Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences

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A total of 76 sweet cherry genotypes were screened with 34 microsatellite primer pairs previously developed in peach. Amplification of SSR loci was obtained for 24 of the microsatellite primer pairs, and 14 of them produced polymorphic amplification patterns. On the basis of polymorphism and quality of amplification, a set of nine primer pairs and the resulting 27 informative alleles were used to identify 72 genotype profiles. Of these, 68 correspond to unique cultivar genotypes, and the remaining four correspond to three cultivars that could not be differentiated from the two original genotypes of which they are mutants, and two very closely related cultivars. The mean number of alleles per locus was

3.7 while the mean heterozygosity over the nine polymorphic loci averaged 0.49. The results demonstrate the usefulness of cross-species transferability of microsatellite sequences allowing the discrimination of different genotypes of a fruit tree species with sequences developed in other species of the same genus. UPGMA cluster analysis of the similarity data divided the ancient genotypes studied into two fairly well-defined groups that reflect their geographic origin, one with genotypes originating in southern Europe and the other with the genotypes from northern Europe and North America.

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Introduction

Sweet cherry (*Prunus avium* L., Rosaceae, $2n = 16$) is cultivated in temperate regions of the world for its edible fruit. Cherries are thought to have originated around the Caspian and Black Seas and currently they are found across mainland Europe and in western Asian areas (Webster, 1996). Sweet cherry cultivation spread through Europe in times of the Roman Empire, although they were not planted extensively until the 16th century (Watkins, 1976). The spread of sweet cherry cultivation across western Europe was probably based on domestication of wild individuals that were well adapted to each area of cultivation. A limited number of these cultivars were taken, by European settlers, to North America and spread throughout the continent. However, since sweet cherries were not commercially exploited until a century ago (Hedrick *et al*, 1915), most sweet cherry cultivars grown today are only a few generations from these landraces (Iezzoni *et al*, 1990).

Current breeding objectives have triggered a constant release of genetically improved genotypes (Iezzoni *et al*,

1990; Brown *et al*, 1996). Precise identification of the existing sweet cherry cultivars is essential for sweet cherry orchard establishment, efficient germplasm collection management, and selection of genotypes in cherry breeding programmes. Moreover, efficient characterisation methods are needed to assess the precious germplasm still present in eastern Europe and to study variability in wild stands scattered through Eurasia.

Cultivar identification has traditionally been based on morphological traits although DNA markers allow a more precise identification of plant genotypes and are of great interest for the characterisation of fruit trees. However, little work has been dedicated to the identification of sweet cherry cultivars. Thus, Granger *et al* (1993) and Beaver *et al* (1995) used isozymes to identify unique genotype profiles from sweet cherry cultivars whilst Gerlach and Stösser (1997) differentiated sweet cherry cultivars using RAPD markers.

Microsatellite markers (SSR; Litt and Luly, 1989; Tautz, 1989) are becoming the markers of choice for fingerprinting purposes due to their high polymorphism and reproducibility (Gupta and Varshney, 2000; Wünsch and Hormaza, in press). Among temperate fruit tree species, most microsatellite flanking sequences have been cloned and sequenced in peach (*Prunus persica* (L.) Batsch; Cipriani *et al*, 1999; Sosinski *et al*, 2000; Testolin *et al*, 2000; Aranzana *et al*, 2002) and apple (*Malus × domestica* Borkh.; Guilford *et al*, 1997; Gianfranceschi *et al*, 1998; Hokanson *et al*, 1998), where they have been used in genotype identification and the determination of genetic relatedness. One drawback of microsatellites is the

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difficulty of their initial isolation. However, once the primer sequences have been identified, SSR markers can be easily applied and the flanking sequences are frequently conserved among species and genera (Downey and Iezzoni, 2000; Hormaza, 2002; Yamamoto *et al*, 2001). However, studies of cross-species transferability in temperate fruit tree species have included limited numbers of genotypes and studies at a larger scale are required to validate the widespread use of the currently available SSR sequences. Thus, in this work, we have tested 34 microsatellite primer pairs developed in peach to characterise and study the genetic similarities among 76 sweet cherry genotypes, including old cultivars obtained from diverse geographical regions, and new genotypes released from different cherry breeding programmes.

