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Genetic Characterization and Relatedness among California Almond Cultivars and Breeding Lines Detected by Randomly Amplified Polymorphic DNA (RAPD) Analysis

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Abstract. **Almond [*Prunus dulcis* (Mill.) D.A. Webb, syn. *P. amygdalus*, Batsch; *P. communis* (l.) Archangeli] represents a morphologically and physiologically variable group of populations that evolved primarily in central and southwest Asia. California cultivars have been developed from highly selected subgroups of these populations, while new breeding lines have incorporated germplasm from wild almond and closely related peach species. The genetic relatedness among 17 almond genotypes and 1 peach genotype was estimated using 37 RAPD markers. Genetic diversity within almond was found to be limited despite its need for obligate outcrossing. Three groupings of cultivar origins could be distinguished by RAPD analysis: bud-sport mutations, progeny from interbreeding of early California genotypes, and progeny from crosses to genotypes outside the California germplasm. A similarity index based on the proportion of shared fragments showed relatively high levels of 0.75 or greater within the almond germplasm. The level of similarity between almond and the peach was 0.424 supporting the value of peach germplasm to future almond genetic improvement.**

World production of almond [*Prunus dulcis* (Mill.) D.A. Webb, syn. *P. amygdalus*, Batsch; *P. communis* (l.) Archangeli] was 1 billion pounds in 1995, exceeding all other temperate tree nut crops. California produced 733 million pounds, with the three major cultivars—'Nonpareil', 'Carmel', and 'Mission'—accounting for 47%, 17%, and 7%, respectively, of total California acreage. 'Nonpareil' and 'Mission' were among the first almond cultivars to be widely grown in California. 'Carmel' originated in 1966 in a 'Nonpareil' planting and is reported to be a bud-sport mutation of 'Nonpareil' (Gradziel, 1997). Most California almond cultivars have originated as chance seedlings, selected and planted by orchardists and nurserymen (Kester and Gradziel, 1996). Isozyme (Hauagge et al., 1987), pollen self-incompatibility (S)-allele analysis (Kester et al., 1994), and where available, records of origin (Gradziel, 1997), support 'Nonpareil' and 'Mission' as parents to most present cultivars. Due to the small genetic pool of the California almond, cultivars are often difficult to differentiate based solely on tree and fruit morphology. Almond's outbreeding nature, enforced by a gametophytic self-incompatibility, however, results in high levels of genetic heterozygosity for individuals within the population, which might be used for cultivar differentiation (Grassely, 1972). An accurate knowledge of parentage and cultivar origin may also lead to a better understanding of the inheritance and potential manipulation of important genetic traits, particularly pollen cross-compatibility (Kester, 1981; Kester and Micke, 1984; Kester et al., 1994), graft incompatibility on plum rootstocks (Kester and Gradziel, 1996; Micke, 1996), and expression of the genetic disorder known as bud failure (Kester and Gradziel, 1996).

Isozyme analysis has been useful in almond cultivar identification (Arulsekar et al., 1986; Cerezo et al., 1989; Hauagge et al., 1987; Jackson and Clarke, 1991). However, such variation has remained restricted to a few polymorphic enzyme systems that are encoded by a limited number of loci. In almond, only ten loci have been studied using the isozymes Gpi, Pgm, Lap, Aat, Ida, and 6Pgd (Hauagge et al., 1987, Arus et al., 1994).

Recently the development of molecular genetics has resulted in various DNA based procedures for the detection of genetic polymorphism. Before the development of polymerase chain reaction (PCR), restriction fragment-length polymorphism (RFLP) was extensively used for genetic diversity, linkage mapping, and fingerprinting studies (Tanksley et al., 1989). In addition to the special care needed in handling the radioactive probes, difficulties in extracting high quality DNA from many tree crop species has limited application of RFLP procedures (Warburton and Bliss, 1996).

The development of PCR and related RAPD techniques overcame many of the limitations of RFLPs (Williams et al., 1990; Welsh and McClelland, 1990). RAPD technology has been used successfully for identifying cultivars (Hu and Quiros, 1992), estimating genetic diversity in crops such as peach (Warburton and Bliss, 1996), for pedigree relationships in barley (Tinker et al. 1993), and constructing genetic linkage maps for crop species (Chaparro et al., 1994; Koller et al., 1993; Warburton et al., 1996).

