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Origin of *Prunus* × *yedoensis* 'Somei-yoshino' based on sequence analysis of *PolA1* gene

Nakamura I.[(1)]*, Takahashi H.[(1)], Ohta S.[(2)], Moriizumi T.[(3)], Hanashiro Y.[(4)], Sato Y.-I.[(5)], Mii M.[(1)]

[(1)] *Graduate School of Horticulture, Chiba University, Matsudo, Matsudo 271-8510, Japan.*

[(2)] *Department of Agriculture, Shizuoka University, Ohya, Shizuoka 422-8529, Japan.*

[(3)] *Bex Co. Ltd., Itabashi, Tokyo 173-0004, Japan.*

[(4)] *Ocean Exposition Commemorative National Government Park Management Foundation, Kunigami, Okinawa 905-0206, Japan.*

[(5)] *Future Center, Kyoto Sangyo University, Kamigamo, Kita-ku, Kyoto 603-8555, Japan.*

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Abstract: *Prunus* × *yedoensis* 'Somei-yoshino' is the most popular cultivar of flowering cherry in Japan. Although the origin of this cultivar has been considered hybrid between *P. pendula* f. *ascendens* and *P. lannesiana* var. *speciosa*, the paternity of *P. lannesiana* has not been clearly proven by molecular analysis. To reveal the origin of 'Somei-yoshino,' we analyzed sequences of intron 19 and exon 20 of *PolA1*, a single-copy nuclear gene encoding the largest subunit of RNA polymerase I. One of two exon 20 sequences found in 'Somei-yoshino' was the same as that of *P. pendula*, whereas the other sequence was shared with several taxa in seven wild species, including *P. jamasakura* and *P. lannesiana*. 'Somei-yoshino' contained two different haplotypes of the intron 19 sequences; one was the same as that of *P. lannesiana*, which is endemic to the Izu and Boso Peninsula in Japan. While another haplotype of 'Somei-yoshino' was different from that of *P. pendula* by two SNPs but identical to one of two haplotypes of *P. pendula* 'Komatsu-otome,' which is a cultivar found in the Ueno Park, Tokyo. These results indicated that 'Somei-yoshino' probably originated by the hybridization of cultivars derived from *P. pendula* and *P. lannesiana*.

1. Introduction

The subgenus *Cerasus* of the genus *Prunus* includes more than 50 species, most of which are distributed in temperate areas in the Northern Hemisphere, especially in China, where 33 wild species occur (Yu and Li, 1986). In Japan, nine native species are recorded: *P. jamasakura* Sieb. ex Koidz., *P. sargentii* Rehder, *P. verecunda* (Koidz,) Koehne, *P. incisa* Thumb. ex Murray, *P. nipponica* Matsum., *P. apetala* (Sieb. et Zucc.) Fr. et Sav., *P. lannesiana* (Carr.) Wilson var. *speciosa* (Koidz.) Makino, and *P. pendula* f. *ascendens* (Makino) Ohwi. In addition, three wild species, *P. pseudo-cerasus* Lindl., *P. cerasoides* D. Don and *P. campanulata* Maxim., have been popularly cultivated since their introduction from China, Taiwan, and Nepal, respectively (Kawasaki, 1991).

Several classifications based on morphological observations have been proposed for Japanese flowering cherries (Kawasaki, 1991; Kobayashi, 1992; Ohba, 1992), and they have been classified into five sections: *Apetalae* (*P. apetala*), *Incisae* (*P. incisa* and *P. nipponica*), *Sargentiella* (*P. jamasakura*, *P. sargentii*, *P. verecunda*, and *P. lannesiana*), *Phyllomahaleb* (*P. maximowiczii*), and *Microcalymma* (*P. pendula*). The phylogenetic relationships among these taxa have been investigated using restriction fragment length polymorphism (RFLP) analysis of chloroplast DNA (Kaneko *et al*., 1986), randomly amplified polymorphic DNA (RAPD) analysis (Shimada *et al*., 2001), and analyses of rDNA ITS sequences (Lee and Wen, 2001), SSR markers for nuclear DNA (Ohta *et al*., 2005), and plastid subtype identity (PSID) sequences (Ohta *et al*., 2006).

