Parentage Analysis in Pear Cultivars Characterized by SSR Markers

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Summary

Parentage of 14 pear cultivars, including 8 cultivars derived from intraspecific crosses and 6 from interspecific crosses, was analyzed using 20 SSR (simple sequence repeat) markers. In 10 out of 14 cultivars, the parent–offspring relationships were reconfirmed because the hybrids inherited SSR alleles from their parents without any discrepancy. There were 4 questionable parent–offspring relationships with respect to 7 or more SSR loci. ‘Housui’ (syn. ‘Hosui’) is not an offspring from a cross of ‘Ri-14’ × ‘Yakumo’ because of discrepancies at 7 loci. Likewise, ‘Chojuro’ and ‘Nijisseiki’ could not be confirmed as parents of ‘Tanzawa’ because of discrepancies at 9 loci. ‘Max Red Bartlett’ was reconfirmed as a parent of ‘Oharabeni’ because all SSR loci matched. ‘Okusankichi’ was not its parent because of discrepancies at 7 loci. Our analyses revealed that ‘Le Conte’ is not a parent of ‘Ninomiya’, whereas ‘Chojuro’ is. In this study, 20 SSR markers were effectively utilized to determine parentage of Japanese pear, and 15 SSR markers could be utilized for interspecific *Pyrus* hybrids.

Key Words: Housui, microsatellite, parent–offspring relationship, *Pyrus*, simple sequence repeat.

Introduction

Pears have been cultivated in Europe, as early as 1000 B.C. (Hedrick et al., 1921). In Japan, the Japanese pear (*Pyrus pyrifolia* (Burm.) Nakai) has been cultivated since the eighth century (Kajiura, 1966). A large number of cultivars had been generated from chance seedlings and bud sports before an organized breeding program started in Japan in 1915 (Kajiura and Sato, 1990). Thereafter, many pear cultivars have been developed by controlled crossbreeding during the last 100 years. Discrepancies in the parent–offspring relationships have been found for some pear cultivars based on morphological traits (Kajiura and Sato, 1990). For example, the parentage of ‘Housui’ thought to be a hybrid between ‘Ri-14’ and ‘Yakumo’, is questioned because of discrepancies in the inheritance of the fruit skin color and self-incompatible genes (Machida et al., 1982). There are many other cultivars whose exact parent–offspring relationships are unknown and unidentifiable based on their morphological characteristics.

S alleles at a single self-incompatible locus have been used for the assessment of parentage in pear. Machida et al. (1982) found that there was discrepancy in the self-incompatible trait in the parentage of ‘Housui’, as well

as in 7 progenies among the 44 seedlings obtained from ‘Ri-14’ × ‘Yakumo’. Subsequently, the *S* genotype of ‘Housui’ was proved as *S*₃*S*₅ (Terai et al., 1997; Ishimizu et al., 1998). RFLPs (restriction fragments length polymorphisms) have been used to identify Japanese pears, including the parentage of 10 cultivars, with only 2 multi-locus probes from human DNA (Teramoto et al., 1994). However, it is necessary to develop many more probes to identify closely related Japanese pear cultivars. Genetic relationships of pear cultivars were reported by using *S* genotypes, isozymes (Jang et al., 1992) and RAPD markers (Banno et al., 2000; Teng et al., 2002). However, only a limited information was noted in parentage analysis in pear. Therefore, it is necessary to use a large number of co-dominant markers in order to evaluate the parentage of pear cultivars.

Recently, SSRs (simple sequence repeats, also called as microsatellites) have become the markers of choice in both animal and plant species because of their abundance, high degree of polymorphism, and suitability for automation (Weber and May, 1989). SSR provides a more reliable method for DNA fingerprinting because of its co-dominant inheritance and large number of alleles per locus. In humans and animals, SSR markers of a single locus have been used in parentage analyses and criminal investigations (Hughes, 1992; Glowatzki-Mullis et al., 1996).

