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SSRs isolated from apple can identify polymorphism and genetic diversity in pear

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Abstract Apple simple sequence repeats (SSRs) were intergenerically applied to the characterization of 36 pear accessions, including 19 Japanese pears (*Pyrus pyrifolia*), 7 Chinese pears (*P. bretschneideri*, *P. ussuriensis*), 5 European pears (*P. communis*), 3 wild relatives (*P. calleryana*), and 2 hybrids between *P. pyrifolia* and *P. communis*. All of the tested SSR primers derived from apple produced discrete amplified fragments in all pear accessions. Nucleotide repeats were detected in the amplified bands by both Southern blot and sequencing analysis, and nucleotide sequences of pear were compared with those of apple. The differences in fragment size among pear or between pear and apple were, in many cases, due to the differences in repeat number. Interestingly, the DNA sequence of flanking regions in apple was highly conserved in pear. Hybrids from *P. pyrifolia*×*P. communis* showed one fragment inherited from each parent in all scorable cases, which suggested that each primer pair amplified fragments originating from the same locus. A total of 79 alleles were detected from seven SSR loci in pear, and all pear varieties except for the mutants could be differentiated. In conclusion, SSRs isolated from apple are highly conserved in pear and could be utilized as DNA markers in the latter genus.

Keywords *Malus*×*domestica* Borkh. · Pedigree analysis · *Pyrus* spp. · Sequence similarity · Simple sequence repeats

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Introduction

SSRs (simple sequence repeats, also designated as microsatellites) have become the genetic markers of choice in mammalian and many plant species due to their abundance, high degree of polymorphism, and suitability for automation (Weber and May 1989). SSR markers have several advantages over other molecular markers, which ensure a more reliable method for DNA fingerprinting. They show codominant inheritance and a large number of alleles per locus and are abundant in genomes. In addition, since the use of SSRs is based on the polymerase chain reaction (PCR) method, the technique is simple and only a small amount of DNA is required. It is widely held that SSRs isolated from a source genome can be transferred to different individuals of the same species or the same genus. For example, cross-species amplification has been observed to be prevalent in *Brassica* (Szewc-McFadden et al. 1996), *Actinidia* (Weising et al. 1996), and *Prunus* species (Downey and Iezzoni 2000). However, there are very few reports on the use of SSRs across genera in the same family or across families.

Pear (*Pyrus* spp.) is one of the most important fruit crops, having been cultivated in Europe and Asia for at least 2–3 thousand years, and is presently commercially grown in all temperate regions encompassing more than 50 countries of the world (Bell 1990; Bell et al. 1996). The genus *Pyrus* contains at least 22 well-recognized primary species, all indigenous to Europe, Asia, and the mountainous regions of North America. In addition, there are at least nine natural or artificial interspecific hybrids between primary species, which are also classified into different species (Bell et al. 1996). Genetic resources have not been fully identified due to the low morphological diversity, lack of differentiating characters among species, and widespread crossability. Therefore, estimation of genetic diversity among *Pyrus* spp. is often very difficult. Morphological characters (Kikuchi 1948; Shen 1980; Westwood 1982) and isozyme analysis (Chevreau et al. 1997; Jang et al. 1991;) have been the

two major tools used to assess the genetic variation in *Pyrus* spp. However, isozyme markers and morphological characters are still limited in number.

SSR markers have been isolated and used for genetic linkage maps and cultivar identification in species belonging to the family Rosaceae, such as apple (*Malus*×*domestica* Borkh., Gianfranceschi et al. 1998; Guilford et al. 1997; Maliepaard et al. 1998), *Prunus* spp. (Cipriani et al. 1999), and so on. However, there are no reports on SSRs isolated from pear, although pear is an important fruit species belonging to the same family Rosaceae and the same sub-family Pomoideae as apple (*Malus* spp.). Furthermore, there have been very few reports on the genetic relationship between pear and apple using molecular markers. In the study presented here, we attempted to transfer SSR primers derived from apple to pear.

