Full Paper

Integrated high-density consensus genetic map of *Pyrus* and anchoring of the ‘Bartlett’ v1.0 (*Pyrus communis*) genome

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Abstract

Genetic maps are essential tools for pear genetics and genomics research. In this study, we first constructed an integrated simple sequence repeat (SSR) and single nucleotide polymorphism (SNP)-based consensus genetic map for pear based on common SSR markers between nine published maps. A total of 5,085 markers, including 1,232 SSRs and 3,853 SNPs, were localized on a consensus map spanning 3,266.0 cM in total, with an average marker interval of 0.64 cM, which represents the highest density consensus map of pear to date. Using three sets of high-density SNP-based genetic maps with European pear genetic backgrounds, we anchored a total of 291.5 Mb of the ‘Bartlett’ v1.0 (*Pyrus communis* L.) genome scaffolds into 17 pseudo-chromosomes. This accounted for 50.5% of the genome assembly, which was a great improvement on the 29.7% achieved originally. Intra-genome and inter-genome synteny analyses of the new ‘Bartlett’ v1.1 genome assembly with the Asian pear ‘Dangshansuli’ (*Pyrus bretschneideri* Rehd.) and apple (*Malus × domestica* Borkh.) genomes uncovered four new segmental duplication regions. The integrated high-density SSR and SNP-based consensus genetic map provided new insights into the genetic structure patterns of pear and assisted in the genome assembly of ‘Bartlett’ through further exploration of different pear genetic maps.

Key words: pear, genetic map, genome assembly, SNP, SSR

1. Introduction

Pear (*Pyrus* spp.) is an important and popular rosaceous fruit crop cultivated in temperate regions for its economic and nutritional value. In 2014, the world production of pear was 25.8 million tonnes, from a harvest area of 1.6 million ha (http://faostat3.fao.org, 31 December 2016, date last accessed), making it the second most important fruit crop in the Maloideae, after apple. Improving pear germplasm is necessary for fruit breeders to meet consumer preferences and adapt to variable cultivation conditions. Increasingly, traditional breeding approaches are complemented by molecular breeding to take advantage of its efficiency in seedling selection and breeding for complex traits¹ using genomic
selection. Essential molecular breeding tools include molecular markers, genetic maps, and whole genome sequences. These tools have many applications in pear, including genetic diversity analyses and quantitave trait loci (QTL) mapping for production traits and fruit quality.  

Prototypes of pear genetic maps used marker techniques, such as random amplified polymorphic DNA (RAPD),13 amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR).14–17 Among different types of markers, SSR markers have been widely used in constructing pear genetic maps because of their co-dominant and transferable nature,18 thus can be used as common markers to compare and construct consensus maps. From 2007, various pear genetic maps were constructed based on SSR markers.16,17,19–21 However, the map with the highest density19 consisted of only 734 markers and covered 1,661.4 cM, which makes it difficult to facilitate map-based gene cloning. The construction of these maps were limited to the available molecular markers, either developed from pear expressed sequence tags (ESTs) or transferred from related species, both of which are labour- and time-consuming.

When the cost of next-generation sequencing (NGS) decreased, pear whole genome sequences were released,22 and single nucleotide polymorphism (SNP) markers were applied rapidly for genetic map construction.10,20,23,24 The first SNP-based genetic map had 857 markers,23 but subsequently a ‘Bayuehong (BYH)’ × ‘Dangshansuli’ map with 3,143 SNP markers was developed with the restriction site-associated DNA sequencing (RADseq) technology and 98 SSR markers.10 A ‘Hosui’ (Pyrus pyrifolia) map of 609 SNP markers and 252 SSR markers was developed from transcriptome data.24 Compared with previous maps of pear, SNP-based maps significantly increased the number of genetic markers covering the whole genome. 

