Genetic Relationships of *Pyrus* Species and Cultivars Native to East Asia Revealed by Randomly Amplified Polymorphic DNA Markers

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ABSTRACT. A total of 118 *Pyrus* sp. (pear) and cultivars native mainly to east Asia were subjected to randomly amplified polymorphic DNA (RAPD) analysis to evaluate genetic variation and relationships among the accessions. Two hundred fifty RAPD markers were scored from 20 decamer primers. RAPD markers specific to species were identified. Clustering analysis revealed two divisions; one comprising cultivars of *P. communis* L., and the other including all accessions of *Pyrus* native to east Asia. The grouping of the species and cultivars by RAPD data largely agrees with morphological pear taxonomy. However, some noted incongruence existed between two classification methods. *Pyrus calleryana* Dcne. clustered together with *P. koehneii* Schn. *P. fawcetti* Schn. and *P. dimorphophylla* Makino. *Pyrus betulaefolia* Bge. clustered with *P. ×hopeiensis* Yu and *P. ×phaeocarpa* Rehd. A noncultivated clone of *P. aromatic* Kikuchi et Nakai grouped with *P. aromatica* cultivars. *Pyrus hondoensis* Nakai et Kikuchi and cultivars of *P. ussuriensis* Max. formed a single group. Some accessions from Korea (named Korean pear) had species-specific RAPD markers and comprised an independent group. Most of the Chinese white pears clustered together with most of the Chinese sand pears. Based on the present results, the new nomenclature *P. pyrifolia* var. *sinensis* (Lindley) Teng et Tanabe for Chinese white pear was suggested. Most accessions of Japanese pears fell into one main group, whereas pear cultivars from Kochi Prefecture of Japan subclustered with some Chinese sand pears and one accession from Korea. Our results infer that some local Japanese pear cultivar populations may have been derived from cultivars native to Kochi Prefecture in Shikoku region, and that the latter may have been introduced from ancient China and/or Korea.

The genus *Pyrus*, with the common name pear, belongs to the subfamily Pomoideae, and the family Rosaceae. The basic *Pyrus* stock is believed to have arisen during the Tertiary period in the mountainous regions in western and southwestern People’s Republic of China (Rubtsov, 1944). From the geographical point of view, pears are traditionally divided into two native groups: Occidental pears and Oriental pears (Layne and Quanme, 1975; Lee, 1948; Rubtsov, 1944). The exact number of species in the genus *Pyrus* varies among taxonomists. According to Rubtsov (1944), the Occidental pears include over 20 species found in Europe, northern Africa, Asia Minor, Iran, central Asia, and Afghanistan; the majority of cultivars grown in these areas have originated primarily from *Pyrus communis*. The Oriental pears include 12 to 15 species, distributed from the Tian-Shan and Hindu Kush Mountains eastward to Japan. In a detailed taxonomic study of *Pyrus*, Challice and Westwood (1973) suggested 21 primary species and four geographic groups of species, of which 10 species native to east Asia were assessed. These east Asian pears are distributed primarily in China, Japan, and Korea.

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Except for a few cultivars of *P. aromatica*, the majority of pear cultivars in Japan are usually grouped into *P. pyrifolia* (Japanese pear or nashi). There are two different viewpoints about the origin of the native pear cultivars in Japan. Kikuchi (1948) proposed that Japanese pear cultivars have been domesticated from wild *P. pyrifolia* which occurs in southern Japan. On the other hand, some researchers insisted that some local cultivars in Japan have come from ancient China and the Korean Peninsula (Kajiura et al., 1979; Shirai, 1929). It has been proven that fruit traits in pear cultivars distributed in the Kyushu area and the coast of the Japan Sea are much more similar to Chinese pear cultivars than to those in other areas of Japan (Kajiura and Suzuki, 1980). However, until now there has been little evidence to affirm that Japanese pear cultivars are closely related to pears in China and Korea. On the other hand, Japanese cultivars originating from the Kanto region may be related genetically to the other hand, Japanese cultivars originating from the Kanto region may be related genetically to *P. pyrifolia* (Kajiura et al., 1983) or *P. hondoensis* (Kawata et al., 1995). Therefore, the taxonomic position of pear cultivars in Japan is yet to be determined.

It has been known that *P. ussuriensis*, *P. pyrifolia*, and *P. fauriei* occur in the Korean Peninsula. Pears grown in Korea were classified into many species by Japanese taxonomists (e.g., Uyeki, 1921, 1925). However, most of these so-called species should be treated as cultivars in the strict sense. It remains unclear how these types of pears are assigned to the known species.

Identification of pear cultivars and phylogenetic analysis of pear species have depended traditionally upon an evaluation of morphological characteristics (Kikuchi, 1948; Rehdor, 1915; Yu, 1979; Yu and Kuan, 1963; Yuan and Du, 1980), which is a practiced skill and is made more difficult by the poor morphological diversity among pear species and cultivars. Therefore, some other markers such as phenolic compounds (Challice and Westwood, 1973; Kajiura et al., 1983), isozyme analysis (Jang et al., 1992; Lin and Shen, 1983), pollen ultrastructure (Westwood and Challice, 1978; Zou et al., 1986), and sugar composition (Kajiura et al., 1979) have been used to distinguish pear species and cultivars. The major drawback of these techniques is that their expression is influenced by the developmental stage and may also be vulnerable to environmental influence. In addition, they are limited by the number of informative markers.

