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Review

Genetic structure and diversity of the wild Ussurian pear in East Asia

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The Ussurian pear is the most important cultivated pear in the northern part of China. Cultivated Ussurian pears are considered to have derived from *Pyrus ussuriensis* Maxim. which is native to the northeast of China. In Japan, two varieties of *P. ussuriensis*, *P. ussuriensis* var. *aromatica* and var. *hondoensis* are native to the northern area and the central area of the main island respectively. In order to reveal the origin of *Pyrus ussuriensis* var. *aromatica* distributed in the northern area of main island of Japan, more than 40 explorations have been performed in Japan and in China, and more than 30 natural habitats were recognized. These natural habitats are at risk of extinction because of human development and forest degradation caused by climate change. Population structure and genetic diversity of *P. ussuriensis* in China and *P. ussuriensis* var. *aromatica* in Japan have been investigated using both morphological and molecular markers in order to define appropriate conservation units, and to provide a good focus for conservation management. Distant evolutionary relationships between *P. ussuriensis* Maxim. in China and *P. ussuriensis* var. *aromatica* in Japan inferred from population genetic structure and phylogenetic analysis are also discussed.

Key Words: *Pyrus ussuriensis*, *Pyrus ussuriensis* var. *aromatica*, Ussurian pear, conservation, population genetics, introgression, SSR.

Introduction

Pears, which are cultivated throughout the temperate regions and have spread all over the world, belong to the genus *Pyrus* in the tribe Pyreae of subfamily Spiraeoidae in the Rosaceae (Campbell *et al.* 2007). The genus *Pyrus* is considered to have originated during the Tertiary period (65–55 million years ago) in the mountain regions of southwestern China (Bell *et al.* 1996). In East Asia, the major cultivated pears are classified traditionally into three species, *Pyrus ussuriensis* Maxim., *P. bretschneideri* Rehder, and *P. pyrifolia* (Burm. f.) Nakai (Gu and Sponberg 2003, Yü and Ku 1974). Several types of cultivar generally corresponding to these botanical classifications have been recognized in horticulture; the Ussurian pear referred to as *P. ussuriensis*, the Chinese white pear referred to as *P. bretschneideri*, the Chinese sand pear, and the Japanese pear referred to as *P. pyrifolia* (Bell *et al.* 1996, Yü 1979).

P. ussuriensis (Ussurian pear) known with aromatic fruit volatiles is the most important cultivated pear in the northern part of China (Kang *et al.* 2010, Li *et al.* 2013, 2014, Qin *et al.* 2012). More than 150 cultivars are known to have originated from this species. Cultivated Ussurian pears are considered to be derived from *P. ussuriensis* which is native to the northern part of China (Pu and Wang 1963).

Ussurian pear is native to the north eastern part of China, the far east of Russia, the northern part of the Korean Peninsula, and the north eastern part of the main island of Japan (Gu and Sponberg 2003, Maximowicz 1859). In China, wild *P. ussuriensis* trees grow in Heilongjiang, Jilin, Liaoning, Inner Mongolia, Heibei, Shandong, and Shanxi (Jiao *et al.* 2000, Wuyun *et al.* 2013). The CAAS report (Report of the Chinese Academy of Agricultural Science 1963) described how wild Ussurian pear trees were distributing from Shuangfeng to Jiameng in Heilongjiang Province, especially abundant in the Yichun forest farms of Heilongjiang Province.

However the forests in Heilongjiang and Jilin are being harmed by human development, so that the habitats of the wild Ussurian pear are decreasing. On the other hand, in Inner Mongolia, there has been little rainfall for the past 30 years, which has led to serious drought damage, desertification and

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soil degeneration (Wuyun *et al.* 2013). Consequently wild Ussurian pears in Inner Mongolia are decreasing. According to research by Ma *et al.* (2011) and Wuyun *et al.* (2013), there were over 1,000 wild Ussurian pear trees in six natural habitats in 2009, but about two thirds of those trees have now been killed by drought after a shortage of rainfall for more than 100 days in 2010 (Wuyun *et al.* 2013). Therefore, conservation is urgently required.

The characteristics, which can distinguish wild Chinese Ussurian pear from other *Pyrus* species are as follows; (1) Morphological features: short peduncle and pedicel, usually 1 to 2 cm (Gu and Sponberg 2003). (2) Cold resistance : this species can endure a temperature of –52°C. (3) After ripening, their eating quality can improve (Yü 1979). But the classification of cultivated Ussurian pears in China is problematic. Although floristic studies adopted the short fruit pedicel (<2 cm) in order to discriminate *P. ussuriensis* from *P. pyrifolia* and *P. bretschneideri* (Gu and Sponberg 2003), many cultivars with longer fruit pedicels have been classified as *P. ussuriensis* in horticultural literature (Iketani *et al.* 2012, Yü 1979).

In Japan, in the early 20th century, as two new native species of *P. ussuriensis* with aromatic volatiles in the fruit were described from two distantly located regions (Nakai 1918): *P. hondoensis* Nakai et Kikuchi from the central area and *P. aromatica* Kikuchi et Nakai from the northern part of the main island in Japan. Later Ohwi (1965) recognized these as two varieties of *P. ussuriensis* i.e., *P. ussuriensis* var. *hondoensis* and *P. ussuriensis* var. *aromatica*, respectively. As indicated by its Latin name, 'aromatica', good aroma is an obvious characteristic of *aromatica* fruit (Katayama *et al.* 2013). Taxonomically, the presence of calyx in the fruit is also considered an important characteristic of var. *aromatica* (Ohwi 1965). In this review, we follow the nomenclature of Ohwi (1965), i.e., *P. ussuriensis* Maxim. var. *aromatica* (Nakai et Kikuchi) Rehd. [for the details of nomenclature, refer to Katayama and Uematsu (2006)] (Ohwi 1965) and *P. ussuriensis* Maxim. var. *hondoensis* (Nakai et Kikuchi) Rehd. (Rehder 1920).

Wild populations of *P. ussuriensis* var. *hondoensis* and var. *aromatica*, which are distributed in the central part and northern part of the main island of Japan respectively, have different habitats: they grow in deciduous forests at altitudes of 900–1800 m and 500–1100 m asl, respectively. Recent taxonomic studies recognized var. *aromatica* as convarietal with var. *ussuriensis* because Asian continental (var. *ussuriensis* sensu stricto) and Japanese (var. *aromatica*) populations of *P. ussuriensis* could not be distinguished morphologically, whereas var. *hondoensis* is distinguished by smaller leaves and serrated (not setoso serrated) leaf margins (Iketani and Ohashi 2001, 2003). Still the origins of *P. ussuriensis* var. *hondoensis* and var. *aromatica* in Japan remain unclear.

The morphological discriminating feature between *P. ussuriensis* in Japan and *P. pyrifolia* is the persistency of calyces of its mature fruit, which are deciduous in *P. pyrifolia* (Rehder 1940). However, calyx persistency is unstable, because interspecific hybrids between *P. ussuriensis* and *P. pyrifolia* with morphologically intermediate traits, arise easily because of cross-compatibility.