In this study we use RAPD markers to assess possible origins and level of genetic diversity for selected almond genotypes. The use of such molecular techniques for tree crop breeding is also discussed.

Materials and Methods

Plant materials. A total of 18 accessions including 14 almond cultivars, 3 advanced breeding lines, and 1 peach (*Prunus persica* L. Batsch) rootstock were used in this study (Table 1). Materials were obtained from the USDA National Clonal Germplasm Re-

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Table 1. Almond cultivars and selections included in this study with their possible parentage.

Cultivar	Putative origin
1) Padre	'Mission' x 'Swanson'
2) Sonora	('Nonpareil' x ('Nonpareil' x 'Eureka')) sib
3) Peerless	Unknown origin, possible 'Swanson' sport
4) Thompson	'Nonpareil' x 'Mission'
5) Trusito	Old Italian variety (PI 223477)
6) Ne Plus Ultra	Sib of 'Nonpareil'
7) Carmel	Bud mutation of 'Nonpareil'
8) UCD,8011-22	'Nonpareil' x (*P. Webbii* x ('Nonpareil' x 'Eureka') x (P. Persica x 'Mission'))
9) SB6, 56-89	('Nonpareil' x (*P. persica* x 'Mission')) selfed
10) SB13, 25-75	(Almond x *P. Mira* x) BC_1 x ('Arbuckle' x almond seedling)
11) Nonpareil	California seedling selection first planted in 1879
12) McKenespy Nonpareil	Mutation of 'Nonpareil'
13) BF-Nonpareil	Mutation of 'Nonpareil'
14) Tardy Nonpareil	Mutation of 'Nonpareil'
15)Weststeyn	Mutation of 'Nonpareil'
16) Mission	Texas seedling selection brought to California about 1900
17) Shaw Mission	Mutation of 'Mission'
18) Nemared	Peach rootstock as outgroup

pository and the Foundation Plant Materials Service, at Davis, Calif., and from the Univ. of California breeding collection maintained in Winters, Calif. DNA from bud-sport mutations of 'Nonpareil' and 'Mission' were used as internal checks.

DNA EXTRACTION. Young almond leaves were collected in early summer. Leaves were stored at 4 °C in the dark for 2 to 3 d before DNA extraction to deplete the starch and polysaccharide levels as much as possible. Total DNA was extracted from leaf tissue following the method described by Gepts and Clegg (1989) with minor modifications as described below. Five grams of young leaves were ground in liquid Nitrogen and mixed with 12 mL of extraction buffer (50 mM Tris-HCl, pH 9.5; 0.7 M NaCl, 10 mM EDTA, 1% SDS, and before use, 1% β-mercaptoethanol and 5% PVPP). The ground leaf samples were incubated at 65 °C for 30 min, and extracted twice with equal volume of chloroform-isoamyl alcohol (24:1). The aqueous phase was recovered and mixed with 2/3 volume of cold isopropanol to precipitate the total nucleic acid. The precipitate was washed twice with the buffer 10 mM ammonium acetate in 76% ethanol and air dried. The precipitate was dissolved in the TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA), and further purified by cesium chloride-ethidium bromide density gradient in an ultra-centrifuge. The purified genomic DNA was dissolved in the TE buffer, quantified using a spectrophotometer and stored at 4 °C. The DNA was diluted to a concentration of 10 ug/ml in sterile water and used for PCR amplification.

PCR AMPLIFICATION AND ELECTROPHORESIS. Sixty decamer oligonucleotides (Operon Technologies, Alameda, Calif.) were used for PCR amplification following the procedure of Williams et al. (1990) with some modifications. Amplification reactions were carried out in 25 µL volumes containing 10× PCR buffer (10 mM tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin) (Perkin Elmer Cetus, Norwolk, Conn.), 50 µM each of dATP, dGTP, dCTP, dTTP (Perkin Elmer Cetus), 0.4 µM Primer, 0.75 units of AmpliTaq DNA Polymerase (Perkin Elmer Cetus) and 50 ng of genomic DNA. Each reaction mixture was overlaid with one drop of mineral oil to prevent evaporation. DNA amplification reactions were performed in a Perkin Elmer Cetus DNA thermal cycler programmed as follows: 1 cycle of 2 min at 94 °C, 40 cycles of 45 s at 92 °C, 1 min at 36 °C, and 2 min at 72 °C (for denaturing, annealing and primer extension, respectively). The last cycle was followed by a final incubation for 5 min at 72 °C and the PCR products were stored at 4 °C before analysis. The amplified products were separated in a 2% agarose gel electrophoresis using 0.5× TBE buffer, and stained with ethidium bromide. The stained gels were photographed under UV light (Fig. 1). The molecular sizes of the amplification products were estimated using a 123 bp DNA ladder (Life Technologies). All PCR reactions were repeated at least three times with two different isolations of DNA. Only bands that were bright and reproduced in all three amplification reactions, and were in the size range of 300 bp to ≈2.5 kb were scored for analysis. Each RAPD fragment useful for discrimination between genotypes was denoted by the primer used and its approximate size in base pairs.

