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AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*

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Abstract We report the sequences of 17 primer pairs of microsatellite loci, which we have cloned and sequenced from two genomic libraries of peach [*Prunus persica* (L) Batsch] 'Redhaven', enriched for AC/GT and AG/CT repeats respectively. For ten of these microsatellite loci we were able to demonstrate Mendelian inheritance in a segregating back-cross population; the remainder did not segregate. The polymorphism of the microsatellites was evaluated in a panel of ten peach genotypes, including true-to-type peaches, nectarines and one canning-peach. Fifteen microsatellites (88%) were polymorphic showing 2–4 alleles each. The mean heterozygosity, averaged over all loci, was 0.32 and significantly higher than that reported in the literature for isozymes and molecular markers, such as RFLPs and RAPDs. We have also assayed the cross-species transportability and found that ten microsatellite (59%) gave apparently correct amplification in all *Prunus* species surveyed, namely *P. domestica* (European plum), *P. salicina* (Japanese plum), *P. armeniaca* (apricot), *P. dulcis* (almond), *P. persica* var. *vulgaris* (peach), *P. persica* var. *laevis* (nectarine), *P. avium* (sweet cherry) and *P. cerasus* (sour cherry), with three of them also being amplified in *Malus* (apple). The remaining microsatellites gave less-extensive amplification. Because of their appreciable polymorphism and wide cross-species transportability, most of these new markers can be integrated into the linkage maps which are currently being constructed in peach, as well as in other stone fruit crops, such as almond, apricot, cherry and plum.

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Introduction

Peach [*Prunus persica* (L) Batsch] is a self-fertile and naturally self-pollinating fruit species with very low genetic variability (Hesse 1975; Scorza et al. 1985; Scorza and Okie 1990). Its genetic base was dramatically narrowed in the second half of the 19th century when a few seedlings of "Chinese Cling"-type peach were introduced from Southern China into the United States and extensively propagated. Subsequently, these genotypes were repeatedly used as parents in breeding programs because of their large fruit size, flesh firmness and quality, thus leading to the high levels of inbreeding and co-ancestry now found amongst peach cultivars (Scorza et al. 1985). A consequence of the narrow genetic base of peach is that few phenotypic and isozyme polymorphims have been found in this species (Monet et al. 1985; Durham et al. 1987; Messeguer et al. 1987; Mowrey et al. 1990 a, b) and this has discouraged any attempt to use markers in breeding programs until the introduction, a decade ago, of molecular markers.

Molecular markers, i.e. markers based on the length polymorphism of DNA sequences, have revolutionised plant breeding and are now being applied to a variety of topics such as the selection of genotypes assisted by trait-linked markers, mapping Mendelian and quantitative traits loci, fingerprinting individuals and the genetic analysis of both populations and individuals (Rafalski et al. 1996). Such molecular markers have served to flank both phenotypic and isozyme markers in the analysis of the peach genome because of their virtually unlimited number. Among the array of DNA markers made available by the introduction of the techniques of DNA detection and amplification, only

RFLPs (restriction fragment length polymorphisms) and RAPDs (random amplified polymorphic DNAs) have been adopted in the genetic analysis of the peach, mainly with the aim of constructing linkage maps (Arús et al. 1994; Chaparro et al. 1994; Dirlewanger and Bodo 1994; Rajapakse et al. 1995; Quarta et al. 1998).

Peach has a small genome: flow cytometry analysis gives a value of 0.61 pg DNA/diploid nucleus (Baird et al. 1994), which corresponds to approximately 0.3×10^9 bp in the haploid genome. At the beginning of the decade, it was considered that the development of a linkage map would be easy, but problems soon arose as DNA markers revealed low polymorphism. Dirlewanger and Bodo (1994) reported that only 7% of the 524 RAPD primers assayed gave bands which segregated in the mapping population while Chaparro et al. (1994) found that only 16% of the 522 RAPD primers tested yielded polymorphism in their cross. Similarly, Rajapakse et al. (1995) demonstrated that only 29 out of 159 (18%) genomic and cDNA clones gave RFLP markers useful for mapping in their population. Quarta et al. (1998) working with a back-cross progeny carrying genes of both *P. persica* and *Prunus ferganensis* achieved better results as they found 38% of the 213 genomic and cDNA probes assayed to be polymorphic.

