
EMPaS	*P. avium*	Genomic	7	7	4	4 (57)	Vaughan and Russell 2004
EPDCU	*P. dulcis*	cDNA	14	13	7	7 (50)	P. Arús (personal communication)
EPPB	*P. persica*	cDNA	16	16	5	5 (31)	E. Dirlewanger (personal communication); Dirlewanger et al. 2006
EPPCU	*P. persica*	cDNA	81	74	21	18 (22)	GDR database (http://www.bioinfo.wsu.edu/gdr/)
M	*P. persica*	cDNA	9	8	0	0 (0)	Yamamoto et al. 2002
MA	*P. persica*	cDNA	44	35	16	12 (27)	Yamamoto et al. 2002
MD	*P. persica*	cDNA	5	4	1	1 (20)	Yamamoto et al. 2005
PacC25	*P. armeniaca*	cDNA	1	1	0	0 (0)	Decroocq et al. 2003
PaCITA	*P. armeniaca*	Genomic	6	6	0	0 (0)	Lopes et al. 2002
Pce	*P. cerasus*	Genomic	7	7	3	3 (43)	Cantini et al. 2001; Struss et al. 2002
Pchcms	*P. persica*	cDNA	3	3	2	1 (33)	Sosinski et al. 2000
Pchgms	*P. persica*	Genomic	12	10	3	2 (17)	Sosinski et al. 2000
PMS	*P. avium*	Genomic	8	8	4	4 (50)	Cantini et al. 2001; Struss et al. 2002
PS	*P. cerasus*	Genomic	5	5	3	2 (40)	Cantini et al. 2001; Sosinski et al. 2000
UCD-CH	*P. avium*	Genomic	5	4	2	2 (40)	Struss et al. 2003
UDA	*P. dulcis*	Genomic	29	25	5	5 (17)	Testolin et al. 2004
UDAp	*P. armeniaca*	Genomic	33	29	9	4 (12)	Messina et al. 2004
UDP	*P. persica*	Genomic	25	20	7	6 (24)	Cipriani et al. 1999; Testolin et al. 2000
		Total	433	380 (88%)	131 (30%)	111 (26)	

[a] SSR markers that did not amplify a product with EF or NY DNA are listed in Supplemental Table 1.
[b] SSR markers that were monomorphic in EF and/or NY are listed in Supplemental Table 2.
[c] Percentage of markers that were mapped of the total markers tested.

the genetic T × E map (http://www.bioinfo.wsu.edu/gdr/). Intron regions in the genes were predicted by comparing each sequence to the *Populus trichocarpa* (poplar) genome available in the Joint Genome Institute database using the *P. trichocarpa v1.1* browser (http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Poptr1_&advanced=1). ESTs showing high similarity to poplar genomic sequences (Basic Local Alignment Search Tool nucleotide vs nucleotide at the cutoff E-value of $1e^{-5}$) were selected to design primer flanking the putative introns using Primer3 v 0.3.0 (http://frodo.wi.mit.edu/). For the genes that showed lower similarity to poplar, primers were designed irrespective of putative intron position. Products were amplified from genomic DNA of each parent using the primers and resulting fragments were directly sequenced at the Molecular and Cellular Imaging Center, in Wooster, OH, USA. Sequences were aligned to each other and compared to the original EST using Sequencher software v 4.2 (Gene Codes Corporation) to detect SNPs and/or insertions–deletions (InDels). The presence of double peaks in an otherwise high-quality chromatogram revealed the presence of a potential SNP. The sudden decay of high-quality chromatogram revealed a potential InDel. CAPS and dCAPS markers were developed following standard protocols (Konieczny and Ausubel 1993; Michaels and Amasino 1998).

A marker was also derived from a cherry ortholog of the tomato *FW2.2* (Frary et al. 2000) using the following strategy. Two translated poplar scaffold sequences (scaffolds 2121 and 678) for which the translated sequence showed homology to tomato FW2.2 were aligned to find conserved protein sequences using the Block Maker server (http://blocks.fhcrc.org/blocks/make_blocks.html). Highly conserved regions were used to design the degenerate primers PR8 (ACTTACTGGTGCCCATGCATHACNTTYGG) and PR9 (CGAATCCTCTATG CTTCAGCTCTC KRTAYTCYTG) using the Consensus-Degenerate Hybrid

Oligonucleotide Primers server (http://blocks.fhcrc.org/blocks/codehop.html). A test PCR using the gradient function on the MJ Research thermocycler was conducted to find the appropriate annealing temperature for amplification. PCR reactions were performed using Phusion HF DNA Polymerase (New England, USA, BioLabs) under conditions recommended by the manufacturer. PCR products were phosphorylated and ligated into the *Hinc*II site of pUC118. The ligation products were transformed into *Escherichia coli* DH5α electrocompetent cells. Five clones from each parent harboring the expected size of the PCR products were selected; the plasmids were isolated and inserts were sequenced. Sequences were aligned using Sequencher software v 4.2 in order to develop locus-specific primers (PR26F and PR26R). These primers were used to amplify the alleles from each parent and the resulting product was cloned into the *Hinc*II site of pUC118. Eighteen clones from each parent were sequenced for detection of the SNP that distinguished the haplotypes.

Genotyping platforms

AFLP and SRAP fragments were run on a 6% denaturing polyacrylamide gel in a 50-cm Sequi-Gen GT vertical sequencing apparatus (Bio-Rad Laboratories, Hercules, CA, USA) for 2.5 h at 70 W with 1× Tris–borate–ethylene diamine tetraacetic acid (TBE) buffer. After electrophoresis, the bands were visualized by silver staining (Promega Corporation, Madison, WI, USA). SSR and InDel markers were size-separated by electrophoresis on a 6% denaturing polyacrylamide gel using two different platforms. Fragments separated on the Sequi-Gen GT vertical sequencing apparatus were visualized by silver staining. Fragments separated on the LI-COR IR^2 DNA Analyzer system (LI-COR. Lincoln, NE, USA) were detected after excitation of the fluorescently labeled fragments. These fluorescent fragments were generated using either the IRDye800 or IRDye700 universally labeled M13 (−29) primer in the PCR reaction following the M13-tailed PCR protocol (Schuelke 2000). The CAPS and dCAPS markers were separated on a 3–4% agarose gel in 0.5× TBE buffer.

Chi square analysis and linkage map construction

All fragments were scored as present or absent. Segregating fragments present in one parent and absent in the other parent were tested for fit to a 1:1 ratio, while segregating fragments present in both parents were tested for fit to a 3:1 ratio. Chi square goodness-of-fit tests were performed using functions in Excel 2002 (Microsoft Corp., Redmond, WA, USA).

Linkage analysis was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001) using a minimum logarithm of odds score of 3.0, a maximum recombination fraction of 0.4, and centimorgan distances calculated by the Kosambi (1944) function. Linkage groups were drawn using MapChart 2.1 (Voorrips 2002) with distances presented in centimorgan.

