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Genetic Characterization of Flowering Cherries (*Prunus* subgenus *Cerasus*) Using *rpl16–rpl14* Spacer Sequences of Chloroplast DNA

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Genetic variations among flowering cherries (*Prunus* subgenus *Cerasus*) were analyzed by spacer sequences between ribosomal protein L16 (*rpl16*) and ribosomal protein L14 (*rpl14*) genes of chloroplast DNA, these sequences were named plastid subtype ID (PS-ID), by using a total of 40 individuals from 11 species and 3 cultivars. Nucleotide sequences of ca. 420 bp were identified as part of *rpl16* gene and PS-ID regions. One mutation site was found in partial nucleotide sequences of *rpl16* gene. Five different A-repeat types were found at PS-ID region, which were denoted as 9A-T-10A, 10A-T-9A, 13A, 14A, and 15A, respectively. One base change also existed in the downstream of A-repeat. Many individuals (20/22) in species that originated from Japan, except for *P. pendula* f. *ascendens*, were 14A type, whereas all 9 individuals of *P. pendula* f. *ascendens* were 10A-T-9A type. Therefore, the maternal line of cultivars related to *P. pendula* f. *ascendens* can be revealed by the analysis of PS-ID region. In addition, *P. pendula* f. *ascendens* differs from other Japanese taxa based on morphological traits. The difference is supported from the nucleotide sequences of PS-ID in this study. The A-repeat types of cultivars, i.e., 'Someiyoshino', 'Ichiharatoranoo', and 'Shirotae', were 10A-T-9A type, 14A type, and 14A type, respectively, which suggests that the female parent of the 'Someiyoshino' was *P. pendula* f. *ascendens*. The results of 'Ichiharatoranoo' and 'Shirotae' analyses were not contradictory to the morphological taxonomy. PS-ID region was highly variable and useful for evaluating genetic variation and elucidating the origin of cultivars.

Key Words: *Cerasus*, chloroplast, flowering cherry, genetic variation, *rpl16–rpl14* spacer.

Introduction

Flowering cherries are classified into *Prunus* subgenus *Cerasus* in the family Rosaceae (Koehne, 1913; Rehder, 1940). There are several taxonomic systems in *Caresus* (Kawasaki, 1991, 2002; Koehne, 1913; Poyarkova, 1941; Rehder, 1940). In most studies, section *Microcerasus* was classified into subgenus *Cerasus*. However, species of section *Microcerasus* differ from section *Cerasus* on phylogenetic relationships (Bortiri et al., 2002; Lee and Wen, 2001). Therefore, we followed Kawasaki's classification (2002) in treating the section *Microcerasus* as out of subgenus *Cerasus*. Species of subgenus *Cerasus* are mainly distributed among the temperate climate zones on the Northern Hemisphere. Many species of subgenus *Cerasus* are found in East Asia. Nine species and 4 varieties are in Japan (Kawasaki, 1991), whereas 33 species and 6 varieties are distributed in China (Yü and Li, 1986). More than 200 flowering cherry cultivars are documented to exist in Japan (Kobayashi, 1992), which have different flower color, form, size, number of petals and so on. Many cultivars are thought to have originated from Japanese native taxa (Kawasaki, 1993; Koidzumi, 1913; Miyoshi, 1916). Some cultivars, such as 'Kanzakura' (*Prunus* × *kanzakura* Makino 'Kanzakura') and 'Kawazuzakura' (*P.* × *kanzakura* Makino 'Kawazu-zakura') were bred by cross-pollination with *P. campanulata* endemic to Taiwan. 'Taizanfukun' (*P.* × *miyoshii* Ohwi 'Ambigua') and 'Myoshoji' (*P.* × *introrsa* Yagi ex Ohwi 'Myoshoji') were bred by cross-pollination with *P. pseudo-cerasus*, a native of China (Kawasaki, 1993).

Classification has been made on morphological studies in *Prunus* subgenus *Cerasus* (Hayashi, 1980; Kawasaki, 1991; Kobayashi, 1992; Kubota, 1974; Ohba, 1992), and the origin of cultivars based on them (Kawasaki, 1993; Koidzumi, 1913; Miyoshi, 1916). The origin of 'Someiyoshino' (*P.* × *yedoensis* Matsum. 'Yedoensis'),