The low levels of polymorphism observed mean that microsatellite DNA, which consists of stretches of tandemly repeated copies of 1–6 nucleotide motifs (Rafalski et al. 1996), could represent an interesting source of polymorphic markers as it harbours considerable length variation between individuals and is extremely abundant in eukaryotic genomes (Tautz 1989; Weising et al. 1989; Morgante and Olivieri 1993; Wang et al. 1994). Until recently, microsatellites have not been widely used in plants because of the high cost of their isolation, but the introduction of procedures such as automatic sequencing and library enrichment has rendered the process quicker and more efficient and has placed these markers within the reach of plant geneticists (Gupta et al. 1996). Moreover, microsatellite DNA often has flanking regions that are highly conserved in related species and this renders primer pairs designed in one species useful for amplification of the same DNA region in related genomes (see Huang et al. 1998 and Peakall et al. 1998 for reviews). Microsatellites have recently been isolated and characterised in several crops, including fruit crops such as grape (Thomas and Scott 1993; Bowers et al. 1996), kiwifruit (Weising et al. 1996; Huang et al. 1998), *Citrus* (Kijas et al. 1995, 1997), apple (Guilford et al. 1997), and avocado (Sharon et al. 1997).

With the aim of producing polymorphic markers for use in preparing a genetic map of peach and related species of *Prunus*, we have isolated and characterised several AC/GT and AG/CT microsatellites from peach, checked their segregation and inheritance, screened them for genetic polymorphism using a panel of different genotypes, and investigated their transportability to the main stone fruit crops.

Materials and methods

Construction of genomic libraries enriched in SSR, and plaque screening

Total DNA was extracted from approximately 1.0 g of young leaves of the peach 'Redhaven' according to a slight modification of the procedure described by Doyle and Doyle (1990), and purified with 2-butoxyethanol according to Manning (1991). DNA was digested with the *Tsp*509I restriction enzyme and fragments in the size range of 200–800 bp were recovered from a 2% agarose gel after electrophoresis. The genomic DNA library was enriched in AC/GT microsatellite repeats and positive plaques screened and sequenced according to the procedure previously described (Huang et al. 1998).

PCR-amplification and product electrophoresis

The pairs of primers were designed to be 18–22-bp long with an annealing temperature between 55 and 63°C (optimum 60°C). PCR reactions were performed in a volume of 25 µl containing 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 100 ng of genomic DNA and 0.1 U of *Taq* polymerase (Pharmacia) using the following temperature profile: 95°C for 5 min, then 35 cycles of (94°C for 45 s, 57°C for 45 s and 72°C for 45 s), finishing with 72°C for 8 min. PCR products were separated by electrophoresis in 1% agarose gels (FMC BioProducts) and then stained with ethidium bromide.

Segregation analysis in peach progeny

The segregation analysis of microsatellites was carried out on 16 individuals originating from the back-cross *P. persica* IF7310828 × (*P. persica* IF7310828 × *P. ferganensis*). The progeny, together with the parents, were kindly supplied by R. Quarta of the Institute of Fruitculture of Rome (Italy). PCR-amplifications and electrophoreses were carried out as described above except that PCR products were separated in 3% Metaphore (FMC BioProducts) agarose gels.