Results and discussion

Transferability and polymorphism of SSR markers

All but 32 of the SSR markers placed on the T × E genetic map and for which primer sequences were publicly available were tested on EF, NY, and a subset of six progeny to determine their suitability for linkage map construction. To fill gaps in the maps, additional SSR markers with known map locations from other *Prunus* maps were also tested to determine if they were heterozygous within EF and/or NY. In total, 433 SSR markers that were derived from six *Prunus* species and 24 genomic and cDNA libraries were screened (Table 1). Of the 43 SSR primers derived from cherry sequence (*P. avium* and *P. cerasus*), only one failed to amplify a fragment from NY and EF. However, the amplification success rate was considerably less with the other *Prunus* species primers. In total, 12% of the screened SSR markers failed to amplify products (see Supplemental Table 1). Primer pairs derived from genomic or cDNA sequences performed similarly, as 87% and 89% of the primer pairs tested, respectively, amplified a product from cherry DNA.

In addition to the reduced amplification rates, the number of SSR markers suitable for comparative mapping was further reduced due to the low level of heterozygosity in the parents (Table 1). Of the amplified SSR products, only 35% and 34% of the primer pairs designed from genomic or cDNA sequences, respectively, were heterozygous in EF and/or NY. Therefore, of the 433 SSR primer pairs tested, 307 either did not amplify a product or were homozygous. Some of the monomorphic SSR primer pairs could be suitable in other cherry germplasm; therefore, a list of the monomorphic SSR markers is provided in the supplemental materials (see Supplemental Table 2). In addition, 15 primer pairs that identified 18 loci on the T × E map were not used as they provided complex patterns that could not be reliably scored (see Supplemental Table 3 for a list of these markers).

Taken together, the success rate for identifying SSR markers from the T × E map and other *Prunus* maps that could be placed on either the EF or NY map was only 26%. Several factors are likely contributing to this low success rate: the limited number of mapped cherry SSR markers, the reduced transferability of SSR markers from other *Prunus* species to cherry, and, lastly, the low level of heterozygosity in the mapping parents. As reported previ-

Table 2 Primer sequences and diagnostic restriction enzymes for two InDel markers and 21dCAPS markers developed from EST sequences

Marker name	Accession no.	Marker type	Enzyme	Sequence of forward primer[a]	Sequence of reverse primer
PR33	BU042407	Indel	–	CGTTACAGATTGGTTGACCTGTGA	ATCAGGCTATGCACACTCTT
PR41	AJ854221	dCAPS	*Hinf* I	CATTGCAAAATCTCACAGAA	CAATTATGATCTAAATAGAGGACT
PR51	AJ854216	dCAPS	*Hinf* I	GGTGTCATAAGGAATTCCCCGACT	TGCAAGATATTCTTCCCAGT
PR56	BU044092	CAPS	*Tru*1 I	TTGGTCTCGTGGTGGATAGA	TTGGCTGTGACACCCTTCTT
PR69	BU046536	CAPS	*Dde* I	AGCCTTTGCGTGAACAACTT	CGATGCTGGAAAGAAGAAGC
PR70	BU039366	dCAPS	*Hinf* I	CTGGGACCGAAGCACTTCTA	GATGCTCAGGGCTGAAAAAG
PR72	BU043855	dCAPS	*Hinc* II	AATGAGCAGAAACTGTTACGTTGA	GCAAGACATCTCCCAATTGAT
PR74	BU039994	CAPS	*Rsa* I	GGTGGTTTGTTGGGTTGAAA	TCGAGGATGGGATTCAAAAG
PR84	BU040863	dCAPS	*Cac*8 I	GGATGCATTTGAAAGGGATTT	ATTGGGTACTCTTGAGGGGCTGG
PR85	BU042636	dCAPS	*Dde* I	AGGATTCCATTTGGTGCTCA	TCTGTGTACTTCCACTATCTCCTT
PR86	BU048031	dCAPS	*Bam*H I	AGTACAATTAGTTGTGGGCGGATC	TGCTTTGCTTCAGCATGTTC
PR90	BU041499	dCAPS	*Hinc* II	GCTTTTATGCGTTTCGGTTG	ATCACAACAAATACAGTACGTCAA
PR93	BU042551	dCAPS	*Bsa*J I	TAGAACTTGTAGGGGTTGTCCCTG	CACCCAGCCTACCTCTCGTA
PR96	BU039798	dCAPS	*Tai* I	GGTTGCATGTTGATTGATGG	TCATGCTAATATTAACAGATAACG
PR98	BU039221	CAPS	*Rsa* I	GCACTGGGCTTCTTGTTGTA	CGACCACATTGCGAAGTAGA
PR101	BU043277	Indel	–	CACGACGTTGTAAAACGACTGCG ATGTGTTTGGTTGTCT	GTCCAGATGAGCCTTCAAGC
PR103	BU039816	dCAPS	*Bcc* I	CCCAGGCTTTAAGTGTGCTC	GAGTGAGGAGGAGGCTACCCTCCA
PR110	BU041732	dCAPS	*Bsa*J I	GGAAGTGGGGCAAAAACTGT	GGTACAATGCAGATATTAACCAGG
PR117	BU042018	dCAPS	*Bsa*J I	TCAAAGTTGTCACGTACTAACCTA	TGGTCCACGTCCATTGATTA
PR121	BU044081	dCAPS	*Apo* I	CCCTGACGTTCTGGATGATT	TGCAAGTTAGTTCTCTAGGCAAAATT
PR122	BU048663	dCAPS	*Bcc* I	TGTTTTCCCATTTGGTTATCACC	AAAGTGCAGACATCCTTGGAG
PR126	BU042394	dCAPS	*Hpa* II	TAGATTTTGATTTCCATAAAACCG	CCTAAACAGATATGACCGTTGC
PR127	BU045325	dCAPS	*Taq* I	GCTCAATGATGCCTTATGC	GACCAACATAATACTTCTCAAATC
PR25/22	AF482011	Indel	–	CACGACGTTGTAAAACGACAT AAAAATGGCCGTAGGTG	TCCATATCATCTCATCTCCAC
PR26	NA	dCAPS	*Bgl* I	CACGACGTTGTAAAACGACTATTCTTG ACAAATTGCCCAACTGG	TTGATGCATGGCAATGTTAAG
PR27	AY100638	dCAPS	*Bgl* II	CACGACGTTGTAAAACGACATGATC GCTCCGGTCTACACCGCCGAGAT	CCAAAACGAAAATTCCCAAGT
Inv 5	AAL05427.2	InDel	–	CACGACGTTGTAAAACGACAACCA GAACGTAATTGGATG	TGGAGACATATCTAACCTACCA

[a] Primer sequences (5′ to 3′) read left to right. For those primer sequences that are longer than 25 nucleotides, the primer sequence is continued on a second line.

ously, amplification of cherry DNA with primers that were derived from other *Prunus* species resulted in the lowest level of success within the genus (Dirlewanger et al. 2006; Dondini et al. 2007). For example, the success of SSR amplification in peach relative to the source DNA was as follows: 95.6% for apricot, 95% for almond, 90% for Japanese plum, and 81.5% for sweet cherry (Dirlewanger et al. 2006). In another study, only 19% of the SSRs derived from cherry were suitable for mapping in apricot (Dondini et al. 2007). Cherry is in the subgenus *Cerasus* which is phylogenetically distant from the other *Prunus* species that are placed within the subgenera *Prunophora* (= *Prunus*; plum and apricot) and *Amygdalus* (peach and almond). An analysis of chloroplast DNA polymorphisms placed the subgenera *Prunuophora* and *Amygdalus* in a separate clade, referred to as the Pruno-Amygdaloid (peach-almond–apricot–plum) clade, that is more distantly related to the *Cerasus–Laurocerasus–Padus* (cherry–laurel-cherry) clade (Shaw and Small 2004).

