Tree Genetics & Genomes (2013) 9:19–36
DOI 10.1007/s11295-012-0522-7

ORIGINAL PAPER

High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.)

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Received: 30 August 2011 / Revised: 4 May 2012 / Accepted: 7 May 2012 / Published online: 24 June 2012
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Abstract Single nucleotide polymorphisms (SNPs) were used to construct an integrated SNP linkage map of peach (*Prunus persica* (L.) Batsch). A set of 1,536 SNPs were evaluated with the GoldenGate® Genotyping assay in two mapping populations, Pop-DF, and Pop-DG. After genotyping and filtering, a final set of 1,400 high quality SNPs in Pop-DF and 962 in Pop-DG with full map coverage were selected and used to construct two linkage maps with JoinMap®4.0. The Pop-DF map covered 422 cM of the peach genome and included 1,037 SNP markers, and Pop-DG map covered 369 cM and included 738 SNPs. A consensus map was constructed with 588 SNP markers placed in eight linkage groups (n=8 for peach), with map coverage of 454 cM and an average distance of 0.81 cM/marker site. Placements of SNPs on the "peach v1.0" physical map were compared to placement on the linkage maps and several differences were observed. Using the SNP linkage map of Pop-DG and phenotypic data collected for three harvest seasons, a QTL analysis for fruit quality traits and chilling injury symptoms was carried out with the mapped SNPs. Significant QTL effects were detected for mealiness (M) and flesh bleeding (FBL) QTLs on linkage group 4 and flesh browning (FBr) on linkage group 5. This study represents one of the first examples of QTL detection for quality traits and chilling injury symptoms using a high-density SNP map in a single peach F1 family.

Communicated by A. Abbott

Electronic supplementary material The online version of this article (doi:10.1007/s11295-012-0522-7) contains supplementary material, which is available to authorized users.

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Keywords Single nucleotide polymorphism · *Prunus* · Linkage map · SNP effects · "Lovell" sequence · Peach v1.0 · Quantitative trait loci · Chilling injury symptoms

Introduction

Peach is the model species for *Prunus* sp. and other Rosaceous crops. A high quality draft peach sequence (peach v1.0) has been generated and assembled for the dihaploid "Lovell" genome by the International Peach Genome Initiative (IPGI). At least three other peach genomes have been sequenced and deposited into the NCBI database, including the parents for the crosses used in the present study (Ahmad et al. 2011). Genetic maps have been constructed for most of the *Prunus* crop species and are reviewed in Dirlewanger et al. (2004) and Abbott et al. (2007). Peach has characteristics useful for tree genetic studies and for breeding, including self-compatibility, a short juvenile period, and a small genome (Shulaev et al. 2008).

The development and use of single nucleotide polymorphisms (SNPs) has become practical due to advancements in sequencing technologies of plant genomes such as rice (*Oryza sativa*), *Arabidopsis* (*Arabidopsis thaliana*), barrel medic (*Medicago truncatula*), maize (*Zea mays*), and wine grape (*Vitis vinifera*). SNPs have a low mutation rate and are evolutionarily stable from generation to generation across the genome (Batley and Edwards 2009). SNP markers have several advantages for genetic mapping over other molecular markers. SNPs have fewer detection/evaluation errors than simple sequence repeats (SSRs) and map quantitative trait loci (QTL) with greater precision than is possible with restriction fragment length polymorphisms (RFLPs) or SSRs (Ball et al. 2010; Yu et al. 2011). SNPs can be used to investigate the structure and evolutionary history of populations, as a tool for association mapping experiments, to identify QTLs (marker/trait association), and to construct high-resolution genetic maps (Akhunov et al. 2009; Aranzana et al. 2005; International HapMap Consortium et al. 2007; Zhao et al. 2007). SNPs are often transferable across species within a genus (Grattapaglia et al. 2011). A microarray chip in peach (ChillPeach) with a set of several putative SNPs was developed by Ogundiwin et al. (2008). Ahmad et al. (2011) describe the development of a 6,654 peach SNP panel from which a 1,536 SNP set was selected for the present study. An additional 8,144 peach SNP set has recently been described by Verde et al. (2012).

The first peach linkage map was constructed from isozyme and random amplified polymorphic DNA markers (RAPDs) (Chaparro et al. 1994). Subsequently, several linkage and QTL maps were constructed on F_1, F_2, and BC_1 peach and interspecific *Prunus* sp. cross progenies shown at http://www.rosaceae.org/. The locations of specific trait loci are shown on these maps, including fruit quality, tree architecture, and resistance/tolerance to abiotic and biotic stresses (Abbott et al. 1998; Aranzana et al. 2002; Blenda et al. 2007; Bliss et al. 2002; Dettori et al. 2001; Dirlewanger et al. 2007; Dirlewanger et al. 2004; Dirlewanger et al. 1999; Foolad et al. 1995; Foulongne et al. 2003; Joobeur et al. 1998; Lu et al. 1998; Ogundiwin et al. 2009; Quarta et al. 2000; Quarta et al. 1998; Rajapakse et al. 1995; Shimada et al. 2000; Verde et al. 2006; Yamamoto et al. 2001; Yamamoto et al. 2005). Despite a number of active peach genetics projects and breeding programs, only one-half of the known single-gene traits have been mapped (Dirlewanger et al. 2007).

Fruit quality in peach is a function of changes in biochemical and sensory changes in color, flavor, and texture as fruits develop, grow, and ripen, and postharvest maintenance of these qualities as the fruits senesce. The genetic dissection of these complex processes will permit a more systematic approach to plant improvement than has been possible previously. An important component of fruit quality peach deterioration in storage is the presence of postharvest chilling injury symptoms (Crisosto et al. 1995). The three major symptoms of chilling injury, which can occur during cold storage or after subsequent ripening, are mealiness, flesh browning, and flesh bleeding. These symptoms are not usually noticed until fruit reaches the consumer. Mealiness is a textural disorder where fruits become soft and dry with non-juicy and grainy flesh. Flesh browning is a discoloration of the flesh that can occur in mealy and non-mealy fruits, though it is often associated with mealiness (Crisosto et al. 1999). Flesh bleeding is the term used to describe the occurrence and spread of red pigmentation throughout the fruit flesh. In chilling-injured fruit, flesh bleeding may originate from the red pigmentation commonly observed in the flesh close to the pit of many peach and nectarine cultivars. Several QTL studies have been conducted in peach, the majority have been focused on mapping important fruit quality traits, since fruit quality is considered the primary selection criteria by peach breeders (Abbott et al. 2002, 2008). All of these studies were based on linkage maps constructed using low-throughput molecular markers, e.g., restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs) (Dirlewanger et al. 1999; Dettori et al. 2001; Ogundiwin et al. 2008). These maps are mostly low-density maps, and QTLs placed on the maps are unable to provide precise and complete information about the numbers and the locations of the genes or QTLs controlling the traits.

