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European and Asian Pears: Simple Sequence Repeat–Polyacrylamide Gel Electrophoresis-based Analysis of Commercially Important North American Cultivars

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Abstract. The genus *Pyrus* (pear) includes species and cultivars of great diversity. We have tested the feasibility of a polyacrylamide gel eletrophoresis (PAGE)-based +/– simple sequence repeat (SSR) screen as a means of defining relationships amongst pears of commercial importance in North America. The screen included 28 pear accessions, including economically important cultivars, numbered selections from breeding programs and interspecific hybrids. It relied on 18 SSR primer pairs, each of which produced polymorphic banding patterns in all the genotypes examined. Fragments were scored for presence or absence within genotypes. The results show that amplification and analysis of a small number of SSR loci enable identification of cultivars and reasonable definition of genetic relationships in North American pears. Seven primer pairs were sufficient to distinguish the 28 pear cultivars. Analyses using both distance and parsimony criteria grouped cultivars in a manner consistent with known pedigrees and sites of origin.

The genus *Pyrus,* containing >22 species, is a highly diverse source of pome fruit cultivated throughout the temperate world. Cultivars and rootstocks used for commercial production are maintained true-to-type through vegetative propagation. In nature, however, pear is an out-crossing perennial, leading to high levels of heterozygosity and allelic diversity within the genus. *Pyrus communis* L., the common (European) pear, encompasses >5000 cultivars (Monte-Corvo et al., 2001), only a small percentage of which are cultivated commercially (Bell et al., 1996). Members of this species are morphologically distinguishable from the major Asian cultivated species, *P. pyrifolia* (Burm.) Nak. [syn. *P. serotina* (Rehd.)].

Early efforts to identify cultivars by means of phenotypic data (Kikuchi 1948; Shen 1980; Westwood 1982) proved useful for a limited number of cultivars in certain conditions. However, the phenotypic variability seen amongst accessions of tree fruit grown in different areas with slightly different environments and production practices demonstrates a number of problems with that approach (Kresovich and McFerson 1992, Hokanson et al., 1998). Isozyme markers have also been used for analyses of genetic relatedness. They tend to detect a relatively low level of polymorphism and may depend on the physiology of the plant at the time of analysis (Arulsekar et al., 1986; Chevreau et al., 1997; Chung and Ko 1995; Messeguer et al., 1987). Since the early 1990s, molecular (DNA) markers have become popular tools for investigating the origins and extent of genetic diversity within a population.

For pears, DNA-based markers are particularly useful for germplasm identification, diversity analysis and verification of rootstock identity. DNA analyses, including the use randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers, have previously been used for molecular fingerprinting in pear (Botta et al., 1998; Monte-Corvo et al., 2001; Oliveira et al., 1999; Yamamoto et al., 2001, 2002a). Relationships among cultivars of Japanese (Iketani et al., 2001, Yamamoto et al., 2001, 2002a, 2002b, 2002c) and European (Botta et al., 1998; Oliveira et al., 1999; Monte-Corvo et al., 2001) pears have been investigated, but little information is available on the genetic relationships amongst cultivars of commercial importance in North America, both those developed there and those introduced from Europe and Asia.

The present study evaluates the genetic diversity and relationships of 28 cultivars across 4 species of the genus *Pyrus* including 2 interspecific hybrids. This study focused on cultivars originating in North America, Asia, and Europe, and currently grown in North America either commercially or for breeding purposes. At the same time, the method itself was evaluated by including some recently-introduced cultivars with well-documented pedigrees, to test the concordance of known relationships with inferred relationships.

Amplification and analysis of a small number of SSR loci permitted identification of cultivars and reasonable definition of genetic relationships in North American pears. A small set of SSR markers which uniquely identify each cultivar have been identified. Genetic relationships predicted by SSR patterns are largely congruent with expectations from geographic origin and available pedigree information. Cultivars generated in Ontario grouped together (e.g., 'AC Harrow Gold', 'AC Harrow Crisp', 'Harvest Queen', and 'Harrow Delight'), as did selections derived from shared interspecific parents (NY10352 and NY10353). Japanese cultivars grown in North America were genetically distinct from *P. communis* and other *Pyrus* species.

Materials and Methods

In total, 28 pear cultivars were selected from a collection of >200 maintained in the germplasm collections of Agriculture and Agri-Food Canada and the University of Guelph at Vineland Station in southern Ontario (latitude 41° 10–12'N, longitude 79° 21–24'W). They were chosen primarily for their commercial or breeding importance; a few cultivars of well-documented origins also provided a means of assessing the experimental approach. The origins and Latin names of each of the cultivars are presented in Table 1.