More than 250 cultivars of flowering cherries, including *Prunus* × *yedoensis* Matsum. 'Somei-yoshino' (Iketani *et al*., 2006), have been created through repeated natural and artificial hybridizations among wild *Cerasus* species (Kawasaki, 1993). 'Somei-yoshino' was first proposed to have arisen as a hybrid between *P. lannesiana* var. *speciosa* and *P. pendula* f. *ascendens* (Wilson, 1916). Takenaka (1962, 1965) produced hybrids between the two species and noted that they had similar morphological characters to 'Somei-yoshino.' However, these hybrid plants, such as 'Amagi-yoshino' and 'Izu-yoshino,' were taller and produced many more flowers with white petals than 'Somei-yoshino.' Based on SSR marker analysis, Iketani *et al*. (2007) pointed out clonal status of 'Somei-yoshino,' which has been propagated by grafting.

* Corresponding author: inakamur@faculty.chiba-u.jp

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Previously, Kaneko *et al*. (1986) showed that *P. pendula* might be the maternal parent of 'Somei-yoshino' based on RFLP patterns of chloroplast DNA. A recent analysis of PSID sequences provided additional support that 'Somei-yoshino' had the 10A-T-4A haplotype specific to *P. pendula*, but not the 14A haplotype for *P. lannesiana* and *P. jamasakura* (Ohta *et al*., 2006). Recently, Roh *et al*. (2007) proposed that variations for nuclear ISSR markers and two plastid DNA sequences of the *P. yedoensis* population on Korean Jeju Island overlapped those of 'Somei-yoshino.' Their data, however, did not prove the paternal origin of 'Somei-yoshino.' Utilizing the associations of nuclear DNA markers dispersed over the genome, it is especially difficult to identify the paternal parent in *Cerasus* species. Because the *Cerasus* species have complete self-incompatibility, those DNA markers recombine every generation.

To resolve the paternity of 'Somei-yoshino,' we decided to compare a relatively short sequence within a single-copy gene, because a short DNA sequence is thought to be a block consisting of many closely linked DNA markers, for which recombination is difficult. Sang (2002) stated that the sequences of single- or low-copy nuclear genes are particularly helpful for understanding the inter- and intraspecific relationships of various plant groups. Recently, we were interested in *PolA1* as a candidate single-copy gene, which encoded the largest subunit of the RNA polymerase I complex. The DNA sequences of intron 19 of the *PolA1* gene were highly polymorphic whereas the exon 20 sequences showed species-specific variations in the genera *Petunia* (Zhang *et al*., 2008), *Oryza* (Takahashi *et al*., 2009), and *Triticum* (Takahashi *et al*., 2010).

The present study was initiated to reveal the origin of 'Somei-yoshino' through the analysis of intron 19 and exon 20 sequences in *PolA1* gene.

2. Materials and Methods

Plant material

Most of the DNA samples used in this study were provided from the Faculty of Agriculture, Shizuoka University, and some DNA samples were extracted from leaves of the clonally propagated plants that were maintained in the Tama Forest Science Garden, Tokyo, Japan. A total of 42 individuals of nine wild species native to Japan (Table 1) were analyzed; *P. apetala* (three individuals), *P. incisa* (four), *P. nipponica* (four), *P. jamasakura* (eight), *P. sargentii* (two), *P. verecunda* (four), *P. lannesiana* (three), *P. maximowiczii* (three), and *P. pendula* (six), and three alien wild species, *P. campanulata* (two), *P. cerasoides* (two), and *P. pseudo-cerasus* (one) (Table 1). Two cultivars, 'Somei-yoshino,' *P. pendula* f. *ascendens* 'Komastuotome' Hayashi & Nshida (Hayashi, 1989), and five Edohigan trees were collected in Ueno Park, Tokyo, Japan. One individual of apricot (*Prunus armeniaca* L.) was also analyzed as out-group material.

Genomic DNA isolation and PCR amplification

Genomic DNA was extracted from *ca*. 50 mg of young leaves using a modified CTAB method (Doyle and Doyle, 1987). The forward primer designated as 19ex5P (5'- CTCGCTGGACGGGGTGAGATGAATG-3') and the reverse primer designated as 21ex3P (5'-ATTTACTGGCAATCCAAGACAGAT-3') were designed based on *PolA1* gene (GenBank accession No. NM_125397) of *Arabidopsis thaliana* and EST (GenBank accession No. BQ641151) of almond (*Prunus dulcis* Mill.), respectively. DNA fragments containing intron 19 and exon 20 sequences of *PolA1* gene were amplified by PCR using a pair of 19ex5P and 21ex3P primers (Fig. 1). The reaction mixture of 25 µl contained 10-50 ng of genomic DNA, 1 unit of *Ex Taq* DNA polymerase (TaKaRa Co., Japan), 2.5 µl of 10× buffer (100 mM Tris-Cl, 500 mM KCl, and 15 mM $MgCl_2$, pH8.0), 2 µl of 2.5mM dNTPs, 1 µl of 2.0 µM each primer (19ex5P and 21ex3P), and 17.5 µl of distilled water. PCR was performed with a condition of 35 cycles of 94°C for 1 min denaturation, 58°C for 1 min annealing, and 72°C for 2 min elongation in PTC200 Thermocycler (MJ Research Co., USA).