Illumina GoldenGate genotyping assays were used in the present study to develop oligonucleotide pool assays (OPAs), with 90 % high quality SNPs, to genotype two mapping populations derived from crosses between "Dr. Davis"×"Georgia Belle" (Pop-DG) and "Dr. Davis"×"F8,1-42" (Pop-DF), a peach introgression line derived from an initial almond×peach interspecific hybrid. SNP maps were generated

for both populations and a consensus SNP map obtained by combining the data from both populations. The SNP map, obtained from Pop-DG, was used to perform QTL analysis of fruit quality traits and chilling injury symptoms.

Materials and methods

Mapping population

Two mapping populations were used in this study. Pop-DF was obtained from controlled crosses between the peach cultivar "Dr. Davis" (female) and the hybrid "F8,1-42"(peach × almond). "F8,1-42" has both almond ("Nonpareil") and peach ("Jungerman" and "Everts") cultivars in its lineage, resulting in a unique phenotype that is freestone with non-melting flesh. Flesh firmness at maturity is comparable to the standard canning clingstone peach cultivar "Dr. Davis". The endoPG genotype (f_1f_1) which confers the clingstone, nonmelting phenotype (Peace et al. 2005) is identical in both "Dr. Davis" and "F8,1-42", indicating that the unique freestone trait was probably transferred to "F8,1-42" from almond. Consequently, "F8,1-42" is the closest breeding line to the desired but previously unavailable freestone, firm flesh genotype. "Dr. Davis" is a clingstone, nonmelting, non-mealy, yellow flesh cultivar expressing low flesh browning potential and low fruit aromatics. The initial mapping population consisted of 117 F_1 individuals. After SNP screening, 69 progeny individuals were used for map construction. The second mapping population, Pop-DG, is an intra-specific cross between "Dr. Davis" (female) and "Georgia Belle" (male) cultivars. "Georgia Belle" is considered a founder for many modern freestone cultivars expressing freestone, melting, white flesh, high flavor, mealy texture, and considerable flesh browning. This second population consisted of 55 F_1 individuals and was previously mapped with 211 SSRs, SRAPs and other markers (Ogundiwin et al. 2009). For genotyping of Pop-DF, five separate DNA samples of "Dr. Davis" and three DNA samples of "F8,1-42" were analyzed; the same replicates of "Dr. Davis" and two "Georgia Belle" samples were used for genotyping Pop-DG.

Peach DNA isolation and quantification

DNA was extracted from new leaves by the CTAB method (Doyle and Doyle 1987), modified by the addition of 1 % 2-mercaptoethanol to the isolation buffer. A preliminary quantification of DNA was determined after electrophoresis on 1 % agarose gels by comparison against a λ *Hin*dIII standard of known concentration (Peace et al. 2005) (note: the GoldenGate® Genotyping assay requires using at least 15 μl of 50 ng/μl DNA per sample).

The samples were sent to the DNA Technologies Core at the UC Davis Genome Center (http://dnatech.genomecenter.ucdavis.edu/dna_quant.html), and a second DNA quantification was done on a Molecular Devices Analyst plate reader with PicoGreen (Invitrogen Molecular Probes).

Discovery and selection of SNPs

A set of 6,654 high-quality SNPs was developed under the framework of the "Integration of genomic tools for next generation peach and almond cultivar development" USDA-NRI project at UC Davis. SNPs were obtained by generation of whole genome sequence for "Dr. Davis", "F8,1-42", and "Georgia Belle" using both Roche 454 and Ilumina-Solexa technologies (Ahmad et al. 2011). Assembly and alignment were done according to Ahmad et al. (2011). The SNPs were selected to be evenly distributed across the genome from a larger SNP set of 6,654 peach SNPs (approximately 1 SNP/40,000 nucleotides). SNPs for mapping Pop-DF were selected so that the SNPs were heterozygous in only one parent (providing 1:1 segregation ratios), with the heterozygous SNPs distributed evenly between the two parents. When applied to Pop-DG, a large number of these SNPs were found to be heterozygous for both parents, providing 1:2:1 segregation ratios. SNPs were also evaluated with a "design" score generated by Illumina, which attempts to predict the probability of success when used in a GoldenGate® assay. SNPs with scores = 1.1 were used for mapping.

SNPs were named starting with "UCD" for the University of California, Davis, followed by "SNP" for the marker type and the number of their order in the OPA (UCD_SNP_XX). Once the final SNPs were selected, their reproducibilities and heritabilities were examined for each population. Linkage groups were constructed from the final set of most informative markers and individuals from each population. SNP descriptions are provided in supplemental files 1 and 2, referenced to the SNP entries in the NCBI SNP database at http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=UCDAVISBIOINFO, and addressed in the range NCBI-dbSNP:275372743 to NCBI-dbSNP:275395485.

Development of the oligonucleotide pool (OPA) for the GoldenGate® assay

Oligonucleotides were designed, synthesized, and assembled into this OPA by Illumina Inc. The peach "GS0012410-OPA.opa" consisted of 1,536 SNPs and was used to genotype the Pop-DF and Pop-DG populations. This is a unique set of SNP oligonucleotides developed for this project but could be applied to any number of peach mapping/evaluation projects.

SNP genotyping

The SNPs were evaluated in an Illumina GoldenGate® Genotyping assay (Fan et al. 2003) with the iScan readout at the UC Davis Genome Center. The results were analyzed with GenomeStudio™ Genotyping Module v1.0 from Illumina (Fig. 1). The SNPs were manually edited and removed from the analysis if clustering (segregation) errors in the parents/progeny distributions were observed, as described in the Infinium® Genotyping Data Analysis from Illumina® Technical Note (www.illumina.com), except that the GenCall score threshold level for inclusion of data into clusters was changed from 0.15 to 0.25 (Higher GenCall scores indicate that the data points are more reliable and can be included in a specific cluster with more confidence). The deviations from expected allelic Mendelian inheritance ratios at all loci for each progeny were measured as parent-parent-child (P-P-C) heritability for each progeny individual. P-P-C heritability is defined by GenomeStudio as the number of SNPs placed as correct by Mendelian inheritance divided by the total number of SNPs for each progeny individual. (note: this is not quantitative heritability of a trait as conventionally defined).

