Available online at www.sciencedirect.com
SCIENCE DIRECT®
Scientia Horticulturae 103 (2004) 131–144
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Molecular characterization of variability and relationships among seven cultivated and selected wild species of *Prunus* L. using amplified fragment length polymorphism

Mallikarjuna K. Aradhya[a,*], Clay Weeks[a], Charles J. Simon[b]

[a] *USDA Germplasm Repository, One Shields Avenue, University of California, Davis, CA 95616, USA*
[b] *Plant Genetic Resources Unit, USDA-ARS, Cornell University, Geneva, NY 14456-0462, USA*

Received 25 August 2003; received in revised form 2 March 2004; accepted 4 May 2004

Abstract

Analysis of genetic variability and differentiation within and among seven cultivated species and seven wild species of *Prunus* using amplified fragment length polymorphism revealed four well-supported groups corresponding to the four sections *Amygdalus*, *Armeniaca*, *Cerasus* and *Prunophora* described within the genus. The section *Armeniaca* showed significant differentiation from its sister section *Prunophora* within the subgenus *Prunus*. Within-species estimates of molecular variation indicated that apricots (0.0529) were the most variable among the species assayed followed by hexaploid plums (0.0359), almonds (0.0330), cherries (0.0310), and diploid plums (0.0303) with moderate levels of variability and peaches (0.0263) were the least variable. The overall distribution pattern of molecular variation within the genus indicated that about 32% of the total variance was accounted for by the within-species variance component, irrespective of partitioning based on either sections or subgenera. The remaining 68% of the variation found among species was hierarchically structured between components due to differentiation among species within and among sections (17.02 and 50.81%, respectively) or among species within and among subgenera (29.53 and 39.05%, respectively). Although cluster and principal components analyses indicate that the gene pools corresponding to the four sections to be homogenous, partitioning of molecular variation suggests considerable differentiation among the taxa within sections.
Published by Elsevier B.V.

Keywords: AFLP; AMOVA; Breeding barrier; Gene pool; Genetic differentiation; Ploidy; *Prunus*

* Corresponding author. Tel.: +1-530-752-6504; fax: +1-530-752-5974.
E-mail address: aradhya@ucdavis.edu (M.K. Aradhya).

0304-4238/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.scienta.2004.05.004

1. Introduction

The genus *Prunus* L. mainly occurs in the temperate regions of the northern hemisphere with some extensions into the southern hemisphere in both the Old and New Worlds (Krussman, 1986; Robertson, 1974). It comprises many species, which are economically important as sources of fruits, nuts, oil, timber, and ornamentals. The fruit and nut bearing species include almonds (*P. dulcis* (Miller) D.A. Webb), apricots (*P. armeniaca* L.), cherries (diploid sweet cherry *P. avium* L. and tetraploid tart cherry *P. cerasus* L.), peaches (*P. persica* (L.) Batsch), and plums (hexaploid *P. domestica* L. and diploid *P. salicina* L.). Cherries and plums are adapted to the cooler temperate regions of the world, while peaches and apricots are grown in warmer temperate, sub-tropical, and tropical highlands, but require adequate winter chilling. Almonds are adapted to regions with Mediterranean climate with periods of winter chilling for normal production.

Prunus is a large, diverse genus with a basic chromosome number $x = 8$, within the subfamily Amygdaloideae (Prunoideae) of the family Rosaceae (Rehder, 1940), and probably originated in Central Asia (Watkins, 1976). The subfamily Amygdaloideae is unique among the rosaceous subfamilies, in bearing a fleshy fruit called a drupe with a hard endocarp, often called the stone. The taxonomic classification within the genus *Prunus* is mainly based on fruit morphology and has been controversial. The revised classification by Rehder (1940), which describes five subgenera; *Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunus* to accommodate variation within the genus, is a widely accepted taxonomic treatment. However, from the genetic improvement perspective, the subgenus *Amygdalus*, to which peaches and almonds belong, and the subgenus *Prunus*, which includes section *Prunophora* comprised of diploid Japanese plums and hexaploid European plums and section *Armeniaca* containing apricots, are considered to be a single gene pool (Watkins, 1976). The subgenus *Cerasus* comprising diploid sweet cherry and tetraploid tart cherry constitutes a distinct group distantly related to the other two subgenera, *Amygdalus* and *Prunus*, included in the study. Nevertheless, breeding barriers exist among taxa possessing different ploidy levels, even within the same section, but hybrids are generally successful when both parents have the same ploidy level (Okie and Weinberger, 1996). The subgenera *Padus* and *Laurocerasus* are more isolated within the genus *Prunus*.

Knowledge of the genetic diversity and relationships among the cultivated species of *Prunus* is important to recognizing gene pools, to identifying pitfalls in germplasm collections, and to develop effective conservation and management strategies. Traditional taxonomic classifications provide rough guidelines to species relationships, but molecular evaluations provide further insight into the genetic structure and differentiation within and among taxa useful for geneticists, plant breeders, and gene bank managers.

Although cultivated species of *Prunus* have been examined for intraspecific diversity and differentiation (Cipriani et al., 1999; Testolin et al., 2000; Mohanty et al., 2001; Wang et al., 2001; Aranzana et al., 2002; Hormaza, 2002; Dirlewanger et al., 2002; Aranzana et al., 2003), genetic characterization of diversity and relationships at the interspecific level is limited to a few molecular phylogenetic studies. They include a few studies on the systematic relationships within *Prunus* using allozyme polymorphisms (Mowrey and Werner, 1990), chloroplast DNA variation (Uematsu et al., 1991; Badenes and Parfitt, 1995), ITS sequence variation of nuclear ribosomal DNA (Lee and Wen, 2001), and ITS and chloroplast

trnL–trnF spacer sequence variation (Bortiri et al., 2001). In the present study, we used the amplified fragment length polymorphism (AFLP) approach to elucidate the genetic structure and differentiation within and among seven cultivated species representing the three economically important subgenera, *Prunus*, *Amygdalus*, and *Cerasus*, within the genus *Prunus*, and examine the implications for their conservation, management, and utilization.