Based on initial comparisons of DNA extracted from young and mature leaves, newly expanded young leaves were collected shortly after bud break in the early spring, frozen in liquid nitrogen and stored at –80 °C until DNA was isolated. Genomic DNA was extracted using a DNeasy Plant Maxi Kit (Qiagen Inc., Mississauga, Ont.). About 100 mg of frozen leaf tissue was homogenized in liquid nitrogen before addition of extraction buffer. A minimum of two extractions was performed for each cultivar. Concentrations of DNA in the extracts were determined by A_{260} absorption or A_{260}/A_{280} ratios, and the quality confirmed by electrophoresis of samples alongside known

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Table 1. List of pear cultivars used in this study, with available information regarding pedigrees and geographic origin.

No.	Cultivars	Scientific name	Pedigree/known background	Origin
1	Nijisseiki	*Pyrus pyrifolia* Burm.	Chance seedling	Japan
2	Hosui	*Pyrus pyrifolia* Burm.	(Kikusui × Yakumo) × Yakumo	Japan
3	Kosui	*Pyrus pyrifolia* Burm	Kikusui × Wase Kozo	Japan
4	Niitaka	*Pyrus pyrifolia* Burm	Amanogawa × Imamura-Aki	Japan
5	Shinko	*Pyrus pyrifolia* Burm	Seedling of cultivar Nijisseiki	Japan
6	Anjou	*Pyrus communis* L.	Europe (France)	
7	Bartlett	*Pyrus communis* L.	Chance seedling	Europe (England)
8	Bosc	*Pyrus communis* L.	Europe (Belgium)	
9	Clapp Favorite	*Pyrus communis* L.	Flemish Beauty × Bartlett	Europe
10	Flemish Beauty	*Pyrus communis* L.	Chance seedling	Europe (Belgium)
11	Magness	*Pyrus communis* L.	Seckel seedling × Comice	U.S. (Md.)
12	Moonglow	*Pyrus communis* L.	U.S. (Mich.) 437 × RCW	U.S. (Md.)
13	Obican Vodenac	*Pyrus communis* L.	East Europe (Yugoslavia)	
14	Timpurii de Dimbovita	*Pyrus communis* L.	East Europe	
15	Harrow Delight	*Pyrus communis* L.	Bartlett × Purdue 80-51[z]	Canada (Ont.)
16	Harvest Queen	*Pyrus communis* L.	Bartlett × Michigan 572[z]	Canada (Ont.)
17	AC Harrow Crisp	*Pyrus communis* L.	Bartlett × US 56112-146[y]	Canada (Ont.)
18	AC Harrow Gold	*Pyrus communis* L.	Harrow Delight × Harvest Queen[x]	Canada (Ont.)
19	NY10352	*Pyrus* hybrid	*P. communis* × (*P. communis* × *P. ussuriensis)*	U.S. (N.Y.)
20	NY 10353	*Pyrus* hybrid	*P. communis* × (*P. communis* × *P. ussuriensis)*	U.S. (N.Y.)
21	Kieffer	*Pyrus* hybrid	*P. pyrifolia* × *P. communis*	U.S. (Pa.)
22	OHF 69	*Pyrus communis* L.	Old Home × Farmingdale	North America
23	OHF 87	*Pyrus communis* L.	Old Home × Farmingdale	North America
24	OHF 333	*Pyrus communis* L.	Old Home × Farmingdale	North America
25	Winter Nelis	*Pyrus communis* L.	Europe (Belgium)	
26	Catillac	*Pyrus communis* L	Europe (France)	
27	PI 312151	*Pyrus ussuriensis* M.	Asia (Uzbekistan)	
28	*Pyrus fauriei*	*Pyrus fauriei* Schneid.	Korea	

[z]Quamme and Spearman, 1983.
[y]Hunter et al., 2002a.
[x]Hunter et al., 2002b.

quantities of standards. The samples were diluted to 5 ng·µL^{-1} with TE buffer (10 mM Tris, 1mM EDTA pH 8.0) and kept at –20 °C for subsequent PCR amplification.

In total, 26 SSR primer pairs from apple (Gianfranscheschi et al., 1998; Guilford et al., 1997), peach (Sosinski et al., 2000), and pear (Yamamoto et al., 2002a, 2002b, 2002c) were tested, using five cultivars randomly selected from those under study. Based on the reproducibility of polymorphic patterns obtained with these cultivars, 18 primer pairs were selected for analysis of the 28 selected genotypes. The names and sources of the primers, along with annealing temperatures, are presented in Table 2.

