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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.)

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Abstract We report the sequence of 41 primer pairs of microsatellites from a CT-enriched genomic library of the peach cultivar 'Merrill O'Henry'. Ten microsatellite-containing clones had sequences similar to plant coding sequences in databases and could be used as markers for known functions. For microsatellites segregating at least in one of the two *Prunus* F_2 progenies analyzed, it was possible to demonstrate Mendelian inheritance. Microsatellite polymorphism was evaluated in 27 peach and 21 sweet cherry cultivars. All primer pairs gave PCR-amplification products on peach and 33 on cherry (80.5%). Six PCR-amplifications revealed several loci (14.6%) in peach and eight (19.5%) in sweet cherry. Among the 33 single-locus microsatellites amplified in peach and sweet cherry, 13 revealed polymorphism both in peach and cherry, 19 were polymorphic only on peach and one was polymorphic only on cherry. The number of alleles per locus ranged from 1 to 9 for peach and from 1 to 6 on sweet cherry with an average of 4.2 and 2.8 in peach and sweet cherry, respectively. Cross-species amplification was tested within the *Prunus* species: *Prunus avium* L. (sweet cherry and mazzard), *Prunus cerasus* L. (sour cherry), *Prunus domestica* L. (European plum), *Prunus amygdalus* Batsch. (almond), *Prunus armeniaca* L. (apricot), *Prunus cerasifera* Ehrh. (Myrobalan plum). Plants from other genera of the Rosaceae were also tested: *Malus* (apple) and *Fragaria* (strawberry), as well as species not belonging to the Rosaceae: *Castanea* (chestnut tree), *Juglans* (walnut tree) and *Vitis* (grapevine). Six microsatellites gave amplification on all the tested species. Among them, one had an amplified region homologous to sequences encoding a MADS-box protein in *Malus* × *domestica*. Twelve microsatellites (29.3%) were amplified in all the Rosaceae species tested and 31 (75.6%) were amplified in all the six *Prunus* species tested. Thirty three (80.5%), 18 (43.9%) and 13 (31.7%) gave amplification on chestnut tree, grapevine and walnut tree, respectively.

Keywords *Prunus* · Peach · Cherry · Rosaceae · Microsatellites · SSR · Fingerprinting · Genetic diversity · Synteny

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Introduction

Peach [*Prunus persica* (L.) Batsch] and sweet cherry (*Prunus avium* L.) are two species belonging to the *Prunus* genus which includes other economically important species such as sour cherry (*Prunus cerasus* L.), apricot (*Prunus armeniaca* L.), almond (*Prunus amygdalus* Batsch), European plum (*Prunus domestica* L.) and myrobalan plum (*Prunus cerasifera* Ehrh). They are members of the Rosaceae, which ranks as the third most-agronomical important plant family in temperate regions and includes fruit (apple, strawberry), forest (mazzard) and ornamental species (rose). *P. persica* and *P. avium* have a diploid (2n = 16) small-size genome, with 262 and 338 M base pairs/1C, respectively, about twice the size of the *Arabidopsis thaliana* genome (145 M base pairs/1C) (Arumuganathan and Earle 1991).

Peach is a self-fertile and naturally self-pollinating fruit species, while most sweet cherry cultivars are self-incompatible with many cross-incompatibility groups. In commercial orchards, cross-compatible cultivars belonging to different pollen incompatibility groups and flowering simultaneously must be co-cultured to ensure fruit set.

French peach and, to a less extent, cherry orchards are characterized by a great diversity of varieties with a fast turnover. The ability to distinguish peach and cherry cultivars would be greatly enhanced by the use of molecular markers. A previous study analyzed the genetic distances between ten peach varieties using isoenzymes (Reynders and Monet 1987) but, because of their common parental genetic background, the polymorphism was too low to distinguish them (Hesse 1975; Scorza et al. 1985). Due to self-incompatibility, the genetic diversity among cultivars is higher in cherry than in peach and isozymes gave satisfying results in distinguishing cherry cultivars (Granger et al. 1993). Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been evaluated for their potential use in peach cultivar identification (Dirlewanger et al. 1998a), but their dominant expression, poor reproducibility (RAPDs), or the complexity of the method (AFLPs), limits their use as routine markers for this purpose.

In our search for molecular markers useful for cultivar identification in peach and cherry, and for synteny analysis in *Prunus*, the microsatellites or simple sequences repeats (SSRs) seem the best candidates. These short tandem repeats of DNA, with a polymorphism based on different numbers of a repeated motif at a given locus, are becoming the markers of choice in many plant-breeding programs since they are multi-allelic and codominant markers, PCR-based, easily reproducible, randomly and widely distributed along the genome, and amenable to automation (Rafalski et al. 1996). Several microsatellite markers have already been reported in peach: 26 from AC- and AG-enriched libraries (Cipriani et al. 1999; Testolin et al. 2000) and ten from a non-enriched cDNA and genomic libraries (Sosinski et al. 2000). In the present paper we report 41 additional microsatellites from peach and explore their potential as reliable markers for germplasm characterization in peach and cherry. We also demonstrate that most of them are transportable within *Prunus* species, in other genera belonging to the Rosaceae and even in other families. In the future, SSRs will be used to complete already existing *Prunus* maps (Dirlewanger et al. 1998b; Joobeur et al. 1998) and to compare and merge in *Prunus* linkage maps.

Materials and methods

Construction of a genomic library enriched for microsatellites

Genomic DNA was extracted from the peach cultivar ‘Merrill O’Henry’ and was enriched for CT repeats according to the procedure described by Billote et al. (1999) with modifications introduced by Aranzana et al. (2002).

Plaque screening for positive clones

Filters of the clones were produced by transferring the colonies onto a Hybond–N+ (Amersham International) nylon filter according to standard procedures. Microsatellite-containing plasmids were screened by hybridization with a $(CT)_{15}$ probe according to the Amersham procedure. The probe was end-labeled with γ ^{33}P-ATP, using T4 polynucleotide kinase (Promega). The filters were then exposed to Kodak X-Omat AR X-ray film overnight at –80 °C and the microsatellite-containing clones were identified by observation of a hybridization signal.

Microsatellite identification

Microsatellite-containing clones were sent to Genome Express (Grenoble, France) for extracting, purifying and sequencing the inserts. Primer pairs were designed for PCR amplification in the microsatellite flanking regions using PRIMER software package v.0.5 (S. Lincoln and co-workers, Whitehead Institute of Biochemical Research, Cambridge, Mass.). The primer pairs were designed to be 17–24-bp long with an annealing temperature between 57 °C and 60 °C and to give an expected product size of 100–250 bp. Microsatellite markers were coded as ‘BPPCT X’, where the acronym stays for Bordeaux *Prunus persica* microsatellites isolated from the library enriched in CT repeats, and X being a three-digit number specific of each microsatellite. Homologous proteins to the amino-acid sequence derived from the microsatellite nucleotide sequence were searched in the NCBI internet site (http://www.ncbi.nlm.nih.gov) with the Blastx algorithm (Altschul et al. 1997).

