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ORIGINAL PAPER

Construction of an integrated consensus map of the apple genome based on four mapping populations

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Abstract An integrated consensus genetic map for apple was constructed on the basis of segregation data from four genetically connected crosses (C1=Discovery × TN10-8, C2=Fiesta × Discovery, C3=Discovery × Prima, C4=Durello di Forli × Fiesta) with a total of 676 individuals using CarthaGene® software. First, integrated female–male maps were built for each population using common female–male simple sequence repeat markers (SSRs). Then, common SSRs over populations were used for the consensus map integration. The integrated consensus map consists of 1,046 markers, of which 159 are SSR markers, distributed over 17 linkage groups reflecting the basic chromosome number of apple. The total length of the integrated consensus map was 1,032 cM with a mean distance between adjacent loci of 1.1 cM. Markers were proportionally distributed over the 17 linkage groups (χ^2=16.53, *df*=16, *p*=0.41). A non-uniform marker distribution was observed within all of the linkage groups (LGs). Clustering of markers at the same position (within a 1-cM window) was observed throughout LGs and consisted predominantly of only two to three linked markers. The four integrated female–male maps showed a very good colinearity in marker order for their common markers, except for only two (CH01h01, CH05g03) and three (CH05a02z, NZ02b01, Lap-1) markers on LG17 and LG15, respectively. This integrated consensus map provides a framework for performing quantitative trait locus (QTL) detection in a multi-population design and evaluating the genetic background effect on QTL expression.

Keywords *Malus* × *domestica* Borkh. · Integrated consensus genetic map · Female–male map · SSR marker

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Introduction

Several complete or partial genetic linkage maps of apple have already been published. They were used to study sex-related differences in recombination rates (Hemmat et al. 1994) to locate genes of interest like scab resistance major genes (Gardiner et al. 1996; Gianfranceschi et al. 1996; Maliepaard et al. 1998; Vinatzer et al. 2001; Xu and Korban 2002; Hemmat et al. 2003; Belfanti et al. 2004; Gygax et al. 2004; Bus et al. 2005) and quantitative trait loci (QTLs; Durel et al. 2003; Liebhard et al. 2003b; Calenge et al.

2004), rosy leaf curling aphid resistance gene (Roche et al. 1997), the self-incompatibility locus (Maliepaard et al. 1997), QTLs for growth and development (Conner et al. 1998; Kenis and Keulemans 2007; Segura et al. 2007), QTLs for fruit skin color (Cheng et al. 1996), fruit quality (King et al. 2000; Costa et al. 2005) and vitamin C content (Davey et al. 2006). Liebhard et al. (2003a) constructed a saturated map for the apple genome, which consisted of different types of markers (amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), simple sequence repeats (SSRs), SCAR). Silfverberg-Dilworth et al. (2006) constructed a map, which is currently used as reference map, by adding a new set of SSR markers to the previous linkage map. A new map including several major genes and summarizing the position of 247 SSR was also recently published by Fernandez-Fernandez et al. (2008).

Each of these maps was based on a single population. In particular, QTLs for scab resistance have been identified in the following three single apple progenies: Prima × Fiesta (Durel et al. 2003), Fiesta × Discovery (Liebhard et al. 2003b), and Discovery × TN10-8 (Calenge et al. 2004). Similar QTL mapping studies have been engaged in three other genetically related progenies (unpublished results) within the framework of the European Durable Apple Resistance in Europe (DARE) project (Lespinasse et al. 2000a). The map intervals of these QTLs are still wide. However, a more precise position is required for a further efficient marker-assisted selection. Integrated analysis of all progenies may narrow down the QTLs' confidence intervals. To carry out such an integrated analysis, one single integrated consensus map is required.

Integrated consensus maps have been constructed for many cultivated plant species like loblolly pine (Sewell et al. 1999), soybean (Cregan et al. 1999; Song et al. 2004), rapeseed (Lombard and Delourme 2001), wheat (Daryl et al. 2004), grapevine (Doligez et al. 2006), and lettuce (Truco et al. 2007). Composite maps derived from multiple populations provide several advantages. In particular, they increase marker density and genome coverage, and the order of common markers is more precise. Integrated consensus maps were used in comparative studies among related species in assigning linkage groups to chromosomes (Beavis and Grant 1991) and have provided evidence of chromosomal rearrangements between homologous linkage groups (Dirlewanger et al. 2004; Pelgas et al. 2006; Nicolas et al. 2007). Integrated consensus maps were also used for QTL detection in multiple populations (Symonds et al. 2005; Blanc et al. 2006).

So far, an integrated consensus map from different mapping populations has not been constructed for apple. However, such a map could potentially lead to more precise estimates of the effects and location of a common QTL and could be used to examine differences in QTL effects in different populations (Walling et al. 2000; Li et al. 2005), whereas single population-based QTL analyses do not provide direct comparisons of a QTL among different populations. In addition, pooled analysis on the basis of integrated consensus map is expected to increase the power of QTL detection (Rebai and Goffinet 1993; Walling et al. 2000; Blanc et al. 2006). The integration of mapping data from several crosses on a single integrated map would be useful to determine the relative positions of transferable markers. Finally, the merging together of a large number of markers belonging to different maps into a single high density map could facilitate the development of markers that are tightly linked to any QTL or major genes of interest.

Here, we report on the integration of the genetic maps from four separated, but genetically connected, populations. The aim was to get a single framework map in order to further perform integrated QTL analyses in the multi-population design.

Materials and methods

Mapping populations

Four F1 populations, with a total number of 676 individuals, were used to construct an integrated consensus map of the apple genome. F1 populations were considered for mapping since apple is an outbreeding plant species (Maliepaard et al. 1997). The first population (C1) was derived from the cross "Discovery × TN10-8" and consisted of 149 genotypes. The second population (C2) was derived from "Fiesta × Discovery", which consisted of 204 genotypes. The third population (C3) was derived from "Discovery × Prima", which consisted of 149 genotypes. The fourth population was derived from "Durello di Forli × Fiesta", which consisted of 174 genotypes. The C1, C2, C3, and C4 populations were obtained at INRA—Angers (France), PRI—Wageningen (The Netherlands), BAZ—Dresden (Germany), and UNIBO—Bologna (Italy), respectively. They belonged to a half-diallel design involving the five parents: Discovery, Durello di Forli, Fiesta, Prima, and TN10-8.

Available linkage mapping data

Marker data were generated and initially mapped by different partners during the European DARE project (Lespinasse et al. 2000a) for the four populations, except the C3 population for which most of the SSR markers were generated after DARE. The segregation datasets consisted predominantly of AFLP and SSR markers, but few

isozymes, RAPD and nucleotide binding sites-leucine-rich repeat (NBS-LRR) loci, were also available depending on the population.

AFLP reactions were performed in each population as described in Vos et al. (1995), with minor modifications according to the partners. For the C3 population, AFLP amplification was adapted to a dual-laser automated DNA sequencer (LI-COR 4200). A multiplexed PCR reaction with infrared-dye-labeled primers of two different wavelengths as described by Myburg et al. (2001) was performed using a total amplification volume of 10 µl. At least ten common AFLP primers (E32M48, E32M50, E32M58, E32M59, E33M47, E33M50, E34M57, E34M58, E34M60, and E35M47) defined by Keygene (http://www.keygene.com) were surveyed in each individual population. This common set was the series of ten primer pairs out of 37 that gave the best overall genome coverage and the least clustering in C2 population. Amplicon were visualized by the mean of radioactive labeling (C2 population), silver staining (C1 and C4 populations), or infrared-dye labeling (C3 population). AFLP markers were named according to primer name and amplicon size (e.g., E34M53-282), but when the amplicon size was not available, the number of the AFLP band on the gel was added to the primer name (e.g., E39M62-n16). Sizes were estimated by means of external ladders, depending on the partner: 100-bp ladder Sigma Aldrich™ for C1, 10-bp ladder SequaMark™ (Research Genetics Inc) for C2, and 100-bp ladder Invitrogen™ for C4. In some cases, notably in the C1 population, the *Eco*RI primer was extended by a fourth nucleotide in order to increase selective amplification (e.g., E32AM59-183, A being the additional nucleotide).

SSR reactions were performed as described in Liebhard et al. (2002), with minor modifications according to the partner. In the framework of the current survey, additional SSR markers were tested to increase the number of bridges between homologous linkage groups (LGs) of the four populations. Thus, all LGs from individual parental maps contained at least two common markers, allowing correct orientation of all LGs relative to the other homologous LGs.

Identification of *Malus* NBS-LRR resistance gene analogs (RGA) and their mapping in the C3 population were described by Thiermann (2002). Briefly, multiple combinations of degenerate primers designed from motifs in the NBS of group 1- (toll interleucin receptor-like region) and group 2- (coiled coil domain) type NBS-LRR genes (Leister et al. 1996; Pan et al. 2000) were used to amplify specific RGA sequences. About 40 NBS-LRR-RGAs were finally mapped in the C3 map by using the SSCP technique.

Isozymes were generated according to the protocol of Chevreau et al. (1999), and RAPD markers were generated according to Liebhard et al. (2003a).