DATA ANALYSIS. RAPD bands were scored from photographs as 1 (present) or 0 (absent) for all markers and for all individuals in the study. A similarity matrix was generated by the NTSYS-pc version 1.7 (Rohlf, 1992) based on the simple matching algorithm of Sokal and Sneath (1963). This algorithm considers RAPD bands as phenotypic rather than genetic characters, and considers individuals which either possess a band in common or lack a common

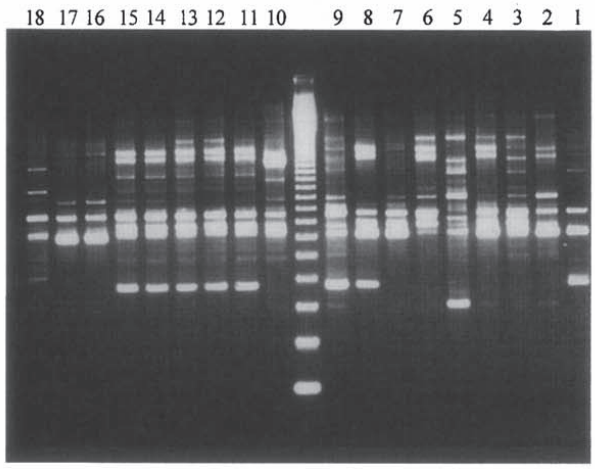

Fig. 1. RAPD band profiles generated by the Operon primer OP-A08 for the almond genotypes included in this study. The cultivar numbers corresponds to the cultivars in shown in Table 1. Molecular weight marker 123-bp ladder shown between profiles 9 and 10 with fourth band from bottom representing the 480-bp band.

Table 3. Coefficient of similarity values for the almond genotypes based on the proportion of shared fragments generated by using simple matching algorithm of the NTSYS program.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1) Padre	1.000																	
2) Sonora	0.555	1.000																
3) Peerless	0.631	0.857	1.000															
4) Thompson	0.594	0.829	0.883	1.000														
5) Trusito	0.529	0.578	0.650	0.666	1.000													
6) Ne Plus Ultra	0.484	0.756	0.820	0.789	0.571	1.000												
7) Carmel	0.742	0.717	0.780	0.700	0.594	0.666	1.000											
8) UCD,8011-22	0.666	0.647	0.666	0.628	0.562	0.645	0.727	1.000										
9) SB6,56-89	0.666	0.648	0.615	0.526	0.628	0.588	0.555	0.709	1.000									
10) SB13,25-75	0.685	0.615	0.731	0.650	0.648	0.500	0.684	0.545	0.555	1.000								
11) Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000							
12) Mckenespy Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000						
13) BF-Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000					
14) Tardy Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000	1.000				
15) Weststeyn	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000	1.000	1.000			
16) Mission	0.486	0.780	0.790	0.809	0.564	0.789	0.700	0.514	0.578	0.550	0.550	0.550	0.550	0.550	0.550	1.000		
17) Shaw Mission	0.486	0.780	0.790	0.809	0.564	0.789	0.700	0.514	0.578	0.550	0.550	0.550	0.550	0.550	0.550	1.000	1.000	
18) Nemared	0.413	0.363	0.457	0.470	0.451	0.333	0.500	0.370	0.266	0.500	0.500	0.500	0.500	0.500	0.500	0.294	0.294	1.000

band as a match (genetically similar). Nei's genetic distance index was not appropriate for this analysis as it is based on allelic frequencies (genetic data) which is not available using RAPD markers in highly heterozygous populations. With most RAPD markers, heterozygous individuals cannot be distinguished from homozygous dominant individuals. The simple matching similarity coefficients were used to cluster individuals using the SAHN procedure of NTSYS which uses the unweighted pair group method with arithmetic averages (UPGMA). Results were used to construct the final dendrogram. 'Nemared' peach was used as an out-group for comparison.