Map construction

A variation of the "two-way pseudo-testcross" approach of Grattapaglia and Sederoff (1994) was used to construct the genetic linkage map from the selected group of SNP markers. SNPs, homozygous in one parent and heterozygous in the other, were chosen to be evenly distributed across the genome using the "peach v1.0" reference genome (released by the International Peach Genome Initiative (IPGI) in 2010 and available in http and ftp repositories at phytozome v7.0 (http://www.phytozome.net/peach.php)), with approximately equal numbers of heterozygous loci distributed in each parent. Linkage analysis was performed with JoinMap®4 (Stam 1993; Van Ooijen and Voorrips 2001). A minimum LOD of 3.0 and a maximum recombination fraction of 0.45 were used as thresholds (Ogundiwin et al. 2009). Segregation distortion was tested using the chi-square goodness-of-fit test ($p>0.05$). Recombination fractions were converted to map distances in centimorgans (cM) using the Kosambi mapping function (Kosambi 1943). The marker data type was coded as cross-pollination (CP) in JoinMap®4. Markers that had suspect linkages, as defined by a recombination frequency >0.7 and a LOD >1 (Van Ooijen JW, personal communication), were removed from further analysis. The maps were calculated without adding the marker order into the scaffolds. Once both maps were obtained, the Combine Groups for Map Integration function from the JoinMap®4 menu was used to find the consensus SNP map using the regression mapping algorithm. (note: map integration is not straightforward if both populations do not have all loci in common (JoinMap®4 Manual (Van Ooijen and Voorrips 2001). The final charts of each linkage group were produced using MapChart 2.2 (Voorrips 2002)).

Comparison of the position of the SNPs in the physical and genetic map

The 1,536 SNPs included in the OPA were ordered by reference to their physical position on the "peach v1.0" genome. The set of SNPs mapped in each linkage group for both populations were aligned with their position in the physical map to evaluate the colliniarity among the linkage and physical maps, using MapChart 2.2 (Voorrips 2002).

Phenotypic data and QTL analysis in Pop-DG

Pop-DG segregated for two Mendelian morphological traits: freestone melting flesh/clingstone non-melting flesh (*F–M* locus), white/yellow flesh color (*Y* locus). These traits were scored visually.

The three major variables used to describe chilling injury (CI) symptoms, mealiness (M), flesh browning (FBr), and flesh bleeding (FBL), were evaluated as described by Martínez-García et al. (2012). Parents and progeny individuals were monitored for three successive years. At commercial maturity, 15 sound fruits were harvested per tree and immediately taken to the laboratory for evaluation. Each fruit was treated with a fungicide dip, 1.2 gL^{-1} of iprodione, cold-stored under CI-inducing condition (5°C for 2 weeks), and then ripened at room temperature to assess CI symptom expression. Each tree was classified as producing fruit that were either freestone or clingstone, melting or non-melting flesh, and white or yellow flesh.

The rank sum test of Kruskal–Wallis (K–W) (test non-parametric equivalent of the one-way analysis of the variance) and χ^2 contingency table were performed for freestone-melting flesh (F-M) and yellow flesh (Y) traits against each SNP, separately, to establish the strength of linkage at individual SNP loci. The K-W test ranks all individuals according to the quantitative trait, while it classifies them according to their marker genotype. A stringent significance level (*P* value) similar or higher than 0.005 was used to identify markers significantly associated with both traits by the Kruskal–Wallis test (Van Ooijen 2004).

QTL analysis of M, FBr, and FBL was performed with MapQTL® 5.0 (Van Ooijen 2004). Permutation test, interval mapping (IM), and multiple QTL model (MQM) mapping strategies were used to detect significant associations between chilling injury symptoms and marker loci in the data sets. The likelihood value for presence of a QTL was expressed as a LOD (log of odds) score. The significant thresholds for genome wide (including all groups) and