Construction of the genetic maps for each individual population

Linkage maps were constructed over again using the software CarthaGene version 0.999R (De Givry et al. 2005; http://www.inra.fr/mia/T/CarthaGene/). CarthaGene permits the ordering of a large set of markers, derived from different populations, using a true multipoint maximum-likelihood criterion. First, framework individual parental maps were built independently within each cross using a double pseudo-testcross strategy (Grattapaglia and Sederoff 1994). AFLP markers that were not informative enough (scored on less than 50% of individuals within a single progeny, or <ab × ab> dominant segregation type) were discarded. When AFLP markers clustered exactly at the same position, only the one scored on the greatest number of individuals was maintained for analysis. In addition, because the goal was not to get an ultra-dense integrated consensus map but to generate a framework map for further integrated QTL detections, AFLP markers separated by less than 5 cM were also dropped. For each individual parental map, the phase was inferred by duplicating all markers, converting all duplicated markers into "mirror" markers (i.e., inverting the coding of all duplicated markers: "0" into "1" and "1" into "0"), building the linkage groups from all available markers (i.e., original and mirror markers), and finally choosing one of out the two mirror linkage groups for each pair of the 17 so-built linkage groups. This strategy is simply taking profit of recombination frequencies among all pairs of markers (original and inverted ones) to infer the phase of the significantly linked markers. Then, an integrated female–male map was constructed for each population using common SSR markers as "bridge markers".

For individual parental and integrated female–male maps, LGs were built with a minimum logarithm of odds (LOD) score of 3.0 and a maximum distance of 30 cM. Map distances were calculated using the Kosambi function (Kosambi 1944). As described by Doligez et al. (2006), a raw marker order within LG was first determined using a heuristic procedure that incrementally includes each marker by determining its insertion point as the one that yielded the highest loglikelihood ("build 5" command). This raw marker order was then improved using an optimization algorithm called "taboo search" with the "greedy 3 1 1 15" command. This "greedy" command tries to improve the loglikelihood of the best-known order for the markers using a dedicated search algorithm. Here, the search was repeated three times, with minimum and maximum length of the taboo list varying stochastically during the search from 1% to 15% of the neighborhood marker list (http://www.inra.fr/mia/T/CarthaGene/). Finally, local marker order was refined by testing all possible marker orders within a sliding

window of size 5, using the "flips 5 2 1" command. Once the two individual parental maps were built for a given population, the integrated female–male genetic map was constructed by using the "dsmergen" command of CarthaGene as described below.

We discarded loci showing inconsistent positions between parental and integrated female–male maps within each progeny, i.e., loci mapped in the middle of a LG in one parent but at the end of the LG in the female–male map (which was assumed to be due to genotyping errors). We also removed all loci mapping at the end of any LG (>10 cM far away from any other marker) and all loci that caused large increases in the distance between flanking loci.

Comparison of individual parental maps

A chi-square homogeneity test was performed to compare the recombination rates between individual parental maps. Analyses were performed only for Discovery and Fiesta that are of great interest in apple breeding programs and involved in three and two crosses, respectively. For the sake of simplicity, only pairs of adjacent SSR markers that were simultaneously common to the homologous LGs and linked at a LOD≥2 were analyzed. Three LGs (LG11, LG12, and LG15) randomly chosen were surveyed. Because of missing data, the number of individuals involved in each recombination rate was not constant within a mapping population. This could lead to discrepancies in chi-square-estimated values from one marker pair to another.

The colinearity of parental maps was evaluated on the basis of their common SSR and isozyme markers.

Construction of the integrated consensus genetic map

Before building the integrated consensus map, we aligned the integrated female–male maps to visualize "bridging" markers and unified their names since the nomenclature of common markers should be strictly identical from one population to another. The nomenclature of SSR markers were according to the HiDRAS SSR-data base (http://www.hidras.unimi.it). Next, we constructed an integrated consensus genetic map, merging all four progeny datasets, using the "dsmergen" command of CarthaGene. This merging method uses all available information in the loaded datasets and estimates a single recombination rate for each given marker pair based on all available meioses, irrespective of which crosses the genotypic data have been derived from. Firstly, independent maximum-likelihood multipoint parameter estimations are performed on each dataset. Then, an overall maximum-likelihood marker ordering is sought (De Givry et al. 2005). The "build 5", "greedy 1 0 1 20" and the "flips 5 2 1" commands were used for marker ordering within each LG. Graphical representations of the maps were drawn using the MapChart software (Voorrips 2002). For the integrated consensus map, positions of markers along the linkage groups were only given with an entire centimorgan unit since using decimals would not make sense considering the very small likelihood differences between the maximum-likelihood marker ordering represented here and the alternative marker orders giving slightly smaller likelihood values.

Table 1 Main features (number of markers, map length, and mean distance between adjacent loci) of the integrated female–male maps

	Number of markers	Map length (cM)	MD (cM)
C1	255	772	3.0
C2	423	888	2.1
C3	278	1,038	3.7
C4	334	833	2.5

MD Mean distance between adjacent loci

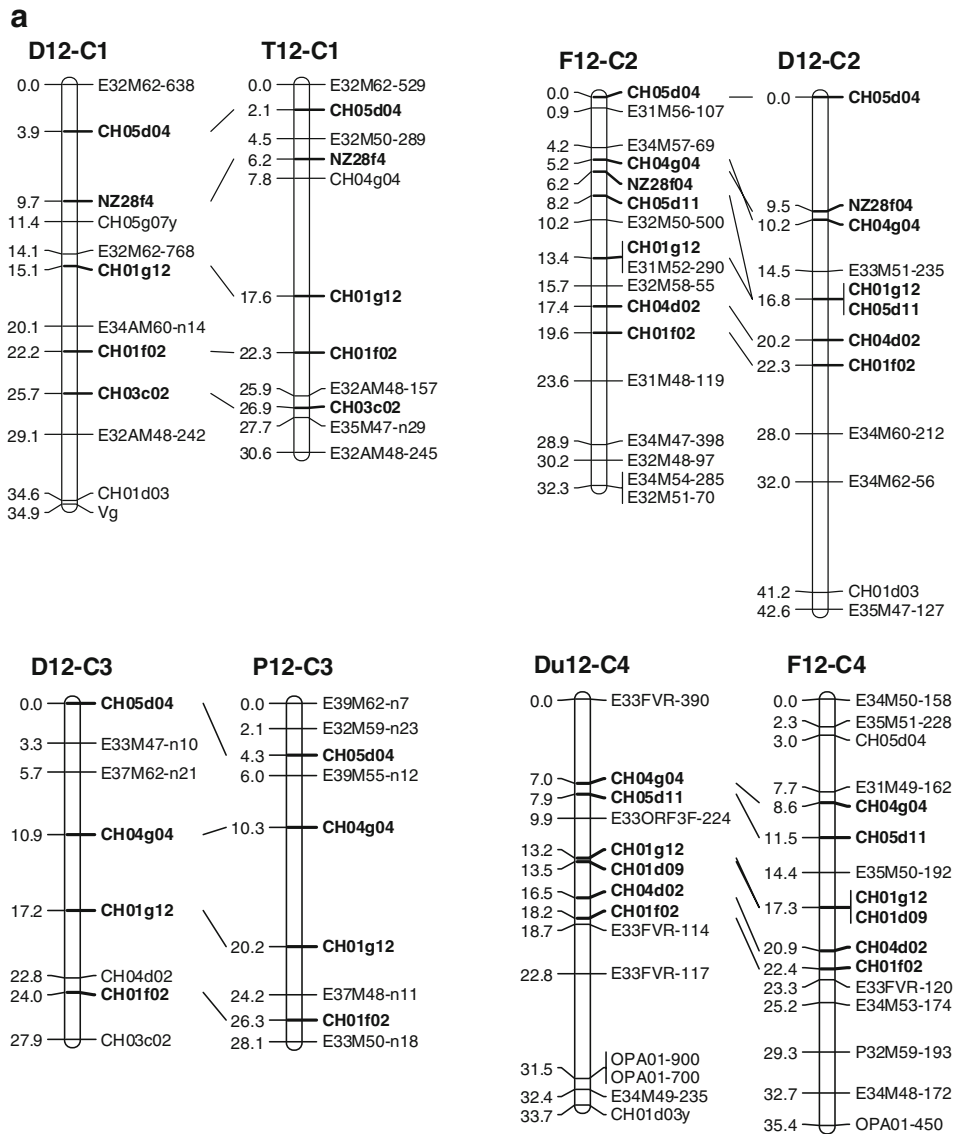
Marker distribution

Marker distribution along each LG of the integrated consensus map was evaluated by comparing the difference between the expected positions of markers under a uniform distribution and the observed ones with the critical D value of the Kolmogorov–Smirnov statistic (α=5%). The observed distribution of each LG was built as follows: let L_i and N_i be the length and the number of markers of LG_i, respectively; P_{ij} is the position (in cM) of marker j along LG_i; the observed cumulative distribution is made up of each j/N_i value. We also tested homogeneity of markers distribution over the linkage groups (with regard to their length) by a chi-square test.