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the most familiar caltivar, has often been discussed for its morphological traits (Takenaka, 1963), reference investigation (Funatsu, 1960), restriction analysis of chloroplast DNA (Kaneko et al., 1986), and DNA fingerprinting (Innan et al., 1995), whereas that of many cultivars remains unclear (Kawasaki, 1993). Therefore, our attempt in this study was to clarify the origin of cultivars by the molecular genetic approach. The sequence analysis of chloroplast DNA has been effectively utilized for phylogenic study. Phylogeny of some genera that belong to Rosaceae has been examined similarly (Potter et al., 2002; Shaw and Small, 2004; Wu et al., 2002). PS-ID is a spacer sequence between ribosomal protein L16 (*rpl16*) and ribosomal protein L14 (*rpl14*) genes of chloroplast DNA. Nakamura et al. (1997) reported that PS-ID region was variable enough to address plastid subtypes of higher plants. Maternal origins were examined by using PS-ID region in Japanese rice populations (Ishikawa et al., 2002).

In this study, we sequenced chloroplast PS-ID region of *Prunus* subgenus *Cerasus* in attempt to discriminate Japanese species of *Prunus* subgenus *Cerasus*. Three cultivars were used to examine whether the PS-ID region was applicable for the elucidation of their origin.

Materials and Methods

Plant materials

Forty individuals that represent 11 species and 3 cultivars in subgenus *Cerasus* were examined (Table 1). Among them, 9 species are distributed in Japan. The other 2 species, *P. campanulata* and *P. pseudo-cerasus* are endemic to China. 'Someiyoshino', 'Ichiharatoranoo' (*P. jamasakura* Siebold ex Koidz 'Ichihara') and 'Shirotae' (*P. lannesiana* (Carrière) E. H. Wilson 'Sirotae') were also studied. Twelve individuals were collected from wild populations, whereas 28 individuals were obtained from botanical gardens. Leaves for DNA extraction from all individuals were frozen or dehydrated immediately after collection, whereas branch specimens were analyzed for morphological characteristics in co-operation with the late Mr. T. Kawasaki. All specimens are kept in the herbarium of Forestry and Forest Products Research Institute, Tama Forest Science Garden (TFA).

DNA extraction

Total DNA was extracted from frozen 50 mg of leaves by soaking them in ethanol to dehydrate overnight and then pulverizing them by using Multi Beads Shocker (Yasui Kikai, Japan). Samples of dehydrated leaves (30 mg) were crushed into powder in a 1.5 mL tube. DNA was washed with IB buffer (10% polyethyleneglycol, 0.35 M sorbitol, 0.1 M Tris-HCl, pH 7.5–8.0, 1% 2-mercaptoethanol) and LB buffer (0.35 M sorbitol, 0.1 M Tris-HCl, pH 7.5–8.0, 1% 2-mercaptoethanol). Then, 800 μL of DNA extraction buffer (Lefort and Douglas, 1999) was added to the washed sample and incubated for 15 min at 65°C. Four hundred μL of chloroform/isoamylalcohol (24:1, v/v) was added to the mixture and centrifuged (10000 rpm, 5 min, 4°C) to isolate DNA solution. Upon removing the residue, 700 μL of isopropanol was added and the supernatant was centrifuged (15000 rpm, 5 min, 4°C) to obtain DNA pellet. The pellet was dissolved in TE (10 mM Tris-HCl, pH 7.5–8.0, 2 mM EDTA) to which 300 μL of phenol/chloroform (1:1) was added and centrifuged (15000 rpm, 10 min, 4°C) to remove proteins. On adding 900 μL of ethanol and 30 μL of 3 M sodium acetate, the solution was centrifuged (15000 rpm, 10 min, 4°C) to precipitate DNA that was washed with 70% ethanol. Subsequently, the DNA solution was eluted through a Sephadex column (G-50 DNA Grade M, Amersham, USA) at 4°C.

Sequencing of PS-ID regions

PS-ID is a link region between *rpl16* and *rpl14* (Fig. 1). The A primer (5'-AAAGATCTAGATTTCGTAAA-CAACATAGAGGAAGAA-3') and B primer (5'-ATCTGCAGCATTTAAAAGGGTCTGAGGTTGAA-TCAT-3') were used for PCR amplification. The A primer was used to amplify not only PS-ID region but also part of *rpl16* gene.

PCR amplification was performed in a 25 μL solution of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each of dNTPs, 10 pM of each primer, 10 ng of genomic DNA, and 0.5 unit of Taq polymerase (TaKaRa, Japan). The PCR profile consisted of an initial denaturation for 4 min at 94°C followed by 33 cycles of 1 min at 94°C, 1 min at 52°C, 1 min at 72°C, and a final extension of 4 min at 72°C.

