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 unacceptably 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 supplementary collection trips to eastern Europe, the Caucasus Mountains, and the surrounding countries.

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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 (NCCR) 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 NCCR 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 NCCR.

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 Zvet 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 Zvet et al., 1983, 1989). T. Dimitrovska 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 NCCR 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 analyses. 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.), 1 X Promega magnesium free buffer [10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton X-100 (Sigma, St. Louis), 0.25 mM MgCl₂, and 0.25 mM dNTP (Promega)]. PCR amplifications were carried out using a PTC-200 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. caucasicus 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       | 300693        | CPYR 38.001      | P. communis ssp. communis (cv. Bartlett) | M.N. Westwood | J. Stair | 1770 | Aldermaston, England | 0.968 |
| A       | 205464        | CPYR 706.002     | P. communis | H. Waterworth | England, UK | 0.945 |
| A       | 324130        | CPYR 711.001     | P. communis | H. Waterworth | Rome, Italy | 0.780 |
| A       | 541389        | CPYR 1221.001    | P. communis ssp. communis | M.N. Westwood | | 0.963 |
| A       | 541437        | CPYR 1489.001    | P. communis ssp. communis | M.N. Westwood | Europe | 0.956 |
| A       | 541449        | CPYR 1584.001    | P. communis (Institut) | M.N. Westwood | | 0.919 |
| A       | 541450        | CPYR 1585.001    | P. communis (Institut) | M.N. Westwood | | 0.962 |
| A       | 541451        | CPYR 1586.001    | P. communis (Institut) | M.N. Westwood | | 0.915 |
| A       | 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       | 617607        | CPYR 2532.001    | P. communis | H. Barrett | Botanical garden, St. Petersburg, Russia | 1920s | 0.870 |
| A'      | 541441        | CPYR 700.001     | P. communis ssp. communis | P. Fridlund | Collection at Prosser, Wash. | | 0.589 |
| B       | 324030        | CPYR 2039.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Stavropol, Russia | 44.833 | 42.167 | 660 | 0.954 |
| B       | 324029        | CPYR 2038.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Stavropol, Russia | 44.833 | 42.167 | 660 | 0.952 |
| B       | 337439        | CPYR 689.001     | P. communis ssp. caucasicus | H.J. Brooks | H.J. Brooks | 1967 | Stavropol, Russia | 44.833 | 41.833 | 600 | 0.885 |
| B       | 321247        | CPYR 1576.001    | P. communis hybrid with a Asian species | H. Waterworth | | 1966 | Alma-ata, Kazakhstan | 0.811 |
| B       | 322286        | CPYR 685.001     | P. communis ssp. caucasicus | H.J. Brooks | H.J. Brooks | 1967 | Ukraine | 44.55 | 34 | 0.799 |
| B       | 483386        | CPYR 1540.001    | P. communis ssp. communis | T. van der Zwet | | 1977 | Baligrad, Poland | 49.3 | 22.4 | 0.642 |
| B       | 483381        | CPYR 1535.002    | P. communis | T. van der Zwet | | 1977 | Czechoslovakia | 50.083 | 14.416 | 0.633 |
| B       | 506373        | CPYR 1674.002    | P. communis ssp. communis | T. van der Zwet | | 1977 | Serbia | 43.83 | 21 | 0.517 |
| B'      | 322710        | CPYR 902.001     | P. communis ssp. caucasicus | H.J. Brooks | H.J. Brooks | 1967 | Askiniya-Nova Bot Garden, Ukraine | 0.277 |
| C       | 313929        | CPYR 680.001     | P. communis ssp. caucasicus | H. H. Waterworth | | 1966 | Vavilov Institute, Russia | 0.916 |
| C       | 541563        | CPYR 681.001     | P. communis ssp. caucasicus | M.N. Westwood | J. Magness | Russia | 0.653 |
| C       | 337438        | CPYR 688.001     | P. communis ssp. caucasicus | H.J. Brooks | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.833 | 41.833 | 600 | 0.609 |
| C       | 337441        | CPYR 690.001     | P. communis ssp. caucasicus | H.J. Brooks | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 43.3 | 41.633 | 1600 | 0.942 |
| C       | 440629        | CPYR 717.001     | P. communis ssp. caucasicus | D.R. Dewey | D.R. Dewey, A.P. | 1977 | Caucasus Mountains, Russia | 45.033 | 41.967 | 0 | 0.963 |
| C       | 440629        | CPYR 718.001     | P. communis ssp. caucasicus | D.R. Dewey | D.R. Dewey, A.P. | 1977 | Caucasus Mountains, Russia | 45.033 | 41.967 | 0 | 0.688 |
| C       | 440629        | CPYR 719.001     | P. communis ssp. caucasicus | D.R. Dewey | D.R. Dewey, A.P. | 1977 | Caucasus Mountains, Russia | 45.033 | 41.967 | 0 | 0.955 |
| C       | 440629        | CPYR 720.001     | P. communis ssp. caucasicus | D.R. Dewey | D.R. Dewey, A.P. | 1977 | Caucasus Mountains, Russia | 45.033 | 41.967 | 0 | 0.955 |
| C       | 440629        | CPYR 721.001     | P. communis ssp. caucasicus | D.R. Dewey | D.R. Dewey, A.P. | 1977 | Caucasus Mountains, Russia | 45.033 | 41.967 | 0 | 0.830 |
| C       | 324028        | CPYR 1192.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.75 | 41.833 | 600 | 0.730 |
| C       | 324032        | CPYR 1193.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.833 | 42.167 | 660 | 0.580 |
| C       | 324037        | CPYR 1194.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 43.583 | 41.833 | 1600 | 0.688 |
| C       | 324042        | CPYR 1195.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 43.667 | 41.917 | 1200 | 0.927 |
| C       | 541564        | CPYR 1602.001    | P. communis ssp. caucasicus | M.N. Westwood | Polish researchers | Caucasus Mountains, Russia | 0.898 |
| C       | 324031        | CPYR 2040.001    | P. communis ssp. caucasicus | M.N. Westwood | H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.833 | 42.167 | 660 | 0.787 |