Results and discussion

The main goal of this study was to construct an integrated consensus map of apple, combining segregation datasets from four mapping populations. Common SSR markers

Fig. 1 **a** Comparison of markers order of parental maps within each C population (*C1* Discovery × TN10-8, *C2* Fiesta × Discovery, *C3* Discovery × Prima, *C4* Durello di Forli × Fiesta; e.g., LG12). Markers positions (cM Kosambi) and names are written at the *left side* and *right side* of each LG, respectively. *Lines* between LGs connect their common markers (*bold character*) by their position. **b** Comparison of markers order among the three Discovery maps and the two Fiesta maps (e.g., LG12). Markers positions (cM Kosambi) and names are written at the *left side* and *right side* of each LG, respectively. *Lines* between LGs connect their common markers (*bold character*) by their position ▶


a
D12-C1
0.0 E32M62-638
3.9 CH05d04
9.7 NZ28f4
11.4 CH05g07y
14.1 E32M62-768
15.1 CH01g12
20.1 E34AM60-n14
22.2 CH01f02
25.7 CH03c02
29.1 E32AM48-242
34.6 CH01d03
34.9 Vg
T12-C1
0.0 E32M62-529
2.1 CH05d04
4.5 E32M50-289
6.2 NZ28f4
7.8 CH04g04
17.6 CH01g12
22.3 CH01f02
25.9 E32AM48-157
26.9 CH03c02
27.7 E35M47-n29
30.6 E32AM48-245
F12-C2
0.0 CH05d04
0.9 E31M56-107
4.2 E34M57-69
5.2 CH04g04
6.2 NZ28f04
8.2 CH05d11
10.2 E32M50-500
13.4 CH01g12
E31M52-290
15.7 E32M58-55
17.4 CH04d02
19.6 CH01f02
23.6 E31M48-119
28.9 E34M47-398
30.2 E32M48-97
32.3 E34M54-285
E32M51-70
D12-C2
0.0 CH05d04
9.5 NZ28f04
10.2 CH04g04
14.5 E33M51-235
16.8 CH01g12
CH05d11
20.2 CH04d02
22.3 CH01f02
28.0 E34M60-212
32.0 E34M62-56
41.2 CH01d03
42.6 E35M47-127
D12-C3
0.0 CH05d04
3.3 E33M47-n10
5.7 E37M62-n21
10.9 CH04g04
17.2 CH01g12
22.8 CH04d02
24.0 CH01f02
27.9 CH03c02
P12-C3
0.0 E39M62-n7
2.1 E32M59-n23
4.3 CH05d04
6.0 E39M55-n12
10.3 CH04g04
20.2 CH01g12
24.2 E37M48-n11
26.3 CH01f02
28.1 E33M50-n18
Du12-C4
0.0 E33FVR-390
7.0 CH04g04
7.9 CH05d11
9.9 E33ORF3F-224
13.2 CH01g12
13.5 CH01d09
16.5 CH04d02
18.2 CH01f02
18.7 E33FVR-114
22.8 E33FVR-117
31.5 OPA01-900
OPA01-700
32.4 E34M49-235
33.7 CH01d03y
F12-C4
0.0 E34M50-158
2.3 E35M51-228
3.0 CH05d04
7.7 E31M49-162
8.6 CH04g04
11.5 CH05d11
14.4 E35M50-192
17.3 CH01g12
CH01d09
20.9 CH04d02
22.4 CH01f02
23.3 E33FVR-120
25.2 E34M53-174
29.3 P32M59-193
32.7 E34M48-172
35.4 OPA01-450
b
D12-C1
0.0 E32M62-638
3.9 CH05d04
9.7 NZ28f4
11.4 CH05g07y
14.1 E32M62-768
15.1 CH01g12
20.1 E34AM60-n14
22.2 CH01f02
25.7 CH03c02
29.1 E32AM48-242
34.6 CH01d03
34.9 Vg
D12-C2
0.0 CH05d04
9.5 NZ28f04
10.2 CH04g04
14.5 E33M51-235
16.8 CH01g12
CH05d11
20.2 CH04d02
22.3 CH01f02
28.0 E34M60-212
32.0 E34M62-56
41.2 CH01d03
42.6 E35M47-127
D12-C3
0.0 CH05d04
3.3 E33M47-n10
5.7 E37M62-n21
10.9 CH04g04
17.2 CH01g12
22.8 CH04d02
24.0 CH01f02
27.9 CH03c02
F12-C2
0.0 CH05d04
0.9 E31M56-107
4.2 E34M57-69
5.2 CH04g04
6.2 NZ28f04
8.2 CH05d11
10.2 E32M50-500
13.4 CH01g12
E31M52-290
15.7 E32M58-55
17.4 CH04d02
19.6 CH01f02
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30.2 E32M48-97
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E32M51-70
F12-C4
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8.6 CH04g04
11.5 CH05d11
14.4 E35M50-192
17.3 CH01g12
CH01d09
20.9 CH04d02
22.4 CH01f02
23.3 E33FVR-120
25.2 E34M53-174
29.3 P32M59-193
32.7 E34M48-172
35.4 OPA01-450

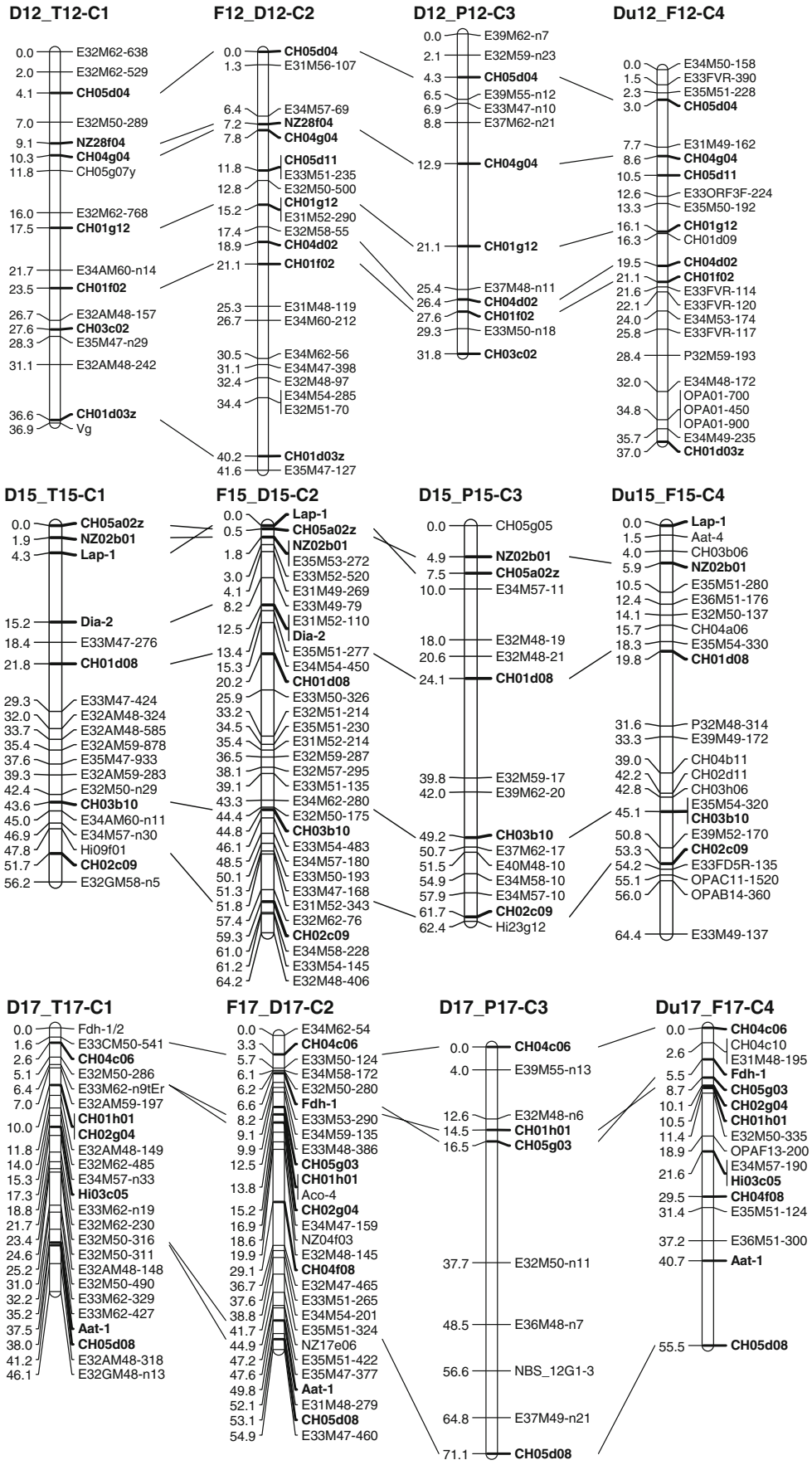

Fig. 2 Alignment of integrated female–male maps of the four crosses (*C1* Discovery × TN10-8, *C2* Fiesta × Discovery, *C3* Discovery × Prima, *C4* Durello di Forli × Fiesta) for LG12, LG15, and LG17. Markers positions (cM Kosambi) and names are written at the *left side* and *right side* of each LG, respectively. Markers in common to at least two LGs are written in *bold character*. *Lines* between adjacent LGs connect their common markers by their position

Table 2 Recombination rates between locus pairs present simultaneously in the 3 populations where Discovery is involved

LGs	Locus pairs	Discovery (C1, *N*=149)	Discovery (C2, *N*=204)	Discovery (C3, *N*=149)	Chi square (*df*=2)	*p*
LG11	CH02d08–CH04a12	0.07	0.08	0.08	0.39	0.82
LG12	CH05d04–CH01g12	0.20	0.26	0.24	0.74	0.69
	CH01g12–CH01f02	0.13	0.09	0.11	2.65	0.27
LG15	CH01d08–CH03b10	0.27	0.37	0.30	0.22	0.89
	CH03b10–CH02c09	0.12	0.21	0.18	1.19	0.55

between homologous LGs were used as bridges to merge the four populations' datasets.