The PCR products were purified and then cycle sequence reaction was performed by using BigDye Terminator Cycle Sequence Kit (Applied Biosystems, USA) with A primer or A3 primer (5'-GGTAGCCGT-TGTTAAACCAGGTCGAATACTTTATG-3') and B primer. Sequencing was performed by using a PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). Sequences of all individuals were compared by using DNASIS ver. 3.7 (Hitachi Software, Japan).

Results and Discussion

Variation at rpl16 and PS-ID regions

Nucleotide sequences of ca. 420 bp were identified as part of *rpl16* gene and PS-ID regions from species of *Prunus* subgenus *Cerasus* (Fig. 2). Nucleotide sequences of 1st to 331st position (denoted by capital letters in Fig. 2) were part of *rpl16* gene and the downstream region from 332nd nucleotide (denoted by small letters in Fig. 2) were part of the PS-ID region.

One mutation site (C/T, 19th nucleotide) was found in 2 individuals of *P. maximowiczii* (Cherry-Ohta 158 and Cherry-Ohta 076) at partial nucleotide sequences of *rpl16* gene (Table 1, Fig. 2). Five different sequences, i.e., A9TA10, A10TA9, A13, A14, and A15 were found in PS-ID regions (Table 2). These were denoted as 9A-T-10A, 10A-T-9A, 13A, 14A and 15A, respectively. The

Table 1. Taxonomy and locality of samples of flowering cherry used in this study.

Scientific name	Characteristics	Voucher number	Locality[z]	A-repeat type	Nucleotide at		DDBJ accession number
					19 bp	408 bp	
Prunus apetala (Siebold et Zucc) Franch. et Sav.	incised double serrate, grown in limestone ground, and very small petals (about 0.8 cm)	Cherry-Ohta 164	Hachioji, Tokyo (TFSG)	14A	T	A	AB201292
		Cherry-Ohta 063	Kawaguchiko, Yamanashi[y]	14A	T	A	
		Cherry-Ohta 093	Chino, Nagano[y]	13A	T	A	AB201293
P. incisa Thunb.	incised double serrate, and small petals (about 1.0 cm)	Cherry-Ohta 160	Amatsukominato, Chiba (TFSG)	14A	T	A	
		Cherry-Ohta 165	Fujiyoshida, Yamanashi (TFSG)	14A	T	A	
		Cherry-Ohta 131	Fujimi, Nagano[y]	14A	T	A	
		Cherry-Ohta 048	Gotenba, Shizuoka[y]	14A	T	A	
P. nipponica Matsum.	incised double serrate, and distributed in sub-alpina zone	Cherry-Ohta 113	Ashiyasu, Yamanashi[y]	14A	T	A	
		Cherry-Ohta 140	Fujimi, Nagano[y]	14A	T	A	
P. pendula Maxim. f. *ascendens* (Makino) Ohwi	large tree, no leaves at the blooming time, pot-shaped calyx tube, and large number of lateral veins	Cherry-Ohta 152	Morioka, Iwate (TFSG)	10A-T-9A	T	C	AB201294
		Cherry-Ohta 159	Kurobe, Toyama (TFSG)	10A-T-9A	T	C	
		Cherry-Ohta 148	Sagamiko, Kanagawa[y]	10A-T-9A	T	C	
		Cherry-Ohta 150	Takekawa, Yamanashi (TFSG)	10A-T-9A	T	C	
		Cherry-Ohta 155	Oya, Hyogo (TFSG)	10A-T-9A	T	C	
		Cherry-Ohta 149	Ochiai, Okayama (TFSG)	10A-T-9A	T	C	
		Cherry-Ohta 163	Oguchi, Kagoshima (TFSG)	10A-T-9A	T	C	
		Cherry-Ohta 015	IFES	10A-T-9A	T	C	
		NIG 001	NIG	10A-T-9A	nd[x]	C	
P. jamasakura Siebold ex Koidz.	red young leaves spread out at the blooming time	Cherry-Ohta 154	Hachioji, Tokyo (TFSG)	14A	T	A	
		Cherry-Ohta 001	Mori, Shizuoka[y]	15A	T	A	
		Cherry-Ohta 011	IFES	14A	T	A	
		NIG 002	NIG	14A	nd[x]	A	
P. sargentii Rehder	rose pink petals (resembles *P. jamasakura*)	Cherry-Ohta 010	IFES	14A	T	A	
		Cherry-Ohta 162	Mamurogawa, Yamagata (TFSG)	14A	T	A	
P. verecunda (Koidz.) Koehne	late brooming time (resembles closely *P. jamasakura*)	Cherry-Ohta 183	Yahiko, Niigata[y]	14A	T	A	
		Cherry-Ohta 084	Inasa, Shizuoka[y]	14A	T	A	
		Cherry-Ohta 016	IFES	14A	T	A	
P. lannesiana (Carrière) E. H. Wilson var. *speciosa* (Koidz.) Makino	large petals (about 2.0 cm), and large leaves (8–13 cm long)	Cherry-Ohta 166	Miyake, Tokyo (TFSG)	14A	T	A	
		Cherry-Ohta 017	IFES	14A	T	A	
		NIG 003	NIG	14A	nd[x]	A	
P. maximowiczii Ruprecht	no cut at the top of petals	Cherry-Ohta 158	Chichibu, Saitama (TFSG)	14A	C	A	AB201295
		Cherry-Ohta 139	Fujimi, Nagano[y]	14A	T	A	
		Cherry-Ohta 076	Shizuoka, Shizuoka[y]	14A	C	A	
P. pseudo-cerasus Lindl.	edible fruits, tetraploid (2n = 32), and distributed in China	Cherry-Ohta 009	IFES	9A-T-10A	T	C	AB201296
P. campanulata Maxim.	deep purplish red petals, and distributed in Taiwan	Cherry-Ohta 161	Taiwan (TFSG)	15A	T	A	AB201297
		Cherry-Ohta 014	IFES	14A	T	A	
		Cherry-Ohta 101	TBG	10A-T-9A	T	C	
P. × *yedoensis* Matsum. 'Yedoensis'	cultivar	NIG 004	NIG	10A-T-9A	nd[x]	C	
P. jamasakura Siebold ex Koidz. 'Ichihara'	cultivar	NIG 005	NIG	14A	nd[x]	A	
P. lannesiana (Carrière) E. H. Wilson 'Sirotae'	cultivar	NIG 006	NIG	14A	nd[x]	A	