SSR markers have been used for parentage analyses in

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grape (Bowers and Meredith, 1997; Bowers et al., 1999; Sefc et al., 1997) and peach (Testolin et al., 2000). In grape, ‘Cabernet Sauvignon’ was confirmed as the progeny of ‘Cabernet Franc’ and ‘Sauvignon Blanc’ (Bowers and Meredith, 1997; Sefc et al., 1997). In peach, 9 cultivars were reconfirmed as parents because there was no discrepancy in the inheritance of SSR markers (Testolin et al., 2000). SSR markers are, therefore, more reliable than any other molecular marker for parentage analysis.

In a previous study, we reported that 35 SSR markers have been isolated in pear and these SSR markers could be utilized for all tested *Pyrus* species (Yamamoto et al., 2002a, b). We also revealed that 9 SSRs developed from pear could be used for genetic identification of Asian pear varieties (Kimura et al., 2002). In this study, the parentage of 14 cultivars derived from the Japanese, European and Chinese pears and interspecific hybrids was examined by using 20 SSR markers.

Materials and Methods

Plant materials and DNA extraction

Fourteen pear cultivars and their parents were used in this study (Table 1). Eight cultivars are intraspecific hybrids, whereas 6 cultivars are results of interspecific hybridization. Plant materials were obtained from the National Institute of Fruit Tree Science (Tsukuba and Morioka, Japan), the Yamagata Prefectural Horticultural Experiment Station (Sagae, Japan) and the National Agricultural Research Center for Hokkaido Region (Sapporo, Japan). Genomic DNA was isolated from fresh young leaves, using the modified CTAB protocol

described by Yamamoto et al. (2001).

SSR amplification and parentage analysis

Twenty SSR markers (KA4b, KA14, KA16, KU10, BGA35, BGT23b, NH001c, NH002b, NH004a, NH005b, NH007b, NH008b, NH009b, NH010a, NH011b, NH012a, NH013a, NH014a, NH015a and NH017a) were used (Yamamoto et al., 2002a, b). PCR amplification was performed in a 20 μ l solution of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01 % gelatin, 0.2 mM of each dNTP, 10 pmoles of each forward primer labeled with a fluorescent chemical (FAM, TET or HEX), and unlabelled reverse primer, 10 ng of genomic DNA, and 0.5 unit of Taq polymerase (Life Technologies, USA). For the SSR loci of KA4b, KA14, KA16, KU10, BGA35 and BGT23b, amplification was conducted as described by Yamamoto et al. (2002b), whereas PCR was utilized on 14 SSR loci (NH001c, NH002b, NH004a, NH005b, NH007b, NH008b, NH009b, NH010a, NH011b, NH012a, NH013a, NH014a, NH015a and NH017a) according to Yamamoto et al. (2002a). The PCR products were separated and detected, using a PRISM 377 DNA sequencer (PE Applied Biosystems, USA). The size of the amplified bands was calculated based on an internal standard DNA (GeneScan-350TAMRA, PE Applied Biosystems, USA) with GeneScan software (PE Applied Biosystems, USA). Parentage was analyzed based on the inheritance of one allele at a single SSR locus transmitted from the parent to the offspring.

Table 1. Fourteen pear cultivars and their reported parents used in this study.

Cultivar	Female parent	Male parent	Species	Citation
Intraspecific cross				
Aurora	Marguerite Marillat	Bartlett	<i>P. communis</i> × <i>P. communis</i>	Brooks and Olmo (1997)
Ballade	Bartlett	La France	<i>P. communis</i> × <i>P. communis</i>	Abe et al. (1996)
Gorham	Bartlett	Josephine de Malines	<i>P. communis</i> × <i>P. communis</i>	Brooks and Olmo (1997)
Pierre Corneille	Beurre Diel *	Doyenne du Comice	<i>P. communis</i> × <i>P. communis</i>	Hedrick (1921)
Gion	Chojuro	Nijisseiki	<i>P. pyrifolia</i> × <i>P. pyrifolia</i>	Kajiura and Sato (1990)
Housui	Ri-14	Yakumo	<i>P. pyrifolia</i> × <i>P. pyrifolia</i>	Kajiura and Sato (1990)
Shinseiki	Nijisseiki	Chojuro	<i>P. pyrifolia</i> × <i>P. pyrifolia</i>	Kajiura and Sato (1990)
Tanzawa	Chojuro	Nijisseiki	<i>P. pyrifolia</i> × <i>P. pyrifolia</i>	Kajiura and Sato (1990)
Interspecific cross				
Oharabeni	Okusankichi	Max Red Bartlett	<i>P. pyrifolia</i> × <i>P. communis</i>	Takahashi et al. (1981)
Taiheiyo	Okusankichi	La France	<i>P. pyrifolia</i> × <i>P. communis</i>	Kajiura and Sato (1990)
Ninomiyaabaili	Yali	Shinchu	<i>P. bretschneideri</i> × <i>P. pyrifolia</i>	Kajiura and Sato (1990)
Kangyoku	Chojuro	Mishirazu	<i>P. pyrifolia</i> × (<i>P. pyrifolia</i> × <i>P. communis</i>)	Kajiura and Sato (1990)
Ninomiya	Chojuro	Le Conte	<i>P. pyrifolia</i> × (<i>P. communis</i> × <i>P. pyrifolia</i>)	Kajiura and Sato (1990)
Zaosu	Pingguoli	Mishirazu	(<i>P. pyrifolia</i> × <i>P. ussuriensis</i>) × (<i>P. pyrifolia</i> × <i>P. communis</i>)	Qu (1980)