PCR amplification and visualization of the microsatellites

Peach microsatellites were PCR-amplified as follows: PCR buffer (20 mM Tris-HCl, 50 mM KCl), 1.5 mM of $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 0.7 U of *Taq* DNA polymerase (Gibco BRL) and 20 ng of peach genomic DNA in a 15-μl final volume. PCR reactions were performed on a GeneAmp 9600 thermal cycler (Perkin-Elmer Cetus) by an initial denaturation for 1 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 57 °C, 2 min at 72 °C; and a final extension of 4 min at 72 °C. Five microliters of the PCR products were separated on a 2% agarose gel and stained with ethidium bromide to check the PCR amplification and determine approximately the size of the amplified fragments. The PCR products were then denatured by the addition of 1 vol of 95% formamide/dye solution (loading dye: 95% deionized formamide, 10 mM NaOH, 0.05% xylene cyanol, 0.05% bromophenol blue), heating for 5 min at 94 °C, chilled on ice and then 1.5 μl of the denatured preparation were loaded on a 6% polyacrylamide sequencing gels containing 7.5 M of urea in 0.5 × TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). Gels were run for 2 h at 80 W. Following electrophoresis, the gel was silver-stained according to the protocol described by Cho et al. (1996). Fragment sizes were estimated with the 10-bp ladder-DNA sizing markers (GibcoBRL, Life TEchnologies).

Segregation analyses

Segregation analyses of microsatellites were performed on 20 individuals originated from the intraspecific peach F_2 population derived from a cross between ‘Jalousia’ and ‘Fantasia’, named (J × F), and 20 individuals originated from the interspecific F_2 *P. amygdalus* cv ‘Texas’ × *P. persica* cv ‘Earlygold’, named (T × E). Those two F_2 populations were already used to build linkage maps (Dirlewanger et al. 1998b; Joobeur et al. 1998).

Analysis of microsatellite information content in peach and cherry

The information content of microsatellite loci was estimated by the observed heterozygosity (Ho), the expected heterozygosity (He = $1–\Sigma\ pi^2$, where pi is the frequency of the i^{th} allele), and by the discrimination power (DP, Kloosterman et al. 1993), according to

Table 1 List of peach and sweet cherry cultivars tested in this study, their main agronomic characteristics and origin

Cultivars	Flower type	Petiole gland shape	Fruit type	Flesh colour	Origin
Peach (*Prunus persica*)					
Belle de Montélimar	Non-showy	Reniform	Peach	White	France
Bradember	Showy	Reniform	Clingstone nectarine	Yellow	USA (N. & C. Bradford)
Catherina	Showy	Reniform	Clingstone peach	Yellow	USA/France (C. Bailey/INRA)
Colomba	Showy	Globose	Peach	White	France (INRA)
Desertgold	Showy	Reniform	Peach	Yellow	USA (USDA)
Fantasia	Showy	Globose	Nectarine	Yellow	USA (USDA)
Ferbar	Showy	Reniform	Peach	White	France (INRA)
Ferjalou	Showy	Globose	Peach	Yellow	France (INRA)
Fuzalode	Showy	Reniform	Nectarine	White	France (INRA)
Grabelle	Non-showy	Reniform	Peach	Yellow	USA (G. Merrill)
Julie	Showy	Reniform	Peach	White	France (A. Maillard)
Klamt	Non-showy	Globose	Clingstone peach	Yellow	USA (L.D. Davis)
Maygold	Non-showy	Globose	Peach	Yellow	USA (USDA)
Merriam	Non-showy	Reniform	Clingstone peach	Yellow	USA (L.D. Davis)
Michelini	Showy	Reniform	Peach	White	Italy (A.Michelini)
Nemared	Showy	Reniform	Peach	White	USA (USDA)
Redhaven	Non-showy	Reniform	Peach	Yellow	USA (S. Johnston)
Robin	Showy	Globose	Peach	White	USA (D.L. Armstrong)
Royale de Barsac	Showy	Reniform	Peach	Yellow	France
Sénateur Cazeneuve	Showy	Globose	Peach	Yellow	France
Sensation	Showy	Reniform	Peach	Yellow	France (A. Maillard)
Sibelle	Showy	Reniform	Peach	Yellow	France (A. Maillard)
Snow Queen	Non-showy	Reniform	Nectarine	White	USA (D.L. Armstrong)
Summergrand	Showy	Reniform	Nectarine	Yellow	USA (F.W. Anderson)
Symphonie	Showy	Reniform	Peach	Yellow	France (A. Maillard)
Zainara	Non-showy	Globose	Peach	White	USA (F. Zaiger)
Zaitibe	Non-showy	Reniform	Peach	White	USA (F. Zaiger)

Cultivars	Maturity	Peduncle length	Fruit type	Fruit size	Fruit colour	Origin
Sweet cherry (*Prunus avium*)						
Bianca di Verona	Late	Medium	Bigarreau	Medium	White	Italy
Bing	Late	Medium	Bigarreau	Large	Red	USA (USDA)
Brooks	Early	Short	Bigarreau	Large	Red	USA (University of Davis)
Burlat	Early	Medium	Bigarreau	Medium	Red	France (Mr Burlat)
Sato Nishiki	Late	Short	Bigarreau	Medium	White	Japan
Enjidel C473	Intermediate	Short	Bigarreau	Large	Red	France (Mr Argot)
Fercer	Intermediate	Medium	Bigarreau	Large	Black	France (INRA)
Ferprime	Early	Short	Bigarreau	Medium	Red	France (INRA)
Garnet	Late	Short	Bigarreau	Large	Black	USA (California)
Géant d'Hedelfingen	Late	Medium	Bigarreau	Medium	Black	Germany
Graffioni	Intermediate	Medium	Bigarreau	Large	White	Italy
Grosse Schwarzkorpel	Late	Long	Bigarreau	Large	Black	Germany
Kordia	Intermediate	Long	Bigarreau	Large	Black	Germany (G. Schmidt)
Lapins	Intermediate	Medium	Bigarreau	Medium	Black	USA (Summerland)
Napoléon	Intermediate	Medium	Bigarreau	Large	White	Germany
Pico Color	nc[a]	nc	Bigarreau	nc	nc	Spain
Regina	Late	Long	Bigarreau	Medium	Black	Germany
Reverchon	Early	Medium	Bigarreau	Large	Black	Italy
Sumini	Intermediate	Short	Bigarreau	Large	Red	USA (Summerland)
Van	Intermediate	Short	Bigarreau	Medium	Black	Canada
Witzenhausener Frühe	nc	nc	Bigarreau	nc	nc	Germany

[a] nc, not confirmed in our collection

the formula as above, for which the allele frequency was replaced by the genotype frequency. The three indexes were evaluated on a set of 27 peach and 21 sweet cherry cultivars (Table 1). Peaches and sweet cherries were chosen to cover a broad range of the morphological variation of each species. The peach cultivars include a showy or non-showy flower type, a reniforme or globose petiole gland shape, a peach, nectarine, clingstone or freestone type of fruit and a white or yellow flesh color. Sweet cherries were chosen for their most-variable agronomic characteristics, i.e. the maturity date, the peduncle length, the fruit size and color. Both, peaches and sweet cherries originated from Europe or the USA.