PCR reactions were performed in a Techne Flexigene DNA thermal cycler (Techne Inc., Minneapolis, Minn.). Reaction mixtures contained 10 ng of genomic DNA, 10 µL Taq PCR Master Mix (Qiagen Inc.), and 50 pmol of each primer brought to a total volume of 20 µL with nuclease-free distilled water. Amplifications were carried out for 35 to 40 cycles, depending on the primer pair. In general, the amplification protocol consisted of initial denaturation for 2 min at 94 °C followed with 1 min at 94 °C, 1 min at the appropriate annealing temperature (see Table 2), and 2 min at 72 °C, with a final elongation step of 7 min at 72 °C. Three primer sets (CH01F02, CH02B10, and KA4b) required touchdown protocols (Mellersh and Sampson, 1993) for optimal amplifications. In those cases, the annealing temperature was reduced by 0.5 or 1.0 °C per cycle for the initial 6 or 8 cycles, followed with 35 to 40 amplification cycles at the target annealing temperature. The amplified products were separated by vertical polyacrylamide gel eletrophoresis (PAGE) in a 22 cm tall gel using 10% polyacrylamide (acrylamide: bis = 29:1). The gels were stained with ethidium bromide (1 µg·µL^{-1}) for 15 min followed with 20 min of destaining in water. To ensure reproducibility, all PCR reactions were conducted at least twice using DNA samples from different extractions.

The lengths of all amplified fragments obtained with 18 primer pairs and 28 cultivars

Table 2. Primer sets used in this study.

Primer	Source	Annealing temp (°C)	Fragments[z] (no.)	Fragment size (bp)
CH01F02	Gianfranceschi et al., 1998	64 ~ 58 (–1 °C/cycle)	49	116–399
CH02B10	Gianfranceschi et al., 1998	64 ~ 58 (–1 °C/cycle)	50	117–368
KA4b	Yamamoto et al., 2002a	58 ~ 54 (–0.5 °C/cycle)	34	77–439
NB105a	Yamamoto et al., 2002b	47	52	128–482
NB109a	Yamamoto et al., 2002b	55	34	140–375
NH001c	Yamamoto et al., 2002c	48	49	103–403
NH006b-1	Yamamoto, personal communication	50	54	101–473
NH010a	Yamamoto, personal communication	49	37	107–441
NH013a	Yamamoto et al., 2002c	55	45	149–486
NH021a	Yamamoto et al., 2002b	55	55	134–397
NH025a	Yamamoto et al., 2002b	51	33	78–387
NH027a	Yamamoto et al., 2002b	47	54	118–463
NH029a	Yamamoto et al., 2002b	55	42	87–382
NZ02b1	Guilford et al., 1997	50	46	160–564
NZ05g8	Guilford et al., 1997	50	22	102–345
NZ28f4	Guilford et al., 1997	52	32	98–253
PS12A02	Sosinski et al., 2000	52	24	162–380
RLG1-1	Yamamoto et al., 2002b	49	51	143–500

[z]Number of different fragments produced amongst all pear genotypes.

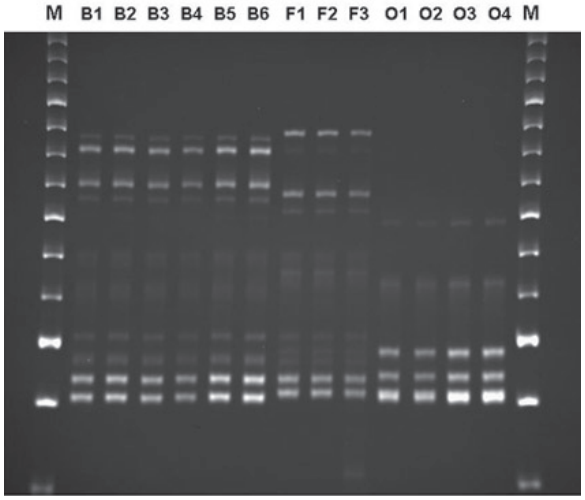

Fig. 1. Polyacrylamide gel containing ethidium bromide-stained simple sequence repeat (SSR) fragments obtained from multiple accessions of 'Bartlett' (B1-B6), 'Flemish Beauty' (F1-F3), and 'Old Home' (O1-O4) with primer pair NZ05g8, demonstrating the absence of detectable intracultivar polymorphisms.

were calculated using Geldoc software (Perkin Alpha Innoteck Corp., San Leandro, Calif.), then verified visually for accuracy, comparing every fragment against standard 25 bp molecular size markers (Invitrogen Canada Inc., Burlington, Ont.).

