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Diversity of Wild *Pyrus communis* Based on Microsatellite Analyses

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Abstract. **Edible european pears (*Pyrus communis* L. ssp. *communis*) are derived from wild relatives native to the Caucasus Mountain region and eastern Europe. Microsatellite markers (13 loci) were used to determine the relationships among 145 wild and cultivated individuals of *P. communis* maintained in the National Plant Germplasm System (NPGS). A Bayesian clustering method grouped the individual pear genotypes into 12 clusters. *Pyrus communis* ssp. *caucasica* (Fed.) Browicz, native to the Caucasus Mountains of Russia, Crimea, and Armenia, can be genetically differentiated from *P. communis* ssp. *pyraster* L. native to eastern European countries. The domesticated pears cluster closely together and are most closely related to a group of genotypes that are intermediate to the *P. communis* ssp. *pyraster* and the *P. communis* ssp. *caucasica* groups. Based on the high number of unique alleles and heterozygosity in each of the 12 clusters, we conclude that genetic diversity of wild *P. communis* is not fully represented at the NPGS. Additional diversity may be present in seed accessions stored in the NPGS and more pear diversity could be captured through supplementary collection trips to eastern Europe, the Caucasus Mountains, and the surrounding countries.**

Edible european pears were selected and bred from wild *P. communis* trees with small, nearly round, hard, gritty, sour, and astringent fruit (Hedrick, 1921). Large and medium-fruited edible pears were cultivated by Greeks and Romans as long as 2500 years ago and French monks and German botanists maintained ancient cultivars until the 16th and 17th centuries. Most modern cultivars originated from breeding efforts in Belgium and England in the 1700s (Hedrick, 1921; Lombard et al., 1980).

In the 1930s, Nicolai Vavilov recognized that Asia Minor (Trans-Caucasia, Iran, and Turkmenistan) represented a center of diversity for wild *P. communis* (Vavilov, 1994). The Caucasus Mountains provide diverse habitats that support highly variable germplasm (Vavilov, 1994). Over the past 50 years, seeds from wild *P. communis* trees were collected from natural or naturalized stands in the Caucasus Mountains, Crimea (Ukraine), Armenia, Turkey, the Balkans, and other European countries. While these individuals tend to have unacceptable fruiting qualities, they may provide valuable genetic diversity for the breeding of disease resistance to fire blight (*Erwinia amylovora* Burrill), pear psylla (*Cacopsylla pyricola* Foerster), and wooly pear aphid (*Eriosoma pyricola* Bak. and David.). Resistance to diseases and pests is a priority in pear breeding programs (Bell, 1982, 1992; van der Zwet et al., 1983; Westwood and Westigard, 1969). Multiple subspecies designations have been described for types of *P. communis*. Domesticated cultivars of *P. communis* ssp. *communis* have hybrid ancestry with wild *P. communis* subspecies and *P. nivalis* Jacquin, the snow pear. Also, *P. communis* ssp. *pyraster* and *P. communis* ssp. *caucasica* are thought to be most likely ancestors of the cultivated european pear (Challice and Westwood, 1973).

Phenotypically, *P. communis* ssp. *pyraster* and *P. communis* ssp. *caucasica* are similar (Aldasoro et al., 1996). These subspecies have been largely classified according to their geographical distribution. *P. communis* ssp. *pyraster* originates from areas west and south of the Black Sea such as the Balkan countries, Turkey and other European countries while wild forms of *P. communis* from areas around and east of the Caucasus Mountains (southwestern Russia, Crimea, Georgia, Armenia, and Azerbaijan) are classified as *P. communis* ssp. *caucasica* (Fig. 1). *P. nivalis* originates from Europe and Asia Minor. Selections and hybrids of *P. nivalis* species have been grown for hundreds of years throughout western Europe and especially in France and England for perry production (Challice and Westwood, 1973). Taxonomist Alfred Rehder considered *P. korshinskyi* Litv. (synonym *P. bucharica* Litv.) a related species of *P. communis*. *P. korshinskyi* is native to central Asia and may have arisen from hybridization between *P. communis* and *P. regelii* Rehder (Rehder, 1940).

Taxonomic studies of species within the genus *Pyrus* L. show that differentiation is highly correlated with geographical origin. Using ordination analysis of morphological and chemotaxonomic characters, Challice and Westwood (1973) refined the concept of differentiation among the east Asian, west Asian, and European species and speculated on their phylogenetic origin. The authors were able to distinguish between the western European *P. communis* types, east Asian pea pears [*P. betulifolia* Bge., *P. fauriei* Schneider, *P. dimorphophylla* (Mak.) Koidz., *P. calleryana* Dcne.], and larger-fruited east Asian pears [*P. pyrifolia* (Burm. f.) Nakai, *P. hondoensis* Kikuchi & Nakai, *P. ussuriensis* Maxim.]. The east Asian pears grouped with the wild European *P. cordata* (Desv.) Schneider, the wild north African *P. longipes* Henry, and the wild Asian *P. pashia* D. Don (Challice and Westwood, 1973). While work was able to distinguish broad taxonomic boundaries among Asian and European *Pyrus* species, attempts at delineating the subspecies of *P. communis* that are most closely related to domesticated pear were not successful.

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Microsatellites have been used for cultivar identification within both Asian (*P. pyrifolia*) and European (*P. communis*) pear species (Kimura et al., 2002; Yamamoto et al., 2002b, 2002c). In addition, many microsatellite loci show a high degree of synteny between apple (*Malus* Mill.) and *Pyrus* genomes (Yamamoto et al., 2001; Pierantoni et al., 2004), facilitating the use of apple markers in pear and simplifying the process of mapping the pear genome (Hayashi and Yamamoto, 2002; Hemmat et al., 2003; Katayama and Uematsu, 2003; Pierantoni et al., 2004; Yamamoto et al., 2001). Pear genetic linkage maps are valuable for breeding programs (Yamamoto et al., 2002a, 2005) as well as population genetic studies of diversity.