Our difficulty in finding heterozygous SSR markers for these parents is in agreement with that for ‘Lapins,’ a self-fertile cultivar and one of the parents in the INRA mapping population (Dirlewanger et al. 2004a). Of 25 polymorphic single-locus SSR markers derived from almond, five (20%) either did not amplify or produced complex patterns unsuitable for genetic analysis and, of the remaining 20 SSR markers, ‘Lapins’ was heterozygous for only five (25%) of them (Mnejja et al. 2005). By comparison, the observed heterozygosity in sweet cherry has been reported to range from 0.49 to 0.90 (Dirlewanger et al. 2002; Wünsch and Hormaza 2002; Schueler et al. 2003, 2006; Pedersen 2006; Stoeckel et al. 2006; Marchese et al. 2007). However, several of these studies only reported the heterozygosity for SSR markers selected based on prior

Table 3 Locations of the gene-derived markers on the T × E bin or physical maps compared to their locations on the 'Emperor Francis' (EF) and 'NY54' (NY) linkage groups

Marker name	Reference on T × E bin or physical map	Heterozygous parent(s)	EF and/or NY G and cM location(s)	T × E bin or location in physical map	Reference
PR33	EPPCU2407	EF	EF G1, 56.4	1:28[a] (26.5 to 27.9)[b]	Howad et al. 2005
PR41	EPPB4221	EF	EF G3, 43	3:37 (24.8 to 36.4)	E. Dirlewanger, GDR
PR51	EPPB4216	EF & NY	NY G5, 19.6 EF G5, 67.8	5:49 (49.1)	E. Dirlewanger, GDR
PR56	EPPCU4092	NY	NY G6, 80.8	6:80 (78.8 to 79.6)	Howad et al. 2005
PR69	EPPCU6536	EF	EF G2, 8.7	2:08 (0 to 8.1)	Howad et al. 2005
PR70	EPPCU9366	NY	NY G2, 2.8	2:08 (0 to 8.1)	Howad et al. 2005
PR72	EPPCU3855	NY	NY G6, 4.2	6:25 (0 to 24.9)	Howad et al. 2005
PR74	EPPCU9994	NY	NY G3, 30.7	6:25 (0 to 24.9)	Howad et al. 2005
PR84	EPPCU0863	EF	EF G5, 46.9	5:41 (21.7 to 40.7)	Howad et al. 2005
PR85	PP_LEa0013G08f	NY	NY G6, 0	6: 53.6	GDR
PR86	PP_LEa0033P09f	NY	NY G6, 60.8	6: 56.4	GDR
PR90	PP_LEa0009I13f	NY	NY G5, 1.0	6: 63.4	GDR
PR93	PP_LEa0013A14f	NY	NY G6, 80.8	1: 40.5, 6: 39.3	GDR
PR96	PP_LEa0003O11f	EF	EF G2, 21.3	2: 19.2	GDR
PR98	PP_LEa0001K03f	NY	NY G1, 137.8	2: 19.2	GDR
PR101	PP_LEa0015I11f	NY	NY G1, 73.6	2: 19.2, 5: 13.4	GDR
PR103	PP_LEa0003P11f	NY	NY G7, 25.5	2: 19.2	GDR
PR110	PP_LEa0010E22f	EF	EF G3, 49.2	2: 25	GDR
PR117	PP_LEa0011D19f	EF	EF G8[c]	2: 19.2	GDR
PR121	PP_LEa0018B24f	EF	EF G6, 75.9	2: 7.9	GDR
PR122	PP_LEa0036J22f	EF	EF G1, 140.9	2: 7.9	GDR
PR126	PP_LEa0012H09f	EF	EF G7, 49.0	2: 27.8	GDR
PR127	PP_LEa0022D10f	EF	EF G6, 26.4	1: 72.9, 2: 38	GDR
PR25/22	NA	EF	EF G8, 28.6	NA	GDR
PR26	NA	EF	EF G5, 69.3	NA	GDR
PR27	NA	NY	NY G8, 35	NA	GDR
Inv 5	NA	EF	EF G2[c]	NA	GDR

GDR Genome Database for Rosaceae (http://www.bioinfo.wsu.edu/gdr), *NA* not applicable (markers not present in the T × E reference map)
[a] Bin feature name represented by chromosome number: end of the bin (cM).
[b] Start and end of the bin on the corresponding chromosome (cM).
[c] These markers grouped in the linkage groups listed but could not be placed with confidence on the linkage map due to high chi square values.

knowledge of allelic polymorphism. Therefore, when the original numbers of nonamplifying or monomorphic SSR markers used in these studies were reported and therefore could be added to the calculation of heterozygosity, the observed sweet cherry heterozygosity ranged from only 0.22 to 0.32 (Dirlewanger et al. 2002; Wünsch and Hormaza 2002; Schueler et al. 2003). This suggests that the low level of SSR polymorphism in sweet cherry is not unique to EF and NY. However, in natural sweet cherry populations, heterozygote excess has been documented and has been suggested to be due to asexual reproduction and the ability to maintain heterozygosity over clonal generations (Stoeckel et al. 2006). NY was selected as a parent because it is fully compatible with EF and as a representative of wild germplasm. However, of the eight markers used by Stoeckel et al. (2006) to survey wild sweet cherry, six were homozygous in NY.

Gene-derived markers

To increase marker coverage, ESTs were selected that were predicted to map to low marker density regions due to their previous placement on the T × E genetic map or the peach physical map. ESTs have the advantage that they correspond to genes as opposed to the random genomic sequences that are represented by the SSR and AFLP fragments. Moreover, gene-derived markers will aid future studies involving gene function and the observed phenotypes. To increase the chance of finding polymorphisms in genic regions, we attempted to design primers that flank introns based on predictions made from the poplar genomic sequences. From the 101 expressed genes for which we obtained amplification products, a total of 23 CAPS and/or dCAPS and four InDels were developed that were mapped in the EF and NY population (Table 2). Thus, the marker

discovery rate on a per gene basis was 23%. Nine of the resulting EST-derived markers (PR33, PR41, PR51, PR56, PR69, PR70, PR72, PR74, and PR84) were designed from ESTs that had been previously placed on the *Prunus* genetic map, while 14 of the EST-derived markers (PR85, PR86, PR90, PR93, PR96, PR98, PR101, PR103, PR110, PR117, PR121, PR122, PR126, and PR127) were designed from ESTs that had previously been placed on the peach physical map. All together, these 23 newly designed "PR" markers represent successfully converted peach markers that can be utilized in cherry for linkage and comparative mapping. Eleven of the PR EST-derived markers identified two alleles in NY and EF while one PR marker identified two alleles in both parents (Table 3).