Recently, DNA-based molecular markers have been used for cultivar and species identification in many plant species (e.g., Jan et al., 1999; Marquard et al., 1997). Attempts have been made to distinguish Asian pears with restriction fragment length polymorphism (RFLP) of nuclear DNA (Kawata et al., 1995) and chloroplast DNA (Iketani et al., 1998). Both studies have provided new information about east Asian pears. However, conclusive genetic relationships among Asian pear species and cultivars have not been established because of the limited number of entries of Chinese sand pears and relatively small degrees of polymorphism that was found in both studies. The polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD) technique developed by Williams et al. (1990) has been used for identification of plant species and cultivars because of simplicity, versatility, and ability to generate high rates of polymorphism. In related studies on pears, RAPD markers have been used to identify parentage (Banno et al., 2000) and a marker linked to the gene conferring susceptibility to black spot disease [*Alternaria alternata* (Fr.) Keissler] (Banno et al., 1999). Oliveira et al. (1999) and Monte-Corvo et al. (2000) have confirmed that RAPD is useful in pear cultivar identification and genetic classification within the genus *Pyrus*. To our knowledge, no detailed research has been conducted to analyze genetic relatedness among pear species and cultivars native to east Asia using RAPD markers. Therefore, the objective of this study was to use RAPD markers to estimate genetic variation among pear species and cultivars native to east Asia and try to gain new understanding of genetic relationships among east Asian pear cultivars and taxa.

## Materials and Methods

### Plant Material

Plant materials used in this study are listed in Table 1. Two white pear cultivars and all cultivars of Chinese sand pear were collected from the China Pear Germplasm Repository (CPGR), Research Institute of Pomology, Chinese Academy of Agricultural Sciences, located in Xincheng, Liaoning Province, China. Four accessions were from Gansu Pomology Institute (GPI), Gansu Academy of Agricultural Sciences, Gansu Province, China. Young pear leaves were taken in late May 1999 and lyophilized for 72 h. The lyophilized leaves were sealed in plastic bags packed with silicon gel, transported to Japan, and stored at −20°C. Fresh leaves of Japanese pear cultivars and other accessions including three cultivars of *P. communis* were harvested from the pear germplasm collection at Tottori University (TU), Tottori, Japan, and stored at −80°C until needed.

### DNA Extraction and Purification Protocol

In general, 3.5 g of fresh leaves or 1.0 to 1.4 g of lyophilized leaves were ground in liquid nitrogen using a mortar and pestle. The powder was then transferred into 50-mL centrifuge tubes with liquid nitrogen and frozen at −80°C for sample storage before isolating DNA.

Total DNA was extracted following the protocol of Dellaporta et al. (1983), with modifications. Ground tissue was combined with 40 mL of washing buffer (0.1 M HEPES pH 8.0, 0.1% polyvinylpyrrolidone (PVP) (K-40), 1% 2-mercaptoethanol added just before use) and centrifuged at 4°C and 21,400 g for 5 min and the supernatant was discarded. This washing process was repeated three to five times until the aqueous phase showed a clearly greenish color. The pellet was resuspended in 10 mL.

### Table 1. *Pyrus* species and cultivars used in RAPD analysis.

| *Pyrus* sp. or cultivar | Origin | Leaf source |
|------------------------|--------|-------------|
| *P. pyrifolia* Baozhuli | Yunan Province | CPGR |
| Bingzili | Fujian Province | CPGR |
| Cangxu | Sichuan Province | CPGR |
| Chenjiadami | Sichuan Province | CPGR |
| Cangwudashali | Guangxi Province | CPGR |
| Damali | Sichuan Province | CPGR |
| Fuyuanhuangli | Yunan Province | CPGR |
| Haidongli | Yunan Province | CPGR |
| Hengshanli | Taiwan | TU |
| Hongfenlili | Guizhou Province | CPGR |
| Hongpisuli | Sichuan Province | CPGR |
| Hongshaobingli | Sichuan Province | CPGR |
| Huishuijingaii | Guizhou Province | CPGR |
| Huobali | Yunan Province | CPGR |
| Kunningmali | Yunan Province | CPGR |
| Mandingxuei | Fujian Province | CPGR |
| Qiangxiandashali | unknown | CPGR |
| Qubaishali | Fujian Province | CPGR |
| Weiningdahuali | Guizhou Province | CPGR |
Table 1. Continued.

| Pyrus sp. or cultivar | Origin | Leaf source |
|----------------------|--------|-------------|
| Xingyihaizili        | Guizhou Province | CPGR         |
| Yanzhouxuei          | Zhejiang Province | CPGR         |
| Yiwuili              | Zhejiang Province | CPGR         |
| Zongbaoli            | Fujian Province | CPGR         |

Cultivars of Chinese white pear

Duanbajitul         | Sichuan Province | CPGR         |
Enli                | Shandong Province | TU           |
Hongxioli           | Hebei Province | Unknown      |
Jizhuali            | Sichuan Province | CPGR         |
Jinhuai             | Gansu Province | Unknown      |
Laiyangcili         | Shandong Province | TU           |
Lanzhoudoungguoli   | Gansu Province | Unknown      |
Pingli              | Hebei Province | TU           |
Pingguoli           | Jinlin Province | TU           |
Pingzili            | Hebei Province | TU           |
Qubaili             | Hebei Province | TU           |
Wowoli              | Shandong Province | TU           |
Xingjingli          | Unknown      |
Xuehuali            | Hebei Province | Gansu        |
Yali                | Hebei Province | TU           |
Yanbali             | Hebei Province | TU           |
ZhuZhuili           | Unknown      |