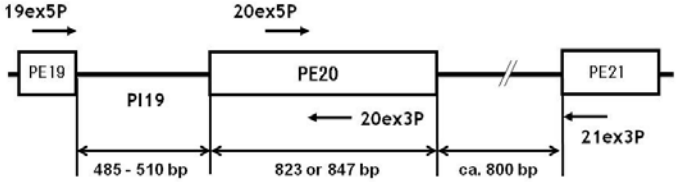


Fig. 1 - DNA fragments containing intron 19 (PI19) and exon 20 (PE20) of *PolA1* gene were amplified using a pair of 19ex5P (PE19) and 21ex3P (PE21) primers. The sequences were determined by direct sequencing using primers for the initial amplification and internal sequence primers, 20ex5P and 20ex3P.

Direct sequencing of PCR products containing the intron 19 and exon 20

The amplified PCR products were subjected to 1.2% agarose gel electrophoresis, purified using QIAquick PCR Purification Kit (Qiagen Co., USA), and directly sequenced with 19ex5P or 21ex3P primer used for the PCR-amplification by ABI3100 Automated DNA Sequencer with a BigDye Terminator Cycle Sequencing Kit (Life Technologies Co., USA). Either 20ex3P (5'-TTGAAGATGTTCAGGTATGGGGAG-3') or 20ex5P (5'-ATAAGTTGAAGAAAATCAC TGTGG-3') primer were also used as an internal sequencing primer. The two internal primers were designed based on the partially determined sequences of *PolA1* exon 20 of *Cerasus* in this study.

The determined sequences of the intron 19 and exon 20 of *PolA1* gene were analyzed using a NCBI web-based Blast server (Altschul *et al*., 1990), and aligned using web server of Mafft ver 6.0 (Kato and Toh, 2008), and then the aligned sequences were subjected to phylogenetic analysis using UPGMA software, with bootstrap analysis using 1,000 replicates, in the Mega 4.0 (Tamura *et al*., 2007).

Table 1 - Samples used in this study

Species	Name [Z]	Locality	PI19 [y]	PE20 [y]
Prunus apetala (Sieb. et Zucc.) Fr. et Sav.	TJ063	Kawaguchiko, Yamanashi	507	nd.
	TJ093	Chino, Nagano	507	847
	TJ164	Hachioji, Tokyo (TFSG)	507	847
P. incisa Thumb.	MM048	Gotenba, Shizoka	507	847
	MM131	Fujimi, Nagano	507	847
	MM160	Amatsukominato, Chiba (TFSG)	507	847
	MM165	Fujiyoshida, Yamanashi (TFSG)	507	nd.
P. nipponica Matsum.	TK077	Shizuoka, Shizuoka	507	nd.
	TK113	Ashiyasu, Yamanashi	507	nd.
	TK140	Fujimi, Nagano	507	847
	TK188	Kusatsu, Gunma	507	847
P. jamazakura Sieb. ex Koidz.	YM001	Morimachi, Shizuoka	507	nd.
	YM011	Ishikawa Forest Exper. Station	485,507	nd.
	YM038	Amagiugashima, Shizuoka	485	823
	YM154	Hachioji, Tokyo (TFSG)	507	nd.
	YM245	Kushikino, Kagoshima	507	847
	YM256	Izumi, Kumamoto	507	847
	YM272	Kinkai, Nagazaki	507	nd.
	YM277	Yayoi, Notsu, Oita	485,507	nd.
P. sargentii Rehder	OY024	Ishikawa Forest Exper. Station	507	nd.
	OY162	Mamurogawa, Yamagata (TFSG)	507	847
P. verecunda (Koidz.) Koehne	KS016	Ishikawa Forest Exper. Station	507	847
	KS136	Fujimi, Nagano	507	nd.
	KS183	Yahiko, Niigata	507	nd.
	KS211	Nishiki, Yamaguchi	507	847
P. lannesiana (Carr.) Wilson var. speciosa (Koidz.) Makino	OS017	Ishikawa Forest Exper. Station	507	847
	OS166	Miyake, Tokyo (TFSG)	507	847
	OSMTD	Matsudo, Chiba	507	nd.
P. maximowiczii Rupr.	MY076	Shizuoka, Shizuoka	507	823
	MY139	Fujimi, Nagano	498	nd.
	MY158	Chichibu, Saitama (TFRG)	507	823
P. pendula Maxim. f. *ascendens* (Makino) Ohwi.	EH015	Ishikawa Forest Exper. Station	506	nd.
	EH149	Ochiai, Okayama (TFRG)	506	nd.
	EH150	Takekawa, Yamanashi (TFRG)	506	823
	EH155	Oya, Hyogo (TFRG)	506	nd.
	EH163	Oguchi, Kagoshima (TFRG)	506	823
	EHFSG	a mountain behind TFRG	506	nd.
P. pseudo-cerasus Lindl. Shinami	SN009	Ishikawa Forest Exper. Station	507	823
P. campanulata Maxim.	KN014	Ishikawa Forest Exper. Station	507	823
	KN101	Tsukubo Botanical Garden	507	nd.
P. cerasoides D. Don.	HM001	Katomandu, Nepal (Shizuoka U.)	507	823
	HM002	Katomandu, Nepal (Shizuoka U.)	507	nd.
P. armeniaca L.	ANZ	Chiba University	510	823
P. × *yedoensis* Matsum. 'Somei-yoshino'		Chiba University	505, 507	823, 847
P. pendula Maxim. 'Komatsu-otome'		Ueno Park, Tokyo	505, 506	823