Comparison of parental and integrated female–male maps

The individual parental maps consisted of different types of markers (AFLP, isozymes, NBS-LRR-RGA, SSR, and RAPD). They were enriched with additional SSR markers in order to generate more bridges between homologous LGs and to flank genomic regions where scab resistance QTLs (Durel et al. 2003; Liebhard et al. 2003a, b; Calenge et al. 2004) had previously been detected. Because of the unexpectedly low number of common AFLP markers, they were not used as bridges in the integration. Adding a few such markers was thought to give little additional value to the quality of the map, whereas they would obscure evaluations on the use of the much more balanced SSRs. Therefore, common AFLP markers were entered under their initial names as given by each partner on the basis of their single population. As AFLPs were tested on different platforms, and sizes were assessed in different ways, initial names of actually common markers were different due to slight differences in size estimates. In addition, sequence data are needed to prove that AFLP markers of the same size represent the same locus (Black 1993; Waugh et al. 1997; Cervera et al. 2001). However, when AFLP markers with similar size come from the same parent and map at the same position, these AFLP markers could reasonably be considered as identical.

For each population, an integrated female–male map was built. The main features (number of markers, map length, and mean distance between adjacent loci (MD)) of these maps are given in Table 1. The C2 population generated the denser integrated female–male map with a MD close to 2 cM. The C3 population generated the longer map distance: more than 1,080 cM, whereas the three other integrated female–male map distances ranged between 770 and 890 cM.

Common SSR and isozyme markers among homologous LGs allowed for a comparison of marker order between parental maps and among the four mapping populations. For each LG (e.g., LG12), a strong colinearity was observed within mapping populations (Fig. 1a). Similarly, marker order was conserved in both Discovery and Fiesta maps (Fig. 1b). A total of 90 markers were common to at least two populations of which 53% (48) were common to the four mapping populations. Marker order was well conserved between integrated female–male maps, except minor changes for four groups of closely linked markers (CH01g05, CH04f06), (Lap-1, CH05a02z, NZ02b01), (CH04c06, Fdh-1), and (CH01h01, CH02g04, CH05g03) on LG14, LG15, and LG17 (twice), respectively. Figure 2 gives examples of the integrated female–male maps alignment for LG12, LG15, and LG17. The well-conserved marker order suggests no evidence of major chromosomal rearrangements in the parental cultivars. The local inconsistency in marker order can be attributed to the tight linkage of the questioned markers pairs, scoring mistakes,

Table 3 Recombination rates between locus pairs present simultaneously in the 2 populations where Fiesta is involved

LGs	Locus pairs	Fiesta (C2, *N*=204)	Fiesta (C4, *N*=149)	Chi square (*df*=1)	*p*
LG11	CH04h02–CH02d08	0.12	0.18	3.82	0.05
	CH02d08–CH04a12	0.09	0.08	0.16	0.69
LG12	CH05d04–CH04g04	0.15	0.11	0.001	0.99
	CH04g04–CH05d11	0.05	0.04	0.002	0.89
	CH05d11–CH01g12	0.05	0.09	2.80	0.09
	CH01g12–CH04d02	0.08	0.07	0.12	0.73
	CH04d02–CH01f02	0.04	0.04	0.20	0.66
LG15	CH05a02z–CH01d08	0.31	NA	–	–
	CH01d08–CH03b10	0.37	0.34	0.94	0.33
	CH03b10–CH02c09	0.21	0.15	0.004	0.95

NA Not available (recombination rate close to 0.5)

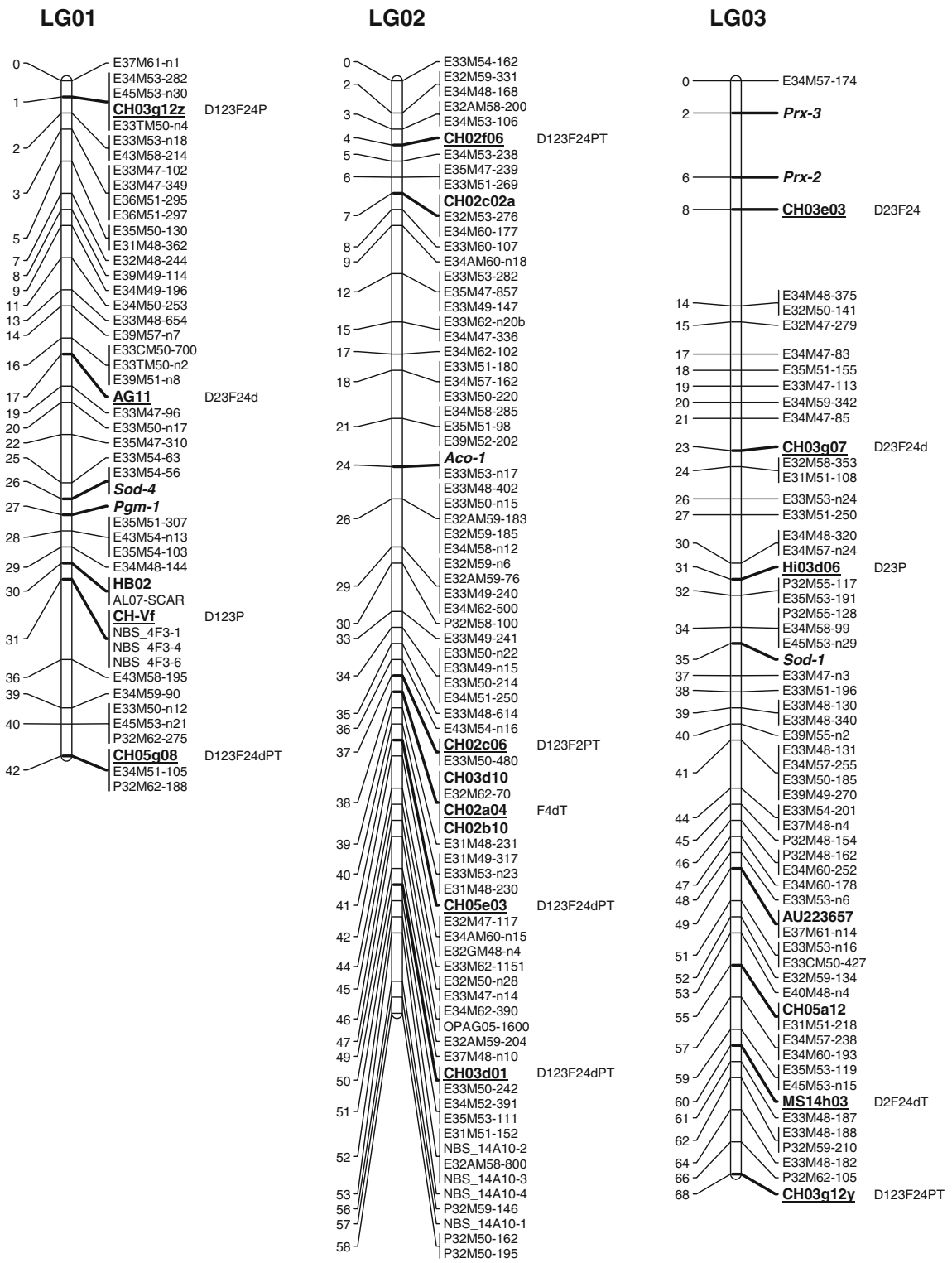

Fig. 3 Integrated consensus genetic map derived from four different apple populations, Discovery × TN10-8 (C1), Fiesta × Discovery (C2), Discovery × Prima (C3), and Durello di Forli × Fiesta (C4). Markers positions (cM Kosambi) and names are written at the *left side* and *right side* of each LG, respectively. SSR markers are written in *bold*; isozymes are in *bold and italic*. Markers used as "bridges" between populations are *underlined* and their parental origin, Discovery (*D*), Fiesta (*F*), Durello di Forli (*d*), Prima (*P*), or TN10-8 (*T*) is indicated. Since Discovery is involved in three populations (C1, C2, and C3) and Fiesta in two populations (C2 and C4), *D* and *F* are followed by a number indicating the populations the marker is derived from (e.g., CH03g12z D123F24P means that CH03g12z was mapped on Discovery (population C1, C2 and C3), on Fiesta (population C2 and C4), and on Prima