Materials and methods

Plant material and genomic DNA extraction

Genomic DNA was extracted from 76 sweet cherry genotypes (Table 1) maintained at the SIA-DGA experimental orchards located at the Campus de Aula Dei in Zaragoza, Spain, using a modified version (Hormaza, 1999) of the DNA extraction protocol described by Doyle and Doyle (1987). Since low quality DNA was extracted from old leaves and fresh leaves are only available in spring, genomic DNA was extracted from eight to 10 winter buds collected during November and December. DNA from fresh leaves collected in spring was compared with that obtained from winter buds for 10 genotypes. Extracted DNA was quantified spectrophotometrically and diluted to 10 ng/μl before PCR amplification.

PCR amplification and product electrophoresis

Extracted genomic DNA was PCR-amplified using 34 pairs of microsatellite primers developed in peach (Tables 2 and 3). PCR reactions were performed in 20 μl volumes containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 4 mM MgCl₂, 0.1 mM each dNTP, 0.2 μM each primer, 40 ng genomic DNA, 0.45 units *Taq* polymerase (Life Technologies, Rockville, MD, USA) and a drop of mineral oil. Reactions were carried out on a PTC-100 (MJ Research) thermocycler using the following temperature profile: an initial step of 2 min at 94°C, 35 cycles of 45 sec at 94°C, 45 sec at 57°C and 1 min at 72°C, and a final step of 5 min at 72°C. For primers pchms1 and pchms3, the annealing temperature was lowered to 40°C. PCR products were separated by electrophoresis using 3% 'Metaphor' agarose gels (FMC Bioproducts) in 1 × TBE buffer at 5 V/cm, stained with ethidium bromide and visualised under UV light. Band scoring was carried out using a standard 10 bp DNA ladder (Life Technologies). To confirm the results, microsatellite products that revealed differences smaller than 5 bp in agarose gels, were also separated using 10% polyacrylamide gel electrophoresis in 1 × TBE buffer at a constant current of 40 mA, stained with ethidium bromide and visualised under UV light.

Data analysis

Heterozygosity was calculated as the number of heterozygous individuals over the total number of individuals analysed for each locus and averaged over the polymorphic loci. Genetic relationships among the genotypes

were calculated using UPGMA cluster analysis of the similarity matrix obtained from the proportion of shared fragments (Nei and Li, 1979). Cophenetic coefficients were computed for the dendrograms after the construction of a cophenetic matrix. All those analyses were computed with the programme NTSYS-pc 2.02 (Exeter Software, Stauket, New York, NY, USA). The robustness of the dendrogram obtained from UPGMA cluster analysis was assessed with bootstrap analysis running 2000 iterations using the WinBoot program (Yap and Nelson, 1996).

Results

Repeatable amplification was obtained for 24 (70%) of the 34 microsatellite primer pairs tested, 13 (38%) were polymorphic and nine of these (Table 2) were selected for the identification of the 76 sweet cherry genotypes. As expected, no differences were recorded between the amplification patterns among the samples taken from fresh leaf tissue in spring and dormant winter buds. Non-denaturing polyacrylamide gel electrophoresis confirmed the results using 'Metaphor' agarose gel electrophoresis but revealed no additional bands. In fact, polyacrylamide electrophoresis showed greater numbers of artefact and stutter bands, making identification of microsatellite alleles more difficult than with agarose gel electrophoresis.

Out of the 24 primer pairs that amplified either poly- or monomorphic loci, 11 (45%) of them showed alleles in the same size range as those reported in peach for the same primer pairs (Tables 2 and 3). Four of the 13 polymorphic loci were discarded, because either size differences among bands were too small to be accurately scored (Pchms1, UPD98-024, UPD98-410) or complex band patterns (UPD98-411) did not allow identification of unique loci. Three primer pairs (Pchms3, UPD98-409, UPD98-412) amplified alleles common to all genotypes analysed. The number of alleles per locus ranged from two to seven, ranging from 90 bp to 290 bp. Heterozygosity ranged from 0.04 to 0.94 for loci UPD98-409 and UPD96-005 respectively, the mean heterozygosity being 0.49. Heterozygosity for the 23 ancient and/or of unknown pedigree cultivars was 0.49, whilst heterozygosity for the other 53 genotypes from more recent breeding programmes was 0.47.