* Beurre Diel was not used in this study.

Results and Discussion

SSR amplification

In Japanese pear cultivars, all 20 SSR loci tested produced 1 or 2 discrete amplified fragment(s), which were used for determining the parentage of 4 Japanese pear cultivars, 'Gion', 'Housui', 'Shinseiki' and 'Tanzawa'.

In European pear cultivars, 16 of the 20 SSR loci produced 1 or 2 discrete amplified fragments, whereas the 4 SSR loci (NH008b, NH010a, NH012a and NH017a) did not. The KA4b locus previously generated a null allele in European pears (Yamamoto et al., 2002b) so it was not used. Therefore, 15 SSR loci were used to determine the parentage of 4 European pear, 'Aurora', 'Ballade', 'Gorham' and 'Pierre Corneille'. The five SSR loci (KA4b, NH008b, NH010a, NH012a and NH017a) which could not be used in European pears were designed from the sequences of 'Housui'. This suggests that these primer sequences might not be completely conserved between *P. pyrifolia* and *P. communis* or that the flanking regions around repeats might have sometimes mutated (Yamamoto et al., 2002b).

In the 5 interspecific hybrids except for 'Ninomiya-baili', 15 SSR loci produced 1 or 2 discrete amplified fragments, whereas the 5 SSR loci (KA4b, NH008b, NH010a, NH012a and NH017a) did not because of weak amplification or the possibility of a null allele. Thus, 18 SSR loci, except for the KA4b and NH012a, were used to determine the parentage of 'Ninomiya-baili', whereas 15–18 SSR loci were tested on 6 interspecific hybrids, 'Taiheiyō', 'Oharabeni', 'Ninomiya-baili', 'Kangyoku', 'Ninomiya' and 'Zaosu'.

Segregation of SSR genotypes were reported in 3 F_1 populations of pear, i.e., the interspecific F_1 progenies of 'Bartlett' and 'Housui', the intraspecific population between Japanese pears '314-32' $\times$ 'Tsukuba 46', and the three-way interspecific hybrids of 'Shinsei' $\times$ '282-12' [*P. pyrifolia* $\times$ (*P. pyrifolia* $\times$ *P. communis*)] (Yamamoto et al., 2002a, c). In these populations, segregation of 5 SSRs (KA4b, KA14, KA16, KU10 and BGA35) fitted the monogenic inheritance (Yamamoto et al., 2002b). The other SSR marker fitted the expected segregation in the F_1 population of 'Bartlett' and 'Housui', and/or could be mapped in the genetic linkage maps of 'Bartlett' and 'Housui' (Yamamoto et al., 2002c). Thus, all SSRs used in this study were apparently derived from a single locus and highly useful for parentage analysis in pear.