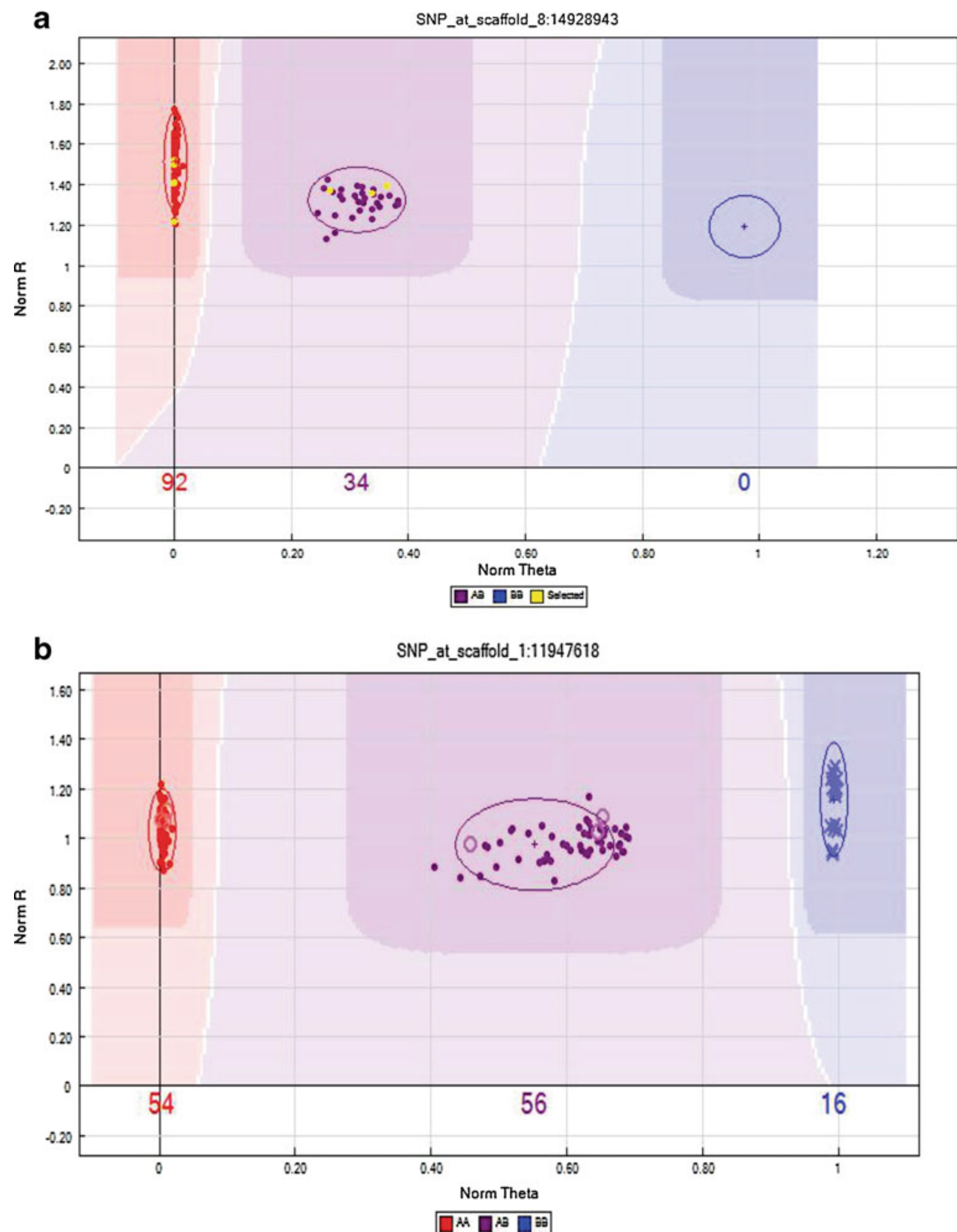

Fig. 1 Scoring genotyping data using GenomeStudio® Genotyping module. **a** UCD_SNP_548 shows a typical score with two distinct clusters (AA, AB), progeny distribution expected for a segregation ratio 1:1. **b** UCD_SNP_1252 shows a segregation ratio 1:1 (AA o AB) but also typical parent-parent-child heritability errors with the presence of cluster BB (in this case, 16 samples presented an unexpected genotype)

individual per linkage group were calculated by permutation test (with a 10,000-permutation test). The genome-wide significance threshold at P value 0.05 (or 5 %) and 0.01 (or 1 %) was used to detect significant QTLs or highly significant QTLs, respectively. The linkage-group-wide significance level of 5 %, a less stringent threshold than used for previous analyses, was used to detect suggestive QTLs (QTLs that may be associated with phenotype but are not strongly supported, statistically). Suggestive QTLs are included for future study by peach researchers. When many SNPs markers were mapped in the same position (perfect linkage) only one marker, representing each specific haplotype block, was used in the QTL analysis to reduce computing time, all makers in each haplotype block show the same percentage of variance explained. A previous QTL study was conducted in the same population by Ogundiwin et al. (2007).

Results

SNP genotyping

The output of the Illumina GenomeStudio® Genotyping module should consist of two distinct progeny clusters with 1:1 AA or BB and AB allele segregation ratio 1:1 (Fig. 1a), identifying one parent as homozygous for A or B alleles and

the other as heterozygous for A and B alleles (Ahmad et al. 2011). In some cases, the output graphs showed three clusters, which indicate that both parents were heterozygous (AB). Cluster separation scores were used to indicate SNP quality (SNPs with a cluster separation score lower than 0.3 were removed) and data points ambiguously located between these clusters were scored as missing data (data with a GenCall score lower of 0.25). Acceptable SNPs had scores ≥0.5 in GenTrain, Heterozygote Excess values ≥−0.3, and Minor Allele Frequency values ≥0.08 (all scores are listed in supplemental file 1 and 2).

Pop-DF results: 1,400 of the 1,536 SNPs represented on the Illumina OPA (~91.14 %) were selected for mapping after screening with the Illumina retention scores. Of these, 528 SNPs (37.71 %) were homozygous for "Dr. Davis" and heterozygous for "F8,1-42", while 837 SNPs (59.79 %) were heterozygous for "Dr. Davis" and homozygous for "F8,1-42". Thirty five SNPs (2.50 %) were heterozygous in both parents.

Pop-DG results: 962 of 1,536 SNPs tested (~62.6 %) were selected for mapping after screening with the Illumina retention scores. Of these, 130 SNPs (13.51 %) were homozygous for "Dr. Davis" and heterozygous for "Georgia Belle", and 422 SNPs (43.87 %) were heterozygous for "Dr. Davis" and homozygous for "Georgia Belle". A significant proportion of SNPs (408 or 42.41 % versus the 2.5 % from Pop-DF) were heterozygous in both parents. A larger number of heterozygotes was observed in Pop-DG because the original SNP selection criteria of homozygote/heterozygote parental SNPs was applied to the Pop-DF parents and many of the "F8,1-42" homozygous SNPs were heterozygous in "Georgia Belle". Two SNPs, representing 0.21 % of the total set, were homozygous in both parents and were removed from the study.

The P-P-C heritability values varied from 0.69 to 1.00 (Fig. 2) in Pop-DF. The lowest values of P-P-C heritability ranged from 0.69 (for progeny DF-222) to 0.92 (for DF-234). The lower heritabilities for some SNPs were due to SNP call errors (Fig. 1b), with values from 430 to 101, respectively, may be caused by the inclusions of "Dr. Davis" selfed progeny in Pop-DF that were not eliminated in a prior screen, or a low-level of contamination with foreign pollen. Scoring errors (where the allelic values were miscalled in a progeny individual) were observed for 800 heterozygous SNPs for "Dr. Davis". The maximum number of errors was 26 observed in one SNP (UCD_SNP_70) followed by a group of 12 SNPs that showed 19 errors (UCD_SNP_42, UCD_SNP_123, UCD_SNP_130, UCD_SNP_339, UCD_SNP_425, UCD_SNP_496, UCD_SNP_599, UCD_SNP_627, UCD_SNP_1076, UCD_SNP_1241, UCD_SNP_1316, and UCD_SNP_1378). Based on the P-P-C heritability results, progeny with low heritability and high error numbers were removed, leaving 69 individuals with a heritability score of 1 and either zero or one error. These individuals were considered to have highly reliable and consistent inheritance of all loci (SNPs) used for mapping.