Many pear trees with wide morphological variations were discovered in and around the Kitakami Mountains including some that were morphologically intermediate between *P. ussuriensis* and *P. pyrifolia* (Katayama and Uematsu 2006). Traditional methods i.e., morphological comparisons, suggested the possible presence of true native populations. However, it was difficult to conclude which is true native because of such a divergent morphology.

To overcome the difficulties in identifying biological taxa or horticultural cultivars only by morphological characters, molecular approaches using nuclear DNA markers, e.g., simple sequence repeat (nSSR), AFLP, or chloroplast DNA (cpDNA) markers representing structural rearrangements and cpSSR have been applied to cultivated and wild trees such as *Malus*, *Sorbus* or *Pyrus*. These methods have revealed the origins, genetic diversity and population genetic structures (Coart *et al.* 2003, 2006, Fernandez-Fernandez *et al.* 2006, Gianfranceschi *et al.*1998, Iketani *et al.* 2010, Katayama and Uematsu 2003, Katayama *et al.* 2007, 2012, Kato *et al.* 2013, Liebhard *et al.* 2002, Liu *et al.* 2012, 2013, Nishitani *et al.* 2005, Robertson *et al.* 2004, Sawamura *et al.* 2004, Song *et al.* 2014, Terakami *et al.* 2012, Volk *et al.* 2006, Yamamoto *et al.* 2001, 2002a, 2002b, Zhong *et al.* 2014).

SSR markers have been adopted to study relationships between cultivated Chinese Ussurian pears and a few wild Ussurian pears (Cao *et al.* 2012). Wuyun *et al.* (2013) revealed the genetic diversity of Chinese wild Ussurian pear using hypervariable regions of cpDNA. A large-scale ecological and population investigation for *P. ussuriensis* in China carried out by nSSR and cpSSR markers indicated suitable conservation units and possible conservation strategies (Wuyun *et al.* 2015).

A wide range of genetic diversity was revealed for var. *aromatica* distributed in the northern part of Japan using nSSR and cpDNA markers (Katayama *et al.* 2007). Based on population genetic structure analysis using nSSR markers, Iketani and Katayama (2012) and Iketani *et al.* (2010) demonstrated a possible introgression between native var. *aromatica* and prehistorically naturalized *P. pyrifolia* in the Kitakami Mountains in the northern part of Japan. These results provided useful information for the conservation of genetic resources of the native Ussurian pear in China and in Japan.

In this review, recent advances in molecular studies for the conservation of native Ussurian pears in both China and Japan are introduced. Based on new population structure data, conservation units requiring urgent action to save wild resources are identified. In addition, in order to clarify the origin of var. *aromatica* in Japan, genetic relationships between *P. ussuriensis* in China and var. *aromatica* in Japan are discussed using new population structure data obtained from recent intensive explorations for var. *aromatica*.

Population genetic structure of *P. ussuriensis* in China

The distribution of wild Ussurian pears (*P. ussuriensis* Maxim.) in Inner Mongolia, Jilin, and Heilongjiang provinces was surveyed eleven times between 2009 and 2011 (Ma *et al.* 2011, Wuyun *et al.* 2013). A total of 1,518 pear trees from thirteen populations were found in these areas (**Fig. 1**). Even though the natural habitats of wild Ussurian pears in China have been seriously damaged, we found more than 1500 wild individuals. The number of wild Ussurian pear trees has been decreasing due to desertification in Inner Mongolia and because of continuous land development by humans in Jilin and Heilongjiang (Ma *et al.* 2011, Wuyun *et al.* 2013).

In Inner Mongolia, more than 1,000 wild Ussurian pear trees were found in the six natural habitats of Reshui, Qinshan and Tuohe forestry stations in Keshiketengqi, Chifeng City (**Table 1**). These habitats are located at relatively high altitudes (1,000 to 1,200 m above sea level). Trees were distributed over mountains or along valleys. The local people call these habitats ‘Shanli gu’, which in Chinese means ‘mountain pear valley’ (Ma *et al.* 2011).

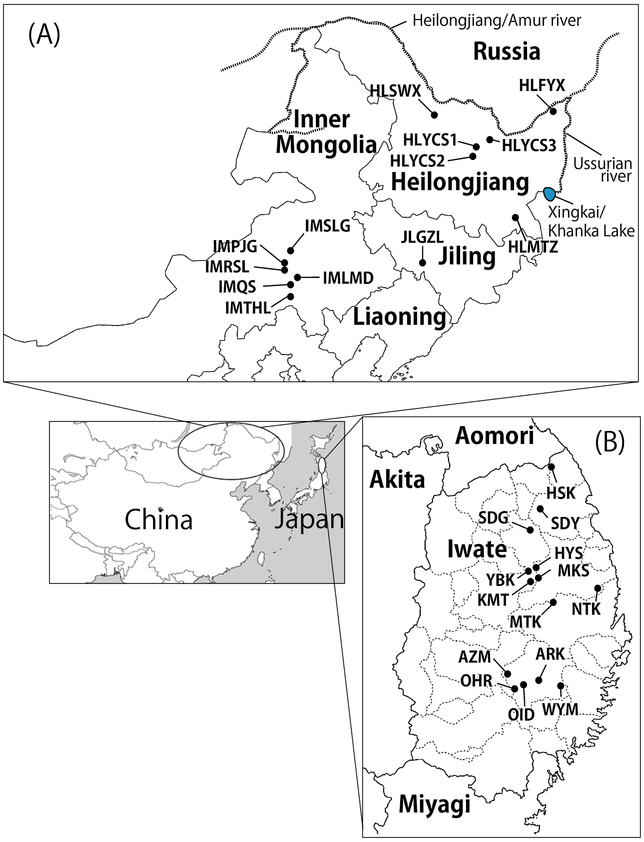

Fig. 1. Geographical distribution of populations originating from Inner Mongolia, Heilongjiang and Jilin province in China (A) and from the Kitakami Mountains in Japan (B). Dotted line in (A) mean Heilongjiang (Amur) and Ussurian rivers along international boundary. Nine populations, SDY, SDG, YBK, KMT, MKS, MTK, NTK, AZM, and ARK in (B) were collected for this review. For detailed information of the other populations, refer to Iketani *et al.* (2010) and Wuyun *et al.* (2015).

In Heilongjiang province, more than 500 trees were recognized in six populations (two in Sunwu county, three in Yichun city and a single population in Fuyuan county) (Ma *et al.* 2011). The area of each population was bigger than that of the Inner Mongolian habitat. In Jilin province, 12 wild trees were distributed within about 10 km distance between Lishu and Gongzhuling. The wild Ussurian pear habitats in Heilongjiang and Jilin provinces are located at lower altitudes (300 to 600 m asl) compared to the Inner Mongolian habitat. The tree distribution in Heilongjiang and Jilin was completely different from that in Inner Mongolia i.e., in Heilongjiang and Jilin, trees were usually found close to places of human activity such as around a farmhouse, at a corner of a field or on the edge of a forest but were rarely found in the mountain area (Wuyun *et al.* 2013).