Genotypes showing a single amplified fragment were considered homozygous for that particular locus (Callen *et al*, 1993). Inheritance of microsatellites was checked for the genotypes with known pedigree and whose parental genotypes were present in our collection. In all cases, bands present in the progeny were always present in at least one of the parental genotypes. Rare alleles were amplified with primer pairs UPD98-409, UPD98-412, UPD97-402 and UDP98-022. All the sampled cherry genotypes present a common allele of 130 bp with primer pair UPD98-409 and only 'Ambrunes', 'Pico Negro' and 'Pico Colorado' show a second band of 160 bp. The cultivars 'Marmotte', 'Hedelfinger' and 'Blanca de Provenza' share a distinct allele with primer pair UPD98-412. Generally, old cultivars, eg 'Burlat', 'Marmotte' or 'Hedelfinger', and several Spanish cultivars contributed most of the rare alleles.

The combination of nine selected primers and the resulting 27 informative markers allowed the unambiguous

Table 1 Sweet cherry cultivars included in this study with the SSR genotypes obtained after amplification with nine SSR primer pairs (primer details in Table 2)

<i>Cultivar</i>	<i>Origin*</i>	<i>Pchgms1</i>	<i>Pchcms3</i>	<i>Pchcms5</i>	<i>UDP96-005</i>	<i>UDP98-409</i>	<i>UPD98-021</i>	<i>UPD98-022</i>	<i>UPD97-402</i>	<i>UPD98-412</i>
Ambrunes	U, S	140	180/160	290	150/125	160/130	110/100	110/90	125	130
Arcina	BP, F	140	180	260	150/130	130	110/100	100/90	140/125	130
Belge	U, CE	190/140	180	260	150/120	130	110/100	110/90	130	130
Bing	BP, US	190/140	180	260	150/120	130	110/100	110/90	130	130
Blanca de Provenza	U	140	180/160	290/260	120	130	100	105/90	145/125	130/100
Brooks	BP, US	140	180	290	150/120	130	110/100	90	135/125	130
Burlat	U, F	140	180	290	150/130	130	110/100	100/90	130/120	130
Burlat C-1	BP, I	140	180	290	150/130	130	110/100	100/90	130/120	130
Celeste (Sumpaca)	BP, C	140	180	290	150/130	130	110/100	100	135/125	130
Chinook	BP, C	190/140	180	260	150/120	130	110/100	110	145/130	130
Compact Stella	BP, C	190/140	180	290/260	150/120	130	110/100	110/100	145/125	130
Coralise (Gardel)	BP, F	140	180	260	150/130	130	110/100	100/90	135/125	130
Corum	BP, US	190/140	180	290/260	150/120	130	110/100	110/100	135/125	140/130
Cristalina (Sumnue)	BP, C	190/140	180/160	260	150/120	130	110	110/100	135/125	130
Cristobalina	U, S	190/140	180	290/260	150/115	130	110/100	110/90	135/125	130
Duroni 3	U, I	140	180	290/260	150/115	130	110/100	100/90	125	130
Earlise (Riverdel)	BP, F	140	180	290/260	150/130	130	100	90	130/120	130
Earlstar	BP, I	190/140	180	290	150/120	130	100	100/90	145/125	130
Early Van Compact	BP, C	190/140	180	290/260	150/120	130	110	100/90	135/125	130/120
Ferrovía	U, I	140	180	290/260	150/120	130	110	100	145/130	140/130
Garnet (Magar)	BP, US	140	180	260	120	130	110/100	110/90	130	130
Gil Peck	BP, US	190/140	180/160	260	150/120	130	110	110	145/130	130