This study is aimed at examining the differentiation and diversity of *P. communis* ssp. *pyraster* and *P. communis* ssp. *caucasica* within an orchard collection at the National Plant Germplasm System (NPGS) National Clonal Germplasm Repository (NCGR) in Corvallis, Ore. The purpose of this work is to describe the extent of *P. communis* genetic diversity collected from Asia Minor and eastern Europe and use these data for germplasm management including suggestions for future collecting trips.

Materials and Methods

Plant materials. A total of 145 *P. communis* individuals were used for this study. Exploration trips to eastern European and Caucasus regions have brought a diverse collection of wild *P. communis* clones and seeds to the United States. Much of this germplasm was channeled to the NCGR by H. Waterworth of the Plant Quarantine Station, Glenn Dale, Md., and M. Westwood, formerly a pomologist at Oregon State Univ. and is documented in the U.S. Dept. of Agriculture's Germplasm Resources Information Network (GRIN). Phenotypic and historical information for each individual is available by querying GRIN using plant introduction (PI) numbers listed in Table 1 (USDA, 2005).

Several historical exploration trips returned to the United States with wild *Pyrus* germplasm from the Trans-Caucasus region. In 1967, H. Brooks traveled to Crimea, Ukraine, and the Caucasus mountains between Teberda, Pyatigorsk, and Stavropol in the former USSR (Brooks, 1968). In 1977, D.R. Dewey and A.P. Plummer from Utah State Univ. collected wild *P. communis* seeds near Stavropol and Svetlograd, in the Russian Federation. Seedlots of *P. communis* were also collected from Armenia by S. Gasparian (Science Research Center of Viticulture, Fruit Growing, and Wine Making in Merdzavan, Armenia) in Fall 2003 and sent to the NCGR.

Collectors gathered seeds of diverse materials from eastern Europe. J.L. Creech and D.H. Scott collected *P. communis* seeds from trees in Moldova (formerly Moldavia, USSR) and Crimea, Ukraine (formerly USSR). T. van der Zwet published details of his collection trips to gather scions from named *P. communis* cultivar trees and landraces in Serbia, Yugoslavia, Romania, Macedonia, Czech Republic, and Poland (van der Zwet et al., 1983, 1989). T. Dimitrovski of the Univ. of Skopje, Macedonia, collected seeds from wild *P. communis* trees from Leva Reka, Stip, and Gorna Bosava-Kavadarci, Macedonia in 1969, 1971, and 1972, respectively, and provided cuttings to the NCGR in the 1980s. Additional *P. communis* seed collections were made in Turkey for M. Westwood by his colleague H. Olez in 1963 and in Kyrgyzstan by Maxine Thompson in 1994. Other individuals of *P. communis* selected for this project were unnamed large-fruited types, rootstocks, and trees without specific collection localities, collected by M. Westwood and A. Rehder from the 1940s to 1960s. Collection details for individuals are provided in Table 1 and Fig. 1.

Phenotypic observations. Many of the wild *P. communis* trees in the NPGS are more than 30 years old. Subspecies designations were assigned by the curator (J.D. Postman) using fruit and foliage characteristics as well as original geographic source data. Data were collected on fruit and leaves during the 2005 season at Corvallis, Ore. Quantitative phenotypic data included fruit size and peduncle length. Qualitative phenotypic data was collected for fruit shape, russetting, lenticel size, leaf shape, and peduncle thickness.

Molecular analysis. Duplicate samples of genomic DNA were isolated from young leaf tissue of 145 *P. communis* individuals using the PUREGENEkit (Gentra Systems, Minneapolis, Minn.). Thirteen microsatellite primers were selected from the literature (Table 2). These markers were unlinked and produced a maximum of two bands per reaction. Forward primers, labeled with either IRD 700 or IRD 800, were obtained from MWG-Biotech (High Point, N.C.). Unlabeled reverse primers were purchased from Integrated Technologies (Coralville, Iowa).

Polymerase chain reactions (PCR) were carried out in 15 μL total volume. For each reaction, 10 to 50 ng DNA template and 0.3 to 0.7 pM of primers were combined with 1.5 units Taq Polymerase (Promega, Madison,Wis.), 1X Promega magnesium free buffer [10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton X-100 (Sigma, St. Louis), 0.25 mM $MgCl_2$, and 0.25 mM dNTP (Promega)]. PCR amplifications were carried out using a PTC200 thermocycler (MJ Research, Reno, Nev.) The PCR program had an initial denaturation step of 2 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at the published primer-specific annealing temperature (Table 2), 15 s at 72 °C and ending with a final extension step of 2 min at 72 °C. Completed PCR reactions were diluted 1:1 in 95% formamide, 50 mM EDTA, bromophenol blue loading dye, and denatured at 95 °C for 3 min. Gels (6.5% LI-COR KB Plus acrylamide; LI-COR, Lincoln, Nebr.) were run in 1X TBE (89 mM Tris, 89 mM boric acid, 20 mM EDTA) buffer for 1 h 45 min at 1500 V, 40 W, 40 mA, and 45 °C on a LI-COR 4200 DNA Sequencer (LI-COR) Digital images were collected from the sequencer using LI-COR Saga Generation2 software and were manually analyzed using the Saga software. Alleles from replicate samples were examined at each locus, and when alleles for replicates were not identical, data for that locus were entered as "missing" in subsequent analyses. Allele sizes were calibrated by comparing values with data collected from *P. pyrifolia* (cultivar Hosui) and three *Malus* ×*domestica* Borkh. individuals (PI 590184, PI 588853, PI 588850).

