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Genetic Relationships among Cultivated Diploid Plums and Their Progenitors as Determined by RAPD Markers

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Abstract. **Diploid plums (*Prunus* L. sp.) and their progenitor species were characterized for randomly amplified polymorphic DNA polymorphisms. Bootstrap analysis indicated the variance of genetic similarities differed little when the sample size was >80 markers. Two species from China (*Prunus salicina* Lindl. and *P. simonii* Carr.) and one species from Europe (*P. cerasifera* Ehrh.) contributed the bulk (72% to 90%) of the genetic background to the cultivated diploid plum. The southeastern plum gene pool was more diverse than those from California, Florida, or South Africa because of the greater contribution of *P. cerasifera* and *P. angustifolia* Marsh. to its genetic background.**

The japanese-type plum (*Prunus* sp.) is the major fresh market plum in the United States. Annual production is ≈220,000 tons with total value of 91 million dollars (U.S. Dept. of Agriculture, 1999). Cultivated japanese-type plums are complex hybrids derived from species of Chinese (*Prunus salicina* and *P. simonii*), European (*P. cerasifera*), and American (*P. americana* Marsh., *P. angustifolia*, and *P. munsoniana* Wight and Hedr.) origin (Byrne, 1989). These interspecific hybrids have resulted from efforts to develop high quality plum cultivars better adapted to various locations (Howard, 1945; Layne and Sherman, 1986; Okie and Ramming, 1999; Okie and Weinberger, 1996; Ramming and Cociu, 1990; Weinberger, 1975).

Plum species are native primarily in the temperate northern hemisphere. *Prunus cerasifera* is found wild and cultivated in Eurasia areas: Asia Minor, Balkans, Iran, and Central Asia with the greatest concentration near the Caspian coast of Daghestan (Kovalev, 1934). *Prunus salicina* has been domesticated in China from ancient times where its wild forms are believed to thrive in the Tsunglin range in Shensi and Kansu (Ramming and Cociu, 1990). *Prunus simonii* is also a Chinese species from the northern area but its wild form is thought to be extinct (Hedrick, 1911). These species from the Old World are combined into the section Euprunus. Plums native to North America are grouped into the section Prunocerasus. Wild forms of these species still are found in many areas (Ramming and Cociu, 1990).

Early domestication of diploid plums in the United States focused on native species such as *P. americana*, *P. angustifolia, P. munsoniana,* and others with the most active work occurring in the Central Plains and the southeastern United States (Hedrick, 1911; Weinberger, 1975). At the turn of the previous century, Luther Burbank revolutionized breeding of diploid plums (Burbank, 1914), when he imported several japanese plum (*P. salicina*) seedlings from Japan and intercrossed these with other plums of Chinese origins (*P. salicina* and *P. simonii*), native plums of several origins (*P. americana*, *P. hortulana* Bailey, and *P. munsoniana*), and plums of Eurasia origins (*P. cerasifera*). Cultivars released by Luther Burbank, almost all multispecies hybrids, later became founding clones of japanese-type plums in the United States (Byrne, 1989).

Japanese-type plums are diploid (2x = 2n = 16) (Weinberger, 1975). The genomic size of only one plum, *P. angustifolia*, has been reported (Baird et al., 1994). Its size is small [0.61 pg/diploid nuclear DNA content (2C)] as are the size of related species such as peach [*P. persica* (L.) Batsch, (Peach Group), 2n = 16] and apricot (*P. armeniaca* L., 2n = 16) (0.54 pg/2C and 0.61 pg/2C, respectively) (Arumuganathan and Earle, 1991). This diploid level and relatively small genomic size make plums suitable for genetic studies with DNA markers.

Comparisons of the genetic diversity of stone fruits using data from pedigrees, isozymes, and randomly amplified polymorphic DNAs (RAPDs) indicated that the cultivated diploid plums (Byrne, 1989; 1990; Byrne and Littleton, 1988; Ortiz et al., 1997) have about the same level of diversity as almonds [*P. dulcis* (Mill.) D. A. Webb] (Byrne, 1990) and more diversity than that found in the cultivated populations of peach (Arulsekar et al., 1986; Byrne, 1990; Byrne and Bacon, 1999; Durham et al., 1987; Gradziel et al., 1993; Ibanez et al., 1993; Messeguer et al., 1987; Mowrey et al., 1990; Perez et al., 1993; Scorza et al., 1985, 1988; Warburton and Bliss, 1996; Werner, 1992), and apricots (Byrne and Littleton, 1989).

Recently, molecular approaches have been used to study genetic relationships in other stone fruit crops such as *Prunus*

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mume Sieb. et Zucc. (Ozaki et al., 1995), plum (Badenes and Parfitt, 1995; Ortiz et al., 1997), peach (Warbuton and Bliss, 1996), and almonds (Bartolozzi et al., 1998).

The number of markers used for estimation of genetic relationships varies from a few to more than a thousand (Smith et al., 1990). The greater the number of markers in an analysis, the more accurate are the estimations. More markers also reduce bias due to undersampling in some genomic regions. However, it is desirable to use as small a set of markers as possible that still yields informative results in order to reduce the cost and time of generating data. The bootstrap sampling method estimates sampling variance of molecular marker data by using a random repetitive sampling scheme of progressively greater numbers of markers (Tivang et al., 1994). The objectives of this study were to 1) determine an appropriate sample size for a genetic relationship study in plums using the bootstrap sampling method, 2) evaluate genetic relationships within and between diploid plum species that contribute to the cultivated japanese-type plum gene pool, 3) determine the genetic relationships among cultivated japanese-type plums from different breeding programs, and 4) estimate species composition of japanese-type plum founding clones based on RAPD profiles of diploid plum species.

Materials and Methods

Plant materials. One hundred fourteen diploid plum genotypes (Table 1) were used in RAPD analysis. These accessions consisted of representatives of the five major progenitor plum species, one local plum species (*Prunus mexicana* S. Wats.), and cultivated plums from four major plum germplasm groups.

DNA extraction. This procedure was modified from that of Doyle and Doyle (1987) for use with 1.5 mL microcentrifuge tube with 60 to 70 mg of young leaf tissue. The DNA concentration was quantified by visual comparison with standard Lambda DNA (Promega, Madison, Wis.) on agarose gel. The DNA stock then was diluted to 2.5 ng·mL^{-1} with sterile Nanopure H_2O (Nanopure II, Barnstead, Boston, Mass.) as a working stock.

RAPD analysis. The RAPD reaction contained 12.5 µL mixture of reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.001% (w/v) gelatin), 2.5 mM $MgCl_2$, 0.8 mM deoxynucleotide triphosphates or dNTPs (200 µM each dATP, dCTP, dTTP, and dGTP) (Promega), 12.5 ng or ≈0.33 µM oligonucleotide decamer primer (Operon Technologies, Alameda, Calif.), 1 unit of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, Calif.), and 6 to 7 ng genomic DNA. Reactions were held in a Falcon 96 well U-bottom microtitre plate (Becton Dickinson, Lincoln Park, N.J.), and each well was overlaid with 50 to 70 µL mineral oil. The RAPD reaction was performed in a MJ PTC-100 thermal cycler (MJ Research, Inc., Watertown, Mass.) under the following conditions: 1 min at 92 °C; 1 min at 35 °C; and 2 min at 72 °C for 41 cycles with the fastest ramp time.