Polymorphism evaluation and allele-sizing in peach cultivars

Microsatellites were also assayed for their polymorphism in a panel of ten peach cultivars, including five true-to-type peaches ('May Crest', 'Spring Crest', 'Iris Rosso', 'Tardivo Zuliani', 'Redhaven'), four nectarines ('Armking', 'Spring Red', 'Independence', 'Caldesi 2000'), and one clingstone peach ('Andross'). PCR reactions were carried out as described above, except that one of the two primers for each pair was radioactively labelled using γ-^{33}P-ATP and polynucleotide kinase (Sambrook et al. 1989). The labelled PCR products were separated on a 6%-denaturing polyacrylamide gel (Long Ranger™, FMC BioProducts) containing 7 M urea and run with 1.2 × TBE buffer at a constant power of 55 W. The gels were then dried and autoradiographed on X-ray film using standard procedures. A reference molecular-size marker was prepared from pUC18 plasmid DNA, which was sequenced using the M13 universal primer (Pharmacia), radioactively labelled as above, and the four sequencing reactions loaded onto the gel. The information content of the microsatellite loci was estimated by the number of alleles per locus and the heterozygosity (Nei 1973).

Assay of amplification in different *Prunus* species

All microsatellite primers were used to amplify the DNA of eight different species of *Prunus*, namely *P. domestica* (European plum) 'Stanley', *P. salicina* (Japanese plum) 'Midnight Sun', *P. armeniaca* (apricot) 'Cricot', *P. dulcis* (almond) 'Pizzuta d'Avola', *P. persica* var. *vulgaris* (peach) 'Redhaven', *P. persica* var. *laevis* (nectarine) 'Spring Red', *P. avium* (sweet cherry) 'Maggese Bianca', and *P. cerasus* (sour cherry) 'Montmorency'. A sample of *Malus* × *domestica* (apple) 'Golden Delicious' was also included.

Results

Microsatellite identification and characterisation

As a result of the enrichment procedure, about 50% of plaques contained the target repeat. With some sequences being discarded at the anchor PCR, because the microsatellite was either too close to or too far from the cloning site, and others being discarded because primers could not be designed, 37% of the clones recovered from plaques gave SSRs that were successfully amplified in both plasmid and the 'Redhaven' genomic DNA template by means of PCR. The bands produced were of the expected size, indicating that no multiple insert was sequenced. No insert was found to be duplicated.

Nearly half the AC/GT microsatellites were perfect, that is they were simple and with no interruption in the repeat sequence, and half were compound, with a run of a different motif adjacent to that expected. AG/CT microsatellites were all perfect (Table 1).

Segregation analysis

Ten of the seventeen loci examined segregated in the *P. persica* × (*P. persica* × *P. ferganensis*) population. In all cases, segregation analysis gave results consistent with those expected assuming Mendelian inheritance and no case of distorted segregation was observed (Table 2).

Table 1 Primer sequences, repeat motif, and PCR product sizes of 17 microsatellites sequenced from two DNA libraries of peach [*P. persica* (L.) Batsch] 'Redhaven', enriched with (AC/GT) and (AG/CT) repeats respectively

Locus code	Primer sequences (5′ → 3′)	Repeat motif	Length (bp)[a]
From the (AC/GT)*n* enriched library			
UDP96-001	5′AGTTTGATTTTCTGATGCATCC 5′TGCCATAAGGACCGGTATGT	(CA)17	120
UDP96-003	5′TTGCTCAAAAGTGTCGTTGC 5′ACACGTAGTGCAACACTGGC	(CT)11(CA)28	143
UDP96-005	5′GTAACGCTCGCTACCACAAA 5′CCTGCATATCACCACCCAG	(AC)16TG(CT)2CA(CT)11	155
UDP96-008	5′TTGTACACACCCTCAGCCTG 5′TGCTGAGGTTCAGGTGAGTG	(CA)23	165
UDP96-010	5′CCCATGTGTGTCCACATCTC 5′TTGATGATTCCATGCGTCTC	(GT)21(GAGT)4(GA)18	131
UDP96-013	5′ATTCTTCACTACACGTGCACG 5′CCCCAGACATACTGTGGCTT	(AG)22(TG)8TT(TG)10	198
UDP96-015	5′CCTTGACCTATTTGTTCGTCA 5′ACTAGTCAAACAATCCCCCG	(CA)31	174
UDP96-018	5′TTCTAATCTGGGCTATGGCG 5′GAAGTTCACATTTACGACAGGG	(AC)21	253
UDP96-019	5′TTGGTCATGAGCTAAGAAAACA 5′TAGTGGCACAGAGCAACACC	(TG)18(AG)7	216
From the (AG/CT)*n* enriched library			
UDP97-401	5′TAAGAGGATCATTTTTGCCTTG 5′CCCTGGAGGACTGAGGGT	(GA)19	130
UDP97-402	5′TCCCATAACCAAAAAAAACACC 5′TGGAGAAGGGTGGGTACTTG	(AG)17	136
UDP97-403	5′CTGGCTTACAACTCGCAAGC 5′CGTCGACCAACTGAGACTCA	(AG)22	150
UDP98-405	5′ACGTGATGAACTGACACCCA 5′GAGTCTTTGCTCTGCCATCC	(AG)9	104
UDP98-406	5′TCGGAAACTGGTAGTATGAACAGA 5′ATGGGTCGTATGCACAGTCA	(AG)15	101
UDP98-407	5′AGCGGCAGGCTAAATATCAA 5′AATCGCCGATCAAAGCAAC	(GA)29	212
UDP98-408	5′ACAGGCTTGTTGAGCATGTG 5′CCCTCGTGGGAAAATTTGA	(CT)14	100
UDP98-409	5′GCTGATGGGTTTTATGGTTTTC 5′CGGACTCTTATCCTCTATCAACA	(AG)19	129