Wuyun *et al.* (2015) calculated genetic diversity and genetic structure using nSSR and cpSSR markers for a total of 153 individuals of wild Ussurian pear belonging to thirteen populations (six populations from Inner Mongolia, six from Heilongjiang, and one from Jilin). As a result, the genetic diversity of individuals was revealed to be low as a result of habitat fragmentation (Wuyun *et al.* 2015). The genetic diversity of the populations from Inner Mongolia (group 1) and the north east of Heilongjiang (group 2 for HLYCS3 and HLFYX) was especially low and there was the possibility of inbreeding. Wild Ussurian pears were divided into five groups based on the Bayesian clustering method using 20 nSSR markers. The populations from Inner Mongolia, the north east of Heilongjiang (group 3 for HLYCS2) especially represented unique genotypes. Two areas, one being the whole area of Inner Mongolia and the other, the north east of Heilongjiang, require urgent conservation such as *ex situ* conservation, because of their genetic particularity and vulnerability. Four conservation units which include two units corresponding to the two urgently required areas mentioned above, were recognized based on the clustering of nSSR markers, and by geographic factors (Wuyun *et al.* 2015). Furthermore, to investigate the origin of Chinese wild ussurian pear, the relationships between 25 haplotypes represented by 16 cpSSR markers in wild and cultivated Ussurian pears in China were analyzed by a median-joining network model. Haplotype E which was the most common haplotype in the north east of Heilongjiang with haplotype network was considered to be the divergent center of wild Ussurian pears in China (Wuyun *et al.* 2015). The wild Ussurian pear in China might have diverged from the north east of Heilongjiang. However, in order to reveal the origin of the Ussurian pear, further analysis, using more wild Ussurian pears collected from the north east and west of Heilongjiang and far east of Russia, is required.

Table 1. Distribution of 27 populations of *P. ussuriensis* Maxim. in China and *P. ussuriensis* var. *aromatica* in Japan

Population name	Location	Number of individual	Latitude (°N)	Longitude (°E)	Altitude (m)
China					
IMQS[a]	Qingshan forest farm, Keshiketengqi, Chifeng city, Inner Mongolia	18	43°14′61″–43°30′00″	117°49′96″–117°82′00″	950.50–1157.00
IMTHL[a]	Tuohe forest farm, Keshiketengqi, Chifeng city, Inner Mongolia	26	43°04′00″–43°07′00″	117°52′89″–117°89′00″	1046.31–1157.00
IMPJG[a]	Pijianggu, Keshiketengqi, Chifeng citiy, Inner Mongolia	20	43°36′00″–43°60′00″	117°46′53″–117°78′00″	1053.51–1106.00
IMRSL[a]	Reshui forest farm, Keshiketengqi, Chifeng city, Inner Mongolia	6	43°36′16″–43°36′18″	117°46′74″–117°46′77″	1003.00–1124.00
IMLMD[a]	Lamadong, Keshiketengqi, Chifeng city, Inner Mongolia	10	43°25′00″–43°42′00″	117°82′00″–117°83′00″	1108.21–1163.25
IMSLG[a]	Shanligu, Keshiketengqi, Chifeng city, Inner Mongolia	11	43°38′01″–43°64′00″	117°46′46″–117°77′00″	1132.62–1271.00
HLYCS1[a]	Wumahe, Youhao, Yichun, Heilongjiang	4	47°23′07″–47°59′01″	128°12′05″–128°48′01″	230.00–248.00
HLYCS2[a]	Cuiluan, Yichun, Heilongjiang	9	47°23′07″–47°59′01″	128°12′05″–128°44′50″	300.00–500.00
HLYCS3[a]	Meixi, Yichun, Heilongjiang	10	47°53′48″–47°53′86″	129°31′70″–129°31′94″	250.21–267.69
HLFYX[a]	Fuyuan, Jiamusi, Heilongjiang	10	48°32′84″–48°32′85″	134°32′37″–134°32′44″	103.23–115.48
HLSWX[a]	Sunwu, Heihe, Heilongjiang	7	49°03′20″–49°39′54″	127°40′00″–127°56′59″	120.00–400.00
HLMTZ[a]	Muling, Mudanjiang, Heilongjiang	10	44°41′00″–44°41′40″	130°32′17″–130°28′17″	302.00–355.00
JLGZL[a]	Gongzhuling, Jilin	12	43°10′00″–44°09′05″	124°01′00″–125°18′00″	400.00–600.00
QZL[a]	Ussurian pear cultivars in China	29			
Japan					
HSK[b]	Hashikami Mountain, Hashikami, Aomori	12	40°24′29″–40°24′41″	141°35′13″–141°35′18″	510.00–620.00
SDY[c]	Sodeyama, Kuzumaki, Iwate	13	39°59′41″–40°01′21″	141°30′56″–141°31′54″	560.00–1070.00
HYS[b]	Hayasaka highland, Iwate	42	39°50′13″–39°51′49″	141°29′44″–141°30′59″	840.00–970.00
SDG[c]	Sodegawa, Morioka, Iwate	12	39°54′06″–39°59′31″	141°25′55″–141°27′45″	520.00–900.00
YBK[c]	Yabukawa, Morioka, Iwate	25	39°46′51″–39°50′58″	141°18′58″–141°26′59″	620.00–700.00
KMT[c]	Karamatusawa, Morioka, Iwate	26	39°44′20″–39°50′35″	141°24′03″–141°25′48″	640.00–780.00
MKS[c]	Mukainosawa, Morioka, Iwate	18	39°47′29″–39°50′10″	141°27′13″–141°28′02″	600.00–780.00
MTK[c]	Matsukusa, Miyako, Iwate	27	39°38′12″–39°48′22″	141°26′14″–141°32′38″	620.00–1100.00
NTK[c]	Natsukari upland, Miyako, Iwate	26	39°42′40″–39°43′27″	141°39′13″–141°40′17″	640.00–880.00
AZM[c]	Azumane pasture, Hanamaki, Iwate	20	39°27′40″–39°28′07″	141°24′35″–141°25′06″	630.00–860.00
OHR[b]	Ohora pasture, Tono, Iwate	20	39°24′50″–39°25′06″	141°26′10″–141°27′07″	580.00–740.00
OID[b]	Oide pasture, Tono, Iwate	10	39°28′29″–39°28′38″	141°30′13″–141°31′19″	420.00–520.00
ARK[c]	Arakawa pasture, Tono, Iwate	12	39°27′15″–39°29′35″	141°32′57″–141°36′07″	800.00–980.00
WYM[b]	Wayama pasture, Kamaishi, Iwate	11	39°23′31″–39°23′44″	141°40′58″–141°41′30″	600.00–800.00
Local[b]	local varieties from *P. ussuriensis*	14			

[a] Refer to Wuyun *et al.* (2013, 2015) for more information.
[b] Refer to Iketani *et al.* (2010) for more information.
[c] Wild populations collected for this review.

True native populations of *P. ussuriensis* var. *aromatica* in Japan

Iketani *et al.* (2010) investigated the native populations of *P. ussuriensis* (var. *aromatica* and var. *hondoensis*) in Japan using Bayesian statistical inference. Five hypothetical ancestral groups were suggested as a result of analysis using 226 individuals from six regions including Asian Continental local cultivars and wild individuals. Although all of var. *hondoensis* in Chubu region showed true native genotype, many individuals belonging to the groups of var. *aromatica* collected from in and around the Kitakami Mountains in northeastern Japan including wild trees and local landraces, were shown to be genetically admixed with *P. pyrifolia*. This result clearly indicated introgression between native var. *aromatica* and prehistorically naturalized *P. pyrifolia* trees. In addition, commonly observed trees harbouring intermediate characteristics supported this introgression (Iketani and Katayama 2012, Iketani *et al.* 2010).