[z] TFSG: Forestry and Forest Products Research Institute, Tama Forest Science Garden. IFES: Tree Park, Ishikawa-ken Forest Experiment Station. NIG: National Institute of Genetics. TBG: Tsukuba Botanical Garden, National Science Museum.
[y] Individuals collected from wild populations.
[x] No data at the region.

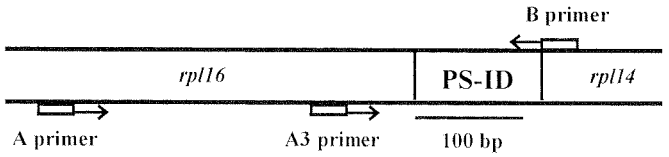

Fig. 1. Primer sites for amplifying the PS-ID (Plastid subtype ID) region located in an intergenic region between *rpl16* (ribosomal protein L16) and *rpl14* (ribosomal protein L14).

	1	11	21	31	41
Cherry-Ohta 158	TATTTGCTTC	GGTAGATACG	CTCTTCAAGC	GCTTGAACCC	GCTTGGATCA
Cherry-Ohta 161	TATTTGCTTC	GGTAGATATG	CTCTTCAAGC	GCTTGAACCC	GCTTGGATCA
Cherry-Ohta 152	TATTTGCTTC	GGTAGATATG	CTCTTCAAGC	GCTTGAACCC	GCTTGGATCA
	51	61	71	81	91
Cherry-Ohta 158	CATCTAGACA	AATAGAAGCA	GGGCGGCGAG	CAATGACACG	AAACGCACGC
Cherry-Ohta 161	CATCTAGACA	AATAGAAGCA	GGGCGGCGAG	CAATGACACG	AAACGCACGC
Cherry-Ohta 152	CATCTAGACA	AATAGAAGCA	GGGCGGCGAG	CAATGACACG	AAACGCACGC
	101	111	121	131	141
Cherry-Ohta 158	CGCGGTGGAA	AAATATGGGT	ACGCATATTT	CCAGACAAAC	CCGTTACAGT
Cherry-Ohta 161	CGCGGTGGAA	AAATATGGGT	ACGCATATTT	CCAGACAAAC	CCGTTACAGT
Cherry-Ohta 152	CGCGGTGGAA	AAATATGGGT	ACGCATATTT	CCAGACAAAC	CCGTTACAGT
	151	161	171	181	191
Cherry-Ohta 158	AAGACCTACA	GAAACACGTA	TGGGTTCGGG	TAAAGGATCT	CCCGAATATT
Cherry-Ohta 161	AAGACCTACA	GAAACACGTA	TGGGTTCGGG	TAAAGGATCT	CCCGAATATT
Cherry-Ohta 152	AAGACCTACA	GAAACACGTA	TGGGTTCGGG	TAAAGGATCT	CCCGAATATT
	201	211	221	231	241
Cherry-Ohta 158	GGGTAGCTGT	TGTTAAACCC	GGTAGAATAC	TTTATGAAAT	GAGCGGAGTA
Cherry-Ohta 161	GGGTAGCTGT	TGTTAAACCC	GGTAGAATAC	TTTATGAAAT	GAGCGGAGTA
Cherry-Ohta 152	GGGTAGCTGT	TGTTAAACCC	GGTAGAATAC	TTTATGAAAT	GAGCGGAGTA
	251	261	271	281	291
Cherry-Ohta 158	GCAGAAAATA	TAGCCAGAAG	GGCAATTTCA	ATAGCGGCAT	CCAAAATGCC
Cherry-Ohta 161	GCAGAAAATA	TAGCCAGAAG	GGCAATTTCA	ATAGCGGCAT	CCAAAATGCC
Cherry-Ohta 152	GCAGAAAATA	TAGCCAGAAG	GGCAATTTCA	ATAGCGGCAT	CCAAAATGCC
	301	311	321	331	341
Cherry-Ohta 158	TATACGAACT	CAATTCATTC	TTTCGGGATA	Gggatgtaga	aacaaaggaa