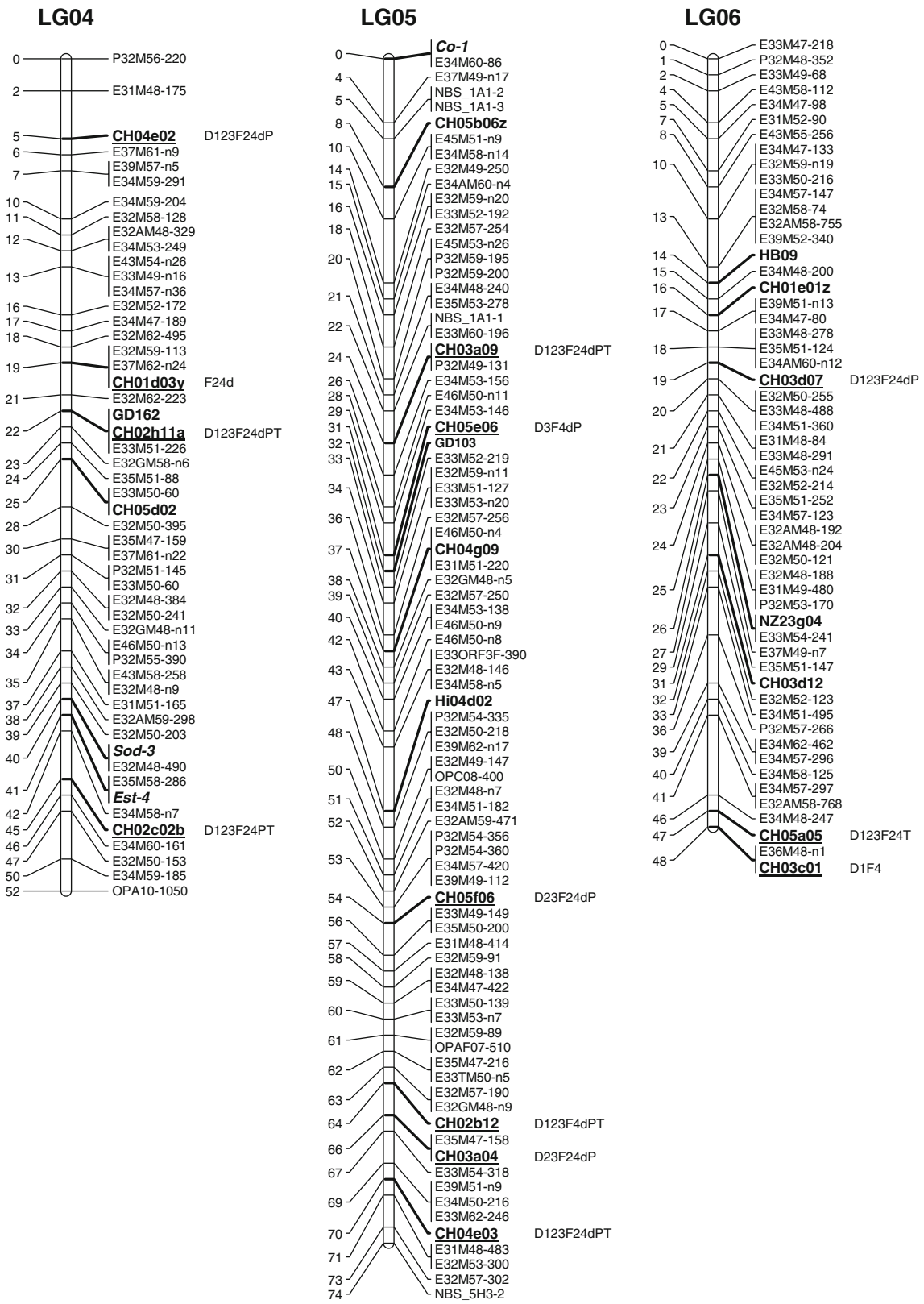

Fig. 3 (continued)

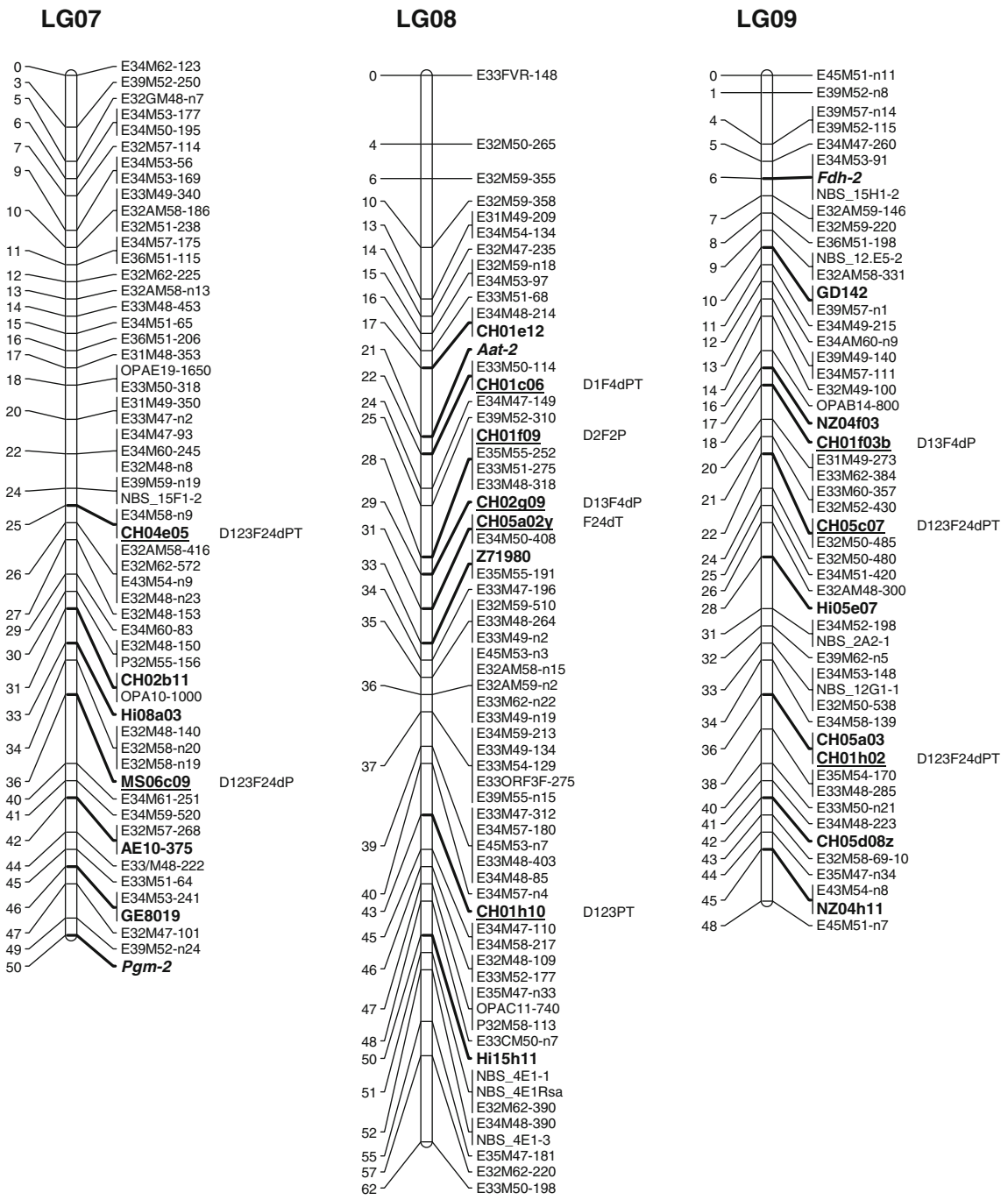

Fig. 3 (continued)

missing data, and differences in segregation information among markers and populations (Maliepaard et al. 1997) rather than an effective chromosomal translocation. Marker orders can also change in the integrated female–male map if a large difference in recombination rate is observed between parental maps. Silfverberg-Dilworth et al (2006) also reported some cases of inversion in marker order when they were very close together. Small sizes of populations can lead to sample bias giving incongruence of markers position as observed in wheat (Daryl et al. 2004) and grapevine (Doligez et al. 2006). The sizes of our populations were larger, thus reducing the risk of such incongruence.