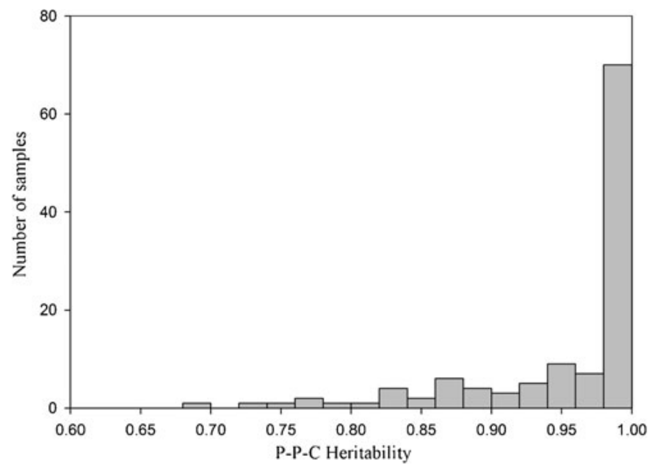

Fig. 2 Parent-parent-child heritability frequency from genomestudio for all individuals in Pop-DF

Linkage map

Linkage results from JoinMap®4.0 showed that in Pop-DF, 122 out of 1,400 (8.7 %) SNP markers showed segregation distortions (different from 1:1 or 1:2:1). As a result, these 122 SNPs were excluded from further map construction in Pop-DF. In Pop-DG, segregation distortions were not observed for any of the markers. To minimize spurious linkages, we used LODs of 5 or higher. The linkage groups were named and oriented by reference to their positions on the "peach v1.0" genome scaffolds, using the majority of the markers present in each linkage group to align the 0 reference position on both maps.

Pop-DF SNP map

A final set of 1,278 markers was used for linkage analysis in the Pop-DF population. The markers were organized into nine linkage groups, since linkage group G2 is represented by two unlinked segments (Fig. 3). Two hundred forty-one SNP markers could not be mapped with JoinMap®4. The total map distance was 442.24 cM, with 298 map positions (sites) for 1,037 SNPs. Approximately, 71 % of the mapped SNPs shared map positions in this population, due to the absence of recombination between some SNPs (co-segregating markers). Marker sites denote the unique recombination positions at which markers are found. Multiple markers

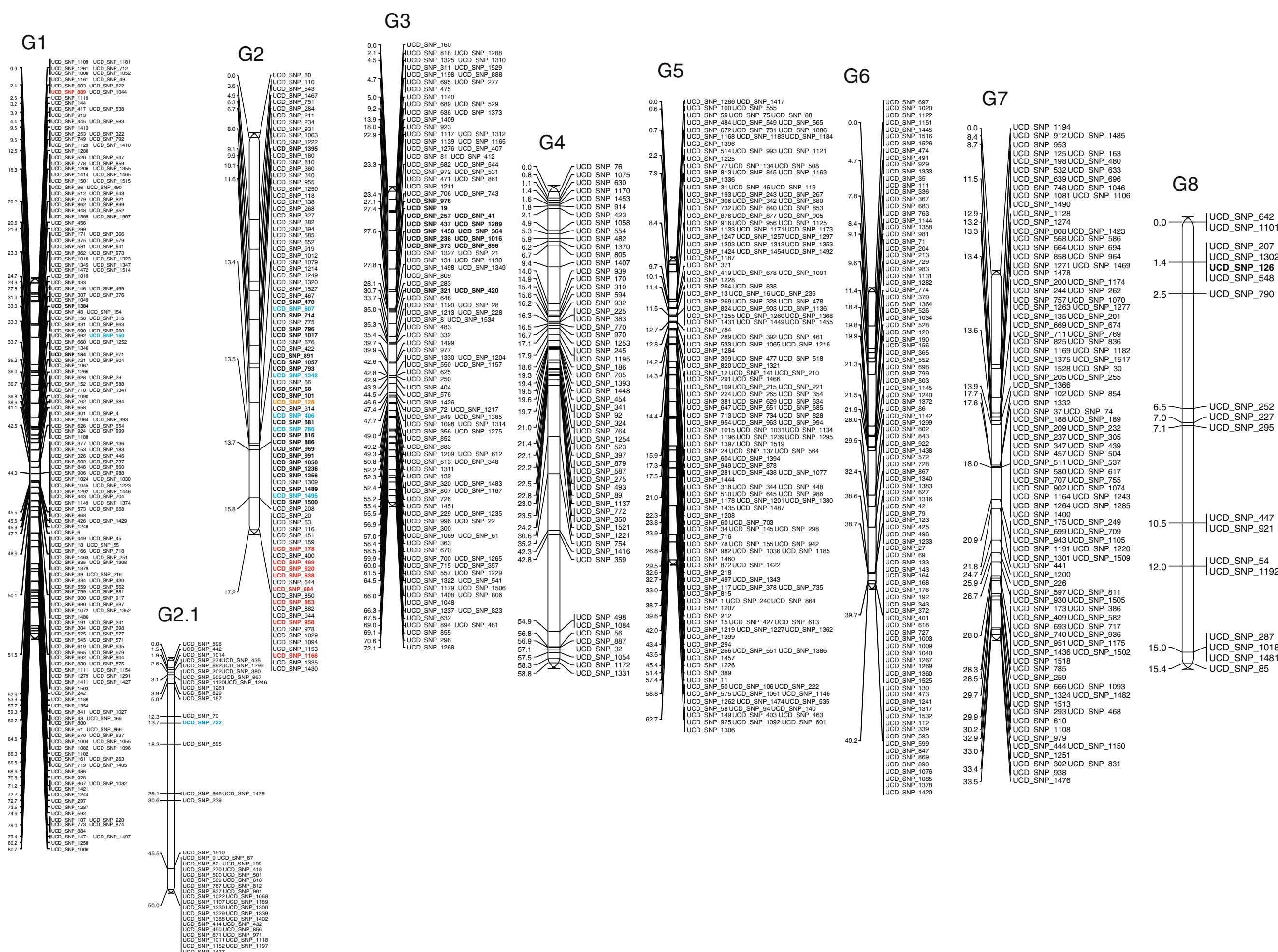

Fig. 3 A SNP map of Pop-DF constructed using JoinMap®4.0. Numbers on the left side show the distance in centiMorgans from the top of each chromosome. Detailed information about these markers is available in supplemental file 1. In linkage group G1, markers shown in *blue* come from scaffold three, markers shown in *bold* come from scaffold 15, and markers shown in *red* come from scaffold 127. In G2, markers in *bold* come from scaffold 4, *red* from scaffold 7, *blue* from scaffold 12, and *orange* from scaffold 8. In G3, markers in *bold* come from scaffold 4 and G8 markers in *bold* come from scaffold 53

are found at each marker site, where no recombination occurred between them (also can be considered to be haplotype blocks). The average marker site density for the 298 marker sites in the map was 1.48 cM/marker site. In general, marker site density was variable among linkage groups, from 1.01 cM/marker site in G7 and G2 to 3.57 cM/marker site in G2.1. The largest gap was 14.98 cM observed in G2.1. Two more large gaps of 13.54 and 8.44 cM were observed in G4 and G7, respectively. The G1 linkage group was the longest, 80.67 cM, and linkage group 8 was the shortest, 15.43 cM. The other linkage groups were from 17.22 (G2.1) to 70.33 (G4) cM in length.

