

# Genetic Linkage Maps of Japanese and European Pears Aligned to the Apple Consensus Map

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## Abstract

Genetic linkage maps of the Japanese pear (*Pyrus pyrifolia* Nakai) cultivar 'Housui' and the European pear (*Pyrus communis* L.) cultivar 'Bartlett' were constructed based on Amplified Fragment Length Polymorphism markers (AFLPs), Simple Sequence Repeat markers (SSRs) (from pear, apple and *Prunus*), isozymes, and phenotypic traits by using their F<sub>1</sub> progenies. The map of the female parent 'Bartlett' consisted of 256 loci including 178 AFLPs, 76 SSRs (32 pear, 39 apple, 5 *Prunus* SSRs), 1 isozyme and a self-incompatibility locus on 19 linkage groups over a total length of 1020 cM. The map of 'Housui' contained 180 loci including 110 AFLPs, 64 SSRs (29 pear, 29 apple, 6 *Prunus* SSRs), 2 phenotypic traits and 4 other markers on 20 linkage groups encompassing a genetic distance of 995 cM. These 2 pear maps were aligned using 37 co-dominant markers that showed segregating alleles in both parents. Out of 80 tested SSR markers developed from apple, more than four-fifth could produce discrete amplified fragments in pear. Thirty-eight apple SSR markers showed 39 segregating loci in the linkage map of 'Bartlett', while 27 markers produced 29 loci in 'Housui'. All pear linkage groups could be successfully aligned to the apple consensus map by at least 1 apple SSRs, suggesting that positions and linkages of SSR loci were well conserved between pear and apple. The self-incompatibility locus (S locus) was mapped to linkage group 17 in Japanese and European pears as well as apple. Our results are the first major effort in comparative mapping of pear and apple.

## INTRODUCTION

Pear (*Pyrus* spp.) is one of the most important fruits, which has been cultivated in Asia and Europe for 2000 to 3000 years. Presently, pear is cultivated commercially in all the temperate regions in more than 50 countries around the world. However, there is very little information on genome research, i.e., genetic linkage maps, development of molecular markers, and genetic analysis on phenotypic traits.

In this study, we constructed genetic linkage maps of the European pear cultivar 'Bartlett' and the Japanese pear cultivar 'Housui' using AFLP markers and SSR markers developed in pear, apple and *Prunus*. Both maps are aligned to the apple consensus map by using apple SSRs as anchor loci. Genetic synteny between pear and apple is discussed.

## MATERIALS AND METHODS

### Plant Material

An F<sub>1</sub> population of 'Bartlett' (*Pyrus communis* L.) x 'Housui' (*P. pyrifolia* Nakai) (63 individuals) was used for constructing genetic linkage maps. Genomic DNA was isolated from young leaves by a CTAB-based extraction method (Yamamoto et al., 2002c).

## DNA Markers

AFLP analysis was done with 40 sets obtained by 8 Mse I primers (M/CAA, CAC, CAG, CAT, CTA, CTC, CTG, CTT) and 5 Eco RI primers labelled with FAM (E/AC, AG, TG, TC, GC). The AFLP 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 using GeneScan software (PE Applied Biosystems).

Fifty-four SSRs developed from pear were used for the detection of microsatellite loci (Yamamoto et al., 2002a, 2002b, 2002c), and were denoted by KA, HG, NB, NH, BG or RLG (Fig. 1, Fig. 2). Eighty-four SSRs developed in apple were screened and used for genetic mapping (Gianfranceschi et al., 1998, Guilford et al., 1997, Liebhard et al., 2002). A total of 65 SSR markers developed in *Prunus* (peach, cherry) were also tested. SSR amplification was performed as described by Yamamoto et al. (2002c).

## Linkage Analysis

JoinMap ver. 2.0 was used for the construction of genetic linkage maps of 'Bartlett' and 'Housui'. The Kosambi function was used to convert recombination units into genetic distances. The mapping analysis was conducted using a minimum LOD score of 6.0. The same numbering system of linkage groups was performed as that of apple (Maliepaard et al., 1998) by using common apple SSRs as anchor loci (Liebhard et al., 2002).

## RESULTS AND DISCUSSION

### Genetic Linkage Maps of Japanese and European Pears

The genetic linkage map of 'Bartlett' was constructed with 256 DNA markers, including 39 apple SSRs, 32 pear SSRs, 5 *Prunus* SSRs, 1 isozyme, 1 S locus and 178 AFLPs (Fig. 1). The obtained map consisted of 19 linkage groups that covered more than 1020 cM with an average distance of 4.0 cM between each pair of loci. The size of the linkage groups ranged from 88 cM (Linkage Group 4 (LG4)) to 11 cM (LG18). The segregation of almost all SSR markers fitted the expected ratio of monogenic inheritance. The segregation of many markers on LG14 was largely distorted. The self-incompatibility locus (S locus) was found in bottom of LG17.