Cultivars of *P. ussuriensis* in China

Beijingbailli       | Beijing         | TU           |
Jianbailli          | Liaoning Province | TU          |
Nanguoli            | Liaoning Province | TU          |

Cultivars originated from Korea

Cheongdangnobae     | South Korea     | TU           |
Hanheungli-Kou      | North Korea     | TU           |
Hanheungli-Otsu     | North Korea     | TU           |
Hoeryongbace        | North Korea     | TU           |
Happsinke           | Central Korea   | TU           |

Cultivars of *P. pyrifolia* in Japan

Akaho               | Kanagawa Pref. | TU           |
Akitaizawa-2 (UC)   | Akita Pref.    | TU           |
Amanogawa           | Kochi Pref.    | TU           |
Asahiruyu           | Niigata Pref.  | TU           |
Awayuki             | Unknown        |
Chojuro             | Kanagawa Pref. | TU           |
Doitsu              | Unknown        |
Edoya               | Kanagawa Pref. | TU           |
Gozenashi           | Unknown        |
Hakatao             | Fukuoka Pref.  | TU           |
Hakuteiruyu         | Niigata Pref.  | TU           |
Hatsushimo          | Unknown        |
Heishi              | Kanto Region   |
Imamuraaki          | Kochi Pref.    | TU           |
Inugoroshi          | Akita Pref.?   | TU           |
Iwatemumaku (PA)    | Iwate Pref.    | TU           |
Kinchaku            | Unknown        |
Konpeito            | Ishikawa Pref. | TU           |
Kosainashi          | Unknown        |
Kozo                | Kanagawa Pref. | TU           |
Koyo                | Gunma Pref.    |
Kunitomi            | Niigata Pref.  | TU           |
Meigetsu            | Ishikawa Pref. | TU           |
Miyadani            | Tottori Pref.  | TU           |

Pyrus sp. or cultivar | Origin | Leaf source |
|----------------------|--------|-------------|
Nanagajisi (UC)      | Nagano Pref. | TU           |
Nijisiikei           | Chiba Pref. | TU           |
Okuroku              | Kanagawa Pref. | TU          |
Okusankichi          | Niigata Pref. | TU          |
Ohtazairai           | Shimane Pref. | TU          |
Rokugatsu            | Kanto Region? | TU          |
Ruisannashi          | Niigata Pref. | TU          |
Saizounashi          | Akita Pref. | TU           |
Sannashi             | Iwate Pref.  |
Sekaiichi            | Saitama Pref. | TU          |
Sekiryu              | Chiba Pref.  |
Shikishima           | Chiba Pref.  |
Shimanezai           | Shimane Pref. | TU          |
Shimoichikoboku      | Nara Pref.   |
Shinchu              | Kanagawa Pref. | TU          |
Sotoorishime (PA)    | Akita & Yamagata | TU        |
Taihaku              | Chiba Pref.  |
Taihei               | Kanagawa Pref. | TU          |
Tanponashi           | Shimane Pref. | TU           |
Tosanashiki          | Kochi Pref.  |
Tsukushiunashii      | Kyushu Region | TU           |
Umajirou             | Kochi Pref.  |
Tottorijsu (UC)      | Tottori Pref. | TU           |
Yokogoshi            | Niigata Pref. | TU           |

Cultivars of *P. communis*

Bartlett            | England     | TU           |
La France            | France      | TU           |
Passe Grasstone      | France      | TU           |

Wild pears originating from east Asia

*P. ussuriensis*   | Northeast China | CPGR         |
*P. pyrifolia*     | South China   | TU           |
*P. betulaefolia*-1 | Northeast China | CPGR         |
*P. betulaefolia*-2 | Gansu, China | GPI          |
*P. betulaefolia*-3 | Ningxia, China | CPGR         |
*P. betulaefolia*-4 | Unknown, China | TU           |
*P. hopeiensis*    | Hebei, China | CPGR         |
*P. phaeocarpa*    | North China  |
*P. calleryana-1*  | South China, | TU           |
*P. calleryana-2*  | Liaoning, China | CPGR     |
*P. koehnei*       | South China, Taiwan | TU  |
*P. fauriei*       | Korea        |
*P. hondoensis*    | Middle Japan  |
*P. aromatic*      | Northeastern Japan | TU   |
*P. dimorphophylla*-4 | Mie Pref. Japan | TU     |
*P. dimorphophylla*-5 | Mie Pref. Japan | TU     |
*P. dimorphophylla*-6 | Mie Pref. Japan | TU     |

*Classification of species and cultivars originating from China is based on Pu et al. (1989), Pu and Wang (1963), and Yu (1979). Classification of species originating from Japan is based on Kikuchi (1948). UC = uncultivated and PA = *P. aromatic*.

*Geographic origin of cultivars in China is based on Pu et al. (1989), Pu and Wang (1963); those in Japan are based on Jang et al. (1992), Kajiura and Sato (1990), and Kikuchi (1948).

*Leaf sources are: CPGR = China Pear Germplasm Repository, Research Institute of Pomology, Chinese Academy of Agricultural Sciences; TU = Tottori University. The pear germplasm collection at Tottori University has been established mainly based on the extensive pear collection at the Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Tsukuba City, Ibaraki Prefecture, Japan. GPI = Gansu Pomology Institute, Gansu Academy of Agricultural Sciences, Gansu Province, China.