Cherry-Ohta 161	TATACGAACT	CAATTCATTC	TTTCGGGATA	Gggatgtaga	aacaaaggaa
Cherry-Ohta 152	TATACGAACT	CAATTCATTC	TTTCGGGATA	Gggatgtaga	aacaaaggaa
	351	361	371	381	391
Cherry-Ohta 158	aggggtcttt	tgtatgaaaa	aaaaaaaaaa	------ttgc	aggttttttg
Cherry-Ohta 161	aggggtcttt	tgtatgaaaa	aaaaaaaaaa	a-----ttgc	aggttttttg
Cherry-Ohta 152	aggggtcttt	tgtatgaaaa	aaaaaataaa	aaaaaattgc	aggttttttg
	401	411			
Cherry-Ohta 158	ttttgaaaaa	ataatattt			
Cherry-Ohta 161	ttttgaaaaa	ataatattt			
Cherry-Ohta 152	ttttgaacaa	ataatattt			

Fig. 2. Nucleotide sequences of *rpl16* (ribosomal protein L16) and PS-ID (Plastid subtype ID) regions in 3 individuals of *Prunus* subgenus *Cerasus*. Individuals of Cherry-Ohta 158, Cherry-Ohta 161 and Cherry-Ohta 152, are classified as *P. maximowiczii*, *P. campanulata* and *P. pendula* f. *ascendens*, respectively. Nucleotide sequences of the *rpl16* and PS-ID regions are shown in capital and small letters, respectively. Substitution sites and A repeat regions are shown in boxes and underline, respectively.

most frequent type was 14A in which 23 individuals of wild species were involved. It was observed in all wild species except for *P. pendula* f. *ascendens* and *P. pseudocerasus*. The 9A-T-10A type, 10A-T-9A type, 13A type, and 15A type were occurred 1, 10, 1, and 2 individuals, respectively. The 10A-T-9A type was found in 10 individuals of wild species, of which 9 were in *P. pendula* f. *ascendens*. As for the PS-ID region, 1 base change (C/A, 408th nucleotide) existed in the downstream of A-repeat, which corresponded to the sequences of 9A-T-10A type and 10A-T-9A type (Table 1). Five haplotypes were distinguished from the sequence of PS-ID region (81–88 bp long). Only one site of base change existed in part of *rpl16* gene (331 bp long). These results

Table 2. Five A-repeat types in PS-ID observed in subgenus *Cerasus*.

Type	Sequence
9A-T-10A	AAAAAAAAATAAAAAAAAAA
10A-T-9A	AAAAAAAAAATAAAAAAAAA
13A	AAAAAAAAAAAAA
14A	AAAAAAAAAAAAAA
15A	AAAAAAAAAAAAAAA

indicated that PS-ID region is highly variable.