The genetic linkage map of 'Housui' contained 180 loci (110 AFLPs, 29 pear SSRs, 29 apple SSRs, 6 *Prunus* SSRs, 2 phenotypic traits and 4 other markers) shown in Fig. 2. The map consisted of 20 linkage groups encompassing a genetic distance of 995 cM. Genetic linkage maps of these 2 pears were aligned using 37 co-dominant markers that showed segregating alleles in both parents.

### Synten between Pear and Apple

Thirty-six SSRs originating from apple were mapped in genetic linkage maps of the European pear 'Bartlett' and the apple consensus map (Table 1). Only 2 SSR loci (CH01b12, CH02d08) were assigned to different linkage groups between pear and apple. The other 34 apple SSR loci could be positioned in presumably homologous linkage groups of pear. All pear linkage groups could be successfully aligned to the apple consensus map by at least 1 apple SSR (or isozyme AAT-1 for LG8), suggesting that positions and linkages of SSR loci were well conserved between them, and that the genome organization of apple and pear is highly similar. The self-incompatibility locus was found at the bottom of LG17 in Japanese and European pears as well as apple. Our results are the first major effort in comparative mapping of pear and apple.

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## Tables

Table 1. Mapped linkage group of apple SSR loci in genetic maps of pear and apple.

SSR Locus	Linkage group	
	Pear (Bartlett)	Apple <sup>a</sup>
CH05g08	#1-2	#1
CH03g12	#1,3	#1,3
CH02b10	#2	#2
CH02f06	#2	#2
MS14h03	#3	#3
CH02d08	#3	#11
NZ05g8	#4	#4
CH02c02b	#4	#4
MS06c09	#4	
CH02b12	#5	#5,10
CH05e06	#5	#5
CH03d12	#6	#6
CH05a05	#6	#6
CH04e05	#7	#7
CH05c07	#9	#9
CH01h02	#9	#9,17
CH02b03b	#10	#10
CH01f07a	#10	#10
CH02c11	#10	#10
CH04h02	#11	#11
CH03d02	#11	#11
CH04g07	#11	#11
NZ28f4	#12	#12
CH01f02	#12	#12
CH04d02	#12	#12
CH05d04	#12	#12
CH03a08	#13	#13
CH05f04	#13	#13
CH01g05	#14	#14
MS01a05	#14	#14
CH03g06	#14	#14
NZ02b1	#15	#15
CH02d11	#15	#15
CH01d08	#15	#15
CH02c09	#15	#15
CH05c06	#16	#16
CH01b12	#17	#4,12,13
CH01h01	#17	#17

<sup>a</sup> Liebhard et al., 2003; van de Weg (pers. commun.)