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| Cluster no. | Species | Donor/collector | Year | Source | Lat. | Long. | Elev. | Q fit |
|------------|---------|-----------------|------|--------|------|-------|-------|-------|
| C PI 324035 CYPR 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 CYPR 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 CYPR 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 CYPR 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 CYPR 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 CYPR 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 CYPR 2056.001 | *P. communis* ssp. *caucasica* | M.N. Westwood H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.133 | 43 | 930 | 0.540 |
| C PI 295838 CYPR 2060.001 | *P. communis* ssp. *caucasica* | J.L. Creech, D.H. Scott | 1977 | Crimea, Ukraine | 44.83 | 34.5 | 642 | |
| C PI 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 617998 CYPR 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 CYPR 2813.001 | *P. communis* ssp. *caucasica* | S. Gasparian | 2003 | Armenia | 40.117 | 44.833 | 725 | |
| C PI 638006 CYPR 2814.001 | *P. communis* ssp. *caucasica* | S. Gasparian | 2003 | Armenia | 40.8425 | 44.3625 | 611 | |
| C PI 638007 CYPR 2815.003 | *P. communis* ssp. *caucasica* | S. Gasparian | 2003 | Armenia | 40.8425 | 44.3625 | 834 | |
| C 2 PI 337437 CYPR 687.001 | *P. communis* ssp. *caucasica* | H.J. Brooks H.J. Brooks | 1967 | Caucasus Mountains, Russia | 44.833 | 41.833 | 630 | 0.385 |
| C 2 PI 324027 CYPR 2037.001 | *P. communis* ssp. *caucasica* | M.N. Westwood H.J. Brooks | 1967 | Caucasus Mountains, Russia | 45.083 | 41.917 | 570 | 0.445 |
| C 2 PI 293834 CYPR 2066.001 | *P. communis* ssp. *caucasica* | J.L. Creech, D.H. Scott | 1977 | Crimea, Ukraine | 45.083 | 41.917 | 540 | 0.700 |
| C 2 PI 617998 CYPR 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 CYPR 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 CYPR 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 CYPR 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 CYPR 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 CYPR 694.001 | *P. communis* ssp. *caucasica* | D.R. Dewey D.R. Dewey, A.P. Plummer | 1977 | Sverlograd, Russia | 45.067 | 43.033 | 300 | 0.955 |
| E PI 440631 CYPR 695.001 | *P. communis* ssp. *caucasica* | D.R. Dewey D.R. Dewey, A.P. Plummer | 1977 | Sverlograd, Russia | 45.067 | 43.033 | 300 | 0.965 |
| E PI 440632 CYPR 697.001 | *P. communis* ssp. *caucasica* | D.R. Dewey D.R. Dewey, A.P. Plummer | 1977 | Sverlograd, Russia | 45.067 | 43.033 | 300 | 0.906 |