Estimation of genetic diversity in peach and sweet cheery

Genetic diversity analysis with SSRs was performed on the same set of peach and sweet cherry cultivars previously described. For single-locus microsatellites, the presence of an allele was scored

Table 2 Primer sequences, repeat motif, and PCR product sizes of 41 microsatellites sequenced from the CT-enriched genomic library of peach cultivar 'Merrill O'Henry'

SSR name	Primer sequence (5′→ 3′)	Repeat motif	Length (bp)[a]
BPPCT 001	AAT TCC CAA AGG ATG TGT ATG AG CAG GTG AAT GAG CCA AAG C	$(GA)_{27}$	159
BPPCT 002	TCG ACA GCT TGA TCT TGA CC CAA TGC CTA CGG AGA TAA AAG AC	$(AG)_{25}$	229
BPPCT 004	CTG AGT GAT CCA TTT GCA GG AGG GCA TCT AGA CCT CAT TGT T	$(CT)_{22}$	200
BPPCT 005	GCT AGC AGG GCA CTT GAT C ACG CGT GTA CGG TGG AT	$(AG)_{10}$	143
BPPCT 006	GCT TGT GGC ATG GAA GC CCC TGT TTC TCA TAG AAC TCA CAT	$(AG)_{19}$	117
BPPCT 007	TCA TTG CTC GTC ATC AGC CAG ATT TCT GAA GTT AGC GGT A	$(AG)_{22}$ $(CG)_2$ $(AG)_4$	149
BPPCT 008	ATG GTG TGT ATG GAC ATG ATG A CCT CAA CCT AAG ACA CCT TCA CT	$(GA)_{36}$	148
BPPCT 009	ATT CGG GTC GAA CTC CCT ACG AGC ACT AGA GTA ACC CTC TC	$(CT)_{14}$	171
BPPCT 010	AAA GCA CAG CCC ATA ATG C GTA CTG TTA CTG CTG GGA ATG C	$(AG)_4$ $GG(AG)_{10}$	131
BPPCT 011	TCT GAG GGC TAG AGT GGG C TGT TTC AGG AGT CGA ACA GC	$(CT)_{16}$	172
BPPCT 012	ACT TCC ATT GTC AGG CATC A GGA GCA ACG ATG GAG TGC	$(CT)_{13}$ $CC(CT)_7$	164
BPPCT 013	ACC CAC AAA TCA AGC ATA TCC AGC TTC AGC CAC CAA GC	$(AG)_{28}$	183
BPPCT 014	TTG TCT GCC TCT CAT CTT AAC C CAT CGC AGA GAA CTG AGA GC	$(AG)_{23}$	215
BPPCT 015	ATG GAA GGG AAG AGA AAT CG GTC ATC TCA GTC AAC TTT TCC G	$(AG)_{13}$	150
BPPCT 016	GAT TGA GAG ATT GGG CTG C GAG GAT TCT CAT GAT TTG TGC	$(AG)_{14}$	96
BPPCT 017	TTA AGA GTT TGT GAT GGG AAC C AAG CAT AAT TTA GCA TAA CCA AGC	$(GA)_{28}$	174
BPPCT 018	CTC AAC TGC TGT CCT CAC TTC CAT GTC TGA TCC TAA CCC CA	$(AG)_{18}$	222
BPPCT 019	TGA TAC CAC CAT CCA ATC TAG C TTG CTG GGA CAT GGT CAG	$(CT)_{28}$	194
BPPCT 020	CGT GGA TGG TCA AGA TGC ATT GAC GTG GAC TTA CAG GTG	$(AG)_{14}$ $GG(AG)_7$ $AT(AG)_8$	200
BPPCT 021	TGC ATG AGA AAC TTG TGG C CCA AGA GCC TGA CAA AGC	$(GA)_{24}$	240
BPPCT 022	TTG CGT CTC GCA GGT TAT A CTA CCC CTG CCA CAA GCT	$(AG)_{22}$	132
BPPCT 023	TGC AGC TCA TTA CCT TTT GC AGA TGT GCT CGT AGT TCG GAC	$(CT)_{21}$	224
BPPCT 024	GAG GAA TGT GCC TCT TCT GG CTC CCG TAC GCG TTT ACC	$(AG)_{15}$	96
BPPCT 025	TCC TGC GTA GAA GAA GGT AGC CGA CAT AAA GTC CAA ATG GC	$(GA)_{29}$	197
BPPCT 026	ATA CCT TTG CCA CTT GCG TGA GTT GGA AGA AAA CGT AAC A	$(AG)_8$ $GG(AG)_6$	134
BPPCT 027	CTC TCA AGC ATC ATG GGC TGT TGC CCG GTT GTA ATA TC	$(GA)_{11}$	249
BPPCT 028	TCA AGT TAG CTG AGG ATC GC GAG CTT GCC TAT GAG AAG ACC	$(TC)_{15}$	164
BPPCT 029	GGA CGG ACA GAA ATG AAG GT CCT TAA CCC ACG CAA CTC C	$(GA)_{12}$ $(CAGA)_4$	159
BPPCT 030	AAT TGT ACT TGC CAA TGC TAT GA CTG CCT TCT GCT CAC AC C	$(AG)_{25}$	175
BPPCT 031	CTG GGG AGA AGA AGT GGC GCT TTC ATG CCA CCT CTC TA	$(GA)_{35}$	119
BPPCT 032	TTA AGC CAC AAC ATC CAT GAT AAT GGT CTA AGG AGC ACA CG	$(AG)_{10}$ $CG(AG)_{13}$	203
BPPCT 033	GTA GCC GGA GCC GTG TAT CTA GAA CCC TAT AAA CAC ATG GC	$(AG)_{32}$	180
BPPCT 034	CTA CCT GAA ATA AGC AGA GCC AT CAA TGG AGA ATG GGG TGC	$(GA)_{19}$	228
BPPCT 035	TGA AGG ATG GCT CTG ATA CC AAT TCA TCT ACT TCT TCC TCA AGC	$(GA)_{33}$	113

Table 2 (continued)

