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Relationships among Peach, Almond, and Related Species as Detected by Simple Sequence Repeat Markers

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Abstract. The genetic relationships among peach [*Prunus persica* (L.) Batsch], almond [*P. dulcis* (Mill.) D.A. Webb or *P. amygdalus* (L.) Batsch] and 10 related *Prunus* species within the subgenus *Amygdalus* were investigated using simple sequence repeat (SSR) markers. *P. glandulosa* Pall. was included as an outgroup. Polymorphic alleles were scored as present or absent for each accession. The number of alleles revealed by the SSR analysis in peach and almond cultivars ranged from one to three whereas related *Prunus* species showed a range of one to 10 alleles. Results demonstrated an extensive genetic variability within this readily intercrossed germplasm as well as the value of SSR markers developed in one species of *Prunus* for the characterization of related species. Mean character difference distances were calculated for all pairwise comparisons and were used to construct an unrooted dendogram depicting the phenetic relationships among species. Four main groups were distinguished. Peach cultivars clustered with accessions of *P. davidiana* (Carr.) Franch. and *P. mira* Koehne. The second group contained almond cultivars. A third group included accessions of *P. argentea* (Lam) Rehd., *P. bucharica* Korschinsky, *P. kuramica* Korschinsky, *P. pedunculata* Pall, *P. petunikowii* Lits., *P. tangutica* (Spach) Batal., and *P. webbii* (Spach) Vieh.. *P. glandulosa* and *P. scoparia* Batal. were included in a fourth group.

Peach (*Prunus persica*) and almond (*P. dulcis*) syn. (*P. amygdalus*) are two species of genus *Prunus* subgenus *Amygdalus* (Rosaceae, subfamily Prunoideae) that are commercially grown worldwide. These species originated in Southeastern and Central Asia respectively, and represent divergent evolution under two distinct environments, being warmer and more humid in the case of peach, and colder and xerophytic for almond (Watkins, 1976). Related *Prunus* species are found growing wild from eastern China to the mountainous areas and deserts of western China, Kurdistan, Turkestan, Afghanistan and Iran (Browick and Zohary, 1996; Faust and Timon, 1995; Grasselly, 1976; Hesse, 1975; Kester and Gradziel, 1996; Kester et al., 1991; Scorza and Sherman, 1996).

The direct use of these related *Prunus* species as a rootstock for peach and almond, mainly under non-irrigated native conditions, has been reported by several authors (Denisov, 1988; Grasselly, 1976; Hesse, 1975). Interspecific crosses (peach x almond, peach x *P. davidiana*, and *P. webbii* x peach) have also been used as peach and almond rootstocks (Berhnard, 1949; Brooks and Olmo, 1982; Kester and Hansen, 1966). Hesse (1975) and Scorza and Sherman (1996) suggested the value of closely related *Prunus* species in peach breeding. Related species have also been reported as having potential in almond breeding to improve the quality of kernels and as sources of self-compatibility (Gradziel and Kester, 1998; Gradziel et al., 2001; Kester and Gradziel, 1996; Kester et al., 1991).

Studies of germplasm diversity and genetic relationships can be used to assess the value of these species in cultivar development. Early studies of *Prunus* involved isozymes (Mowrey and Werner, 1990) and restriction fragment analysis of chloroplast DNA (Badenes and Parfitt, 1995; Uematsu et al., 1991). Recent studies analysed variation in DNA sequences of internal transcribed spacers (ITS) in nuclear ribosomal DNA (Lee and Wen, 2001) and chloroplast DNA (Bortiri et al., 2001). Analysis was from a taxonomic perspective, assaying a wide range of species.

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Simple sequence repeat (SSR) markers (microsatellites) are characterized by high polymorphism and abundance, with co-dominant inheritance, and are often transferable across closely related species (Gupta et al., 1996). These molecular markers are ideal for assessing genetic variability in related species and understanding the genetic relationships among them (Westman and Kresovich, 1997). Recently, SSR primers generated in different *Prunus* species have been reported (Aranzana et al., 2002; Cantini et al., 2001; Cipriani et al., 1999; Dirlewanger et al., 2002; Downey and Iezzoni, 2000; Joobeur et al., 2000; Sosinski et al., 2000; Wang et al., 2002). These include highly informative markers that are required for accurate estimation of the amount and nature of genetic variability.