Two allelic variants were identified for four candidate genes using newly designed primer pairs based on cherry sequence (Table 2). The *SorT1* (PR25/22) and *Inv5* allelic variants were based on InDels, while the *SorT2* (PR27) and *FW2.2* (PR26) variants were based on SNPs.

AFLP and SRAP markers

To further increase marker density, AFLP and SRAP markers were employed. By using eight different *Eco*RI and *Mse*I selective primer combinations, 72 polymorphic fragments were identified (Table 4). Depending upon the selective nucleotides used, the number of polymorphic fragments ranged from four to 18 with an average of nine polymorphic fragments per primer combination. This was higher than the average of 6.8 reported in peach by Lu et al. (1998) who reported that the use of *Eco*RI and *Mse*I with two and three selective nucleotides, respectively, offered the best compromise between the number of polymorphic fragments produced and the ease of scoring.

Table 4 Enzymes used for digestion, selective nucleotide combinations used as primers, number of polymorphic fragments, and number of mapped fragments generated by amplified fragment length polymorphism analysis in the development of the 'NY 54' × 'Emperor Francis' sweet cherry genetic linkage maps

Selective nucleotides		Number of polymorphic fragments	Number of mapped fragments
*Eco*RI	*Mse*I		
AA	CTT	6	5
AA	CAC	9	9
AA	CCC	8	7
AA	CCT	6	4
AA	CAA	4	2
AT	CTC	18	15
AT	CCC	9	7
AC	CTA	12	12
	Total	72	61 (84.7%)

Two SRAP primer combinations identified seven polymorphic fragments. Six of these seven fragments were identified using the reverse primer em2 which had the selective nucleotides TGC at the 3′ end.

Map construction

The EF and NY linkage maps consisted of the expected eight linkage groups and totaled 711.1 and 565.8 cM, respectively (Table 5, Fig. 1). This compares well to a recent intra-specific map of peach which totaled 621.2 cM (Dirlewanger et al. 2006). In addition, the centimorgan size for the EF linkage map is consistent with the predicted genome size of sweet cherry which is slightly larger than peach (6.6×10^8 vs 5.3×10^8) (Dickson et al. 1992). The nomenclature and orientation of the linkage groups were according to that assigned to the T × E map (Joobeur et al. 1998). The average distances between markers were 4.94 and 6.22 cM for the EF and NY linkage maps, respectively. The largest gaps between markers were present on G1, the longest *Prunus* linkage group, and were 29.5 and 33.5 cM for EF and NY, respectively (Table 5). The maps consisted of a total of 197 markers, of which 102 were SSRs, 61 were AFLPs, seven were SRAP markers, and 27 were gene-derived markers. Of the 27 gene-derived markers, 25 were mapped (Fig. 1). *Inv5* and PR117 could not be mapped with high accuracy due to skewed segregation of these markers.

A total of 49 (34%) and 16 (18%) skewed loci were placed on the EF and NY linkage groups, respectively. Linked markers that significantly deviated from their chi-square expectations identified regions of the linkage groups that were skewed towards favoring a particular homolog. This was especially apparent for EF G2, G6, and G8 and NY G1. The percentage of skewed loci in EF was higher than that reported from other intra-specific *Prunus* crosses: 15% to 18% in peach (Lu et al. 1998; Dettori et al. 2001), 10% in almond (Joobeur et al. 2000), and 11% to 14% in apricot (Hurtado et al. 2002; Vilanova et al. 2003). The region flanking the self-incompatibility locus (*Si*) on G6 for both EF and NY did not contain skewed markers unlike the homologous region on other *Prunus* linkage maps (Foolad et al. 1995; Joobeur et al. 1998; Bliss et al. 2002; Foulongne et al. 2003; Vilanova et al. 2003; Lambert et al. 2004). In addition, our ability to map *Si* for both EF and NY is in contrast to the partially incompatible 'Regina' × 'Lapins' population (S_1 S_3 × S_1 S_4') where the *S*-locus would only be placed on the 'Regina' map as all the progeny would have the S_4' allele from 'Lapins.'

Because the NY and EF populations consisted of reciprocal crosses, we were able to examine the influence of gamete sources from each parent on the observed marker distortion. A 30-cM span from the top of EF G2 to the marker UDAp-461 was the only region where linked

Table 5 Number and type of marker, length, marker density and marker gap length for the 'Emperor Francis' (EF) and 'NY 54' (NY) maps and their individual linkage groups

	Parental maps			Linkage groups														
Marker type	Total	EF	NY	EF1	NY1	EF2	NY2	EF3	NY3	EF4	NY4	EF5	NY5	EF6	NY6	EF7	NY7	EF8	NY8
SSR	102	84	51	13	7	15	8	13	5	8	5	11	4	11	10	8	8	5	4
AFLP	61	41	23	5	4	4	4	6	2	3	0	4	0	6	4	2	3	11	6
SRAP	7	4	3	0	0	0	0	0	0	0	0	1	0	1	0	2	1	0	2
Gene-derived	27	15	14	2	2	2	1	2	1	0	0	3	2	4	6	1	1	1	1
Total	197	144	91	20	13	21	13	21	8	11	5	19	6	22	20	13	13	17	13
Map statistics																			
Length (cM)		711.11	565.8	143.0	147.9	96.0	91.5	82.4	30.7	84.5	30.9	83.5	20.8	75.7	84.1	83.6	73.2	62.4	86.7
Marker density (markers/cM)		0.2	0.16	0.14	0.09	0.22	0.14	0.25	0.26	0.13	0.16	0.23	0.29	0.29	0.24	0.16	0.18	0.27	0.15
Average marker distance (cM)		4.94	6.22	7.2	11.4	4.6	7.0	3.9	3.8	7.7	6.2	4.4	3.5	3.4	4.2	6.4	5.6	3.7	6.7
Largest gap (cM)		29.5	33.5	29.5	33.5	18.3	25.0	16.3	10.2	13.2	18.1	11.1	11.4	7.1	21.4	16.3	11.1	9.2	16.7
Gaps>15 cM (n)		18	11	2	5	1	1	1	0	0	1	0	0	0	1	2	0	0	1
Average cM/ linkage group		88.9	70.7																

markers were distorted in both reciprocal crosses. In all other cases, segregation distortion was only present from the pollen parents (data not presented), in that one of the two alleles in the pollen parent was present in more than 50% of the offspring. This observation is similar to that described by Foulongne et al. (2003) in which gametophytic selection causing distorted segregation in a peach × *P. davidiana* F_2 population was assumed to occur only among male gametes.