[Z] Nos. are according to Ohta *et al*. (2006).
[y] length (bp).
Nd= not determined.

3. Results

Polymorphisms of the PolA1 intron 19 sequences

Using total DNA extracted from 43 individuals in ten species as template, *ca*. 2.2-kb-long DNA fragments containing intron 19 and exon 20 of the *PolA1* gene were clearly amplified by PCR (Fig. 2). The *PolA1* intron 19 sequences of most *Cerasus* species were 507 bp in length (Table 1). All six individuals of *P. pendula* contained 506 bp because of a one-base insertion at position 49 and a two-base deletion at position 349-350. One individual (YM038) of *P. jamasakura* and one individual (MY139) of *P. maximowiczii* had shorter intron 19 lengths of 485 and 498 bp, respectively. Two individuals (YM011 and YM277) of *P. jamasakura* had two intron 19 sequences of different lengths (485 and 507 bp), although these sequences could not be confirmed.

The DNA sequences described in this paper have been deposited in DDBJ DNA database (accession nos. LC010372- LC010416).

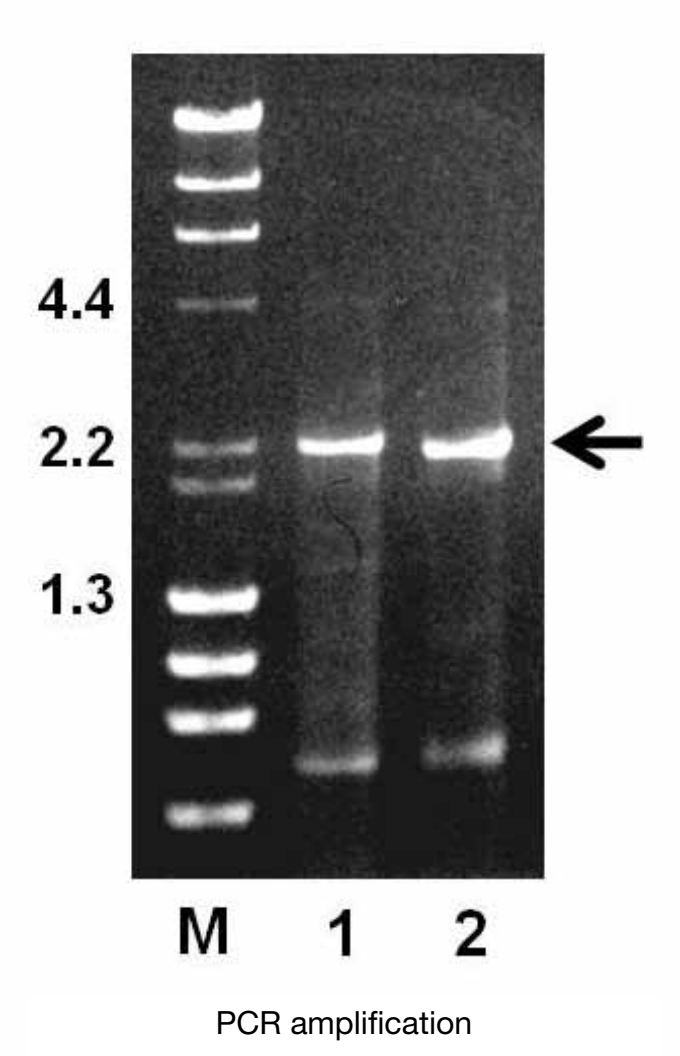


Fig. 2 - PCR products (arrow) of DNA fragments containing intron 19 and exon 20 of *PolA1* gene in the *Cerasus* species, 1: *Prunus lannesiana* var. *speciosa*, 2: *P. pendula* f. *ascedens*, M: marker (λDNA/*Hin*dIII plus øx144 DNA/*Hae*III).

Polymorphisms of the PolA1 exon 20 sequences

Prunus pendula, P. maximowiczii, P. pseudo-cerasus, P. campanulata, P. cerasoides, and *P. armeniaca* (outgroup) contained an 823-bp exon 20 (Table 1), whereas, seven species (*P. apetala*, *P. incisa*, *P. nipponica*, *P. jamasakura*, *P. sargentii, P. verecunda*, and *P. lannesiana*) showed a long 847-bp-long exon 20 with a 24-bp insertion. One individual (YM038) of *P. jamasakura* had the short exon 20 (823 bp), and two individuals (YM011 and YM277) possessed both long and short exons 20 (Table 1).