Parentage analysis of European pear

The parentage of 4 European pear cultivars, using 15 SSR loci, revealed that 'Aurora' is a derivative from a cross of 'Marguerite Marillat' $\times$ 'Bartlett', 'Ballade' from 'Bartlett' $\times$ 'La France', 'Gorham' from 'Bartlett'

	NH002b : 170/ 184	
	NH004a : 90 / 96	
Bartlett	NH009b : 143/ 149	Gorham (offspring)
(female)	NH011b : 168/ 172	
	NH014a : 70/ 72	
	NH015a : 107/ 119	
	NH002b : <u>170</u> / <u>174</u>	
Josephine	NH004a : <u>90</u> / 90	Gorham (offspring)
de Malines	NH009b : <u>143</u> / <u>143</u>	
(male)	NH011b : 162/ <u>172</u>	
	NH014a : <u>82</u> / 82	
	NH015a : 106/ <u>107</u>	

Fig. 1. Parentage analysis of 'Gorham' and its parents 'Bartlett' and 'Josephine de Malines' by 6 SSR loci. Putative alleles transmitted from 'Bartlett' and 'Josephine de Malines' are indicated by bold type and underlines, respectively.

$\times$ 'Josephine de Malines', and 'Pierre Corneille' from 'Beurre Diel' $\times$ 'Doyenne du Comice'. In the parentage of 'Gorham', 12 SSR loci showed polymorphisms between its parent cultivars 'Bartlett' and 'Josephine de Malines'. Thirty-nine alleles were generated from 15 SSR loci in the parent of 'Gorham'.

The inheritance of SSR alleles for 'Gorham' and its parents revealed that those of 184 bp and 170 bp at NH002b were inherited from 'Bartlett' and 'Josephine de Malines', respectively (Fig. 1). Similarly, the alleles of 90 bp and 90 bp at NH004a, 149 bp and 143 bp at NH009b, 172 bp and 172 bp at NH011b, 72 bp and 82 bp at NH014a, 119 bp and 107 bp at NH015a were also inherited from 'Bartlett' and 'Josephine de Malines', respectively. In 'Aurora' and 'Ballade', 7 and 13 SSR loci showed polymorphisms between parents, respectively. One allele of a parent was consistently transmitted to the offsprings for all SSR loci. Four cultivars, 'Aurora', 'Ballade', 'Gorham' and 'Pierre Corneille' were reconfirmed as offsprings of their cited parents because there was no discrepancy in the inheritance of all polymorphic SSR alleles (Fig. 1, 2).

Parentage analysis of Japanese pear

An analysis of 4 Japanese pears, 'Gion', 'Housui', 'Shinseiki' and 'Tanzawa', using 20 SSR loci, revealed that 'Gion' is a hybrid between 'Chojuro' $\times$ 'Nijisseiki' and that 'Shinseiki' is a product of 'Nijisseiki' $\times$ 'Chojuro' in that 11 SSR loci showed polymorphisms between parents. One allele at all polymorphic SSR loci was transmitted from parents to their offsprings and no discrepancy in the inheritance of alleles at 20 SSR loci (Fig. 2). Therefore, 'Gion' and 'Shinseiki' were reconfirmed as offsprings of their cited parents.

In 'Housui', 35 alleles were generated from 20 SSR loci. Nine alleles observed in 'Housui' were not observed in either 'Ri-14' or 'Yakumo'. For example, the SSR alleles of 251 bp and 253 bp at KU10, 192 bp at BGT23b, 180 bp at NH002b, 341 bp at NH005b, 222 bp

Fig. 2. Amplified SSR fragments of 6 sets of pears with parent-offspring relationships at NH014a locus. Lanes 1 to 18 show amplified products of the following varieties. Lane 1: 'Aurora' ('Marguerite Marillat' × 'Bartlett'); 2: 'Marguerite Marillat'; 3: 'Bartlett'; 4: 'Ballade' ('Bartlett' × 'La France'); 5: 'Bartlett'; 6: 'La France'; 7: 'Gion' ('Chojuro' × 'Nijisseiki'); 8: 'Chojuro'; 9: 'Nijisseiki'; 10: 'Shinseiki' ('Nijisseiki' × 'Chojuro'); 11: 'Chojuro'; 12: 'Nijisseiki'; 13: 'Oharabeni' ('Okusankichi' × 'Max Red Bartlett'); 14: 'Okusankichi'; 15: 'Max Red Bartlett'; 16: 'Ninomiya' ('Chojuro' × 'Le Conte'); 17: 'Chojuro'; 18: 'Le Conte'.

