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T. Yamamoto · T. Kimura · M. Shoda · T. Imai
T. Saito · Y. Sawamura · K. Kotobuki · T. Hayashi
N. Matsuta

Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears

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Abstract Genetic linkage maps of the European pear (*Pyrus communis* L.) cultivar 'Bartlett' and the Japanese pear (*Pyrus pyrifolia* Nakai) cultivar 'Housui' were constructed based on AFLPs, SSRs from pear, apple and *Prunus*, isozymes and phenotypic traits by using their F_1 progenies. The map of the female parent Bartlett consisted of 226 loci including 175 AFLPs, 49 SSRs, one isozyme and one S locus on 18 linkage groups over a total length of 949 cM, while that for 'Housui' contained 154 loci including 106 AFLPs, 42 SSRs, two phenotypic traits and the other four markers on 17 linkage groups encompassing a genetic distance of 926 cM. These maps were partially aligned using 20 codominant markers which showed segregating alleles in both parents. Compared with the reports of apple genetic maps, these pear maps were not saturated but were near saturation. Distorted segregation was observed in two and one regions of the genome of Bartlett and Housui, respectively. The position of 14 SSRs originating from apple could be successfully determined in pear maps, which enabled us to compare the two maps. Some SSRs developed from *Prunus* (peach, cherry) were also mapped. The relationships between pear and the other species belonging to the Rosaceae were discussed based on the position of SSRs.

Keywords AFLP · Genetic mapping · *Pyrus communis* · *Pyrus pyrifolia* · Simple sequence repeats

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T. Yamamoto (✉) · M. Shoda · T. Imai · T. Saito · Y. Sawamura
K. Kotobuki · T. Hayashi · N. Matsuta
Department of Breeding, National Institute of Fruit Tree Science,
Fujimoto 2-1, Tsukuba, Ibaraki 305-8605, Japan
e-mail: toshiya@ affrc.go.jp
Tel.: +81-298-38-6462, Fax: +81-298-38-6437

T. Kimura
National Center for Seeds and Seedlings, Tsukuba,
Ibaraki 305-0852, Japan

Introduction

Pears (*Pyrus* spp.) have been cultivated for more than 2-thousand years and are among the most important fruits in all the temperate regions in about 50 countries of the world (Bell 1990; Bell et al. 1996). The Japanese pear (*Pyrus pyrifolia* Nakai), the European pear (*Pyrus communis* L.) and the Chinese pears (*Pyrus bretschneideri* Rehd. and *Pyrus ussuriensis* Maxim.) are major edible species commercially grown for fruit production. The Japanese pear is cultivated in East Asian countries, compared to the European pear distributed in Europe, North America, and temperate regions of the southern hemisphere. All the species of *Pyrus* are intercrossable and there are no major incompatibility barriers to interspecific hybridization in *Pyrus*, in spite of the wide geographic distribution of the genus (Westwood and Bjornstad 1971). Although interspecific hybrids among *Pyrus* spp. have been tested to improve disease and pest resistances, fruit quality and adaptability were generally low (Bell et al. 1996). Since fruit quality and adaptability are presumably controlled by QTLs, it is rather difficult to analyze these traits without molecular markers. Therefore, it is considered that molecular markers will greatly help to conduct backcrossing to improve the good fruit quality and adaptability without losing disease and pest resistance genes. However, molecular markers are very limited in pears.

SSRs (simple sequence repeats, also designated as microsatellites) offer several advantages over other molecular markers, which provide a more-reliable method for genetic mapping because of their codominant inheritance, large number of genotypes (alleles) per locus, and abundance in genomes. In pear, SSR markers have been isolated and used for cultivar identification (Yamamoto et al. 2002a, b) but their position has not been determined in genetic linkage maps. It was reported that SSR markers have been isolated and used for genetic linkage maps in species belonging to the same family Rosaceae, such as apple (Guilford et al. 1997; Gianfranceschi et al. 1998; Maliepaard et al. 1998) and peach (Cipriani et al.

1999). It was revealed that apple SSRs could be effectively used as codominant DNA markers in pear (Yamamoto et al. 2001).

In our previous study, genetic linkage maps of the Japanese pear varieties 'Kinchaku' and 'Kousui' were constructed using RAPD markers (Iketani et al. 2001). The former map consisted of 120 loci in 18 linkage groups covering a length of approximately 770 cM, in which two disease-related genes, resistant to pear scab and susceptibile to black spot, were detected. However, no genetic linkage map of the related species European pear has been reported yet.

In this study, we constructed genetic linkage maps of European and Japanese pears based on AFLPs, SSRs, isozymes and phenotypic traits using F_1 progenies obtained from an interspecific cross. SSR markers originating from pear and the other species of the same family Rosaceae, such as apple, peach and cherry, were tested and mapped. The relationships between pear species as well as between pear and the other Rosaceae fruit species were examined.

Materials and methods

Plant materials and DNA extraction

Sixty three F_1 individuals obtained from an interspecific cross between the European pear cultivar (*P. communis* L.) 'Bartlett' and the Japanese pear (*P. pyrifolia* Nakai) cultivar 'Housui', were used in this study. The double pseudo-testcross strategy (Grattapaglia and Sederoff 1994) was adopted to construct genetic linkage maps because of the self-incompatibility character in pear (Kikuchi 1929; Ishimizu et al. 1998a). All the plant materials were obtained from the National Institute of Fruit Tree Science (Ibaraki, Japan). Genomic DNA was isolated from young leaves by a CTAB-based extraction method (Hasebe and Iwatsuki 1990; Yamamoto et al. 2001).