Amplifications for each DNA sample and primer were repeated two to four times independently in order to verify that the RAPD markers were reproducible and consistent. RAPD markers that were ambiguous or inconsistent in any run were not included in the analysis.

Gel electrophoresis. After the completion of polymerase chain reaction (PCR), RAPD products were size-separated with 2% agarose gel electrophoresis in TBE buffer (Sambrook et al., 1989). The horizontal electrophoresis system (model #A4; Owl Scientific, Woburn, Mass.) was used with an agarose gel cast into a size of 20.0 × 20.4 × 0.5 cm (W × L × H).

Gel-loading buffer type IV (Sambrook et al., 1989) was added to each RAPD sample. Electrophoresis was run at a constant voltage between 45-125 V until the loading buffer migrated to the end of a gel. The gels were stained with 0.5 µg·mL^{-1} ethidium bromide solution for 10-20 min and destained with water for 20 min. The size of RAPD markers were estimated by comparing to the standard marker included in the gel. The gel was photographed on a UV transilluminator using a Polaroid camera model PDC-34 with film type 667 (Polaroid, Cambridge, Mass.). Each amplified RAPD marker was identified by the primer used to produce it and the approximate size in base pairs (bp).

Bootstrap sampling method. Six diploid plums, 'Burbank' (*P. salicina*), 'Simon' (*P. simonii*), 'AU-Roadside' (cultivated hybrid), 'Methley' (*P. salicina* x *P. cerasifera*), ANG-GH (*P. angustifolia*), and MEX-1537 (*P. mexicana*) (Table 1) were screened with 45 primers. The RAPD conditions were as described earlier except that the Taq polymerase enzyme was the Stoffel fragment (Perkin Elmer, Foster City, Calif.). At least two independent RAPD reactions were carried out for each plum sample and primer (Operon Technologies, C10, E6, G6, J5, K3, K18, and N10) combination. Only those RAPD markers that were unambiguous and reproducible were included in the data.

Two-hundred bootstrap samples each of size N, where N is number of RAPD markers and N = 10, 20, 30, ... , 150 with increments of 10, were drawn from the preliminary data. Genetic relationships using Simple Matching (Romesburg, 1990) based on N markers were calculated for each of 200 bootstrap samples, then sampling variance associated with N sample size was calculated and plotted against the N sample size.

Data analysis. Scoring of RAPD markers was based on reproducible and consistent RAPD bands from all replicated assays. Because each DNA sample was amplified at least twice, markers that were present in all amplifications were scored as present and markers that were absent in all amplification were scored as absent. Markers that were present in one amplification but absent in another were considered as ambiguous markers and were not included in the analysis.

Pairwise calculations of similarity coefficients using Nei and Li (1979) and the simple matching between all diploid species and cultivated plums were done using NTSYS-pc version 1.8 (Exeter Software, Setauket, N.Y.). Cluster analysis using the unweighed pair-group method with arithmetic average (UPGMA) based on the similarity coefficients of RAPD data were performed to construct dendrograms representing genetic relationships among diploid plums and cultivated japanese-type plums. Species specific diagnostic markers, i.e., markers which unambiguously identified a species, and the species specific frequency of the RAPD markers were used to assess the species composition of cultivated japanese-type plums.

Results and Discussion

Bootstrap sampling method. 'Burbank' and ANG-GH were chosen for estimation of genetic similarities with the rest of the samples in the bootstrap sampling method. These two showed the greatest dissimilarity between each other and between the rest of the plums. Sampling variance of genetic similarities for each bootstrap sample size (N) were plotted against the number of markers (Fig. 1A and B). Increasing sample size from 10 to 40 RAPD markers resulted in a rapid decrease of the sampling variance. However, variance decreased little when sample size was >80 markers. Boonprakob and Byrne (1995) showed that

Table 1. Diploid plums (*Prunus* sp.) used in genetic relationship study.

Sample	Type[z]	Origin[y]	Parents[x]	Notes
Prunus salicina				
Abundance	CU	JP	*P. salicina*	Seed from Japan
Burbank	CU	JP	*P. salicina*	Seedling from Japan
Cambridge	CU	NZ	*P. salicina*	PI 133881, DPRU 824, collected from New Zealand
George Wilson	CU	NZ	*P. salicina*	PI 139152, DPRU 844, donated from New Zealand
IR-2	SP	N/A	*P. salicina*	DPRU 468
Ivanovka	SP	MU	*P. salicina*	DPRU 1560, collected from Manchuria
Japanese Greengage	CU	SA	*P. salicina*	PI 134008, DPRU 833, collected from South Africa
Kelsey	CU	JP	*P. salicina*	Seed from Japan (Hedrick, 1911)
Mel Westwood	SP	N/A	*P. salicina*	DPRU 384
Norine	CU	AU	*P. salicina*	PI 129635, DPRU 863, collected from Australia
Ouishi-Nakate	SP	JP	*P. salicina*	DPRU 1718, collected from Japan
Rena	SP	AU	*P. salicina*	DPRU 373, donated from Australia
Satsuma	SP/CU	JP	*P. salicina*	Seedling from Japan
Taiwan	SP	TW	*P. salicina*	Seed from Taiwan plum Huang -ju
Prunus simonii				
Simon	SP/CU	CN	*P. simonii*	DPRU 545
SIM-376	SP	N/A	*P. simonii*	PI 91527, DPRU 376
SIM472	SP	N/A	*P. simonii*	DPRU 472
Prunus cerasifera				
Allred	CU	N/A	*P. cerasifera*	Red leaf, hybrid?
Burrell's Red Myrobalan	SP	N/A	*P. cerasifera*	PI 370146, DPRU 1580, collected from United Kingdom
Clark Hill RL	CU	N/A	*P. cerasifera*	Ornamental, hybrid?
Coffee's Myrobalan	RS	NZ	*P. cerasifera*	PI 47932, DPRU 718, collected from New Zealand
De Caradeve	SP	N/A	*P. cerasifera*	DPRU 1511
Early Gem	CU	AU	*P. cerasifera*	DPRU 831, Australia
Early Yellow	CU	N/A	*P. cerasifera*	DPRU 1512
Kok Sultan	SP	RU	*P. cerasifera*	PI 128561, DPRU 805, collected from USSR
Mirabi	RS	FR	*P. cerasifera*	
Myrobalan	SP/CU	N/A	*P. cerasifera*	DPRU 579
Marianna 4001	RS	CA	*P. cerasifera*	Hybrid with *P. munsoniana*?
CER-816	SP	USSR	*P. cerasifera*	DPRU 816, collected from former Soviet Union, PI 213564
Yellow Cherry	CU	AU	*P. cerasifera*	PI 133584, DPRU 790, Australia
Prunus angustifolia				
ANG-PC	SP	SC	*P. angustifolia*	
ANG-GH	SP	TX	*P. angustifolia*	
ANG-604	SP	TX	*P. angustifolia*	Overton, Texas, 88604
ANG-609	SP	TX	*P. angustifolia*	Overton, Texas, 87609
ANG-AR	SP	AR	*P. angustifolia*	Conway, Ark., 91437
ANG-9518	SP	LA	*P. angustifolia*	Webster Parish, La., LR95-18
ANG-9521	SP	LA	*P. angustifolia*	Richland Parish, La., LR95-21
ANG-9522	SP	LA	*P. angustifolia*	Richland Parish, La., LR95-22
ANG-9523	SP	LA	*P. angustifolia*	Madison Parish, La., LR95-23
ANG-9524	SP	LA	*P. angustifolia*	Tensas Parish, La., LR95-24
ANG-9529	SP	TX	*P. angustifolia*	Harrison County, Texas, LR95-29
ANG-2161	SP	GA	*P. angustifolia*	Warner Robins, Ga., BY93M2161
ANG-KY1	SP	KY	*P. angustifolia*	Near Livingstone, Ky., KY-1
Prunus mexicana				
MEX-1537	SP	TX	*P. mexicana*	
MEX-LA1	SP	LA	*P. mexicana*	La.
MEX-LA2	SP	LA	*P. mexicana*	Collected in Louisiana
MEX-424	SP	TX	*P. mexicana*	College Station, Texas, 88424
MEX-432	SP	TX	*P. mexicana*	20 miles east College Station, Texas, 88432
MEX-441	SP	TX	*P. mexicana*	Silkwood Dr., Bryan, Texas, 88441
MEX-951	SP	TX	*P. mexicana*	Anderson County, Texas, LR95-1
MEX-957	SP	LA	*P. mexicana*?	Ouachita Parish, La., LR95-7
MEX-958	SP	LA	*P. mexicana*?	Ouachita Parish, La., LR95-8
MEX-9516	SP	LA	*P. mexicana*	Lincoln Parish, La., LR95-16
Lee Jones	SP	TN	*P. mexicana*	Lee Jones, Millington, Tenn.