Variation of intra- and inter-species

An intraspecific variation of part of *rpl16* gene (19th C/T) was observed only in *P. maximowiczii* (Table 1). Two individuals of *P. maximowiczii* (Cherry-Ohta 158 and Cherry-Ohta 076) contained a C nucleotide at the mutation site (Table 1, Fig. 2). The other one showed a T nucleotide. *P. maximowiczii* is morphologically different from other Japanese species, based on phenotypic traits, such as having no cut at the tip of petals, raceme, and remaining large bract. Therefore, it was thought that two individuals of *P. maximowiczii* have a different genetic background. An intraspecific variation of A-repeat type at PS-ID was seen in 3 species, i.e., *P. apetala*, *P. jamasakura* and *P. campanulata* (Table 3). Out of 3 individuals of *P. apetala*, Cherry-Ohta 093 was a 13A type, while Cherry-Ohta 164 and Cherry-Ohta 063 were of the 14A type. Among 4 individuals of *P. jamasakura*, Cherry-Ohta 001 was a 15A type, while Cherry-Ohta 154, Cherry-Ohta 011, and NIG 002 were in the 14A type. All 3 individuals of *P. campanulata* showed different A-repeat types. Cherry-Ohta 101, Cherry-Ohta 014, and Cherry-Ohta 161 were identified as types, 10A-T-9A, 14A, and 15A, respectively. There were no intra-specific variations in other wild species used in this study except for *P. pseudo-cerasus*. All 9 individuals of *P. pendula* f. *ascendens* were 10A-T-9A type, and one individual of *P. pseudo-cerasus* (Cherry-Ohta 009) was 9A-T-10A type. In addition, the individuals with 14A were 4 *P. incisa*, 2 *P. nipponica*, 2 *P. sargentii*, 3 *P. verecunda*, 3 *P. lannesiana* var. *speciosa*, and 3 *P. maximowiczii*.

All 9 individuals of *P. pendula* f. *ascendens*, that were 10A-T-9A type, had a base change of A to C at 408th nucleotide (Table 1). Only one individual of wild species (Cherry-Ohta 101; *P. campanulata*) was the same haplotype as *P. pendula* f. *ascendens*. These results suggest that the maternal line of cultivars, related to *P. pendula* f. *ascendens*, will be revealed by the analysis of their PS-ID regions. Hence, when a cultivar, related to *P. pendula* f. *ascendens*, was 10A-T-9A type, its maternal parent would be *P. pendula* f. *ascendens*. There are many morphological differences between *P. pendula* f. *ascendens* and *P. campanulata* such as the existence of hair on leaves and petioles, the number of lateral veins in the leaf blades, the shape of calyx tube, and petal color (Kawasaki, 1993). Therefore, cultivars of the 10A-T-9A type of A-repeat can be distinguished as being derivatives of *P. pendula* f. *ascendens* or *P. campanulata* as their maternal parentage. Thus, a cultivar, related to *P. pendula* f. *ascendens*, that was not 10A-T-9A type, *P. pendula* f. *ascendens* would be its male parent. Kaneko et al. (1986) distinguished *P. pendula* f. *ascendens* from some Japanese native taxa of subgenus *Cerasus* based on restriction endonuclease analysis of chloroplast DNA, but their study did not include *P. jamasakura*, *P. incisa*, *P. campanulata* and so on. Furthermore, the difference between *P. pendula* f. *ascendens* and some Japanese native taxa was only one restriction site. In this study, *P. pendula* f. *ascendens* was distinguished from Japanese native taxa of subgenus *Cerasus*, including all taxa except for local varieties, based on A-repeat type and 1 site of base change at PS-ID region. Intraspecific variations were also observed in some species classified into different sections such as, *P. apetala*, *P. jamasakura*, and *P. campanulata* (Table 3). Especially, 3 individuals from *P. campanulata*, species from a foreign country,

Table 3. Variation of repeat types observed in PS-ID in each taxon of subgenus *Cerasus*.

Taxon	A-repeat type[z]				
	9A-T-10A	10A-T-9A	13A	14A	15A
P. apetala	0	0	1	2	0
P. incisa	0	0	0	4	0
P. nipponica	0	0	0	2	0
P. pendula f. *ascendens*	0	9	0	0	0
P. jamasakura	0	0	0	3	1
P. sargentii	0	0	0	2	0
P. verecunda	0	0	0	3	0
P. lannesiana var. *speciosa*	0	0	0	3	0
P. maximowiczii	0	0	0	3	0
P. campanulata	0	1	0	1	1
P. pseudo-cerasus	1	0	0	0	0

[z] Number of individuals showing each repeat type is indicated.

had different A-repeat sequences. Therefore, it was thought that adding the analysis of PS-ID region was much better than the analysis only by restriction endonuclease to formulate the relationships among subgenus *Cerasus*.