AFLP analysis

AFLP was performed with AFLP Analysis System II (Life Technologies) according to the supplier's protocol, except for the use of *Eco*RI primers labelled with a fluorescent chemical. Two hundred and fifty nanograms of genomic DNA were digested with two restriction enzymes (*Mse*I, *Eco*RI) and then the DNA fragments were ligated to adaptors. Pre-amplification reactions were performed with a pre-amp primer mix. Selective amplification was performed with 40 primer combinations of eight *Mse*I primers (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG and M-CTT) and five FAM-labelled *Eco*RI primers (E-AC, E-TG, E-TC, E-AG and E-GC). The PCR products were separated and detected using a PRISM 377 DNA sequencer (PE Applied Biosystems). The size of the amplified bands was determined based on an internal standard DNA (GeneScan-500ROX, PE Applied Biosystems) using GeneScan software (PE Applied Biosystems).

SSR markers from pear

Fifty five SSR markers originating from Housui or Bartlett were used for the detection of the microsatellite loci. Thirty eight SSRs developed from enriched genomic libraries (Yamamoto et al. 2002a, Table 1) were denoted by NH and NB (Table 2). In this study, 19 out of 38 SSRs were newly developed from genomic libraries of Housui and Bartlett enriched with (AG)/(TC) using the magnetic beads method (Yamamoto et al. 2002a) and are listed in Table 1. Nine and seven SSRs were developed by RAHM (random amplified hybridization microsatellites) and 5′-anchored PCR methods, respectively (Yamamoto et al. 2002b). One SSR marker RLG1 was derived from a resistance gene-like DNA sequence. A set of oligonucleotide sequences, 5′-AAGTCTGGGAGGATTCA-

Table 1 Newly developed SSR loci derived from pear

SSR loci	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Origin	Motif	Type of marker	Map position	
						Bartlett	Housui
NB102a	tgt tat cac ctg agc tac tgcc	ctt cct ctt tat ttg ccg tct t	Bartlett	$(AG)_6$	mloc/sloc[a]		H06-2
NB103a	ttg tag gga aaa tga tgc ca	gtg ttg ata ctc tct ctc tc	Bartlett	$(AG)_{31}$	sloc	Ba13	
NB105a	aaa caa ccg act gag caa cat c	aaa atc tta gcc caa aat ctc c	Bartlett	$(AG)_{15}AT(AG)_{10}$	sloc	Ba4	
NB106a	gta cgt cga cat gag aga g	tct ctt gtt cct tcc tgc ac	Bartlett	$(AG)_{19}$	sloc	Ba9	
NB109a	atg ctc tat aaa acc cac cta cc	aga ggg acc att gtg tta ttg tat	Bartlett	$(AG)_{18}$	sloc	Ba3	Ho3
NB110a	tga tga tgt gat ttg atg gag tg	cca ttc cat tgt acg gat t	Bartlett	$(AG)_{20}$	mloc	Ba1/9	
NB111a	cca agc tgt gat tat agg aag	agg ctg aaa gat tgt aag gt	Bartlett	$(AG)_{19}$	mloc	Ba4	Ho4
NB113a	atg aaa tat gtc gtg ttg ccc tta g	ccc ttc ctc agc atg ttt cct aga c	Bartlett	$(AG)_{14}$	sloc	Ba3	Ho3
NH019b	gag atg gag tag taa aga aga agg	acg aca tag tga aaa cag aag	Housui	$(AG)_{18}$	sloc		Ho15
NH020a	gga tca gcc aag agg agg tg	cga gat gca gag gac gac g	Housui	$(AG)_{10}(GA)_{10}$	sloc/mloc	Ba13	Ho5
NH021a	atc tca att ttc tcg gta acc a	ctg ata tct ctc tgc act ccc t	Housui	$(AG)_{21}$	mloc	Ba11	
NH022a	cat gaa gtg cgt aga agt tgg tgt	ccc aat aat gat aag ttc cca aag	Housui	$(GT)_9(AG)_{15}$	sloc		Ho11
NH023a	gat gct aga agg aag gaa tga tgg	ctt ttc aac ccc ttc acc ttc tc	Housui	$(AG)_{19}$	sloc	Ba3	Ho3
NH024b	acc aaa agt aga gag aga ga	ccc aac cag act aaa aga ga	Housui	$(AG)_{30}$	mloc		Ho3/4
NH025a	ctg gac aca aac att caa gag gg	cac acc aga aac tcc aaa aca gg	Housui	$(AG)_{21}(GA)_4$	sloc	Ba6	Ho6-1
NH026a	cgt aat act cgt agt gca tga tg	gct tct gga cta tca cta ttt ctt c	Housui	$(AG)_{30}$	sloc		Ho10
NH027a	taa tgt gtt ggg gag aga gag	gct ctt gtt cct tgc tcc taa	Housui	$(GA)_{21}$	sloc	Ba6	
NH029a	gaa gaa aac cag agc agg gca	cct ccc gtc tcc cac cat att ag	Housui	$(AG)_8$	sloc	Ba9	
NH030a	gca aca gat agg agc aaa gag gc	tcc aaa gtt caa cac aga tca aga g	Housui	$(AG)_{18}$	mloc		Ho3

[a] Multi-loci in Bartlett and a single-locus in Housui

Table 2 Description of SSR markers used for mapping in the Bartlett × Housui population

SSR marker origin	Terminology	No. of SSR markers	SSRs detecting more than two loci	No. loci mapped		Source
				Bartlett	Housui	
Pear genomic	KA, HG, NB, NH, BG, NB, RLG	43	4	32	29	Table 1, Yamamoto et al. 2002a, b
Apple genomic	CH	11	0	9	7	Gianfranceschi et al. 1998
Apple genomic	NZ	3	0	3	0	Guilford et al. 1997
Peach cDNA	M	3	0	2	1	Yamamoto et al. 2002c
Peach genomic	UDP	3	1	1	3	Cipriani et al. 1999, Testolin et al. 2000
Cherry genomic	PS	1	1	2	2	Sosinski et al. 2000

AGT-3′ and 5′-CATCCACAAAGTCCCTATCA-3′, was used for the amplification of the RLG1 locus. One specific inter-microsatellite marker SIMS18 was developed from a fragment obtained from a 5′-anchored PCR. A set of oligonucleotide sequences, 5′-TCTCTCTCTACAGGAAAAGC-3′ and 5′-CCCCTCCCCATTACAC-3′, was used for the amplification of the inter-microsatellite region.