Materials and methods

Plant material

Thirty-six pear accessions and two apple cultivars were used in this study. Pear accessions included 19 Japanese pears (*Pyrus pyrifolia* Nakai), 7 Chinese pears (*P. bretschneideri* Rehd., *P. ussuriensis* Maxim.), 5 European pears (*P. communis* L.), 2 hybrids between *P. pyrifolia* and *P. communis*, and 3 accessions of the wild relative *P. calleryana* Decne. Classification of the Asian species was done following Iketani et al. (1998). Two apple (*Malus*×*domestica* Borkh.) cultivars, Cox's Orange Pippin and Golden Delicious, were used as references. All varieties were obtained from the National Institute of Fruit Tree Science (Ibaraki, Japan).

Sixty-three F_1 plantlets obtained from the interspecific cross between the Japanese pear Housui and the European pear Bartlett were used to examine the segregation and inheritance of the SSR loci in pear.

DNA extraction

Genomic DNA was isolated from young leaves by a CTAB-based extraction method (Hasebe and Iwatsuki 1990; Yamamoto et al. 2000). One gram of fresh leaf tissue was homogenized in liquid nitrogen. The resulting tissue powder was suspended in 10 ml of CTAB extraction buffer (2% CTAB, 1.4 *M* NaCl, 20 m*M* EDTA, 0.1 *M* Tris-HCl, pH 8.0), and 1 ml lysis buffer (10% sodium *N*-lauroyl sarcosinate, 20 m*M* EDTA, 0.1 *M* Tris-HCl, pH 8.0), 0.2 ml of 2-mercaptoethanol and 100 mg polyclar AT (insoluble polyvinyl pyrrolidone, GAF Chemicals, Japan) were added to the suspension, which was incubated at 60°C for 1 h. The suspension was then purified twice by a chloroform: isoamyl alcohol (24:1) solution and precipitated with 2-propanol. The recovered DNA was dissolved in TE buffer (10 m*M* Tris-HCl, 1 m*M* EDTA, pH 7.5) and purified using a QIAGEN-tip 20 column (Qiagen, Germany).

Microsatellite PCR amplification

Nine SSR primers derived from apple, i.e. 02b1, 05g8, 28f4 (Guilford et al. 1997), CH01B12, CH01E12, CH01F02, CH01H01, CH01H10, CH02B12 (Gianfranceschi et al. 1998), were used for PCR amplification in pear. All the SSR loci contained (AG)/(TC) repeats. The PCR amplification was performed according to the conditions reported, but forward primers were labelled with a fluorescent chemical (FAM or TET or HEX). PCR products were separated and detected using a PRIZM 377 DNA sequencer (PE Applied Biosystems). The size of the amplified bands was calculated using an internal DNA standard (GeneScan-350TAMRA, PE Applied Biosystems) and the GeneScan software (PE Applied Biosystems).

Southern blot analysis

The presence of the microsatellite repeat in the amplified fragments was searched by means of Southern blot hybridization. DNA bands amplified with non-labelled primers were separated on 4% NuSieve GTG agarose gels (FMC BioProducts) at 50 V for 80 min. DNA was transferred onto nylon membrane (HybondN$^+$, Amersham, UK) by capillary blotting. Hybridization was performed using 5′-biotin-$(AG)_{15}$ as a probe dissolved in a hybridization buffer (5×SSC, 0.1% sodium *N*-lauroyl sarcosinate, 0.02% sodium dodecylsulfate, 1% blocking reagent) at 50°C for 16 h. The membranes were then washed twice with a washing solution (2× SSC, 0.1% sodium dodecylsulfate) at room temperature. Detection was performed using BrightStar BioDetect kit (Ambion) according to the manufacturer's instructions.

Cloning and sequencing amplified fragments

More than 35 amplified fragments derived from apple cv. Cox's Orange Pippin, pear cv. Housui, and the other pear accessions were separated on 2–4% agarose gels at 50 V for 80 min. The targeted bands were isolated and purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech.) and then cloned into pCR2.1 vector (Original TA Cloning Kit, Invitrogen) according to the manufacturers' instructions. Sequencing of the cloned fragments was carried out using a PRIZM 377 DNA sequencer (PE Applied Biosystems) and BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems). Three to ten clones for each fragment were sequenced in order to obtain reliable sequences. The nucleotide sequences of pear and apple were aligned using Genetyx ver 9.0.