The DNA sequences described in this paper have been deposited in DDBJ DNA database (accession nos. LC010540 - LC010565).

Phylogenetic tree of the PolA1 intron 19 and exon 20 sequences

The 41 sequences determined for intron 19 and the 23 sequences for exon 20 were aligned using Mafft and subjected to phylogenetic analysis using the UPGMA method in MEGA 4.0 with 1,000 bootstap replicates. In the phylogenetic tree for exon 20, the nine Japanese wild species were classified into two groups, Jamasakura and Pendula (Fig. 3). *Prunus campanula* belonged to a distantly-related clade. In the Jamasakura group, *P. jamasakura*, *P. nipponica*, *P. incisa*, *P. lannesiana*, and *P. apetala* shared a long exon 20 and formed a closely-related clade, and ten individuals of these five species contained the same sequence for the exon 20. One individual (KS016) of *P. verecunda* and one individual (OY162) of *P. sargentii* also shared an identical exon 20. One individual (YM038) possessed the short exon 20 and was distantly related to the other individuals in the Jamasakura group. The remaining two species, *P. pendula* and *P. maximowiczii*, formed the Pendula

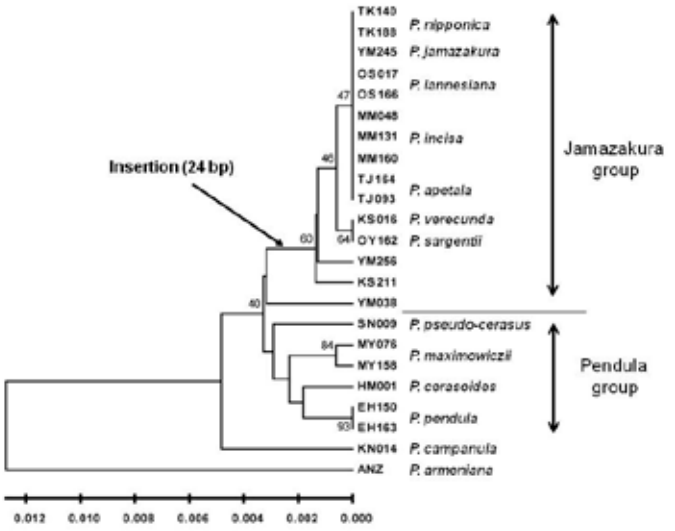


Fig. 3 - Phylogenetic UPGMA tree of the exon 20 sequences in the *PolA1* genes from 22 individuals in nine *Cerasus* species. Individuals used are listed in Table 1.

group together with *P. pseudo-cerasus* and *P. cerasoides*. Unlike the species in the Jamasakura group, the four species in the Pendula group were clearly differentiated from one another (Fig. 3).