Microsatellite amplification was performed in a 20 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 pmol of each forward primer labelled with fluorescent chemical (FAM or TET or HEX) and unlabelled reverse primer, 10 ng of genomic DNA, and 0.5 units of *Taq* polymerase (Life Technologies). Amplification was performed with 35 cycles at 94 °C for 1 min, 50–55 °C for 1 min and 72 °C for 2 min, for denaturation, annealing and primer extension, respectively. The PCR products were separated and detected using a PRISM 377 DNA sequencer. The size of the amplified bands was determined based on an internal standard DNA (GeneScan-350TAMRA, PE Applied Biosystems) with GeneScan software.

SSR markers from apple, peach and cherry

Thirty one SSR markers developed from apple (Guilford et al. 1997; Gianfranceschi et al. 1998) were screened in our pear mapping population. Among them, 14 SSRs could produce reproducible amplified bands and showed a segregation of genotypes in the population. Eleven SSRs reported by Gianfranceschi et al. (1998) and three SSRs reported by Guilford et al. (1997) were denoted by CH and NZ (Table 2), respectively.

About 60 peach (*Prunus persica* L.) SSR markers developed by three different groups were tested for scoring. Twenty six markers were developed by an Italian group (Cipriani et al. 1999; Testolin et al. 2000), and designated as UDP. Twenty three SSRs obtained from genomic or cDNA in our group (Yamamoto et al. 2002c) were denoted by M. We also tested ten SSRs from Clemson University (Sosinski et al. 2000).

Five SSR markers derived from sweet cherry (*Prunus avium* L.) were denoted by PS (Sosinski et al. 2000). Amplification and detection of the microsatellite fragments from apple, peach and cherry primers were performed under the same conditions as those described in "SSR markers from pear".

Isozyme analysis

Isozyme analysis was performed with extracts from unexpanded young leaves in an acrylamide gel, according to the protocols described in Chevreau and Laurens (1987) and Glaszmann et al. (1988) except for the extraction buffer. Extraction was performed in the buffer containing 0.3 M Tris–HCl, 0.1 M NaCl, 0.2 M ascorbic acid, 0.1% mercaptoethanol, 2% Triton X-100 and 2% polyvinylpyrrolidone. Seven isozymes, malate dehydrogenase (MDH, E.C.1.1.1.37), diaphorase (DIA, E.C.1.6.99.-), aspartate aminotransferase (AAT, E.C.2.6.1.1), shikimate dehydrogenase (SDH, E.C.1.1.1.25), endopeptidase (ENP, E.C.3.4.9.9), glutamate dehydrogenase (GDH, E.C.1.4.1.2) and isocitrate dehydrogenase (IDH, E.C.1.1.1.42), were studied for polymorphism.

S genotype and phenotypic traits in leaves

Self-incompatibility genotypes (S genotypes) of the parents and F_1 individuals were identified by the PCR-RFLP method (Ishimizu et al. 1999). A set of oligonucleotide sequences, 5′-TTTACGCAGCAATATCAG-3′ and 5′-AC(A/G)TTCGGCCAAATAATT-3′, was used for amplification of the S alleles. DNA fragments were separated on 2% agarose and 7% polyacrylamide gels. After electrophoresis, the gels were stained with an ethidium bromide solution and then visualized with ultraviolet light.

Several phenotypic traits in leaves were investigated in our mapping population. The color of unexpanded leaves was scored for parents and F_1 individuals. Ten to twenty of the completely expanded leaves were used to score their size, shape, trichome, petiole and serration.

Linkage analysis

JoinMap ver. 2.0 (Stam and van Ooijen 1995) was used for the construction of genetic linkage maps of Bartlett and Housui, including SSRs, AFLPs, isozyme markers and other traits. The Kosambi function was used to convert recombination units into genetic distances. The mapping analysis was conducted using a minimum LOD score of 4.0. In this study, we did not use markers of a/b × a/b and a/- × a/- types, because of the difficulty of use and inaccuracy. Distorted markers analyzed by the chi-square test were used for the construction of linkage maps. However, some distorted markers were discarded, when they caused any inconsistencies.