Giorgia	BP, I	140	180	290/260	150/120	130	110	100/90	125	130/120
Hartland	BP, US	140	180	260	150/120	130	110	100/90	140/130	130
Hedelfinger	U, CE	140	180	290/260	150/120	130	110/100	110/100	135/125	130/100
Lambert	BP, US	190/140	180/160	290/260	150/120	130	110/100	110	125	130
Lamida	BP, US	140	180	260	150/120	130	110	110	145/125	130
Lapins	BP, C	190/140	180	290	150/120	130	110/100	100/90	135/125	130
Larian	BP, US	190/140	180	260	150/120	130	110	110/100	145/130	130
Marmotte	U, F	190/140	180	290/260	150/120	130	110/100	110/100	135/125	130/100
Marvin (Niram)	BP, US	140	180	290/260	120	130	110/100	100/90	130/125	130
Moreau	U, F	140	180	290/260	150/115	130	100	100/90	145/125	130
Napoleon	U, CE	190/140	180	260	150/120	130	110/100	110/100	135/125	130
Newstar	BP, C	140	180	290/260	150/120	130	110/100	100	135/125	130/120
Pico Colorado	U, S	140	180/160	290/260	150/130	160/130	110/100	110	140/125	130
Pico Negro	U, S	140	180/160	290/260	150/115	160/130	100	110/90	140/120	130
Precoco Bernard	U, F	140	180	290/260	150/130	130	100	100/90	145/130	130
Rainier	BP, US	190/140	180	290/260	120	130	110/100	90	130	130
Ramon Oliva	U, F	140	180/160	290	150/130	130	100	90	120	130
Reverchon	U, I	140	180	290/260	150/115	130	110/100	100	125	130
Royalton	BP, US	190/140	180	260	150/130	130	100	110/100	135/125	130
Ruby	BP, US	190/140	180	260	120	130	110/100	110/90	130	130
Sam	BP, C	140	180/160	290/260	150/120	130	100	110/100	145/125	130
Samba (Sumste)	BP, C	190/140	180	290/260	150/120	130	110	110/90	135/130	130
Santina	BP, C	190/140	180/160	290/260	150/130	130	100	110/100	135/125	130
Skeena	BP, C	190	180	290/260	150/120	130	100	90	145/125	130
Somerset	BP, US	190/140	180	260	150/120	130	110/100	100	135/125	130/120
Sonata (Sumleta)	BP, C	190	180	290	150/120	130	100	100/90	145/125	130/120
Spalding	BP, US	190/140	180	260	150/130	130	110/100	110/100	135/125	140/130
Star	BP, C	190/140	180/160	260	150/120	130	110/100	110/100	135/125	130
Stark. Hardy Giant	BP, US	140	180	260	150/120	130	110/100	100/90	135/125	130
Sue	BP, C	140	180/160	260	150/120	130	110	110/100	135/125	140/130
Sumesi (12S.8.33)	BP, C	140	180	290/260	150/120	130	110/100	110/100	145/130	130
Summit	BP, C	140	180/160	290/260	150/120	130	110/100	100	125	130
Sunburst	BP, C	140	180	290	150/120	130	110/100	100/90	145/130	130/120
Sweetheart	BP, C	190/140	180	290/260	150/120	130	110/100	100/90	135/125	130/120
Sylvia	BP, C	140	180/160	290	150/120	130	110/100	100	145/125	130
Taleguera Brillante	U, S	140	180/160	290/260	150/115	130	100	100/90	135	130
Tigre	U, F	140	180	290	150/115	130	110	110/90	120	130
Van	BP, C	190/140	180	290/260	150/120	130	110	100/90	135/125	130/120
Van Spur	BP, C	190/140	180	290/260	150/120	130	110	100/90	135/125	130/120
Vega	BP, C	190/140	180	260	150/130	130	110	100/90	135/125	140/130
Vic	BP, C	140	180	260	150/120	130	110/100	100/90	135/125	140/130