Continued next page.

Table 1. Diploid plums (*Prunus* sp.) used in genetic relationship study (continued).

Sample	Type[z]	Origin[y]	Parents[x]	Notes
Prunus americana				
AMR-438	SP	MA	*P. americana*	
AMR-LA1	SP	LA	*P. americana*, hybrid?	
AMR-LA2	SP	LA	*P. americana*, hybrid?	
AMR-9526	SP	LA	*P. americana*	Tensas, La., LR95-26
Brown	SP	MS	*P. americana*	Larry Brown, Miss.
AMR-2200	SP	KY	*P. americana*	Bone Lick, Ky., BY90M2200
AMR-2490	SP	NC	*P. americana*	Old Oxford, N.C., BY93M2490
AMR-4866	SP	SC	*P. americana*	S.C., SL4866
AMR-SD1	SP	SD	*P. americana*	Mitchell, S.D., 93-1-29
AMR-SD2	SP	SD	*P. americana*	Mitchell, S.D., 93-8-3
AMR-WI1	SP	WI	*P. americana*	River Falls, Wis., 93-14-2
AMR-WI2	SP	WI	*P. americana*	Menomonie, Wis., 94-4-7
AMR-WI3	SP	WI	*P. americana*	Ellsworth, Wis., 94-2-1
Southeastern gene pool				
AU-Amber	CU	AL	Methley x Unknown	
AU-Producer	CU	AL	Unknown	
AU-Roadside	CU	AL	Ozark Premier x Unknown	
AU-Rosa	CU	AL	Unknown	
AU-Rubrum	CU	AL	Crimson mutation	
Bruce	CU	TX	Abundance x *P. angustifolia*	
Byrongold	CU	GA	(Gaviota F_2) x (Ozark Premier x *P. angustifolia*)	
BY4-601	SE	GA	Queen Ann x Santa Rosa	
Homeside	CU	AL	Unknown	
Robusto	CU	GA	(Queen Ann x Barstow) x (Ozark Premier x *P. angustifolia*)	
Rubysweet	CU	GA	Mariposa x Morris	
Ozark Premier	CU	MO	Burbank x Methley	
Segundo	CU	GA	(Queen Ann x Santa Rosa) x (Ozark Premier x *P. angustifolia*)	
California gene pool				
Angeleno	CU	CA	Queen Ann x Unknown	
Blackamber	CU	CA	Friar x Queen Rosa	
Catalina	CU	CA	Angeleno x unknown	
Ebony Sun	CU	CA	Unknown	
Eldorado	CU	CA	Unknown, developed by Luther Burbank	

they could separate unambiguously 25 samples representing six diploid plum species using 92 RAPD markers. Therefore, the threshold for number of RAPD markers for a genetic relationship study in plums is ≈90 markers.

RAPD ANALYSIS. The eight primers used amplified 168 reproducible and unambiguous RAPD markers which ranged in size between 340 to 4000 bp with 85% within 500 to 2000 bp. These results agreed with the reported size range of RAPD markers (Tingey et al., 1992).

Among 168 RAPD markers, only six were present in all samples and three markers were unique to the individual samples, ANG-GH (*P. angustifolia*), AMR-WI2 (*P. americana*), and AMR-2200 (*P. americana*). Fifty-seven markers (34%) were unique to plums from the section Euprunus, and 55 markers (33%) were unique to plums in the section Prunocerasus. There were 51 markers (30%) amplified in samples from both sections. Five markers were not present in any of the sampled plum species, but were present in cultivated interspecific hybrid plums. Only two markers from each section were invariable. These RAPD results confirmed a high level of polymorphisms in diploid plums (Table 2).

The species had from 49 to 86 RAPD markers and exhibited five to 17 markers that were unique to the species (Table 2). These species specific markers are useful in identifying the species background of the cultivated plums. *Prunus simonii* possessed the fewest markers of which most were invariable (96%) and six were unique to the species. This species is thought to be extinct in the wild in China (Hedrick, 1911) and only three samples were available. Of these three, two (Simon and SIM-376) were indistinguishable. This lack of variability is partly due to the small sample number available. All the other species had polymorphisms in 45% to 79% of the RAPD markers. The least polymorphic of these were *P. angustifolia* (45%) and *P. salicina* (58%). All sampled diploid plums could be unambiguously identified into their taxonomic group by sets of invariable and variable RAPD markers that were unique for each species. Within species, all clones could be distinguished by RAPD markers, except 'Simon' and SIM-376.

GENETIC RELATIONSHIPS AMONG THE DIPLOID PLUM SPECIES. The average Nei and Li (1979) similarity coefficients within and between plum species indicated that similarities within species were higher than those between species (Table 3). Plum species within the same section were more similar to each other than plums between sections.

Cluster analysis using these data divided the plums into their respective sections and species as expected from their taxonomic classification (Rehder, 1986). Within the section Euprunus, *P. salicina* plums and *P. simonii* plums were more similar to each other than to *P. cerasifera* plums (Table 3 and Fig. 2). About three-quarters of RAPD markers were shared between *P. salicina*

Table 1. Continued.