Results and Discussion

A total of 60 decamer primers were evaluated; 21 primers produced 37 reproducible bands among the 18 accessions used in this study (Table 2). About two-thirds of the primers either did not amplify discrete products (only smears) and/or were not informative (i.e., produced either monomorphic or no bands) in distinguishing the groups tested.

Genetic diversity and relatedness among germplasm. The similarity coefficients among almond cultivars/selections ranged from 1.00 between bud-sport mutations to 0.484 between 'Ne Plus Ultra' and 'Padre' (Table 3). A dendrogram constructed from similarity data shows separate groupings with affinities to 'Nonpareil' and 'Mission', reflecting their historical importance to almond cultivar development in California (Fig. 2). 'Nonpareil', along with 'Ne Plus Ultra' originated from a single seedling orchard planted by A.T. Hatch of Suisun, Calif., in 1879 and are known as the "Hatch" cultivars (Kester and Gradziel, 1996; Wood, 1925). 'Mission' originated in Houston, Texas, sometimes being referred to as 'Texas' or 'Texas Prolific', and was first introduced into California about 1900 (Gradziel, 1997). 'Nonpareil' rapidly became the main cultivar in California due to its good tree and nut

Table 2. List of the 20 primers and their sequences that produced polymorphic markers among the almond genotypes studied.

Primer	Sequence	Polymorphic fragments (no.)	Fragment size (base pairs)
OP-A01	CAGGCCCTTC	2	860, 1800
OP-A04	AATCGGGCTG	3	369, 400, 640
OP-A05	AGGGGTCTTG	1	630
OP-A08	GTGACGTAGG	1	480
OP-A09	GGGTAACGCC	2	400, 738
OP-A10	GTGATCGCAG	1	850
OP-A11	CAATCGCCGT	1	870
OP-A14	TCTGTGCTGG	5	440, 520, 700, 960, 1290
OP-A18	AGGTGACCGT	1	970
OP-A19	CAAACGTCGG	1	1230
OP-B01	GTTTCGCTCC	1	680
OP-B02	TGATCCCTGG	1	450
OP-B04	GGACTGGAGT	1	1230
OP-B05	TGCGCCCTTC	2	1476, 1960
OP-B10	CTGCTGGGAC	4	680, 860, 1400, 1900
OP-B13	TTCCCCCGCT	1	500
OP-C05	GATGACCGCC	2	740, 1500
OP-C06	GAACGGACTC	3	260, 320, 680
OP-C10	TGTCTGGGTG	1	500
OP-C11	AAAGCTGCGG	2	984, 1500
Total		37	

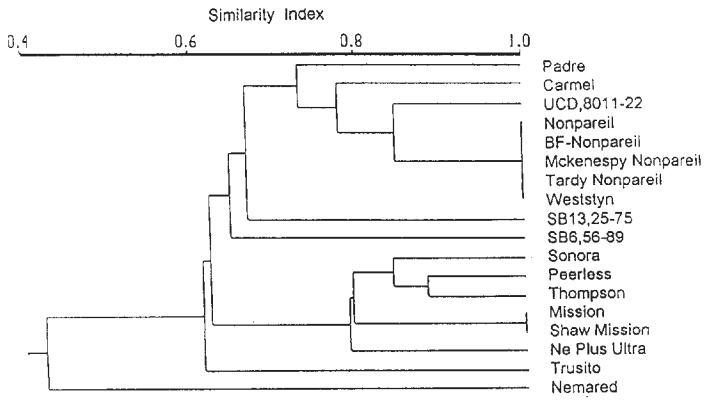

Fig. 2. An UPGMA dendogram based on the similarity index among 17 almond genotypes and a peach outgroup for 37 RAPD markers.

qualities. Because almond is self-incompatible, 'Mission' and 'Ne Plus Ultra' were used as the main pollinizers for 'Nonpareil'. 'Ne Plus Ultra', although a Hatch origin cultivar like 'Nonpareil' shows greater similarity to 'Mission'. 'Carmel' has been reported to be a bud-sport of 'Nonpareil', but based on the RAPD marker data, it is clearly distinct. The lower similarity indices (Table 3) and more divergent dendrogram branch points (Fig. 2) of the genotypes derived from hybridizations with peach (i.e., UCD,8011-22, SB6,56-89 and SB13,25-75) demonstrate the greater genetic variability of this material.