2. Materials and methods

2.1. Plant material and DNA isolation

One hundred and thirteen diverse accessions representing seven cultivated and seven wild species of *Prunus* from the three subgenera and four sections described within the genus *Prunus* were sampled from the germplasm collection maintained at the USDA Germplasm Repository, Davis, CA (Table 1). Total DNA was extracted by following a two step protocol, which involved homogenization of plant tissue and pelleting of nuclei, followed by lyses of nuclei and chloroform–isoamyl alcohol extraction (Paterson et al., 1993). The supernatant was further extracted with equal volumes of phenol/chloroform (1:1, v/v) followed by a second extraction with equal volume of chloroform/isoamyl alcohol (24:1, v/v). Nucleic acid was precipitated with one volume of chilled isoproponol and centrifuged at low speed to pellet the DNA. The pellet was washed twice with 75% ethanol containing 10 mM ammonium acetate, air dried, dissolved in 500 μL of TE buffer, and treated with RNase A (Sigma) at the rate of 10 μg/ml at 37 °C for 1 h.

2.2. AFLP analysis

Details of AFLP assay, adapter and primer sequences, PCR conditions for preselective and selective amplifications, and selective primer designation were according to Vos et al. (1995) and Vuylsteke et al. (1999). Genomic DNA was restricted with *Eco*RI/*Mse*I enzyme combination, double-stranded adapters specific to each site were ligated, and preselective amplification was performed with primers complementary to the adaptors with an extra selective base on each primer (*Eco*RI-A/*Msc*I-C). Selective amplification was carried out with five primer combinations involving two *Mse*I (M) and three *Eco*RI (E) primers [M60(CTC)/E33(AAG), E36(ACC), E38(ACT) and M61(CTG)/E33(AAG), E38(ACT)]. Fragments were resolved using capillary electrophoresis on an ABI Prism 310 genetic analyzer with the data collection software version 1.2 (PE/Applied Biosystems). AFLP fragment analysis was performed with GeneScan, Version 3.1 and Genotyper, Version 2.5, and the data were assembled in binary format.

2.3. Data analysis

Genetic relationships within and among taxa were computed based on the proportion of fragments shared between two accessions for all possible pair-wise comparisons using Nei and Li distance (Nei and Li, 1979). The resulting distance matrix was subjected to a cluster analysis (CA) following the unweighted pair group method using arithmetic averages (UP-

Table 1
Prunus germplasm accessions included in the study

No.	Accession no.	Cultivar	Source
Almond (2×) – *P. dulcis* (Mill) D.A. Webb.			
1	DPRU 204	"Pioneer' (peach × almond)	USA
2	DPRU 1458.7	Unknown	Pakistan
3	DPRU 201	'Vesta'	USA
4	DPRU 1456.1	'Badam'	Pakistan
5		'Tarragona'	Spain
6		'Marcona'	Spain
7	DPRU 207	'Eureka'	USA
8	DPRU 210	'Languedoc'	France
9	DPRU 1597	'Durkheim Jv'	Germany
10	DPRU 2333.17	'Double nut soft shell'	China
11	DPRU 209	'Profuse'	USA
12	DPRU 2334.21	'Multiple Fruit'	China
13	DPRU 2336.11	'Rough shell'	China
14	DPRU 2335.18	'Late high yield'	China
15	DPRU 2330.5	'Eagle's beak'	China
16	DPRU 2337.6	'Double nut'	China
17	DPRU 1457.1	Unknown	Pakistan
18	DPRU 1456.8	Unknown	Pakistan
Apricot (2×) – *P. armeniaca* L.			
1	DPRU 343	'Shirpaivan'	Turkistan
2	DPRU 345	'Hulan'	Manchuria
3	DPRU 1788.3	Unknown	Turkey
4	DPRU 1882.2	'Mirsanjeli Late'	Uzbekistan
5	DPRU 729	'Palummella'	Italy
6	DPRU 1807.1	Unknown	USSR
7	DPRU 1787.4	Unknown	Turkey
8	DPRU 1435.4	Unknown	Pakistan
9	DPRU 1381.1	'Habiju'	Pakistan
10	DPRU 1380.4	'Habiju'	Pakistan
11	DPRU 1379.1	Unknown	Pakistan
12	DPRU 1377.1	'Kabuli'	Pakistan
13	DPRU 1372.2	'Khubani'	Pakistan
14	DPRU 1045	'NJ-A64'	USA
European plum (6×) – *P. domestica* L.			
1	DPRU 706	'Early Jewel'	Australia
2	DPRU 558	'Warwickshire Drooper'	UK
3	DPRU 1527	'Jefferson'	USA
4	DPRU 1594	'Kinstendilsva'	Bulgaria
5	DPRU 1632	'Prune d'ente 707'	France
6	DPRU 1649	'Pozegaca D-6'	Yugoslavia
7	DPRU 1630	'Ruth Gerstetter'	Germany
8	DPRU 927	'Reine Claude de Bavay	Belgium
9	DPRU 1255	'Pearl'	US
10	DPRU 1516	'Lohr Pflaume'	Unknown
11	DPRU 1524	'Arch Duke'	UK
12	DPRU 1529	'Saint Catherine'	USA/France
13	DPRU 1537	'Moyer Perfecto	USA
14	DPRU 720.4	*P. cerasifera*	Uzbekistan