Results

Marker analysis

AFLPs

Forty primer combinations produced 184 and 115 polymorphic AFLP fragments in the F_1 population originating from Bartlett and Housui, respectively. Only two primer combinations (E-TG/M-CTA, E-AG/M-CTT) could not generate discrete polymorphic bands. The number of polymorphic fragments per primer combination ranged from 0 (E-TC/M-CTT) to 15 (E-TG/M-CTG) in Bartlett, with an average number of 4.6. In Housui, polymorphic bands per primer combination varied from 0 (E-AG/M-CTA, E-TG/ M-CTG) to 9 (E-AG/M-CAG), with an average number of 2.9. About 10% of the frag-

Table 3 Segregation and map position of SSR loci

SSR loci	Origin	Genotype[a] (Bartlett × Housui)	Segregation	χ^2-value[b]	Map position	
					Bartlett	Housui
KA4b	Pear	an × cd	ac:ad:nc:nd=24:16:14:9	7.41ns	Ba8-1	Ho8
KA14	Pear	ab × cc	ac:bc=33:30	0.14ns	Ba10	
KA16	Pear	ab × cc	ac:bc=30:33	0.14ns	Ba11	
KA20	Pear	ab × cc	ac:bc=28:35	0.78ns	Ba9	
HGT6	Pear	nn × an	an:nn=35:27	1.03ns		Ho1
HGA8b	Pear	aa × ab	an:nn=37:26	1.92ns		Ho3
BGT23b	Pear	ab × cd	ac:ad:bc:bd=34:4:22:3	42.7***	Ba2	Ho2
BGT24	Pear	aa × ab	aa:ab=30:33	0.14ns		Ho5
NH001c	Pear	ab × cc	ac:bc=47:16	15.3***	Ba7	
NH002b	Pear	ab × cc	ac:bc=35:28	0.78ns	Ba2	
NH004a	Pear	ab × cd	ac:ad:bc:bd=19:8:21:15	6.27ns	Ba7	Ho7
NH005b	Pear	aa × bc	ab:ac=31:32	0.02ns		Ho11
NH006b-1	Pear	an × nn	an:nn=25:38	2.68ns	Ba1	
NH006b-2	Pear	nn × an	an:bn=34:29	0.40ns		Ho1
NH006b-3	Pear	nn × ab	an:bn=29:34	0.40ns		Ho9
NH007b	Pear	ab × cc	ac:bc=33:30	0.02ns	Ba10	
NH008b	Pear	nn × ab	ab:bn=25:38	0.40ns		Ho1
NH009b	Pear	ab × cc	ac:bc=33:30	0.14ns	Ba11	
NH010a	Pear	an × bb	ab:bn=25:38	2.68ns	Ba8-1	
NH011a	Pear	ab × cc	ac:bc=27:36	1.29ns	Ba12	
NH013a	Pear	ab × cd	ac:ad:bc:bd=19:17:6:21	8.56*	Ba8-2	Ho8
NH014a	Pear	ab × ac	aa:ac:ab:bc=17:18:12:16	1.32ns	Ba1	Ho1
NH015a	Pear	ab × cd	ac:ad:bc:bd=15:20:13:15	1.70ns	Ba1	Ho1
NH017a	Pear	nn × ab	an:bn=33:30	0.14ns		Ho5
NB101a	Pear	ab × cc	ac:bc=25:38	2.68ns	Ba13	
RLG1-1	Pear	an × nn	an:nn=31:30	0.02ns	Ba8-2	
RLG1-2	Pear	nn × an	an:nn=30:31	0.02ns		Ho15
RLG1-3	Pear	nn × an	an:nn=35:26	1.33ns		Ho4
CH01B12	Apple	nn × ab	an:bn=29:34	0.40ns	Ba1	
CH01E12	Apple	an × bn	ab:an:bn:nn=31:17:6:9	24.0***		Ho6-2
CH01F02	Apple	ab × cc	ac:bc=29:34	0.40ns	Ba11	
CH01H01	Apple	ab × cn	ac:an:bc:bn=13:14:18:18	1.32ns	Ba1	Ho1
CH01H02	Apple	ab × cd	ac:ad:bc:bd=22:18:10:13	5.38ns	Ba9	Ho9
CH01H10	Apple	an × ab	(aa+an):ab:bn=34:10:19	2.97ns		Ho11
Ch02B03b	Apple	an × bc	ab:ac:bn:cn=14:13:17:19	1.44ns	Ba5	Ho5
CH02B10	Apple	ab × cd	ac:ad:bc:bd=10:17:11:25	9.06*	Ba2	Ho2
CH02B12	Apple	ab × nn	an:bn=31:32	0.02ns	Ba13	
CH02D11	Apple	ab × cd	ac:ad:bc:bd=18:13:7:25	11.1*	Ba6	Ho6-2
CH02F06	Apple	ab × nn	an:bn=34:29	0.40ns	Ba2	
NZ28f4	Apple	ab × cd	ac:ad:bc:bd=14:17:11:21	3.48ns	Ba11	
NZ05g8	Apple	ab × cc	ac:bc=36:27	1.29ns	Ba12	
NZ02b1	Apple	ab × cc	ac:bc=30:33	0.14ns	Ba6	
M7a	Peach	ab × cc	ac:bc=35:28	0.78ns	Ba5	
M9a	Peach	ab × nn	an:bn=30:33	0.14ns	Ba10	
M11a	Peach	aa × ab	aa:ab=41:22	5.73*		Ho7
UDP96-019-1	Peach	nn × an	an:nn=29:34	0.40ns		Ho6-2
UDP96-019-2	Peach	nn × an	an:nn=35:28	0.78ns		Ho9
UDP98-018	Peach	nn × an	an:nn=29:34	0.40ns		Ho13
UDP98-411	Peach	ab × nn	an:bn=33:30	0.14ns	Ba3	
PS12A02-1	Cherry	ab × nn	an:bn=34:29	0.40ns	Ba6	
PS12A02-2	Cherry	an × nn	an:nn=27:36	1.29ns	Ba8-2	
PS12A02-3	Cherry	nn × an	an:nn=35:28	0.78ns		Ho6-1
PS12A02-4	Cherry	nn × an	an:bn=37:26	1.92ns		Ho8

[a] 'n' indicates a null allele
[b] *,**,*** indicate distortions at the 5%, 1%, 0.1% levels

ments from Bartlett (19 out of 184) showed a distorted segregation, compared to approximately 12% (14/115) in Housui.