Data analyses. We used complimentary approaches to cluster, estimate diversity and display genetic differentiation in the set of pear individuals using SSR data. Initially a Bayesian clustering analysis was conducted using the software STRUCTURE (Pritchard et al., 2000). This approach uses a model-based clustering algorithm to identify clusters of individuals that have distinctive allelic frequencies. Individuals are assigned to clusters based on their allelic frequencies without a priori information such as geographic origin or parentage. The model assumes k groups, linkage equilibrium among markers, and Hardy-Weinberg equilibrium within a group. The parameter k was determined by simulating a range of values of k and the posterior probability of each value was assessed. Posterior probabilities were estimated using a Markov Chain, Monte Carlo (MCMC) method based on 50,000 iterations of each chain following a 30,000 iteration burn-in period. Each MCMC chain for each value of k (ranging from 1

Table 1. *Pyrus communis* accessions maintained by the U.S. National Plant Germplasm System (NPGS) that were included in SSR analyses have been organized by assigned cluster (as determined using Bayesian clustering analyses). Plant introduction (PI) and Corvallis, Ore. *Pyrus* local (CPYR) identification numbers are provided. *Pyrus communis* ssp. *communis* assignments were based on fruit size and *P. communis* ssp. *caucasica* and *P. communis* ssp. *pyraster* assignments were based on collection location. Collection information includes donor (to the NPGS), collector, collection date, general source location, specific latitude and longitude, and approximate elevation. Q fit describes the membership coefficient of an individual for its assigned cluster.

Cluster	Accession no.	Local ident. no.	Species	Donor	Developer/ collector	Year	Source	Lat.	Long.	Elev.	Q fit
A	PI 300693	CPYR 38.001	*P. communis* ssp. *communis* (cv. Bartlett)	M.N. Westwood	J. Stair	1770	Aldermaston, England				0.968
A	PI 205464	CPYR 706.002	*P. communis*	H. Waterworth			England, UK				0.945
A	PI 324130	CPYR 711.001	*P. communis*	H. Waterworth			Rome, Italy				0.780
A	PI 541389	CPYR 1221.001	*P. communis* ssp. *communis*	M.N. Westwood							0.963
A	PI 541437	CPYR 1489.001	*P. communis* ssp. *communis*	M.N. Westwood			Europe				0.956
A	PI 541449	CPYR 1584.001	*P. communis* (rootstock)	M.N. Westwood							0.919
A	PI 541450	CPYR 1585.001	*P. communis* (rootstock)	M.N. Westwood							0.962
A	PI 541451	CPYR 1586.001	*P. communis* (rootstock)	M.N. Westwood							0.915
A	PI 293833	CPYR 2067.001	*P. communis* ssp. *pyraster*	M.N. Westwood	J.L. Creech, D.H. Scott		Moldova	48.833	28.833		0.852
A	PI 617607	CPYR 2532.001	*P. communis*	H. Barrett		1920's	Botanical garden, St. Petersburg, Russia				0.870
A[z]	PI 541441	CPYR 700.001	*P. communis* ssp. *communis*	P. Fridlund			Collection at Prosser, Wash.				0.389
B	PI 324030	CPYR 2039.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Stavropol, Russia	44.833	42.167	660	0.954
B	PI 324029	CPYR 2038.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Stavropol, Russia	44.833	42.167	660	0.952
B	PI 337439	CPYR 689.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Stavropol, Russia	44.833	41.833	600	0.885
B	PI 312147	CPYR 1576.001	*P. communis* hybrid with a Asian species	H. Waterworth		1966	Alma-ata, Kazakhstan				0.811
B	PI 322286	CPYR 685.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Ukraine	44.55	34		0.799
B	PI 483386	CPYR 1540.001	*P. communis* ssp. *communis*	T. van der Zwet			Baligrod, Poland	49.3	22.4		0.642
B	PI 483381	CPYR 1535.002	*P. communis*	T. van der Zwet			Czechoslovakia	50.083	14.416		0.633
B	PI 506373	CPYR 1674.002	*P. communis* ssp. *communis*	T. van der Zwet			Serbia	43.83	21		0.517
B[z]	PI 322710	CPYR 902.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Askiniya-Nova Bot Garden, Ukraine				0.277
C	PI 313929	CPYR 680.001	*P. communis* ssp. *caucasica*	H. H. Waterworth		1966	Vavilov Institute, Russia				0.916
C	PI 541563	CPYR 681.001	*P. communis* ssp. *caucasica*	M.N. Westwood	J. Magness		Russia				0.653
C	PI 337438	CPYR 688.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Caucasus Mountains, Russia	44.833	41.833	600	0.609
C	PI 337441	CPYR 690.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Caucasus Mountains, Russia	43.3	41.633	1600	0.942
C	PI 440629	CPYR 717.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	45.033	41.967	0	0.963
C	PI 440629	CPYR 718.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	45.033	41.967	0	0.688
C	PI 440629	CPYR 719.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	45.033	41.967	0	0.955
C	PI 440629	CPYR 720.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	45.033	41.967	0	0.955
C	PI 440629	CPYR 721.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	45.033	41.967	0	0.830
C	PI 324028	CPYR 1192.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.75	41.833	600	0.730
C	PI 324032	CPYR 1193.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.833	42.167	660	0.580
C	PI 324037	CPYR 1194.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.583	41.833	1600	0.688
C	PI 324042	CPYR 1195.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.667	41.917	1200	0.927
C	PI 541564	CPYR 1602.001	*P. communis* ssp. *caucasica*	M.N. Westwood	Polish researchers		Caucasus Mountains, Russia				0.898
C	PI 324031	CPYR 2040.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.833	42.167	660	0.787