SSR name	Primer sequence (5′→ 3′)	Repeat motif	Length (bp)[a]
BPPCT 036	AAG CAA AGT CCA TAA AAA CGC GGA CGA AGA CGC TCC ATT	$(AG)_{11}$	253
BPPCT 037	CAT GGA AGA GGA TCA AGT GC CTT GAA GGT AGT GCC AAA GC	$(GA)_{25}$	155
BPPCT 038	TAT ATT GTT GGC TTC TTG CAT G TGA AAG TGA AAC AAT GGA AGC	$(GA)_{25}$	135
BPPCT 039	ATT ACG TAC CCT AAA GCT TCT GC GAT GTC ATG AAG ATT GGA GAG G	$(GA)_{20}$	154
BPPCT 040	ATG AGG ACG TGT CTG AAT GG AGC CAA ACC CCT CTT ATA CG	$(GA)_{14}$	135
BPPCT 041	CAA TAA GGC ATT TGG AGG C CAG CCG AAC CAA GGA GAC	$(AG)_{21}$	220
BPPCT 042	AAC CCT ACT GGT TCC TCA GC GAC CAG TCC TTT AGT TGG AGC	$(CT)_{25}$	243

[a] The length was determined from the sequencing results for the isolated Merryl O'Henry clones

as 0.5 if the individual was heterozygous, 1 if the parent was homozygous, and 0 when the allele was not present. All analyses were performed with NTSYS-pc 2.0 package (Rohlf 1997). Genetic similarity was measured with the SIMGEND program, which computes similarity coefficients for genetic data. Genetic distance was calculated according to Rogers (1972). Trees were produced by clustering the data with the unweighted pair group method using arithmetic average (UPGMA) with the SAHN-clustering and TREE programs.

Cross-species amplification

All microsatellite primers were used to amplify the DNA of the following *Prunus* species: *P. avium* (four mazzard clones from France), *P. cerasus* (sour cherry 'Montmorency' and 'Griotte du Nord'), *P. domestica* (European plum 'Najbolja' and 'Pozegaca'), *P. amygdalus* (almond 'Texas' and 'Garfi'), *P. armeniaca* (apricot 'Screara' and 'Stark Early Orange'), *P. cerasifera* ('P2175' and 'P2980' clones, INRA, France). Cross-genus amplification was also tested in *Malus* × *domestica* (apple 'Discovery' and 'TN10-8' clone), *Fragaria* × *ananassa* (strawberry 'Capitola' and 'CF1116' clone, Ciref, France) and, outside the Rosaceae, in *Castanea* (chestnut tree *Castanea crenata* × *Castanea sativa* 'Marigoule' and *Castanea sativa* 'CA 97' clone, France), *Juglans regia* (walnut-tree 'Franquette' and 'UK 6.2' clone, Ukraine), and *Vitis* (grapevine, *Vitis vinifera* 'Cabernet Sauvignon' and *Vitis riparia* 'Riparia Gloire'). PCR amplifications and agarose gel electrophoresis were performed according to the procedure previously described for peach and cherry.

Results

Microsatellite identification and characterization

One hundred and ninety one recombinant plasmids from the CT-enriched DNA library were sequenced. One hundred and sixty nine (88.5%) plasmids gave non-ambiguous sequences with an average size of 488 bp. Among them, 162 (84.8%) contained microsatellite sequences, 104 were unique sequences (61.5%) and 58 were present in several copies. The most-redundant sequences were found eight and nine times. For 17 of the 104 unique sequences, the number of nucleotides before or after the microsatellite was too small to allow the design of primers suitable for PCR amplification. Thus, 87 (83.6%) among the 104 unique sequences were usable to design primer pairs for microsatellite amplification. Among those, 42 primer pairs were designed and 41 gave PCR amplification products (Table 2). Most microsatellites (34) were simple, with a number of repeats ranging from 10 to 36, and an average of 20 repeats per microsatellite. For ten microsatellite sequences, a protein homologous to the derived amino-acid sequence was found in the Genbank database (http://www.ncbi.nlm.nih.gov) (Table 3). These peach sequences were introduced into the Genbank database and accession numbers are given in Table 3. Two of the deduced amino-acid sequences were found to be similar to proteins from *Malus* × *domestica* species: a MADS-box protein and a Constans-like protein; another sequence corresponded to a hypothetical protein from *Oryza sativa* and the remaininder had similarities with proteins from *Arabidopsis thaliana*.

Segregation analysis

Segregation patterns were analyzed for the microsatellites for which segregation occurred in the F_2 progenies (J × F) or (T × E) (Table 4). With microsatellites BPPCT 009 and BPPCT 021, two loci were identified for each in the J × F progeny and only one in the T × E progeny.

Fifteen microsatellite loci among the 43 analyzed (34.9%) segregated in the intraspecific peach F_2 J × F progeny. The percentage of segregating SSR loci was higher than that observed with RFLPs, where only 22.8% of the probes were able to detect polymorphism (Dirlewanger et al. 1998b). In the interspecific almond 'Texas' almond × 'Earlygold' peach, 30 microsatellite loci among the 39 tested (76,9%) were segregating.

In the F_2 J × F population, all the polymorhic SSRs segregated according to a Mendelian ratio. In the T × E F_2, nine microsatellite loci (30%) presented a distorted segregation ratio. This is in agreement with the very high level of distorted markers (46%) already observed in this progeny (Joobeur et al. 1998).

Table 3 Proteins similar to the amino-acid derived sequences from some of the microsatellite containing nucleotide sequences. Only proteins with an expected value lower than 1E-08 were retained and the higher score was shown

SSR name	Accession number	Homologous protein [species]	Accession number	Expect value[a]	% Identity[b] (% positives)
BPPCT 010	AF374939	Acetyl-CoA C-acetyltransferase [*Arabidopsis thaliana*]	BAA97003	6E-32	75% (92%)
BPPCT 013	AF374940	Hypothetical protein [*Oryza sativa*]	AAG13534	8E-43	54% (69%)
BPPCT 014	AF374941	MADS-box protein 3 [*Malus* × *domestica*]	AAD51422	8E-23	98% (98%)
BPPCT 016	AF374942	Probable serine/threonine-specific protein kinase [*Arabidopsis thaliana*]	T01086	2E-11	57% (80%)
BPPCT 020	AF374943	CONSTANS-like protein 2 [*Malus* × *domestica*]	AAC99310	2E-08	80% (82%)
BPPCT 027	AF374937	Hypothetical protein F22K18.250, clathrin coat assembly protein AP50 [*Arabidopsis thaliana*]	T05579	5E-09	79% (87%)
BPPCT 028	AF374944	Gene id: MZN14.14 an unknown protein [*Arabidopsis thaliana*]	BAB02183	3E-21	45% (54%)
BPPCT 034	AF374945	Putative O-sialoglycoprotein endopeptidase [*Arabidopsis thaliana*]	AC002387	3E-09	79% (93%)
BPPCT 040	AF374946	Vacuolar sorting receptor protein homolog F18F4.210 [*Arabidopsis thaliana*]	T04895	2E-17	58% (68%)
BPPCT 041	AF374938	Gene id: MDF20.26 an unknown protein [*Arabidopsis thaliana*]	BAB09249	3E-09	58% (75%)