In the phylogenetic tree for intron 19, *P. pendula* was positioned as the most distantly-related clade (Fig. 4) because this species contained a unique insertion (position 49) and a unique deletion (positions 349-350) (Fig. 5). Except for *P. pendula*, the individuals in the Jamasakura and Pendula groups formed two independent clades. Although the species in the Pendula group were clearly differentiated from one another, concurring with the results for exon 20, most individuals of the six species in the Jamasakura group, except for YM038, shared similar sequences at intron 19, and 14 individuals of the six species possessed the same intron 19 sequence. Three individuals of *P. lannesiana* had the same sequence and belonged to an independent sub-clade (Fig. 4).

Analysis of the origin of P. × yedoensis 'Somei-yoshino'

When the two allelic sequences of exon 20 of 'Somei-yoshino' were determined, one was identical to that of *P. pendula*, and the other was the same as that shared by five species in the Jamasakura group. For the intron 19 sequence, the (O) haplotype of *P. lannesiana* was distinguished from the haplotypes of *P. jamasakura* by three unique single-nucleotide polymorphisms (SNPs) (positions 25, 101, and 171), which were also found in one of two 'Somei-yoshino' haplotypes (Fig. 5). By contrast, the other (K) haplotype was found to differ from the (E) haplotype of *P. pendula* by one base deletion at position 49 and one base substitution of C to A at position 392 (Fig. 5). Consequently, we found that *P. pendula* f. *ascendens* 'Komatsu-otome' possessed two haplotypes (K and E): one was the same haplo-

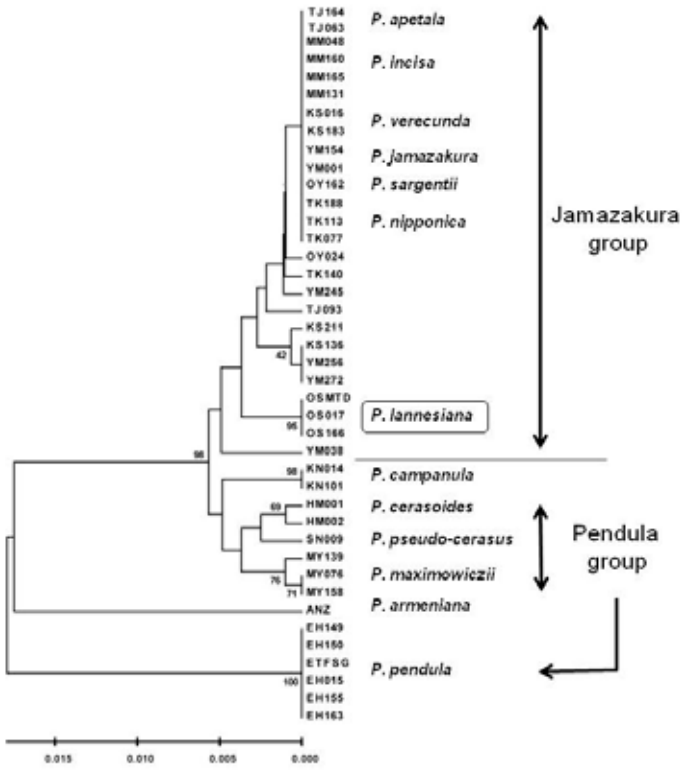
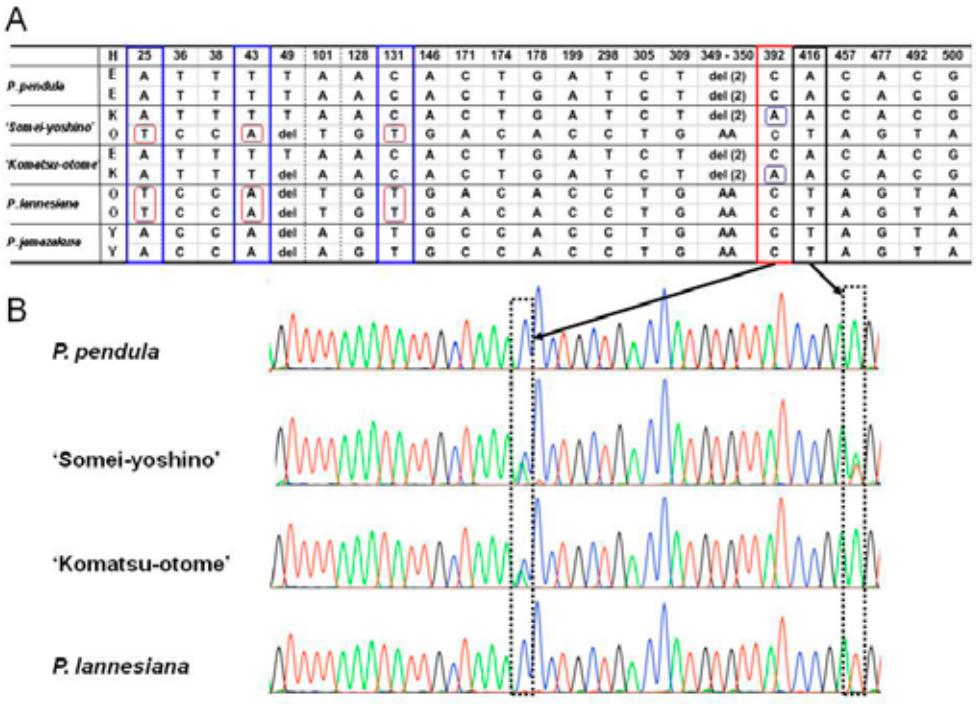