Table 1 (*Continued*)

No.	Accession no.	Cultivar	Source
Japanese plum (2×) – *P. salicina* L.			
1	DPRU 2127	'Mammoth Cardinal'	USA
2	DPRU 1718	'Ouishi-nakate'	Japan
3	DPRU 1596	'Wilson'	Australia
4	DPRU 792	'Purple King'	New Zealand
5	DPRU 777	'Patterson's Late'	Australia
6	DPRU 2129	'Nubiana'	USA
7	DPRU 2274	'Gold Hill'	Australia
8	DPRU 1233	'Red Gold'	South Africa
9	DPRU 800	'Sumomo'	India
10	DPRU 844	'George Wilson'	New Zealand
11	DPRU 791	'Victory'	New Zealand
12	DPRU 468	Unknown	Unknown
13	DPRU 1235	'Laetitia'	South Africa
Peach (2×) – *Prunus persica* L. Batsch			
1	DPRU 528	'Belle of Georgia'	USA
2	DPRU 942	'Foster'	USA
3	DPRU 737	'Kiang-Si'	Spain
4	DPRU 535	'Rutger's red leaf'	USA
5	DPRU 537	'Hiley'	USA
6	DPRU 533	'Chui Lum Tao'	China
7	DPRU 534	'Amarillo Tardio'	Spain
8	DPRU 1188	'Henneuse #2 (plumcot)	Unknown
9	DPRU 542	'Red Slovenia'	Czechoslovakia
10	DPRU 980	'Calmar'	USA
11	DPRU 585	'Shanghai peach'	China
12	DPRU 1132	'Stanwick'	Syria
13	DPRU 1179	'Salway'	UK
Sour cherry (4×) – *P. cerasus* L.			
1	DPRU 66	'George Glass'	Unknown
2	DPRU 36	'Kentish'	UK
3	DPRU 741	'Morello rootstock'	Unknown
4	DPRU 1647	'Oblancinka'	Hungary
5	DPRU 1697	'M-172'	Hungary
6	DPRU 1714	'Visin Local 38/13'	Unknown
7	DPRU 2239	'Pandy 35'	Romania
8	DPRU 2244	'Pandy 38'	Romania
9	DPRU 2247	'Sumadinka'	Yugoslavia
10	DPRU 2367	'Kelleris 14'	Serbia
11	DPRU 23	'Shubinka'	USSR
12	DPRU 1583	'Rosii de Istrita'	Romania
13	DPRU 26	'Spanische Glaskirsche	Germany
14	DPRU 1707	'Espera'	Poland
15	DPRU 1587	'Stevns'	Denmark
Sweet cherry (2×) – *P. avium* L.			
1	DPRU 105	'Montearly'	USA
2	DPRU 2044	'Guigne Douce de Champ de L'air	Unknown
3	DPRU 2362	'Big Burlat'	Italy

Table 1 (*Continued*)

No.	Accession no.	Cultivar	Source
4	DPRU 1600	'H-Bistrita'	Romania
5	DPRU 43	'Sweet September'	USA
6	DPRU 50	'Yellow Spanish'	Unknown
7	DPRU 57	'Corum'	USA
8	DPRU 54	'Walpurgis'	unknown
9	DPRU 56	'Durone II'	Italy
10	DPRU 8	'Bolium'	UK
11	DPRU 1539	'Merton Bigarreau'	UK
12	DPRU 75	'Black Eagle'	UK
13	DPRU 11	'Flamentiner'	Germany
Myrobalan plum (2×) – *P. cerasifera* Ehrh.			
1	DPRU 795	*P. cerasifera* (PI 91456)	Unknown
2	DPRU 720.11	*P. cerasifera* (PI 502569)	USSR
3	DPRU 880	*P. cerasifera* (PI 73613)	Uzbekistan
4	DPRU 563	*P. cerasifera*	Unknown
Other species			
1	DPRU 581	*P. davidiana*	China
2	DPRU 582	*P. kansuensis*	NW China
3	DPRU 1724.3	*P. serotina*	Ecuador
4	DPRU 2222	*P. virginiana*	USA
5	DPRU 2316.4	*P. tomentosa*	China
6	DPRU 597	*P. speciosa* (PI500127)	Japan
7	DPRU 848	*P. spinosa* (PI 141219)	UK
8	DPRU 418	*P. mahaleb* (PI 193702)	Unknown
9	DPRU 421	*P. mahaleb* (PI 193699)	Unknown

GMA) algorithm. Bootstrap analysis (500 replicates) was performed to assess the relative support for different groups. A three-dimensional projection of AFLP variation within and among taxa was obtained through principal components analysis (PCA) to further support the CA results.

The analysis of molecular variance (AMOVA) was performed on the AFLP data using the WINAMOVA (Version 1.55) program (Excoffier et al., 1992). Prior to the analysis, a χ^2-test for homogeneity of variances, commonly known as the Bartlett's test, was performed to verify the homogeneity of variances among species, sections and subgenera. Hierarchical partitioning of molecular variation within and among sections, and subgenera was performed using the nested analysis module, which adapts inter-genotypic distances to compute the conventional sum of square deviations (SSDs). The total SSD is then partitioned into variation within and among species, sections, and subgenera, and the corresponding mean square deviations (MSD) were obtained by dividing each SSD by the appropriate degrees of freedom. Further, individual variance components were extracted by equating the MSDs to their expectations. The variance components from the analysis were used to estimate the population subdivisions (Φ statistics, similar to Wright's *F*-coefficients representing correlation between AFLP phenotypes) within and among sections and subgenera of *Prunus*. This approach consists of three different Φ coefficients corresponding to the

total population level (T), subdivisions (S and G corresponding to sections and subgenera) and individuals (I) in our study. Φ_{ST} and Φ_{GT} are correlations between random AFLP phenotypes drawn from within sections and subgenera, respectively, relative to that from the whole population. Φ_{IS} and Φ_{IG} are correlations between random AFLP phenotypes drawn from within species relative to that from within the sections and subgenera, respectively. Φ_{IT} is the correlation among random AFLP phenotypes within species relative to that from the entire population without regard to either sections or subgenera. Significance of variance components was tested with 500 random permutations for each analysis.