Sample	Type[z]	Origin[y]	Parents[x]	Notes
Freedom	CU	CA	Laroda x (Queen Ann x Late Santa Rosa)	
Gaviota	CU	CA	*P. salicina* x *P. americana*	
Grand Rosa	CU	CA	Eldorado x Unknown	
Laroda	CU	CA	Gaviota x Santa Rosa	
Mariposa	CU	CA	*P. salicina* x Unknown	Chance seedling
Midnite Sun	CU	CA	Unknown	
Red Beaut	CU	CA	(Eldorado x Burmosa) x Unknown	
Roysum	CU	CA	Late Santa Rosa mutant	Based on morphological characters
Santa Rosa	CU	CA	Unknown	
Shirley	CU	?	Unknown	
Simka	CU	CA	Unknown	Chance seedling
Wickson	CU	CA	Burbank x Simon	
Florida gene pool				
Gulfblaze	CU	FL	Unknown	Polycrosses
Gulf Gold	CU	FL	Unknown	Polycrosses
Gulf Ruby	CU	FL	Ozark Premier x Taiwan	
FLA86-1	SE	FL	Unknown	Polycrosses
FLA86-2	SE	FL	Unknown	Polycrosses
FLA86-4	SE	FL	Unknown	Polycrosses
FLA86-7	SE	FL	Unknown	Polycrosses
FLA87-4	SE	FL	Unknown	Polycrosses
FLA87-6	SE	FL	Unknown	Polycrosses
FLA87-10	SE	FL	Unknown	Polycrosses
FLA3-5	SE	FL	Unknown	Polycrosses
South African gene pool				
Harry Pickstone	CU	SA	Gaviota x (Methley x Wickson)	
Laetitia	CU	SA	Golden King x Unknown	
Methley	CU	SA	*P. salicina* x *P. cerasifera*	
Redgold	CU	SA	Golden King x Wickson	
Reubennel	CU	SA	Gaviota x (Methley x Wickson)	
Wilson	CU	AU	*P. cerasifera* x *P. salicina*	

[z]SP = wild species, CU = cultivated, RS = rootstock, SE = selection.
[y]Site of origin or collection. AL = Alabama, AR = Arkansas, AU = Australia, CA = California, CN = China, FL = Florida, FR = France, GA = Georgia, JP = Japan, KY = Kentucky, LA = Louisiana, MA = Massachusetts, MO = Missouri, MS = Mississippi, MU = Manchuria, NC = North Carolina, NZ = New Zealand, SA = South Africa, SC = South Carolina, SD = South Dakota, TN = Tennessee, TW = Taiwan, TX = Texas, USSR = former Soviet Union, WI = Wisconsin, N/A = not available.
[x]Pedigrees from Byrne and Littleton, 1988; Byrne, 1989; Hedrick, 1911; Howard, 1945; Okie and Ramming, 1999; and Topp and Sherman, 1990.

and *P. simonii* plums, while about one-half of RAPD markers were shared between *P. salicina* and *P. cerasifera* plums. These results agree with the natural geographic distribution of these species. *Prunus salicina* and *P. simonii* plums are believed to have originated in China; while *P. cerasifera* plums are distributed in Europe (Kovalev, 1934).

Within the section Prunocerasus, American plums and Mexican plums were more closely related to each other than to *P. angustifolia* (Table 3 and Fig. 2). This is consistent with taxonomic classification (Rehder, 1986). American plums are naturally distributed in northern states and intergrade with Mexican plums in the southwestern United States. In some areas where these ranges overlap, interspecific hybrids have been found (Charlie Graham, personal communication). Gene flow between these two species seems to occur in nature. In contrast, *P. angustifolia* plums do not appear to intercross with these plums in wild stands. While geographic distribution of *P. angustifolia* plums overlap with American and Mexican plums, *P. angustifolia* plums come into anthesis earlier in the season and grow in different niches. *Prunus angustifolia* plums are found in sandy soils and open areas, whereas Mexican plums are found in woods and American plums are found in upland and rich soil.

Two subclusters were formed within the cluster of *P. salicina* showing diversity within sampled clones. One sub-cluster included 'Japanese Greengage', 'Rena', 'Ouishi-Nakate', 'George Wilson', and 'Norine', which were different from the other subclusters because they possessed five RAPD markers that were common in *P. cerasifera*. Among *P. cerasifera* samples, four clones ('Allred', CER-816, CER-4001, and 'Clark Hill RL') joined the cluster distantly because these clones possessed four to 10 markers commonly found in *P. salicina*. Three of these ('Allred', CER-4001, and 'Clark Hill Red Leaf') have been reported as possible interspecific hybrids (Table 1). Genetic variability of *P. cerasifera* samples would be less and comparable to that of *P. salicina* if these four clones were excluded from the group.

Genetic variability within *P. angustifolia* was lower than in the other two native species in this study (Table 3 and Fig. 2). This was surprising since the sampled clones represented a wide range of geographic distribution of the species.

Sampled *P. mexicana* showed the most diversity among native plums (Table 3 and Fig. 2). This greater diversity was a result of three clones, MEX-957, MEX-958, and MEX-9516. MEX-957 and MEX-958 were tentatively identified as possible interspe-

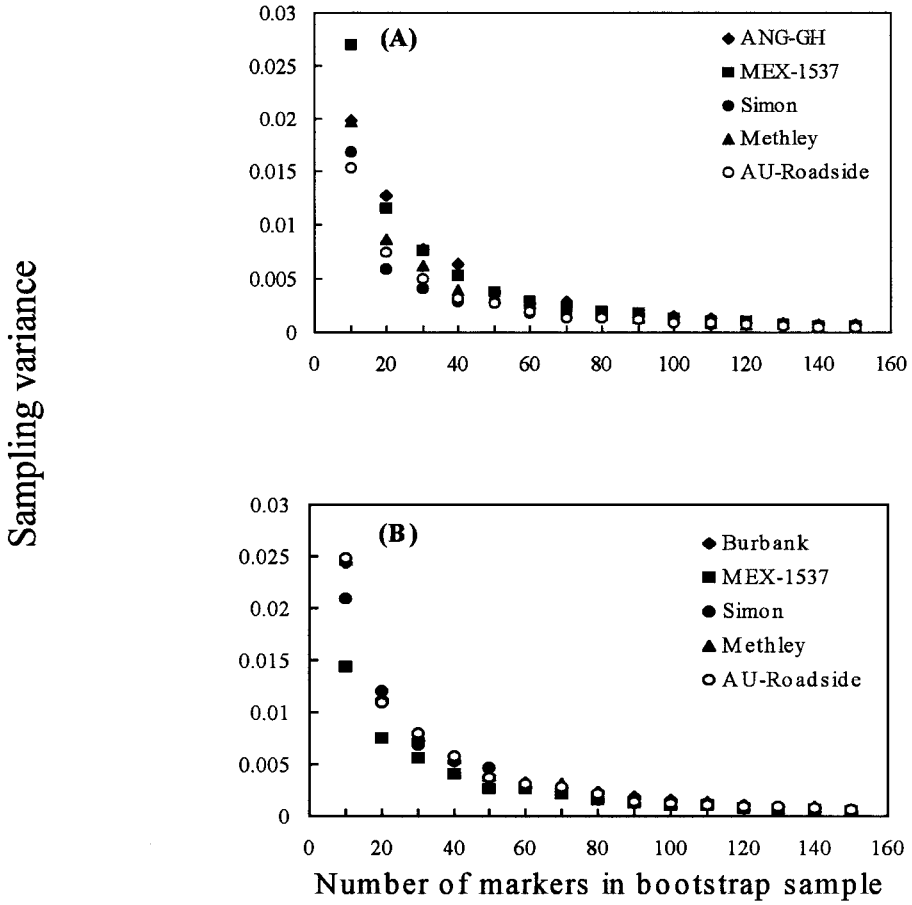

Fig. 1. Plots between sampling variance of similarity coefficient and number of markers (N) of the bootstrap sampling method. (**A**) 'Burbank' and (**B**) ANG-GH were compared to other samples for sampling variance of similarity coefficient.

cific hybrids at the time of sampling, but later were classified as *P. umbellata* Ell. Excluding these three clones, genetic diversity of *P. mexicana* was comparable to that of *P. americana*.