The objective of this research was to establish the genetic relationships among peach, almond and 10 related *Prunus* species using SSR markers, possibly facilitating their use in interspecific introgression and cultivar improvement.

Materials and Methods

Plant material and DNA isolation. Evaluated germplasm included six peach cultivars (Chinese Cling, Bailey, Fay Elberta, Halford, Siberian C, and Tzim Pee Tao) and five almond cultivars (Ferragnes, Garden Prince, Mission, Ne Plus Ultra and Nonpareil), which are representative of the diversity in these species (Table 1). Two accession of each of 10 related *Prunus* species within the subgenus *Amygdalus* (*P. argentea*, *P. bucharica*, *P. davidiana*, *P. kuramica*, *P. mira*, *P. pedunculata*, *P. petunikowii*, *P. scoparia*, *P. tangutica*, and *P. webbii*) were also included. An accession of *P. glandulosa* was included as an outgroup for statistical analysis (Table 2).

Total genomic DNA was isolated using the procedure described by Gepts and Clegg (1989). DNA was quantified using a fluorometer DyNAQuant 200 (Amershan-Pharmacia, Piscataway, N.J.).

SSR analysis. Eighteen SSR markers developed in sweet cherry or peach (Table 3) were screened for DNA polymorphism. The primers were synthesized by Gibco-BRL (Gibco BRL, Carlsbad,

Table 1. Origin, parentage and main agronomic characteristics of the peach and almond cultivars assayed.

Cultivar	Origin	Parentage	Main agronomic characteristics
Chinese Cling	China	Unknown	Clingstone peach used for processing
Bailey	US	Unknown	Freestone peach used as rootstock
Fay Elberta	US	Elberta (OP)[z]	Freestone peach used for the fresh market
Halford	US	Lovell (OP)[z]	Cling peach used for processing
Siberian C	China	Unknown	Clingstone peach used as rootstock
Tzim Pee Tao	China	Unknown	Freestone peach used as rootstock
Ferragnes	France	Cristomorto x Ai	Soft-shell and self-incompatible almond
Garden Prince	US	Peach x almond	Paper-shell and self-compatible almond
Mission	US	Unknown	Semi-hard shell and self-incompatible almond
Ne Plus Ultra	US	Unknown	Soft-shell and self-incompatible almond
Nonpareil	US	Unknown	Paper-shell and self-incompatible almond

[z]OP = open pollinated, pollen parent unknown.

Calif.). The PCR reaction was done in 25-μL volumes and the reaction mixture contained 10 μM Tris-HCl (pH 8.2), 50 mM KCl, 1.9 mM $MgCl_2$, 100 μM of each dNTP, 0.125 μM of each primer (forward and reverse), one unit of Taq DNA polymerase enzyme (Roche Applied Science. Indianapolis, Ind.), and 50 ng of genomic DNA. The cycling parameters were one cycle of 95 ºC for 3 min; 35 cycles of 94 ºC for 1 min, 57 ºC for 1 min, and 72 ºC for 2 min; followed by 10 min at 72 ºC. Annealing temperature used (57 ºC) is the temperature recommended for these primers in peach (Cipriani et al., 1999; Testolin et al., 2000). In addition, Hormaza, et al. (2002) and Serrano et al. (2002) used a similar temperature of 55 ºC in the application of these pairs of primers in a wide range of *Prunus* species. The PCR reactions were carried out in a 96-well block Robocycler (Stratagene Inc., La Jolla, Calif.). Amplified PCR products were separated in a 3% Metaphor (Biowittaker, Rockland, Maine) agarose gel (1× TBE buffer), stained with ethidium bromide and photographed. A 123-bp ladder (Gibco BRL) was used as a molecular size standard.