All unambiguous fragments were scored as either present (1) or absent (0) for each genotype in the data table. A matrix of distances (D) was constructed, using the percentage of fragments that differed between each genotype:

$$D = \left[1 - \left(\frac{fragments\ shared}{fragments\ scored}\right)\right]$$

Based on the distance measures, the Fitch package in PHYLIP (Felsenstein, 2000) was used to generate trees, which were evaluated using the Fitch-Margoliash algorithm (Fitch and Margoliash, 1967) implemented in PHYLIP. The optimal tree was selected from 10 runs. Each run used the default parameters and a different order of cultivars in constructing trees. A second tree was estimated by maximum parsimony criteria, implemented with the MIX program of PHYLIP using default settings. All fragments were considered characters of equal weight. Support for clades within the parsimony tree was estimated with bootstrap resampling (1000 permutations).

Results and Discussion

While extractions from leaves harvested in the spring yielded high-quality DNA, older toughened leaves did not provide DNA suitable for amplification. All primer pair/sample combinations produced identical fragments in two or more amplifications. The electrophoretic system could generally resolve 100 to 300 bp fragments differing by 2 bp, as determined from standards of known lengths (data not shown). Eighteen primer pairs which produced useful polymorphic fragments within the sample collection were used in the study (Table 2).

To test for potential within-cultivar variations, DNA from cultivars for which multiple accessions were available were examined using the primer pair NZ05g8 (Guilford et al., 1997). There were no within-cultivar differences seen among six accessions of 'Bartlett', three accessions of 'Flemish Beauty', and four accessions of 'Old Home' (Fig. 1). A comparison of fragments for the one known set of progeny (Clapp Favorite) and parents (Flemish Beauty and Bartlett) demonstrated that all major fragments in the progeny cultivar were detectable in one or both parents (data not shown).

Although information on expected product sizes was not available for most combinations of primer pair and cultivar, six such fragment sizes were available. In all but one of the six cases the expected products were seen: CH01F02 (Gianfranceschi et al., 1998) amplified fragments of 163 and 176 bp from 'Bartlett' and 165 bp from 'Hosui'; K4Ab (Yamamoto et al., 2002a) amplified fragments of 95 and 107 bp from 'Hosui', 97 bp from 'Bartlett', and 97 and 107 bp from 'Niitaka'. The exception was the combination of K4Ab with 'Winter Nelis', which failed to produce the expected fragment of 137 bp (Yamamoto et al., 2002a).

The amplification products of the NZ05g8 primer pair for all 28 cultivars (Fig. 2) illustrates the variability found in amplified PCR fragments. Overall, scorable fragment lengths ranged from 77 to 500 bp. Some primer pairs generated high molecular weight fragments (>500 bp), which were difficult to score and compare accurately; these were not included in the analysis. For a single individual the number of scorable fragments obtained with a single set of primers ranged from one to 16. Microsatellite amplifications generated nearly 800 different readable fragments with an average of 42 fragments per marker.

The 18 SSR primer pairs provided sufficient information to distinguish all 28 cultivars from one another (Table 3). Only one 177 bp fragment, detected by the RLG1-1 primer pair, was shared by all cultivars under study. A 108 bp fragment obtained with the NH010a marker was found in all the cultivars except 'Kieffer', 'Catillac' and the rootstock selection OHF 69. The primer pair CH01F02, distinguishing only three cultivars, offered the least information, while NH006b1 produced unique identifying markers for nine cultivars within our sample. A total of seven primer pairs (NB105a, NB109a, NH001c, NH006b1, NH013a, NZ02b1, and PS12A02) was sufficient to effectively differentiate all 28 cultivars. These SSR primer pairs are therefore potentially powerful tools for cultivar identification as well as breeding and genetic studies of pear.

In our analysis, we can distinguish neither allelic pairs nor individual loci; each fragment is counted as a character of the cultivar from which it was amplified. As a result, an SSR primer pair with a large number of fragments has a greater weight in cultivar classification than an SSR primer pair with a small number of fragments. In addition, heterozygous loci counted as two separate characters. Many of the primer–cultivar combinations produced two fragments that were abundant and similar in size. A second parsimony tree was generated, based on these potentially allelic fragments. Bootstrap support for the clades generated in this way, however, were lower than for those obtained in the original analysis (data not shown). The decreased bootstrap support for groups based on this subset of the data argued against preselection of fragments when, as in the current case, fragments are not assignable to specific loci.

Relationships among the 28 pear cultivars were inferred using both distance (Fig. 3, Table 4) and parsimony (Fig. 4) criteria. Both analyses reveal a varying degree of genetic relatedness for cultivars belonging to different species and from different geographic origins. For the most part, cultivars are classified into clades as would be predicted from what is known about their genetic backgrounds.