High-quality genetic maps are not only valuable tools for QTL mapping, but can also serve to anchor genome assembly scaffolds into pseudo-chromosomes.25 The draft genome of ‘Dangshansuli’ (Pyrus bretschneideri Rehd.)22 was reported as highly heterozygous, and assembled with a ×194 coverage of BC-by-BC (bacterial artificial chromosome) and NGS. The assembly consisted of 512.0 Mb sequences and 2,103 scaffolds, corresponding to 97.1% of the estimated genome size. From the assembly, 386.7 Mb (or 75.5%), was anchored into 17 pseudo-chromosomes corresponding to the basic chromosome number of pear (k = 17), using a genetic map of 2,005 SNP markers. Concurrently, the draft genome of the European pear ‘Bartlett’ (Pyrus communis L.) was assembled with ×11.4 coverage NGS data and consisted of 142,089 scaffolds covering 577.3 Mb in the assembled genome,26 accounting for most of the expected 600 Mb genome size. However, using 2,279 genetically mapped loci (1,391 apple and 888 pear SNPs), only 171.4 Mb (29.7%) of these scaffolds were anchored to linkage groups (LGs), due to the relatively fragmented scaffold sequences and an insufficient genetic map used to anchor the scaffolds. Therefore, it is important and necessary to develop higher quality genetic maps to help improve the scaffolds anchoring ratio of the ‘Bartlett’ v1.0 genome assembly.

For this study, we collected nine published pear genetic maps to build an integrated SSR and SNP-based pear consensus genetic map, which was used for map comparisons. Additionally, a set of three high-quality SNP-based pear genetic maps with European pear genetic backgrounds was used for anchoring the ‘Bartlett’ v1.0 genome scaffolds. The new ‘Bartlett’ v1.1 genome assembly was further used for synteny analyses.

2. Materials and methods

2.1. Construction of a new genetic map for ‘Old Home (OH)’ × ‘Louise Bon Jersey (LBJ)’

A new OHxLBJ genetic map was constructed using SNPs derived from genotyping-by-sequencing (GBS).27 The two European pear cultivars ‘OH’ and ‘LBJ’ were used to create a segregation population of 297 progenies at The New Zealand Institute for Plant & Food Research (PFR).5,23 Total DNA was extracted from young leaves of sixty F1 trees in their third year of growth after grafting using the CTAB method.28 The GBS libraries were prepared using restriction enzyme BamHI following a modified version of the protocol developed by Elshire et al.27 with the following modifications: one microgram of DNA was used for the restriction digestion; adapter annealing was done following the protocol of Ko et al.29; the adapter ligation step was performed following digestion without drying out the DNA/adapter mixture; high fidelity enzyme (AccuPrime Taq DNA polymerase High Fidelity, Life Technologies) was used for amplification; the libraries were amplified and cleaned up prior to pooling. The pooled GBS libraries were sequenced on an Illumina HiSeq2000 platform in parallel in two lanes in single-end mode with reads length of 101 bases. The average read count per library was 1.85 million and 1.86 million for the two lanes. The reads were trimmed from 3’ and 64 bases long fragments were kept after de-multiplexing. The Phred score for bases in the trimmed reads were higher than 20, so no read was discarded after trimming. The sequencing quality was checked with FastQC version 0.11.2.30 Genotyping was carried out using TASSEL/GBS v5 pipeline,31 using the ‘Bartlett’ v1.0 genome assembly as a reference genome. For SNP discovery, we requested at least eight supporting reads per site. Mapping of tags (64-base long unique genomics sequences) to the reference genome was performed using Bowtie2 v2.2.51 in—end-to-end and—very-sensitive modes. To get the flanking sequences, 250 nucleotides at either side of an SNP site were extracted from the reference genome. Additionally, SNP markers were considered of good quality when they segregated in a Mendelian manner: ABxAAB (1:1 ratio), ABxAAB (1:2:1 ratio) and AABB (1:1 ratio).33 All SNPs with missing data for >10 individuals were removed from the analysis. A merged genetic map for both parents was constructed using the package Onemap34 version 2.0-4 in R-studio (R Core Team, 2016). A LOD (logarithm of odds) score of five or higher was used for grouping and the genetic maps were calculated using the Kosambi function. LG numbers were assigned by comparing markers in the new OHxLBJ with the SNP array-based OHxLBJ map published by Montanari et al.23

2.2. Collection of pear genetic maps and genome resources

Nine previously published genetic maps were selected to construct an integrated pear consensus genetic (I-PCG) map using common SSR markers among maps as shown in Fig. 1A.10,16,17,19–21,23,24 These maps were generated from four pear populations, including ‘BYH’ × ‘Dangshansuli’ (two maps), ‘Bartlett’ × ‘Hosui’ (four maps), ‘Shinsei’ × 282-12 (‘Hosui’ × ‘La France’ (one map), and ‘Akiakari’ × ‘Taikaku’ (two maps). ‘BYH’ is a descendant of European pear ‘Clapp’s Favorite’ and Chinese pear ‘Zaosuli’. ‘Dangshansuli’ is a native Chinese pear cultivar. The convention of SSR marker names was not exactly the same, especially for those SSRs indicating multiple loci. For instance, TsuGNH235-1 and TsuGNH235_m1 should be the same marker. Thus, we scanned all
SSR marker names and renamed them if necessary. The map IDs were named based on their cultivar abbreviations and publication abbreviations (detail information is listed in Table 1).