[a] From the sequenced clones

Table 2 Segregation analysis of microsatellite markers in the *P. persica* × (*P. persica* × *P. ferganensis*) backcross (see text for more information about the cross)

Locus	Cross[a]	Segregation	Expected ratio	Observed ratio	χ^{2} [b]
UDP96-001	aa × ab	aa : ab	1 : 1	9 : 7	0.25 n.s.
UDP96-003	ab × ab	aa : ab : bb	1 : 2 : 1	2 : 12 : 2	4.00 n.s.
UDP96-005	aa × aa	None			
UDP96-008	aa × ab	aa : ab	1 : 1	6 : 10	1.00 n.s.
UDP96-010	ab × a0[c]	(aa + a0) : (ab + b0)	1 : 1	8 : 8	0.00 n.s.
UDP96-013	bb × ab	ab : bb	1 : 1	7 : 9	0.25 n.s.
UDP96-015	bb × ab	ab : bb	1 : 1	7 : 9	0.25 n.s.
UDP96-018	ab × ab	aa : ab : bb	1 : 2 : 1	5 : 11 : 0	5.38 n.s.
UDP96-019	aa × aa	None			
UDP97-401	bb × ab	ab : bb	1 : 1	9 : 7	0.25 n.s.
UDP97-402	aa × aa	None			
UDP97-403	aa × aa	None			
UDP98-405	aa × aa	None			
UDP98-406	ab × ab	aa : ab : bb	1 : 2 : 1	4 : 8 : 4	0.00 n.s.
UDP98-407	aa × aa	None			
UDP98-408	aa × aa	None			
UDP98-409	aa × ab	aa : ab	1 : 1	9 : 7	0.25 n.s.

[a] At any given locus, the fast-migrating allele has been designated as a, the next fastest as b, etc.
[b] At $P = 0.05$
[c] The allele a shows weak amplification in homozygous genotypes and is not amplified when b is present, because of the strong competition of b

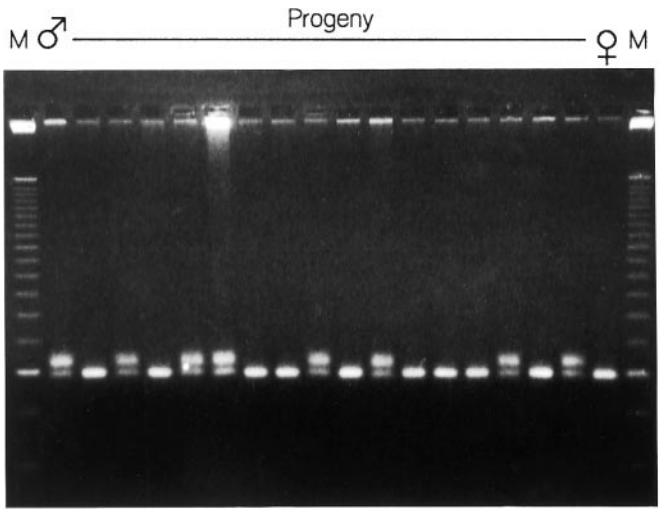

Fig. 1 Allelic segregation of the microsatellite locus UDP96-001, sequenced from peach, in the back-cross *P. persica* IF7310828 × (*P. persica* IF7310828 × *P. ferganensis*). *M* = 25-bp ladder