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Table 1. *Continued.*

Cluster	Accession no.	Local ident. no.	Species	Donor	Developer/ collector	Year	Source	Lat.	Long.	Elev.	Q fit
C	PI 324035	CPYR 2043.001	*P. communis ssp. caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.967	42.917	990	0.840
C	PI 324038	CPYR 2045.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.583	41.833	1600	0.942
C	PI 324043	CPYR 2049.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.667	41.917	1200	0.970
C	PI 324044	CPYR 2050.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	43.667	41.917	1200	0.947
C	PI 324047	CPYR 2053.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.167	42.5	540	0.851
C	PI 324048	CPYR 2054.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.167	42.5	540	0.916
C	PI 324050	CPYR 2056.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	44.133	43	930	0.540
C	PI 293838	CPYR 2060.001	*P. communis* ssp. *caucasica*	M.N. Westwood	J.L. Creech, D.H. Scott		Crimea, Ukraine	44.83	34.5		0.642
C	PI 617598	CPYR 2522.001	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.885
C	PI 617598	CPYR 2522.003	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.951
C	PI 617598	CPYR 2522.004	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.714
C	PI 617598	CPYR 2522.005	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.971
C	PI 617598	CPYR 2522.006	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.918
C	PI 617598	CPYR 2522.007	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.891
C	PI 617598	CPYR 2522.008	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.882
C	PI 617598	CPYR 2522.009	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.798
C	PI 638005	CPYR 2813.001	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.117	44.833		0.725
C	PI 638006	CPYR 2814.001	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.611
C	PI 638007	CPYR 2815.003	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.834
C[z]	PI 337437	CPYR 687.001	*P. communis* ssp. *caucasica*	H.J. Brooks	H.J. Brooks	1967	Caucasus Mountains, Russia	44.833	41.833	600	0.385
C[z]	PI 324027	CPYR 2037.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Caucasus Mountains, Russia	45.083	41.917	570	0.445
C[z]	PI 293834	CPYR 2066.001	*P. communis* ssp. *caucasica*	M.N. Westwood	J.L. Creech, D.H. Scott		Crimea, Ukraine				0.470
C[z]	PI 617598	CPYR 2522.002	*P. communis* ssp. *korshinskyi*	M. Thompson	M. Thompson	1994	Experiment station, Ak-Terek, Kyrgyzstan	41.25	72.833	1600	0.479
D	PI 440630	CPYR 727.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	44.683	42.483	400	0.867
D	PI 440630	CPYR 728.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	44.683	42.483	400	0.952
D	PI 440630	CPYR 729.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	44.683	42.483	400	0.970
D	PI 541321	CPYR 731.001	*P. communis*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Caucasus Mountains, Russia	44.683	42.483	400	0.971
E	PI 440631	CPYR 694.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Svetlograd, Russia	45.067	43.033	300	0.955
E	PI 440631	CPYR 695.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Svetlograd, Russia	45.067	43.033	300	0.965
E	PI 440632	CPYR 697.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Svetlograd, Russia	45.067	43.033	300	0.906

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Table 1. *Continued*.

Cluster	Accession no.	Local ident. no.	Species	Donor	Developer/ collector	Year	Source	Lat.	Long.	Elev.	Q fit
E	PI 440632	CPYR 697.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Svetlograd, Russia	45.067	43.033	300	0.906
E	PI 440632	CPYR 698.001	*P. communis* ssp. *caucasica*	D.R. Dewey	D.R. Dewey, A.P. Plummer	1977	Svetlograd, Russia	45.067	43.033	300	0.961
E[z]	PI 293842	CPYR 2059.001	*P. communis* ssp. *pyraster*	M.N. Westwood	J.L. Creech, D.H. Scott		Moldova	48.833	28.833	300	0.374
F	PI 638004	CPYR 2812.002	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.731
F	PI 638005	CPYR 2813.002	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.945
F	PI 638005	CPYR 2813.003	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.536
F	PI 638005	CPYR 2813.004	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.944
F	PI 638007	CPYR 2815.001	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.974
F	PI 638007	CPYR 2815.002	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.727
F	PI 638008	CPYR 2816.001	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.958
F	PI 638008	CPYR 2816.002	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.947
F	PI 638008	CPYR 2816.003	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.980
F	PI 638008	CPYR 2816.004	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.978
F	PI 638008	CPYR 2816.005	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.832
F	PI 638008	CPYR 2816.006	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.955
F	PI 638008	CPYR 2816.007	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.974
F	PI 638008	CPYR 2816.008	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.976
F	PI 638008	CPYR 2816.009	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.976
F	PI 638008	CPYR 2816.010	*P. communis* ssp. *caucasica*	S. Gasparian		2003	Armenia	40.8425	44.3625		0.980
G	PI 541291	CPYR 693.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.928
G	PI 541292	CPYR 710.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey				0.907
G	PI 541323	CPYR 712.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey				0.915
G	PI 369881	CPYR 986.001	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1971	Macedonia	41.733	22.183		0.729
G	PI 369881	CPYR 986.002	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1971	Macedonia	41.733	22.183		0.522
G	PI 369881	CPYR 986.005	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1971	Macedonia	41.733	22.183		0.708
G	PI 541391	CPYR 1248.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.937
G	PI 541392	CPYR 1249.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.927
G	PI 541393	CPYR 1250.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.578
G	PI 541394	CPYR 1251.002	*P. communis ssp. pyraster*	M.N. Westwood			France				0.684
G	PI 541395	CPYR 1252.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.875
G	PI 541435	CPYR 1465.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.936
G	PI 541436	CPYR 1466.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.841
G	PI 617521	CPYR 1467.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey	38.2	28.6		0.681
G	PI 506380	CPYR 1671.001	*P. communis ssp. pyraster*	T. van der Zwet			Valsoara, Romania				0.585
G	PI 541490	CPYR 2057.001	*P. communis ssp. pyraster*	M.N. Westwood	H. Olez	1963	Turkey				0.868
H	PI 349026	CPYR 989.001	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.923
H	PI 349026	CPYR 989.002	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.957
H	PI 349026	CPYR 989.003	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.831
H	PI 349026	CPYR 989.004	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.901
H	PI 349026	CPYR 989.005	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.971
H	PI 349026	CPYR 989.006	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.597
H	PI 349026	CPYR 989.007	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.557

continued next page

Table 1. *Continued*.