*Introduced from Oregon State Univ., Corvallis, Ore.
extraction buffer (0.1 M Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 0.5 M NaCl). Then, 1.0 mL of 20% sodium dodecyl sulfate (SDS) was added and mixed thoroughly by vigorous shaking. The extract was incubated at 70 °C for 15 min. The solution was then allowed to cool to about 25 °C, after which the extract was emulsified in one third volume of 5 M potassium acetate by gentle inversion, and incubated at 0 °C (on ice) for 20 min, followed by centrifugation at 4 °C and 21,400 g for 30 min. The supernatant was poured into a clean 50-mL tube containing 1 volume of isopropanol and shaken gently at about 25 °C for about 30 min to precipitate the DNA. The DNA was hooked and put into a 15 mL tube (in most cases) or pelleted after a 5 min centrifugation at 1,300 g. DNA was washed twice with 5 mL of 70% ethanol. The tube was air dried for 20 min. The DNA was redissolved in 1 to 3 mL Tris-EDTA buffer; then 2 to 5 µL of RNase (10 mg/mL) was added, and the solution was incubated at 37 °C for 60 min and stored at 4 °C.

An aliquot of DNA-TE solution was added to a 1.5 mL tube and mixed with 1 volume of 25 phenol : 24 chloroform : 1 isoamylalcohol (PCI) (by volume) and centrifuged at 12,500 g for 3 min. The upper phase was carefully transferred to a new 1.5 mL tube. This process was repeated three to five times until the white layer disappeared between the aqueous phase and PCI phase. The sample was mixed with one tenth volume of 3 M NaCl). Then, 1.0 mL of 20% sodium dodecyl sulfate (SDS) was added and mixed thoroughly by vigorous shaking. The extract was incubated at 70 °C for about 30 min to precipitate the DNA. The DNA was washed twice with 5 mL of 70% ethanol. The tube was air dried for 20 min. The DNA was redissolved in 1 to 3 mL Tris-EDTA buffer; then 2 to 5 µL of RNase (10 mg/mL) was added, and the solution was incubated at 37 °C for 60 min and stored at 4 °C.

Table 2. List of the primers used in the RAPD analysis, their sequence, number of scorable polymorphic bands, and bands specific to species or cultivar.

| Primer | Sequence (5'-3') | Scorable polymorphic bands (no.) | W | Duli | Uss | Cal | Aro | Chi | Cili | K |
|--------|------------------|----------------------------------|----|------|-----|-----|-----|-----|-----|----|
| OPA-07 | GAAACCGGTG       | 11                               | 0  | 0    | 0   | 0   | 0   | 1   | 0   |    |
| OPA-09 | GGTAAACGCC       | 21                               | 1  | 1    | 0   | 1   | 0   | 1   | 0   |    |
| OPA-10 | GTGATCCGAG       | 4                                | 0  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OPA-11 | CAATCGGCCT       | 3                                | 1  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OPA-12 | TC GCCGATAG       | 21                               | 4  | 1    | 1   | 0   | 0   | 0   | 0   |    |
| OPA-16 | AGCCACGCAG       | 15                               | 1  | 0    | 0   | 0   | 0   | 0   | 1   |    |
| OPA-18 | AGGTACGCGT       | 10                               | 3  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OPA-19 | CAACGCTGCG       | 17                               | 2  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OPA-20 | GTTTCGATCC       | 8                                | 1  | 0    | 0   | 0   | 0   | 1   | 0   |    |
| OP-26-02 | TGATGGTGTT      | 11                               | 1  | 0    | 0   | 0   | 0   | 1   | 0   |    |
| OP-26-05 | GAACCAACATC     | 14                               | 2  | 0    | 0   | 0   | 0   | 0   | 1   |    |
| OP-26-08 | TGGTAAGGGG     | 15                               | 2  | 1    | 2   | 0   | 0   | 0   | 0   |    |
| OP-26-13 | GTTTTCGCGA      | 14                               | 1  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OP-26-15 | GATCCGATAC     | 11                               | 0  | 0    | 0   | 1   | 0   | 0   | 0   |    |
| OP-26-16 | GATCAGTCTA      | 10                               | 1  | 0    | 1   | 0   | 0   | 0   | 0   |    |
| OP-26-18 | GATCTCAAGC     | 5                                | 1  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OP-26-20 | GATCAAATGCC    | 15                               | 1  | 0    | 0   | 0   | 0   | 0   | 0   |    |
| OP-26-22 | GATCGCAATGG     | 15                               | 1  | 1    | 1   | 0   | 0   | 0   | 0   |    |
| OP-26-24 | GATCATAGGCC    | 14                               | 2  | 1    | 0   | 0   | 1   | 0   | 0   |    |
| OP-26-25 | GATCTAAGGC     | 16                               | 2  | 1    | 0   | 0   | 0   | 0   | 1   |    |

W = P. communis, Duli = Chinese name of P. ussuriensis, Uss = P. calleryana, Aro = P. aromatica, Chi = Chinese sand pear and white pear, Cili = Laiyang Cili, and K = Korean pear.
including A study. A cultivar specific band was found in however, this specific band was not found in the cultivars share a common progenitor species. white pears (42% of total), which infers that cultivars from the Korean Peninsula occurred and separated by electrophoresis at 85 to 90 V for ≥3 h, and photographed on an ultraviolet transilluminator. The sizes of band amplification products were determined by comparison with Lambda (λ) DNA digested with EcoRI and HindIII restriction enzymes.