3. Results and discussion

3.1. The AFLP profile

The AFLP technique is effective, economical and combines the reliability of restriction fragment length polymorphism (RFLP) and the power of PCR. It generally produces polymorphisms several folds higher than RFLP or any other PCR based marker system. The five AFLP primer combinations used to assay 113 accessions representing seven species each of cultivated and wild *Prunus* revealed a total of 199 polymorphic fragments. The number of polymorphic fragments ranged from 23 for the primer combination M60(CTC)/E33(AAG) to 50 for M60(CTC)/E38(ACT) with an average of 40 fragments/primer combination. There was extensive polymorphism within and among species with several species-specific fragments. Multivariate analyses of the data suggested that AFLPs are highly discriminatory and extremely useful markers for classification and analysis of genetic structure and differentiation in the genus *Prunus*. However, extremely polymorphic markers such as AFLPs often display trans-specific polymorphisms thus elevating interspecific genetic similarities as compared to within species similarities. Such trans-specific polymorphisms causing high levels of homoplasy have been attributed to either incomplete lineage sorting (Avise et al., 1990) or retention of ancestral polymorphisms in derived lineages.

3.2. Genetic relationships within and among sections and subgenera

The pair-wise genetic distances computed based on the proportion of shared fragments ranged from 0.01 to 0.5 with an overall mean distance of 0.17 indicating considerable similarity within and between species. As mentioned above, such low genetic distances are characteristic of AFLPs, which generate notoriously high levels of homoplasy, especially among distantly related species. Nevertheless, the distinct advantage of high levels of polymorphisms representing the entire genome as revealed by AFLPs has the potential to generate a more realistic species tree as compared to a particular gene tree. This is especially true among closely related, potentially interbreeding species, where there is a high probability of reticulate evolution occurring. With the use of appropriate statistical tools (Excoffier et al., 1992; Yeh and Boyle, 1997), AFLPs can also be used to analyze genetic structure and differentiation within and among species.

The UPGMA cluster analysis revealed four distinct well-supported clusters corresponding to the four sections, *Amygdalus*, *Armeniaca*, *Cerasus*, and *Prunophora* (Fig. 1). Within

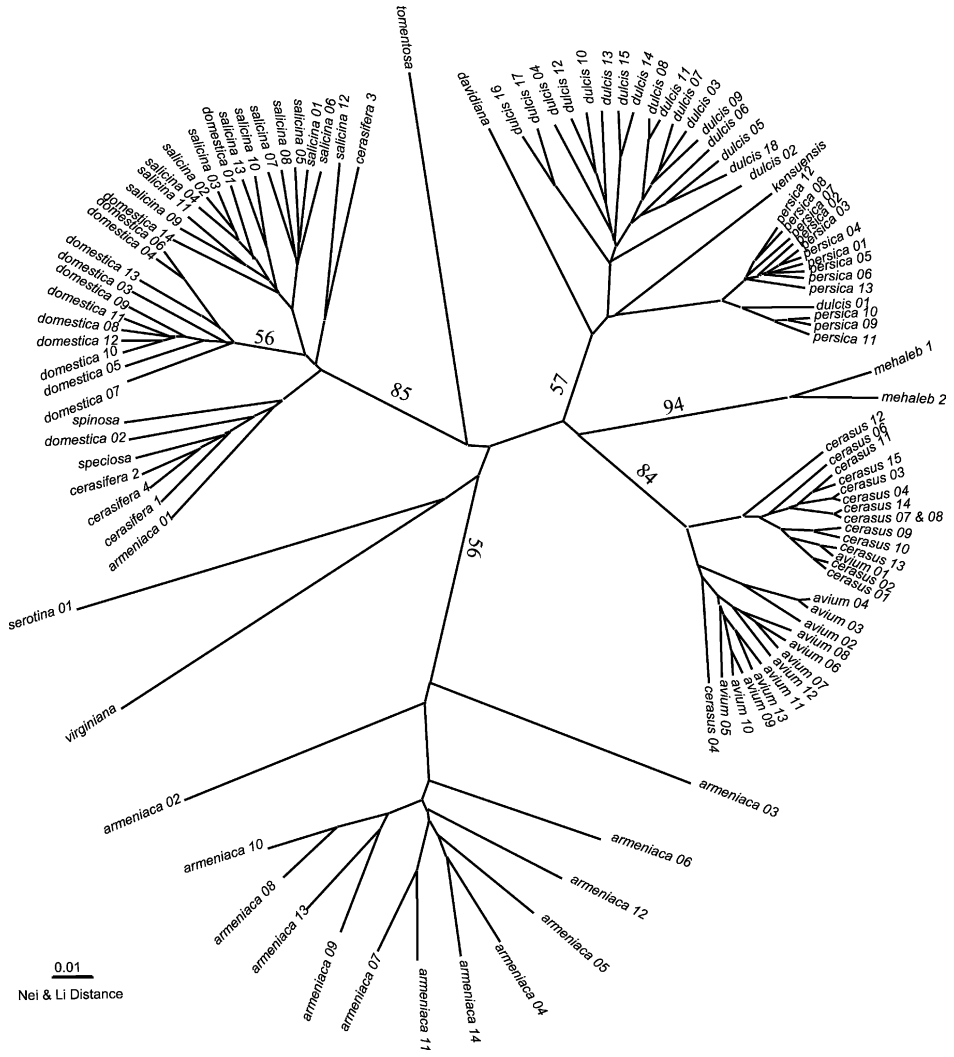

Fig. 1. Phenogram depicting genetic relationships within and among species of *Prunus*. Numbers associated with species names refer to numbers in column 1 of Table 1.