Cluster	Accession no.	Local ident. no.	Species	Donor	Developer/ collector	Year	Source	Lat.	Long.	Elev.	Q fit
H	PI 349026	CPYR 989.008	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.910
H	PI 349026	CPYR 989.009	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.964
H	PI 349026	CPYR 989.010	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.841
H	PI 349026	CPYR 989.011	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.884
H	PI 349026	CPYR 989.012	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Izvor, Macedonia	41.555	41.833		0.912
H[z]	PI 483383	CPYR 1537.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet		Poland				0.327
I	PI 541440	CPYR 699.001	*P. communis ssp. pyraster*	P. Fridlund			Unknown				0.838
I	PI 349027	CPYR 991.001	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.929
I	PI 349027	CPYR 991.002	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.967
I	PI 349027	CPYR 991.003	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.964
I	PI 349027	CPYR 991.004	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.922
I	PI 349027	CPYR 991.005	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.946
I	PI 349027	CPYR 991.006	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Leva Reca, Macedonia				0.927
I	PI 502178	CPYR 1638.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet		Macedonia	41.883	21.667		0.559
I[z]	PI 541434	CPYR 1421.001	*P. communis*	M.N. Westwood							0.435
I[z]	PI 506379	CPYR 1684.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet		Romania				0.310
J	PI 541567	CPYR 881.001	*P. communis ssp. pyraster*	P. Fridlund, M.N. Westwood			Collection at Prosser, Wash.				0.967
J	PI 349028	CPYR 993.001	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.947
J	PI 349028	CPYR 993.002	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.909
J	PI 349028	CPYR 993.003	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.974
J	PI 349028	CPYR 993.004	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.922
J	PI 349028	CPYR 993.005	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.958
J	PI 349028	CPYR 993.006	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.977
J	PI 349028	CPYR 993.007	*P. communis ssp. pyraster*	M.N. Westwood	T. Dimitrovski	1969	Mavrovo, Macedonia	41.417	20.833		0.957
J[z]	PI 502173	CPYR 1633.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet		Poland	50	22		0.287
K	PI 541319	CPYR 714.001	*P. communis ssp. pyraster*	P. Fridlund, M.N. Westwood			Europe				0.952
K	PI 541452	CPYR 1592.001	*P. communis ssp. pyraster*	M.N. Westwood			Europe				0.837
K	PI 541453	CPYR 1607.001	*P. communis ssp. pyraster*	M.N. Westwood			France				0.852
K	PI 502180	CPYR 1640.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet	1978	Macedonia	41.833	21.667		0.689
K	PI 293837	CPYR 2064.001	*P. communis* ssp. *caucasica*	M.N. Westwood	J.L. Creech, D.H. Scott		Crimea, Ukraine	44.833	34.5		0.918
K[z]	PI 324172	CPYR 1191.001	*P. communis* ssp. *caucasica*	M.N. Westwood	H.J. Brooks	1967	Crimea, Ukraine	44.5	34.166		0.410
K[z]	PI 293841	CPYR 2068.001	*P. communis ssp. pyraster*	M.N. Westwood	J.L. Creech, D.H. Scott		Kholorash Region, Moldova				0.369
L	PI 132094	CPYR 1288.001	*P. communis ssp. pyraster*	M.N. Westwood	A. Rehder	1939	Iran				0.972
L	PI 541568	CPYR 1289.003	*P. communis ssp. pyraster*	M.N. Westwood	A. Rehder		Hungary				0.974
L	PI 541569	CPYR 1292.001	*P. communis ssp. pyraster*	M.N. Westwood	A. Rehder		Hungary				0.956
L[z]	PI 322285	CPYR 684.001	*P. communis* ssp. *communis*	H.J. Brooks	H.J. Brooks	1967	Crimea, Ukraine	44.517	34		0.495
L[z]	PI 377611	CPYR 883.001	*P. communis ssp. pyraster*	N.W. Callan	T. Dimitrovski	1972	Macedonia	41.35	22.067		0.498
L[z]	PI 483385	CPYR 1539.001	*P. communis ssp. pyraster*	T. van der Zwet	T. van der Zwet		Poland				0.267

[z]Q fit is <0.5.

Table 2. Microsatellite loci were used to amplify pear DNA. The original cited name, range of allele sizes (in base pairs), number of amplified alleles, genetic mapping information, and original citations are provided for each locus.

Microsatellite	Allele size (bp)	Alleles amplified (no.)	Chromosomal linkage group no.	Source
NH009b	134–154	9	13	Yamamoto et al., 2005
NH015a	95–127	14	17	Yamamoto et al., 2005
CH01D08	226–300	14	15	Liebhard et al., 2002, 2003
CH01D09	118–172	23	12	Liebhard et al., 2002, 2003
CH01F07A	176–226	19	10	Liebhard et al., 2002, 2003
CH01H01	97–123	11	17	Liebhard et al., 2002, 2003
CH02B10	113–159	18	2	Liebhard et al., 2002, 2003
CH02D12	213–255	23	11	Liebhard et al., 2002, 2003
CH05E03	151–221	33	2	Liebhard et al., 2002, 2003
GD12	136–170	18	10	Hemmat et al., 2003
GD142	136–188	26	3	Hemmat et al., 2003
GD96	141–175	18	1	Hemmat et al., 2003
GD147	120–148	9	4	Hemmat et al., 2003

Table 3. Descriptive information is provided for each of the 12 clusters of genotypes identified by Bayesian clustering analyses. Summary statistics are given for each cluster as a whole. Summary statistics include the number of individuals in each cluster (N), Nei's gene diversity, the number of alleles unique to the identified cluster and the total number of alleles scored across all microsatellite loci. Allelic richness is a scaled measure of allelic diversity that controls for cluster size.