Of the three wild populations (the first is HSK, the second is HYS, and the third is, referred to as TNO by Iketani *et al.* (2010), including ARK, OHR, and OID in this review) from the Kitakami Mountains, only HYS had a higher proportion of true native individuals than those of the other gene pools. Their present habitats were found in human-disturbed secondary forests or pasturelands in the Kitakami Mountains. Native individuals have likely sustained a genetic influence from cultivated or escaped trees. Thus, this group may have maintained a highly native genetic structure through sheer chance (Iketani *et al.* 2010). Based on this result, var. *aromatica* in the Kitakami Mountains has been registered as a threatened species in the Japanese National Red List of Threatened Plants (Ministry of Environment, Japan 2007).

New individuals and habitats around the Kitakami Mountains (**Table 1**, **Fig. 1**) have been found as a result of more intensive explorations conducted after a report by Iketani *et al.* (2010). Only about one hundred trees distributed in the central area of the Kitakami Mountains including three populations of HYS, KMT, and MKS as blue cluster (**Fig. 3**), were identified as true native populations by means of population genetic structure analysis using the same nSSR and cpDNA markers as Iketani *et al.* (2010) and Katayama *et al.* (2012) (Unpublished data).

Diversity in floral and fruit morphology of *P. ussuriensis* in China

In order to demonstrate the relationships between populations of *P. ussuriensis* in China based on floral and fruit morphology, principal component analysis (PCA) was carried out using six floral and five fruit characteristics (Wuyun *et al.* 2015). The wild populations in Inner Mongolia and Heilongjiang showed a tendency towards small flowers, short peduncles, and slender petals. Short peduncle length agreed with the length of 1 to 2 cm for *P. ussuriensis* described by Gu and Sponberg (2003). In contrast, most Ussurian cultivars had large flowers, long peduncles, and round petals. The flowers of two populations (HLMTZ and HLFYX) from Heilongjiang showed an intermediate flower morphology i.e., relatively large flowers and long peduncles (Wuyun *et al.* 2015). Based on these results, the fruit morphology of wild Ussurian pear in China could be divided into 2 groups; (1) five populations from Inner Mongolia and one population from Heilongjiang had globular shape and short peduncles; (2) two populations from Heilongjiang had an intermediate elliptical shape and relatively long peduncles.

Differences in floral and fruit morphology between *P. ussuriensis* in China and var. *aromatica* in Japan

To investigate the origin of var. *aromatica* in the Kitakami Mountains, Japan, floral and fruit morphologies of var. *aromatica* were newly compared with those of *P. ussuriensis* in China in this review. Five flower morphologies i.e., flower diameter, petal length, petal width, petal length/petal width, and peduncle length, and five fruit morphologies i.e., fresh weight, fruit diameter, fruit length, peduncle length, and calyx existence, were measured to perform PCA analysis, for seven populations (IMQS, IMTHL, IMLMD, IMSLG, HLYCS3, HLFYX, and HLMTZ), and 26 Ussurian cultivars from China, and eleven populations (HSK, SDY, HYS, SDG, YBK, KMT, MKS, MTK, AZM, OHR, and ARK) from Japan. For each population, twenty flowers were used for measurements (**Fig. 2**). The flower and fruit morphology

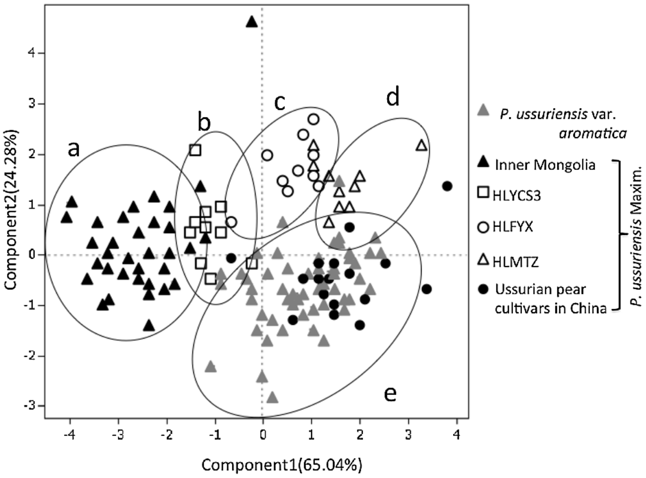

Fig. 2. Principal component scores plotted for principal component 1 and 2. The representative populations marked by circles were calculated from the measurements of five floral morphological characters. Circle a; Inner Mongolia, b; HLYCS3, c; HLFYX, d; HLMTZ, and e; Chinese Ussurian pear cultivars and *P. ussuriensis* var. *aromatica* in Japan.

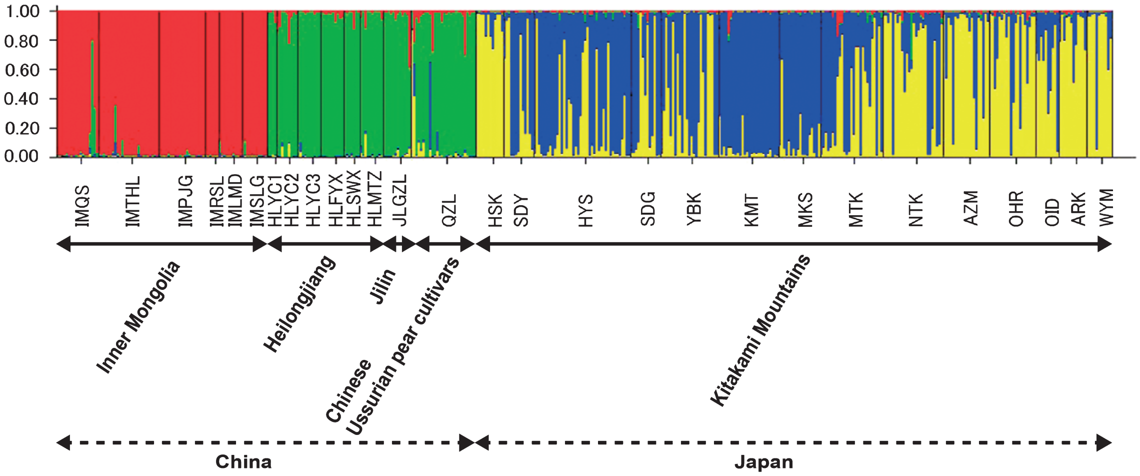

Fig. 3. Percentage membership of genotypes to clusters (q value) inferred at K = 4 (independent model) applying all samples without local varieties originated from *P. ussuriensis* var. *aromatica* to STRUCTURE analysis. Each genotype is represented by a vertical line divided into colored segments. The length of the vertical lines indicates the proportion of genome attributed to the inferred clusters.

of wild *P. ussuriensis* from China were considerably different from those of var. *aromatica* from Japan, which showed similarities to Chinese Ussurian cultivars (**Fig. 2**). Although recent taxonomic studies recognized var. *aromatica* as convarietal with var. *ussuriensis* (Iketani and Ohashi 2001, 2003), in this review we use the nomenclature of Ohwi (1965) i.e., two varieties (var. *hondoensis* and var. *aromatica*) of *P. ussuriensis*, because wild *P. ussuriensis* from China and var. *aromatica* from Japan could be distinguished morphologically. Most individuals of the Chinese Ussurian cultivars and var. *aromatica* from Japan had large flowers, relatively long peduncles, and round petals. In general cultivars have larger flowers compare to wild. But on this occasion, var. *aromatica* thought to be wild had large flowers and long peduncles. This might arise as a result of the introgression from cultivated Japanese pear to var. *aromatica*. There might have been an ancestral population of Japanese var. *aromatica* with small flowers, short peduncles and thin petals (characteristics of wild *P. ussuriensis* in China) but the present populations might have lost those original features due to intensive introgression.