Results and discussion

Microsatellite PCR amplification and Southern blot analysis

Discrete reproducible bands were obtained for pear varieties with all SSR primers. The same primers yielded bands in the two apple cultivars, which were almost of the same size as those reported by Guilford et al. (1997) and Gianfranceschi et al. (1998). The fragments amplified with the 05g8, CH01B12, CH01E12, CH01F02, CH01H01, CH01H10, and CH02B12 primers showed the exact same size as those reported for Cox's Orange Pippin. The other two primers 02b1 and 28f4, produced 4-base longer and 1-base shorter fragments than reported for Cox's Orange Pippin, respectively. This difference presumably originated from differences in the detection system because the bands obtained from Golden Delicious also exhibited the same shifts with these two primers. The size of the fragments obtained from apple was different from that of pear.

The Japanese pear cultivar Kousui and the apple cultivar Golden Delicious were used for the analysis of the presence of the SSR repeat. When PCR products were probed with the biotin-labelled $(AG)_{15}$, all apple bands produced positive signals. In pear, positive signals were observed in all fragments except for the CH01B12 fragment of Kousui. Similarly, almost all fragments obtained from the 36 pear varieties exhibited positive signals in all SSRs but CH01B12. These results suggested that, in most cases, the AG/TC repeat was conserved in pear.

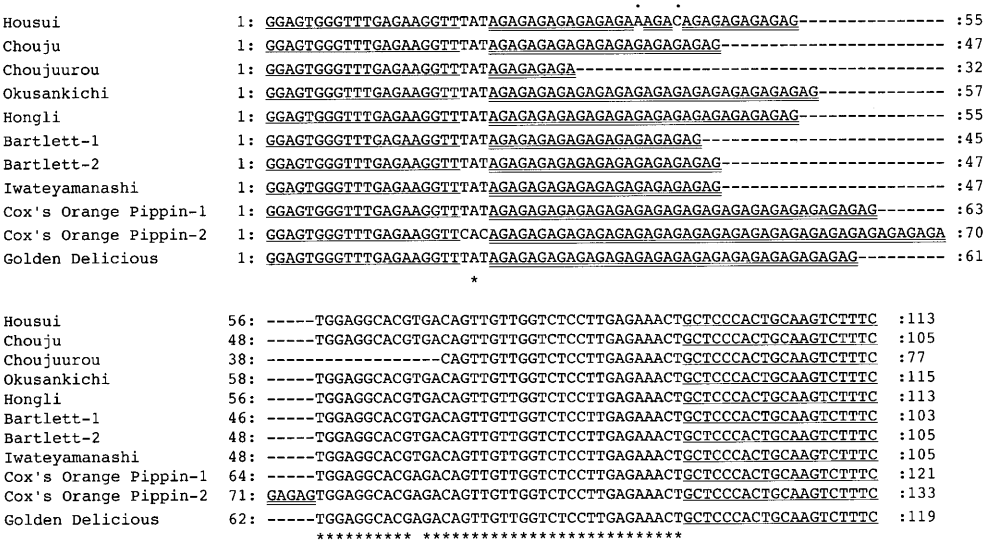

Fig. 1 Comparison of nucleotide sequences of the microsatellite locus CH01H01 among pear and apple varieties. *Single underlines*, *double underlines*, and *asterisks* indicate primer sequences, AG-repeats, and consensus, respectively. *Dots above letters* within the AG-repeats represent an interrupted repeat

Nucleotide sequence of pear fragments

Sequencing of SSR alleles in Kousui and/or Housui pears showed that eight out of the nine loci assayed contained the AG motif, which was 10–25 repeats long. However, the sequence of $(AG)_3A_3(AG)A_2(AG)A_{12}AG$ was found in Kousui in the 134-bp fragment of CH01B12, which did not show a positive signal in Southern blot analysis. In addition, nucleotide identity of amplified fragments between pear and apple was relatively low (approx. 68%). We concluded that the origin of the pear fragment was different from that of apple and that this SSR primer could not be utilized for analysis in pear. Thus, we deleted it from further analyses.