The linkage positions of 54 SNP markers in the linkage maps differed from their positions as defined from the "peach v1.0" scaffold (Table 1). The "peach v1.0" draft assembly consists of eight major scaffolds corresponding to the eight peach linkage groups and a number of minor scaffolds, which are DNA tracts that could not be connected to any of the eight major scaffolds during the assembly process. From the construction of the linkage maps, the approximate location of some of these minor assemblies in the eight major scaffolds can be deduced as shown in Table 1.

Linkage group G1 had one SNP from scaffold three (UCD_SNP_150), two SNPs from scaffold 15 (UCD_SNP_184, 1384), and one SNP from scaffold 127 (UCD_SNP_889). In linkage group G2, 20 SNPs from scaffold four changed position (UCD_SNP_68, 101, 470, 681, 714, 793, 796, 816, 886, 891, 969, 991, 1017, 1050, 1057, 1236, 1256, 1395,1489, and 1500), as did eight SNPs from scaffold seven (UCD_SNP_178, 499, 620, 638, 684, 863, 958, and 1166), one SNP from scaffold eight (UCD_SNP_128), and five SNPs from scaffold 12 (UCD_SNP_406, 607, 786, 1495, and 1342). In linkage group

Table 1 Discrepancies in placement of SNPs between mapped positions and reported locations on “peach v1.0” assembly (the number of SNPs found on physical map scaffolds different than those expected from the SNP locations in the linkage maps are shown)

Linkage group:	Pop-DF				Pop-DG				Consensus Map				
	G1	G2	G3	G8	G1	G2	G3	G7	G1	G2	G3	G7	G8
Scaffold3	1	–	–	–	–	–	–	–	1	–	–	–	–
Scaffold4	–	20	14	–	–	–	6	–	–	1	6	–	–
Scaffold7	–	8	–	–	–	–	–	–	–	–	–	–	–
Scaffold8	–	1	–	–	–	–	–	–	–	–	–	–	–
Scaffold12	–	5	–	–	–	2	–	–	–	2	–	–	–
Scaffold15	2	–	–	–	2	–	–	–	3	–	–	–	–
Scaffold53	–	–	–	1	–	–	–	–	–	–	–	–	1
Scaffold62	–	–	–	–	–	–	–	1	–	–	–	1	–
Scaffold127	1	–	–	–	–	–	–	–	–	–	–	–	–

G3, 14 SNPs changed from scaffold four (UCD_SNP_19, 41, 238, 257, 321, 364, 373, 420, 437, 896, 1016, 1289, and 1450) and in linkage group G8, one SNP changed from scaffold 53 (UCD_SNP_126).

Pop-DG SNPs map

There were 11 linkage groups for Pop-DG because linkage groups G2, G4, and G6 had two unlinked segments (Fig. 4).