each of these clusters there was evidence for differentiation within and among species. The section *Armeniaca* within the subgenus *Prunus*, which includes the apricot, *P. armeniaca* has considerably differentiated from the other cultivated species of *Prunus*. This observation is further supported by the fact that Watkins (1976), while discussing the evolutionary trends in the genus *Prunus*, suggested apricots to be farther from the center of the genus than plums. He further speculated that factors which contributed to self-fertility in apricots may also have contributed to its isolation within the genus. On the contrary, Kostina (1969) while describing the ecogeographic variation within cultivated apricot collection

suggested that self-fertility was found only in the narrowly variable European group derived from a relatively few forms introduced from the Irano-Caucasian region during the past 2000 years. As regards to plums, the myrobalan plum (*P. cerasifera*), the blackthorn (*P. spinosa*), and *P. speciosa* along with two other accessions, one of which was *P. cerasifera*, but mislabeled as *P. domestica* (*domestica* 02), and one hybrid (plum × apricot) accession (*armeniaca* 01) have formed a separate group within the section *Prunophora*. Interestingly, the Nanking cherry (*P. tomentosa*) accession belonging to the section *Microcerasus* within the subgenus *Cerasus* occupied the basal sister position within the plum group. Members of *Microcerasus*, based on their breeding and grafting behavior, are considered to be closer to plums than to cherries (Ramming and Cociu, 1991). It is widely believed that the European plum has arisen as a natural allopolyploid between myrobalon and blackthorn plums in the Caucasus Mountains, where their distributions overlap (Crane and Lawrence, 1952; Zhukovsky, 1965), but a recent study on the evolutionary relationships within the subgenus *Prunus* based on RFLP variation in cpDNA genes suggested that European plum may have originated from polyploid forms of myrobalan plum (Reynders and Salesses, 1991). Based on randomly amplified polymorphic DNA analyses, Shimada et al. (1999) and Casas et al. (1999) demonstrated high levels of genetic affinity among diploid, tetraploid, and hexaploid plums with only marginal variation accounting for differentiation. Despite high levels of genomic similarities among diploid and hexaploid plum species, breeding barriers do exist among them. However, there are reports of successful introduction of genes for productivity, fruit characteristics, climatic adaptation and disease-pest resistance from other wild diploid species into the Japanese plum, *P. salicina*, through interspecific hybridization and selection (Cullinan, 1937; Howard, 1945; Okie and Weinberger, 1996).

The diploid sweet cherry *P. avium* and the tetraploid tart cherry *P. cerasus*, within the subgenus *Cerasus* formed two distinct clusters except for two accessions (*avium* 1 and *cerasus* 5), which are intermixed. Considerable level of ploidy-imposed breeding barrier exists between the sweet and tart cherries. However, sweet cherry is considered to be a progenitor of tart cherry (Oldén and Nybom, 1968) and they do hybridize and occasionally produce viable hybrids commonly known as 'Duke cherry', often grown in Europe. The two *P. mahaleb* accessions belonging to the section *Mahaleb* within the subgenus *Cerasus* formed a basal sister group to the section *Cerasus*. Surprisingly, the two tetraploid American wild cherry species, *P. serotina* and *P. virginiana* classified under the subgenus, *Padus* formed a group basal to the sections, *Amygdalus*, *Cerasus*, and *Prunophora*. Overall, cherries were closer to the sections *Amygdalus* and *Armeniaca* than to the section *Prunophora*.

The two cultivated species within the section *Amygdalus* formed separate but closely related groups except for a *P. davidiana* accession which showed affinity towards almond and an accession (*dulcis* 1) called 'Pioneer', which is a peach × almond hybrid, closely aligned with peach group. Peaches and almonds readily hybridize to produce vigorous and fertile hybrids generating a wide array of segregants ranging from peach to almond parental types (Armstrong, 1957; Kester and Asay, 1975).

In the PCA, the first three principal axes accounted for 30, 10, and 7% of the total variation, respectively indicating the complex multidimensional nature of AFLP variation. The three-dimensional projection of accessions along the first three principal axes revealed the overall genetic relationships among the taxa somewhat similar to the CA (Fig. 2). The four sections, *Amygdalus*, *Armeniaca*, *Prunophora*, and *Cerasus*, produced tight clusters and