Three high-density SNP-based genetic maps (Fig. 1B) with European pear genetic backgrounds were used for anchoring the ‘Bartlett’ v1.0 genome scaffolds, while other genetic maps with Asian pear (P. bretschneideri or P. pyrifolia) genetic backgrounds were not involved in the anchoring because of the large differences in SNPs and their low efficiency of transferability among different species. These three maps included the newly created genetic map OHxLBJ, a ‘BYH’/C2 ‘Dangshansuli’ consensus map,10 and a European pear and interspecific Asian pear hybrid consensus genetic (PH-CG) map attained from the 10 genetic maps published by Montanari et al.23 derived from five populations, including OHxLBJ, PremP003 × ‘Moonglow’ (T003xM), PEAR1 × PEAR2 (T042xT081),2 PEAR1 × PEAR2 (T042xT081),2 POP356 (T052xT003), and POP356 (T052xT064). The BYH map was a subset of BYHxDS-JXB10 with marker names starting with Pyb and Pybd, representing SNPs derived from ‘BYH’ and both parents, respectively.

Pseudo-chromosome sequences of ‘Dangshansuli’ genome v1.0 were retrieved from the Pear Genome Project website (http://pearge.njau.edu.cn/default.asp?d=4&m=2, 31 December 2016, date last accessed); scaffold sequences of the ‘Bartlett’ v1.0 genome (https://www.rosaceae.org/species/pyrus/pyrus_communis-genome_...
Table 2. Number of pairwise common SSR markers for nine pear genetic maps used for creating the integrated SSR and SNP-based pear consensus genetic (I-PCG) map

| Map ID          | BYHxDS-JXB | HS-TGG | BYHxDS-PMBR | BL-BS | AK-BS | TH-BS | LF-BS | BL-BS | HS-JJSHS |
|-----------------|------------|--------|-------------|-------|-------|-------|-------|-------|----------|
| HS-TGG          | 42         | –      | –           | –     | –     | –     | –     | –     | –        |
| BYHxDS-PMBR     | 63         | 45     | –           | –     | –     | 65    | –     | –     | –        |
| BL-BS           | 52         | 153    | 65          | –     | –     | –     | –     | –     | –        |
| TH-BS           | 31         | 80     | 38          | 139   | –     | –     | –     | –     | –        |
| AK-BS           | 26         | 87     | 34          | 105   | 115   | –     | –     | –     | –        |
| LF-BS           | 43         | 56     | 59          | 95    | 62    | 46    | –     | –     | –        |
| BL-BS           | 44         | 53     | 45          | 112   | 54    | 39    | 95    | –     | –        |
| HS-JJSHS        | 36         | 94     | 36          | 61    | 39    | 36    | 55    | 53    | –        |

A new pear genetic map and an improved European pear genome

2.3. Construction and comparison of pear consensus genetic maps

MergeMap, a software that uses common markers from different maps as bridging markers to merge maps, was used to create the I-PCG map from nine published maps (Fig. 1A) and the PH-CG map (Fig. 1B). To prepare input data for MergeMap, we firstly removed conflict markers that presented in inconsistent LGs. Secondly, each individual LG in the input genetic maps for MergeMap was filtered using three criteria: number of markers in a LG not <3, LG length not <5 cM, and maximum common markers of a LG with other maps not <3. Thirdly, for the I-PCG map, each individual map was set as equal weights in the map merging process, while for the PH-CG map, individual maps were set based on the genetic background of European pear. Lastly, MergeMap tried to resolve conflicts in map positions among the individual maps by deleting a minimum set of marker occurrences. Genetic map comparison between the I-PCG and individual maps were visualized by the Strudel software.

Most maps for constructing the I-PCG map consisted of 17 LGs, which was the same as the basic chromosome number of pear (x = 17), except for two maps (HS-TGG and AK-BS) (Table 1). To confirm whether these maps were appropriate for merging into one consensus map, we first calculated the number of common SSR markers between these maps (Table 2). Each map was found to have at least 26 SSR markers in common with any other map. For instance, the number of common SSR markers for AK-BS vs. TH-BS, AK-BS vs. BL-BS, and AK-BS vs. TH-BS vs. BL-BS was 115, 105 and 139, respectively.