Fig. 3. Amplified SSR fragments of 'Housui', 'Ri-14' and 'Yakumo' at 3 SSR loci, KU10, NH002b and NH013a. Lanes 1 to 9 show amplified products of the 3 varieties at the following SSR loci. Lane 1 to 3: KU10; Lane 4 to 6: NH002b; Lane 7 to 9: NH013a.

at NH013a, 70 bp and 86 bp at NH014a, and 90 bp at NH017a do not match (Table 2, Fig. 3). Therefore, neither 'Ri-14' nor 'Yakumo' was confirmed as parents of 'Housui'.

Morphological traits of 'Housui' support our data

because 'Housui' has a corked, brown, russeted skin, whereas seedlings of 'Ri-14' × 'Yakumo' have a smooth skin without any russeting (Machida et al., 1982).

Furthermore, 'Ri-14' (S_1S_2) and 'Yakumo' (S_1S_4) are not parents of 'Housui' because 'Housui' is cross-compatible with 'Nijisseiki' (S_2S_4) and 'Suisei' (S_1S_4) (Machida et al., 1982). Ishimizu et al. (1998) determined the S genotype of 'Housui' is S_3S_5 by a combination of two-dimensional gel electrophoresis and N-terminal sequence analysis for S proteins. 'Housui', a leading cultivar in Japan, has been used as a parent of a large number of pear cultivars so that it is important to know the exact parentage and the genetic background of 'Housui'.

In 'Tanzawa' (Anonymous, 1927), 9 alleles, 110 bp at NH001c, 185 bp at NH002b, 78 bp at NH004a, 204 bp at NH008b, 185 bp at NH011b, 105 bp at NH012a, 222 bp at NH013a, 78 bp at NH014a and 109 bp at NH015a were not observed in either 'Chojuro' or 'Nijisseiki' (Table 3) which leads us to believe that they are not parents of 'Tanzawa'.

Parentage analysis of interspecific hybrids

Analyses of the parentage of 6 cultivars, 'Oharabeni', 'Taiheiyo', 'Ninomiya', 'Kangyoku', 'Ninomiya' and 'Zaosu', revealed that in 'Taiheiyo', 15 SSR loci showed polymorphisms between the parents 'Okusankichi' and 'La France'. Fifty-one alleles were generated from these 15 SSR loci in the parent cultivars. One SSR

Table 2. SSR genotypes of 'Housui' and its reported parents.

Cultivar	SSR marker (bp)						
	KU10	BGT23b	NH002b	NH005b	NH013a	NH014a	NH017a
Housui (F ₁)	251/253	192/222	180/180	341/345	205/222	70/ 86	90/101
Ri-14 (female parent)	239/239	210/222	176/185	324/324	209/209	76/ 96	101/101
Yakumo (male parent)	239/239	222/222	178/178	324/345	205/209	76/ 96	101/101

Table 3. SSR genotypes of 'Tanzawa' and its reported parents.

Cultivar	SSR marker (bp)								
	NH001c	NH002b	NH004a	NH008b	NH011b	NH012a	NH013a	NH014a	NH015a
Tanzawa (F ₁)	110/122	180/185	78/ 78	184/204	175/185	105/108	205/222	78/ 96	103/109
Choujuro (female parent)	122/124	178/180	100/107	200/200	175/183	108/108	205/209	70/ 86	103/138
Nijisseiki (male parent)	122/126	174/176	100/113	184/204	175/183	108/108	205/209	96/ 96	103/103

Table 4. SSR genotypes of 'Oharabeni' and its reported parents.

Cultivar	SSR marker (bp)						
	KU10	NH001c	NH004a	NH009b	NH013a	NH014a	NH015a
Oharabeni (F ₁)	233/251	122/124	78/ 90	149/159	166/209	70/ 72	107/138
Okusankichi (female parent)	239/253	110/122	107/113	141/155	205/220	66/ 78	109/109
Max Red Bartlett (male parent)	233/233	122/158	90/ 96	143/149	166/232	70/ 72	107/119

Table 5. SSR genotypes of 'Ninomiya' and its reported parents.