Orthologous SSR markers also permitted a comparison of meiotic recombination rates between the maps of the two parents: Discovery and Fiesta. Because of the great number of all possible 2×2 statistical combinations among this set of markers, the pairwise tests were only performed on three LGs. For the subset of SSR locus pairs analyzed, no significant (α=5%) difference was observed between

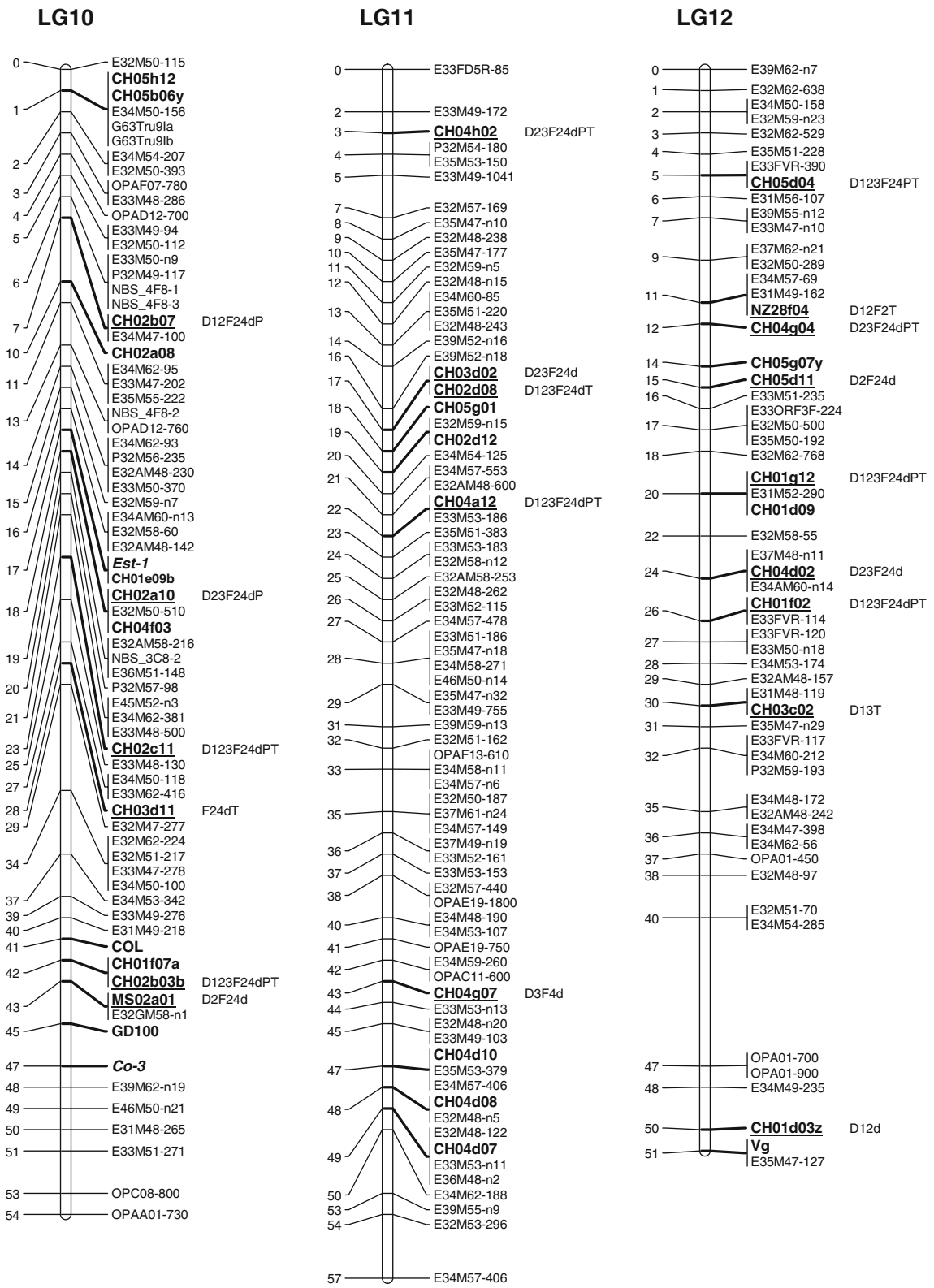

Fig. 3 (continued)

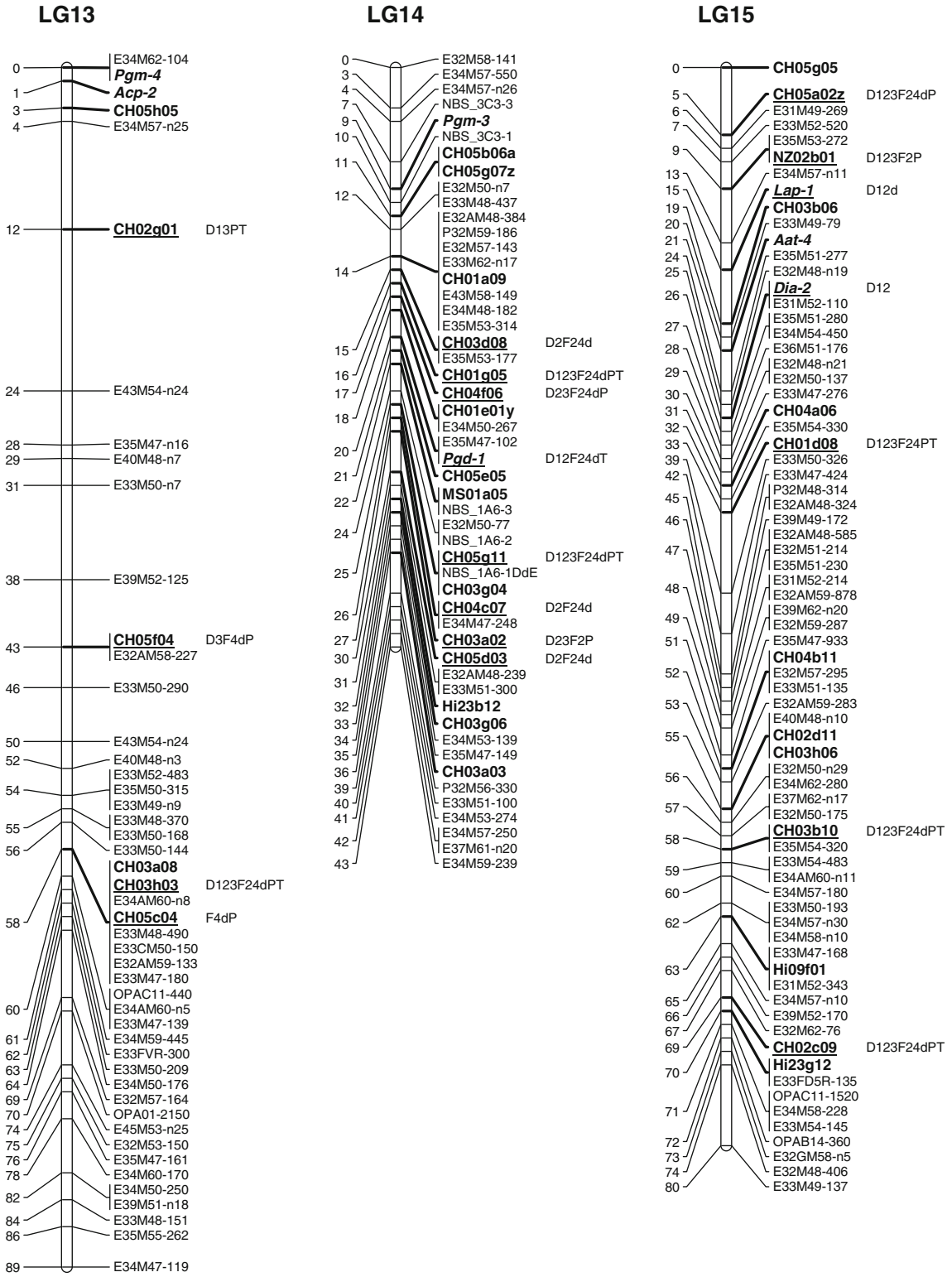

Fig. 3 (continued)

Discovery maps (Table 2). Conversely, the recombination rate of two locus pairs (CH04h02–CH02d08, CH05a02z–CH01d08) differed between the Fiesta maps of the C2 and C4 populations (Table 3). The recombination rate of the latter locus pair was 0.31 in the C2-Fiesta map, whereas it was not estimable for the C4-Fiesta map, suggesting a value greater than 0.5. Given the very limited number of locus pairs tested, it seems reasonable to think that more cases of