Cluster	Origin	N	Gene diversity	Unique alleles (no.)	Alleles at each SSR locus (no.)													Total alleles (no.)	Allelic richness
					CH01D08	CH01d09	CH01F07A	Ch01h01	CH02b10	CH02d12	CH05e03	GD12	GD96	GD142	GD147	NH009b	NH015a		
A	Domesticated	11	0.64	3	7	6	6	4	6	6	8	5	5	6	2	2	7	70	3.28
B	Various	9	0.75	5	5	7	8	7	8	8	8	7	8	8	5	6	7	92	3.99
C	Caucasus, Ukraine	38	0.68	11	7	16	10	7	8	17	22	11	13	15	7	4	8	145	2.90
D	Caucasus	4	0.49	0	1	4	3	2	2	4	2	3	3	4	4	1	3	36	3.64
E	Caucasus	5	0.60	0	2	6	3	2	4	8	2	2	4	4	4	2	4	47	3.52
F	Armenia	17	0.57	3	3	6	5	6	5	9	7	4	5	4	2	3	4	63	2.52
G	Turkey, Macedonia	16	0.75	8	8	11	8	6	5	10	11	4	11	16	5	6	6	107	3.93
H	Macedonia	13	0.67	7	3	8	7	6	7	11	9	4	5	9	7	5	6	87	3.47
I	Macedonia	10	0.70	7	5	8	6	5	8	7	5	4	6	8	4	5	6	77	3.35
J	Macedonia	9	0.62	0	3	6	5	3	6	8	5	1	2	4	5	4	5	57	3.93
K	Europe, various	7	0.74	3	5	8	5	4	8	9	9	4	7	4	5	3	8	79	3.14
L	Various	6	0.62	3	4	8	5	5	4	3	5	2	3	6	4	5	7	61	2.82

to 40) was run 10 times. The method allows for individuals with ancestry from more than one group. These individuals are fractionally assigned to multiple groups using a membership coefficient (Q) which sums to 1 across all groups. Individual assignments can vary across runs when there is a weak genetic basis for assigning an individual to a cluster. To address this variation, we ran 100 separate MCMC chains at the most probable value of *k* to look for similarity among assignments (Rosenberg et al., 2002).

Descriptive statistics, including variation between groups (F_{st}), and diversity within groups including Nei's gene diversity (Nei, 1987), number of polymorphic alleles and allelic richness (El Mousadik and Petit, 1996) were estimated from genotypic data using the software package GDA (Lewis and Zaykin, 2001) and FSTAT (Goudet, 1995). Pairwise F_{st} values were tested for significance using a permutation test. Analysis of molecular variance (AMOVA) was carried out using the software ARLEQUIN ver. 2.0 (Schneider et al., 2000).

Groups were plotted as nodes in a minimum spanning network using MINSPNET, a module within ARLEQUIN (Schneider et al., 2000) using pairwise F_{st} values as the distance metric. The network display has a number of advantages over a bifurcation tree structure, especially when the pedigrees of the individuals are reticulate (characterized by ancestral interspecific hybridization). The minimum spanning tree was manually drawn using computer outputs.

Results

Genotypic data were collected for 145 *P. communis* individuals. Microsatellites provided between 9 and 33 alleles per locus (Table 2). A total of 235 microsatellite alleles were scored within the dataset.

Phenotypic observations. Morphological data were used in an attempt to differentiate individuals according to *P. communis* ssp. *communis* (domesticated), *P. communis* ssp. *pyraster* (south and west of the Black Sea), and *P. communis* ssp. *caucasica* (north and east of the Black Sea) (Fig. 1). Floral and fruit characteristics could not distinguish between *P. communis* ssp. *pyraster* and *P.*

communis ssp. *caucasica* [data not shown, but available online (USDA, ARS, National Genetic Resources Program, 2005)]. The *P. communis* ssp. *communis* individuals (including hybrids between cultivated and wildtypes) have larger fruit than individuals in the other subspecies of *P. communis*. In 2005, accessions PI 300693, PI 541389, PI 541437, PI 293833, PI 324030, PI 324029, PI 337439, PI 322286, and PI 506373 had large fruit, characteristic of cultivated-type pears.

Genetic analysis. Posterior probabilities of Bayesian clustering analysis across a range of k identified (k = 12) as the most probable clusters within the dataset. Among the subsequent 100 separate MCMC chains run with k = 12, individual assignments to groups were highly correlated (>0.85) among runs. All clusters showed varying degrees of admixture reflecting the relatedness of individuals (such as sibling collections) (Table 3). Sixteen individuals (11%) had membership coefficients, Q, less than 0.5. While the affinity of these individuals to their assigned cluster was low, their placement in a cluster reflects consistent assignments among runs and represents the best fit of the data. Phenotypic observations of fruit and leaf characteristics of these individuals revealed that seven of these 16 individuals exhibited large fruit or serrated leaf margins. Hybridization between wildtype *P. communis* and domesticated *P. communis* ssp. *communis* could result in large fruit, while hybridization between *P. communis* and another *Pyrus* species (such as *P. nivalis*) could result in serrated leaf margins and low membership coefficients.

Genetic diversity data were calculated by cluster. Nei's gene diversity ranged from 0.49 to 0.74, revealing a high level of heterozygous individuals. Each of the 12 clusters identified was significantly differentiated with an average F_{st} value of 0.18. AMOVA results indicated that the within cluster variance component accounted for 82% of total variation, indicative of a highly variable, outcrossing species (Table 3).