2.4. Anchoring ‘Bartlett’ scaffolds and synteny analyses of intra- or inter-genomes

SSR primer sequences and SNP flanking sequences were mapped onto ‘Bartlett’ scaffolds using isPcr and blat programs, respectively. In either condition, only the best match of each marker was kept, and markers were removed if there were two equally good best matches. The ALLMAPS software, with the strength of computing a scaffold ordering that maximizes collinearity across a collection of maps, was used for anchoring the ‘Bartlett’ genome with the guidance of three high-density SNP-based genetic maps with European pear genetic backgrounds as mentioned above. The multiple collinearity scan toolkit (MCScanX) was used to perform intra-genome synteny analyses for the ‘Bartlett’ genome, and inter-genome analyses for the ‘Bartlett’ versus ‘Dangshansuli’ genomes, and ‘Bartlett’ genome versus the genome of apple cultivar ‘Golden Delicious’.

3. Results

3.1. Merging multiple genetic maps leads to a high quality and density integrated SSR and SNP-based consensus map of pear

Nine published pear genetic maps (Table 1) constructed mainly using SSR and SNP markers were integrated into a consensus genetic map I-PCG, using SSR markers in common and the software MergeMap. The number of markers for these maps ranged from 105 for HS-JJSHS to 3,241 for BYHxDS-JXB. Among these, BYHxDS-JXB had the most SNP markers (3,143) and BYHxDS-PMBR had the most SSR markers (734). The integrated SSR and SNP-based pear consensus genetic map (I-PCG map) consisted of 17 LGs made up of 5,085 markers in total (1,232 SSRs, and 3,853 SNPs), spanning 3,266 cM, with an average marker interval of 0.64 cM (Table 3; markers listed in Supplementary Table S1). As shown in Fig. 2, a large proportion (73.8%) of the markers in the map were SNPs, from a minimum of 63.3% on LG 16 (abbreviated as LG16, same as below), to 90.2% on LG5. SSR markers were interspersed with SNP markers in almost all parts of the 17 LGs, except for a few regions with very low SNP diversity (6.2% to 20.9%).

The PH-CG map was found to have at least 26 SSR markers in common with any other map. For instance, the number of common SSR markers for AK-BS vs. TH-BS, AK-BS vs. BL-BS, and AK-BS vs. BL-BS was 115, 105 and 139, respectively.

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Table 3. Summary of the integrated SSR and SNP-based pear consensus genetic (I-PCG) map

| Linkage group | Number of markers | Number of SSRs | Number of SNPs | Number of common SSR markers | Length (cM) | Average marker interval (cM) | Length of the locus cluster | Marker number in the locus clusters |
|---------------|------------------|----------------|----------------|-----------------------------|------------|-------------------------------|-----------------------------|-----------------------------------|
| 1             | 124              | 38             | 86             | 20                          | 147.9      | 1.20                          | 15                          | 32                                |
| 2             | 367              | 90             | 277            | 29                          | 198.6      | 0.54                          | 100                         | 324                               |
| 3             | 374              | 82             | 292            | 29                          | 247.6      | 0.66                          | 90                          | 278                               |
| 4             | 169              | 51             | 118            | 15                          | 87.7       | 0.52                          | 55                          | 146                               |
| 5             | 318              | 69             | 249            | 19                          | 158.8      | 0.50                          | 75                          | 257                               |
| 6             | 206              | 37             | 169            | 20                          | 96.6       | 0.47                          | 55                          | 178                               |
| 7             | 273              | 56             | 217            | 20                          | 168.0      | 0.62                          | 65                          | 212                               |
| 8             | 265              | 66             | 199            | 32                          | 224.2      | 0.85                          | 55                          | 173                               |
| 9             | 373              | 96             | 277            | 34                          | 244.3      | 0.66                          | 80                          | 274                               |
| 10            | 401              | 86             | 315            | 25                          | 230.1      | 0.58                          | 100                         | 326                               |
| 11            | 247              | 86             | 161            | 19                          | 206.4      | 0.84                          | 45                          | 146                               |
| 12            | 255              | 58             | 197            | 21                          | 165.3      | 0.65                          | 50                          | 161                               |
| 13            | 285              | 84             | 201            | 33                          | 211.4      | 0.74                          | 75                          | 198                               |
| 14            | 562              | 131            | 431            | 37                          | 269.3      | 0.48                          | 135                         | 471                               |
| 15            | 207              | 76             | 131            | 21                          | 180.3      | 0.88                          | 50                          | 130                               |
| 16            | 334              | 94             | 240            | 32                          | 244.3      | 0.73                          | 75                          | 222                               |
| 17            | 334              | 94             | 240            | 32                          | 244.3      | 0.73                          | 75                          | 222                               |
| Total         | 5,085            | 1,232          | 3,853          | 425                         | 3,266      | 0.64                          | 1,185                       | 3,776                             |