Relationships among Prunus subgenus Cerasus

P. pendula f. *ascendens* showed a different haplotype from other Japanese species. It differs from other Japanese taxa based on morphological traits, such as its pot-shaped calyx tube, elliptical shape of leaves, and large number of lateral veins in the leaf blades. Thus, *P. pendula* f. *ascendens* is commonly considered to be genetically far apart from other Japanese taxa (Kawasaki, 1991; Kobayashi, 1992); this is also supported from the nucleotide sequence of PS-ID in this study. However, it will be necessary to investigate more regions to establish exact relationships among subgenus *Cerasus*.

Three individuals from *P. campanulata* and 1 individual from *P. pseudo-cerasus*, that are the species from a foreign country, were different A-repeat types, whereas 20 individuals among 22 individuals in wild species that originated in Japan, except for *P. pendula* f. *ascendens*, were the 14A type. These results suggested that Japanese species have a smaller variation than do the foreign species. Our results indicate that some haplotypes existed in Japanese and foreign species, and that the analysis of PS-ID region is useful to elucidate the origin of the Japanese flowering cherry (*Prunus* subgenus *Cerasus*). It is interesting that the same base change occurred in the downstream of 2 types of A-repeat (the 9A-T-10A and the 10A-T-9A) when discussing the relation among haplotypes.

Analysis of cultivars

The A-repeat types of 'Someiyoshino', 'Ichiharatoranoo', and 'Shirotae' were 10A-T-9A type, 14A type and 14A type, respectively (Table 1). 'Someiyoshino' showed the same haplotype as *P. pendula* f. *ascendens*, characterized by 10A-T-9A type and the base change at the 408th nucleotide (Table 1). Hence, it is considered a hybrid between *P. pendula* f. *ascendens* and *P. lannesiana* var. *speciosa* based on morphological traits, crossing experiment, and reference investigation (Funatsu, 1960; Takenaka, 1963). It was suggested that the female parent of the 'Someiyoshino' is *P. pendula* f. *ascendens*, because they have the same haplotype at PS-ID region. This is in agreement with restriction endonuclease analysis of chloroplast DNA by Kaneko et al. (1986). However, our result is contradictory in that 'Someiyoshino' is an offspring of *P. lannesiana* var. *speciosa* as a female plant (Funatsu 1960). The question whether 'Someiyoshino' reported by Funatsu (1960) is identical to that used in this study remains further study. 'Ichiharatoranoo' and 'Shirotae' are thought to have originated from *P. jamasakura* and *P. lannesiana* var. *speciosa*, respectively (Kawasaki, 1993) as they are 14A types at PS-ID region. These results are not contradictory to the in morphological taxonomy.

Since many cultivars are related to *P. pendula* f. *ascendens*, such as 'Kohigan' (*P.* × *subhirtella* Miq. 'Subhirtella'), 'Koshinohiganzakura' (*P. koshiensis* Koidz.) and 'Kabazakura' (*P.* × *media* Miyoshi 'Media'), the analysis of the PS-ID region seems applicable for the elucidation of the origin of the flowering cherries.

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Literature Cited

Bortiri, E., S. H. Oh, F. Y. Gao and D. Potter. 2002. The phylogenetic utility of nucleotide sequences of sorbitol 6-phosphate dehydrogenase in *Prunus* (Rosaceae). Amer. J. Bot. 89: 1697–1708.

Funatsu, K. 1960. Who raised *Prunus yedoensis* Matsum. Collecting and Breeding 28: 95 (In Japanese).

Hayashi, Y. 1980. One hundred of selective flowering cherries (In Japanese). New Science, Tokyo.

Ishikawa, R., Y.-I. Sato, T. Tang and I. Nakamura. 2002. Different maternal origins of Japanese lowland and upland rice populations. Theor. Appl. Genet. 104: 976–980.

Innan, H., R. Terauchi, N. T. Miyashita and K. Tsunewaki. 1995. DNA fingerprinting study on the intraspecific variation and the origin of *Prunus yedoensis* (Someiyoshino). Jpn. J. Genet. 70: 185–196.

Kaneko, T., T. Terachi and K. Tsunekawa. 1986. Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. II. Restriction analysis of ctDNA of 11 *Prunus* species. Jpn. J. Genet. 61: 157–168.

Kawasaki, T. 1991. The distribution of *Prunus* subgenus *Cerasus* in East-Asia and classification of Japanese wild species. Sakura Science 1: 28–45 (In Japanese with English summary).

Kawasaki, T. 1993. Flowering cherries of Japan (In Japanese). Yama-kei Publishers, Tokyo.

Kawasaki, T. 2002. The classification of *Prunus* subgenus *Cerasus*. Forestry Technology 719: 22–25 (In Japanese).