SSRs from pear

Among the 55 SSR markers originating from the pear cultivars Bartlett or Housui, 43 SSR markers generated 49 polymorphic loci (Tables 1, 3). Thirty four out of 38 SSRs developed from the enriched genomic libraries showed a segregation in the F_1 mapping population of Bartlett and Housui. Characteristics of 19 SSR markers newly developed from enriched genomic libraries were shown in Table 1. Twelve and five out of 19 SSR markers produced segregation of single-locus and multiple-loci types, respectively. The other two SSR markers showed segregation of a single locus for one parent and

multiple loci for another parent. Four SSRs obtained by the RAHM approach (KA4b, KA14, KA16, KA20) and four SSRs obtained by the 5′-anchored PCR method (HGA8b, HGT6, BGT24, BGT23b) generated polymorphisms in at least one parent and showed a segregation in the F_1 progenies. Bartlett's alleles at 32 loci and Housui's alleles at 29 loci segregated in the F_1 mapping population. Segregation was observed for alleles of both Bartlett and Housui at 12 loci. Three SSR loci showed a distorted segregation.

SSRs from apple, peach and cherry

Among the 31 SSR markers developed from apple, 14 SSR loci produced reproducible amplified bands and showed a segregation of genotypes in our mapping population (Table 3). Seven and 12 loci showed a segregation in F_1 individuals for the alleles from Bartlett and Housui, respectively. Among them, five SSRs showed a segregation for the alleles of both Bartlett and Housui. Three SSR loci showed a distorted segregation.

About 60 peach SSR markers were evaluated in the pear population. Only six SSR primers produced discrete amplified fragments and showed segregating genotypes. Three SSR markers were obtained from cDNA clones in our group (Yamamoto et al. 2002c) and the other three SSRs were derived from the Italian group (Cipriani et al. 1999; Testolin et al. 2000).

One SSR marker PS12A02 derived from sweet cherry showed four putative loci, in which two and two loci were found in Bartlett and Housui, respectively.

Isozymes

Seven isozymes produced ten loci in Bartlett and Housui. Six loci, *MDH1*, *MDH2*, *DIA1*, *DIA2*, *AAT1* and *SDH1*, showed a segregation in the F_1 mapping population. Among them, segregation at five loci (*MDH2*, *DIA1*, *DIA2*, *AAT1* and *SDH1*) was observed for the alleles of one parent, which fitted the expected ratio of 1:1. *MDH1* generated a segregation of the a/b × a/b type, fitting the expected ratio of 1:2:1.

Phenotypic traits

The PCR-RFLP method was used to identify the S genotypes of the parents and their F_1 hybrids. Three fragments 113, 263 and 367 bp in size, were obtained for the male parent Housui after digestion with the S5 specific enzyme *Alw*NI. The former two fragments were restricted products of the S5 allele and the latter undigested band originated from the S3 allele. The female parent Bartlett produced 1.0- and 1.4-kbp bands, presumably amplified from S alleles, which we describe as Sb1 and Sb2, respectively. Segregation of S genotypes in the F_1 individuals was S3/Sb1:S3/Sb2:S5/Sb1:S5/Sb2 = 20:18:10:15, which fitted to the expected ratio of 1:1:1:1 based on the Chi-square test.

Several phenotypic traits in leaves, i.e. young leaf color, size, shape, trichome, petiole and serration, were investigated in our mapping progenies. All the traits except for the young leaf color showed continuous distributions (data not shown), which suggested that the traits were presumably controlled by QTLs. The red- or green-leaf color trait of young leaves could be scored. In the Japanese pear Housui young leaves showed a red color pigmentation, compared to the green color of leaves in the European pear Bartlett. In 35 F_1 individuals the young leaves showed a red color, while in 28 plantlets the leaves showed a green color. Segregation of young leaf color fitted the ratio of 1:1, suggesting a monogenic inheritance. Here we represent Lc/lc (red/green) as a gene symbol.

Map construction

A genetic linkage map of the European pear Bartlett was constructed based on AFLP markers, SSRs developed from pear and the other species, isozymes and the self-incompatibility locus. The terminology of SSR loci derived from different origins was denoted in Table 2. This map consisted of 226 markers, including 175 AFLPs, 32 pear SSR loci, 12 apple SSRs, three peach SSRs, two cherry SSRs, one isozyme and one S locus. Seven AFLP markers showing large distortions were discarded during the mapping stages because their presence caused inconsistencies. Four markers, i.e. one apple SSR (CH01E12), one isozyme (*SDH1*) and two AFLPs, were not assigned to any linkage groups. Eighteen linkage groups were identified that covered 949 cM with an average distance of 4.2 cM between each pair of loci (Fig. 1). The size of the linkage groups ranged from 90.3 cM (Ba11 group) to 10.7 cM (Ba17 group).

Another genetic linkage map of the Japanese pear Housui was also constructed. This map consisted of 154 loci, including 106 AFLPs, 42 SSRs (29, 7, 4 and 2 were derived from pear, apple, peach and cherry), three isozymes and two phenotypic traits (self-incompatibility, leaf color). Nine DNA markers (one apple SSR and eight

Fig. 1 Genetic linkage maps of the European pear 'Bartlett' and the Japanese pear 'Housui'. Linkage groups are designated as Ba1 to Ba17 for Bartlett and as Ho1 to Ho16 for Housui. Genetic distances and loci are listed on the right and/or the left sides, respectively. The designation of the AFLP is based on the primer combination and the sizes expressed as bp (*Eco* primer/*Mse* primer-size). SSR loci from pear, apple, peach and cherry are denoted as indicated in Table 2. The self-incompatibility locus is denoted by S in the groups Ba1 and Ho1. Isozyme locus AAT1 is included in the group Ba15. Isozyme loci MDH2, DIA1 and DIA2 are included in the group Ho14. *Asterisks* indicate distorted segregations of markers in the chi-square test. Distortions at 5%, 1% and the 0.1% level are indicated as *, ** and ***, respectively. The groups Ba10 and Ho10 are connected by two SSR loci which show significant linkage (recombination value 0.091) in the European pear La France