The Nemared peach is shown to be a distinct out-group with similarity indices ranging from 0.266 to 0.500. In addition, 'Trusito', an old self-fertile and very hard-shelled Italian cultivar not previously used in California, is the most divergent of the almond material with uniformly low similarity indices ranging from 0.529 to 0.666 compared to other cultivars in this study. Similarity indices within this range, however, are common between many of the genotypes tested due to divergent origins and the expected high heterozygosity among the self-sterile almonds. Outbreeding within a limited gene pool can lead to similarity values not fully reflecting known parentage, as with 'Padre', a 'Mission' x 'Swanson' cross showing greater similarity to 'Nonpareil', and the 'Nonpareil' x 'Eureka' backcross 'Sonora' showing greater similarity to 'Mission'.

Comparison between peach and almond RAPD markers. Within the *Prunus* genus, almond is closely related to peach which evolved in the warmer and more humid climates of eastern Asia rather than the colder, xerophytic central and southwest Asia center of origin of almond (Watkins, 1979). A previous study of genetic diversity in peaches using RAPD markers (Warburton and Bliss, 1996) included the California almond breeding line 6A-11, a 'Nonpareil' cross, as an outgroup. The level of similarity between the peaches and almond was found to be 0.5, which is close to the value of 0.424 found in this study between Nemared peach and the 17 almond varieties (Table 3). In contrast, while the average level of similarity within the almond cultivars in this study is relatively high (0.75 or greater), the average similarity within U.S. peach cultivars was >0.90. As with almonds, peaches in the United States have been reported to be derived from a limited number of original introductions. The self-pollinating nature of peach would further limit genetic variability of progeny from this germplasm. Thus, it appears that the heterozygous nature of the naturally outcrossing almond is maintaining a higher degree of variability within the gene pool than has been possible for peach.

Cultivar characterization and origin. All cultivars are distinguishable by unique RAPD profiles generated by multiple primers except the putative bud-sport mutations previously described (Table 4). These RAPD profiles support three distinct

Table 4. Polymorphic RAPD fragments scored to characterize the almond genotypes. The peach cultivar 'Nemared' is used an out group in the genetic similarity study; 0 = absence of the band; 1 = presence of the band.

	Primer														
	A01	A01	A04	A04	A04	A05	A08	A09	A09	A10	A11	A14	A14	A14	A14
	Size														
Genotype	860	1800	369	400	640	630	480	400	738	850	870	440	520	700	960
Padre	0	0	1	1	1	0	1	0	0	0	0	1	1	1	1
Sonora	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0
Thompson	1	1	1	1	0	0	0	0	1	1	1	1	0	1	1
Peerless	0	1	1	1	0	0	0	0	1	1	1	1	0	1	1
Ne Plus Ultra	0	1	1	1	0	0	0	0	1	1	1	1	0	1	0
Carmel	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1
Nonpareil	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1
McEn Nonpareil	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1
BF Nonpareil	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1
Tardy Nonpareil	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1
Weststeyn	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1
Mission	1	0	1	1	0	0	0	0	0	1	1	1	0	1	0
Shaw Mission	1	0	1	1	0	0	0	0	0	1	1	1	0	1	0
Trusito	1	1	1	1	0	0	0	1	1	0	1	1	1	1	1
SB13, 25-75	0	0	0	1	0	0	0	1	1	0	1	1	1	1	1
SB6, 56-89	0	1	1	1	1	0	1	1	0	0	0	1	1	1	0
UCD,8011-22	0	1	1	1	1	0	1	0	1	0	0	1	0	1	0
Nemared	0	0	1	0	1	1	0	1	1	0	1	0	0	0	1

origins for the almonds tested: bud-sport mutations; progeny from the interbreeding of early California cultivars; and progeny from outcrosses to non-Californian material.