The *P. americana* clones from University of Wisconsin-River Falls (AMR-SD1, AMR-SD2, AMR-WI1, AMR-WI2, and AMR-WI3) were the most similar to each other and formed the core of the cluster. The genetic diversity among these *P. americana* clones was greater than that of the sampled *P. salicina* or *P. cerasifera* (when four distantly related clones were not included) genotypes.

Genetic relationships of cultivated japanese-type plums. The cultivated japanese-type plums, as the name indicate, were more similar to japanese plums (*P. salicina*) than to other species; thus, clustering with *P. salicina* samples. The dendrogram produced by cluster analysis included cultivated plums and two other diploid plum species (*P. salicina* and *P. simonii*) (Fig. 3). Other species were not included because they were not clustered as closely to cultivated plums as these two species. Except for three clusters which separated out before *P. simonii*, the other commercial plums formed clusters after *P. simonii* but before *P. salicina*. The three clusters that separated apart from most of the commercial plums were 'Methley', a *P. salicina* x *P. cerasifera* hybrid, a group of *P. angustifolia* hybrids ('Bruce', 'Segundo', and 'Robusto'), and

Table 2. RAPD marker distribution in the four cultivated gene pools of diploid plums (*Prunus sp.*).

	Number of RAPD markers in each group				
	Assayed	Cultivated gene pools			
Parameter	plums	CA[z]	SE[y]	FL[x]	SA[w]
No. plums assayed	69	17	13	11	5
Total markers	167	69	79	66	69
Shared markers	51	36	44	34	39
Markers not in species	---	2	2	2	3
	Number of diagnostic markers in each group				
Plum	Species or	Cultivated gene pools			
group	section group	CA	SE	FL	SA
Section Euprunus (30)[v]	57	30	27	28	26
P. salicina (14)	7	4	3	7	3
P. simonii (3)	6	1	0	1	1
P. cerasifera (13)	17	2	1	1	0
Section Prunocerasus (37)	59	1	6	2	1
P. angustifolia (13)	7	0	3	0	0
P. americana (13)	12	0	0	1	1
P. mexicana (11)	5	0	0	0	0

[z]California gene pool: 'Angeleno', 'Blackamber', 'Catalina', 'Ebony Sun', 'Eldorado', 'Freedom', 'Gaviota', 'Grand Rosa', 'Laroda', 'Mariposa', 'Midnite Sun', 'Red Beaut', 'Roysum', 'Santa Rosa', 'Shirley', and 'Simka'.
[y]Southeastern U.S. gene pool: 'AU-Amber', 'AU-Producer', 'AU-Roadside', 'AU-Rosa', 'AU-Rubrum', 'Bruce', 'Byrongold', BY4-601E, 'Homeside', 'Ozark Premier', 'Robusto', 'Rubysweet', and 'Segundo'.
[x]Florida gene pool: 'Gulfblaze', 'Gulf Gold', 'Gulf Ruby', FLA3-5, FLA86-1, FLA86-2, FLA86-4, FLA86-7, FLA87-4, FLA87-6, and FLA87-10.
[w]South African gene pool: 'Harry Pickstone', 'Laetitia', 'Methley', 'Redgold', 'Reubennel', and 'Wilson'.
[v]Number in parentheses is the sample size from each species.

Table 3. Average and SE of Nei and Li (1979) similarity coefficients of diploid plum (*Prunus sp*.) samples within and between species.

Plum species	*P. salicina*	*P. simonii*	*P. cerasifera*	*P. americana*	*P. angustifolia*	*P. mexicana*
P. salicina	0.85					
	(± 0.004)					
P. simonii	0.73	0.99				
	(± 0.006)	(± 0.007)				
P. cerasifera	0.54	0.44	0.80			
	(± 0.005)	(± 0.012)	(± 0.009)			
P. americana	0.36	0.31	0.36	0.79		
	(± 0.002)	(± 0.003)	(± 0.003)	(± 0.006)		
P. angustifolia	0.35	0.29	0.38	0.59	0.91	
	(± 0.002)	(± 0.002)	(± 0.004)	(± 0.002)	(± 0.004)	
P. mexicana	0.35	0.32	0.29	0.62	0.54	0.73
	(± 0.003)	(± 0.007)	(± 0.003)	(± 0.005)	(± 0.006)	(± 0.010)

'Catalina', a commercial plum grown in California of unknown origin.

The dendrogram from UPGMA cluster analysis could be divided into five main clusters (Fig. 3). The least similar cluster (V) of cultivated plums consisted of four clones: 'AU-Roadside', 'Byrongold', BY4-601, and 'Rubysweet'. These clones, except for BY4-601 are known to have either *P. angustifolia* or *P. cerasifera* in their parentage. BY4-601 is reported to be a hybrid between 'Queen Ann' and 'Santa Rosa'. RAPD data suggest that this is erroneous.

The next least similar cluster (Cluster IV) included plums from the Florida breeding program. These plums were unique from other japanese-type plums because they were bred with the 'Taiwan' plum and selected for low chill adaptation (Sherman et al., 1992). 'Taiwan' possessed one unique RAPD marker (E6-1200). This marker was found in all Florida plums, except FLA87-4 and FLA3-5. Another marker, J5-640, amplified only in 'Taiwan' and 'Ivanovka', was also found in many Florida plums including FLA3-5 but not FLA87-4. These results showed the introgression of 'Taiwan' plum into the Florida gene pool. The only Florida plum which did not cluster with its group was FLA87-4.

Cluster III included 11 clones. 'AU-Amber' and 'Shirley' are half sibs of 'Methley' while 'Wilson' is an interspecific hybrid between *P. cerasifera* and *P. salicina* as was 'Methley'. In 'AU-Amber', 39 markers out of 52 total (75%) and in 'Shirley', 38 markers out of 51 total (74.5%) were shared with 'Methley'. Among these shared markers, 32 were common in both.

Thirteen markers present in 'Shirley' but absent in 'Methley' should be present in 'Abundance' if the reported pedigree was correct. Eleven of these markers were present; while two were absent from 'Abundance'. If these were RAPD artifacts, which would represent ≈4% error, the reported pedigree would be correct.