Fig. 4 - Phylogenetic UPGMA tree of the intron 19 sequences in the *PolA1* genes from 40 individuals in nine *Cerasus* species. Individuals used are listed in Table 1.



	H	25	36	38	43	49	101	128	131	146	171	174	178	199	298	305	309	349 - 350	392	416	457	477	492	500
P. pendula	E	A	T	T	T	T	A	A	C	A	C	T	G	A	T	C	T	del (2)	C	A	C	A	C	G
	E	A	T	T	T	T	A	A	C	A	C	T	G	A	T	C	T	del (2)	C	A	C	A	C	G
'Somei-yoshino'	K	A	T	T	T	T	A	A	C	A	C	T	G	A	T	C	T	del (2)	A	A	C	A	C	G
	O	T	C	C	A	del	T	G	T	G	A	C	A	C	C	T	G	AA	C	T	A	G	T	A
'Komatsu-otome'	E	A	T	T	T	T	A	A	C	A	C	T	G	A	T	C	T	del (2)	C	A	C	A	C	G
	K	A	T	T	T	del	A	A	C	A	C	T	G	A	T	C	T	del (2)	A	A	C	A	C	G
P. lannesiana	O	T	C	C	A	del	T	G	T	G	A	C	A	C	C	T	G	AA	C	T	A	G	T	A
	O	T	C	C	A	del	T	G	T	G	A	C	A	C	C	T	G	AA	C	T	A	G	T	A
P. jamazakura	Y	A	C	C	A	del	A	G	T	G	C	C	A	C	C	T	G	AA	C	T	A	G	T	A
	Y	A	C	C	A	del	A	G	T	G	C	C	A	C	C	T	G	AA	C	T	A	G	T	A

Fig. 5 - Haplotypes (H) of the intron 19 sequences in the *PolA1* genes among 'Somei-yoshino' (KO), *P. pendula* (EE), 'Komatsu-otome' (KE), *P. lannesiana* (OO), and *P. Jamasakura* (YY), A: polymorphic bases and their positions in two haplotypes are shown, B: Sequence charts were produced using 21ex3P primer and converted to the complementary charts using the 4Peak software.

type of 'Somei-yoshino' and the other was identical to that of a wild *P. pendula* individual. Except for cultivars derived from 'Somei-yoshino,' we found that 'Komatsu-otome' and two other trees (Nos. 142, 145) in Ueno Park had the same haplotype as 'Somei-yoshino' (Fig. 6).

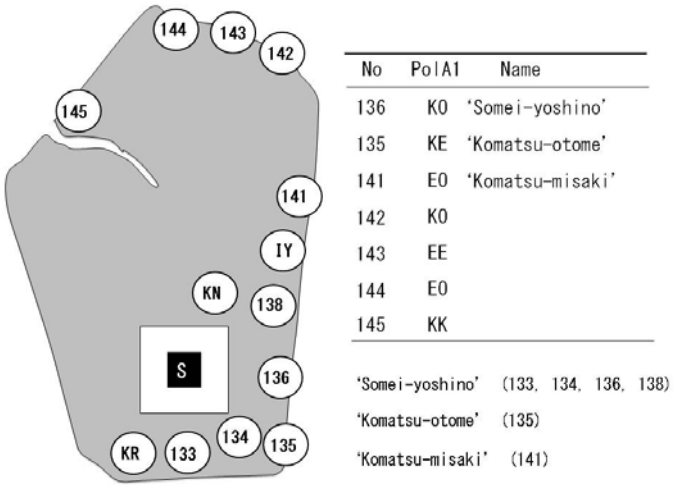


Fig. 6 - Haplotype of the *PolA1* intron 19 of trees (Nos. 135-145) found around "Komatsu no miya" statue (S) in the Ueno Park. 'Somei-yoshino' (133, 134, 136, 138) and 'Komatsu-otome' (135) share a haplotype K, *P. pendula* (E), *P. lannensiana* (O). IY, KN and KZ are flowering cherry cultivars, 'Ichi-yo,' 'Kanzan,' and 'Kanzakura,' respectively.