Cultivar	SSR marker (bp)										
	KA14	KA16	BGA35	BGT23b	NH001c	NH007b	NH009b	NH011b	NH013a	NH014a	NH015a
Ninomiya (F ₁)	181/187	137/151	130/130	202/204	122/124	130/152	145/159	168/175	205/213	82/ 86	105/138
Chojuro (female parent)	181/181	137/148	122/130	192/202	122/124	121/152	159/159	175/183	205/209	70/ 86	103/138
Le Conte (male parent)	179/181	127/133	124/124	200/236	120/158	140/142	149/153	172/181	222/232	70/ 88	107/109

allele from each parent was transmitted to the offspring 'Taiheiyō'; e. g., the alleles of 180 bp and 170 bp at NH002b observed in 'Taiheiyō' were inherited from 'Okusankichi' and 'La France', respectively, thus confirming its parentage.

In 'Ninomiya-baili', 'Kangyoku' and 'Zaosu', 16, 15 and 15 SSR loci showed polymorphisms between parents, respectively, generated 53, 48 and 47 alleles, respectively. One allele from each parent was consistently transmitted to their offsprings. 'Ninomiya-baili', 'Kangyoku' and 'Zaosu' were reconfirmed as offsprings of 'Yali' × 'Shinchu', 'Chojuro' × 'Mishirazu' and 'Pingguoli' × 'Mishirazu', respectively.

Analyses of 'Oharabeni' and its parents 'Okusankichi' and 'Max Red Bartlett' indicate that the alleles of 239 bp and 253 bp at KU10, 107 bp and 113 bp at NH004a, 141 bp and 155 bp at NH009b, 205 bp and 220 bp at NH013a, 66 bp and 78 bp at NH014a and 109 bp at NH015a of 'Okusankichi' were not transmitted to

'Oharabeni' (Table 4, Fig. 2). Hence, 'Okusankichi' is not its parent, whereas 'Max Red Bartlett' was reconfirmed as a parent because there was no discrepancy in the inheritance of all used SSR markers. 'Oharabeni' was heretofore thought to be a chance seedling of 'Okusankichi' with 'Max Red Bartlett' as its parent because of its proximity in the orchard, the red color trait of young leaves, and its segregation pattern (Takahashi et al., 1981). However, our data indicate that 'Okusankichi' was not one of its parents.

Likewise, the analyses of 'Ninomiya' and its parents 'Chojuro' and 'Le Conte' indicate that the alleles of 127 bp and 133 bp at KA16, 124 bp at BGA35, 200 bp and 236 bp at BGT23b, 120 bp and 158 bp at NH001c, 140 bp and 142 bp at NH007b, 149 bp and 153 bp at NH009b, 172 bp and 181 bp at NH011b, 222 bp and 232 bp at NH013a, 70 bp and 88 bp at NH014a, and 107 bp and 109 bp at NH015a are not present in 'Ninomiya' (Fig 2). In contrast, one allele of 'Chojuro' was consist-

Table 6. Analysis of 14 parent–offspring relationships by SSR loci.

Cultivar	Genetic origin (Cross combination)	Comment
Gion	Chojuro × Nijisseiki	Both Chojuro and Nijisseiki confirmed as parents
Housui	Ri-14 × Yakumo	Neither confirmed as parents of Housui because of discrepancy at 7 loci
Shinseiki	Nijisseiki × Chojuro	Both Nijisseiki and Chojuro confirmed as parents
Tanzawa	Chojuro × Nijisseiki	Neither confirmed as parents of Tanzawa because of discrepancy at 9 loci
Aurora	Marguerite Marillat × Bartlett	Both Marguerite Marillat and Bartlett confirmed as parents
Ballade	Bartlett × La France	Both Bartlett and La France confirmed as parents
Gorham	Bartlett × Josephine de Malines	Both Bartlett and Josephine de Malines confirmed as parents
Pierre Corneille	Beurre Diel × Doyenne du Comice	Doyenne du Comice confirmed as a parent
Taiheiyo	Okusankichi × La France	Both Okusankichi and La France confirmed as parents
Oharabeni	Okusankichi × Max Red Bartlett	Okusankichi did not confirmed as a parent because of discrepancy at 7 loci Max Red Bartlett confirmed as a parent
Ninomiya	Yali × Shinchu	Both Yali and Shinchu confirmed as parents
Kangyoku	Chojuro × Mishirazu	Both Mishirazu and Chojuro confirmed as parents
Ninomiya	Chojuro × Le Conte	Chojuro confirmed as a parent Le Conte did not confirmed as a parent because of discrepancy at 10 loci or more loci
Zaosu	Pingguoli × Mishirazu	Both Pingguoli and Mishirazu confirmed as parents