The differences in the size of the fragments between pear and apple as well as among pear varieties were mainly due to differences in the repeat number of the AG units (Fig. 1). The nucleotide sequence of the regions flanking the AG repeats recorded in apple was highly conserved in pear. When we compared the flanking regions of Kousui and/or Housui pear with those of apple, at least 90% of identity was observed between the two species. No pear sequence showed any significant homology to the registered structural genes. Interestingly, deletions (or insertions) were found in the flanking region around the AG repeat when the latter was compared between the two species.

Segregation and inheritance of SSRs

Segregation and inheritance of SSR loci were examined using 63 F_1 plantlets derived from the interspecific cross between Housui (*Pyrus pyrifolia*) and Bartlett (*P. communis*) (Table 1). Six SSRs (02b1, 05g8, 28f4, CH01F02, CH01H01, CH01H10) fitted the expected segregation, which suggested that the fragments examined were derived from the same locus in both species. Segregation at the CH01E12 locus was largely distorted. In the case of CH02B12, Housui had two alleles (136/– bp; here we represent null allele by –) and Bartlett apparently had four (160/133/113/101 bp). However, the segregation from Housui fitted the 1:1 ratio. As this SSR requires further analysis, we discarded it for cultivar identification in pear. In addition, null alleles were found for four loci. These results suggested that apple SSRs, in general, could be successfully used in pear for mapping.

Table 1 Seregation of SSRs observed for interspecific hybrids of pear (*ns* not significant)

SSR	Parents' genotype[a]		Segregation in F_1 plants	χ^2-value
	Housui	Bartlett		
02b1	aa (256/256)	bc (258/254)	ab:ac=30:33	0.14 ns
05g8	aa (107/107)	bc (111/103)	ab:ac=36:27	1.29 ns
28f4	ab (113/105)	cd (102/98)	ac:ad:bc:bd=14:11:17:21	1.82 ns
CH01E12	ab (263/–[b])	cd (238/–[b])	ac:ad:bc:bd=31:6:17:9	21.0*
CH01F02	aa (165/165)	bc (176/163)	ab:ac=29:34	0.40 ns
CH01H01	ab (113/–[b])	cd (105/103)	ac:ad:bc:bd=13:18:14:18	1.19 ns
CH01H10	ab (107/97)	ac (107/–[b])	(aa+ac):ab:bc=34:10:19	2.97 ns
CH02B12	ab (136/–[b])	cdef (160/133/113/104)	a:b=29:34	0.40 ns

* Distortion at 5% level

[a] The numbers indicate the size of the alleles (in base pairs)

[b] –, Null allele

Genetic diversity and cultivar identification of pear

Seven SSRs (02b1, 05g8, 28f4, CH01E12, CH01F02, CH01H01, CH01H10) were used for the evaluation of genetic diversity and for cultivar identification in 36 pear varieties (Tables 2, 3). The seven SSR primers could produce 79 fragments (approx. equal to alleles), with an average of 11 alleles per locus with a range from 9 (CH01H10) to 14 (CH01E12, CH01F02). Although segregation analysis showed the existence of null alleles, we did not take null alleles into consideration because of their detection difficulty. Allelic differences were observed within species as well as among species. For example, CH01H01 showed 12 putative alleles ranging from 77 bp to 135 bp in size. Six alleles were present in *P. pyrifolia*, of which the alleles of 77 bp, 115 bp, and 123 bp were only observed in this species. The same SSR also showed six, three, two, and two alleles in *P. bretschneideri*, *P. ussuriensis*, *P. communis*, and *P. calleryana*, respectively. CH01F02 exhibited 14 alleles ranging from 159 to 208 bp in length, of which five, four, six, six, and five alleles were found for *P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, *P. communis*, and *P. calleryana*, respectively. Only two alleles, corresponding to the 105-bp fragment from 28f4 and the 163-bp fragment from CH01F02, were commonly observed in all tested species. These results indicated that apple SSRs could be utilized for the evaluation of genetic diversity in *Pyrus* spp.