Four *P. salicina* clones ('Japanese Greengage', 'George Wilson', 'Norine', and 'Rena') were closely related to each other and joined into Cluster III. These plums appear to have *P. cerasifera* background. The RAPD data revealed that these plums had between eight to nine markers that were present at higher frequency in *P. cerasifera* than in *P. salicina*. Given that 'George

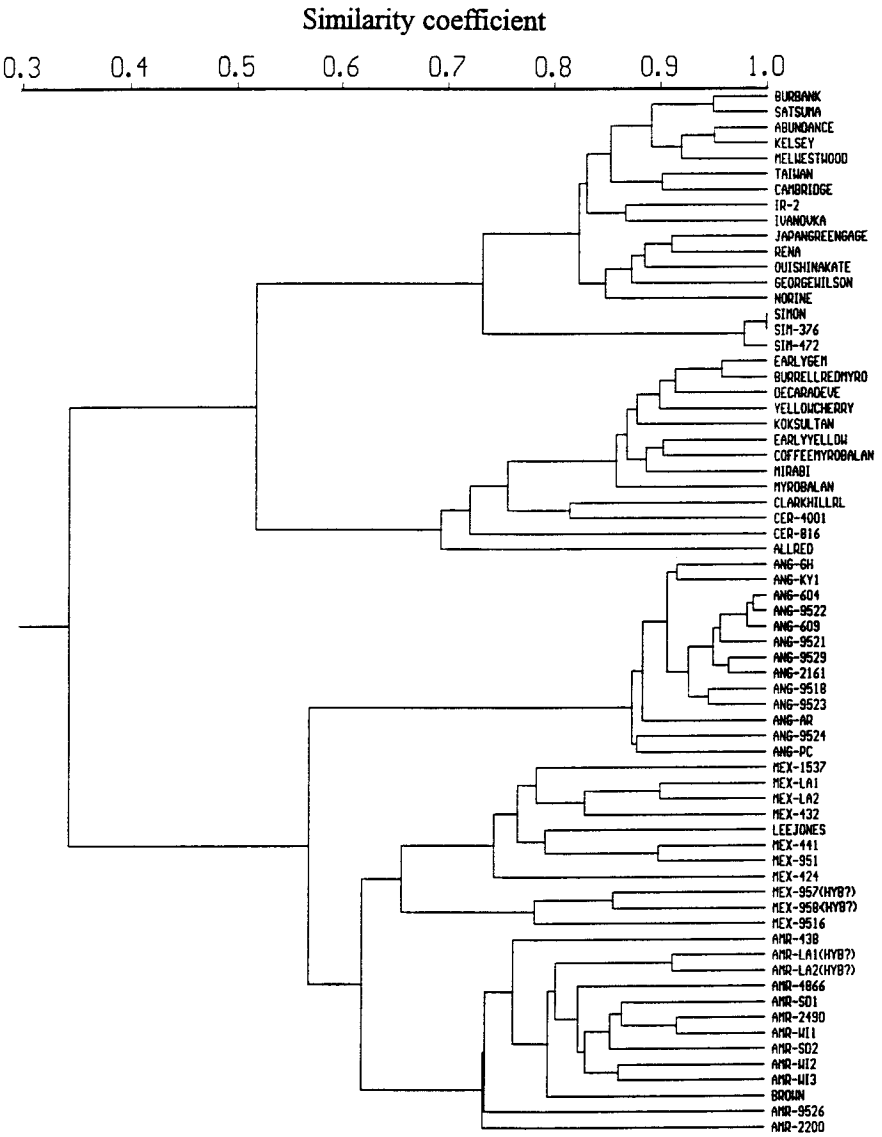

Fig. 2. Dendrogram of diploid plum (*Prunus* sp.) species using UPGMA cluster method on Nei and Li (1979) similarities of RAPD markers.

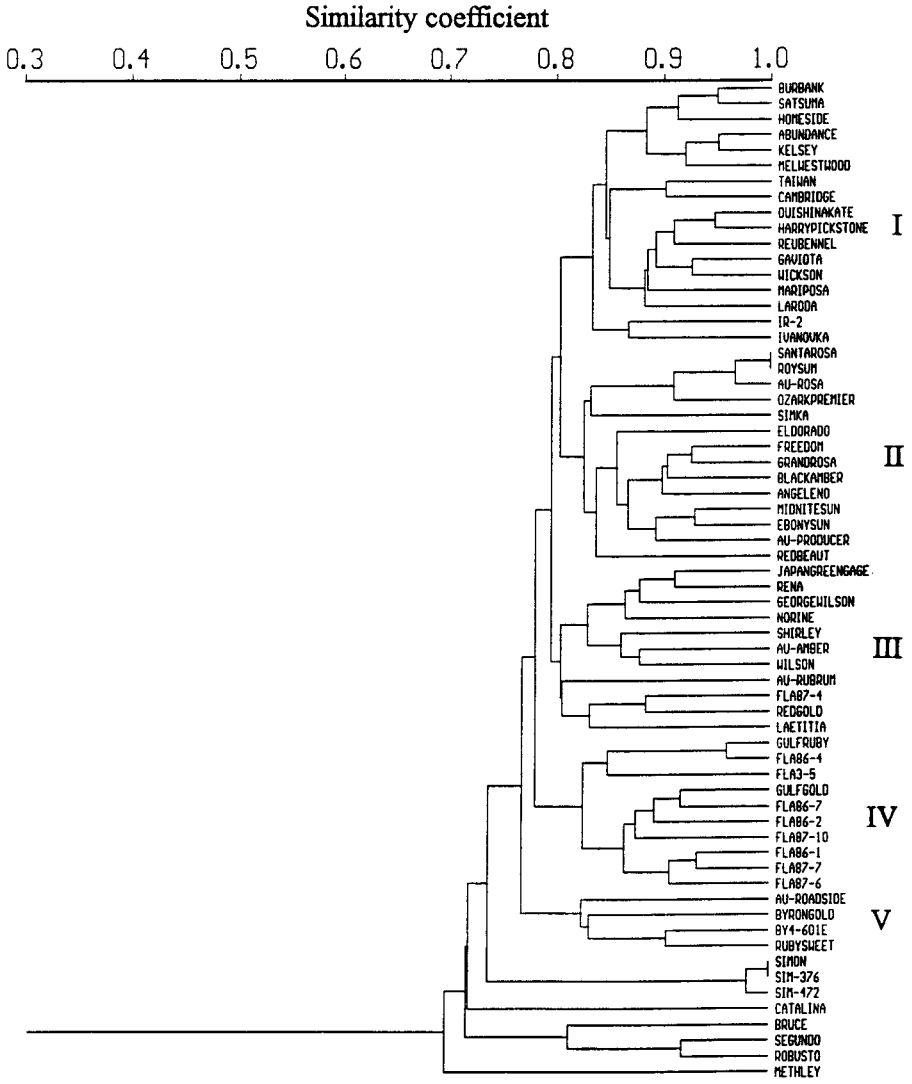

Fig. 3. Dendrogram of cultivated japanese-type plums (*Prunus* sp.) using UPGMA cluster method on Nei and Li (1979) similarities of RAPD markers.

Wilson' and 'Norine' had small and thin leaves similar to *P. cerasifera* and clustered with cultivated plums of known *P. cerasifera* background, it is likely that these four clones are not pure *P. salicina* but rather mixed with *P. cerasifera*.