Six loci (35%) segregated 1 : 1 as a result of heterozygosity in the F_1 hybrid (Table 2, Fig. 1) and were suitable for mapping in that parent. Three loci (18%) segregated 1 : 2 : 1, being heterozygous for the same alleles in both parents, and were useful as allelic bridges for merging and orientating linkage subgroups and as anchor markers for finding linkage groups homologous in the two parents of the cross and constructing integrated genetic maps.

Length polymorphism of microsatellites in *P. persica*

Microsatellite polymorphism was assayed in ten cultivars representative of all three types of peaches (true peach, nectarine and canning-peach). Fifteen of the microsatellites studied (88%) were polymorphic, showing 2–4 alleles; the other two microsatellites (UDP98-405 and UDP98-408) revealed no polymorphism amongst the cultivars examined (Table 3). An example of the resolution achieved in the polyacrylamide gels using radioactively labelled primers is given in Fig. 2. The mean heterozygosity, averaged over all loci, was 0.32 (range 0.0–0.68).

Cross-species transportability

Most microsatellite loci gave apparently correct PCR-amplification in different species of *Prunus*. PCR amplification was considered to be successful when a number of sharp bands compatible with the ploidy level of that species and in the expected size range were obtained on agarose gels. Using such a criterion, ten microsatellite loci (59%) gave amplification in all *Prunus* species surveyed (Fig. 3), and three also gave amplification in apple (Table 4). Two loci gave amplification only within the subgenus *Amygdalus*, to which peach belongs, but not in the other subgenera surveyed. One locus (UDP96-010) gave amplification only in the true peaches.

Discussion

The library enrichment procedure adopted here made searching for microsatellites substantially more efficient, since the percentage of inserts containing a microsatellite repeat was considerably higher than that usually found in non-enriched libraries, where positive clones seldom reach 1% of the screened plaques. The

Table 3 Allelic sizes and frequencies, and heterozygosity in ten peach [*P. persica* (L) Batsch] cultivars at 17 SSR loci[a]

Locus	Alleles[b]				Heterozygosity[c]
	1	2	3	4	
UDP96-001	127	129			
	0.95	*0.05*			*0.10*
UDP96-003	131	139	147	149	
	0.40	*0.20*	*0.35*	*0.05*	*0.68*
UDP96-005	151	167			
	0.15	*0.85*			*0.26*
UDP96-008	148	170			
	0.35	*0.65*			*0.46*
UDP96-010	127	143			
	0.10	*0.90*			*0.18*
UDP96-013	189	205	207	213	
	0.10	*0.20*	*0.05*	*0.65*	*0.52*
UDP96-015	163	179	183	185	
	0.10	*0.50*	*0.35*	*0.05*	*0.38*
UDP96-018	240	258	272		
	0.10	*0.70*	*0.20*		*0.46*
UDP96-019	215	225	227		
	0.05	*0.85*	*0.10*		*0.26*
UDP97-401	131	137			
	0.15	*0.85*			*0.26*
UDP97-402	139	151			
	0.65	*0.35*			*0.46*
UDP97-403	155	157	159		
	0.20	*0.70*	*0.10*		*0.46*
UDP98-405	114				
	1.00				n.p.
UDP98-406	105	123			
	0.90	*0.10*			*0.18*
UDP98-407	188	210	212		
	0.30	*0.10*	*0.60*		*0.54*
UDP98-408	109				
	1.00				n.p.
UDP98-409	134	136			
	0.90	*0.10*			*0.18*
Average heterozygosity					*0.32*