Figure 2. Distribution of SNP and SSR markers on 17 linkage groups (LGs) of the integrated SSR and SNP-based pear consensus genetic (I-PCG) map. Red bars indicate SSR markers, and black bars indicate SNP markers. X-axis indicates LG number, and Y-axis LG length in centiMorgans (cM). This figure is available in black and white in print and in colour at DNA Research online.
Figure 3. Alignment of the integrated SSR and SNP-based pear consensus genetic (I-PCG) map and the nine individual maps. Red bars on the left denote linkage groups (LGs) of the I-PCG map, and blue/green bars on the right represent the corresponding LGs for individual maps. Blue and green colours indicate forward and reverse orientation, respectively. Purple lines connecting the I-PCG map and individual maps indicate common markers (present in at least two individual maps), and grey lines indicate non-common markers. LG length is relative to total length. This figure is available in black and white in print and in colour at DNA Research online.
Figure 3. Continued.
In total, 425 common SSR markers were detected on the nine individual maps, as mentioned above, to construct the I-PCG map (Table 3, Fig. 3). Of these, 200 (47.1%) were derived from apple, 148 (34.8%) from pear genomic sequences, and 77 (18.1%) from pear EST sequences. There was a large proportion of markers from apple because few SSR markers were developed from pear before the whole genome sequences were released. Common markers on the 17 LGs varied from 15 on LG4 to 37 on LG15. Of these markers, 70% were common between two or three maps, and five markers were common on all nine individual maps: NB113a (LG3), CH03g12-2 (LG3), CH02c11 (LG10), CH02b03b (LG10), and CH04h02 (LG11).

### 3.2. Construction of a new GBS SNP map for the ‘OH’ × ‘LBJ’ population

The new OHxLBJ map, constructed using SNPs derived from the GBS approach, consists of 9,151 markers and spans 1,664.6 cM with an average of 0.18 cM distance between markers (Supplementary Table S4 for a summary and Supplementary Table S5 for a detailed list of markers). The SNPs are located on 1,719 ‘Bartlett’ assembly scaffolds. The information on the LG assignment from the OHxLBJ SNP array map enabled the assignment of LGs on the new GBS-based OHxLBJ pear genetic map in this study. The lengths of the LGs showed a wide range, from 12.3 cM (LG8) to 255.0 cM (LG17). In total, five LGs were >100 cM, and another three LGs were <50 cM, indicating that some LGs were saturated, while other LGs were partially covered. In addition, we noticed high-level marker co-segregation in this map; for instance, 403 SNP markers on LG1 were located in 24 unique genetic positions, suggesting 16.8 markers per unique genetic position for LG1. Overall, there were 428 unique genetic positions on this GBS-based OHxLBJ map, and on average 21.4 markers per unique genetic position, with an average interval of 4.05 cM.

### 3.3. Improvement of ‘Bartlett’ genome assembly anchoring by multiple high-density maps

Since genetic maps were commonly used for anchoring scaffolds into pseudo-chromosomes, we evaluated the potential application of the I-PCG map and three high-density SNP-based pear genetic maps with European pear background to improve the ‘Bartlett’ v1.0 genome assembly. The PH-CG map consisted of 1,740 markers and spanned 2,773.3 cM (Supplementary Table S2 for a summary, Table 4.

| Table 4. High-density SNP-based pear genetic maps with European pear genetic background used for anchoring the ‘Bartlett’ v1.0 genome scaffolds |
|---|---|---|---|---|---|
| Map ID | Number of SNPs | Number of linkage groups | Length (cM) | Average marker interval (cM) | References |
| OHxLBJa | 9,151 | 17 | 1,664.6 | 0.18 | This study |
| BYHb | 2,863 | 17 | 2,080.0 | 0.73 | 10 |
| PH-CGc | 1,740 | 17 | 2,773.3 | 1.61 | 23 |

Population: ‘Old Home’ × ‘Louise Bon Jersey’.

bBayuehong × ‘Dangshansuli’.

cConsensus genetic map derived from five populations: ‘Old Home’ × ‘Louise Bon Jersey’, PremP003 × ‘Moonglow’ (T003xM), PEAR1 × PEAR2 (T042xT081), POP369 (T052xT003), and POP356 (T052xT064).