[a] The Expect value is a parameter that describes the number of hits one can "expect" to see just by chance when searching a database of a particular size

[b] % Identity with the most similar amino-acid sequence

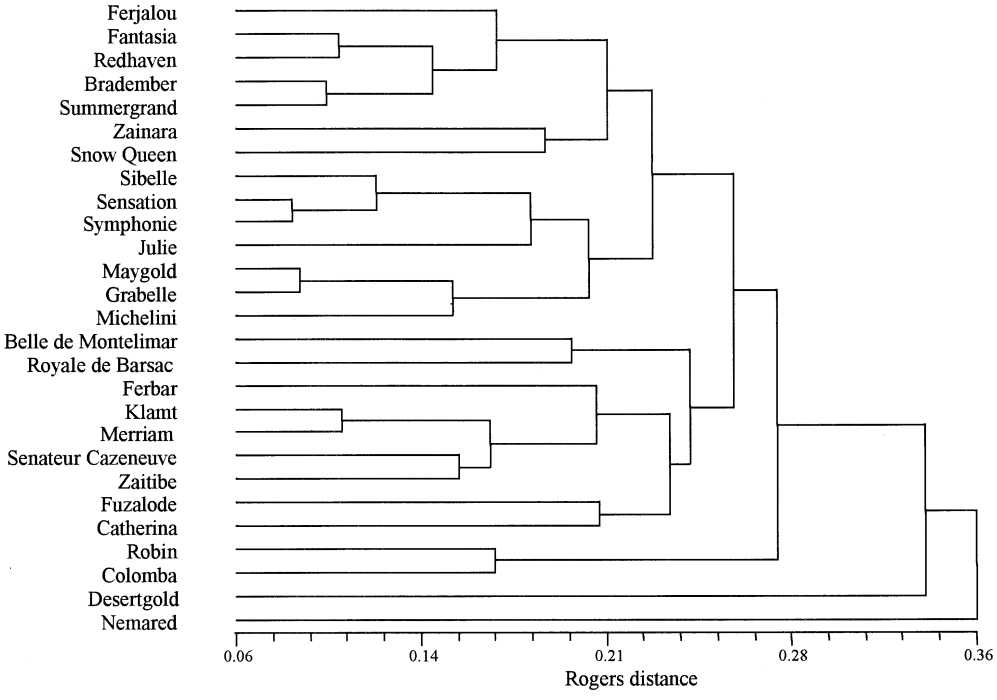

Fig. 1 Dendrogram constructed from single-locus SSR data, using SIMGEN program, Rogers distance, and UPGMA clustering with the 27 peaches

In the J × F F_2 progeny, 11 loci were segregating according to a 1:2:1 ratio and four with a 3:1 ratio. In the T × E F_2 progeny, 22 were segregating according to a 1:2:1 ratio and nine with a 3:1 ratio, the null allele coming from the almond. Interestingly, among 27 microsatellites giving no polymorphism in J × F progeny, 21 revealed polymorphism when tested in T × E progeny. Conversely, among 11 microsatellites revealing no polymorphism in T × E, five gave polymorphism in J × F progeny.

Microsatellite polymorphism and discrimination power

All the 41 primer pairs tested gave PCR-amplification products on peach and 33 on sweet cherry (80.5%) (Table 5). Six PCR-amplifications revealed several loci in peach (14.61%) and eight (19.5%) in sweet cherry. Among the 33 single-locus microsatellites common to peach and sweet cherry, 13 revealed polymorphism both in peach and cherry, 19 were polymorphic only in peach and one was polymorphic only in cherry (Table 5). The number of alleles per locus ranged from 1 to 9 for peach

Table 4 Segregation analysis of microsatellite loci in *P. persica* ('Jalousia' × 'Fantasia') intraspecific and in *P. amygdalus* ('Texas') × *P. persica* ('Earlygold') interspecific F_2 crosses

SSR locus (allele size in the J × F or T × E progenies)	'Jalousia' × 'Fantasia' F_2			'Texas' × 'Earlygold' F_2		
	Expected ratio[a]	Observed ratio	χ^{2} [b]	Expected ratio[a]	Observed ratio	χ^{2} [b]
BPPCT 001	m			1:2:1	3:15:2	5.10 ns
BPPCT 002	m			1:2:1	3:15:2	5.10 ns
BPPCT 004	m			1:2:1	2:16:2	7.20*
BPPCT 005	m			m		
BPPCT 006	m			1:2:1	8:8:4	2.40 ns
BPPCT 007	m			1:2:1	1:16:3	7.60*
BPPCT 008	m			1:2:1	5:13:2	2.70 ns
BPPCT 009a (J × F:152, T × E:160/152)	3:1	13:7	1.07 ns	1:2:1	6:12:2	2.40 ns
BPPCT 009b (J × F:145/135)	1:2:1	3:12:5	1.20 ns	–		
BPPCT 010	m			1:2:1	6:14:0	6.80*
BPPCT 011	m			m		
BPPCT 012	m			3:1	13:7	1.07 ns
BPPCT 013	m			1:2:1	3:15:2	5.10 ns
BPPCT 014	m			1:2:1	5:12:3	1.20 ns
BPPCT 015	1:2:1	5:9:5	0.05 ns	1:2:1	13:6:1	17.60**
BPPCT 016	m			1:2:1	1:12:7	4.40 ns
BPPCT 017	1:2:1	5:9:6	0.30 ns	1:2:1	4:11:5	0.30 ns
BPPCT 018	1:2:1	5:12:3	1.20 ns	m		
BPPCT 019	m			1:2:1	6:13:1	4.30 ns
BPPCT 020	m			3:1	18:2	2.40 ns
BPPCT 021a (J × F:240)	3:1	13:7	1.07 ns	–		
BPPCT 021b (J × F:200, T × E:200/192)	3:1	15:5	0.00 ns	1:2:1	1:11:8	6.10*
BPPCT 022	m			m		
BPPCT 023	1:2:1	4:12:4	0.80 ns	3:1	11:9	4.27*
BPPCT 024	m			3:1	13:7	1.07 ns
BPPCT 025	1:2:1	3:12:5	1.20 ns	3:1	17:3	1.07 ns
BPPCT 026	m			m		
BPPCT 027	m			1:2:1	3:12:5	1.20 ns
BPPCT 028	1:2:1	4:12:4	0.80 ns	1:2:1	0:12:8	7.20*
BPPCT 029	m			m		
BPPCT 030	m			m		
BPPCT 031	3:1	16:4	0.27 ns	m		
BPPCT 032	m			3:1	15:5	0.00 ns
BPPCT 033	m			1:2:1	2:15:3	5.10 ns
BPPCT 034	m			3:1	13:7	1.07 ns
BPPCT 035	1:2:1	4:11:4	0.47 ns	m		
BPPCT 036	m			3:1	11:9	4.27*
BPPCT 037	1:2:1	3:11:6	1.00 ns	1:2:1	7:9:4	1.10 ns
BPPCT 038	1:2:1	4:11:2	1.94 ns	1:2:1	7:10:3	1.60 ns
BPPCT 039	m			1:2:1	4:13:3	1.90 ns
BPPCT 040	m			1:2:1	10:9:1	8.30*
BPPCT 041	1:2:1	4:8:8	2.70 ns	nt		
BPPCT 042	m			nt		