Table 2. Genetic characteristics within 27 populations of the *P. ussuriensis* Maxim in China and *P. ussuriensis* var. *aromatica*

Population name	nSSR markers					
	n	Na	Ne	Ho	He	FIS
China						
IMQS	18	4.00	2.65	0.357	0.548	0.25836 **
IMTHL	26	4.14	2.39	0.378	0.524	0.14861 *
IMPJG	20	3.57	2.25	0.363	0.502	0.12909
IMRSL	6	2.57	1.78	0.300	0.425	0.21569
IMLMD	10	3.71	2.53	0.357	0.534	0.30663 **
IMSLG	11	3.29	2.20	0.322	0.534	0.30736 **
HLYCS1	4	2.86	2.26	0.369	0.553	0.22857
HLYCS2	9	5.14	3.57	0.470	0.669	0.34583 **
HLYCS3	10	4.00	2.78	0.365	0.573	0.25865 **
HLFYX	10	3.14	2.12	0.457	0.496	0.03571
HLSWX	7	4.29	3.42	0.490	0.664	0.32344 **
HLMTZ	10	4.00	2.58	0.344	0.582	0.21918 *
JLGZL	12	5.71	3.65	0.436	0.638	0.25691 **
QZL	29	9.00	4.38	0.594	0.745	0.08914 *
Japan						
HSK	12	5.86	4.24	0.738	0.782	0.02067
SDY	13	6.29	3.74	0.692	0.692	–0.09174
HYS	42	9.57	4.16	0.663	0.703	0.02208
SDG	13	7.29	4.65	0.703	0.779	0.12292 *
YBK	25	8.00	4.75	0.697	0.762	0.07003
KMT	26	6.43	3.24	0.603	0.64	0.05127
MKS	18	6.43	3.73	0.690	0.678	0.0068
MTK	27	7.86	3.97	0.649	0.726	0.05971
NTK	26	7.71	4.52	0.676	0.752	0.07558
AZM	20	7.71	5.00	0.707	0.778	0.04843
OHR	20	8.71	5.52	0.786	0.801	–0.02814
OID	10	5.29	3.61	0.757	0.721	–0.10388
ARK	12	6.71	4.32	0.869	0.791	–0.08946
WYM	11	7.86	4.89	0.741	0.798	0.0538
Local	14	8.57	5.98	0.816	0.829	–0.07692

Number of alleles (Na), effective number of alleles (Ne), Observed heterozygosity (Ho), and expected heterozygosity (He) and fixation index (F_{IS}). Significances of F_{IS} values are given by * $P < 0.05$, ** $P < 0.01$. QZL and Locals means the populations of Chinese Ussurian pear cultivars and local varieties of Ussurian pear in Japan.

Genetic diversity of Ussurian pears in China and Japan based on nSSR data

In this review, the genetic diversity within populations of wild Ussurian pears in China inferred from the values of Na, Ne, Ho, and He was much lower when these values were compared to those of Japanese var. *aromatica* (**Table 2**). In addition, the genetic diversity of Chinese wild Ussurian pear is low compared to that of Callery pear (*P. calleryana*), wild apple (*Malus sieversii* and *M. sylvestris*), African fruit tree of Rosaceae (*Prunus africana*), wild grapevine from Spain, and many perennial and outcross species (Cornille *et al.* 2012, De Andres *et al.* 2012, Nina *et al.* 2008, Nybom 2004, Richards *et al.* 2009).

In spite of low genetic diversity within populations, Wuyun *et al.* (2015) showed 20.05% variation among populations of wild Ussurian pears in China by analysis of molecular variance (AMOVA). This value was relatively high compared to those of other species such as *P. pyrifolia*, *P. calleryana*, and *P. pashia* (Jiang *et al.* 2009, Liu *et al.* 2012, 2013). The high value (20.05%) of AMOVA might reflect the differentiation among populations or restricted gene flow. Alternatively, low genetic diversity might reflect the restrictive gene flow among populations. Therefore both might suggest the same thing i.e., restrictive gene flow and/or differentiation among populations of Chinese wild Ussurian pears. Gene flow is important to maintain genetic diversity as a counter to the effect of genetic drift arising from habitat fragmentation such as forest degradation caused by climate change in Inner Mongolia or human development in Heilongjiang (Wuyun *et al.* 2015). But these results suggest that Chinese wild Ussurian pears do have restricted gene flow. Moreover most of the wild populations in China showed high values for F_{IS} (**Table 2**) which can be attributed to non-random mating i.e., inbreeding caused by habitat fragmentation and limited gene flow. The self-incompatibility (S-) locus can usually prevent crossing between closely-related individuals and self-pollination, but in small populations, inbreeding such as sister brother mating can occur

The genetic diversity within populations of Japanese var. *aromatica* inferred from the values of Na, Ne, Ho, and He was higher than that of Chinese wild Ussurian pears (**Table 2**). This might reflect introgression from *P. pyrifolia* to var. *aromatica* as described by Iketani *et al.* (2010). On the other hand, the genetic differentiation among populations in var. *aromatica* estimated by AMOVA was as low as 7.20% (Tachibana *et al.* 2009). This value was similar to that of *P. communis* (Volk *et al.* 2006), but lower than that of Chinese wild Ussurian pears (20.05%). The difference in AMOVA values between Japanese var. *aromatica* populations and Chinese wild Ussurian pear populations might reflect differences in geographical distance between populations i.e., closely distributed in Japan or widespread in China.

The pairwise F_{ST} values obtained for each of the Chinese wild Ussurian pear populations were higher than the F_{ST} values obtained for each of the var. *aromatica* populations in Japan (data not shown). This result agrees with the result obtained by analysis of molecular variance (AMOVA) i.e., high value for Chinese wild Ussurian pear populations vs. low value for Japanese var. *aromatica* populations.