All of the pear varieties except the mutant cultivars could be differentiated from each other using seven apple SSRs. Gold Nijisseiki and Osa Gold showed SSR patterns identical to that of the original Nijisseiki cultivar. Gold Nijisseiki is a Nijisseiki mutant carrying black spot disease resistance induced by gamma-ray irradiation of the original cultivar (Sanada et al. 1988). Osa Gold is a black spot resistant and self-compatible mutant originating from Osanijisseiki (Masuda et al. 1998), which in turn is a mutant of Nijisseiki. In addition, Max Red Bartlett, a bud sport of Bartlett, showed the same SSR genotypes as the original cultivar. The cultivar pedigree was investigated using several parent-offspring pairs. The accession 282–12, which is a hybrid between Housui and La France, had one allele coming from each parent. Similarly, parent-offspring compatibility was found for the following pairs: La France (female parent) vs. Silver Bell (offspring), Bartlett (female parent) vs. Le Lectier (offspring), Okusankichi (female parent) vs. Hougetsu (offspring), Housui (female parent) vs. Chikusui (offspring), and Kousui (pollen donor) vs. Akiduki (offspring). On the contrary, Syuugyoku patterns were markedly incompatible with that of its parent Kousui. This SSR analysis suggested that Syuugyoku may not have had Kousui as a parent.

Genetic similarity between apple and pear

Röder et al. (1995) noted that only a few microsatellites of wheat (*Triticum aestivum*) could be used for rye

Table 2 Number of putative alleles of seven SSRs observed for *Pyrus* species

SSR	Number of alleles					
	P. pyrifolia	*P. bretscheneideri*	*P. ussuriensis*	*P. communis*	*P. calleryana*	Total
02b1	2 (251, 256)[a]	4 (252, 254, 256, 257)	4 (250, 251, 253, 256)	4 (254, 255, 258, 293)	3 (251, 252, 254)	10
05g8	4 (107, 109, 111, 121)	4 (105, 113, 118, 133)	5 (105, 107, 115, 122, 133)	4 (103, 105, 107, 111)	5 (103, 105, 107, 113, 133)	10
28f4	3 (103, 105, 113)	4 (103, 105, 109, 111)	6 (96, 98, 103, 105, 112, 113)	3 (98, 102, 105)	3 (103, 105, 107)	10
CH01E12	7 (247, 248, 249, 252, 261, 263, 265)	3 (241, 242, 247)	6 (241, 247, 252, 260, 263, 269)	2 (238, 246)	3 (237, 239, 263)	14
CH01F02	5 (163, 165, 168, 169, 178)	4 (163, 165, 167, 169)	6 (163, 165, 170, 182, 186, 187)	6 (159, 163, 167, 176, 180, 208)	5 (163, 165, 170, 174, 180)	14
CH01H01	6 (77, 105, 113, 115, 121, 123)	6 (111, 113, 121, 125, 129, 135)	3 (105, 111, 113)	2 (103, 105)	2 (99, 111)	12
CH01H10	4 (97, 107, 109, 117)	2 (117, 119)	5 (97, 105, 119, 121, 123)	3 (105, 107, 121)	5 (107, 117, 119, 123, 129)	9

[a] The size of the alleles (in base pairs) are in parenthesis

Table 3 Cultivar identification of pear using apple SSRs. Two apple cultivars are included as references