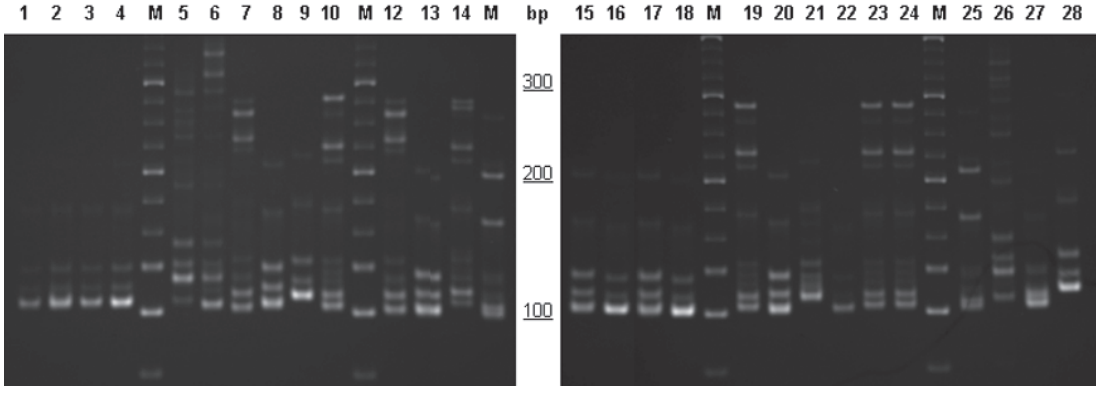

Fig. 2. The pattern of amplified simple sequence repeat (SSR) fragments obtained from pear cultivars under study using the primer pair NZ05g8, separated by polyacrylamide gel eletrophoresis (PAGE) and visualized by staining with ethidium bromide as described in the text. See Table 1 for the names of cultivars designated by numbers 1 to 28.

Table 3. Simple sequence repeat (SSR) fragments most useful for cultivar identification, designated by primer name and fragment size, separated by a hyphen.

Cultivar name	Identifying fragments (bp)	Cultivar name	Identifying fragments (bp)
Nijisseiki	NH006b1-198, NH021a-150, NH021a-313, NH027a-321	AC Harrow Crisp	NH001c-356, NH001c-391
Hosui	NH006b1-453, NH025a-382	AC Harrow Gold	NH001c-379, NH001c-399, NZ28f4-199
Kosui	NH001c-287	NY 10352	NB109a-320
Niitaka	NH006b1-292, NH021a-297, NH025a-387	NY 10353	NH001c-229, NH006b1-189, NH006b1-226, NH025a-84,
Shinko	CH02B10-299, NH001c-199, NH001c-215		NH025a-336
	NH021a-209, NH025a-182, RLG1-1-220	Kieffer	NB109a-155, NB109a-265, NZ05g8-180, PS12A02-245,
Anjou	CH01F02-387, KA4b-416, NH006b1-221, NH006b1-280,		PS12A02-295
	NH021a-204, NZ05g8-312, NZ05g8-345	OHF 69	NH027a-198, PS12A02-212
Bartlett	NH013a-192	OHF 87	NB105a-354, NH006b1-389, NH021a-231, NH021a-253
Bosc	NB105a-237, NH029a-292, NH029a-382	OHF 333	NB105a-482, NH027a-406, RLG1-1-485
Clapp Favorite	NB109a-230	Winter Nelis	CH02B10-333, KA4b-85, NZ28f4-211, NZ28f4-221,
Flemish Beauty	NH001c-221, NH001c-343		PS12A02-285
Magness	KA4b-160, NH013a-299, NH029a-279, NZ28f4-229	Catillac	CH01F02-292, NB109a-200, NB109a-265, NH006b1-156,
Moonglow	KA4b-81, KA4b-227, KA4b-305, KA4b-388, NH001c-403,		NH006b1-174, NH006b1-195, NH021a-246, NH027a-118,
	PS12A02-220		NH027a-224, NZ02b1-273, NZ05g8-150
Obican Vodenac	CH01F02-226, CH02B10-291, NH001c-204, NH013a-289,	PI 312151	NB109a-275, NH006b1-136, NH010a-145, NH010a-267,
	NH013a-406, NH021a-239, NH021a-287, NH029a-266,		NH013a-149, NH021a-278, NH027a-125, PS12A02-218,
	NH029a-373, NZ02b1-266, NZ02b1-466, NZ28f4-253		RLG1-1-360, RLG1-1-376
Timpurii de Dimbovita	CH02B10-167, CH02B10-338, CH02B10-356, KA4b-106,	*Pyrus fauriei*	KA4b-404, NH006b1-443, NH006b1-473, NH010a-217,
	NB105a-406		NH010a-261, NH021a-134, NH027a-399, NZ02b1-338,
Harrow Delight	NH013a-227, RLG1-1-170, RLG1-1-305		NZ05g8-175, PS12A02-320
Harvest Queen	NZ02b1-293, NZ02b1-333		