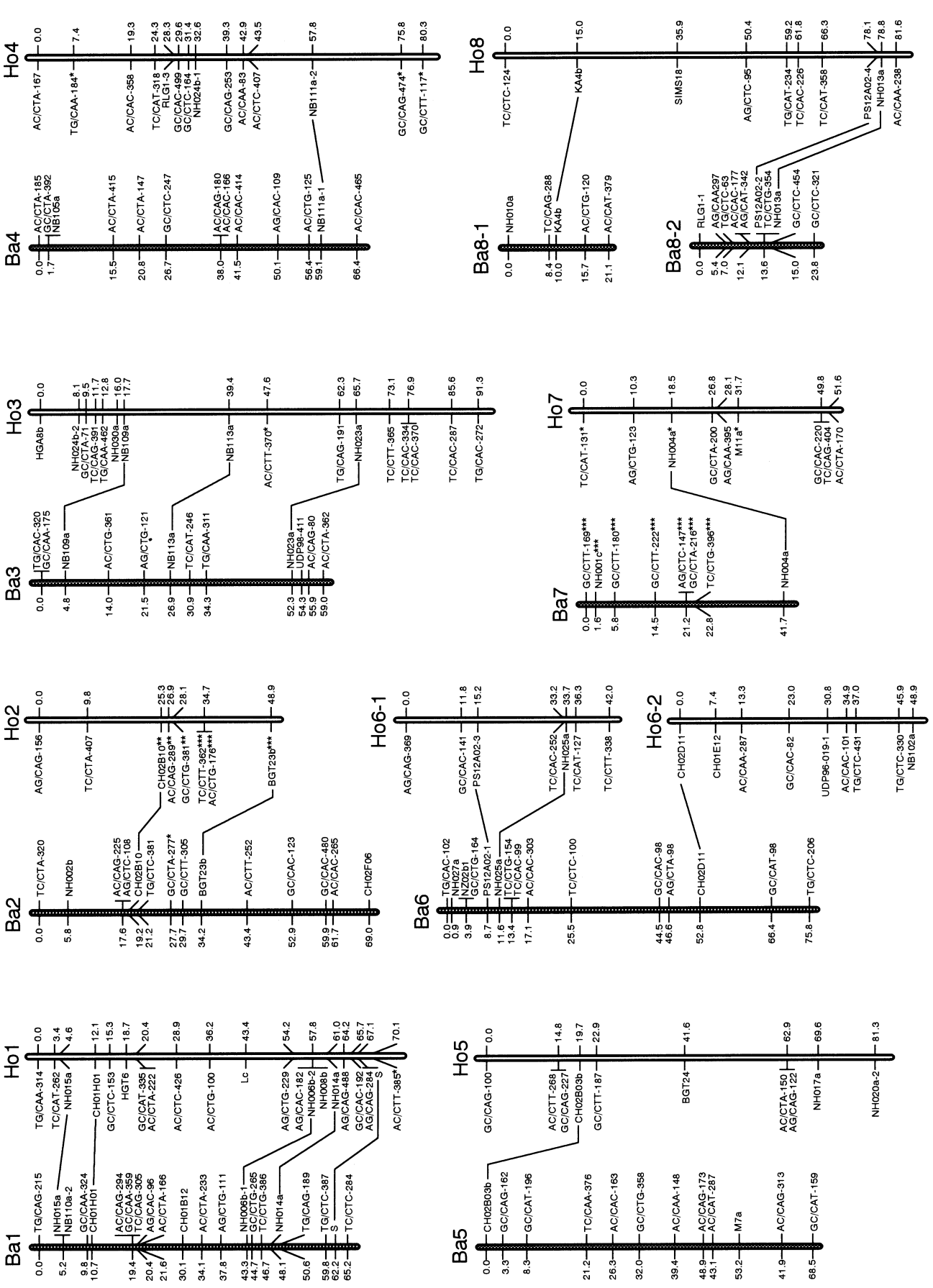

Fig. 1 Legend see page 13

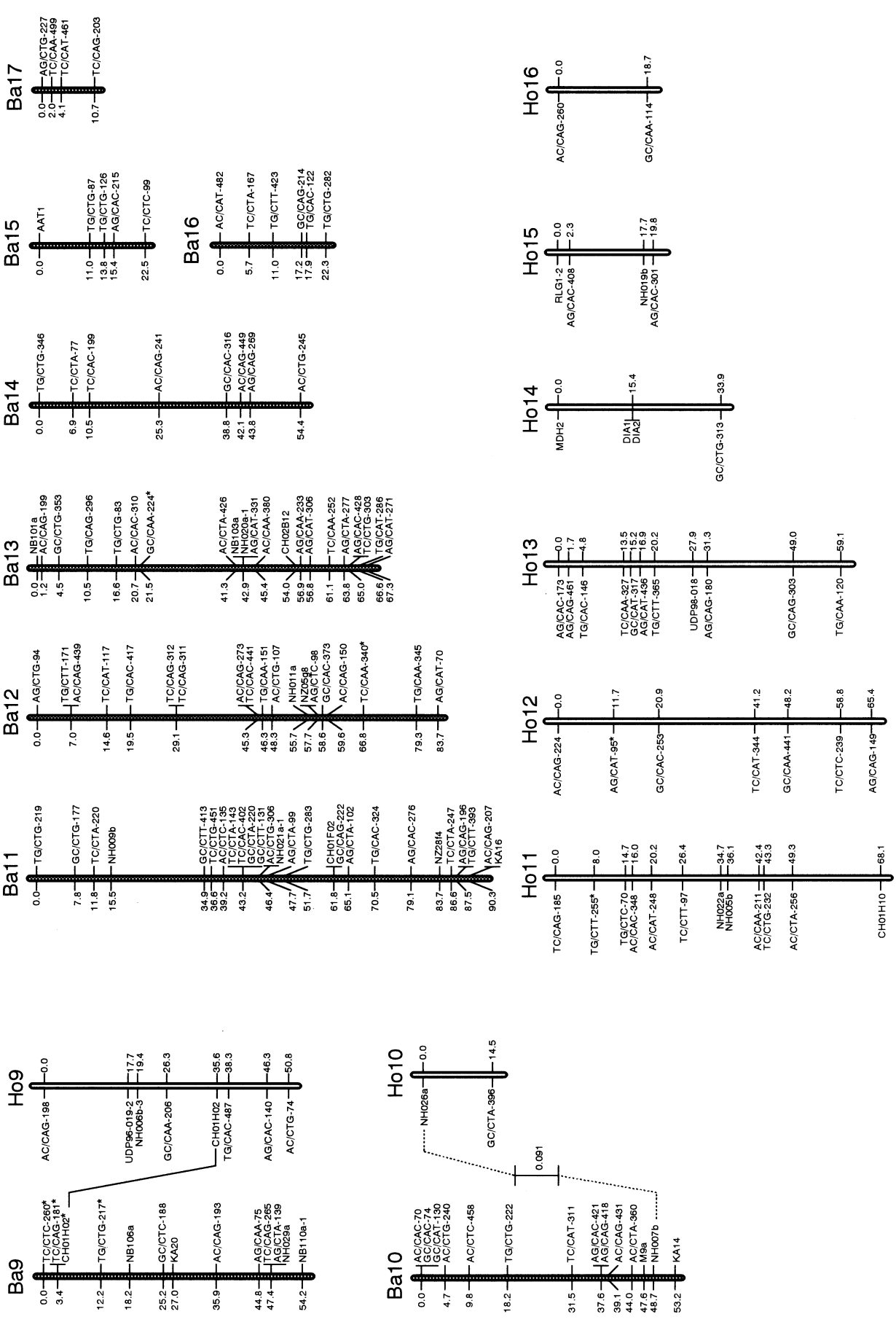

Fig. 1 (continued)

AFLPs) were not assigned to any linkage group. This map consisted of 17 linkage groups ranging from 91.3 cM (Ho3 group) to 14.5 cM (Ho10 group) and encompassed 926 cM with an average distance of 6.0 cM between each pair of loci (Fig. 1).

Ten linkage groups of Bartlett and Housui could be connected together by using SSR loci and the S locus as anchors. The linkage groups 1 of Bartlett (Ba1) and Housui (Ho1) were well consolidated by five anchor loci, NH015a, CH01H01, NH006b, NH014a and S (Fig. 1). The groups 3 of the parents' map were anchored together by three pear SSR loci. The groups 2, 4, 5, 7 and 9 of both maps were aligned by one or two SSR loci. The Ba6 group corresponded to the Ho6-1 and Ho6-2 groups connected with three loci. The Ba8-1 and Ba8-2 groups corresponded to the Ho8 group connected with three loci. The Ba10 group presumably corresponded to the Ho10 group, because the SSR loci NH007b and NH026a showed tight linkage in the map of European pear 'La France' with a recombination value of 0.091 (data not shown).

Two and one distorted regions were found in the genetic maps of Bartlett and Housui, respectively. Seven markers in the Ba7 group showed a distorted segregation at the 0.1% level based on the chi-square test. Four markers in the Ba9 group showed a slight distortion at the 5% level. In Housui, six markers in the Ho2 group were distorted at the 0.1–1% level. However the two SSR loci BGT23b and CH02B10 showed a distortion