The number of alleles represented across each of the 13 molecular markers was high (235), with most markers providing many diverse alleles. We used allelic diversity (El Mousadik and Petit, 1996) which employs a statistical scaling method to make direct comparisons among clusters composed of different numbers of individuals (N). In this data set, the sample size is set to the smallest cluster size where N = 4. Cluster B showed the highest allelic diversity, followed by clusters G and J. Cluster size did not correlate to measures of genetic diversity. The largest cluster (cluster C with N = 38 individuals) had one of the lowest measures of allelic richness based upon Nei's gene diversity score (Table 3).

Clustering of individuals. Outputs were used to create a minimum spanning tree that graphically displays the genetic differentiation among the 12 clusters identified in Bayesian analysis (Fig. 2). Each node in the network corresponds to a particular cluster of genotypes. Distances between each connected cluster on the diagram represent F_{st} values. Each node is displayed as a pie chart where node diameters correlate to the number of individuals within the cluster (Fig. 2). Pie chart shading represents the number of individuals identified as either *P. communis* ssp. *communis*, *P. communis* ssp. *pyraster*, or *P. communis* ssp. *caucasica*. Pairwise F_{st} values among clusters were significant at $\alpha = 0.05$.

The broad structure of the network shows a central cluster (B) connected to genotypes of domesticated lineages (node A) and two other clusters. Each of these clusters (G and C) forms the center of two distinct star groups. Clusters C–F (predominantly *P. communis* ssp. *caucasica*) are genetically differentiated from clusters G–L (predominantly *P. communis* ssp. *pyraster*). These data support the genetic differentiation of *P. communis* ssp. *pyraster* from *P. communis* ssp. *caucasica*.

Clusters A and B both include individuals with large domesticated-type fruit characteristic of *P. communis* ssp. *communis*. Cluster A contains mostly domesticated cultivars including the

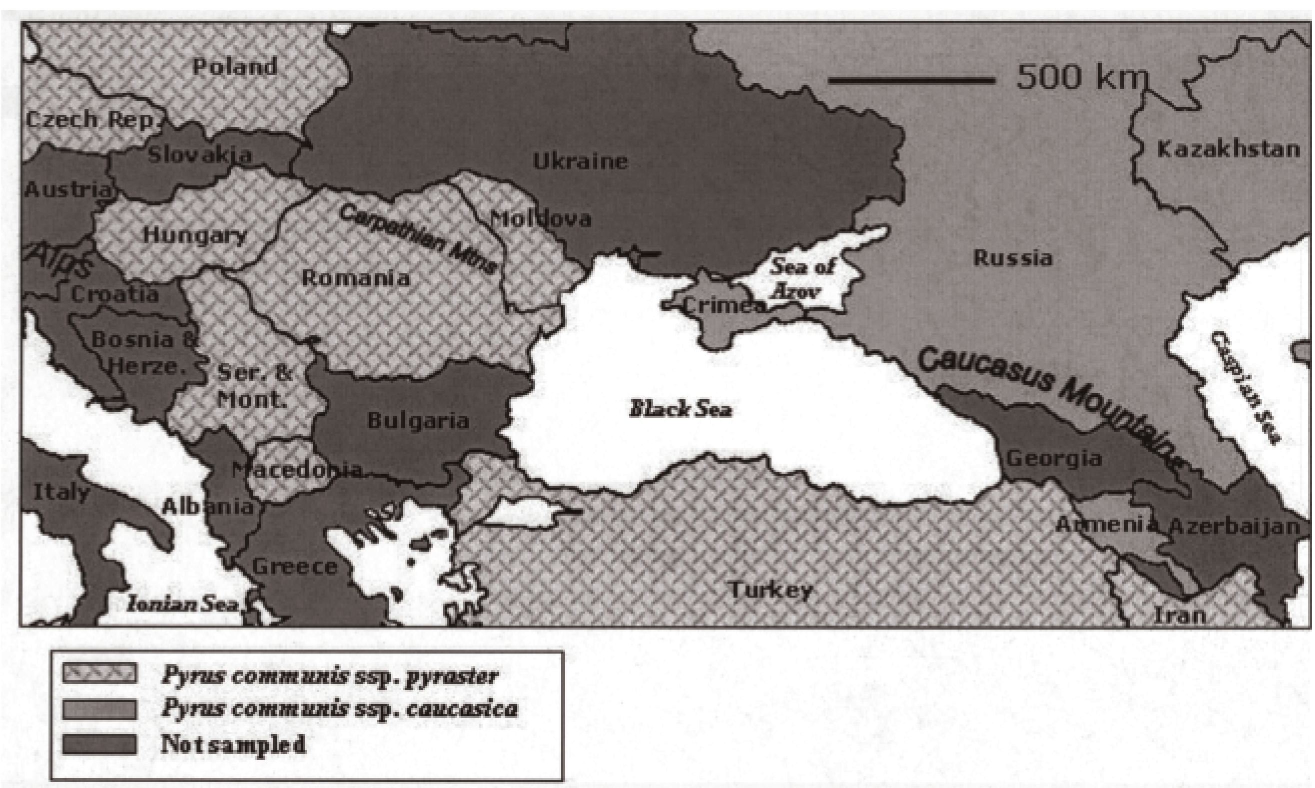

Fig. 1. Map of the countries of origin for the wild *Pyrus communis* individuals from the U.S. National Plant Germplasm System collection.