Only strong and reproducible bands were scored as present (1) or absent (0) for calculating the Dice coefficients (Nei and Li, 1979) of similarity. Ambiguities were scored as missing data. A dendrogram was constructed based on the similarity matrix data by using the unweighted pair-group method with arithmetic average (UPGMA), using the NTSYS-pc program (Rohlf, 1998).

Following Marquard et al. (1997), average similarities of some representative accessions to main species or taxonomic groups were calculated to help understand the genetic relatedness between each accession and taxa. Morphologically ambiguous accessions (e.g., ‘Pingli’) were excluded from the calculation of average similarities. Because ‘Laiyangcili’, ‘Wowoli’, and ‘Enli’ shared very high similarities (≥0.987) each other and may come from the same lineage (see Results and Discussion), only ‘Laiyangcili’ was included in the calculation of average similarities.

Results and Discussion

Characteristics of RAPD Markers. Use of RAPD markers in some plants has resulted in poor levels of reproducibility. However, in this study, with careful optimization and strict control of PCR conditions, reproducible and bright RAPD bands were obtained (Fig. 1). Using 20 selected primers, 250 polymorphic RAPD markers were scored. The markers ranged in size from 300 to 2000 or rarely 2500 base pairs (bp), but were mostly 500 to 1600 bp. The number of markers scored for each primer ranged from 3 (OPA-11) to 21 (OPA-9 and -12), with an average of 12.5 per primer, which is in the scope reported by Oliveira et al. (1999) and Monte-Corvo et al. (2000) in separate studies related to pear identification using RAPD markers.

Certain amplified bands were found to be specific to a given species, i.e., they were present in (or absent from) only one species but absent from (or present in) the remaining species. These bands could be used for species identification (Table 2, Fig. 1). The bands specific to P. communis were observed in RAPD profiles with most primers and bands specific to P. betulaefolia, P. ussuriensis (including P. hondoensis), P. aromatica, P. calleryana (including P. koehnei, P. fauriei, and P. dimorphophylla), and some pear cultivars from the Korean Peninsula occurred with only some primers. Using primer OPA-9, the unique band near 400 bp was observed for 18 Chinese cultivars of sand pears and white pears (42% of total), which infers that both Chinese white pear and sand pear cultivars share a common progenitor species. However, this specific band was not found in the cultivars of Japanese pears used in this study. A cultivar specific band was found in ‘Laiyang Cili’ and its nearest relatives.

Genetic relationships among and within Pyrus species. Similarity values of accessions, estimated by Dice’s coefficient (Nei and Li, 1979), ranged from 0.284 for P. dimorphophylla-1 and ‘La France’ to 0.993 for ‘Enli’ and ‘Wowoli’. Species and cultivars native to east Asia had the lowest affinity to the cultivars of P. communis (Table 3). The accessions, on the other hand, generally had the highest affinity to the taxa to which they have been assigned, based on morphological traits. However, the boundary of Chinese white pear and sand pear was ambiguous (Table 3). The dendrogram resulting from the UPGMA cluster analysis is shown in Fig. 2. The dendrogram clearly separated all accessions into two divisions at the 0.37 level of similarity. The first division included all accessions of pears native to east Asia and was divided further into 11 major groups, and the second division was formed by a single group of three cultivars of P. communis, which is in agreement with the studies of Kawata et al. (1995), Iketani et al. (1998), Oliveira et al. (1999), and Monte-Corvo et al. (2000), who also divided Pyrus into the occidental group and the oriental group using RFLP or RAPD markers. These results support the traditional view that genus Pyrus consists of two geographic species groups: Occidental pears and Oriental pears (Layne and Quamme, 1975; Lee, 1948; Rubtsov, 1944).

Peas are endemic to east Asia and characterized by their small fruit with a diameter of ≈1 cm. In this study, they were separated into two main groups: P. calleryana group (Group I) and P. betulaefolia group (Group XI) (Fig. 2). Species in both groups had distant affinities to the large-fruited species (Table 3). Group I included P. calleryana and its relatives. These species shared the same 3 species-specific RAPD markers (Table 2). Because they have some resemblance to each other morphologically, these pea pears were treated as varieties of P. calleryana by Rehder (1940). In this group, P. calleryana-1 and P. koehnei branched at a similarity of 0.750 and had a close affinity, which is related with their geographic origins (Table 1). Yu (1979) classified P. koehnei as P. calleryana Dcne. var. koehnei (Schneid.) Yu. In addition, the P. fauriei clone and the P. dimorphophylla clone showed distant relationships with the P. calleryana, which supports the view that P. fauriei and P. dimorphophylla should be treated as independent species (Challice and Westwood, 1973;
Westwood, 1968). Genetic polymorphism was observed within *P. dimorphophylla* (Fig. 2), which reflects the observed morphological polymorphism.