Four other clones in Cluster III were 'AU-Rubrum', FLA87-4, 'Redgold', and 'Laetitia'. 'Redgold' and 'Laetitia' were half sibs sharing 'Golden King' as the female parent. 'Golden King' is not available in the United States and its pedigree is unknown. Both 'Redgold' and 'Laetitia' have RAPD markers typical of *P. cerasifera* and thus appear to have a *P. cerasifera* background. 'AU-Rubrum' is believed to be a bud sport of 'Crimson'. The reported pedigree of 'Crimson' as 'Bruce' x 'Methley' is incorrect (Byrne and Littleton, 1988).

Cluster II included 14 clones, all of which except for 'Ozark Premier' and 'AU Producer' are from the California gene pool. 'Santa Rosa' and 'Roysum' were identical. This was consistent with the pedigree record because 'Roysum' was a bud sport of 'Santa Rosa'. 'Grand Rosa' and 'Red Beaut' which are half sibs of 'Eldorado' joined the cluster with 'Eldorado' as did 'Angeleno' and 'Blackamber', which both had 'Eldorado' in their parentage. 'Eldorado' has unknown parentage but is believed to be an interspecific hybrid of *P. salicina* and *P. simonii* (Howard, 1945). The RAPD data showed that 'Eldorado' possesses several markers invariable in *P. simonii*. Three markers amplified uniquely in *P. cerasifera* and *P. americana* were found in 'Eldorado', indicating possible genetic contribution of these two species to 'Eldorado'. Thus, 'Eldorado' might be an interspecific hybrid derived from these four species.

'Ozark Premier' ('Burbank' x 'Methley' hybrid) had three (7%) of 43 markers that were absent from both parents. This sheds doubt on its reported parentage although, these inconsistent markers could be the result of RAPD error due to either a false positive or a false negative.

The previously reported error (Byrne and Littleton, 1988) in the 'Bruce' x 'Santa Rosa' parentage of 'AU Producer' was confirmed by RAPD results. There were five markers out of 49 total in 'AU-Producer' that were absent in both 'Bruce' and 'Santa Rosa'. Since 'AU-Producer' shares numerous markers with 'Methley' and *P. cerasifera*, it may have these in its background.

The four clones ('AU-Rosa', 'Ebony Sun', 'Midnite Sun', and 'Simka') of unknown parentage in this cluster might share parents or might have similar genetic background with the other clones in

Table 4. Average and standard error of genetic similarities [Nei and Li (1979) coefficient] of cultivated plums (*Prunus sp.*) among different gene pools.

Gene pool	California	Southeastern	Florida	South African
California[z]	0.84 ± 0.004			
Southeastern[y]	0.78 ± 0.004	0.77 ± 0.006		
Florida[x]	0.78 ± 0.003	0.75 ± 0.004	0.85 ± 0.006	
South African[w]	0.79 ± 0.005	0.77 ± 0.007	0.78 ± 0.009	0.80 ± 0.028

[z]California gene pool: 'Angeleno', 'Blackamber', 'Catalina', 'Ebony Sun', 'Eldorado', 'Freedom', 'Gaviota', 'Grand Rosa', 'Laroda', 'Mariposa', 'Midnite Sun', 'Red Beaut', 'Roysum', 'Santa Rosa', 'Shirley', and 'Simka'.

[y]Southeastern U. S. gene pool: 'AU-Amber', 'AU-Producer', 'AU-Roadside', 'AU-Rosa', 'AU-Rubrum', 'Bruce', 'Byrongold', BY4-601E, 'Homeside', 'Ozark Premier', 'Robusto', 'Rubysweet', and 'Segundo'.

[x]Florida gene pool: 'Gulfblaze', 'Gulf Gold', 'Gulf Ruby', FLA3-5, FLA86-1, FLA86-2, FLA86-4, FLA86-7, FLA87-4, FLA87-6, and FLA87-10.

[w]South African gene pool: 'Harry Pickstone', 'Laetitia', 'Methley', 'Redgold', 'Reubennel', and 'Wilson'.

Table 5. Species composition of cultivated plum (*Prunus sp.*) gene pools according to RAPD analysis.

	Putative contributing species[z]				
Gene pool	SAL	SIM	CER	AMR	ANG
California	0.36	0.26	0.28	0.10	0
Southeastern U.S.	0.29	0.21	0.22	0.10	0.18
Florida	0.37	0.23	0.21	0.09	0.10
South Africa	0.34	0.27	0.25	0.14	0

[z]SAL = *P. salicina*, SIM = *P. simonii*, CER = *P. cerasifera*, AMR = *P. americana*, and ANG = *P. angustifolia*.

the cluster. 'Freedom' which is an offspring of 'Laroda' must be an outcross because it was placed in a different cluster.

In Cluster I, 10 clones of *P. salicina* formed a large group which included the founding clones of japanese-type plums that were sources of *P. salicina* ('Burbank', 'Satsuma', 'Abundance', and 'Kelsey'). Although they were imported from Japan at different times in late 1800s and early 1900s, they are closely related according to RAPD analysis. These plums were introduced to Japan from China ≈2000 years ago but cultivated in a limited acreage (Masao Yoshida, personal communication) and probably represents only a few introductions from China.

'Homeside' was the only cultivated plum to join in this subcluster with the *P. salicina* founding clones. 'Homeside' was reported to have 'Methley' x 'Ozark Premier' parents but this was dismissed later by isozyme analysis (Byrne and Littleton, 1988) and is confirmed by the six RAPD markers (12.5%) out of 48 amplified in 'Homeside' that were absent from both reported parents. Cluster analysis using RAPD data indicated that 'Homeside' was very similar to both 'Burbank' and 'Satsuma'. There were only two markers present in 'Homeside' but absent in 'Burbank' and 'Satsuma'. These markers were invariable in *P. simonii*. 'Homeside' might have been derived from these by selfing or crossing to related clones. Outcrossing to other plum species would place 'Homeside' into another cluster. Isozyme data are consistent with 'Burbank' as a parent of 'Homeside'.

Two other small groups within this cluster contain the source of low chilling genes used in the Florida breeding program ('Taiwan') and a cultivar collected from Manchuria, the extreme northern limitation of *P. salicina*'s distribution ('Ivanovka'). These are representatives of the extreme in adaptation of *P. salicina*. Their grouping apart from the founding clones indicates much variability of *P. salicina* remains to be explored in China.

Seven other plums ('Mariposa', 'Gaviota', 'Wickson', 'Laroda', 'Reubennel', 'Ouishi-Nakate', and 'Harry Pickstone') form a group within this cluster. 'Reubennel' and 'Harry Pickstone' are siblings and have 'Gaviota' as the maternal parent, as does 'Laroda'. 'Mariposa' had unknown parentage. Forty-two markers (93%) out of 45 total in 'Mariposa' were also amplified in 'Gaviota'. According to cluster analysis on RAPD data, it might be related to 'Gaviota' as an offspring or shared similar genetic background.