Continued

Table 1 Continued

Cultivar	Origin*	Pchgms1	Pchcms3	Pchcms5	UDP96-005	UDP98-409	UPD98-021	UPD98-022	UPD97-402	UPD98-412
Vignola (Duroi 2)	U, I	140	180	260	150/130	130	110	100	140/125	130
Vittoria	U, I	140	180	290/260	150/130	130	110	100/90	145/130	130/120
13N.7.19	BP, C	140	180	290/260	150/120	130	110/100	100/90	135/125	130/120
13S.17.20	BP, C	190/140	180	290/260	150/120	130	110	110/90	130	130
13S.18.10	BP, C	190/140	180	290/260	150/120	130	110/100	110/100	135/125	130
13S.18.15	BP, C	190/140	180	290/260	150/120	130	110	110/100	135/125	130/120
13S.21.7	BP, C	140	180	290	150/130	130	110/100	100	135/125	130
13S.27.17	BP, C	140	180	260	150/120	130	110	100/90	135/125	140/130
13S.3.13	BP, C	190/140	180	290	150/120	130	100	110/100	145/130	130
44W.11.8	BP, C	190/140	180	260	150/120	130	110	110/100	145/130	130
83703007	BP, I	190/140	180	290	150/120	130	100	100	145/120	130
84703002	BP, I	190/140	180	290	150/120	130	110/100	100/90	130/120	130
84704006	BP, I	140	180	290	150/130	130	110/100	100/90	130/120	130

*U = Unknown pedigree; BP = Breeding programme; C = Canada; CE = Central Europe; F = France; I = Italy; S = Spain; US = USA.

Table 2 Microsatellite primer pairs used for the identification of sweet cherry genotypes

Locus	SSR repeat structure in peach	Product size in peach (bp)	Size range in sweet cherry (bp)	No. of alleles in peach	No. of alleles in sweet cherry	H
Pchgms1 ^a	(AC) ₁₂ (AT) ₆	194	140–190	4	2	0.43
Pchcms3 ^a	(CT) ₁₉	220	160–180	2	2	0.21
Pchcms5 ^a	(CA) ₉ (TA) ₈	246	260–290	3	2	0.47
UDP96-005 ^b	(AC) ₁₆ TG(CT) ₂	155	120–150	4	5	0.94
	CA(CT) ₁₁					
UDP98-409 ^b	(AG) ₁₉	129	130–160	5	2	0.04
UPD98-021 ^c	(GA) ₂₂ (CA) ₁₁	145	100–110	2	2	0.52
UPD98-022 ^c	(TG) ₁₂ (AG) ₂₄	139	90–110	5	4	0.75
UPD97-402 ^b	(AG) ₁₇	136	120–150	3	7	0.81
UPD98-412 ^c	(AG) ₂₈	129	100–140	5	4	0.25

^aSosinski *et al* (2000). ^bCipriani *et al* (1999). ^cTestolin *et al* (2000).

Table 3 Microsatellite primers that did not amplify scorable or polymorphic loci in sweet cherry genotypes