Phenetic relationships. Polymorphic alleles were scored as present or absent. Some primer combinations did not generate any products in some accessions, and we analysed the data with the alleles for those markers scored in two different ways. First, we assigned missing values (?) to all alleles in a particular accession for any marker for which no amplification was observed in that accession. Second, we coded all such alleles as absent (0) in those accessions. Mean character difference distances were calculated for all pairwise comparisons in PAUP* test version 4.0b4a (Swofford, 2000). The results were used to construct an unrooted dendrogram using the neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) that depicts the phenetic relationships among the different accessions.

Results

Polymorphism. The number of alleles revealed by the SSR analysis in peach and almond cultivars ranged from one to three (data not shown), whereas all the other *Prunus* species showed a range from one to ten alleles for the different markers assayed. UDP96-003 was the most polymorphic marker with a total of 10 alleles detected in the different *Prunus* species assayed. The SSR markers UDP96-010 and UDP96-015 were specific for peach (*P. persica*) (Table 3).

Amplification was successful for all 18 SSR loci assayed in peach, with 15 of the loci successful in almond (*P. dulcis*). For other species, the number of loci that amplified ranged from 16 in *P. davidiana*, 15 in *P. mira* and *P. webbii,* to 6 in *P. glandulosa* (Table 4).

A total of 42 and 48 polymorphic bands were scored for the six peach and five almond cultivars, respectively. For the remaining *Prunus* species, polymorphism was evaluated from only two accessions, and so were reduced in number. Nevertheless, it was possible to see differences among species. *Prunus argentea*, *P. bucharica*, *P. davidiana*, *P. kuramica*, *P. mira*, and *P. webbii* had more than 20 polymorphic bands. A range of 10 to 16 polymorphic bands was observed in *P. glandulosa*, *P. pedunculata*, *P. petunikowii*, *P. scoparia*, and *P. tangutica* (Table 4).

Phenetic relationships. Phenetic relationships among the peach and almond cultivars and related species, based on the second

Table 2. Species from the genus *Prunus* subgenus *Amygdalus* assayed and their potential use in peach and almond breeding, with *P. glandulosa* of the subgenus Cerasus as an outgroup.

Section	Species	Use in peach and almond breeding[z]
Amygdalus Spach	*P. persica* (L.) Batsch	Self-compatibility and pest and disease resistance in almond
	P. davidiana (Carr.) Franch.	Disease resistance in peach and self-compatibility in almond
	P. mira Koehne	Disease resistance in peach and self-compatibility in almond
	P. dulcis (Mill.) D.A. Webb	Pest and disease resistance in peach
	P. argentea Lam	Self-compatibility and frost resistance in almond
	P. bucharica Korschinsky	Self-compatibility, growth habit and frost resistance in almond
	P. kuramica Korschinsky	Self-compatibility and disease resistance in almond
	P. webbii (Spach) Vieh.	Self-compatibility and growth habit in almond
Chameamygdalus Spach	*P. petunikowii* Lits.	Pest and disease resistance in almond
	P. tangutica Batal.	Pest and disease resistance in almond
Spartioides Spach	*P. scoparia* Batal.	Self-compatibility and drought resistance in almond
Leptopus Spach	*P. pedunculata* Pall.	Pest and disease resistance in almond
Microcerasus Reh.	*P. glandulosa* Pall.	Outgroup

[z]Denisov, 1988; Gradziel et al., 2001; Grasselly, 1976; Hesse, 1975; Kester et al, 1991; Kester and Gradziel, 1996; Scorza and Sherman, 1996.

Table 3. SSR markers assayed and polymorphism observed among the peach, almond and related *Prunus* species assayed.