*Pyrus betulaefolia* is another pear species important for its extensive use as a pear rootstock in east Asia. It is distributed from east to west in North China (Pu and Wang, 1963; Yu, 1979). Samples from different regions showed some genetic variation (Fig. 2). *Pyrus betulaefolia* was presumed to be a hybrid between *P. betulaefolia* and *P. ussuriensis* (Challice and Westwood, 1973; Yu, 1979; Yu and Kuan, 1963), and the origin of *P. hopeiensis* may involve *P. betulaefolia* and *P. ussuriensis* (Yu, 1979; Yu and Kuan, 1963). *Pyrus ×hopeiensis* and *P. ×phaeocarpa* were found to share some markers with *P. betulaefolia* and *P. ussuriensis* (Table 2). These data support the hypothesis that *P. betulaefolia* and *P. ussuriensis* are involved in the ancestry of *P. ×hopeiensis* and *P. ×phaeocarpa*.

‘Pingguoli’ (apple-like pear) is one of the leading cultivars in China. The origin and classification of this cultivar are obscure. It is said that this cultivar was introduced into Jilin Province, China, from North Korea in 1921. In 1998, three stock trees of this cultivar were still alive in China (Teng, unpublished data). In the literature, ‘Pingguoli’ has been assigned either to *P. ×bretschneideri* (Pieniazek, 1967; Pu et al., 1989) or to *P. pyrifolia* (Pu and Wang, 1963; Yu, 1979; Zou et al., 1986), and is believed to be a hybrid between *P. pyrifolia* and *P. ussuriensis* (Pu and Wang, 1963). In the present study, it clustered together with some cultivars native to Korea (Group II) (Fig. 2), which genetically supports a geographic origin in North Korea. Cultivars of this group shared some common RAPD bands with *P. ussuriensis*, which infer that they have some relationships with *P. ussuriensis*. Markers specific only to these cultivars were found with some of the primers (Table 2). On the other hand, this group branched distantly from the majority of Chinese white pears or sand pears and Japanese pears (Fig. 2) and had low affinities to other Asian large-sized pears (Table 3). Morphologically and physiologically, ‘Pingguoli’ belongs to neither typical *P. pyrifolia* (or *P. ×bretschneideri*) nor typical *P. ussuriensis* (Pu and Wang, 1963; Zou et al., 1986). Zou et al. (1986) reported that the round pollen grains of ‘Pingguoli’, which are different from those of other pear species.

Table 3. Average similarity (affinity) of some pear accessions to the taxonomic groups of genus *Pyrus*. Averages were computed from the genetic similarity (Dice coefficient; Nei and Li, 1979) matrix.

| Species | Accession | P. betulaefolia | P. phaeocarpa | P. ussuriensis | P. ×hopeiensis | P. ×phaeocarpa | P. pyrifolia from China | P. pyrifolia from Japan |
|---------|-----------|----------------|---------------|---------------|---------------|---------------|-------------------------|------------------------|
| *P. betulaefolia* | *P. betulaefolia*-1 | 0.750 | 0.620 | 0.590 | 0.470 | 0.480 | 0.520 | 0.455 |
| *P. phaeocarpa* | *P. phaeocarpa* | 0.750 | 0.570 | 0.570 | 0.496 | 0.480 | 0.520 | 0.476 |
| *P. ussuriensis* | *P. ussuriensis* | 0.750 | 0.545 | 0.545 | 0.475 | 0.480 | 0.470 | 0.470 |
| *P. ×hopeiensis* | *P. ×hopeiensis* | 0.750 | 0.545 | 0.545 | 0.545 | 0.545 | 0.545 | 0.545 |
| *P. ×phaeocarpa* | *P. ×phaeocarpa* | 0.750 | 0.545 | 0.545 | 0.545 | 0.545 | 0.545 | 0.545 |

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species, could be transmitted to its progenies. Based on the above facts, ‘Pingguoli’ and other cultivars in Group II should be treated as an independent species. Here, the name ‘Korean pear’ is suggested to represent those pears tentatively.

‘Beijingbaili’, ‘Nanguoli’, and ‘Jianbali’, well-known cultivars of *P. ussuriensis*, clustered together with *P. hondoensis* (Group X), which suggests a close relationship between these two species. Moreover, RAPD markers specific to *P. ussuriensis* were also present in *P. hondoensis* (Table 2). Based on morphological traits, *P. hondoensis* was once classified as a variety of *P. ussuriensis*. On the other hand, these Ussurian cultivars grouped separately from wild *P. ussuriensis* (Group III). A similar result was reported by Kajura et al. (1983), who found that flavonoid aglycone (a kind of flavonoid) existed in wild *P. ussuriensis* and a majority of its cultivars, but not in ‘Beijingbaili’ and ‘Nanguoli’, and proposed that the origin of these two latter cultivars may involve hybridization with other pears in China. It is interestingly noted that ‘Pingli’ and ‘Hongxiaoli’, morphologically classified as *P. ×bretschneideri*, clustered together with *P. ussuriensis* (Group III). They may be hybrid cultivars involving *P. ussuriensis*, because some markers specific to *P. ussuriensis* were also observed in RAPD profiles for ‘Pingli’ and ‘Hongxiaoli’.

*Rhus aromatica* grows wild in Iwate, Aomori, and Akita Prefecture, Japan (Kikuchi, 1948). It clustered together with its cultivars ‘Iwatemukaku’ and ‘Sotoorishime’, and an unidentified accession, ‘Naganojisei’ (Group VII in Fig. 2). ‘Sotoorishime’ bears fruit with smooth skin (green), which is different from the russet fruit of wild *P. aromatica* and ‘Iwatemukaku’. Genetically, ‘Sotoorishime’ was closer to Japanese pears than the two latter types (data not presented), which may infer that this cultivar is not a pure cultivar of *P. aromatica*, but a hybrid with *P. pyrifolia*. Cultivars in this group shared some RAPD markers with *P. ussuriensis* or *P. hondoensis*, but also had their own species-specific RAPD markers (Table 2).