Kobayashi, Y. 1992. Flowering cherry cultivars. Planter 20: 9–14 (In Japanese).

Koidzumi, G. 1913. Conspectus Rosacearum Japonicarum. J. Coll. Sci. Imp. Univ. Tokyo 34: 254–286 (In German).

Koehne, E. 1913. *Prunus* L. subgen. *Cerasus*. p. 226–262. In: C. S. Sargent (ed.). Plantae Wilsonianae vol. 1. Harvard University Press, Massachusetts.

Kubota, H. 1974. Wild flowering cherries. p. 64–99 (In Japanese). In: S. Honda and S. Hayashi (eds.). Flowering cherries of Japan. Seibundo Shinkosya, Tokyo.

Lee, S. and J. Wen 2001. A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. Amer. J. Bot. 88: 150–160.

Lefort, F. and G. C. Douglas 1999. An efficient micro-method

of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. Ann. For. Sci. 56: 259–263.

Miyoshi, M. 1916. Japanische Bergkirschen, ihre Wildformen und Kulturrassen. J. Coll. Sci. Imp. Univ. Tokyo 34: 175 (In German).

Nakamura, I., N. Kameya, Y. Kato, S. Yamanaka, H. Jomori and Y. Sato. 1997. A proposal for identifying the short ID sequence which addresses the plastid subtype of higher plants. Breed. Sci. 47: 385–388.

Ohba, H. 1992. Japanese cherry trees under the genus *Cerasus* (Rosaceae). J. Jpn. Bot. 67: 276–281.

Potter, D., F. Gao, P. E. Bortiri, S. H. Oh and S. Baggett. 2002. Phylogenetic relationships in Rosaceae inferred from chloroplast *mat*K and *trn*L-*trn*F nucleotide sequence data. Plant Syst. Evol. 231: 77–89.

Poyarkova, A. I. 1941. Genus *Cerasus*. p. 547–575 (In Russian). In: V. L. Komarov (ed.). Flora SSSR vol 10, Akad. Nauk. SSSR, Moskva Leningrad.

Rehder, A. 1940. Manual of cultivated trees and shrubs, 2nd edn. Macmillan, New York.

Shaw, J. and R. Small. 2004. Addressing the "hardest puzzle in American pomology:" Phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. Amer. J. Bot. 91: 985–996.

Takenaka, Y. 1963. The origin of the Yoshino cherry tree. J. Hered. 54: 207–211.

Wu, S., Y. Ueda, H. He, S. Nishihara and S. Matsumoto. 2002. Phylogenetic analysis of Japanese Rosa species using *mat*K sequences. Breed. Sci. 50: 275–281.

Yü, T. T. and C. L. Li. 1986. *Cerasus*. p. 1–133 (In Chinese). In: T. T. Yü (ed.). Flora Reipublicae Popularis Sinicae 38, Science Press, Beijing.

葉緑体 *rpl16–rpl14* 領域の塩基配列によるサクラ属サクラ亜属の遺伝的特徴づけ

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サクラ属サクラ亜属 11 種および 3 つの栽培品種から合計 40 個体を用い，葉緑体 *rpl16–rpl14*（PS-ID）領域の塩基配列により遺伝的変異を明らかにした．*rpl16* 遺伝子の一部および PS-ID 領域の合計約 420 塩基対の配列が決定できた．*rpl16* 遺伝子の一部には，1 か所の塩基置換が存在した．PS-ID 領域には 5 種類の A リピートが認められ，それぞれ 9A-T-10A，10A-T-9A，13A，14A および 15A と名付けた．A リピートの下流には，1 か所の塩基置換が存在した．エドヒガンは 9 個体すべてが 10A-T-9A 型であった．一方，エドヒガンを除く日本産の種では，多くの個体（20/22）が 14A 型だった．したがって，PS-ID 領域の分析により，エドヒガンに由来する栽培品種の母系が明らかにできると考えられた．さらに，エドヒガンは形態的に他の日本産の分類群とは異なるが，このことは今回の PS-ID 領域の塩基配列からも支持された．3 つの栽培品種，'染井吉野'，'市原虎の尾' および '白妙' の A リピート型は，それぞれ 10A-T-9A 型，14A 型および 14A 型であった．このことから '染井吉野' の母親はエドヒガンであることが示唆された．'市原虎の尾' および '白妙' の結果も，形態学的な分類と矛盾がなかった．これらの結果から PS-ID 領域は，日本産のサクラ亜属の由来や，栽培品種の由来を解明するのに有効と考えられた．