Locus	Reference	SSR repeat structure in peach	Size range* (bp)
Pchcms1	Sosinski <i>et al</i> (2000)	(CA) ₉ (TA) ₁₄ (GA) ₁₃	130–190
UPD98-024	Testolin <i>et al</i> (2000)	(GT) ₁₉ TC(TG) ₇	70–80
UPD98-411	Testolin <i>et al</i> (2000)	(TC) ₁₆	150–190
UPD98-410	Testolin <i>et al</i> (2000)	(AG) ₂₃	130–160
Pchgms2	Sosinski <i>et al</i> (2000)	(CT) ₂₄	135 n.p.
Pchgms3	Sosinski <i>et al</i> (2000)	(CT) ₁₄	190 n.p.
Pchgms4	Sosinski <i>et al</i> (2000)	(CT) ₂₁	150 n.p.
Pchcms2	Sosinski <i>et al</i> (2000)	(CA) ₈	90 n.p.
UDP96-003	Cipriani <i>et al</i> (1999)	(CT) ₁₁ (CA) ₂₈	85 n.p.
UDP96-008	Cipriani <i>et al</i> (1999)	(CA) ₂₃	135–155 n.p.
UDP96-018	Cipriani <i>et al</i> (1999)	(AC) ₂₁	265 n.p.
UDP96-019	Cipriani <i>et al</i> (1999)	(TG) ₁₈ (AG) ₇	320 n.p.
UDP98-405	Cipriani <i>et al</i> (1999)	(AG) ₉	105 n.p.
UDP98-406	Cipriani <i>et al</i> (1999)	(AG) ₁₅	395 n.p.
UPD98-025	Testolin <i>et al</i> (2000)	(CA) ₁₉	70–95 n.p.
UPD98-407	Cipriani <i>et al</i> (1999)	(GA) ₂₉	n.a.
UPD98-416	Testolin <i>et al</i> (2000)	(AG) ₁₈	n.a.
UDP96-001	Cipriani <i>et al</i> (1999)	(CA) ₁₇	n.a.
UDP97-403	Cipriani <i>et al</i> (1999)	(AG) ₂₂	n.a.
UPD96-010	Cipriani <i>et al</i> (1999)	(GT) ₂₁ (GAGT) ₄ (GA) ₁₈	n.a.
UPD96-013	Cipriani <i>et al</i> (1999)	(AG) ₂₂ (TG) ₈ TT(TG) ₁₀	n.a.
UPD96-015	Cipriani <i>et al</i> (1999)	(CA) ₃₁	n.a.
UPD97-401	Cipriani <i>et al</i> (1999)	(GA) ₁₉	n.a.
UPD98-408	Cipriani <i>et al</i> (1999)	(CT) ₁₄	n.a.
UPD98-414	Testolin <i>et al</i> (2000)	(TC) ₂₄	n.a.

*n.p. = no polymorphism; n.a. = no or weak amplification.

ous differentiation of 72 (94%) genotypes. UPGMA cluster analysis revealed several possible dendrograms and the dendrogram with the highest cophenetic correlation coefficient (0.66) is shown in Figure 1. As expected the genotypes derived by mutation could not be distinguished from the original genotype ('Van Spur' and 'Early Van Compact' from 'Van' and 'Burlat C1' from 'Burlat'). The other genotypes that could not be distinguished are two pairs of genotypes that share a common pedigree ('Celeste' and '13S.21.7' and 'Burlat' and '84704006'). Most genotypes obtained from the same breeding programmes and/or sharing common parents, group together.

Since most of the recently released genotypes share common parents, UPGMA cluster analysis was performed with the 23 genotypes that are not recent releases from breeding programmes. Those genotypes include the ancient cultivars of unknown origin and parental breeding genotypes directly derived from old cultivars. In this case, a single dendrogram with a cophenetic correlation coefficient of 0.70 was obtained (Figure 2). The dendrogram clustered these 23 cultivars into two main groups, although with a low bootstrap value (<50%) support, that separate the genotypes originating from northern

Figure 1 Dendrogram of the 76 sweet cherry cultivars included in this study based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient after amplification with nine pairs of microsatellite primers.

Figure 2 Dendrogram of 23 ancient or directly derived from sweet cherry cultivars based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient after amplification with nine pairs of microsatellite primers.

Europe and North America from those originating from southern Europe.

Discussion

In this work 72 sweet cherry genotypes were characterised with 27 SSR markers using nine primer pairs initially developed in peach. The results confirm the efficiency of microsatellite markers for fingerprinting purposes. Moreover, they demonstrate that microsatellites are highly conserved among peach and sweet cherry allowing the use of the same SSR primers to detect intra-specific variation in related species even though the ability to amplify loci and detect polymorphism usually decreases as the genetic distance increases (Steinkellner *et al*, 1997; White and Powell, 1997; Maguire *et al*, 2000; Roa *et al*, 2000).