Group IV is comprised of Chinese white pears and sand pears, including wild *P. pyrifolia*, and ‘Shimoichikoboku’, a semicultivated pear clone grown in Nara Prefecture in Japan (Fig. 2). It should be noted that ‘Shimoichikoboku’ clustered together with wild *P. pyrifolia* native to China, and showed the closest affinity to the latter (a similarity of 0.763), rather than other Japanese accessions. Taking into consideration the flourishing trade and cultural exchanges between Nara and China during ancient times, it can be hypothesized that ‘Shimoichikoboku’ or its progenitors were probably introduced from ancient China. If this accession is native to Nara Prefecture of Japan, the present result would mean that wild *P. pyrifolia* in both China and Japan is identical. Surprisingly, in this group Chinese white pears and sand pears did not cluster further into separate subgroups based on their presumed taxa (Table 1). In contrast, the subgroup usually formed by a combination of Chinese white pears and sand pears. This clustering could very well represent the true genetic relationship of those clones to each other, as it is based on a rather large number of molecular markers that directly reflect genetic differences at the DNA level. Accessions of Chinese white pears generally had the same affinities to the *P. pyrifolia* group as to Chinese white pear group (Table 3). Previous studies have indicated that Chinese white pears resemble Chinese sand pears in both leaf morphology and fruit texture (Kikuchi, 1948; Pu and Wang, 1963; Yu, 1979), and peroxidase isozymic patterns (Lin and Shen, 1983). All of these data indicate that wild *P. pyrifolia* should be a common progenitor species of both Chinese sand pear and white pear cultivars. Their common RAPD markers (Table 2) further confirmed a close relationship between Chinese white pears and sand pears.

We did not find any RAPD markers specific to *P. betulaefolia* that were present in any accessions of Chinese white pears. This finding does not support the view that *P. betulaefolia* is one of the progenitor species of Chinese white pear (Challilce and Westwood, 1973). Except for ‘Hongxiaoli’ and ‘Pingli’, cultivars native to the north part of Hebei Province, China, and which are hybrids

![Fig. 2. Dendrogram of 118 pear species and cultivars resulting from UPGMA cluster analysis based on Dice’s similarity coefficient (Nei and Li, 1979).](image-url)
involving *P. ussuriensis* (Table 2, Fig. 2), the other accessions of Chinese white pears used in this study were not found to share species-specific RAPD markers with *P. ussuriensis*. It may be appropriate to conclude that the majority of Chinese white pear cultivars originated directly from *P. pyrifolia*. In China, *P. pyrifolia* arose in the Changjiang River valley and bears fruit with either russet skin or smooth green or yellow skin (Kikuchi, 1948; Pu and Wang, 1963; Yu, 1979). The results presented above would infer that through borealization, some *P. pyrifolia* (very probably those with smooth fruit skin) have acquired winter hardness and evolved as new ecotypes, from which cultivars of Chinese white pears have arisen. Taxonomists usually cannot classify distinctly the cultivars of Chinese pears with smooth fruit skin, which are located in the geographic zone of overlap between sand pears and white pears. This is another reason to believe that our inference about the origin of Chinese white pears is reasonable. In the northern part of Hebei Province and southern Liaoning Province, where the distribution of *P. ussuriensis* and white pears overlap, some white pears have hybridized with *P. ussuriensis* and formed types that closely resemble *P. ussuriensis*. So-called wild *P. *\xbretschnedi*eri* grown in northern Hebei Province most probably has no relationship with Chinese white pear cultivars prevailing in North China, because morphological traits of Chinese white pear cultivars differ very much from those of so-called wild *P. *\xbretschnedi*eri*, according to the description of Kikuchi (1946). Until recently, boundaries of cultivated species of *Pyrus* originating from China were poorly understood by researchers external to China. As a result, species names have been misapplied in the taxonomic and agronomic literature. Different authors have often described similar and/or identical genotypes under different names. Based on the above facts, we assign cultivars of Chinese white pears a new name: *P. pyrifolia* var. *sinensis* (Lindley) Teng et Tanabe, which reflects the status of Chinese white pears more exactly than does *P. ussuriensis* var. *sinensis* Kikuchi.

Data from group IV show a close relationship between some pear cultivars that originated in the same geographic location. ‘Eali’, ‘Wowoli’, and ‘Laiyangcili’, which originated in Shandong Province in China were tightly subclustered together with ‘Qiubaili’, a cultivar grown in Hebei Province, where is adjacent to Shandong Province, and distantly related to the large main group of Chinese white pears and sand pears. ‘Eali’, ‘Wowoli’, ‘Laiyangcili’, and ‘Qiubaili’ share a common RAPD band (Table 2). Morophologically, they have specific characteristics, such as fruit skin covered with many large russetted lenticels, and thick coriaceous leaves, which make them clearly distinguishable from other white pears (Kikuchi, 1948). These morphological and genetic results suggest a common lineage among these cultivars. ‘Chenjiadami’ and other cultivars, all native to Sichuan Province (Table 1), subclustered closely. ‘Hengshenli’, a cultivar extensively grown in Taiwan, which is said to have been introduced from southern China (Lin et al., 1991), subclustered with ‘Zongbaoli’ (similarity of 0.792), which is native to Fujian Province.