Bayesian statistical inference of the population structure

The Bayesian model-based clustering programs STRUCTURE 2.3.4 (Pritchard *et al.* 2010) were employed to detect population structure and assign individuals to groups using the 20 nSSR markers as used by Wuyun *et al.* (2015) under the admixture model and the option of correlated allele frequencies between populations of *P. ussuriensis* endemic in China and Japan. The cluster number (K) was set to vary from 1 to 15. The model was run as 10 independent simulations for each K and used a burn-in length of 100,000 and a run length of 1,000,000 MCMC iterations. The relationships between the K value and (i) the data likelihood of K and (ii) the *ad hoc* statistic ΔK as recommended by Evanno *et al.* (2005) were plotted. The ΔK showed largest value at K = 4. The plot of the average log-likelihood values reached a plateau at K = 4 (data not shown). Values of $\ln P(X|K)$ were similar in the independent and correlated models. At K = 4, *P. ussuriensis* in China and var. *aromatica* in Japan were divided into two groups (**Fig. 3**). K = 4 indicates a reasonable result; (1) Inner Mongolia with red cluster (IMQS, IMTHL, IMPJG, IMRSL, IMLMD, and IMSLG), (2) Heilongjiang with green cluster (HLYCS1, HLYCS2, HLYCS3, HLFYX, HLSWX, and HLMTZ), Jilin; JLGZL (green), and QZL; Ussurian pear cultivars in China (green), (3) Northern and Southern areas of Kitakami Mountains with yellow and blue clusters (HSK, SDY, SDG, YBK, MTK, NTK, AZM, OHR, OID, ARK, and WYM) in Japan, (4) central area of the Kitakami Mountains with blue cluster (HYS, KMT, and MKS) in Japan. Populations from Inner Mongolia and Heilongjiang in China were made up of relatively uniform gene pools respectively, though IMQS and QZL, which includes Ussurian cultivars, formed slight admixtures. This result agrees with a previous report indicating low genetic diversity in Chinese Ussurian pear populations (Wuyun *et al.* 2015). STRUCTURE analysis of 20 nSSR markers using 226 individuals of, wild, seemingly wild, and cultivated trees in Japan, revealed var. *aromatica* in Japan was genetically admixed with two genetic clusters: one is the true native var. *aromatica* and the other is a prehistorically introduced *P. pyrifolia* in the Kitakami Mountains. This result agreed well with Iketani *et al.* (2010), who concluded that true native populations of var. *aromatica* existed at HYS. In this review, population structure analysis using more populations from the Kitakami Mountains determined blue dominant clusters representing true native populations (HYS, KMT, and MKS) which occur in the central area of the Kitakami Mountains (**Fig. 3**). Clusters admixed with blue and yellow suggest introgressions from *P. pyrifolia* in the northern and southern areas of the Kitakami Mountains (**Fig. 3**). The genetic structures of *P. ussuriensis* in China and var. *aromatica* in Japan were completely different even though both were constructed from two clusters. Chinese Ussurian cultivars, QZL, were present in the same cluster (green) of wild populations from Heilongjiang and Jilin in China.

Genetic relationships between *P. ussuriensis* in China and *P. ussuriensis* var. *aromatica* in Japan

In this review, twenty seven populations of Ussurian pears from China and Japan, and two groups including cultivars and local varieties were separated into five clades on an unrooted NJ tree shown in **Fig. 4** using 20 nSSR markers as used by Wuyun *et al.* (2015). The tree topology was identical to that inferred by phylogenetic analysis reported previously

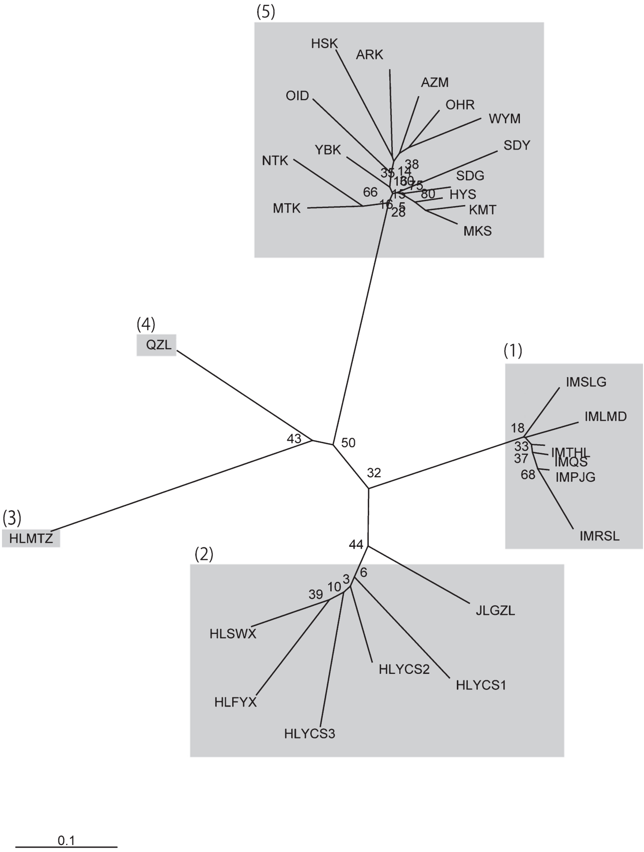

Fig. 4. Unrooted neighbor-joining tree based on Nei's DA distance value among populations. The number of points of divergence represents bootstrap values. The six populations (IMQS, IMTHL, IMPJG, IMRSL, IMLMD, and IMSLG) originating from Inner Mongolia. (2) The 5 populations (HLYCS3, HLFYX, HLSWX, HLYCS2, and HLYCS1) from Heilongjiang. (3) HLMTZ from Mudanjiang, Heilongjiang. (4) The cultivated Ussurian pears (QZL) were combined. (5) The 14 populations (HSK, SDY, HYS, SDG, YBK, KMT, MKS, MTK, NTK, AZM, OHR, OID, ARK, and WYM) originating from Kitakami Mountains in Japan.

(Wuyun *et al.* 2015). The six populations (IMQS, IMTHL, IMPJG, IMRSL, IMLMD, and IMSLG) originating from Inner Mongolia, were all closely related. The five populations (HLYCS3, HLFYX, HLSWX, HLYCS2, and HLYCS1) from Heilongjiang and one population (JLGZL) from Jilin also showed relatively close relationships. The six populations from Inner Mongolia and the five populations from Heilongjiang were distantly positioned from each other.

One population (HLMTZ) from Mudanjiang, Heilongjiang was distantly related to the other populations in Heilongjiang. Based on STRUCTURE analysis, Wuyun *et al.* (2015) indicated that the HLMTZ population was completely different from other populations in Heilongjiang. The present result of PCA for flower morphology shows that the HLMTZ population and some Ussurian pear cultivars share the characteristics of relatively large petals and long peduncles (**Fig. 2**). In Mudanjiang City, where the population of HLMTZ is located, Ussurian pears have a long history of cultivation, so that introgression of Ussurian pear cultivars into wild *P. ussuriensis* might have occurred in HLMTZ over time. On the other hand this population was also distantly related to QZL which consists of Chinese Ussurian cultivars (**Fig. 4**).