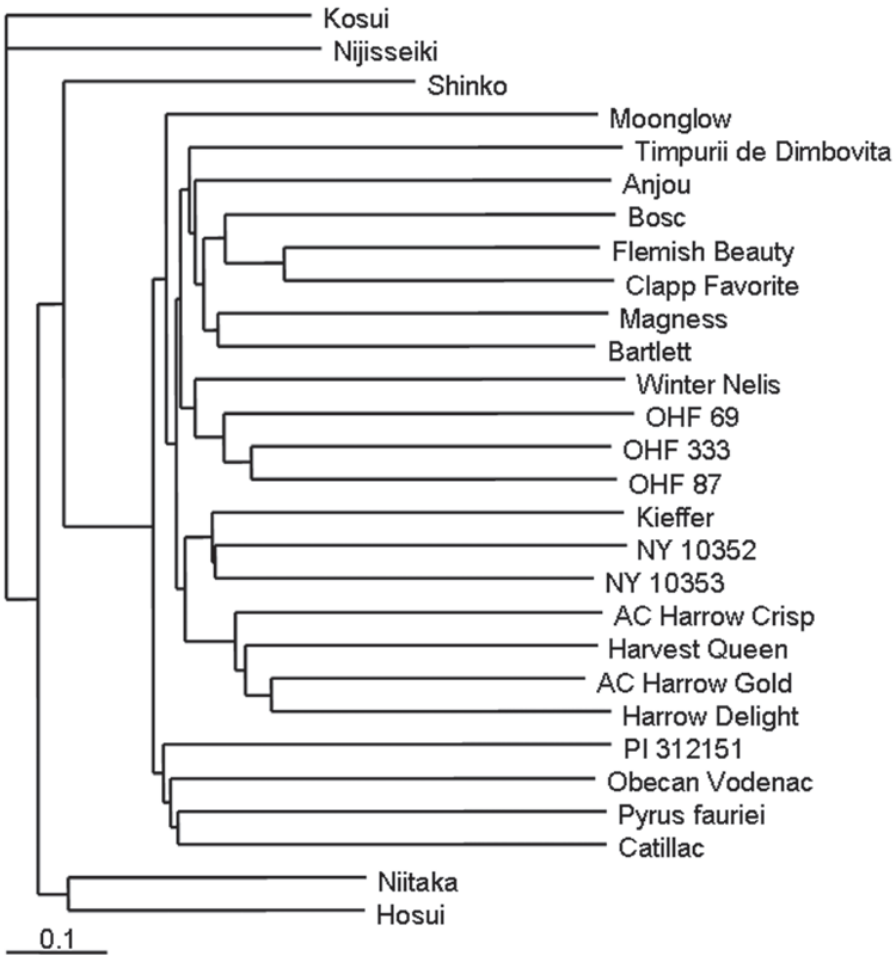

Fig. 3. Tree generated using the Fitch-Margoliash algorithm least squares criteria to identify an optimal tree. Distance is indicated by the horizontal bar at bottom left.

Four Canadian cultivars ('Harrow Delight', 'AC Harrow Crisp', 'AC Harrow Gold', and 'Harvest Queen') are clustered in the same clade with strong support, consistent with their known pedigrees (Hunter et al., 2002a, 2002b; Quamme and Spearman, 1983). The rootstock genotypes OHF 69, OHF 87, and OHF 333 also group together with moderate support. This result is consistent with their origins as seedling selections from a cross between 'Old Home' and 'Farmingdale' (Brooks, 1984).

Within the European (*P. communis*) pears, a number of anticipated subgroups are found in both trees (Figs. 3 and 4). The cultivars of western European origin ('Flemish Beauty', 'Clapp Favorite', 'Bosc', 'Anjou'. and 'Bartlett') belong to the same grouping, reflecting their close genetic interrelationships. The cultivar 'Magness' is part of the same grouping, as expected from its *P. communis* ancestors 'Seckel' and 'Comice'. The interspecific hybrids NY 10352 and NY 10353 were found in a closely related subgroup (bootstrap support 74%). Both NY selections, which originated from the cross *P. communis* × (*P. communis* × *P. ussuriensis* Maxim), are similar to 'Kieffer', an interspecific hybrid between *P.serotina* [sic] and putatively *P. communis* cultivar 'Bartlett' (Hedrick 1921).