For both EF and NY, the marker profile on G8 was unique in that SSR and gene-derived marker polymorphisms were low resulting in only six and seven markers on the EF and NY linkage groups, respectively. However, G8 was supplemented with 11 and six AFLP markers in EF and NY, respectively, resulting in a well-built linkage group. In contrast, in a peach F_2 population (Ferjalou Jalousia × Fantasia), G8 was not identified at all as all of the 181 molecular markers mapped to the first seven linkage groups (Dirlewanger et al. 2006). Similarly, G8 could not be resolved from a BC_2 population from *P. persica* × *P. davidiana* as only one G8 marker was polymorphic (Quilot et al. 2004). Interestingly, two of our candidate gene-derived markers representing sorbitol transporters *SorT*1 (PR25/22) and *SorT*2 (PR27) mapped to EF G8 and NY G8, respectively (Table 4, Fig. 1). The cherry ortholog of *FW2.2* (PR26) was placed on EF G5.

Despite screening many publicly available SSR markers that were placed on the sweet cherry cv. 'Regina' and 'Lapins' partial linkage maps, only 16 SSR markers were in common with those placed on the EF and NY maps (Dirlewanger et al. 2004a). The maximum number of shared markers per linkage group was four for EF G2 and only three other linkage groups shared three common markers (EF G1, NY G2, and EF G5). In most cases, marker order was in agreement. Interestingly, two markers, BPPCT034 and BPPCT002, were in a similar order in NY G2 and 'Lapins' G2 but a reversed order in EF and 'Regina.'

The analysis of the shared markers between the NY and EF parents showed a conservation of marker and gene order for the two genomes. However, the NY parent exhibited several linkage groups smaller than those of EF. In particular, the NY linkage groups 3, 4, and 5 were less than half the centimorgan lengths of the respective EF linkage groups. Based on an analysis of the shared markers, this can be explained by the low overall level of heterozygosity of the NY parent. This analysis allowed us to determine that the partial G3 and G4 identified for NY represented the top segments of these linkage groups, whereas the partial NY G5 represents the bottom segment of G5. Our inability to resolve these genomic regions for NY suggests that NY may be homozygous for substantial regions of these three linkage groups. This was unexpected

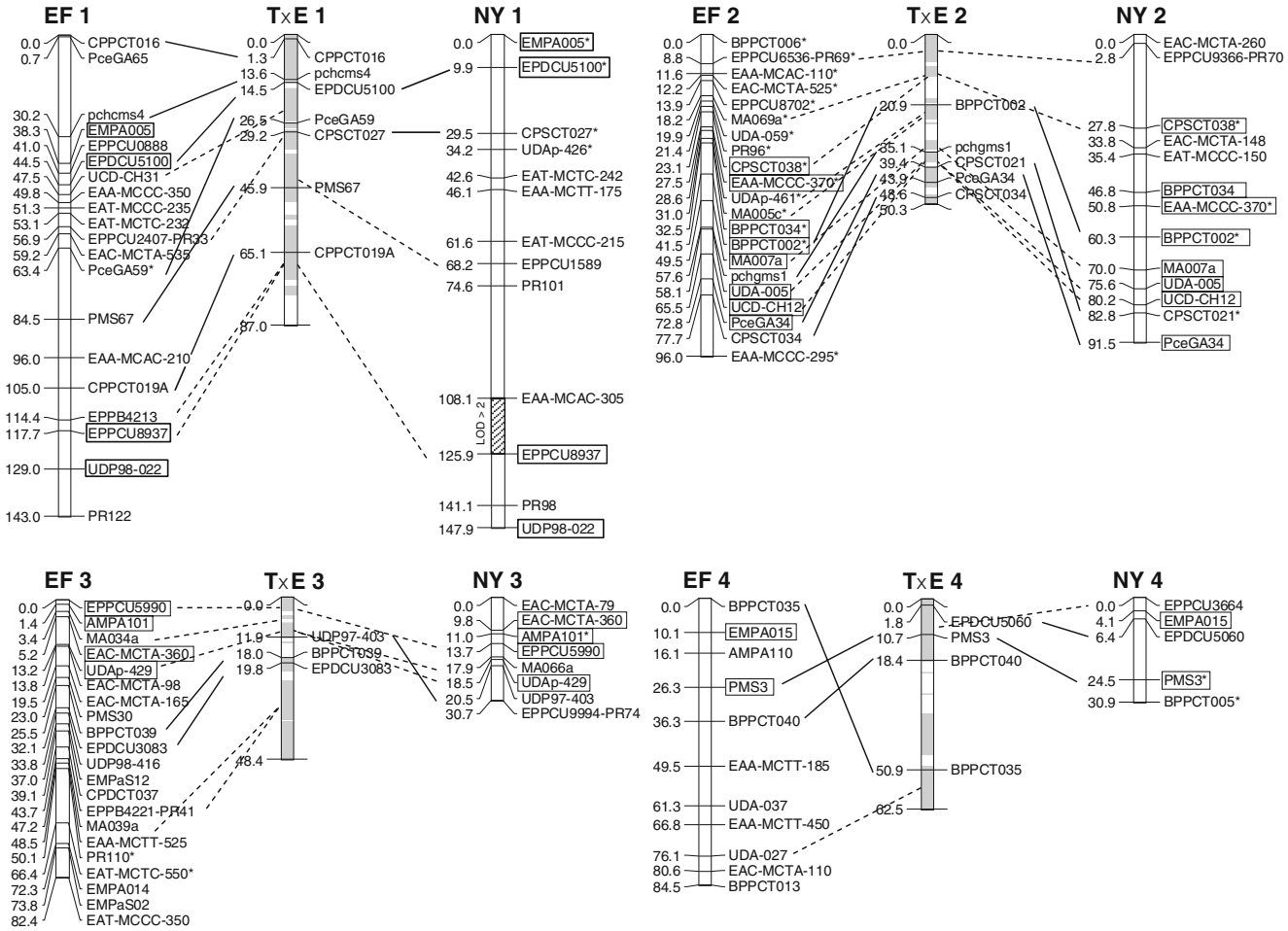

Fig. 1 Alignment of 'Emperor Francis' (*EF*) and 'New York 54' (*NY*) sweet cherry parental maps with the *Prunus* reference and bin map ['Texas' almond × 'Earlygold' peach (*T*×*E*)]. Only common markers present on EF and NY maps are presented on the *Prunus* reference map. *Shaded areas* on the reference map linkage groups indicate *Prunus* bin locations. *Solid lines* indicate homology between mapped markers; *dashed lines* indicate markers present in *Prunus* bins. *Boxed markers* indicate anchor points between the EF and NY parental maps. Markers followed by an *asterisk* indicate significant deviation ($P<0.05$) from the expected chi-square segregation value

in sweet cherry as it is an obligate outcrossing species with an active gametophytic self-incompatibility system. This outcrossing mechanism is controlled by alleles at the *S*-locus on G6; therefore, this region of G6 must by definition be heterozygous. Interestingly, NY G6 is longer than EF G6 (84.1 cM compared to 75.7 cM), suggesting that the self-incompatibility system has maintained heterozygosity for the *S*-locus and linked regions while other regions of the sweet cherry genome may have become homozygous.