# Figures

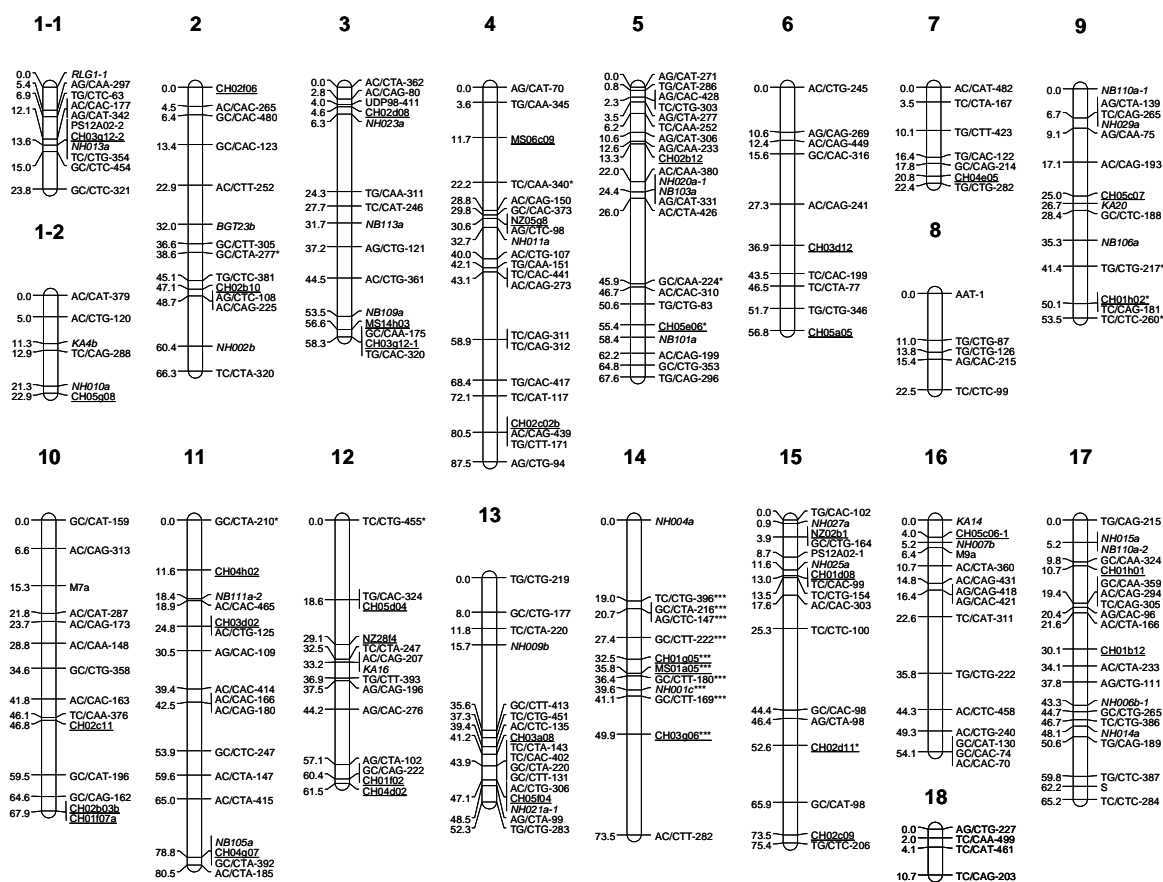


Fig. 1. A genetic linkage map of the European pear 'Bartlett'. Genetic distances and loci are listed on the left and the right sides, respectively. The designation of AFLP markers is based on primer combination and size. SSR loci from apple and pear are underlined and in italics, respectively. The self-incompatibility locus is denoted by S on LG17. Asterisks indicate distorted segregations of markers according to the chi-square test. Distortions at the 5%, 1% and 0.1% level are indicated as \*, \*\* and \*\*\*, respectively.

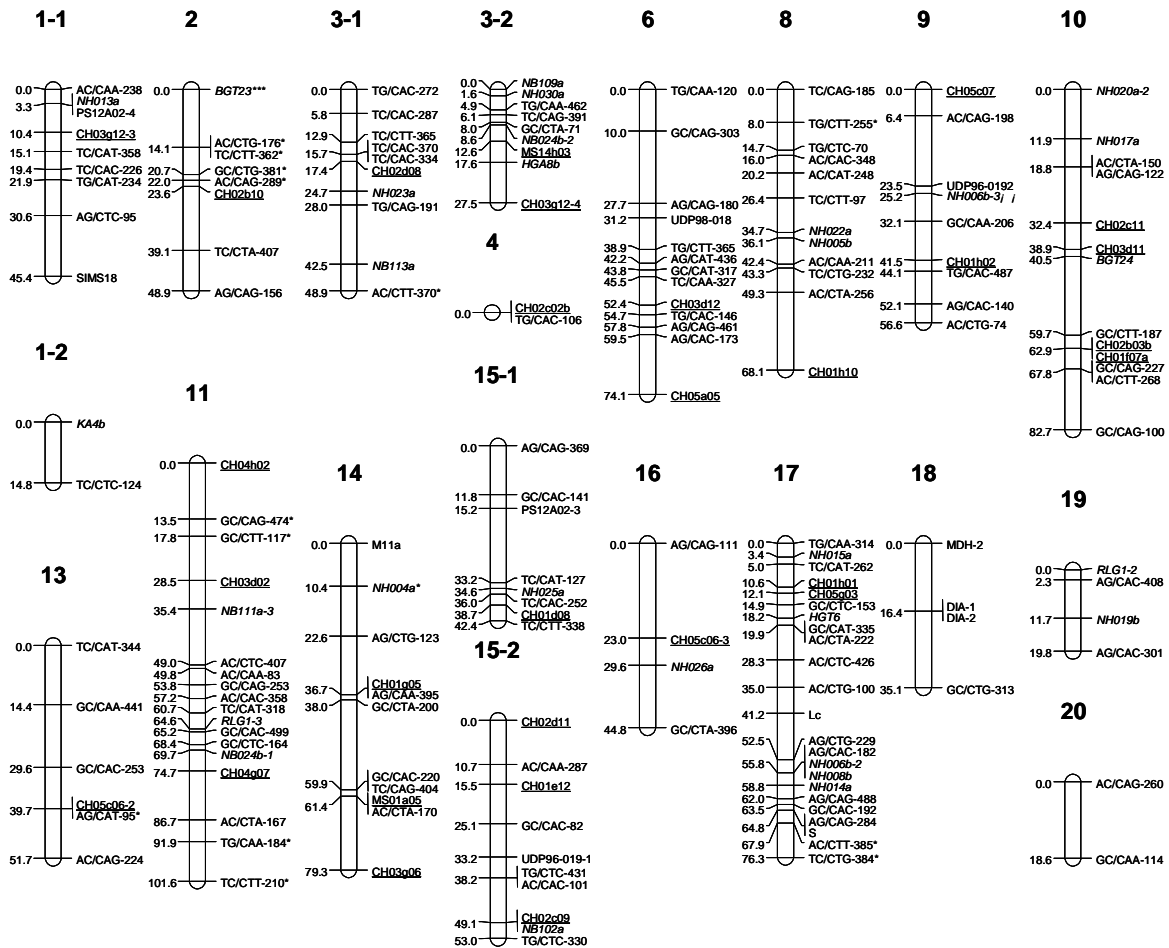


Fig. 2. A genetic linkage map of the Japanese pear 'Housui'. Genetic distances and loci are listed on the left and the right sides, respectively. The self-incompatibility locus and the young leaf color are denoted by S and Lc on LG17, respectively.