The fact that both DNA sources used, leaves and dormant buds, produce DNA of sufficient quality for SSR amplification allows the characterisation of plant material at any time of the year. SSR detection using polyacrylamide gel electrophoresis (PAGE) complicates the identification of microsatellite alleles, mainly due to the presence of ladder-like stutter patterns, with no significant improvement in resolution compared to agarose gel

electrophoresis. Moreover, PAGE is more time-consuming and toxic than agarose gel electrophoresis. An automated detection system would be able to resolve allelic variation at a finer scale and, consequently, the number of alleles detected would even be greater than that reported in this work. However, for routine SSR genotype analysis, agarose gel electrophoresis is a convenient method (Morgante *et al*, 2001).

In this work, amplification of the microsatellite loci in sweet cherry was possible for 24 of the 34 (73%) peach microsatellites, of which 13 (38%) were polymorphic. This efficiency is lower than that recently reported in apricot (*Prunus armeniaca* L.), where 88% of peach SSR primers amplified loci, while 54% of them were polymorphic (Hormaza, 2002) probably due to the different position of apricot and cherry in relation to peach in *Prunus* phylogeny. Thus, morphological studies (Westwood, 1993) supported by cpDNA variation (Badenes and Parfitt, 1995) classifies *Prunus* species into five subgenera (*Prunophora*, *Amygdalus*, *Cerasus*, *Padus* and *Laurocerasus*). The main cultivated *Prunus* species belong to three of them: *Amygdalus* (peach and almond), *Prunophora* (plum and apricot), and *Cerasus* (sour and sweet cherries). The studies on cpDNA variation (Badenes and Parfitt, 1995) confirm the hypothesis (Watkins, 1976) that cherries are genetically more distant from peach than apricot, since the present day subgenus *Cerasus* species diverged earlier from the ancestral *Prunus* species than the other two subgenera.

For the set of sweet cherry genotypes analysed, the mean heterozygosity of the polymorphic loci was 0.49 ranging from 0.04 to 0.94. Although sweet cherry is an outbreeding species, the mean heterozygosity reported here is similar to that reported for other *Prunus* species, including peach (0.47, Testolin *et al*, 2000; 0.45, Sosinski *et al*, 2000) and apricot (0.51, Hormaza, 2002) using SSR markers. A low level of polymorphism in sweet cherry has also been detected by Stockinger *et al* (1996) and Gerlach and Stösser (1997), using RAPD markers, and by Boskovic and Tobutt (1998) and Beaver *et al* (1995) with isozyme markers, probably reflecting a narrow genetic base in sweet cherry germplasm.

Neither 'Burlat C1', a compact mutation of 'Burlat', nor 'Van Spur' or 'Early Van Compact', both mutations of 'Van', could be differentiated from 'Burlat' or 'Van', respectively. The impossibility of differentiating mutants that differ from the original genotype in one or few genes, was expected due to the intrinsic nature of SSRs since it is very unlikely that the microsatellites amplified correspond to the mutated DNA region when they have been randomly isolated from the whole genome. The case of the other two pairs of genotypes 'Celeste' and '13S.21.7', and '84704006' and 'Burlat' can be explained regarding the pedigree information available. 'Celeste' and '13S.21.7' both originated from the cross of 'Van' and 'Newstar', and '84704006' descends from the cross of 'Burlat' and 'Sunburst'. In the first situation, the two genotypes appear to have inherited the same alleles for the loci analysed, and, in the second, the allele segregation has resulted in the same band profile as one of the parental genotypes, 'Burlat'. The use of more SSRs recently developed in *Prunus* (Aranzana *et al*, 2002; Cantini *et al*, 2001) may allow discrimination between these two closely related genotypes.

Since several of the genotypes share common ancestors

various similar dendrograms were possible and they were ranked according to their cophenetic correlation coefficient. The phenogram generated from the SSR data illustrates the identification of the genotypes studied and groups them according to their known pedigree (Figure 1). A better representation of the similarity relationships in this species is obtained when only the 23 genotypes that include the ancient cultivars of unknown origin and parental breeding genotypes directly derived from other old cultivars are used to construct the dendrogram (Figure 2). In this case, a single dendrogram was obtained after UPGMA analysis. Consequently, we believe that this dendrogram has a real biological significance although the scarcity of markers available results in the low cophenetic correlation coefficient and the low bootstrap value support obtained. This situation will likely change when additional SSRs developed in *Prunus* are tested in cherry.