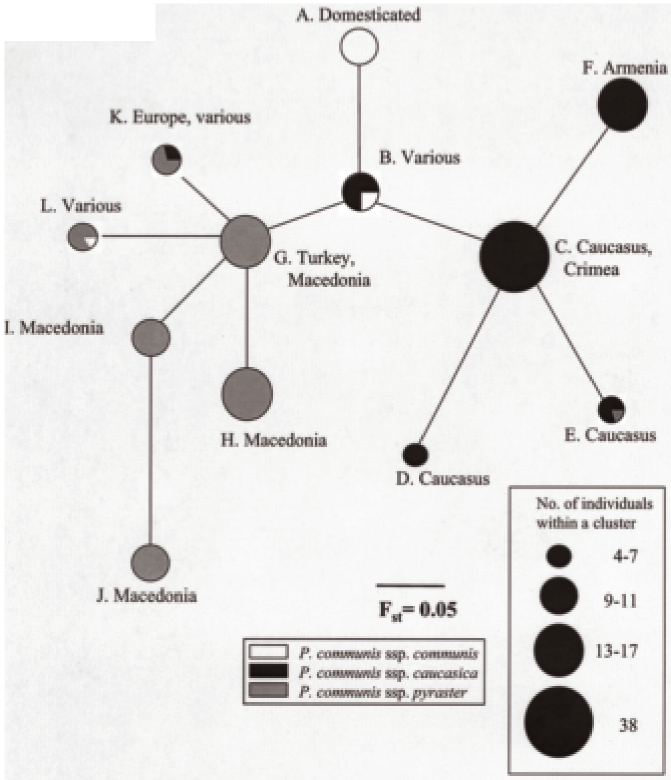

Fig. 2. A minimum spanning network for the wild *Pyrus communis* individuals from the U.S. National Plant Germplasm System collection. Distances among connected nodes represent F_{st} values. Pie chart nodes represent proportions of individuals from *P. communis* ssp. *communis*, *P. communis* ssp. *caucasica*, and *P. communis* ssp. *pyraster* in the clusters identified through Bayesian analysis. The diameter of each node reflects the number of individuals within the cluster.

cultivar Bartlett as well as some domesticated rootstock cultivars. Cluster B contains a mixture of *P. communis* ssp. *communis* and *P. communis* ssp. *caucasica*. Individuals in cluster B were collected by Brooks from the northern side of the Caucasus Mountains and Ukraine and by van der Zwet in Poland, Serbia, and the Czech Republic (Table 1).

Clusters C–F contain individuals classified as *P. communis* ssp. *caucasica* that were acquired from several collection trips in the Armenia, Crimea, and Caucasus Mountain regions. These include collection trips by Creech and Scott in Crimea, Brooks as well as Dewey and Plummer in the Caucasus Mountains of Russia and Thompson in Kyrgyzstan (Table 1). Thompson's seedlot from Kyrgyzstan was originally classified as *P. communis* ssp. *korshinskyi*; however, genotypic data suggest that these individuals should be reclassified as *P. communis* ssp. *caucasica*.

Clusters G–L contain accessions mostly classified as *P. communis* ssp. *pyraster*. These clusters include individuals from European, Balkan, and western Turkish locations. Cluster G contains 10 individuals from Turkey, a sibship of three individuals from Macedonia, and seedling selections from France and Romania. Clusters H, I, and J are primarily comprised of sibling populations from Macedonia. Cluster H has 12 siblings from Izvor, Macedonia, and one individual from Poland. Cluster I includes six siblings from Leva Reca, Macedonia, one individual each from van der Zwet's trips to Macedonia and Romania, and two individuals of unknown origins. Cluster J contains seven siblings from Mavrovo, Macedonia, and two other individuals of unknown origin. Cluster K is comprised of a cultivar of seedling selections donated by Westwood that came from seedlots from France, Europe, Crimea, Macedonia, and Moldova. Cluster L originates from a variety of sources. Three individuals came from A. Rehder and originated in Hungary and Iran. Other cluster L individuals had poor correlations and originated from Crimea, Macedonia, and Poland.

Discussion

Genetic analysis at 13 microsatellite loci revealed significant differentiation between the two wild subspecies considered the progenitors of the domesticated european pear. Morphological variation in fruit and floral characters did not distinguish these subspecies since the results were based on a limited number of characters. Moreover, the variation in trait values among wild populations for characters associated with domestication is often low and may be a poor predictor of genetic potential in these taxa (Tanksley and McCouch, 1997).

Pyrus communis ssp. *caucasica* individuals from the Caucasus, Ukraine, and Armenia group together (Fig. 2, clusters C–F) and may represent the wild center of diversity for domesticated pear, *Pyrus communis* ssp. *communis*. *Pyrus communis* ssp. *pyraster* individuals from Turkey, Macedonia and other European countries cluster separately in the network (Fig. 2, clusters G–L). These individuals display a high level of diversity that could have partially arisen through gene flow and introgressive hybridization with co-occurring congeneric species found in Europe. The differentiation of *P. communis* ssp. *pyraster* from *P. communis* ssp. *caucasica* could result from human migrations from the Trans-Caucasia region through Turkey and into the Balkan region over thousands of years (Gamkrelidze and Ivanov, 1990). In some cases, open pollinated sibling individuals did not differentiate into the same cluster. This is not surprising since seeds could have been fertilized by pollen from highly diverse trees in the wild.

Some individuals do not clearly fit into any of the 12 identified clusters. Hybridization is prevalent in *Pyrus* and some of these individuals appear to have a hybrid lineage, as indicated by the presence of serrated leaves. Many of the wild pears, particularly of European origin, could be escapes from cultivation or hybrids between wild *P. communis* and native european pear species, such as *P. nivalis* (Aldasoro et al., 1996; Paganova, 2003). More accurate determination of this would be possible with increased sampling in the region of the putative hybridization.

The broad diversity of the NPGS wild *P. communis* collection is evident through genetic analyses of molecular markers, but not readily apparent by morphological characterizations. Our study of 145 individual trees represents a conservative estimate of the natural diversity. Both the number and identity of alleles measured through allelic richness indicate that each genetic cluster, regardless of its size, adds measurable molecular diversity to the overall collection. Although leaves and fruits are morphologically similar, further morphological characterization of additional sibling populations will likely reveal novel phenotypic traits of interest to pear breeders. Increased sampling intensity, either by pursuing new collection trips or by sampling additional clones or seeds from previous collection trips from eastern Europe and Asia Minor, is critical to adequately evaluate natural genetic diversity of wild *P. communis*.

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