[a] *m* monomorphic locus, *nt* non tested, – locus not existing
[b] *ns* non-significant value, **P* less than or equal to 0.05, ***P* less than or equal to 0.01

and from 1 to 6 for sweet cherry with an average of 4.2 and 2.8 in peach and sweet cherry, respectively. The microsatellites having the highest number of alleles in peach did not give any amplification in sweet cherry (BPPCT 001, -025, -033) or were multilocus microsatellites in sweet cherry (BPPCT 008 and -015). For single-locus SSRs, the allele-size range of each microsatellite (Table 5) was in agreement with the length of the fragment sequenced from the 'Merrilyl O'Henry' clones (Table 2). The microsatellites BPPCT 027, -028, -037, -040 gave a similar allele-size range in peach and sweet cherry. For 16 microsatellites, the allele-size range in peach was higher than in sweet cherry. On peach, one or two alleles for each microsatellite were present with high frequency, the remaining alleles being generally present only in one or two cultivars (data not shown). Excluding three cases, the most-frequent allele of all loci was present in the 'Redhaven' profile and the rare alleles were often found in 'Nemared' and 'Desertgold'. In cherry, the rare alleles were present in 'Garnet' and 'Grosse Schwarzkorpel'. For polymorphic microsatellites, the expected heterozygosity ranged from 0.07 to 0.79 (mean of 0.41) for peach and from 0.39 to 0.76 (mean of 0.60) for sweet cherry. The observed heterozygosity was lower than the expected for all but three microsatellites in peach due to the presence of null alleles. The discrimina-

Table 5 Number of alleles, size range, observed heterozygosity (Ho), expected heterozygosity (He), discrimination power (DP) of microsatellites, sequenced in peach and screened in peach and cherry cultivars

SSR name	Evaluation on 27 peach cultivars					Evaluation on 21 sweet cherry cultivars				
	No. of alleles	Size range	Ho	He	DP	No. of alleles	Size range	Ho	He	DP
BPPCT 001	8	132–163	0.42	0.66	0.85	N[c]	N	N	N	N
BPPCT 002	5	226–238	0.07	0.21	0.27	4	179–185	0.48	0.55	0.71
BPPCT 004	3	198–206	0.26	0.23	0.40	2	177–179	0.38	0.41	0.57
BPPCT 005	1	143	0.00	0.00	0.00	6	157–199	0.89	0.67	0.79
BPPCT 006	6	115–137	0.26	0.53	0.66	1	99	0.00	0.00	0.00
BPPCT 007	4	143–151	0.50	0.59	0.73	2	111–113	0.00	0.48	0.48
BPPCT 008[a]	8	131–161	0.33	0.55	0.69	m.p.[d]	101–93	–	–	–
BPPCT 009[b]	m.p.	138–181	–	–	–	m.p.	150–180	–	–	–
BPPCT 010	2	129–131	0.11	0.17	0.26	2	117–119	0.59	0.42	0.48
BPPCT 011	4	172–180	0.27	0.49	0.66	N	N	N	N	N
BPPCT 012	2	160–168	0.12	0.17	0.27	4	154–164	0.00	0.64	0.64
BPPCT 013[b]	m.p.	165–200	–	–	–	m.p.	150–242	–	–	–
BPPCT 014	4	200–226	0.30	0.52	0.67	3	190–194	0.47	0.39	0.6
BPPCT 015[a]	9	154–228	0.59	0.77	0.89	m.p.	159–500	–	–	–
BPPCT 016	4	89–103	0.32	0.61	0.73	N	N	N	N	N
BPPCT 017	5	151–181	0.41	0.59	0.76	1	197	0.00	0.00	0.00
BPPCT 018	3	219–223	0.48	0.57	0.76	1	193	0.00	0.00	0.00
BPPCT 019[b]	m.p.	182–254	–	–	–	m.p.	216–500	–	–	–
BPPCT 020	4	198–208	0.56	0.62	0.78	N	N	N	N	N
BPPCT 021[b]	m.p.	200–350	–	–	–	m.p.	220–350	–	–	–
BPPCT 022	4	133–155	0.27	0.33	0.49	1	99	0.00	0.00	0.00
BPPCT 023	5	183–237	0.30	0.51	0.67	N	N	N	N	N
BPPCT 024	3	95–101	0.04	0.18	0.21	1	91	0.00	0.00	0.00
BPPCT 025	9	178–202	0.30	0.60	0.70	N	N	N	N	N
BPPCT 026	3	134–145	0.35	0.53	0.69	6	140–190	0.67	0.72	0.85
BPPCT 027	2	246–248	0.00	0.07	0.07	1	246	0.00	0.00	0.00
BPPCT 028	3	155–167	0.26	0.41	0.58	3	151–173	0.47	0.47	0.63
BPPCT 029[b]	m.p.	157–161	–	–	–	m.p.	99–160	–	–	–
BPPCT 030	6	158–180	0.17	0.15	0.44	1	140	0.00	0.00	0.00
BPPCT 031	2	102–119	0.15	0.33	0.61	N	N	N	N	N
BPPCT 032	3	202–206	0.15	0.20	0.27	1	158	0.00	0.00	0.00
BPPCT 033	8	164–212	0.48	0.79	0.88	N	N	N	N	N
BPPCT 034	4	216–242	0.30	0.26	0.45	6	224–258	0.81	0.7	0.84
BPPCT 035[b]	m.p.	80–160	–	–	–	m.p.	160–170	–	–	–
BPPCT 036	2	250–253	0.00	0.42	0.42	1	246	0.00	0.00	0.00
BPPCT 037	5	146–156	0.48	0.62	0.79	5	142–156	0.85	0.71	0.80
BPPCT 038	7	127–143	0.44	0.58	0.75	5	101–133	0.74	0.70	0.85
BPPCT 039	2	148–158	0.31	0.47	0.64	5	134–150	0.67	0.72	0.87
BPPCT 040	3	121–141	0.00	0.14	0.14	6	122–146	0.90	0.76	0.84
BPPCT 041	2	210–220	0.00	0.07	0.07	1	201	0.00	0.00	0.00
BPPCT 042	3	240–250	0.07	0.07	0.14	1	215	0.00	0.00	0.00