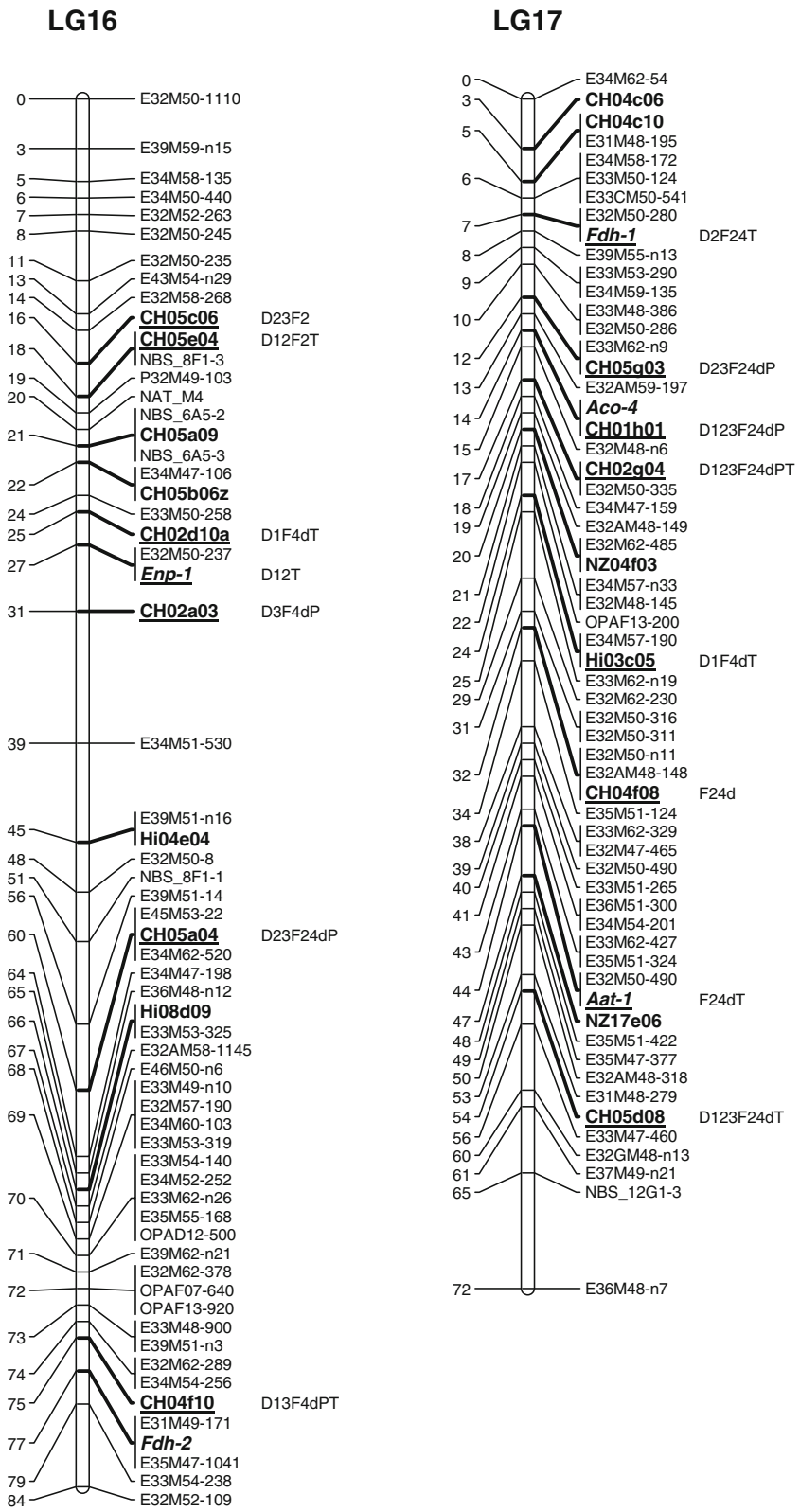

Fig. 3 (continued)

differences in recombination rates could appear with a greater number of analyzed markers. Heterogeneous recombination rates were also reported in many plant species: maize (Fatmi et al. 1993; Causse et al. 1996), pine (Sewell et al. 1999), sunflower (Gentzbittel et al. 1999), fescue (Saha et al. 2005), grapevine (Doligez et al. 2006), and brassica (Nicolas et al. 2007). They have been attributed to diverse environmental and genetic factors. Singularly, differences in recombination rates were partly explained by selection among pollen grains at the haploid level (Lenormand and Dutheil 2005).

The distribution of markers was not compared between individual parental maps because we previously "arbitrary" discarded markers of low informativeness (scored on less than 50% of individuals, <ab × ab> segregation type, clustering at exactly the same position or separated by less than 5 cM) within each individual population. No evidence of marked clustering tendency in a particular parental map was observed. These markers were discarded because they would not bring any additional information in the further QTL analysis. Furthermore, this strategy allowed the optimization of computing time.

Features and usefulness of the integrated consensus map

We constructed the integrated consensus map using CarthaGene® software (De Givry et al. 2005). The integrated consensus map consisted of 1,046 markers, of which 159 are SSR markers, distributed on 17 LGs equivalent to the haploid chromosome number of the apple genome (Fig. 3). Table 4 gives the main characteristics of the integrated consensus map. The density of markers on the map ranged from 0.7 marker per centimorgan on LG02 to 1.4 marker per centimorgan on LG16. The total length of the integrated consensus map was 1,032 cM, with a mean distance between adjacent loci of 1.1 cM. This high marker density should not hide the fact that it is a relative value since AFLP markers come from different populations and were positioned by comparing ones to the others on the basis of the common SSR markers. However, an advantage of such a marker density could be narrowing down the confidence interval of further putative QTLs detected by the mean of integrated analysis.

Markers were proportionally distributed over the 17 linkage groups (χ^2=16.53, df=16, p=0.41) with regard to their length, suggesting a balanced distribution. However, a non-uniform marker distribution was observed within all of the LGs. This could be due to non-random sampling of the genome by the primers used, by uneven distribution of recombination along the LGs (Tanksley et al. 1992), or by clustering of some markers (Radhika et al. 2007). Clustering of markers at the same position (within a 1-cM window) was observed throughout LGs and consisted predominantly of only two to three linked markers. Of the 159 mapped SSR markers, 90 were common to at least two populations simultaneously, generating a great amount of anchorage points between populations.

The validity of the construction of integrated consensus map from individual populations where a difference occurs in recombination frequency was questioned by Beavis and Grant (1991). However, if the marker order between individual maps and the integrated consensus map is

Table 4 Main characteristics of the integrated consensus map

Linkage groups	Number of total markers	Number of SSR markers	Map length (cM)	Markers density (cM/markers)
LG01	48	4	42	0.9
LG02	79	8	58	0.7
LG03	61	7	68	1.1
LG04	52	7	52	1.0
LG05	83	11	74	0.9
LG06	55	7	48	0.9
LG07	55	5	50	0.9
LG08	64	8	62	1.0
LG09	52	10	48	0.9
LG10	71	14	54	0.8
LG11	75	10	57	0.8
LG12	57	12	51	0.9
LG13	48	6	89	1.9
LG14	51	17	43	0.8
LG15	72	13	80	1.1
LG16	62	10	84	1.4
LG17	61	10	72	1.2
Total	1,046	159	1,032	1.1

conserved, the composite map should remain valuable (Lespinasse et al. 2000b). In our study, marker order was well conserved between the integrated consensus map and the integrated female–male maps of the four populations. Similar results were obtained by Doligez et al. (2006) for the integrated map of grapevine. "Small" inconsistencies of marker order can appear when differences in recombination rates are observed among populations (Beavis and Grant 1991; Lombard and Delourme 2001; Loridon et al. 2005). However, the composite map is still a valuable tool for selecting markers and comparing the relative location of traits in different genetic backgrounds.

In conclusion, the map constructed in this study is the first integrated consensus map for apple merging together individual maps from independent experiments. Singularly, AFLP markers belonging to different maps were brought together into a single map. This map will be a reliable tool to perform QTL detection in a multi-population design and evaluate the genetic background effect on QTL expression. It will first be devoted to scab and powdery mildew resistance QTL detection as phenotypic (resistance) data are available for each of the four populations tested in common environments. The construction of the integrated consensus map on the basis of four distinct genetic maps made it possible to determine the relative positions of transferable markers and to get a statistically based estimation on the order of the non-common markers.

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References

Beavis WD, Grant D (1991) A linkage map based on information from four F2 populations of maize (*Zea mays* L.). Theor Appl Genet 82(5):636–644

Belfanti E, Silfverberg-Dilworth E, Tartarini S, Patocchi A, Barbieri M, Zhu J, Vinatzer BA, Gianfranceschi L, Gessler C, Sansavini S (2004) The *HcrVf2* gene from a wild apple confers scab resistance to a transgenic cultivated variety. Proc Natl Acad Sci U S A 101(3):886–890

Black WC (1993) PCR with arbitrary primers: approach with care. Insect Mol Biol 2(1):1–6

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet 113(2):206–224

Bus VGM, Rikkerink EHA, Van de Weg WE, Rusholme RL, Gardiner SE, Bassett HCM, Kodde LP, Parisi L, Laurens FND, Meulenbroek EJ, Plummer KM (2005) The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. Mol Breed 15(1):103–116

Calenge F, Faure A, Goerre M, Gebhardt C, Van de Weg WE, Parisi L, Durel CE (2004) Quantitative trait loci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in apple progeny challenged with eight isolates of *Venturia inaequalis*. Phytopathology 94:370–379

Causse M, Sansavini S, Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39:418–432

Cervera MT, Storme V, Ivens B, Gusmao J, Liu BH, Hostyn V, Van Slycken J, Van Montagu M, Boerjan W (2001) Dense genetic linkage maps of three *Populus* species (*Populus deltoides*, *P. nigra* and *P. trichocarpa*) based on AFLP and microsatellite markers. Genetics 158(2):787–809

Cheng FS, Weeden NF, Brown SK (1996) Identification of co-dominant RAPD markers tightly linked to fruit skin color in apple. Theor Appl Genet 93(1):222–227

Chevreau E, Manganaris AG, Gallet M (1999) Isozyme segregation in five apple progenies and potential use for map construction. Theor Appl Genet 98(2):329–336

Conner PJ, Brown SK, Weeden NF (1998) Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. Theor Appl Genet 96(8):1027–1035

Costa F, Stella S, Sansavini S, Van de Weg WE (2005) Functional markers as genetic approach to study ethylene production and fruit softening in apple (*Malus domestica* Borkh.). Acta Hort (ISHS) 682:389–394

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39(5):1464–1490

Daryl JS, Peter I, Keith E (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109(6):1105–1114

Davey MW, Kenis K, Keulemans J (2006) Genetic control of fruit vitamin C contents. Plant Physiol 142(1):343–351

de Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CARTHAGENE: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics 21(8):1703–1704