The close similarity of 'Wickson' and 'Gaviota' was surprising. 'Wickson' was recorded to be a *P. salicina* x *P. simonii* hybrid while 'Gaviota' was thought to be a *P. salicina* x *P. americana* hybrid and probably mixed with other species (Hedrick, 1911; Howard, 1945). 'Gaviota' possesses a marker unique to *P. simonii* and thus it appears that 'Gaviota' has *P. simonii* in its background. In contrast, although 'Gaviota' does possess a few markers that are commonly present in *P. americana* and *P. cerasifera*, it did not contain any that were unique to these species which puts its *P. americana* parentage in question.

The cluster analysis using the UPGMA method on similarity of Nei and Li (1979) based on RAPD markers revealed the dendrogram of cultivated japanese-type plums. Closely related clones with similar pedigree or genetic background grouped to each other fairly well. Some clones with unknown pedigrees could be placed with clones with known parentage allowing inference about their putative pedigree of these unknowns. Cultivated plums with *P. angustifolia* background formed separate clusters, as did 'Methley' which has 50% *P. cerasifera* background. Cultivated plums from the Florida breeding program were unique and formed a separate cluster.

Genetic relationships of cultivated japanese-type plums from different gene pools. The four gene pools of interest were from California, the southeastern United States, Florida, and South Africa (Table 4). The California gene pool combined cultivars released by both public (USDA, Fresno, University of California, Davis) and private (Burbank and others) breeding programs. The southeastern United States included cultivars released mainly from Georgia (USDA, Byron) and Alabama (Auburn University). The Florida gene pool was from clones developed by the University of Florida, Gainesville.

The Florida and California plums showed the most similarity, and the southeastern United States gene pool was the most diverse (Table 4). This indicates that the southeastern United States gene pool had the broadest genetic background; while the Florida and the California gene pools had the narrowest. The southeastern United States gene pool was more diverse because it combined California materials ('Santa Rosa', 'Mariposa', and 'Gaviota') with 'Methley' and several native species (*P. angustifolia* and *P. munsoniana*) (Byrne, 1989).

The Florida gene pool showed the least similarity with the southeastern gene pool. It also had the narrowest genetic background (the highest similarity within its gene pool) (Table 4). The Florida germplasm was unique because the main breeding goal was an adaptation to low chill areas by incorporating low chill traits from one or two Taiwan clones (Sherman et al., 1992). High selection pressure for adaptation to low chill areas combined with just a few founding clones resulted in a narrow genetic background. Its uniqueness was obvious in the cluster analysis in which the Florida plums grouped by themselves and separated from all other plums (Fig. 3). The similarities of the Florida to the California gene pools and the Florida to the South African gene pools were comparable (Table 4). The dendrogram of cluster analysis was consistent with these by combining these three gene pools at about the same similarities (Fig. 3). The dendrogram of cluster analysis showed that some plums from the southeastern gene pool ('AU-Roadside', 'Bruce', 'Byrongold', 'Robusto', 'Rubysweet', 'Segundo', and BY4-601) clustered away from other cultivated plums. These resulted in the lowest similarity between the Florida and the southeastern gene pools.

The four cultivated gene pools had between 66 and 79 RAPD markers each and two to three markers each which were not found among the progenitor species group. Twenty-six to 30 of these

were diagnostic of the section Euprunus versus one to six markers diagnostic of the section Prunocerasus in each group. The other markers were found in both sections. This reflects the importance of the species from the section Euprunus in the development of the cultivated diploid plum. The most prominent species is *P. salicina* which has three (43%) to seven (100%) of the diagnostic markers in each cultivated plum gene pool. The other two species of the Euprunus section (*P. simonii* and *P cerasifera*) are apparent in the gene pools via a few species specific markers although the Southeastern and the South African gene pools lack diagnostic markers for *P. simonii* and *P. cerasifera* respectively. In both cases, these contain other markers that are found at high frequency in the respective species. This combined with pedigree and morphological evidence indicate that all four cultivated gene pools have contributions from these three species in the section Euprunus.

Very few diagnostic markers from the species of the section Prunocerasus appear in the cultivated plum germplasm examined (Table 2). The cultivated gene pool with the greatest evidence of introgression from this plum group is the Southeastern group in which *P. angustifolia* was used as a source of disease resistance (Byrne, 1989). There is direct evidence of a *P. americana* contribution only in the Florida and the South African gene pools but not the California or the Southeastern gene pools. Nevertheless, pedigree records indicate that both of these species have contributed to both these gene pools (Byrne 1989; Hedrick, 1911; Howard, 1945). As expected there is no evidence of the introgression of *P. mexicana* into the cultivated plum germplasm.

Prunus salicina was the species that contributed most to the genetic background of the cultivated plum (Table 5). It was initially imported from Japan and first popularized by Burbank (1914) and subsequently used extensively for its fruit qualities (fruit size, fruit firmness, and fruit color), precocity, and disease resistance. It was possible that other *P. salicina* imported from China and Korea contributed their genetic background to the cultivated plum gene pool because Burbank (1914) mentioned use of these plums in his breeding work.

The contributions of *P. simonii* and *P. cerasifera* to the cultivated plum gene pools were comparable and each constituted about one-fourth of the gene pool (Table 5). *Prunus simonii* had a good fruit firmness character, which was needed for good shipping quality (Hedrick, 1911). *Prunus cerasifera* was used primarily for a rootstock in the United States (Cullinan, 1937; Hedrick, 1911). However, since many plums are self incompatible, this species might have crossed to other plums in breeding plots unintentionally. Hybrids of *P. cerasifera* to others might have shown hybrid vigor and good health and thus were selected. In South Africa, *P. cerasifera* was intentionally used in the development of cultivated plums for resistance to bacterial spot [*Xanthomonas campestris* pv. *pruni* (Smith) Dye]. The southeastern United States gene pool had less of *P. simonii* and *P. cerasifera* background than other gene pools, and this background was replaced by *P. angustifolia* which constituted almost one-fifth of the southeastern gene pool. *Prunus americana,* used for its good flavor and cold hardiness (Burbank, 1914), contributed ≈9% to 14% to the cultivated plum gene pool.

Prunus cerasifera contributed the most to the California and South African gene pools (28% and 25% respectively), while its contribution to the southeastern United States and the Florida gene pools were comparable (21% and 22%). This high contribution of *P. cerasifera* to the California gene pool conflicts with the reported parentage of this group. However, in the case of 'Santa Rosa' and several other clones, the parentage is based on recollection and notes on morphological similarity with suspected parents. It should be noted that the RAPD data are strong for the presence of *P. cerasifera* but not for *P. americana* in the California gene pool.

In conclusion, the most diverse (the southeast United States gene pool) and the least diverse (the Florida gene pool) plum gene pools are derived from five putative species, whereas, the California and South Africa gene pools only have four putative species in their background. In all cases, the greatest contribution was from *P. salicina* (29% to 36%) followed by approximately equal contributions from *P. simonii* (21% to 26%) and *P. cerasifera* (21% to 28%). These Euprunus species contribute the vast bulk (72% to 90%) of the genetic background of cultivated plums. The Prunocerasus plums contribute 28% in the southeast United States gene pool (10% *P. americana* and 18% *P. angustifolia*) to 10 % in the California gene pool (only *P. americana*).

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