[a] Indicates multi-locus SSR in sweet cherry
[b] Indicates multi-locus SSR in peach and sweet cherry
[c] *N* indicates that no amplification was observed
[d] *m.p.* indicates multiple product

tion power ranged from 0.07 to 0.89 (mean of 0.54) in peach and from 0.48 to 0.87 (mean of 0.72) in sweet cheery. Five microsatellites had a discrimination power higher than 0.6 both in peach and sweet cherry (BPPCT 014, -026, -037, -038, -039) and four ranged from 0.4 to 0.6 in peach and sweet cherry (BPPCT 004, -007, -028, -034). All peaches and sweet cherries were distinguished with only three microsatellites: BPPCT 001, -020, -033 or BPPCT 015, -020, -033 were suitable for peach and BPPCT 034, -039, -040 or BPPCT 034, -038, -040 for cherries. Figures 1 and 2 show the dendrograms obtained with the 27 peach and 21 sweet cherry varieties. All peach and cherry varieties were discriminated unambiguously.

Cross-species transportability

Six microsatellites (BPPCT 012, -014, -030, -035, -039, -041) gave amplification on all species tested, that belong to the genera *Prunus*, *Malus* and *Fragaria*, of the Rosaceae and *Castanea*, *Juglans* and *Vitis* oustside of the Rosaceae (Table 6). Among them, one had an amplified region homologous to a MADS-box protein identified in *Malus* × *domestica*. Twelve microsatellites (29.3%) gave amplification in *Prunus*, *Malus* and *Fragaria* and 31 (75.6%) gave amplification only in *Prunus*. Within *Prunus*, apart from peach and sweet cherry, the best results were obtained in European plum in which 40 microsatellites (97.6%) gave amplification. As much as 95.1% SSRs gave amplification in Myrobalan plum and al-

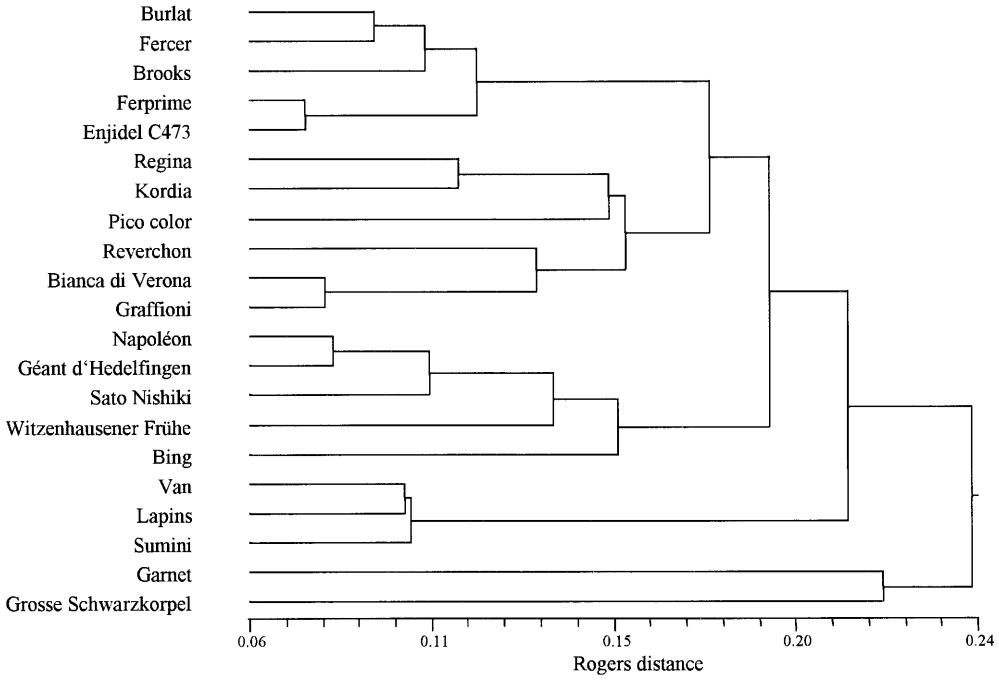

Fig. 2 Dendrogram constructed from single-locus SSR data, using SIMGEN program, Rogers distance, and UPGMA clustering with the 21 cherries

mond, 92.7% in apricot, 90.2% in sour cherry and 80.5% in mazzard. Thirty three (80.5%), 18 (43.9%) and 13 (31.7%) microsatellites gave amplification in chestnut tree, grapevine and walnut tree, respectively. However, out of *Prunus*, the frequency of complex-pattern increases make some of these amplification results difficult to interpret. Complex patterns were observed most frequently in apple and strawberry: 44 and 43% of the SSRs giving amplification in apple and strawberry, respectively.

Discussion

The microsatellite enrichment procedure was very successful; 84.8% of the selected clones contained microsatellite sequences. This percentage is higher than that reported by Cipriani et al. (1999) where 50% of the clones contained the target repeat. The proportion of redundant sequences reached 30% among the 191 recombinant plasmids sequenced. This value may increase if further clones are sequenced from the same enriched library. Most microsatellites sequenced from the CT-enriched library were simple with no interruption in the repeat sequence (83.6%). This result is in agreement with those previously reported in plants. In peach, Cipriani et al. (1999) found that all microsatellites obtained from the CT library were perfect, and nearly half of the AC microsatellites were composite, with a different motif adjacent to that expected. This was also described in other plants (Weising et al. 1996; Guilford et al. 1997; Huang et al. 1998; Sun et al. 1998).

For ten amino-acid sequences deduced from the DNA sequences of the microsatellite-containing clones, a similarity was found with the protein sequences in Genbank. One of them (BPPCT014) was homologous to a highly conserved protein, such as the apple MADS-box protein. MADS-box genes have been identified in a wide range of eukaryotic organisms including yeast, insects, mammals and plants (Shore and Sharrocks 1995). They form a superfamily whose members share a highly conserved domain of 56 amino acids. In plants, they play key roles in plant development, as reported for apple (Yao et al. 1999), and in the development of specific organs, like floral whorls (Coen and Meyerowitz 1991).