*The total size of ‘Bartlett’ v1.0 (Pyrus communis) scaffold sequences was 577,335,413 bp.

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| Table 5. Summary of the new Bartlett v1.1 genome assembly constructed using the ALLMAPS software |
|---|---|---|---|---|---|
| Pseudo-chromosome | Length of pseudo-chromosome (bp) | Number of scaffolds | Length of scaffolds (bp) | Number of markers | Markers from BYH | Markers from PH-CG | Markers from OHxLBJ |
| Chr1 | 10,263,956 | 110 | 10,253,056 | 408 | 2 | 65 | 341 |
| Chr2 | 17,147,276 | 141 | 17,133,276 | 618 | 212 | 107 | 299 |
| Chr3 | 18,593,297 | 184 | 18,574,997 | 996 | 188 | 89 | 719 |
| Chr4 | 14,885,393 | 156 | 14,869,893 | 703 | 95 | 60 | 548 |
| Chr5 | 20,478,923 | 210 | 20,458,023 | 847 | 241 | 97 | 509 |
| Chr6 | 15,384,309 | 167 | 15,367,709 | 784 | 174 | 79 | 531 |
| Chr7 | 16,091,674 | 154 | 16,076,374 | 684 | 110 | 71 | 503 |
| Chr8 | 16,973,589 | 130 | 16,960,689 | 650 | 150 | 58 | 442 |
| Chr9 | 14,040,893 | 127 | 14,028,293 | 353 | 127 | 82 | 144 |
| Chr10 | 20,488,728 | 212 | 20,467,628 | 1,019 | 196 | 105 | 718 |
| Chr11 | 19,300,883 | 200 | 19,280,983 | 859 | 209 | 102 | 548 |
| Chr12 | 16,230,967 | 154 | 16,215,667 | 766 | 144 | 80 | 542 |
| Chr13 | 15,861,480 | 113 | 15,850,280 | 619 | 126 | 87 | 406 |
| Chr14 | 14,746,025 | 131 | 14,733,025 | 553 | 146 | 53 | 354 |
| Chr15 | 28,200,744 | 212 | 28,179,644 | 1,075 | 316 | 127 | 632 |
| Chr16 | 15,009,554 | 113 | 14,995,054 | 554 | 82 | 61 | 411 |
| Chr17 | 19,000,797 | 173 | 18,983,597 | 765 | 150 | 81 | 534 |
| Sum | 291,798,488 | 2,720 | 291,528,188 (50.5%) | 12,253 | 2,668 | 1,404 | 8,181 |

In total, 425 common SSR markers were detected on the nine individual maps, as mentioned above, to construct the I-PCG map (Table 3, Fig. 3). Of these, 200 (47.1%) were derived from apple, 148 (34.8%) from pear genomic sequences, and 77 (18.1%) from pear EST sequences. There was a large proportion of markers from apple because few SSR markers were developed from pear before the whole genome sequences were released. Common markers on the 17 LGs varied from 15 on LG4 to 37 on LG15. Of these markers, 70% were common between two or three maps, and five markers were common on all nine individual maps: NB113a (LG3), CH03g12-2 (LG3), CH02c11 (LG10), CH02b03b (LG10), and CH04h02 (LG11).
Supplementary Table S3 for a detailed list), while the new OHxLBJ consisted of 9,151 markers and spanned 1,664.6 cM (Supplementary Table S4 for a summary and Supplementary Table S5 for a detailed list). A brief overview of the maps is shown in Table 4. Using these maps, a total of 291.5 Mb ‘Bartlett’ v1.1 genome assembly was obtained in 17 pseudo-chromosomes, increasing the assembly ratio to 50.5% (Table 5 for a summary and Supplementary Table S6 for a detailed list), while the new OHxLBJ consisted of 9,151 markers and spanned 1,664.6 cM (Supplementary Table S4 for a summary and Supplementary Table S5 for a detailed list). As shown in Figure 4, the I-PCG map and the new ‘Bartlett’ v1.1 genome assembly displayed good collinearity. The uniformity of the genetic map with the physical maps could also be observed in the ‘Dangshansuli’ and ‘Golden Delicious’. Among these, 3,414 markers were located in corresponding pseudo-chromosomes in at least two of the above three genome assemblies (Supplementary Table S1). Furthermore, misaligned markers between the I-PCG map and ‘Bartlett’ v1.1 genome assembly were enriched in five LG/Chr pairs (at least five markers for each LG/Chr pair): 1–7, 3–11, 13–16 and 6–14. For the ‘Dangshansuli’, they were enriched in six LG/Chr pairs (at least 17 markers for each LG/Chr pair): 1–7, 11–15, 8–15, 3–11, 5–11 and 13–16. For ‘Golden Delicious’, they were enriched in nine LG/Chr pairs (at least 41 markers for each LG/Chr pair): 5–10, 3–11, 1–7, 9–17, 13–16, 8–15, 2–15, 6–14 and 1–15. Overall, comparing the I-PCG map with the ‘Dangshansuli’, ‘Bartlett’ and ‘Golden Delicious’ assemblies confirmed the reliability of the consensus genetic map.