ently transmitted to ‘Ninomiya’ for all SSR loci. ‘Chojuro’ was reconfirmed as a parent because there was no discrepancy in the inheritance of alleles at all used SSR loci (Table 5).

In 10 of the 14 parent–offspring relationships, both female and male cultivars were established as parents because alleles at all SSR loci of offspring cultivars were inherited from their parents without any discrepancy (Table 6). However, the 4 cultivars, ‘Housui’, ‘Tanzawa’, ‘Oharabeni’ and ‘Ninomiya’, showed the inconsistency for some alleles at 7–10 SSR loci. Twenty SSR markers were effectively utilized for the analysis of 14 parent–offspring relationships, including Japanese pears, European pears and interspecific hybrids.

The probability for paternity

In parentage analysis of humans, the probability for paternity is calculated based on the allele inheritance and frequency (Hashiyada et al., 1997; Hummel, 1971; Katsumata et al., 2001). The probability for paternity of 8 pear cultivars without discrepancy on SSR alleles was calculated using the computer program ‘Probability Calculator for Paternity Likelihood and Exclusion, ver. 2.0b’ (<http://www3.kmu.ac.jp/~legalmed/DNA/ppl.html>), by Dr. A. Akane. The probability for paternity in the parentage of 8 pear cultivars ranged from 0.9911 to 0.9999, with an average value of 0.9979. According to the criteria for human parentage, the value of 0.9911 means extremely high possibility as a parent, whereas the value of 0.9999 shows an exact parent–offspring relation.

It is very difficult to evaluate allele frequency in cultivated pears because allele frequency varies according to cultivar and species. However, we conclude that the possibility of a parent–offspring relationship of 8 pear cultivars could be reconfirmed with very high accuracy. For human parentage, the number of SSR loci

is increased until the value reaches more than 0.998 for paternity likelihood. Values greater than 0.998 are obtained by using 4 to 7 SSR loci. In this study, 15 to 20 SSR loci were used to determine pear parentage which should be sufficient.

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SSR マーカーを用いたナシ品種の親子鑑定

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摘 要

20種類の SSR (simple sequence repeat) を用いて、ナシの種内交雑由来 8 品種および種間交雑由来 6 品種の合計 14 品種とその親品種について親子鑑定を行った。その結果、10 品種では、SSR マーカーの遺伝から親子関係に矛盾が認められず親子であることが再確認された。しかし、4 組合せの親子間では親子として矛盾が認められた。‘豊水’は、‘Ri-14’ × ‘八雲’ から育成された (現在両親不詳) が、7 つの SSR 遺伝子座で親子としての不一致が認められ、‘Ri-14’ と ‘八雲’ の子供として認められなかった。‘丹沢’では、その両親とされる ‘長十郎’ と ‘二十世紀’ において 9 つの SSR 遺伝子座で親子としての不一致が認められ、両品種は ‘丹沢’ の両親とは認められなかった。‘大原紅’ の

親子関係では、‘晩三吉’ は 7 つの SSR 遺伝子座で親子としての不一致が認められ、‘マックスレッドバートレット’ は、用いたすべての SSR 遺伝子座で ‘大原紅’ の親であることが確認された。‘二宮’ では、父親とされる ‘ル・コント’ は 10 の SSR 遺伝子座で親子としての不一致が認められ、‘長十郎’ は用いたすべての SSR 遺伝子座で ‘二宮’ の親であることが確認された。SSR マーカーは親子鑑定に有効であり、20 種類の SSR マーカーはニホンナシ品種とその親品種の親子鑑定に利用可能であった。このうち、15 種類の SSR マーカーは、セイヨウナシ品種及び種間交雑由来品種とその親品種の親子鑑定に利用できた。