The dendrogram grouped the 23 ancient cultivars into two main clusters according to their geographical origin and area of cultivation, probably reflecting local selection of outstanding wild genotypes. One group contains genotypes from southern Europe, and the other contains cultivars that mainly originated in northern and central Europe or in North America but derive from ancient cultivars from northern Europe. The cultivar 'Blanca de Provenza', the only cherry tree in our collection that produces cherries with yellow-white coloured skin and flesh, appears separated from the other two clusters together with the Italian cultivar 'Vignola'. Interestingly, within the group that comprises southern European cultivars, the three cultivars that originated in the Spanish Extremadura region ('Ambrunes', 'Pico Negro' and 'Pico Colorado') cluster together. Another subgroup is formed by some French cultivars ('Precoce Bernard', 'Moreau', and 'Burlat'), that were obtained in the French region of Rhone by the end of the nineteenth and the beginning of the twentieth century (Hedrick *et al*, 1915; Société Pomologique de France, 1927, 1947). The cultivar 'Ramon Oliiva', that clusters closer to those three French cultivars, although it is sometimes reported to be of Spanish origin (Bargioni, 1996), seems to have been found originally near Angers in France (Hedrick *et al*, 1915). 'Duron-3' and 'Reverchon', that cluster closely together, were both taken to France from Italy (Ctifl, 1990; Bargioni, 1996), while 'Taleguera Brillante', 'Cristobalina' and 'Tigre' have an unclear origin. 'Tigre' is thought to come from France as well (Société Pomologique de France, 1927) whereas 'Cristobalina' originated in eastern Spain is morphologically different and geographically separated from the other Spanish cultivars studied; furthermore, it is self-compatible (Hugard, 1978) and the earliest variety to flower.

The second main cluster includes the ancient cultivars from Northern and Central Europe like 'Marmotte' (Northern France), 'Napoleon' (Central Europe) and 'Hedelfinger' (Central Europe probably from Germany) and the newer genotypes originating in North America or Europe but derived from cultivars from Northern Europe: 'Star' from the open pollination of 'Deacon', 'Sam' from the open pollination of a seedling of 'Windsor' (found in Ontario, Canada), 'Bing' from a seedling of 'Republican' (probably a seedling of Napoleon) and 'Van' from the open pollination of 'Empress Eugenie' (Northern France) (Hedrick *et al*, 1915; Société Pomolo-

gique de France, 1927; Ctifl, 1990; Bargioni, 1996; Brooks and Olmo, 1952, 1997). A southern European cultivar, 'Ferrovia', from the Italian region of Bari, also clusters in this subgroup.

The possibility of using SSR primers developed in a related species to detect intra-specific variation overcomes the troublesome process of their development, making a whole battery of already isolated SSR *Prunus* primer sequences available for the genetic characterisation of different species in the genus. Molecular characterisation of fruit tree scions and rootstocks is essential for the interests of fruit tree breeders, germplasm collections and the commercial sector such as nurseries. Since most temperate fruit tree species are vegetatively propagated, the identification of genotypes allow a standardisable reference for the identification of any cultivar and control of its propagation, independently of any factors that may limit or influence phenotypic characterisation, such as the environment, the time of year or the age of the tree. Furthermore, the possibility of studying the genetic diversity among different cultivars and populations, will benefit cherry breeding programmes by helping to take decisions on parental genotypes for crosses, and germplasm management to maximise the conserved diversity. This is becoming increasingly important to conserve the existing variability in the wild stands of this species scattered through most European and some Asian countries, especially due to the progressive narrowing of the genetic base. This situation is evidenced by the fact that the variability of traits of potential economic interest is limited in good fruit quality sweet cherry varieties (Iezzoni *et al*, 1990) and that all the self-compatible cultivars currently planted or used in breeding programmes derive from a single cultivar, 'Stella'. Microsatellites seem to be appropriate to fulfil those objectives and it will be of interest to expand these kind of studies to areas where it is necessary to conserve interesting cultivars or genotypes before they disappear.

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