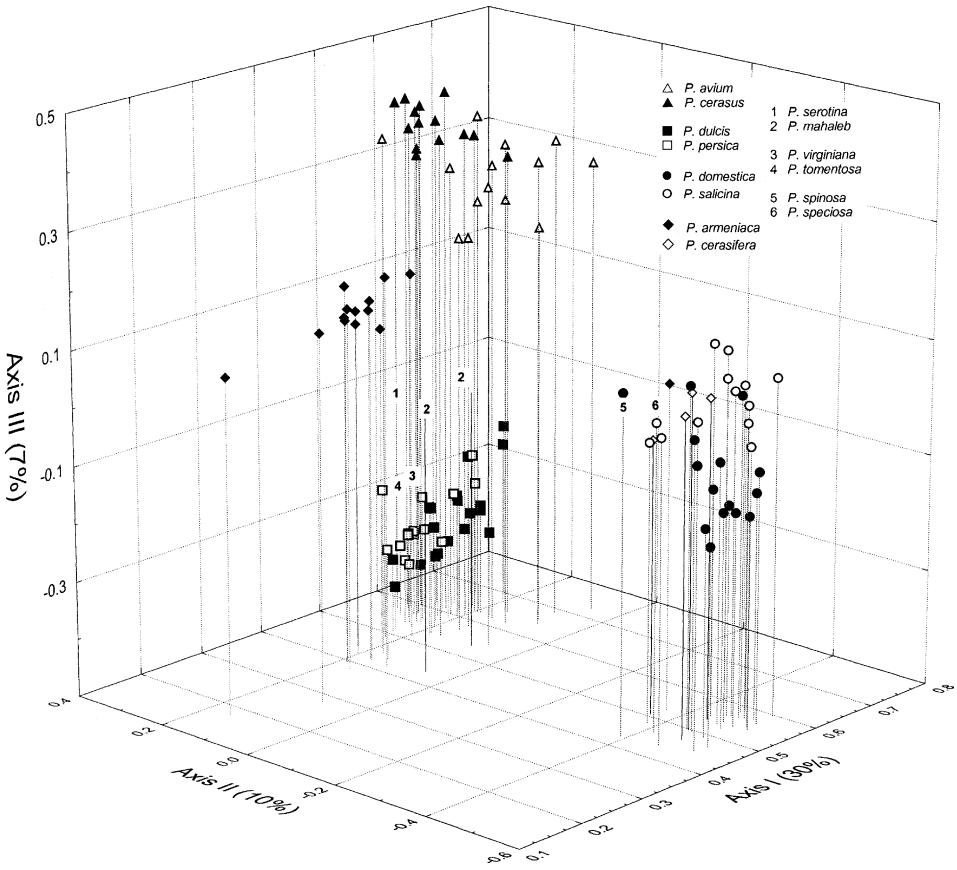

Fig. 2. Three-dimensional projection of AFLP variation among 113 accessions representing seven cultivated and seven wild species of *Prunus*.

exhibited considerable divergence rather more pronounced than in the CA. Surprisingly, the first principal axis which accounted for the most variation (30%) contributed the least for the separation of taxa. The factor loadings along the second axis (10%) contributed for separating plums from the remaining taxa. The third axis accounting for only 7% of the total variation was heavily loaded to discriminate the sections *Amygdalus*, *Armeniaca*, and *Cerasus*. *Cerasus* and *Prunophora* appeared to be the most divergent among the sections within the genus. According to Watkins (1976), members of the subgenus *Cerasus* were considered to be ancient and were the first to diverge from the ancestral *Prunus*. Unlike in the CA, the section *Armeniaca* containing apricots was much closer to the section *Amygdalus* and *Cerasus* than to *Prunophora*. The wild cherries, *P. mahaleb* from the section *Mahaleb* and *P. virginiana* and *P. serotina* from the subgenus *Padus* fell between sections *Amygdalus* and *Armeniaca*. The two multivariate approaches, CA and PCA, used in the analysis of genetic relationships within and among the sections and subgenera of *Prunus*, produced generally comparable results. Nevertheless, they were chosen to complement each other,

because PCA is known to be less sensitive to distances between close neighbors, but represents more accurately distances between clusters, while CA generally reproduces distances between the close neighbors faithfully, but shows distortion among members of different clusters (Sneath and Sokal, 1973).

3.3. Hierarchical partitioning of molecular variation

Within-species molecular variation estimated using the AMOVA procedure indicated that apricots (0.0529) were the most variable among the species assayed, followed by hexaploid plums (0.0359), almonds (0.0330), cherries (0.0310), and diploid plums (0.0303) with moderate levels of variability, and peaches (0.0263) were the least variable. Based on the chloroplast DNA restriction fragment length variation, Uematsu et al. (1991) arrived at similar conclusions that *P. armeniaca* forms the centre of the stone fruit diversity. Surprisingly the diploid plums, which represent a hybrid complex within the section *Prunophora*, showed moderate level of AFLP variation, may indicate a narrow genetic base up on which most of the diploid plum breeding were founded.

The χ^2-test for homogeneity of variances indicated that the variances were homogeneous among species, sections, and subgenera, thus allowing for hierarchical partitioning of variation following the analysis of molecular variance. Total variance was partitioned into components due to differentiation: (1) within and among sections; (2) within and among subgenera described within the genus *Prunus*, using the nested AMOVA procedure (Table 2). The overall distribution pattern of molecular variation within the genus suggests that about 32% of the total variance was accounted for by the within-species component of variance irrespective of portioning either based on sections or subgenera. The remaining 68% of the variation found among species was hierarchically structured between components due

Table 2
Hierarchical partitioning of molecular variation within and among sections and subgenera in *Prunus*

Variance component	Observed partition		Φ-statistics	P[a]
	Variance	Total (%)		
(a) Within and among sections				
Among sections (V_A)	0.0512	50.81	$\Phi_{ST} = 0.508$	<0.002
Among species/sections (V_B)	0.0172	17.02	$\Phi_{IS} = 0.346$	<0.002
Within species (V_C)	0.0324	32.16	$\Phi_{IT} = 0.678$	<0.002
(b) Within and among subgenera				
Among subgenera (V_A)	0.0403	39.05	$\Phi_{GT} = 0.390$	<0.002
Among species/subgenera (V_B)	0.0305	29.53	$\Phi_{IG} = 0.485$	<0.002
With in species (V_C)	0.0324	31.42	$\Phi_{IT} = 0.686$	<0.002