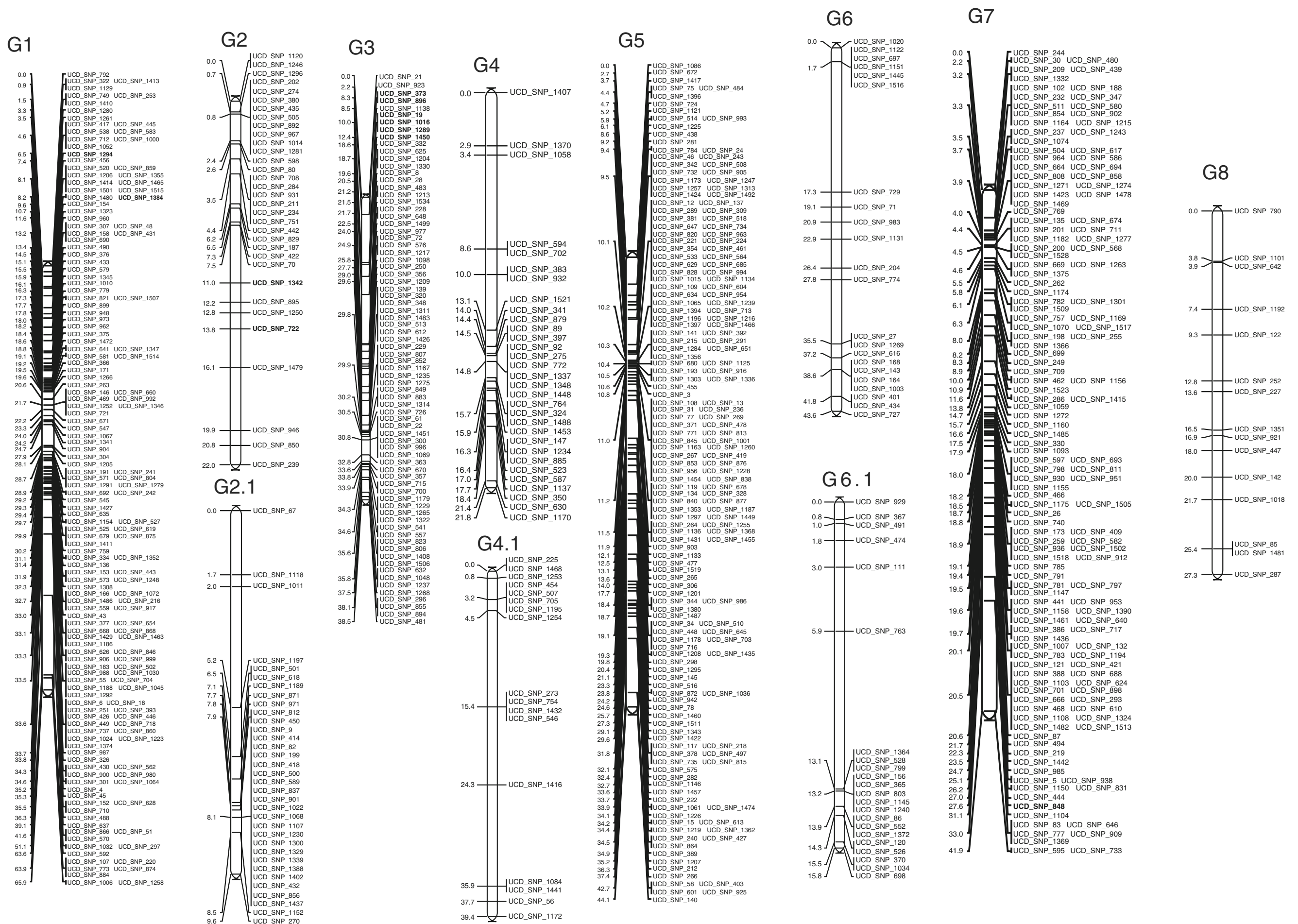

Fig. 4 A SNP map of Pop-DG constructed using JoinMap®4.0. *Numbers* on the *left side* show the distance in centiMorgans from the top of each chromosome. Detailed information about these markers is available in supplemental file 2. In G1, markers shown in *bold* come from scaffold 15. In G2, markers in *bold* come from scaffold 12. In G3, markers in *bold* come from scaffold 4. In G7, markers *bold* come from scaffold 62

The total distance covered by this map was 369.79 cM. A total of 51 % of the SNPs (co-segregating markers) shared common positions in this population, with 363 unique map positions observed for the 738 SNPs mapped. The average density, not counting multiple markers at a single map location, was 1.02 cM/marker site. Marker site density varied among linkage groups, from 0.63 cM/marker site in G4.1 to 2.73 cM/marker site in G5. The largest gap was 15.57 cM in G6; two more large gaps of 12.46 and 11.56 cM were observed in G1 and G4, respectively. Linkage group G1 was the longest at 65.88 cM and linkage group G8, the shortest at 27.28 cM; the rest varied between 9.55 cM (G2.1) and 44.11 cM (G5).

Eleven SNPs markers were found in different locations on the linkage map than would be expected from their positions in the "peach v1.0" scaffold alignments (Table 1); a majority of these positional differences were also found in Pop-DF. Linkage group G1 contained two SNPs from scaffold 15 (UCD_SNP_1384 and UCD_SNP_1294). In linkage group G2, there were two SNPs from scaffold 12 (UCD_SNP_722 and UCD_SNP_1342). In linkage group G3 were six SNPs from scaffold four (UCD_SNP_19, UCD_SNP_373, UCD_SNP_896, UCD_SNP_1016, UCD_SNP_1289, and UCD_SNP_1450) and in linkage group G7, one SNP came from scaffold 62 (UCD_SNP_848).

Consensus SNPs map

The consensus SNP map showed the expected eight linkage groups (G1 to G8) previously observed in peach. The map of 588 SNPs covered 454.80 cM, with 4.1 % of markers co-segregating and 564 unique map positions (Fig. 5). The average density was 0.81 cM/marker site. Marker site density varied among linkage groups, from 0.59 cM/marker site in G1 to 1.91 cM/marker site in G8. The largest gap was 14.25 cM, observed in G2, with three additional large gaps

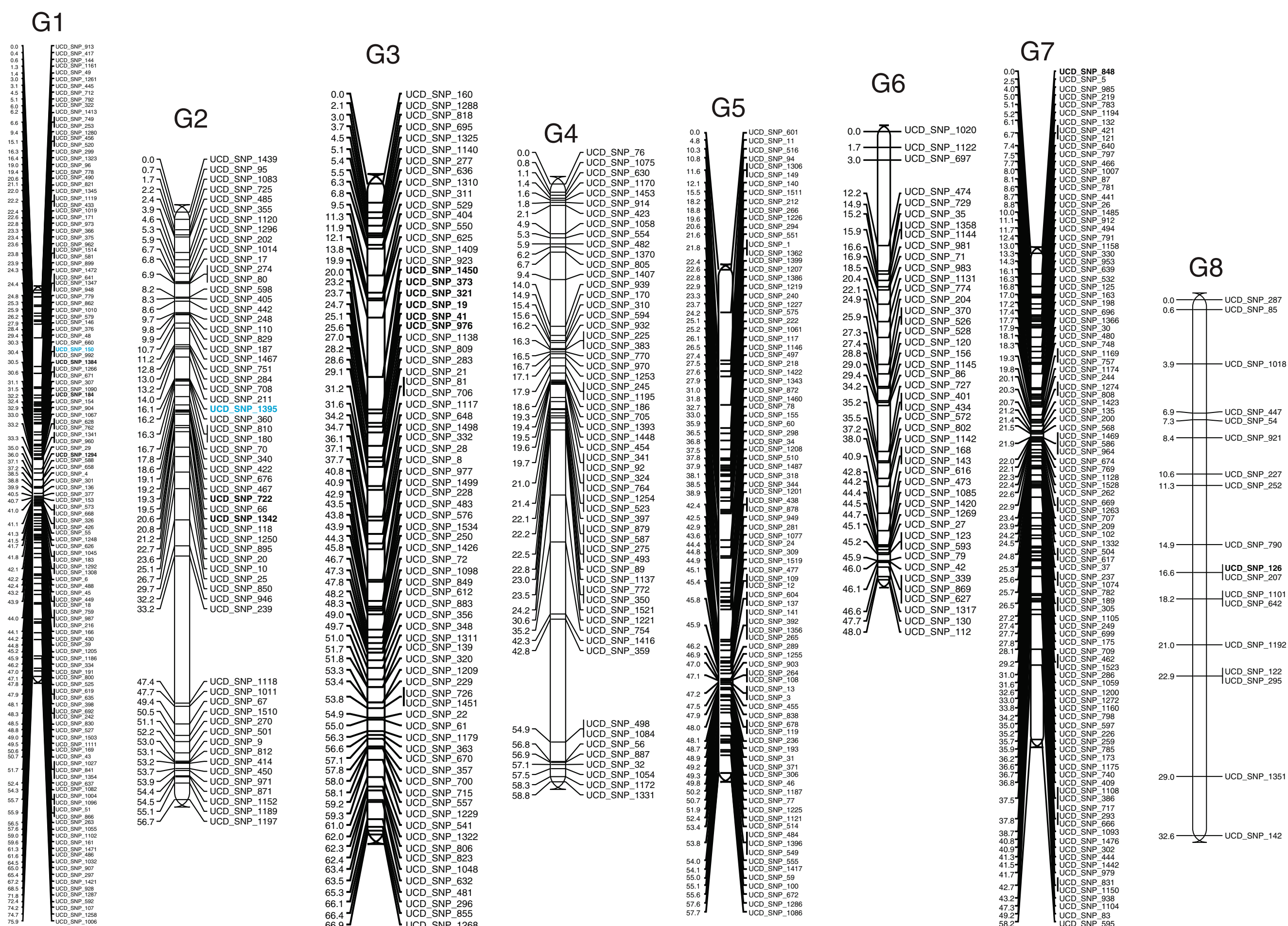

Fig. 5 A consensus SNPs map in peach derived from Pop-DF and Pop-DG was constructed using JoinMap®4.0. *Numbers* on the *left side* show the distance in centiMorgans from the top of each chromosome. Detailed information about these markers is available in supplemental file 1 and 2. In G1, *blue marker* comes from scaffold 3 and *bold markers* come from scaffold15. In G2, *bold markers* come from scaffold 12 and *blue* from scaffold 4. In G3, *bold markers* come from scaffold 4. In G7 and G8, *bold markers* come from scaffold 62 and 53, respectively