Mutations. No differences in RAPD patterns were found within either the 'Mission' mutation group ('Mission' and 'Shaw Mission'), or the 'Nonpareil' group ('Nonpareil', 'McKenespy Nonpareil', 'BF-Nonpareil', 'Tardy Nonpareil', and 'Weststeyn'). A clear difference in 18 of the 37 markers was observed between these two mutation groups. The Shaw 'Mission' differs from 'Mission' by its expression of a distinctive die-back or failure of terminal buds during the initiation of winter dormancy (Kester and Gradziel, 1996, Micke, 1996). This bud failure (BF) trait also distinguishes the McKenespy 'Nonpareil' (no BF expression) and 'BF-Nonpareil' (high BF expression) from standard 'Nonpareil' (variable expression). BF has been shown to be genetically controlled and heritable (Kester and Gradziel, 1996). The Tardy Nonpareil and Weststeyn sports have a nut morphology similar to 'Nonpareil' but flower approximately 10 days later and differ also in tree structure and productivity. The failure to discriminate among these almond clones suggests the mutations are localized and so discernible only in highly saturated genetic maps.

Progeny from interbreeding of early California cultivars. 'Carmel', which has been reported to be a bud sport or mutation of 'Nonpareil' (Gradziel, 1997), has RAPD patterns more consistent with it's origin as a progeny of 'Nonpareil' and 'Mission'. Early California almond cultivars were commonly grafted onto almond seedling rootstocks, with 'Nonpareil' pollinated by 'Mission' being a common seed source. Shoot growth from the seedling rootstock sometimes produced limbs and nuts of good horticultural type. Many current California cultivars are believed to have originated in this manner (Kester et al., 1994) and so it is probable that some novel phenotypes were mistakenly identified as bud-mutations or sports of the budded cultivar. A 'Nonpareil' x 'Mission' origin of 'Carmel' is further supported by earlier isozyme (Hauagge et al., 1987) and S-allele inheritance studies (Kester et al., 1994). RAPD profiles also allow placement of 'Peerless' and 'Thompson' in the 'Nonpareil' x 'Mission' group yet allow their differentiation from each other and from 'Carmel'. 'Ne Plus Ultra', 'Padre' and 'Sonora' have similar RAPD band distribution patterns yet can be differentiated from this group by unique banding patterns such as those at $C05_{-740}$, $A14_{-520}$, and $C11_{-984}$, respectively. Somewhat surprisingly, recent self-incompatibility (S) allele analysis (Kester et al., 1994) indicates that 'Mission' and 'Ne Plus Ultra' have the S_5 self-incompatibility allele in common despite their reported origins in different times and places. The S-locus, which controls the gametophytic self incompatibility response has been reported to be highly polymorphic in nature representing a high multiple allelic series (de Nettancourt, 1977). A common allele for 'Ne Plus Ultra' and 'Mission' thus suggests the possibility of consanguinity for these cultivars and so an increased risk of inbreeding in current California cultivars.

Historical reports suggest that 'Mission' was a seedling of an early American cultivar known as 'Languedoc'. The seed from which 'Ne Plus Ultra', 'Nonpareil' and the other Hatch cultivars were selected has independently been reported to have probably originated from the Languedoc region of France (Kester et al., 1991). Thus the soft-shelled characteristic for which this region was known may have encouraged independent selection from within the same initial germplasm. 'Ne Plus Ultra' has a similarity index of 0.789 with 'Mission' as compared to 0.666 between 'Ne Plus Ultra' and 'Nonpareil' and 0.555 between 'Nonpareil' and 'Mission' (Table 3). The presence of the S_5 incompatibility allele in California cultivars, such as 'Thompson' had previously been interpreted as having 'Mission' as a probable parent. The identification of S_5 in 'Ne Plus Ultra' indicates that some of these cultivars may in effect be sib-mating between 'Nonpareil' and 'Ne Plus Ultra', thus further increasing the risk of inbreeding depression, as well as the probability of passing on the bud-failure and plum rootstock graft-incompatibility traits associated with the original Hatch cultivars. While the similarity index (Table 3) and resultant dendrogram (Fig. 2) show roughly equal similarities between Thompson and 'Mission' and 'Ne Plus Ultra' (0.809 and 0.789,

Primer																					
A14	A18	A19	A20	B01	B02	B04	B05	B05	B10	B10	B10	B10	B13	C05	C05	C06	C06	C06	C10	C11	C11
Size																					
1290	970	1230	1900	680	450	1230	1476	1960	680	860	1400	1900	500	740	1500	260	320	680	500	984	1500
0	1	0	0	0	0	1	1	0	1	1	0	1	0	1	1	0	0	0	1	0	0
1	1	0	1	1	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1	1	1
1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	0	0
1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1
1	1	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	1	1	0	1
0	1	1	0	0	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	0
0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0
0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0
0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0
0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0
0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0
1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	0	1
1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	0	1
0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	1	0	0	0
0	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	1	0	1	1	0	0
1	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	0	0	0	0	0	1
0	0	0	0	0	0	1	1	1	0	1	0	1	0	1	0	0	0	1	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0

Table 5. Possible origins for almond cultivars based on RAPD analysis.