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Table 1. Continued.

| Cluster no. | Local ident. no. | Species | Donor | Developer/collector | Year | Source | Lat. | Long. | Elev. | Qtl |
|------------|------------------|---------|-------|---------------------|------|--------|------|-------|-------|-----|
| E PI 440632 | CPYR 697.001     | P. communis ssp. caucasica | D.R. Dewey | D.R. Dewey, A.P. Plummer | 1977 | Svetlograd, Russia | 45.067 | 45.033 | 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 | 45.033 | 300   | 0.961 |
| F PI 293842 | CPYR 2059.001    | P. communis ssp. pyraster | M.N. Westwood | J.L. Creech, D.H. Scott | 2003 | 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 |

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Table 1. Continued.

| Accession no. | Local ident. no. | Species | Donor | Developer/collector | Year | Source | Lat. | Long. | Elev. | Q fit |
|---------------|------------------|---------|-------|---------------------|------|--------|------|-------|-------|------|
| H 54026 CPYR 989.008 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Izvor, Macedonia | 41.555 | 41.833 | 0.910 |
| H 54026 CPYR 989.009 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Izvor, Macedonia | 41.555 | 41.833 | 0.964 |
| H 54026 CPYR 989.010 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Izvor, Macedonia | 41.555 | 41.833 | 0.841 |
| H 54026 CPYR 989.011 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Izvor, Macedonia | 41.555 | 41.833 | 0.884 |
| H 54026 CPYR 989.012 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Izvor, Macedonia | 41.555 | 41.833 | 0.912 |
| H 548338 CPYR 1537.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | Poland | 0.327 |
| I 541440 CPYR 699.001 | P. communis ssp. pyraster | P. Fridlund | Unknown | | 0.838 |
| I 54027 CPYR 991.001 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.929 |
| I 54027 CPYR 991.002 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.967 |
| I 54027 CPYR 991.003 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.964 |
| I 54027 CPYR 991.004 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.922 |
| I 54027 CPYR 991.005 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.946 |
| I 54027 CPYR 991.006 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Leva Rea, Macedonia | 0.927 |
| I 502178 CPYR 1638.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | Macedonia | 41.883 | 21.667 | 0.559 |
| I 50639 CPYR 1684.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | Romania | 0.310 |
| J 541567 CPYR 881.001 | P. communis ssp. pyraster | P. Fridlund, M.N. Westwood | Collection at Prosser, Wash. | | 0.967 |
| J 54028 CPYR 993.001 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.947 |
| J 54028 CPYR 993.002 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.909 |
| J 54028 CPYR 993.003 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.974 |
| J 54028 CPYR 993.004 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.922 |
| J 54028 CPYR 993.005 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.958 |
| J 54028 CPYR 993.006 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.977 |
| J 54028 CPYR 993.007 | P. communis ssp. pyraster | M.N. Westwood | T. Dimitrovski | 1969 | Mavrovo, Macedonia | 41.417 | 20.833 | 0.957 |
| J 54028 CPYR 1633.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | Poland | 50 | 22 | 0.287 |
| K 54139 CPYR 714.001 | P. communis ssp. pyraster | P. Fridlund, M.N. Westwood | Europe | | 0.952 |
| K 541452 CPYR 1592.001 | P. communis ssp. pyraster | M.N. Westwood | Europe | | 0.837 |
| K 541453 CPYR 1607.001 | P. communis ssp. pyraster | M.N. Westwood | France | | 0.852 |