[a] Probability of obtaining more extreme random variance component and Φ-statistic than the observed values by chance alone. Φ_{ST}, Φ_{GT} and V_A are tested under random permutations of whole populations across sections or subgenera. Φ_{ST} and Φ_{GT} = correlation between random AFLP phenotypes drawn from within sections and subgenera, respectively, relative to that from the whole population. Φ_{IS} and Φ_{IG} = correlation between random AFLP phenotypes drawn from within species relative to that from within the sections and subgenera, respectively. Φ_{IT} = correlation between random AFLP phenotypes within species relative to that from the entire population without regard to either sections/subgenera.

to differentiation among species within and among sections (17.02 and 50.81%, respectively), or among species within and among subgenera (29.53 and 39.05%, respectively). Ploidy-imposed interspecific barriers to gene flow has resulted in considerable genetic differentiation within and among sections ($\Phi_{IS} = 0.346$ and $\Phi_{ST} = 0.508$, respectively), within and among subgenera ($\Phi_{IG} = 0.485$ and $\Phi_{GT} = 390$, respectively), and among species irrespective of either sections ($\Phi_{IT} = 0.678$) or subgenera ($\Phi_{IT} = 0.686$). Surprisingly, the level of genetic differentiation was more pronounced among sections than among subgenera in *Prunus*, probably due to the placement of *P. armeniaca* within the subgenus *Prunus*, which exhibits the highest variability among the species included in the study and shows substantial divergence within the subgenus.

Generally, there was significant divergence (Φ_{ST}, here referred to the correlation between random AFLP phenotypes drawn from within species to that from the whole population) among the species examined with the species pair-wise divergence ranging from 0.748 between *P. cerasus* and *P. salicina* to 0.266 between the two plums, *P. domestica* and *P. salicina* (Table 3). The overall relationships among the cultivated species of *Prunus* included in this study closely agree with earlier systematic studies based on allozyme polymorphisms (Mowrey and Werner, 1990), cpDNA restriction fragment length polymorphisms (Badenes and Parfitt, 1995), and ITS and the cpDNA spacer, *trnL–trnF* sequence polymorphisms (Bortiri et al., 2001). Unlike these earlier studies, which either used variations in chloroplast and/or nuclear DNA or allozyme variation on a limited sampling basis to elucidate the phylogenetic relationships, we have surveyed AFLP variation within and among cultivated species on a broader sampling basis to analyze genetic diversity and relationships within and among cultivated species of *Prunus*. The pattern of differentiation among the cultivated taxa within the genus *Prunus* suggests four gene pools corresponding to the four sections *Amygdalus*, *Armeniaca*, *Cerasus*, and *Prunophora*, within which gene flow can potentially occur as interspecific hybrids within the same ploidy level are viable with some level of fertility.

We should remember that no germplasm collection, especially of perennial crop species, truly represents the range of variability found in their natural gene pools. Nevertheless, evaluation of existing germplasm collections contribute tremendously to the understanding of overall patterns of distribution of genetic variation and allow for drawing some general

Table 3
Species pair-wise genetic differentiation (below diagonal = Φ_{ST}[a] values; above diagonal = probability of having more extreme Φ_{ST} values than observed values by chance alone)

	P. dulcis	*P. persica*	*P. salicina*	*P. domestica*	*P. avium*	*P. cerasus*	*P. armeniaca*
P. dulcis	–	0.000	0.000	0.000	0.000	0.000	0.000
P. persica	0.446	–	0.000	0.000	0.000	0.000	0.000
P. salicina	0.681	0.724	–	0.000	0.000	0.000	0.000
P. domestica	0.659	0.724	0.266	–	0.000	0.000	0.000
P. avium	0.604	0.693	0.648	0.648	–	0.000	0.000
P. cerasus	0.681	0.732	0.748	0.739	0.380	–	0.000
P. armeniaca	0.590	0.601	0.693	0.673	0.651	0.658	–

[a] Φ_{ST} here refers to the correlation between random AFLP phenotypes drawn from within species to that from the whole population (not to be confused with Φ_{ST} in Table 2).

conclusions. The genetic material included in the study represents a subset of the *Prunus* germplasm maintained at the USDA germplasm repository in Davis, which comprises some of the cultivars and selections developed in various plum breeding programs around the world and may not again truly represent the natural gene pools of species under consideration. However, all the seven cultivated *Prunus* species included in the study exhibited moderate levels of AFLP variation, showed considerable differentiation along the sectional and subgeneric boundaries, and allowed for some generalization on the genetic structure and differentiation within the genus *Prunus*.

Taxonomic and genetic identities of accessions and the knowledge of genetic variation and relationships within and among cultivated and wild species is the key to organize germplasm into gene pools in the efficient conservation and management of *Prunus* germplasm. Pattern of distribution of molecular variation within the genus *Prunus* suggest four distinct gene pools corresponding to the four sections. Within-section genetic differentiation was not apparent in the CA and PCA, but partitioning of molecular variation suggests otherwise. From the genetic conservation perspective, although the results suggest four distinct gene pools, the genetic barriers imposed by the differences in the ploidy levels among species call for considering species-wise primary gene pools for conservation and management of diversity in the genus.

References

Aranzana, M.J., Garcia-Mas, J., Carbo, J., Arus, P., 2002. Development and variability of microsatellite markers in peach. Plant Breed. 121, 87–92.

Aranzana, M.J., Carbo, J., Arus, P., 2003. Microsatellite variability in peach [*Prunus persica* (L.) Batsch]: cultivar identification, marker mutation, pedigree inferences and population structure. Theor. Appl. Genet. 106, 1341–1352.

Armstrong, D.L., 1957. Cytogenetic study of some derivations of the F1 hybrid *Prunus amygdalus* × *P. persica*. Ph.D. Dissertation. University of California, Davis.

Avise, J.C., Ankney, C.D., Nelson, W.S., 1990. Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. Evolution 44, 1109–1119.