Cultivar	Thompson	Carmel	Padre	Sonora	Trusito
Nonpareil x Mission	Yes	Yes	Yes	No	No
Nonpareil x Ne Plus Ultra	No	No	No	No	No
Nonpareil x Peerless	No	No	No	No	No
Mission x Ne Plus Ultra	No	No	No	No	No
Mission x Peerless	Yes	No	No	No	No
Ne Plus Ultra x Peerless	No	No	No	No	No

respectively), an examination of individual RAPD bands amplified by different primers provides additional useful information. The primers A01(860), A20(1900) and B01(680) amplified a band in Thompson, but failed to amplify the same band in 'Ne Plus Ultra' and 'Nonpareil'. This observation virtually rules out 'Ne Plus Ultra' as a possible parent with 'Nonpareil' to produce Thompson. The same RAPD bands support the possible parentage of either a 'Nonpareil' by 'Mission' cross or 'Mission' by Peerless cross for the origin of Thompson. Similarly the RAPD analysis also supports a parentage of 'Nonpareil' x 'Mission' for the origin of 'Carmel' and 'Padre' varieties. (Tables 4 and 5).

Progeny from outcrosses to non-californian material. The remaining genotypes, 'Sonora', 'Padre', Trusito, UCD,8011-22, SB13,25-75, and SB6,56-89 demonstrate unique banding patterns which distinguish them from the early California cultivar group. The frequency of unique bands is also in general agreement with their putative genetic origin (Tables 1, 4, and 5). Thus, 'Sonora' and 'Padre', which are the product of controlled outbreeding of 'Nonpareil' and 'Mission', show distinct banding patterns from the 'Nonpareil' x 'Mission' gene pool. Trusito, an old, self-compatible Italian selection can be clearly distinguished from the 'Nonpareil' x 'Mission' gene pool by uniqueness at 4 of the 37 bands evaluated. Trusito, as well as the other self-compatible selections UCD,8011-22, SB13,25-75, and SB6,56-89, can be clearly differentiated from all cultivars tested by unique RAPD bands. Examples include $C06_{-320}$ for Trusito and $A04_{-369}$ for SB13,25-75. The peach outgroup Nemared shows unique bands for 6 of the 37 RAPD bands evaluated. Parentage proposed by this analysis (Table 5) are consistent with previous isozyme (Hauagge et al. , 1987; Arus et al. 1994) and S-allele studies (Kester et al. 1994), and where available, breeding records (Gradziel, 1997).

The results of this study clearly demonstrate that RAPD markers are suitable for the detection of genetic variation in almond. Results also support previous observations that obligate outcrossing in almonds results in maintenance of the genetic heterozygosity in individual gene loci. The limited number of isozyme loci that were tested in earlier studies revealed similar results (Arulsekar et al., 1986; Hauagge et al., 1987). In a recent study of a wild sweet cherry (*P. avium*) species which is also an obligate outcrosser, Mariette et al. (1997) found that the colonizing process of a founder population did not result in the reduction of heterozygosity as revealed by isozyme markers. The maintenance of heterozygosity was attributed to the outcrossing nature of the species. Thus, although the small number of genotypes tested does not represent the total diversity in almond, considerable diversity is shown to be present, particularly when efforts are made to introduce outside germplasm.

The dendrogram constructed on the basis of shared fragments shows good agreement with known pedigree history, though a few genotypes did not reflect expected affinities. These findings may reflect the high heterozygosity within a narrow genetic base. Furthermore, dominance of the RAPD marker might mask heterozygotes resulting in misinterpretation of relatedness.

Despite similar tree and fruit morphologies, the RAPD profiles used in this study were capable of discriminating all genotypes except those originating as bud-sport mutations. Additional primers should provide even greater understanding of the genetic relatedness of almond cultivars.

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