3.4. Comparison of the I-PCG map with genome assemblies of pear and apple revealed both collinearity and misalignments

To further evaluate the collinearity between the pear genetic and physical maps, we compared the I-PCG map with the new ‘Bartlett’ v1.1 (Fig. 6A), ‘Dangshansuli’ and apple genome assemblies (Fig. 6B). In total, 4,036, 4,157 and 3,707 markers were mapped onto the ‘Bartlett’, ‘Dangshansuli’ and ‘Golden Delicious’ genome assemblies, and among these, 3,622, 4,069 and 3,707 markers were mapped on pseudo-chromosomes, respectively (Supplementary Table S1). As shown in Figure 6A, the I-PCG map and the new ‘Bartlett’ v1.1 genome assembly displayed good collinearity. The near-linear arrangement of homologous regions for all 17 corresponding pseudo-chromosomes was observed. Nonetheless, the arrangements of Chr1, Chr2, Chr3 and Chr5 were not as well aligned as other pseudo-chromosomes. Interestingly, inter-genome synteny analyses between ‘Bartlett’ and the apple genome of ‘Golden Delicious’ (Fig. 5C), both in the subfamily Maloideae, showed not only good collinearity, but also homologous relationships, similar to the ‘Bartlett’ intra-genome synteny analyses. This phenomenon was also observed when inter-genome synteny analysis was performed between ‘Dangshansuli’ and ‘Golden Delicious’ (data not shown), indicating the unique genome structure for apple.

4. Discussion

Common methods to build high-density genetic maps with NGS usually start with genotyping individuals in a hybrid population through reduced-genome complexity technologies, such as RADseq, GBS, and SLAF-Seq. In this study, we merged published pear genetic maps by treating common SSR markers presented in maps from multiple sources as bridging markers and constructed an integrated SSR and SNP-based pear consensus genetic map (the I-PCG map). The advantages of merging genetic maps include integrating multiple sources as bridging markers and constructed an integrated SSR and SNP-based pear consensus genetic map (the I-PCG map). The length of the consensus genetic map could be inflated (>3,000 cM in this study) due to the method used to develop the consensus map, i.e. artifact of the merging process, not true recombination. Thus, the consensus genetic map may be more relevant.
to the relative positioning of markers than the absolute distances between them.43

Anchoring scaffold sequences into pseudo-chromosomes requires both high-quality scaffold sequences and high-quality genetic maps.25,44 In this study, we selected three high-quality maps with European pear genetic backgrounds for re-anchoring the ‘Bartlett’ scaffolds. The constructed pseudo-chromosomes captured 291.5 Mb of the ‘Bartlett’ genome sequence and dramatically increased the anchored ‘Bartlett’ genome from 29.7% in the original assembly to 50.5% in version 1.1. The improvement of a larger ratio for ‘Bartlett’ genome assembly was due to the higher density genetic maps used compared with the original assembly, which contained 1,391 Malus SNPs, and 888 P. communis SNPs.26 In this study, we used a 5.4-fold number of SNP markers, including 2,668 from the BYH map, 1,404 from the PH-CG map, and 8,181 from the OHxLBJ map (Table 5), ensuring the capture of more ‘Bartlett’ scaffolds. Synteny analyses of the new ‘Bartlett’ v1.1 assembly indicated an extensive conserved genome collinearity with P. bretschneideri ‘Dangshansuli’ and Malus × domestica ‘Golden Delicious’, indicating that the new ‘Bartlett’ v1.1 is consistent with its closely related species. Furthermore, the homologous pseudo-chromosome pairing relationships demonstrated by intra-genome synteny analyses were similar with previous studies in ‘Dangshansuli’22 and ‘Golden Delicious’,40 indicating that the genome structure of the new ‘Bartlett’ v1.1 genome assembly was correct. However, we noticed that four pseudo-chromosomes of ‘Bartlett’ did not have such good collinearity with ‘Dangshansuli’ and ‘Golden Delicious’ as other pseudo-chromosomes, namely Chr1, Chr2, Chr3, and Chr5, and