Alignment with the T × E *Prunus* reference map and other *Prunus* maps

A total of 82 markers were shared between the EF and NY linkage maps and the T × E linkage and bin maps (Fig. 1). The number of shared markers for the eight linkage groups were as follows: G1, 12; G2, 14; G3, 9; G4, 7; G5, 10; G6, 12; G7, 10; and G8, 8. Nine of these 82 shared markers represented previously mapped peach ESTs, with the prefix EPPCU or EPPB (Table 1) for which cherry InDel, CAPS, or dCAPs markers had been designed. Of these nine markers, only one, PR74 (EPPCU9994), mapped to an alternate linkage group (Table 6) and PR41 (EPPB4221) mapped to an additional group in NY (Table 4) compared to its location on the T × E map. The other previously mapped gene-derived markers, *PrpFT* and the *Si S*-RNase, also mapped to their predicted locations on G6 (Fig. 1; Silva et al. 2005).

Of the 102 SSR markers located on the EF and NY linkage maps, only ten were placed on different linkage groups compared to the T × E map, bin map, apricot map (Dondini et al. 2007), or the interspecific myrobalan plum × (peach × almond) maps (Dirlewanger et al. 2004b; Table 6). BPPCT006 has previously been shown to be a multilocus marker and therefore could be expected to map elsewhere. UDAp-426 and UDAp-471 were placed in different linkage groups in apricot compared to T × E and BPPCT013, UDP96–019 and CPPCT029 were placed in different link-

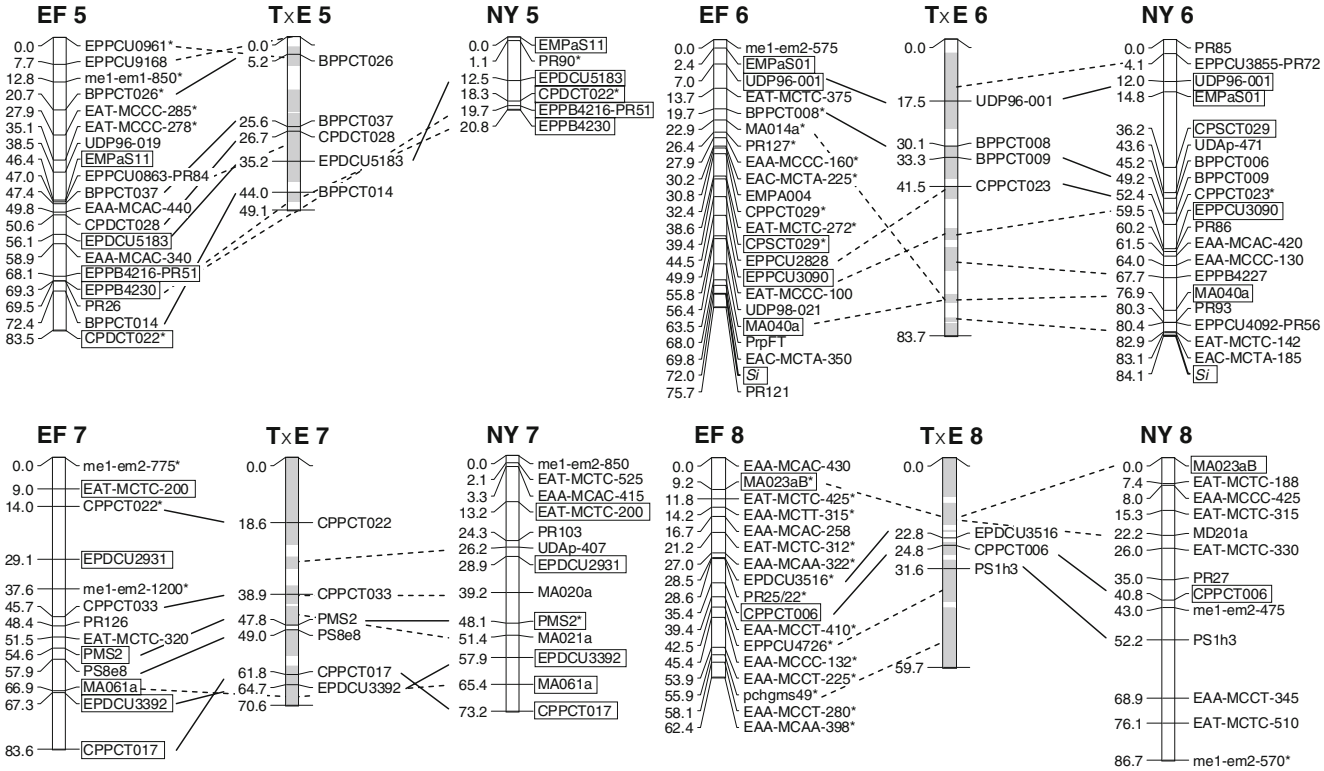

Fig. 1 (continued)

Table 6 List of markers that were placed on different 'Emperor Francis' (EF) and 'NY 54' (NY) linkage groups (G) compared to other *Prunus* maps with the exception of the cherry markers developed from physically mapped peach ESTs

Marker	G in EF and/or NY	G in other *Prunus* maps (reference)
UDAp-426	G1: NY	G7: Bin Map[a, b]; LG1: L, B[c]
BPPCT006	G2: EF; G6: NY	G8: T × E[a]; G1: P2175[d]; G3: B[c]
EPPCU8702	G2: EF	G3: Bin Map[a, b]
UDA-059	G2: EF	G1: Bin Map[a, b]
UDP98–416	G3: EF	G6: GN[d]
EPPCU9994 (PR74)	G3: NY	G6: Bin Map[a, b]
UDA-037	G4: EF	G2: Bin Map[a, b]
BPPCT013	G4: EF	G2: T × E[a]; G2: GN & P2175[d]
UDP96–019	G5: EF	G8: T × E, Bin Map[a, b]; G5: P2175[d]
CPPCT029	G6: EF	G1: T × E[a]; G2: GN[d]
UDAp-471	G6: NY	G1: Bin Map[a, b]; G7: L & B[c]

All the markers are SSRs derived from genomic sequences except for the two EPPCU markers that are derived from EST sequences

[a] T × E Bin Map (Genome Database for Rosaceae, http://www.bioinfo.wsu.edu/gdr)

[b] T × E Bin Map (Howad et al. 2005)

[c] L and B are maps derived from the apricot cultivars 'Lito' and 'BO81604311,' respectively (Dondini et al. 2007)

[d] P2175: map constructed from myrobalan plum, *Prunus cerasifera*, clone number P2175; GN map constructed from the almond × peach F_1 hybrid between Garfi × Nemared (Dirlewanger et al. 2004b)

age groups compared to plum and/or a peach × almond hybrid. It is possible that we have found additional loci in the cherry genome which would be one of the explanations for the discrepancy in marker position. The fact that, of these ten markers, all but two (EPPCU8702 and EPPCU9994) were derived from genomic DNA also suggests that these markers could represent repetitive regions within the *Prunus* genome.