Some predicted relationships were not supported by the trees. 'Catillac', a European cultivar, did not cluster with the other cultivated *P. communis* samples. Both 'Winter Nelis', originating from a *P. communis* seedling selection in Europe, and 'Moonglow', originating from a controlled cross between *P. communis* selections in North America, fail to cluster as might be expected from their *P. communis* backgrounds. Given the failure of the 'Winter Nelis' sample to produce the expected SSR fragment with primer set K4Ab, however, there is some doubt about the authenticity of the 'Winter Nelis' sample. Comparison of our sample with that from a specimen tree in the pear repository should clarify this issue. One subgroup contains two species [*P. fauriei*, PI 312151 (*P. ussuriensis*)] as well as the *P. communis* cultivar 'Catillac'. However, bootstrap support for these classifications is weak.

There was a clear separation of the *P. pyrifolia* Japanese cultivars from European and North American accessions of *P. communis*. According to pedigree information obtained from the Germplasm Resources Information Network (GRIN) of the United States Agriculture Research Service/NCGR-Corvallis *Pyrus* Catalog (www.ars-grin.gov/cor/catalogs/pyrcult), 'Hosui' and 'Kosui' originated from the same maternal parent 'Kikusui' (Table 1). With our SSR data, 'Hosui' groups close to 'Niitaka', although no pedigree relationship is known.

In summary, this study reports reproducible SSR data for 28 cultivars of the genus *Pyrus*

Table 4. Distance matrix of *Pyrus* species and cultivars (indicated by numbers on the first row and column 1, see Table 1 for cultivar names).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
2	0.59	---																									
3	0.55	0.57	---																								
4	0.64	0.55	0.58	---																							
5	0.77	0.67	0.70	0.62	---																						
6	0.89	0.83	0.89	0.83	0.81	---																					
7	0.87	0.87	0.85	0.87	0.82	0.77	---																				
8	0.88	0.87	0.89	0.87	0.87	0.73	0.74	---																			
9	0.90	0.87	0.86	0.92	0.89	0.85	0.74	0.78	---																		
10	0.91	0.86	0.89	0.91	0.88	0.78	0.73	0.68	0.66	---																	
11	0.91	0.90	0.91	0.90	0.89	0.76	0.72	0.76	0.77	0.76	---																
12	0.90	0.88	0.90	0.84	0.84	0.80	0.85	0.82	0.88	0.81	0.77	---															
13	0.87	0.85	0.87	0.86	0.84	0.80	0.84	0.83	0.89	0.81	0.78	0.80	---														
14	0.91	0.87	0.91	0.88	0.91	0.84	0.82	0.82	0.84	0.80	0.76	0.80	0.82	---													
15	0.86	0.85	0.86	0.87	0.85	0.82	0.76	0.80	0.81	0.83	0.81	0.81	0.83	0.85	---												
16	0.89	0.89	0.90	0.88	0.86	0.80	0.74	0.76	0.85	0.77	0.79	0.78	0.86	0.80	0.71	---											
17	0.89	0.92	0.88	0.91	0.88	0.83	0.74	0.81	0.84	0.80	0.82	0.81	0.84	0.86	0.72	0.66	---										
18	0.89	0.88	0.89	0.89	0.85	0.81	0.77	0.80	0.84	0.82	0.77	0.77	0.86	0.79	0.68	0.61	0.64	---									
19	0.91	0.93	0.92	0.91	0.90	0.85	0.81	0.79	0.84	0.74	0.84	0.82	0.84	0.84	0.83	0.79	0.79	0.80	---								
20	0.88	0.87	0.90	0.88	0.86	0.81	0.77	0.81	0.83	0.77	0.84	0.83	0.84	0.79	0.80	0.75	0.74	0.73	0.69	---							
21	0.90	0.90	0.88	0.90	0.85	0.88	0.82	0.90	0.87	0.84	0.88	0.88	0.85	0.87	0.86	0.87	0.82	0.83	0.77	0.76	---						
22	0.90	0.92	0.89	0.90	0.90	0.91	0.86	0.89	0.87	0.85	0.87	0.88	0.89	0.89	0.91	0.89	0.87	0.90	0.88	0.87	0.85	---					
23	0.90	0.89	0.90	0.90	0.88	0.86	0.80	0.83	0.83	0.78	0.85	0.86	0.86	0.86	0.85	0.82	0.81	0.81	0.77	0.81	0.83	0.79	---				
24	0.91	0.90	0.89	0.90	0.89	0.80	0.81	0.82	0.83	0.82	0.82	0.87	0.87	0.88	0.80	0.80	0.72	0.77	0.81	0.79	0.82	0.77	0.67	---			
25	0.89	0.86	0.88	0.87	0.91	0.80	0.86	0.85	0.84	0.86	0.82	0.82	0.84	0.88	0.84	0.87	0.85	0.88	0.90	0.90	0.88	0.88	0.83	0.77	---		
26	0.86	0.86	0.88	0.90	0.88	0.93	0.91	0.93	0.89	0.90	0.90	0.89	0.87	0.94	0.91	0.89	0.89	0.90	0.92	0.88	0.85	0.89	0.90	0.88	0.88	---	
27	0.89	0.90	0.90	0.91	0.87	0.89	0.88	0.89	0.89	0.86	0.88	0.89	0.82	0.90	0.86	0.90	0.86	0.88	0.88	0.86	0.91	0.90	0.86	0.87	0.86	0.87	---
28	0.87	0.84	0.82	0.82	0.84	0.87	0.91	0.89	0.90	0.88	0.88	0.89	0.84	0.91	0.87	0.88	0.89	0.89	0.91	0.88	0.87	0.89	0.89	0.86	0.88	0.84	0.84