[a] Single-banded genotypes have been considered as homozygous at that given locus
[b] Size (bp) is in the first row and the frequency, in italics, in the second row
[c] n.p. = not polymorphic

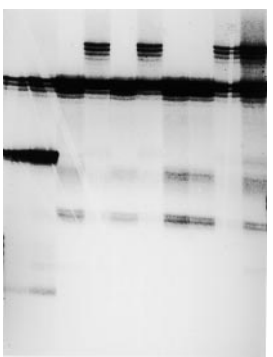

Fig. 2 Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labelled UDP96-018 primer pairs from ten peach cultivars. From left: Maycrest, Springcrest, Iris rosso, Tardivo Zuliani, Armking, Spring Red, Independence, Andross, Caldesi 2000, and Redhaven

efficiency of the enrichment procedure recorded for peach is not far from that we previously reported in *Actinidia* (Huang et al. 1998) and since the procedure appears to be of general application in plants, it is probably the most significant technical development since microsatellites were first proposed as ideal markers for plant genetic analysis and marker-assisted selection.

Microsatellites sequenced from the AC/GT library were often compound, with an AG/CT repeat associated with the expected AC/GT repeat. On the other hand, the microsatellites found in the AG/CT library were all simple and of the expected type. This result is in agreement with those reported for other plants, both in angiosperms, such as *Actinidia* (Weising et al. 1996; Huang et al. 1998), *Malus* (Guilford et al. 1997) and avocado (Sharon et al. 1997), as well as in gymnosperms such as *Picea* (Pfeiffer et al. 1997), and supports the conclusion that in plants AG/CT repeats are more common than AC/GT repeats (Langercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994).

We were able to check the segregation pattern of ten loci and, in all cases, alleles showed co-dominance and segregated according to Mendelian laws. No distortion

Table 4 Cross-species amplification of 17 microsatellite markers sequenced in peach[a]

Locus	Subgenus *Amygdalus*			Subgenus *Prunofora*			Subgenus *Cerasus*		*Malus* Apple
	Peach	Nectarine	Almond	Apricot	European plum	Japanese plum	Sweet cherry	Sour cherry	
UDP96-001	+[b]	+	+	+	+	+	+	+	n.a.[b]
UDP96-003	+	+	+	+	+	+	+	+	n.a.
UDP96-005	+	+	+	+	+	+	+	+	n.a.
UDP96-008	+	+	+	+	+	+	+	+	n.a.
UDP96-010	+	n.a.	n.a.	?[b]	?	n.a.	n.a.	n.a.	n.a.
UDP96-013	+	+	+	n.a.	+	+	n.a.	n.a.	n.a.
UDP96-015	+	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
UDP96-018	+	+	+	+	+	+	+	+	+
UDP96-019	+	+	+	+	+	+	+	+	n.a.
UDP97-401	+	+	+	n.a.	n.a.	n.a.	+	n.a.	n.a.
UDP97-402	+	+	n.a.	+	+	+	+	?	n.a.
UDP97-403	+	+	+	+	+	+	+	+	n.a.
UDP98-405	+	+	+	+	+	+	+	+	?
UDP98-406	+	+	+	+	+	+	+	+	?
UDP98-407	+	+	+	?	?	+	+	+	+
UDP98-408	+	+	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
UDP98-409	+	+	+	+	+	+	+	+	+

[a] Each amplification has been repeated at least twice
[b] + = successful amplification, ? = weak amplification, n.a. = no amplification

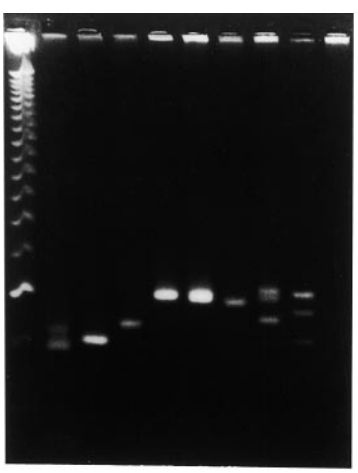

Fig. 3 PCR-amplification and electrophoresis on a 3% Metaphor agarose gel of the microsatellites UDP96-001 in different species of *Prunus* and apple. From left: 25-bp ladder, European plum, Japanese plum, apricot, almond, peach, nectarine, sweet cherry, sour cherry, and apple. Note the apparently correct amplification in all *Prunus* species, but not in apple, and the compatibility of the number of bands with the known ploidy of the different species

was observed in the ratio of the observed phenotypes, despite the population being derived from an initial interspecific cross between *P. persica* and *P. ferganensis*, but this could have been due to the low number of loci examined.