Figure 5. (A) Intra-genome synteny for the ‘Bartlett’ (P. communis) v1.1 pseudo-chromosomes; (B) Inter-genome synteny for the ‘Bartlett’ v1.1 and ‘Dangshansuli’ (P. bretschneideri) pseudo-chromosomes; (C) Inter-genome synteny for the ‘Bartlett’ v1.1 and ‘Golden Delicious’ (Malus × domestica) pseudo-chromosomes. This figure is available in black and white in print and in colour at DNA Research online.
that there were a number of dispersed collinear regions. These were possibly due to differences in the genome structure between 'Bartlett' and the other two genomes. On the other hand, this assembly ratio was still lower than that for 'Dangshansuli', which accounted for 75.5% of the genome. This may be the result of the genetic maps we used not capturing more scaffold sequences, although we used 5.4 times more SNP markers than for the original 'Bartlett' genome assembly. Also, the scaffold N50 length of the 'Bartlett' genome assembly was 88 kb compared with 540.8 kb of the 'Dangshansuli' one, which made it difficult to capture more scaffold sequences. Overall, we significantly improved the 'Bartlett' genome assembly, but more work is required for further improvement, especially a better quality scaffold assembly, which is the target of a recent initiative by an international European pear genome consortium.

The integrated I-PCG map was compared with the improved 'Bartlett' v1.1, 'Dangshansuli' and 'Golden Delicious' genome assemblies. Both collinearity and misalignment of markers were observed for this comparison, indicating that the I-PCG map we constructed was reliable, but both the pear genetic and physical map are far from being complete. The reason for the misalignment may be repeat content or genome-wide duplication in the pear and apple genomes. Furthermore, there were 42.9% misaligned markers for the apple genome of 'Golden Delicious', much higher than the 5.4% and 10.3% for the 'Bartlett' and 'Dangshansuli' genomes, respectively, indicating the much difference between pear and apple. In addition, similar homologous pseudo-chromosome pairing relationships were revealed by both comparisons of the consensus map and synteny analyses of the genome assembly of pear, which was the first step for further integration of genetic and physical maps in the future.

In conclusion, we demonstrated that a set of different pear genetic maps is a valuable resource for map integration and genetic comparison, which has been exploited in anchoring genome scaffolds of European pear 'Bartlett' (P. communis). It was proved that the abundant common markers could be merged into a consensus map, resulting in an integrated SSR and SNP-based pear consensus genetic map with the highest marker density to date. It consists of 5,085 markers and spans 3,266.0 cM, with an average marker interval of 0.64 cM. Meanwhile, the multiple high-density SNP-based maps enabled a significant improvement (291.5 Mb) in scaffold anchoring to pseudo-chromosomes, which accounted for 50.5% of the 'Bartlett' genome. The genetic and physical map comparison further revealed synteny and variation between European pear and Chinese white pear or apple.

Figure 6. Alignment of the integrated SSR and SNP-based pear consensus genetic (I-PCG) map with (A) 'Bartlett' pseudo-chromosomes (Pc), (B) 'Dangshansuli' (Pb) and apple (Md) pseudo-chromosomes. Red bars indicate linkage groups (LGs) of the I-PCG map. Blue and green bars indicate forward and reverse orientation LGs, respectively. LG length is relative to total length. The yellow lines indicate markers located in the same linkage group/pseudo-chromosome number and the grey lines indicate markers in different linkage group/pseudo-chromosome number. This figure is available in black and white in print and in colour at DNA Research online.
Accession number
PRJNA349767.

Data availability
The sequencing data for the OHxLBJ GBS libraries were deposited at NCBI Sequence Read Archive (SRA) database under BioProject accession number of PRJNA349767. The pseudo-chromosome sequences for the European pear (P. communis) ‘Bartlett’ v1.1 genome were deposited at the Pear Genome Project website (http://peargene.njau.edu.cn/default.asp?id=4&m=2).

Conflict of interest
None declared.

Supplementary data
Supplementary data are available at www.dnaresearch.oxfordjournals.org.

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