		Polymorphism		
Marker	Origin	Alleles (no.)	Size range	Reference
PS08E08	Cherry	6	195–212	Sosinski et al., 2000
PS12A02	Cherry	5	178–218	Sosinski et al., 2000
UDP96-001	Peach	9	80–154	Cipriani et al., 1999
UDP96-003	Peach	10	50–180	Cipriani et al., 1999
UDP96-005	Peach	9	110–210	Cipriani et al., 1999
UDP96-008	Peach	6	120–170	Cipriani et al. 1999
UDP96-010	Peach	0	---	Cipriani et al., 1999
UDP96-013	Peach	8	130–245	Cipriani et al., 1999
UDP96-015	Peach	0	---	Cipriani et al., 1999
UDP96-018	Peach	9	220–260	Cipriani et al. 1999
UDP96-019	Peach	7	205–245	Cipriani et al., 1999
UDP97-401	Peach	6	92–160	Cipriani et al., 1999
UDP97-402	Peach	1	134	Cipriani et al., 1999
UDP97-403	Peach	8	120–170	Cipriani et al., 1999
UDP98-405	Peach	9	82–150	Cipriani et al., 1999
UDP98-406	Peach	6	73–140	Cipriani et al., 1999
UDP98-407	Peach	6	178–220	Cipriani et al., 1999
UDP98-408	Peach	9	73–207	Cipriani et al. 1999

analysis, are shown as an unrooted NJ dendrogram in Fig. 1. Four main groups were observed. Group 1 included *Prunus persica* cultivars and accessions of *P. davidiana* and *P. mira*; Group 2 contained the *P. dulcis* cultivars; Group 3 included accessions of *P. argentea*, *P. bucharica*, *P. kuramica*, *P. pedunculata*, *P. petunikowii*, P. *tangutica*, and *P. webbii*; and *Prunus glandulosa* and *P. scoparia* formed Group 4.

Figure 1 resulted from an analysis in which 0's were assigned to all alleles in a particular accession for any marker for which no amplification was observed in that accession. There was a total of 384 such cells in our data matrix, 17.1% of the total (2244 cells). When those cells were instead coded with missing values (?) (results not shown), the four groups described above were again recovered, but the relationships among them were slightly different. Thus, while in Fig. 1 the almond cultivars (Group 2) clustered with Group 3, in the alternate analysis Group 2 clustered with *P. glandulosa* and *P. scoparia* (Group 4). The branching order within Group 3 was also slightly different in the two analyses.

Discussion

Amplification was successful in peach for all markers initially developed in peach. These results agree with reports by Cipriani et al. (1999) of the successful use of these markers. Results showed a high degree of homology for the SSR loci between peach and almond. Of 18 markers with successful amplification in peach, 15 also showed successful amplification in almond (Table 2). These results support a close evolutionary distance between these species as suggested by Watkins (1976).

The level of polymorphism in our peach cultivars was similar to that reported by Cipriani et al. (1999). The range of the amplified band sizes in peach, almond and *Prunus* species (Table 2) was also similar to those reported by Downey and Iezzoni (2000) for black cherry and Cipriani et al. (1999) for peach using the same primer pairs.

Variations in the number of polymorphic SSR marker and the total number of polymorphism (detected alleles) were observed (Table 4), allowing differentiation into two groups, one with a high number of polymorphic bands (higher than 20) (*P. argentea*, *P. bucharica*, *P. davidiana*, *P. kuramica*, *P. mira*, and *P. webbii*), and a second group with a much reduced number of polymorphic bands (between 10 to 13) (*P. glandulosa*, *P. pedunculata*, *P. petunikowii*, *P. scoparia*, and *P. tangutica*). The small sample per species, however, does not allow broader generalizations about the species in general.

Differences in amplification success for SSR markers observed among species are due to the genetic variability between the different species and peach, where the SSRs were developed. A decrease in the amount of polymorphic SSR markers would be expected as genetic distance increases from the designated anchor species.

Results also demonstrated the possibility of cross-species transfer for several SSR markers and consequently the value of markers developed in one species of *Prunus* for the characterization of other species within the subgenus. Results may have value in assessing cross-species genetic compatibility and could be useful

Table 4. SSR markers assayed and polymorphism observed for the Prunus species evaluated.