4. Discussion

Speciation of wild Cerasus species *in Japan*

Comparing the sequences of intron 19 and exon 20 (Figs. 3 and 4), the nine wild species of Japanese *Cerasus* were clearly classified into two groups; the Jamasakura group of seven species (*P. apetala*, *P. incisa*, *P. nipponica*, *P. jamasakura*, *P. sargentii*, *P. verecunda*, and *P. lannesiana*) and the Pendula group of two species (*P. pendula* and *P. maximowiczii*). Although the former group had been grouped into sections, *Apetalae*, *Incisae*, and *Sargentiella* based on morphological differences (Kawasaki, 1966, 1991; Kobayashi, 1992; Ohba, 1992), all seven species shared the same 24-bp insertion within the long exon 20 (847 bp), suggesting that they originated from the same ancestor. The remaining two wild species, *P. pendula* and *P. maximowiczii*, contained the short exon 20 (823 bp), in common with three alien wild species (*P. pseudo-serasus*, *P. campanulata*, and *P. cerasoides*), and with *P. armeniaca* (out-group). The results from the sequence analysis of exon 20 were thought to be more reliable than those for intron 19 for classifying the subgenus *Cerasus*, and polymorphisms found in intron 19 will be useful for discriminating among closely-related taxa and cultivars.

Although the seven species in the Jamasakura group have clearly different phenotypes, such as the apetala flower of *P. apetala* and dwarf stature of *P. incisa* and *P. nipponica*, these species have formed a large hybridizing population because they share the same sequences for intron 19 and exon 20. The short intron 19 found in three individuals (YM011, YM038, and YM277) of *P. jamasakura* might have been derived from an ancestral cryptic species. These results suggest that the classification of seven species in the Jamasakura group remains to be revised based on further molecular information.

Origin of 'Somei-yoshino'

'Somei-yoshino' is the most popular flowering cherry cultivar in Japan and the rest of the world. Ever since Wilson (1916) proposed a hypothesis for the hybrid origin of 'Somei-yoshino,' the biological and geographical origin of this cultivar has been disputed in Japan. In this study, we found that the O haplotype for intron 19 of *P. lannesiana* contained three unique SNPs, and these SNPs were also found in one of the two haplotypes (K and O) in 'Somei-yoshino' (Fig. 5). This indicates that the paternal parent of 'Somei-yoshino' was *P. lannesiana* or its cultivars. As *P. lannesiana* is endemic to the Izu Peninsula and the Izu Oshima Islands, 'Somei-yoshino' may have originated on the the Izu Peninsula (Takenaka, 1962) or in Edo and Tokyo (Iwasaki, 1989), and not on Jeju Island, Korea (Park *et al*., 1984; Roh *et al*., 2007).

The other (K) haplotype of intron 19 in 'Somei-yoshino' was identical to that (E) of *P. pendula*, except for two SNPs (Fig. 5). We also found that one of the two haplotypes (K and E) for intron 19 of 'Komatsu-otome' was the same as that of 'Somei-yoshino.' The original individual of 'Komatsu-otome' grows inside the Ueno Park, Tokyo (Fig. 6) and has a dwarf stature with pinkish flower petals. This implies that the maternal origin of 'Somei-yoshino' is a cultivar related to 'Komatsu-otome.' Out of five trees grown in the same position with 'Somei-yoshino' and 'Komatsu-otome' shown in Figure 6, two trees (Nos. 142, 145) contained K haplotype and three (Nos. 141, 142, 144) were hybrids between *P. pendula* f. *ascendens* and *P. lannesiana* var. *speciosa*.

These results suggest that there were sufficient genetic resources to develop 'Somei-yoshino.' Because *P. pendula* and 'Komatsu-otome' bloom two weeks earlier than *P. lannesiana*, 'Somei-yoshino' and 'Komatsu-misaki' were probably produced in Tokyo through artificial hybridizations between 'Komatsu-otome' or a related cultivar, and *P. lannesiana* or a related cultivar, before the end of the Edo Period (Iwasaki, 1989).

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