Conclusions and Prospects

Based on population structure and phylogenetic analyses, *P. ussuriensis* var. *aromatica* and *P. ussuriensis* (wild Ussurian pear in China) was revealed to be genetically divergent (**Figs. 3**, **4**). They might have differentiated sometime between the Pliocene and the Pleistocene as a consequence of geographical disjunction caused by climate fluctuation represented by the glacial/interglacial. However, although the natural habitat of *P. ussuriensis* var. *aromatica* in Japanese was found, its origin still remains unclear. Also in order to reveal the origin of *P. ussuriensis* in China, more intensive habitat explorations in the north east and west of Heilongjiang in China and the far east of Russia is required.

Molecular studies for genetic diversity and genetic structure of the native Ussurian pears populations both in China and Japan suggested that urgent conservation is required for the following populations i.e., four conservation units from Inner Mongolia and the north east of Heilongjiang (four groups shown in Wuyun *et al.* 2015) and three true native populations such as HYS, KMT, and MKS in the central area of the Kitakami Mountains in Japan shown in this review. Individuals in these populations should be preserved with both of *in situ* and *ex situ* conservations.

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Literature Cited

Bell, R.L., H.A. Quamme, R.E.C. Layne and R.M. Skirvin (1996) Pears. *In*: Janick, J. and J.N. Moore (eds.) Fruit breeding, volume I: tree and tropical fruit. Wiley, New York, pp. 441–514.

Campbell, C.S., R.C. Evans, D.R. Morgan, T.A. Dickinson and M.P. Arsenault (2007) Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): Limited resolution of a complex evolutionary history. Plant Syst. Evol. 266: 119–145.

Cao, Y., L. Tian, Y. Gao and F. Liu (2012) Genetic diversity of cultivated and wild Ussurian pear (*Pyrus ussuriensis* Maxim.) in China evaluated with M13-tailed SSR markers. Genet. Resour. Crop Evol. 59: 9–17.

Coart, E., X. Vekemans, M.M. Smulders, I. Wagner, J. Van Huylenbroeck, E. Van Bockstaele and I. Roldán-Ruiz (2003) Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. Mol. Ecol. 12: 845–857.

Coart, E., S. Van Glabeke, M. De Loose, A.S. Larsen and I. Roldán-Ruiz (2006) Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). Mol. Ecol. 15: 2171–2182.

Cornille, A., P. Gladieux, M.J. Smulders, L. Roldán-Ruiz, F. Laurens, B. Le Cam, A. Nersesyan, J. Clavel, M. Olonova, L. Feugey *et al.* (2012) New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet. 8: e1002703.

De Andres, M.T., A. Benito, G. Perez-Rivera, R. Ocete, M.A. Lopez, L. Gaforio, G. Munoz, F. Cabello, J.M. Martinez Zapater and R. Arroyo-Garcia (2012) Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. Mol. Ecol. 21: 800–816.

Evanno, G., S. Gegnaut and J. Goudet (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611–2620.

Fernandez-Fernandez, F., N.G. Harvey and M. James (2006) Isolation and characterization of polymorphic microsatellite markers from European pear (*Pyrus communis* L.). Mol. Ecol. 6: 1039–1041.

Gianfranceschi, L., N. Seglias, R. Tarchini, M. Komjanc and C. Gessler (1998) Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96: 1069–1076.

Gu, T.C. and S.A. Sponberg (2003) *Pyrus*. *In*: Wu, Z.Y., P.H. Raven and D.Y. Hong (eds.) Flora of China, 9th edn. Science Press. Beijing, pp. 173–179.

Iketani, H. and H. Ohashi (2001) *Pyrus*. *In*: Iwatsuki, K., D.E. Boufford and H. Ohba (eds.) Flora of Japan IIb. Angiospermae Dicotyledoneae Archichlamydeae. Kodansha, Tokyo, pp. 123–124.

Iketani, H. and H. Ohashi (2003) Taxonomy and distribution of Japanese populations of *Pyrus ussuriensis* Maxim. (Rosaceae). J. Jpn. Bot. 78: 119–134.

Iketani, H., T. Yamamoto, H. Katayama, C. Uematsu, N. Mase and Y. Sato (2010) Introgression between native and prehistorically naturalized (archaeophytic) wild pear (*Pyrus* spp.) populations in Northern Tohoku, Northeast Japan. Conserv. Genet. 11: 115–126.

Iketani, H. and H. Katayama (2012) Introgression and long-term naturalization of archaeophytes into native plants underestimated risk of hybrids. *In*: Povilitis, T. (ed.) Topics in Conservation Biology, In-Tech Educational and Publishing, pp. 43–56.

Iketani, H., H. Katayama, C. Uematsu, N. Mase, Y. Sato and T. Yamamoto (2012) Genetic structure of East Asian cultivated pears (*Pyrus* spp.)

and their reclassification in accordance with the nomenclature of cultivated plants. Plant Syst. Evol. 298: 1689–1700.

Jiang, Z., F. Tang, H. Huang, H. Hu and Q. Chen (2009) Assessment of genetic diversity of Chinese sand pear landraces (*Pyrus pyrifolia* Nakai) using simple sequence repeat markers. HortScience 44: 619–626.

Jiao, P.J., T.J. Guo, F.Y. Zhang, J.H. Li, W.P. Lu, Z.G. Liu and Z.G. Guo (2000) Research on the evaluation of main characters of the *Pyrus ussuriensis* Maxim. Special Wild Economic Animal and Plant Research 1: 24–28.

Kang, X., T. Wuyun, Y. Cao and L. Tian (2010) Analysis of aroma components in ripe fruits of *Pyrus ussuriensis*. Hunan Forest. Sci. Tech. 2: 1–4.

Katayama, H. and C. Uematsu (2003) Comparative analysis of chloroplast DNA in *Pyrus* species: physical map and gene localization. Theor. Appl. Genet. 106: 303–310.

Katayama, H. and C. Uematsu (2006) Pear (*Pyrus* species) genetic resources in Iwate, Japan. Genet. Resour. Crop Evol. 53: 483–498.

Katayama, H., S. Adachi, T. Yamamoto and C. Uematsu (2007) A wide range of genetic diversity in pear (*Pyrus ussuriensis* var. *aromatica*) genetic resources from Iwate, Japan revealed by SSR and chloroplast DNA markers. Genet. Resour. Crop Evol. 54: 1573–1585.

Katayama, H., M. Tachibana, H. Iketani, S.L. Zhang and C. Uematsu (2012) Phylogenetic utility of structural alterations found in the chloroplast genome of pear: hypervariable regions in highly conserved genome. Tree Genet. Genomes 8: 313–326.

Katayama, H., M. Ohe and E. Sugawara (2013) Diversity of odor-active compounds from local cultivars and wild accessions of Iwateyamanashi (*Pyrus ussuriensis* var. *aromatica*) revealed by Aroma Extract Dilution Analysis (AEDA). Breed. Sci. 63: 86–95.

Kato, S., A. Imai, R. Nishioka and Y. Mukai (2013) Population genetic structure in a threatened tree, *Pyrus calleryana* var. *dimorphophylla* revealed by chloroplast DNA and nuclear SSR locus polymorphisms. Conserv. Genet. 14: 983–996.

Li, G., H. Jia, R. Wu and Y. Teng (2013) Changes in volatile organic compound composition during the ripening of 'Nanguoli' pears (*Pyrus ussuriensis* Maxim) harvested at different growing locations. J. Hort. Sci. Biotechnol. 88: 563–570.