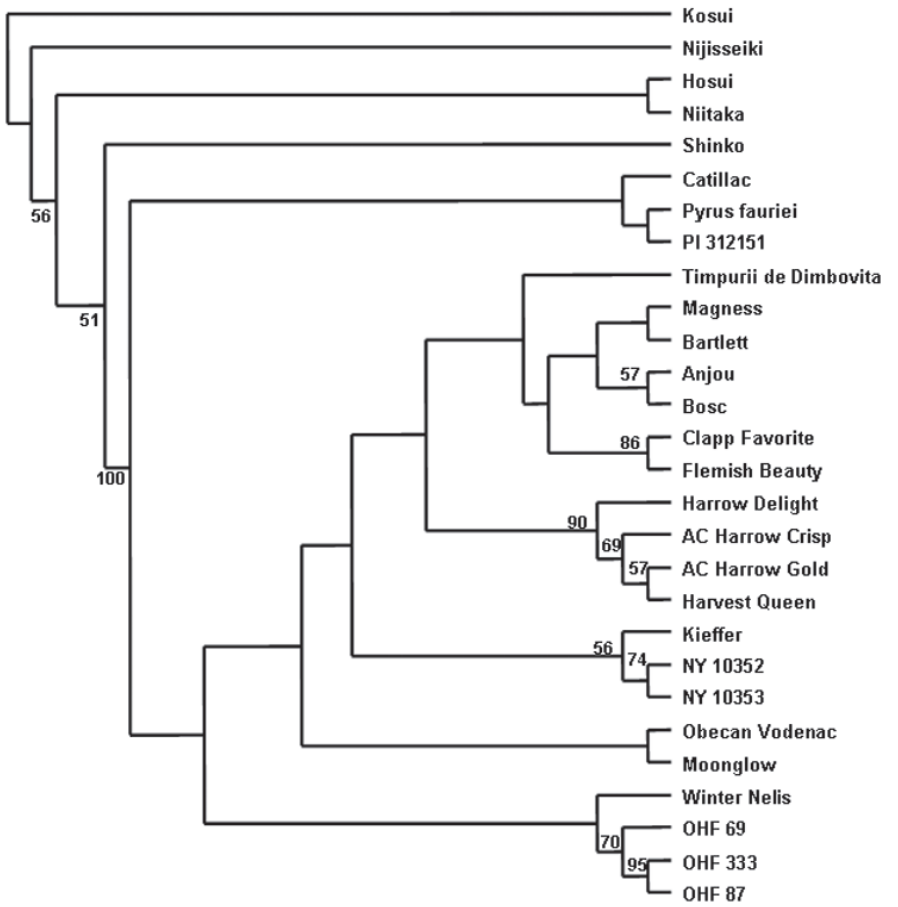

Fig. 4. Parsimony tree based on shared simple sequence repeat (SSR) fragments. Nodes found in >50% of the trees generated from 1000 permuted data sets are indicated by numbers representing the bootstrap support value.

grown in North America. A small number of SSR markers uniquely identified every cultivar within our sample. Athough the statistical support for many clades is weak, the two trees generated by different methods are remarkably consistent. With a few exceptions cultivar relationships based on this method are confirmed by pedigree records. As these 28 accessions are considered important cultivars in pear-growing areas, the data generated in this study enable verification of, and evaluation of genetic distance between, cultivars for use in pear improvement programs.

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