Cultivar name	Species	SSR genotype (bp)						
		02b1	05g8	28f4	CH01E12	CH01F02	CH01H01	CH01H10
Housui	*P. pyrifolia*	256/	107/	113/105	263/	165/	113/	107/97
Shinsei	*P. pyrifolia*	–	121/107	105/	249/	178/165	113/	107/97
Kousui	*P. pyrifolia*	256/	107/	105/	263/	178/165	113/	97/
Chouju	*P. pyrifolia*	256/	121/107	105/	263/	178/	105/77	107/97
Gold Nijisseiki	*P. pyrifolia*	256/	107/	113/105	263/249	178/165	113/	97/
Choujuurou	*P. pyrifolia*	256/	121/107	105/103	265/261	178/	77/	107/97
Okusankichi	*P. pyrifolia*	256/	121/107	113/105	248/	165/	115/	107/97
Nanseichabo	*P. pyrifolia*	251/	121/109	105/	252/	168/163	123/	109/
Nijisseiki	*P. pyrifolia*	256/	107/	113/105	263/249	178/165	113/	97/
Osa Gold	*P. pyrifolia*	256/	107/	113/105	263/249	178/165	113/	97/
Niitaka	*P. pyrifolia*	256/	121/107	105/	265/	178/165	77/	107/97
Atago	*P. pyrifolia*	256/	107/	103/	263/	178/	77/	107/97
Akiduki	*P. pyrifolia*	256/	121/107	105/	263/	178/165	113/	97/
Chikusui	*P. pyrifolia*	256/	121/107	105/	263/	165/	113/	107/
Yasato	*P. pyrifolia*	256/	121/107	113/105	263/	165/	77/	107/97
Hougetsu	*P. pyrifolia*	256/	107/	113/105	247/	165/	113/	107/97
Syuugyoku	*P. pyrifolia*	256/	111/	113/103	263/249	178/169	121/115	117/107
Shinsui	*P. pyrifolia*	256/	121/107	105/	263/249	178/165	113/	107/97
Kinchaku	*P. pyrifolia*	256/	111/	105/	265/261	165/163	113/	109/97
Yali	*P. bretschneideri*	257/254	133/	111/103	242/	167/165	129/125	119/
Laiyangcili	*P. bretschneideri*	257/	113/	109/103	242/	169/165	135/121	117/
Mili	*P. bretschneideri*	256/252	118/105	105/103	242/	169/163	113/111	119/117
Hongli	*P. bretschneideri*	256/252	118/105	109/103	247/241	169/163	113/	119/117
Beijin Baili	*P. ussuriensis*	256/251	133/105	103/98	260/247/241	187/165	113/111	119/
Balixiang	*P. ussuriensis*	253/250	115/107	113/105	252/	186/182	113/	123/121
Iwateyamanashi	*P. ussuriensis*	256/251	122/105	112/96	269/263	170/163	105/	105/97
La France	*P. communis*	293/255	105/103	105/102	246/238	167/159	105/103	107/
Bartlett	*P. communis*	258/254	111/103	102/98	238/	176/163	105/103	107/
Max Red Bartlett	*P. communis*	258/254	111/103	102/98	238/	176/163	105/103	107/
Le Lectier	*P. communis*	293/258	107/103	98/	238/	176/159	105/103	105/
Silver Bell	*P. communis*	293/255	105/	102/	238/	208/180/159	105/103	121/
282-12	*P. communis×pyrifolia*	256/255	107/103	113/105	246/	165/159	103/	97
290-36	*P. communis×pyrifolia*	256/254	111/107	113/102	238/	165/163	113/103	107/
NIFTS61113003-0005	*P. calleryana*	254/251	113/105	107/105	239/	174/170	99/	123/107
Mamenashi 6	*P. calleryana*	252/251	133/103	105/103	237/	165/163	111/	129/117
Mamenashi 14	*P. calleryana*	251/	133/107	107/	263/	180/	111/	119/
Cox's Orange Pippin	*M. domestica*	220/	123/	111/109	249/	206/180	133/121	100/
Golden Delicious	*M. domestica*	231/220	123/	111/97	251/245	180/171	121/119	111/93

(*Secale cereale*) and barley (*Hordeum vulgare*) in the Poaceae family. Kijas et al. (1995) reported that SSRs isolated from an intergeneric hybrid between rangpur lime (*Citrus×limonia*) and trifoliate orange (*Poncirus trifoliata*) could be applied for the genetic analysis of *Citrus* and related genera, where intergeneric hybridization easily occurs. On the other hand, it has been reported that SSR cross-species amplification sometimes fails (Downey and Iezzoni 2000; Weising et al. 1996). Weising et al. (1996) stated that SSRs obtained from *Actinidia chinensis* could produce amplified fragments from one to eight species out of the eight *Actinidia* species tested. These data suggest that intergeneric amplification of SSRs is rather difficult and depends on the genetic relatedness.

Although both pear and apple are classified into the Rosaceae family, and the Pomoideae sub-family, their genetic relationship still remains unclear. There is considerable morphological variation not only in the vegetative tissues but also in reproductive organs in Pomoideae. However, it has been suggested that the genera of Pomoideae should have a close genetic relationship each other based on the analyses of the chromosome number, presence of natural intergeneric hybrids, etc. (Kovanda 1965; Sax 1931) Iketani (1993) who conducted a restriction fragment length polymorphism (RFLP) analysis in *Pyrus* spp. and related genera (*Malus*, *Cydonia*, *Chaenomeles*) probed with several chloroplast clones, and found that there were a few polymorphisms among them. In this study, we demonstrated that apple SSRs could be successfully used in *Pyrus* spp. Our results confirm that *Pyrus* spp. display a close genetic relationship to *Malus* spp.