Li, T., X. Li, D. Tan, Z. Jiang, Y. Wei, J. Li, G. Du and A. Wang (2014) Distinct expression profiles of ripening related genes in the 'Nanguo' pear (*Pyrus ussuriensis*) fruits. Sci. Hortic. 171: 78–82.

Liebhard, R., L. Gianfranceschi, B. Koller, C.D. Ryder, R. Tarchini, E. Van De Weg and C. Gessler (2002) Development and characterisation of 140 new microsatellites in apple (*Malus* × *domestica* Borkh.). Mol. Breed. 10: 217–241.

Liu, J., X. Zheng, D. Potter, C. Hu and Y. Teng (2012) Genetic diversity and population structure of *Pyrus calleryana* (Rosaceae) in Zhejiang province, China. Biochem. Syst. Ecol. 45: 69–78.

Liu, J., P. Sun, X. Zheng, D. Potter, K. Li, C. Hu and Y. Teng (2013) Genetic structure and phylogeography of *Pyrus pashia* L. (Rosaceae) in Yunnan Province, China, revealed by chloroplast DNA analyses. Tree Genet. Genomes 9: 433–441.

Ma, T., T. Wuyun, W. Deng, K. Xiu and L. Jian (2011) Investigation on wild *Pyrus ussuriensis* Maxim. resources of Chifeng area in Inner Mongolia. Guangdong Agricultural Science. 2.

Maximowicz, C.J. (1859) Primitiae florae amurensis. Mémoires présentés a l'académie impériale des sciences de St.-Pétersbourg par divers savants et lus dans ses assemlées, 9: 1–504.

Ministry of the Environment, the Government of Japan (2007) The updated japanese red lists on mammals, brackish-water/freshwater fishes, insects, shellfish, and plants I and II.

Nakai, T. (1918) Notulae ad plantas japonicae et koreae XVI. Bot. Mag. Tokyo 32: 28–37.

Nina, F., B. Carsten and B.G. Katrin (2008) Human disturbance reduces genetic diversity of an endangered tropical tree, *Prunus africana* (Rosaceae). Conserv. Genet. 9: 317–326.

Nishitani, C., T. Shimizu, S. Ohta, T. Kimura, Y. Sawamura, T. Hayashi and T. Yamamoto (2005) Functional genomics in pear I. cDNA libraries derived from various tissues of the Japanese pear 'Housui'. Abstr. Plant and Animal Genome XIII Conference. 198.

Nybom, H. (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol. 13: 1143–1155.

Ohwi, J. (1965) *Pyrus*. *In*: Flora of Japan. Smithsonian Institution, Washington DC, p. 549.

Pritchard, J.K., W. Wen and D. Falush (2010) Documentation for STRUCTURE software: version 2.3. Available from http://pritch.bsd.uchicago.edu/structure.html.

Pu, F. and Y. Wang (1963) Pomology of China. Vol. 3. Pears. Shanghai: Shanghai Science and Technology Press.

Qin, G., S. Tao, Y. Cao, J. Wu, H. Zhang, W. Huang and S. Zhang (2012) Evaluation of the volatile profile of 33 *Pyrus ussuriensis* cultivars by HS-SPME with GC–MS. Food Chem.134: 2367–2382.

Rehder, A. (1920) New species, varieties and combinations from the herbarium and the collections of the Arnold Arboretum. J. Arn. Arb. 2: 42–64.

Rehder, A. (1940) A manual of cultivated trees and shrubs hardy in North American exclusive of the subtropical and warmer temperate regions, 2nd edn. Macmillan, New York, p. 403.

Report of the Chinese Academy of Agricultural Science (1963) China fruit records, Shanghai Technology House.

Richards, C.M., G.M. Volk, A.A. Reilley, A.D. Henk, D.R. Lockwood, P.A. Reeves and P.L. Forsline (2009) Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genet. Genomes 5: 339–347.

Robertson, A., A.C. Newton and R.A. Ennos (2004) Multiple hybrid origins genetic diversity and population genetic structure of two endemic *Sorbus* taxa on the Isle of Arran, Scotland. Mol. Ecol. 13: 123–134.

Sawamura, Y., T. Saito, N. Takeda, T. Yamamoto, T. Kimura, T. Hayashi and K. Kotobuki (2004) Identification of parentage of Japanese pear 'Housui'. J. Japan. Soc. Hort. Sci. 73: 511–518.

Song, Y., L. Fan, H. Chen, M. Zhang, Q. Ma, S. Zhang and J. Wu (2014) Identifying genetic diversity and a preliminary core collection of *Pyrus pyrifolia* cultivars by a genome-wide set of SSR markers. Sci. Hortic. 167: 5–16.

Tachibana, M., H. Katayama, Y. Iketani, T. Yamamoto and C. Uematsu (2009) Evaluation of genetic diversity of Iwate yamanashi (*Pyrus ussuriensis* var. *aromatica*) populations revealed by DNA markers and morphological characters. Breed. Res. 11 (Suppl. 1): 191.

Terakami, S., Y. Matsumura, K. Kurita, H. Kanamori, Y. Katayose, T. Yamamoto and H. Katayama (2012) Complete sequence of the chloroplast genome from pear(*Pyrus pyrifolia*): genome structure and comparative analysis. Tree Genet. Genomes 8: 841–854.

Volk, G.M., C.M. Richards, A.D. Henk, A.A. Reilley, N.V. Bassil and J.D. Postman (2006) Diversity of wild *Pyrus communis* based on microsatellite analyses. J. Amer. Soc. Hort. Sci. 131: 408–417.

Wuyun, T., T. Ma, C. Uematsu and H. Katayama (2013) A phylogenetic network of wild Ussurian pears (*Pyrus ussuriensis* Maxim.) in China revealed by hypervariable regions of chloroplast DNA. Tree Genet. Genomes 9: 167–177.

Wuyun, T., H. Amo, J. Xu, T. Ma, C. Uematsu and H. Katayama (2015)

Population structure of and conservation strategies for wild *Pyrus ussuriensis* Maxim. in China. PLoS ONE 10: e0133686.

Yamamoto, T., T. Kimura, Y. Sawamura, K. Kotobuki, Y. Ban, T. Hayashi and N. Matsuta (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor. Appl. Genet. 102: 865–870.

Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban and N. Matsuta (2002a) Simple sequence repeats for genetic analysis in pear. Euphytica 124: 129–137.

Yamamoto, T., T. Kimura, M. Shoda, T. Imai, T. Saito, Y. Sawamura, K. Kotobuki, T. Hayashi and N. Matsuta (2002b) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theor. Appl. Genet. 106: 9–18.

Yü, T.T. (1979) Taxonomy of the fruit tree in China, Agriculture Press, Beijing, China.

Yü, T.T. and T.C. Ku (1974) *Pyrus*. *In*: Yü, T.T. (ed.) Flora Reipublicae Popularis Sinicae, Vol. 36, Science Press, Beijing, China, pp. 354–372.

Zhong, Y., P. Sun, J. Liu, X. Yue, K. Li and Y. Teng (2014) Genetic diversity and population structure of seedling populations of *Pyrus pashia*. Plant Mol. Biol. Rep. 32: 644–651.