| K 502180 CPYR 1640.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | 1978 | Macedonia | 41.833 | 21.667 | 0.689 |
| K 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 324172 CPYR 1191.001 | P. communis ssp. caucasica | M.N. Westwood, H.J. Brooks | Crimea, Ukraine | 44.5 | 34.166 | 0.410 |
| K 293841 CPYR 2068.001 | P. communis ssp. pyraster | M.N. Westwood, J.L. Creech, D.H. Scott | Kholorash Region, Moldova | | 0.369 |
| L 532094 CPYR 1288.001 | P. communis ssp. pyraster | M.N. Westwood, A. Rehder | Iran | | 0.972 |
| L 541568 CPYR 1289.003 | P. communis ssp. pyraster | M.N. Westwood, A. Rehder | Hungary | | 0.974 |
| L 541569 CPYR 1292.001 | P. communis ssp. pyraster | M.N. Westwood, A. Rehder | Hungary | | 0.956 |
| L 322825 CPYR 684.001 | P. communis ssp. communis | H.J. Brooks, H.J. Brooks | Crimea, Ukraine | 44.517 | 34 | 0.495 |
| L 377641 CPYR 883.001 | P. communis ssp. pyraster | N.W. Callan | T. Dimitrovski | 1972 | Macedonia | 41.35 | 22.067 | 0.498 |
| L 483385 CPYR 1539.001 | P. communis ssp. pyraster | T. van der Zwart | T. van der Zwart | Poland | 0.267 |

<sup>Q fit is <0.5.</sup>
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 |
| CH01P07A       | 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 |
|---------|--------|---|-----------------|----------------------|-----------------------------|---------------------|-----------------|
| A       | Domesticated | 11  | 0.64            | 3                    | 7                           | 6                   | 70              | 3.28 |
| B       | Various    | 9  | 0.75            | 5                    | 5                           | 7                   | 92              | 3.99 |
| C       | Caucasus, Ukraine | 38 | 0.68          | 11                   | 7                           | 16                  | 113             | 2.90 |
| D       | Caucasus   | 4  | 0.49            | 0                    | 1                           | 4                   | 3               | 3.64 |
| E       | Caucasus   | 5  | 0.60            | 0                    | 2                           | 6                   | 8               | 4.72 |
| F       | Armenia    | 17 | 0.57            | 3                    | 3                           | 6                   | 9               | 6.31 |
| G       | Turkey, Macedonia | 16 | 0.75          | 8                    | 8                           | 11                  | 14              | 107             |
| H       | Macedonia, Germany | 13 | 0.67          | 7                    | 3                           | 8                   | 7               | 6.87 |
| I       | Macedonia, Germany | 10 | 0.70          | 7                    | 5                           | 8                   | 5               | 7.77 |
| J       | Macedonia, Germany | 9  | 0.62            | 0                    | 3                           | 6                   | 5               | 5.57 |
| K       | Europe, various | 7  | 0.74            | 3                    | 5                           | 8                   | 5               | 5.77 |
| L       | Various    | 6  | 0.62            | 3                    | 4                           | 8                   | 5               | 5.14 |

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 (Fst), 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 Fst 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).

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 α = 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
communis 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 subsib 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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