Most Japanese pear cultivars fell into Group V (Fig. 2). Among all accessions of Japanese pears, ‘Kopeito’ and ‘Koyuki’, which originated from different regions (Table 1), had the highest affinity, with a similarity of 0.957. This finding is consistent with the result from an isozyme analysis in a separate study (Jang, 1992), that reported the same pattern of peroxidase isozymes for these two cultivars. These results suggest that these two cultivars have a relatively similar genetic background, and may have arisen in the same region. ‘Nijisseiki’, which originated in Chiba Prefecture, is one of the most well-known Japanese cultivars. It had the highest affinity (similarity of 0.859) to ‘Heishi’, a cultivar originating from the Kanto Region, and clustered with ‘Sekaishi’ and ‘Taihaku’, also from the Kanto region. Previous research has suggested that Japanese cultivars native to the Kanto region, including ‘Nijisseiki’, may be related genetically to *P. hondoensis* (Kawata et al., 1995) or *P. aromatica* (Kajiura et al., 1983). However, we did not find RAPD markers from *P. hondoensis* or *P. aromatica* that were present in ‘Nijisseiki’ or other cultivars of Japanese pears (Table 2).

Within Group V, several subclusters were identified (Fig. 2). Some cultivars subclustered according to their origin. ‘Akaho’, ‘Shinchun’, ‘Chojuro’, ‘Kouzou’, ‘Okuroku’, and ‘Taihei’ native to Kanagawa Prefecture clustered together (J2 subgroup). Most cultivars from Kochi Prefecture also subclustered. However, most others did not subcluster according to their geographic distribution. For those cultivars native to Kanagawa Prefecture, it appears likely that there was relatively little widespread movement before recent times. For other Japanese pear cultivars, widespread movement would have been more common.

It was said that wild *P. pyrifolia* was once distributed in the Shikoku and southern Kyushu regions of Japan, where the climate was warmer than in other areas (Kikuchi, 1948). However, cultivation of Japanese pears flourished in Niigata and Gunma Prefectures in North Japan. Most of the native cultivars of Japanese pears originated from Kanagawa, Niigata and Chiba Prefectures, which is incongruent with distribution of wild Japanese pears (Kikuchi, 1948). If cultivars of Japanese pears have been derived from wild *P. pyrifolia* grown in Japan, primitive cultivars in these areas should have been introduced from Kochi Prefecture and other areas where wild *P. pyrifolia* grows. In this study, some accessions from Kochi Prefecture and ‘Tsukishinunashi’ from the Kyushu region subclustered in Kochi subgroup (Fig. 2). ‘Ichiharawasa’ from Kochi Prefecture was included in the J1 subgroup. Some accessions native to areas outside of Kochi Prefecture, such as ‘Taponashi’ in the J1 subgroup, and ‘Inugoroshi’ in the J3 subgroup, shared high similarities with some cultivars in the Kochi subgroup (data not presented). These data suggest that germplasm native to Kochi Prefecture is related to other populations of Japanese pear cultivars.

Most accessions of Japanese pears showed much higher affinities to other cultivars of the same taxa (Japanese pear group) than to cultivars of Chinese sand pears, although both kinds of pears belong to the same species, *P. pyrifolia* (Table 3). Results of our study suggest that intrapopulation genetic variation within Japanese pears is smaller than that within Chinese sand pears, which supports the view of Kikuchi (1948) that Japanese pears are genetically more homogeneous than Chinese pears.

Three of the five cultivars native to Kochi Prefecture were clustered with some Chinese sand pear cultivars and one Korean sand pear cultivar (Fig. 2). These Chinese sand pear cultivars showed similar affinities to both the Chinese sand pear group and the Japanese pear group (Table 3). In addition, ‘Umajiro’, a cultivar originating from Kochi, clustered into Group VI with Chinese sand pears, two cultivars from Fujian Province, and one cultivar from Sichuan Province. These results suggest that at least some old cultivars of Japanese pears may have been introduced from ancient China or Korea. To clarify relationships among cultivars of *P. pyrifolia* grown in China, Korea, and Japan, further studies will be needed using large samples from Korea.

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and from Zhejiang and Fujian Provinces of China. ‘Akitataza-2’, ‘Ohtazairei’, and ‘Shimanezaizai’ fell outside the main group of Japanese pears and formed an independent group (Group VIII). These three cultivars had distant affinities to other Japanese pear cultivars (Fig. 2). Some RAPD markers common to _P. communis_ were also found in ‘Shimanezaizai’, which means this cultivar may be a hybrid between Japanese pear and _P. communis._

‘Hongfenli’, a Chinese sand pear from Guizhou Province was found to be distantly related to other cultivars of _P. pyrifolia_ from both China and Japan (Table 3) and clustered independently as Group IX (Fig. 2), which reflects its genetic uniqueness from other cultivars of _P. pyrifolia._ For this reason, it may be a useful source of genetic diversity.

In summary, results herein indicate that the RAPD technique is useful in distinguishing species and cultivars of the genus _Pyrus_. RAPD markers specific to species or cultivar were identified. The grouping of the species and cultivars based on RAPD data agrees to a large extent with pear taxonomy based on morphological traits. New findings from this study will help to establish correct phylogenetic relationships in the genus _Pyrus_ native to east Asia and to clarify the origin of large-fruited species of _Pyrus_, especially Chinese white pears and Japanese pears.

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