We are currently constructing a genetic linkage map of pear using an F_1 population derived from an interspecific cross of Housui (*Pyrus pyrifolia*) and Bartlett (*P. communis*). The SSRs 02b1, 05g8, 28f4, and CH01H01 have been mapped on our preliminary map of the European pear. These SSRs will be utilized as anchor loci to connect the apple linkage groups to those of pear. We consider that the synteny between apple and pear genomes can be studied in the near future by comparing linkage groups using bridge markers such as SSRs.

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References

Bell RL (1990) Pears (*Pyrus*). In: Moore JN, Ballington JR Jr (eds) Genetic resources of temperate fruit and nut Crops I. International Society for Horticultural Science, Wageningen, the Netherlands, pp 655–697

Bell RL, Quamme HA, Layne REC, Skirvin RM (1996) Pears. In: Janick J, Moore JN (eds) Fruit breeding, vol I: tree and tropical fruits. John Willey & Sons, London, pp 441–514

Chevreau E, Leuliette S, Gallet M (1997) Inheritance and linkage of isozyme loci in pear (*Pyrus communis* L.). Theor Appl Genet 94:498–506

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Downey SL, Iezzoni AF (2000) Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. J Am Soc Hortic Sci 125:76–80

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

Guilford P, Prakash S, Zhu JM, Rikkerink E, Gardiner S, Bassett H, Forster R (1997) Microsatellites in *Malus×domestica* (apple): abundance, polymorphism and cultivar identification. Theor Appl Genet 94:249–254

Hasebe M, Iwatsuki K (1990) *Adiantum capillus-veneris* chloroplast DNA clone bank: as useful heterologous probes in the systematics of the leptosporangiate ferns. Am Fern J 80:20–25

Iketani H (1993) Chloroplast DNA diversity in *Pyrus* and related genera. Gamma Field Symp 32:63–69

Iketani H, Manabe T, Matsuta N, Akihama T, Hayashi T (1998) Incongruence between RFLPs of chloroplast DNA and morphological classification in east Asian pear (*Pyrus* spp.). Genetic Resour and Crop Evol 45:533–539

Jang JT, Tanabe K, Tamura F, Banno K (1991) Identification of *Pyrus* species by peroxidase isozyme phenotypes of flower buds. J Jpn Soc Hortic Sci 60:513–519

Kijas JMH, Fowler JCS, Thomas MR (1995) An evaluation of sequence tagged microsatellite site markers for genetic analysis within *Citrus* and related species. Genome 38:349–355

Kikuchi A (1948) Horticulture of fruit trees, vol 1. Yokendo, Tokyo

Kovanda M (1965) On the generic concepts in the *Maloideae*. Preslia 37:27–34

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.). Theor Appl Genet 97:60–73

Masuda T, Yoshioka T, Sanada T, Kotobuki K, Nagara M, Uchida M, Inoue K, Murata K, Kitagawa K, Yoshida A (1998) A new Japanese pear cultivar 'Osa Gold.' (in Japanese with English summary). Bull Natl Inst Agrobiol Resour 12:1–11

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Sanada T, Nishida T, Ikeda F (1988) Resistant mutant to black spot disease of Japanese pear 'Nijisseiki' induced by gamma-rays. J Jpn Soc Hortic Sci 57:159–166

Sax K (1931) The origin and relationships of the Pomoideae. J Arnold Arbor Har Univ 12:3–22

Shen T (1980) Pears in China. HortScience 15:13–17

Szewc-McFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor Appl Genet 93:534–538

Westwood MN (1982) Pear germplasm of the new national clonal repository: it's evaluation and use. Acta Hortic 124:57–65

Weber JK, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–397

Weising K, Fung RWM, Keeling DJ, Atkinson RG, Gardner RC (1996) Characterisation of microsatellites from *Actinidia chinensis*. Mol Breed 2:117–131

Yamamoto T, Iketani H, Ieki H, Nishizawa Y, Notsuka K, Hibi T, Hayashi T, Matsuta N (2000) Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. Plant Cell Rep 19:639–646