Species	SSR assayed[z] (no.)	SSRs amplified[y] (no.)	Polymorphic SSRs[x] (no.)	Total alleles detected[w] (no.)
P. argentea	18	13	6	21
P. bucharica	18	12	8	26
P. davidiana	18	16	6	24
P. dulcis	18	15	15	48
P. glandulosa	18	6	4	10
P. kuramica	18	13	6	22
P. mira	18	15	8	24
P. pedunculata	18	11	4	16
P. persica	18	18	17	42
P. petunikowii	18	7	3	11
P. scoparia	18	10	2	13
P. tangutica	18	10	4	14
P. webbii	18	15	9	25

[z]Number of SSR markers screened.
[y]Number of SSR that amplified.
[x]Number of polymorphic loci detected by marker.
[w]Total number of alleles detected.

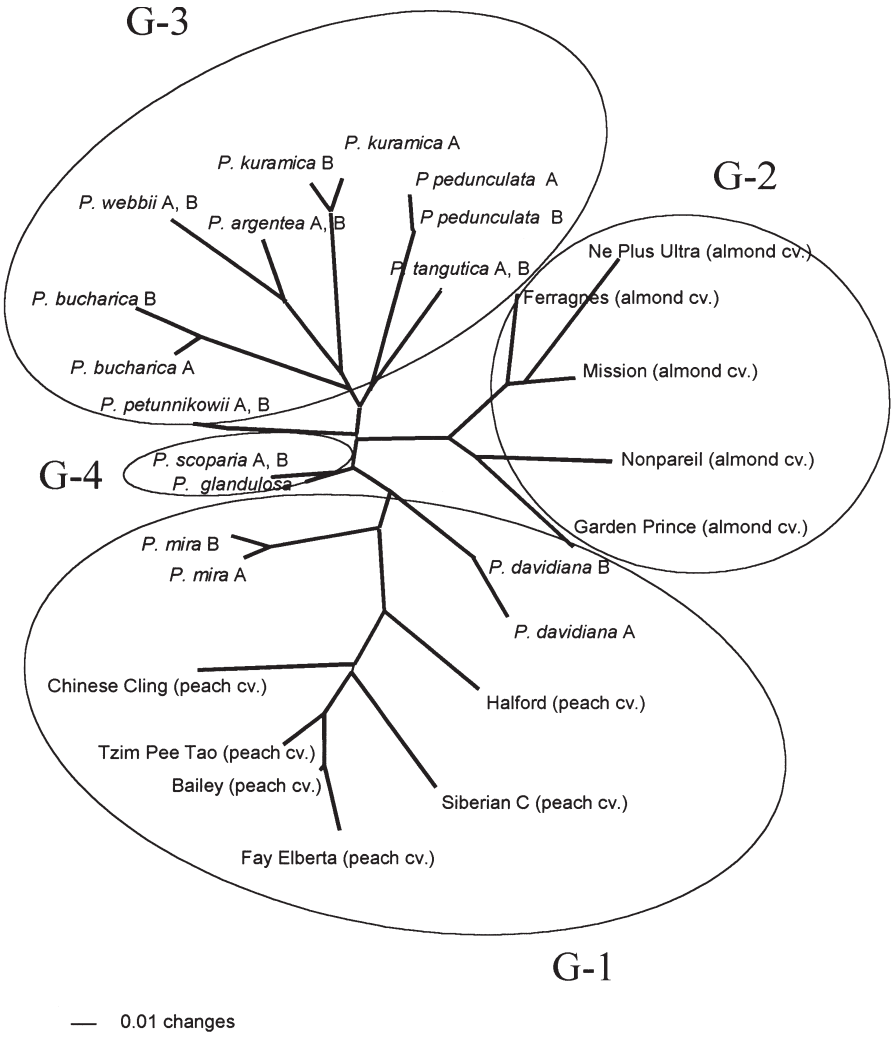

Fig. 1. Unrooted dendogram obtained by neighbor-joining cluster analysis based on the mean character difference distances among the different species. *Prunus glandulosa* was included as an outgroup.

for developing a consensus linkage map for *Prunus* as well as in studies assaying the gene pool value (Downey and Iezzoni, 2000; Joobeur et al., 2000; Westman and Kresovich, 1997).