at the 0.1–1% level in the Housui Ho2 group, while no distorted segregations were observed in the Bartlett Ba2 group for the same loci. The CH01H02 locus did not show any distortion in Housui (Ho9) but showed a slight distortion in Bartlett (Ba9). Similarly, the NH004a locus showed a distortion only for the Housui's alleles.

Discussion

The present study is the first to report a genetic linkage map of the European pear (*P. communis* L.). The genetic maps of the European pear 'Bartlett' and the Japanese pear 'Housui' were partially aligned using 20 codominant markers. In our previous study, two disease-related genes, pear scab resistance caused by *Venturia nashicola* and black spot susceptibility caused by *Alternaria alternata*, were characterized and mapped on the linkage map of the Japanese pear 'Kinchaku' (Iketani et al. 2001). The linkage maps of Japanese pears 'Kinchaku' and 'Kousui' were constructed by using RAPD markers and were estimated to cover more than a half of the total pear genome. Since these maps of Japanese pears consisted of only dominant markers, it was rather difficult to align them together, as well as to anchor the other pear maps. In this study, we therefore developed and mapped a large number of SSR markers in pear and tried to utilize almost all reported SSRs derived from apple and *Prunus* for pear mapping. Ten linkage groups in the maps of Bartlett and Housui could be aligned by using codominant markers. It is necessary to develop a much larger number of codominant markers in order to completely consolidate different pear maps.