Badenes, M.L., Parfitt, D.E., 1995. Phylogenetic relationships of cultivated species from an analysis of chloroplast DNA variation. Theor. Appl. Genet. 90, 1035–1041.

Bortiri, E., Oh, S., Jiang, J., Baggett, S., Granger, A., Weeks, C., Buckingham, M., Potter, D., Parfitt, D.E., 2001. Phylogeny and systematics of *Prunus* (Rosaceae) as determined by sequence analysis of ITS and the chloroplast *trnL–trnF* spacer DNA. Syst. Bot. 26, 797–807.

Casas, A.M., Igartua, E., Balaguer, G., Moreno, M.A., 1999. Genetic diversity of *Prunus* rootstocks analyzed by RAPD markers. Euphytica 110, 139–149.

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

Crane, M.B., Lawrence, W.J., 1952. The Genetics of Garden Plants. MacMillan, London.

Cullinan, F.P., 1937. Improvement of stone fruits. Yearbook Agric. U.S. Dept. of Agric., pp. 665–748.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arus, P., Laigret, F., 2002. Deveopment of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105, 127–138.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Hormaza, J.I., 2002. Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor. Appl. Genet. 104, 321–328.

Howard, W.L., 1945. Luther Burbank's plant contributions. Calif. Agr. Expt. Sta. Bull., p. 691.
Kester, D.E., Asay, R.A., 1975. Almonds. In: Janick, J., Moore, J.N. (Eds.), Advances in Fruit Breeding. Purdue University Press, West Lafayette, IN, pp. 387–419.
Kostina, K.F., 1969. The use of varietal resources of apricots from breeding. Trudy Nikit. Bot. Sada. 40, 45–63 (in Russian).
Krussman, G., 1986. Manual of Cultivated Broad-leaved Trees and Shrubs, vol. III. Timber Press, Portland, pp. 18–85.
Lee, S., Wen, J., 2001. A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. Am. J. Bot. 88, 150–160.
Mohanty, A., Martin, J.P., Aguinagalde, I., 2001. Chloroplast DNA study in wild populations and some cultivars of *Prunus avium* L. Theor. Appl. Genet. 103, 112–117.
Mowrey, B.D., Werner, D.J., 1990. Phylogenetic relationships among species of *Prunus* as inferred by isozymes markers. Theor. Appl. Genet. 80, 129–133.
Nei, M., Li, W., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76, 5269–5273.
Okie, W.R., Weinberger, J.H., 1996. Plums. In: Janick, J., Moore, J.N. (Eds.), Fruit Breeding, vol. 1: Tree and Tropical Fruits. Wiley, New York, pp. 559–609.
Oldén, E.J., Nybom, N., 1968. On the origin of *Prunus cerasus* L. Hereditas 59, 327–345.
Paterson, A.H., Brubaker, C.L., Wendel, J.F., 1993. A rapid method of extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol. Biol. Rep. 11, 122–127.
Ramming, D.W., Cociu, V., 1991. Plums. In: Moore, J.N., Ballington, J.R., Jr. (Eds.), Genetic Resources of Temperate Fruit and Nut Crops. ISHS Acta Horticultura 290, Wageningen, pp. 235–287.
Rehder, A., 1940. Manual of Cultivated Trees and Shrubs, 2nd ed. MacMillan, New York, pp. 452–481.
Reynders, S., Salesses, G., 1991. Study on the relationships within the subgenus *Prunophora*. Restriction maps of the ribosomal genes in *P. cerasifera* and *P. spinosa*. Acta Hort. 283, 27–29.
Robertson, K.R., 1974. The genera of Rosaceae in south-eastern United States. J. Arnold Arbor. Harv. Univ. 55, 303–662.
Shimada, T., Hayama, H., Haji, T., Yamaguchi, M., Yoshida, M., 1999. Genetic diversity of plums characterized by randomly amplified polymorphic DNA (RAPD) analysis. Euphytica 109, 143–147.
Sneath, P.R., Sokal, R.R., 1973. Numerical taxonomy—the principles and practices of numerical classification. Freeman, San Francisco.
Testolin, R., Marrazzo, T., Cipriani, G., Quarta, R., Verde, I., Dettori, M.T., Pancaldi, M., Sansavini, S., 2000. Microsatellite DNA in peach (*Prunus persica* L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. Geneome 43, 512–520.
Uematsu, C., Sasakuma, T., Ogihara, Y., 1991. Phylogenetic relationships in the stone fruit group of *Prunus* as revealed by restriction fragment analysis of chloroplast DNA. Jpn. J. Genet. 66, 59–69.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new concept for DNA fingerprinting. Nucl. Acids Res. 23, 4407–4414.
Vuylsteke, M., Mank, R., Antonise, R., Bastiaans, E., Senior, M.L., Stuber, C.W., Melchinger, A.E., Lubberstedt, T., Xia, X.C., Stam, P., Zabeau, M., Kuiper, M., 1999. Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor. Appl. Genet. 99, 921–935.
Wang, Y., Georgi, L.L., Zhebentyayeva, T.N., Reighard, G.L., Scorza, R., Abbott, A.G., 2001. High-throughput targeted SSR marker development in peach (*Prunus persica*). Genome 45, 319–328.
Watkins, R., 1976. Cherry, plum, peach, apricot and almond. In: Simmonds, N.W. (Ed.), Evolution of Crop Plants. Longman, London, pp. 242–247.
Yeh, F.C., Boyle, T.J.B., 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian J. Bot. 129, 157.
Zhukovsky, P.M., 1965. Main gene centers of cultivated plants and their wild relations within territory of the USSR. Euphytica 14, 177–188.