Prunus davidiana and *P. mira* are shown to be closer to peach than to almond as proposed by Hesse (1975) and Scorza and Sherman (1996). Successful hybridization between *P. dulcis* (almond) and *P. argentea*, *P. bucharica*, *P. kuramica*, and *P. webbii* has been also reported by different authors (Kester et al., 1991; Kester and Gradziel, 1996).

The phenetic relationships inferred among the peach and almond cultivars and related species studied here are in general agreement with previous taxonomic and phylogenetic studies. All of the species except *P. glandulosa* were classified by Rehder (1940) in section *Amygdalus* of subgenus *Amygdalus*. We recovered four major clusters: one including the peach cultivars plus *P. davidiana* and *P. mira*, a second including the almond cultivars, a third including all of the remaining species studied except *P. scoparia* and *P. glandulosa*, which formed the fourth group. The first group includes the fleshy-fruited species commonly referred to as peaches and the second and third groups include dry-fruited species known as almonds. The results of a phylogenetic study of chloroplast and nuclear DNA sequence data (Bortiri et al., 2001), which included some but not all of the species studied here, supported a sister relationship between *P. persica* and *P. davidiana* and a close relationship between those species and *P. dulcis*, *P. bucharica* and *P. argentea*. Mowrey and Werner (1990) and Badenes and Parfitt (1995) also indicated a similar grouping of peach with *P. davidiana* and *P. mira*.

The relative positions of the four clusters varied depending on how we coded alleles for markers for which no amplification was observed in some accessions. The second and third groups (cultivated and wild almonds) clustered together in one analysis but not the other, in which cultivated almonds clustered with *P. scoparia and P. glandulosa*. The variation in the position of group 4, including the last two taxa, probably stems from the fact that they had the highest proportion of markers for which no amplification was observed.

The genetic similarity observed between *P. glandulosa* and *P. scoparia* was surprising. While the latter species is considered a close relative to *P. dulcis*, the former was classified in section *Microcerasus* of subgenus *Cerasus* (Rehder, 1940), though the results of Bortiri et al. (2001) suggest that members of this section are actually closely related to members of subgenus *Prunus*.

Prunus bucharica and *P. kuramica* have been described as the *Prunus* species more closely related to almond (Browick and Zohary, 1996; Grasselly, 1976), and are also described as ancestral species of the modern cultivated almond by Kester et al. (1991). More recently, however, Browicz and Zohary (1996) proposed only *P. fenzliana* as the wild ancestor of almond. Unfortunately, *P. fenzliana* germplasm was not available for this study.

Natural introgression of genes from related *Prunus* species to almond has been reported (see review by Kester et al., 1991). Kester and Gradziel (1996) suggest that the sweet kernel trait, in addition to being a natural variant within *P. dulcis*, may have been independently transferred to *P. dulcis* from *P. bucharica* or *P. kuramica*. *Prunus webbii* may also be the original source of self-compatibility in European almond cultivars including 'Genco', 'Tuono' or 'Cristomorto' with natural introgression of this gene occurring during the centuries of almond cultivation in the Puglia region of Italy (Reina et al., 1985).

Conclusions

Both peach and almond suffer from a limited gene pool available for future breeding progress. Interspecific gene transfer among these *Prunus* species offers a greatly expanded genetic diversity available to breeders, particularly given the relative ease of the initial hybridization and subsequent backcrosses (Gradziel et al., 2001). Further SSR analysis of this germplasm offers opportunities for determining more precise genetic relationships and could be an important tool for marker assisted gene transfer. DNA fingerprinting using SSR analysis could also be very useful for the early selection of the most promising progeny from interspecific crosses or backcrosses, leading to greatly improved breeding efficiency.

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