It was considered that the genetic linkage maps of the European pear Bartlett and the Japanese pear Housui were not saturated but were near saturation because only a few loci could not be assigned to any of the linkage groups. The length of the genetic linkage maps of apple was reported to be 700 to 950 cM (Hemmat et al. 1994; Conner et al. 1997; Maliepaard et al. 1998). Maliepaard et al. (1998) reported that the total length for the maps of 'Prima' and 'Fiesta' with a basic chromosome number (x=17) was 842 and 984 cM, respectively. Pear is very closely related to apple. Both pear and apple belong to the same sub-family Pomoideae in the family Rosaceae and have the same basic chromosome number. Since the map length of Bartlett and Housui was 949 and 926 cM, it is considered that both maps cover almost the whole genomes.

The position of the self-incompatibility locus S was determined in pear maps. The self-incompatible trait has been of special interest for geneticists and breeders in Japanese pear. Nine alleles (S-RNase genes) at the S locus have been cloned and characterized in Japanese pear (Ishimizu et al. 1998b; Sawamura et al. 2002). It was indicated that S-RNase genes from Japanese pear and apple showed high homology based on their nucleotide and/or amino-acid sequences (Ishimizu et al. 1998b). However, very little information on self-incompatible genes was obtained in the other *Pyrus* species such as the European pear and Chinese pear. There have been no reports on the position of the S locus on pear chromosomes and on its positional relationship with apple. In this study, it was revealed that the position of the S locus was conserved between Japanese and European pears in the linkage groups Ba1 and Ho1, which were anchored by NH015a, CH01H01, NH006b and NH014a SSR loci. The S locus and the CH01H01 SSR were assigned to linkage group 17 on the genetic map of the apple progeny Prima × Fiesta (Maliepaard et al. 1998; Drs. Van de Weg and Liebhard, personal communication). These results suggested that our pear linkage group 1 corresponded to apple group 17 and that the position of the S locus was conserved between pear and apple in a putative homologous linkage group.

Four isozyme loci could be positioned on our pear map, i.e. *AAT1* in the linkage group Ba15, and *MDH2*, *DIA1* and *DIA2* in the Ho14 group. A large number of isozyme loci have been characterized and mapped in apple genetic linkage maps (Maliepaard et al. 1998; Chevreau et al. 1999). Chevreau et al. (1997) noted that most of the isozyme systems in pear showed a high similarity to apple isozymes. *AAT1* in group Ba15 might correspond to the *AAT2* locus in apple group 8. The other isozyme loci *MDH2*, *DIA1* and *DIA2* were not compared to apple. Although *DIA2* showed linkages with *ESTC* and *PRXC2* in Prima × Fiesta apple progenies, their linkage group was not identified (Chevreau et al. 1999).

The position of 14 SSR loci originating from apple could be successfully determined in the pear maps. It

was revealed that SSRs isolated from apple are highly conserved in pear and can be utilized as DNA markers in the latter genus (Yamamoto et al. 2001). Since it is assumed that the loci of pear and apple amplified by the same apple SSR primers were presumably derived from the same origin, pear maps could be anchored to apple maps by using apple SSRs. Several pear linkage groups could be aligned with those of apple based on the positions of SSRs in the European apple consensus map (Drs. Van de Weg and Liebhard, personal communication; Drs. Liebhard and Gessler, personal communication). Pear linkage groups 1, 2, 5, 6 and 10 could correspond to apple groups 17, 2, 10, 15 and 9, respectively. Groups Ba11, Ba12, Ba13 and Ho11 might correspond to apple groups 12, 4, 5 and 8, respectively. Linkages between apple SSRs, i.e. CH02B10 vs CH02F06, NZ02b1 vs CH02D11, and CH01F02 vs NZ28f4, were conserved in our pear map. However, discrepancies on linkages of apple SSRs were found in the pear map. CH01B12 and CH01F02, which were mapped in the same linkage group of 'Iduna' and 'A679/2' apple (Gianfranceschi et al. 1998), were not grouped together in the pear map. Since a long AG repeat was not contained in amplified fragments produced by CH01B12 in pear (Yamamoto et al. 2001), the CH01B12 locus in pear could be different from that in apple. Another discrepancy was the position of CH01E12. CH01E12 was mapped in the same group with CH01H10 on the map of Gianfranceschi et al. (1998) as well as on the consensus map. CH01E12 was not grouped with CH01H10 but with CH02D11 in pear group 6. Segregation of the genotype of the pear population at CH01E12 was largely distorted in the ratio of 1:1:1:1. This discrepancy might be due to the different loci, the different genome structure between apple and pear, or the error from a relatively small number of populations. It will be necessary to map and compare the locus in different pear maps. Although apple and pear are closely related, the nuclear DNA content of the pear species was estimated to be about 2/3rds that of apple (Dickson et al. 1992). SSR markers could be useful for comparing the apple and pear genome organization and for comparative mapping of both species.

About one-tenth (7 out of 65) of the *Prunus* (peach, cherry) SSR markers could produce segregating genotypes in the F_1 progenies obtained from a cross between the European and Japanese pears. The cherry SSR PS12A02, included in linkage group 4 of the *Prunus* consensus map (Dr. Dirlewanger, personal communication), produced two and two polymorphic loci for Bartlett and Housui, respectively. These putative loci of the parents could be successfully connected in the pear linkage groups 6 and 8. The peach SSR UDP96-019 identified in group 8 of the *Prunus* map produced two polymorphic loci in the pear groups Ho6-2 and Ho9. UDP98-411 in the Ba3 group was mapped in group 2 of the *Prunus* map. Since pear and *Prunus* belong to a different subfamily of the family Rosaceae, it was considered that, due to the difference in genome composition between them, SSR amplification would sometimes be difficult. We are currently investigating sequence homology to determine whether mapped SSR loci amplified by *Prunus* SSRs showed the same origin.

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