Within each linkage group, an analysis of the 82 SSR and gene-derived markers shared between the EF and NY maps and the T × E linkage and bin maps revealed extensive conservation of marker order (Fig. 1). However, notable exceptions were observed. On EF G4, the marker BPPCT035 was placed at position 0.0 cM, but it is at position 46.4 cM on the T × E map. As the segregating fragment size of BPPCT035 from EF was large (~1,000 bp), it is possible that this marker identified an additional locus in EF. Another exception was MA014a that was placed at 22.9 cM on EF G6 and bin location 6.74 (G6 72 cM to 74.3 cM) on the T × E map. However, by far, the majority of the markers that were derived from other *Prunus* genetic maps mapped in the expected order in sweet cherry. Thus, our results demonstrate significant colinearity of the cherry and other *Prunus* species genomes.

The colinearity exhibited in sweet cherry using markers placed on *Prunus* linkage and bin maps was in stark contrast to our results using 14 EST-derived markers from

the *Prunus* physical map (Table 4, PR85, PR86, PR90, PR93, PR96, PR98, PR101, PR103, PR110, PR117, PR121, PR122, PR126, and PR127). The map positions of these 14 ESTs were based on hybridization to bacterial artificial chromosome (BAC) clones that carry peach genomic DNA inserts (Zhebentyayeva et al. 2006). These BAC clones were then anchored to the *Prunus* reference genetic map. However, most of the ESTs that were anchored to the physical map did not correspond to the expected location (Table 4). Only two (15%) of these ESTs mapped to the expected position (PR86 and PR96), whereas another two ESTs mapped to the predicted linkage group but not at the expected position (PR85 and PR93). As the comparison of the EF and NY linkage groups with the T × E linkage and bin maps identified significant regions of colinearity, the basis for the lack of correspondence for marker position for the physically mapped peach ESTs is not likely due to lack of synteny. Instead, our observations may be due to gene duplication that could have resulted in physical mapping of two paralogs in different positions depending on the mapping strategy that was used. However, only three of the 14 physically mapped peach ESTs that we utilized (PP_LEa0013A14f, PP_LEa0015I11f, and PP_LEa002D10f) have been shown to represent duplicate genes as they mapped to two locations on the peach physical map (Table 3). Nevertheless, our results demonstrate that developing markers for targeted regions in *Prunus* using ESTs placed on the peach physical map is highly inefficient.

Conclusions

In this study, two new intraspecific sweet cherry molecular linkage maps were constructed by leveraging all the marker resources developed for *Prunus* species. The transferability of SSR markers developed from other *Prunus* species to cherry was evaluated and showed a reduced level of amplification from cherry genomic DNA that likely represents the phylogenetic distance between cherry and the other *Prunus* species from which the SSRs were derived. In addition, linkage map construction was further hampered by the low level of polymorphism within EF and NY that was consistent with results from previous cherry linkage mapping studies. Therefore, additional markers were developed by converting peach ESTs into cherry InDel, dCAP, or CAP markers, plus the addition of four new candidate genes. All these markers, combined with AFLP and SRAP markers, permitted the construction of the sweet cherry linkage maps. However, further map development and identification of markers that are tightly linked to traits of importance would need much higher marker density than can be achieved using the available resources. The high level of colinearity observed between the sweet cherry and other *Prunus* genetic maps, especially with the genetically mapped EST markers, offers a future opportunity to continue to develop gene-derived markers that can be utilized across *Prunus* species and ultimately across genera within the Rosaceae.

Acknowledgement This project was supported by the National Research Initiative Competitive Grant 2005-35300-15454 from the US Department of Agriculture Cooperative State Research, Education, and Extension Service.

References

Abbott AG, Rajapakse S, Sosinski B, Lu ZX, Sossey-Alaoui K, Gannavarapu M, Reighard G, Ballard RE, Baird WV, Scorza R, Callahan A (1998) Construction of saturated linkage maps of peach crosses segregating for characters controlling fruit quality, tree architecture and pest resistance. Acta Hortic 465:41–49

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

Aranzana MJ, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF, Arús P (2003) A set of simple-sequence repeat markers covering the *Prunus* genome. Theor Appl Genet 106:819–825

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

Bošković R, Tobutt KR (1998) Inheritance and linkage relationships of isoenzymes in two interspecific cherry progenies. Euphytica 103:273–286

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

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

Choi C, Kappel F (2004) Inbreeding, coancestry, and founding clones of sweet cherries in North America. J Am Soc Hortic Sci 129:535–543

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

Clarke JB, Tobutt KR (2003) Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. Mol Ecol Notes 3:578–580

Decroocq V, Fave MG, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–922

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

Dickson EE, Arumuganathan K, Kresovich S, Doyle JJ (1992) Nuclear DNA content variation within the Rosaceae. Am J Bot 79:1081–1086

Dirlewanger E, Pronier V, Parvery C, Rothan C, Guye A, Monet R (1998) A genetic linkage map of peach (*Prunus persica* L. Batsch) using morphological, RFLP, isoenzyme, RAPD, and AFLP markers. Theor Appl Genet 97:888–895

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P, Laigret R (2002) Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127–138

Dirlewanger E, Graziano E, Joobeur T, Garriga-Caldere F, Cosson P, Howad W, Arús P (2004a) Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc Natl Acad Sci 101:9891–9896

Dirlewanger E, Cosson P, Howad W, Capdeville G, Bosselut N, Claverie M, Voisin R, Poizat C, Lafargue B, Baron O, Laigret F, Kleinhentz M, Arús P, Esmenjaud D (2004b) Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid—location of root-knot nematode resistance genes. Theor Appl Genet 109:827–838

Dirlewanger E, Cosson P, Boudehri K, Renaud C, Capdeville G, Tauzin Y, Laigret F, Moing A (2006) Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. Tree Genet Genomes 3:1–13

Dondini L, Lain O, Geuna F, Banfi R, Gaiotti F, Tartarini S, Bassi D, Testolin R (2007) Development of a new SSR-based linkage map in apricot and analysis of synteny with existing *Prunus* maps. Tree Genet Genomes 3:239–249

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

Foulongne M, Pascal T, Arús P, Kervella J (2003) The potential of *Prunus davidiana* for introgression into peach [*Prunus persica* (L.) Batsch] assessed by comparative mapping. Theor Appl Genet 107:227–238

Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci U S A 97:4718–4723

Gao Z, Maurousset L, Lemoine R, Yoo S-D, van Nocker S, Loescher W (2003) Cloning, expression, and characterization of sorbitol transporters from developing sour cherry fruit and leaf sink tissues. Plant Physiol 131:1–10

Hagen LS, Chaib J, Fady B, Decroocq V, Bouchet JB, Lambert P, Audergon JM (2004) Genomic and cDNA microsatellites from apricot (*Prunus armeniaca* L.). Mol Ecol Notes 4:742–745

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

Horn R, Lecouls A-C, Callahan A, Dandekar A, Garay L, McCord P, Howad W, Chan H, Verde I, Main D, Jung S, Georgi L, Forrest S, Mook J, Zhebentyayeva T, Yu Y, Kim HR, Jesudurai C, Sosinski B, Arús P, Baird V, Parfitt D, Reighard G, Scorza R, Tomkins, Wing R, Abbott AG (2005) Candidate gene database and transcript map for peach, a model species for fruit trees. Theor Appl Genet 110:1419–1428

Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriana G, Monforte AJ, Georgi L, Abbott AG, Arús P (2005) Mapping with a few plants: using selective mapping for micro-satellite saturation of the *Prunus* reference map. Genetics 171:1305–1309

Hurtado MA, Vilanova S, Romero C, Abbott AG, Llacer G, Badenes ML (2002) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.) based on molecular markers. Theor Appl Genet 105:182–191

Ikeda K, Ushijima K, Yamane H, Tao R, Hauck NR, Sebolt AM, Iezzoni AF (2005) Linkage and physical distances between the *S*-haplotype *S-RNase* and *SFB* genes in sweet cherry. Sex Plant Reprod 17:289–296

Joobeur T, Viruel MA, de Vicente MC, Jauregui B, Ballester J, Dettori MT, Verde I, Truco MJ, Messeguer R, Battle I, Quarta R, Dirlewanger E, Arús P (1998) Construction of a saturated linkage map for *Prunus* using an almond × peach F_2 progeny. Theor Appl Genet 97:1034–1041

Joobeur T, Periam N, de Vicente MC, King GJ, Arús P (2000) Development of a second generation linkage map for almond using RAPD and SSR markers. Genome 43:649–688

Konieczny A, Ausubel F (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Lambert P, Hagen LS, Arús P, Audergon JM (2004) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.) compared with the almond 'Texas' × peach 'Earlygold' reference map for *Prunus*. Theor Appl Genet 108:1120–1130

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:445–461

Lopes MS, Sefc KM, Laimer M, Da Camara Machado A (2002) Identification of microsatellite loci in apricot. Mol Ecol Notes 2:24–26

Lu Z-X, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998) Construction of a genetic linkage map and identification of AFLP markers or resistance to root-knot nematodes in peach rootstocks. Genome 41:199–207

Marchese A, Tobutt KR, Raimondo A, Motisi A, Bošković RI, Clarke J, Caruso T (2007) Morphological characteristics, microsatellite fingerprinting and determination of incompatibility genotypes of Sicilian sweet cherry cultivars. J Hortic Sci Biotech 82:41–48

Messina R, Lain O, Marrazzo MT, Cipriani G, Testolin R (2004) New set of microsatellite loci isolated in apricot. Mol Ecol Notes 4:432–434

Michaels SD, Amasino RM (1998) A robust method for detecting single-nucleotide changes as polymorphic markers by PCR. Plant J 14:381–385

Mnejja M, Garcia-Mas J, Howad W, Badenes ML, Arús P (2004) Simple sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. Mol Ecol Notes 4:163–166

Mnejja M, Garcia-Mas J, Howad W, Arús P (2005) Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. Mol Ecol Notes 5:531–535

Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J 14:387–392

Pedersen BH (2006) DNA fingerprints of 51 sweet and sour *Prunus* accessions using simple sequence repeats. J Hortic Sci Biotech 81:118–124

Quilot B, Wu BH, Kervella J, Genard M, Foulongne M, Moreau K (2004) QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild related species *P. davidiana*. Theor Appl Genet 109:884–897

Rajapakse S, Belthoff LE, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995)

Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90:503–510

Schueler S, Tusch A, Schuster M, Ziegenhagen B (2003) Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.)—markers for individual identification and reproductive processes. Genome 46:95–102

Schueler S, Tusch A, Scholz F (2006) Comparative analysis of the within-population genetic structure in wild cherry (*Prunus avium* L.) at the self-incompatibility locus and nuclear microsatellites. Mol Ecol 15:3231–3243

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Shaw J, Small RL (2004) Addressing the "hardest puzzle in American pomology": phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. Am J Bot 91:985–996

Silva C, Garcia-Mas J, Sanchez AM, Arús P, Oliveira MM (2005) Looking into flowering time in almond (*Prunus dulcis* (Mill) D. A. Webb): the candidate gene approach. Theor Appl Genet 110:959–968

Sonneveld T, Robbins TP, Boskovic R, Tobutt KR (2001) Cloning of six cherry self-incompatibility alleles and development of allele-specific PCR detection. Theor Appl Genet 102:1046–1055

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

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

Stoeckel S, Grange J, Fernandez-Manjarres F, Bilger I, Frascaria-Lacoste N, Mariette S (2006) Heterozygote excess in a self-incompatible and partially clonal forest tree species—*Prunus avium* L. Mol Ecol 15:2109–2118

Struss D, Boritzki M, Karle R, Iezzoni AF (2002) Microsatellite markers differentiate eight Giessen cherry rootstocks. HortScience 37:191–193

Struss D, Ahmad R, Southwick SM, Boritzki M (2003) Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. J Am Soc Hortic Sci 128:904–909

Tao R, Yamane H, Sugiura A, Murayama H, Sassa H, Mori H (1999) Molecular typing of *S*-alleles through identification, characterization and cDNA cloning for *S*-RNases in sweet cherry. J Am Soc Hortic Sci 124:224–233

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

Testolin R, Messina R, Lain O, Marrazzo MT, Huang WG, Cipriani G (2004) Microsatellites isolated in almond from an AC-repeat enriched library. Mol Ecol Notes 4:459–461

Van Ooijen JW, Voorrips RE (2001) JoinMap® 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vaughan SP, Russell K (2004) Characterization of novel microsatellites and development of multiplex PCR for large-scale population studies in wild cherry, *Prunus avium*. Mol Ecol Notes 4:429–431

Vilanova S, Romero C, Abbott AG, Llacer G, Badenes ML (2003) An apricot (*Prunus armeniaca* L.) F_2 progeny linkage map based on SSR and AFLP markers, mapping plum pox virus resistance and self-incompatibility traits. Theor Appl Genet 107:239–247

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

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Heredity 93:77–78

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

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

Wünsch A, Hormaza JI (2002) Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences. Heredity 89:56–63

Yamamoto T, Shimada T, Imai T, Yaegaki H, Haji T, Matsuta N, Yamaguchi M, Hayashi T (2001) Characterization of morphological traits based on a genetic linkage map in peach. Breeding Sci 51:271–278

Yamamoto T, Mochida K, Imai T, Shi YZ, Ogiwara I, Hayashi T (2002) Microsatellite markers in peach (*Prunus persica* (L.) Batsch) derived from an enriched genomic and cDNA libraries. Mol Ecol Notes 2:298–301

Yamamoto T, Yamaguchi M, Hayashi T (2005) An integrated genetic linkage map of peach by SSR, STS, AFLP and RAPD. J Jpn Soc Hort Sci 74:203–213

Yamane H, Tao R, Sugiura A, Hauck NR, Iezzoni AF (2001) Identification and characterization of *S*-RNases in tetraploid sour cherry (*Prunus cerasus* L.). J Am Soc Hort Sci 126:661–667

Zhebentyayeva TN, Hort R, Mookj S, Lecouls A-C, Georgi L, Swire-Clark G, Reighard GL, Baird WV, Abbott AG (2006) A physical framework for the peach genome. Acta Hortic 713:83–88