Nine loci (53%) were suitable for mapping in the F_1 hybrid but only four (24%) in *P. persica*. Because of the low level of heterozygosity in peach, segregating populations are generated by selfing the F_1 progeny obtained by crossing unrelated wild genotypes, or by test-crossing the F_1 progeny to one of the parents (Chaparro et al. 1994). Our data showed that test-crossing is a good mating design to produce segregating populations for mapping in peach and this confirms the results obtained by Quarta et al. (1998) using RFLP markers on the same population. Nevertheless, heterozygosity in peach cultivars at microsatellite loci could be higher than expected for a self-pollinating species, with the percentage of heterozygous loci in the cultivars examined varying from 6 to 41% (data not shown). If this is confirmed and linkage maps are constructed in the future using microsatellite markers, then segregating populations for peach mapping could also be obtained by simply test-crossing any peach cultivar, previously assayed for its high degree of heterozygosity, to wild genotypes of any compatible *Prunus*. This non-canonical testcross, called a pseudo-testcross (Grattapaglia and Sederoff 1994; Hemmat et al. 1994), is commonly used in work with out-crossing woody species.

The level of microsatellite heterozygosity found in peach (average 0.32, range 0.0–0.68) deserves further comment. It should be noted that the particular cultivars used in our study are not a random sample of a natural population. Furthermore, only a few genotypes were examined, though this is a less severe constraint since even a few individuals are sufficient for estimating H if a large number of loci is examined (Gorman and Renzi 1979, cited in Nei 1987). This is because the variation in single-locus heterozygosity among loci is very great in many species and the estimation of average heterozygosity can be improved more by the addition of a further locus than by the addition of a further genotype (Nei 1987). Despite these limitations, we believe that the low genetic polymorphism observed in morphological traits and isozymes in peach is due to the genetic drift caused by the introduction into the United States of a few genotypes from China in the 19th century and to their extensive use in breeding. These genotypes founded a new cultivar platform which progressively substituted the

previous one in all Western countries (Scorza et al. 1985; Scorza and Okie 1990). The higher polymorphism of microsatellite loci could have originated *ex novo* from the second half of the past century, given the fast mutation rate of the DNA repeats, as indicated by the first analyses carried out on the human genome (Weber and Wong 1993; Tautz and Schlötterer 1994).

The appreciable polymorphism of some microsatellites renders these molecular markers the most informative of those so far analysed in peach and they are therefore very suitable for studying peach germplasm diversity and parentage, and are useful for a better understanding of the history of present-day peach cultivars. Microsatellites also appear suitable for genome mapping and cultivar fingerprinting as well as for other genetic studies.

The transportability of microsatellites from peach, from which they were isolated, to different species of *Prunus* was extensive but not surprising as similar transportability has been already demonstrated in very divergent taxa, including fruit crop genera such as *Vitis* (Thomas and Scott 1993), *Citrus* (Kijas et al. 1995), and *Actinidia* (Weising et al. 1996; Huang et al. 1998), but see Huang et al. (1998) and Peakall et al. (1998) for more comprehensive lists. Our data show a parallel behaviour of pairs of species such as almond-peach (including nectarine), apricot-plum, sweet- and sour cherry, thus confirming that these pairs of species are closely related phylogenetically. The wide cross-species transportability of most primers designed in peach makes microsatellite markers even more useful, given the current high level of investment in mapping the genomes of related stone fruit species, such as almond, apricot, cherry and plum (Arús et al. 1994).

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