Dirlewanger E, Graziano E, Joobeur T, Garriga-Caldere F, Cosson P, Howad W, Arus P (2004) Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc Natl Acad Sci U S A 101(26):9891–9896

Doligez A, Adam-Blondon AF, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five mapping populations. Theor Appl Genet 113(3):369–382

Durel CE, Parisi L, Laurens F, Van de Weg WE, Liebhard R, Jourjon MF (2003) Genetic dissection of partial resistance to race 6 of *Venturia inaequalis* in apple. Genome 46(2):224–234

Fatmi A, Poneleit CG, Pfeiffer TW (1993) Variability of recombination frequencies in the Iowa Stiff Stalk Synthetic (*Zea mays* L.). Theor Appl Genet 86(7):859–866

Fernandez-Fernandez F, Evans KM, Clarke JB, Govan CL, James CM, Maric S, Tobutt KR (2008) Development of an STS map of an interspecific progeny of *Malus*. Tree Genet Gen. DOI 10.1007/s11295-007-0124-y

Gardiner SE, Bassett HCM, Noiton DAM, Bus VG, Hofstee ME, White AG, Ball RD, Forster RLS, Rikkerink EHA (1996) A detailed linkage map around an apple scab resistance gene

demonstrates that two disease resistance classes both carry the *Vf* gene. Theor Appl Genet 93(4):485–493

Gentzbittel L, Mestries E, Mouzeyar S, Mazeyrat F, Badaoui S, Vear F, Tourvieille de Labrouhe D, Nicolas P (1999) A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. Theor Appl Genet 99(1):218–234

Gianfranceschi L, Koller B, Seglias N, Kellerhals M, Gessler C (1996) Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theor Appl Genet 93(1):199–204

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137(4):1121–1137

Gygax M, Gianfranceschi L, Liebhard R, Kellerhals M, Gessler C, Patocchi A (2004) Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata* jackii. Theor Appl Genet 109(8):1702–1709

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85(1):4–11

Hemmat M, Brown SK, Aldwinckle HS, Mehlenbacher SA, Weeden NF (2003) Identification and mapping of markers for resistance to apple scab from 'Antonovka' and 'Hansen's baccata #2'¢. Acta Hort 622:153–161

Kenis K, Keulemans J (2007) Study of tree architecture of apple (*Malus domestica* Borkh.) by QTL analysis of growth traits. Molecular Breeding 19(3):193–208

King GJ, Maliepaard C, Lynn JR, Alston FH, Durel CE, Evans KM, Griffon B, Laurens F, Manganaris AG, Schrevens E, Tartarini S, Verhaegh J (2000) Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill.). Theor Appl Genet 100(7):1074–1084

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eug 12:172–175

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14(4):421–429

Lenormand T, Dutheil J (2005) Recombination difference between sexes: a role for haploid selection. PLoS Biology 3(3):e63

Lespinasse Y, Durel CE, Parisi L, Laurens F, Chevalier M, Pinet C (2000a) A European project D.A.R.E.—Durable resistance of apple to scab and powdery-mildew. Acta Hort 538:197–200

Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M (2000b) A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100(1):127–138

Li R, Lyons MA, Wittenburg H, Paigen B, Churchill GA (2005) Combining data from multiple inbred line crosses improves the power and resolution of quantitative trait loci mapping. Genetics 169(3):1699–1709

Liebhard R, Gianfranceschi L, Koller B, Ryder CD, Tarchini R, Van De Weg WE, Gessler C (2002) Development and characterisation of 140 new microsatellites in apple (*Malus domestica* Borkh.). Molecular Breeding 10(4):217–241

Liebhard R, Koller B, Gianfranceschi L, Gessler C (2003a) Creating a saturated reference map for the apple (*Malus domestica* Borkh.) genome. Theor Appl Genet 106(8):1497–1508

Liebhard R, Koller B, Patocchi A, Kellerhals M, Pfammatter W, Jermini M, Gessler C (2003b) Mapping quantitative field resistance against apple scab in a 'Fiesta' 'Discovery' progeny. Phytopathology 93:493–501

Lombard V, Delourme R (2001) A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. Theor Appl Genet 103:491–507

Loridon K, McPhee K, Morin J, Dubreuil P, Pilet-Nayel M, Aubert G, Rameau C, Baranger A, Coyne C, Lejeune-Hènaut I, Burstin J (2005) Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor Appl Genet 111(6):1022–1031

Maliepaard C, Jansen J, Van Ooijen JW (1997) Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genet Res 70:237–250

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.) using multi-allelic markers. Theor Appl Genet 97:60–73

Myburg AA, Remington DM, O'Malley DM, Sederoff RR, Whetten RW (2001) High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. BioTechniques 30(2):348–357

Nicolas SD, Mignon GL, Eber F, Coriton O, Monod H, Clouet V, Huteau V, Lostanlen A, Delourme R, Chalhoub B, Ryder CD, Chevre AM, Jenczewski E (2007) Homologous recombination plays a major role in chromosome rearrangements that occur during meiosis of *Brassica napus* Haploids. Genetics 175 (2):487–503

Pan Q, Liu Y-S, Budai-Hadrian O, Sela M, Carmel-Goren L, Zamir D, Fluhr R (2000) Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and *Arabidopsis*. Genetics 155(1):309–322

Pelgas B, Beauseigle S, Acheré V, Jeandroz S, Bousquet J, Isabel N (2006) Comparative genome mapping among *Picea glauca*, *P. mariana* × *P. rubens* and *P. abies*, and correspondence with other Pinaceae. Theor Appl Genet 113(8):1371–1393

Radhika P, Gowda S, Kadoo N, Mhase L, Jamadagni B, Sainani M, Chandra S, Gupta V (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. Theor Appl Genet 115 (2):209–216

Rebai A, Goffinet B (1993) Power of tests for QTL detection using replicated progenies derived from a diallel cross. Theor Appl Genet 86:1014–1022

Roche P, Alston FH, Maliepaard C, Evans KM, Vrielink R, Dunemann F, Markussen T, Tartarini S, Brown LM, Ryder C, King GJ (1997) RFLP and RAPD markers linked to the rosy leaf curling aphid resistance gene (*Sd1*) in apple. Theor Appl Genet 94(3):528–533

Saha MC, Mian R, Zwonitzer JC, Chekhovskiy K, Hopkins AA (2005) An SSR- and AFLP-based genetic linkage map of tall fescue (*Festuca arundinacea* Schreb.). Theor Appl Genet 110(2):323–336

Segura V, Denance C, Durel CE, Costes E (2007) Wide range QTL analysis for complex architectural traits in a 1-year-old apple progeny. Genome 50:159–171

Sewell MM, Sherman BK, Neale DB (1999) A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151(1):321–330

Silfverberg-Dilworth E, Matasci C, Van de Weg WE, Van Kaauwen M, Walser M, Kodde L, Soglio V, Gianfranceschi L, Durel CE, Costa F, Yamamoto T, Koller B, Gessler C, Patocchi A (2006) Microsatellite markers spanning the apple (*Malus domestica* Borkh.) genome. Tree Genetics & Genomes 2(4):202–224

Song Q, Marek L, Shoemaker R, Lark K, Concibido V, Delannay X, Specht J, Cregan P (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109(1):122–128

Symonds VV, Godoy AV, Alconada T, Botto JF, Juenger TE, Casal JJ, Lloyd AM (2005) Mapping quantitative trait loci in multiple populations of *Arabidopsis thaliana* identifies natural allelic variation for trichome density. Genetics 169(3):1649–1658

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB et al (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132(4):1141–1160

Thiermann M (2002) Molekulare charakterisierung dauerhafter, polygen vererbter resistenzquellen für apfelschorf und apfelmehltau, Dissertation Univ. Bremen.

Truco M, Antonise R, Lavelle D, Ochoa O, Kozik A, Witsenboer H, Fort S, Jeuken M, Kesseli R, Lindhout P, Michelmore R, Peleman J (2007) A high-density, integrated genetic linkage map of lettuce (*Lactuca* spp.). Theor Appl Genet 115(6):735–746

Vinatzer BA, Patocchi A, Gianfranceschi L, Tartarini S, Zhang HB, Gessler C, Sansavini S (2001) Apple contains receptor-like genes homologous to the *Cladosporium fulvum* resistance gene family of tomato with a cluster of genes cosegragating with *Vf* apple scab resistance. Mol Plant-Microbe Interactions 14:508–514

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78

Vos P, Hogers R, Bleeker M, Reijans M, Lee Tvd, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23(21):4407–4414

Walling GA, Visscher PM, Andersson L, Rothschild MF, Wang L, Moser G, Groenen MAM, Bidanel J-P, Cepica S, Archibald AL, Geldermann H, de Koning DJ, Milan D, Haley CS (2000) Combined analyses of data from quantitative trait loci mapping studies: chromosome 4 effects on porcine growth and fatness. Genetics 155(3):1369–1378

Waugh R, Bonar N, Baird E, Thomas B, Graner A, Hayes P, Powell W (1997) Homology of AFLP products in three mapping populations of barley. Mol Gen Genet MGG 255(3):311–321

Xu M, Korban SS (2002) A cluster of four receptor-like genes resides in the Vf locus that confers resistance to apple scab disease. Genetics 162(4):1995–2006