The number of alleles detected per locus in peach ranged from 1 to 9 with a mean value of 4.2 alleles per locus. This value is very close to that reported by Testolin et al. (2000) using 50 peach cultivars (range 2–8 with a mean value of 4.5 alleles per locus). In sweet cherry, this value was lower and ranged from 1 to 6 with a mean value of 2.8. As many as 34 out of the 35 single-locus SSRs (97%) were polymorphic in peach, compared to only 14 of 25 (56%) in cherry. These results are surprising considering that a higher level of polymorphism was expected in sweet cherry given its self-incompatibility in contrast with the self-compatibility of peach (Byrne 1990). From the 12 loci that were polymorphic in one species and monomorphic in the other (11 fixed in cherry and 1 in peach), all but two had a shorter amplified fragment in the monomorphic species than the range of sizes in the polymorphic one. This is in agreement with the fact that longer microsatellites are generally more polymorphic (Smulders et al. 1997; Loridon et al. 1998; Testolin et al. 2000). As a more-general consequence, the fact that the primers of a microsatellite developed from a species results in DNA amplification in another, even a closely related one, may not say much about their level of polymorphism in this species.

Table 6 Cross-species amplification using peach microsatellite primers

SSR name	*Rosaceae*								*Castanea* Chestnut tree	*Juglans* Walnut tree	*Vitis* Grape
	Prunus						*Malus* Apple	*Fragaria* Straw-berry			
	Mazzard	Sour cherry	European plum	Myrobalan plum	Almond	Apricot					
BPPCT 001	–[a]	+[b]	++[b]	++	++	++	–	+	++	–	–
BPPCT 002	++	++	++	++	++	++	–	–	+	–	–
BPPCT 004	++	++	++	++	++	++	–	+	++	++	++
BPPCT 005	++	++	++	++	++	++	–	–	–	–	–
BPPCT 006	–	++	++	++	++	+	–	–	+	–	–
BPPCT 007	++	+	++	++	++	++	–	–	–	–	–
BPPCT 008	++	++	++	++	++	++	–	+*	++	++	–
BPPCT 009	+	++	++	++	++	++	–	–	+	–	–
BPPCT 010	++	++	++	++	++	++	–	–	+	–	+
BPPCT 011	+	++	++	++	++	++	–	–	–	–	–
BPPCT 012	++	++	++	++	++	++	++*	+*	++	+*	+*
BPPCT 013	++	++	++	++	++	++	++*	++*	+	++*	–
BPPCT 014	++	++	++	++	++	++	+	+	++	+	++
BPPCT 015	++	++	+	+	++	++*	+	–	–	–	–
BPPCT 016	–	++	++	++	++	++	++*	++*	++*	+*	–
BPPCT 017	++	++	+	++	++	++	–	+	–	–	–
BPPCT 018	+	++	+	++	++	++	–	–	++	–	++
BPPCT 019	+	+	++	++	+	++	–	–	++	–	–
BPPCT 020	–	–	+	+	++	++	+	–	++	–	–
BPPCT 021	++	++	++	++	++	++	–	–	++	–	–
BPPCT 022	++	++	++	–	–	–	–	–	++	–	–
BPPCT 023	–	–	++	++	++	+	–	–	++	–	++
BPPCT 024	++	++	++	+	++	++	–	–	–	–	–
BPPCT 025	–	–	++	++	++	++	–	–	+	–	–
BPPCT 026	++	++	+	++	++	++	++	++	++	–	+
BPPCT 027	+	+	++	++	++	++	+*	++	++	–	+
BPPCT 028	++	++	++	++	++	++	++	++*	++	–	++
BPPCT 029	+	++	++	++	++	++	+	+	–	+	–
BPPCT 030	++	++	++	++	++	++	++*	++*	++	++	++
BPPCT 031	–	–	++	–	–	–	++	+	–	–	+
BPPCT 032	++	++	++	++	++	–	++	++	++	++	++
BPPCT 033	–	+	–	++	++	++	+	++	++	++	+
BPPCT 034	++	++	++	++	++	+	–	–	++	–	++
BPPCT 035	++	++	++	+	+*	++	++*	++*	++	++*	++*
BPPCT 036	++	++	+	++	++	++	–	–	+	–	–
BPPCT 037	++	++	++	++	++	++	+*	++*	++	–	–
BPPCT 038	++	++	++	++	+	++	–	++	++	–	–
BPPCT 039	++	++	++	++	++	++	++*	++*	++	++	++
BPPCT 040	++	++	++	++	++	+	–	–	++	–	++
BPPCT 041	++	++	++	++	++	++	++	+	++	+	++
BPPCT 042	++	++	++	++	+	++	–	–	+	–	–

[a] – no amplification

[b] +, ++ indicates amplification of single product on all the individuals tested (++) or on part of the individuals (+)

* Indicates amplification displaying a complex banding pattern

In peach, the frequency was high for one or two alleles and very low for the others. As already reported by Testolin et al. (2000), the most-frequent alleles were found in 'Redhaven' while the rare alleles were found in 'Nemared'. This was expected since 'Redhaven' is a parent of most peach cultivars (Scorza et al. 1985). In sweet cherry the mean heterozygosity and discrimination power were higher than in peach and this is in agreement with the sweet cherry being self-incompatible as opposed to peach. Several microsatellites present a high discrimination power both in peach and sweet cherry, and will be useful for fingerprinting and synteny analyses in these two species. All peach and cherry varieties were clearly identified.

The transportability of microsatellites from peach to other *Prunus* species was very high, confirming what was previously reported (Cipriani et al. 1999; Sosinski et al. 2000). This demonstrates that microsatellites are very powerful markers for synteny analysis in *Prunus*. Surprisingly, the transportability was high also for chestnut tree, a species that does not belong to the Rosaceae (80.5%). It was rather good for strawberry (51%), another species belonging to the Rosaceae, and less for the apple and grapevine (43.9%) and for the walnut tree (31.7%). Those results demonstrate that cross-species transportability is not related to the genetic distance from peach: the chestnut tree being more distant genetically

from peach than strawberry or apple. The frequency of complex patterns, the highest being observed for apple and strawberry, seems not related to be to the genetic distance from peach but may be linked to the complexity of the DNA content, *Fragaria* × *ananassa* strawberry being octoploid, and *Malus* being an allopolyploid between the *Prunoideae* and *Spiraeoideae* (Maliepaard et al. 1998). The frequencies for the transportability observed here were higher than those reported in other plants. For example, 26% of the barley microsatellite primers amplified microsatellites from oat (Li et al. 2000). Lower values were even found for microsatellite loci sequenced in soybeans, from which only 10% provide useful markers for cowpeas, broad beans or lupins (Peakall et al. 1998).

The microsatellites characterized in this paper will be used to complete the maps constructed in the European *Prunus* mapping project (Arús et al. 1994), and especially for the intraspecific peach (Dirlewanger et al. 1998b) and interspecific almond × peach maps (Joobeur et al. 1998). The synteny analysis already initiated with RFLP markers will also be improved by microsatellites.

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