of 12.04, 9.18, and 8.98 cM in G8, G6, and G7, respectively. Linkage group G1 was the longest, spanning 75.90 cM, and linkage group G8 was the shortest covering 32.60 cM. The length of the other groups varied between 48.02 cM for G6 and 66.85 cM for G3.

Fifteen SNPs had a change in position in the consensus SNP map, compared to their locations in the original scaffold alignments (Table 1). Linkage group G1 included three SNPs (UCD_SNP_184, 1294, 1384) from scaffold 3 and one SNP (UCD_SNP_150) from scaffold 15. Linkage group G2 included two SNPs from scaffold 12 (UCD_SNP_722 and UCD_SNP_1342) and one SNP from scaffold 4 (UCD_SNP_1395). Linkage group G3 had six SNPs from scaffold 4 (UCD_SNP_19, UCD_SNP_41, UCD_SNP_321, UCD_SNP_373, UCD_SNP_976, and UCD_SNP_1450). Linkage group G7 had UCD_SNP_848 from scaffold 62. Group G8 had UCD_SNP_126 from scaffold 53.

Comparison of SNP physical positions in scaffold alignments and genetic positions in linkage maps

Mapped SNPs in Pop-DF suggest the presence of inversions based on their physical positions on the "peach v1.0" alignments. Linkage groups three, four, and six had high numbers of inversions based on physical and genetic maps of Pop-DF. In Pop-DG, the linkage groups with the most possible inversions were two, four, and seven. The linkage groups one, five, seven, and eight show high homology with the "peach v1.0" physical map in both populations (Fig. 6).

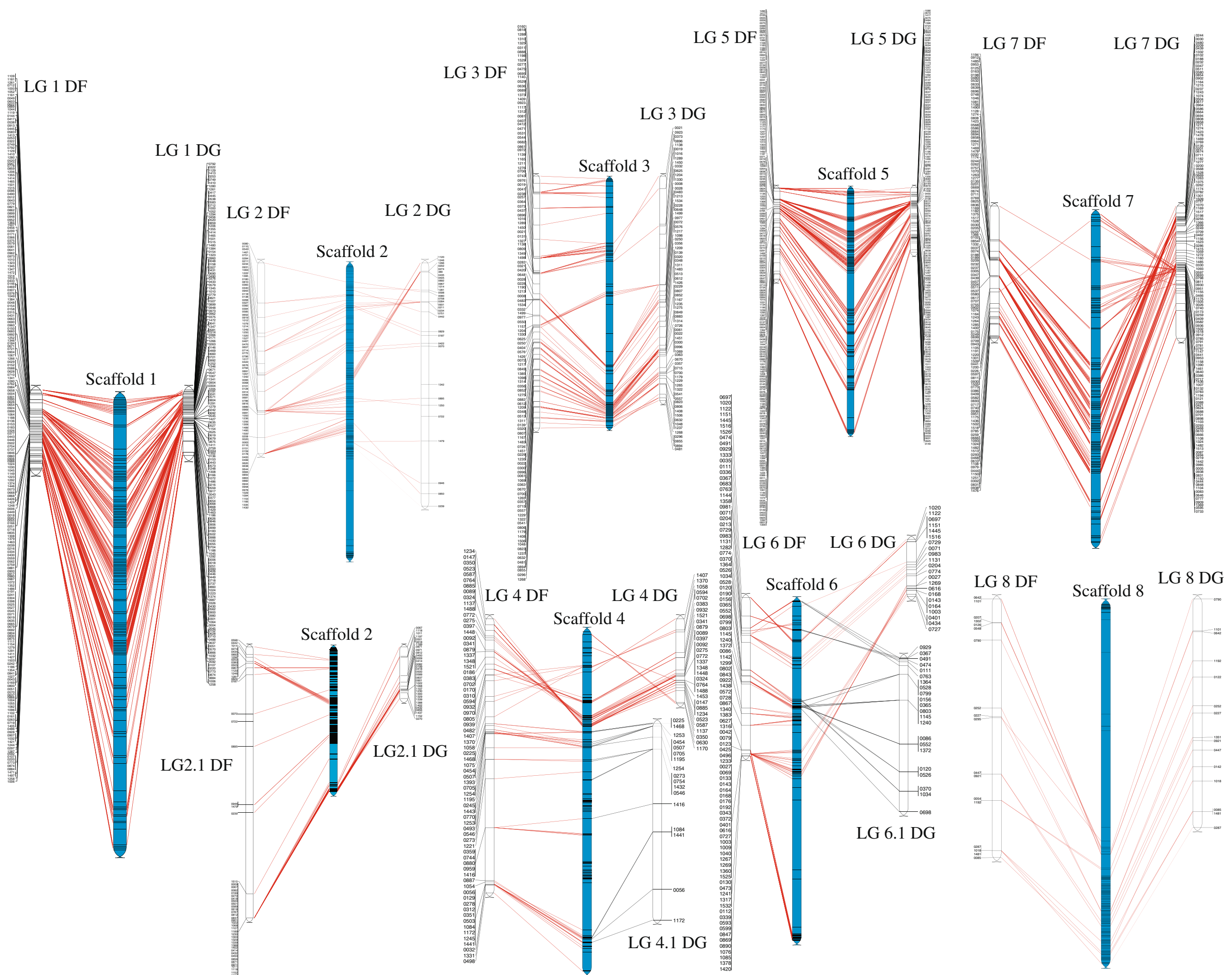

Fig. 6 Comparison of the positions of SNPs in the scaffolds of "peach v1.0" with their position in the genetic maps obtained for Pop-DF and Pop-DG. The physical map for each chromosome is shown in *blue*

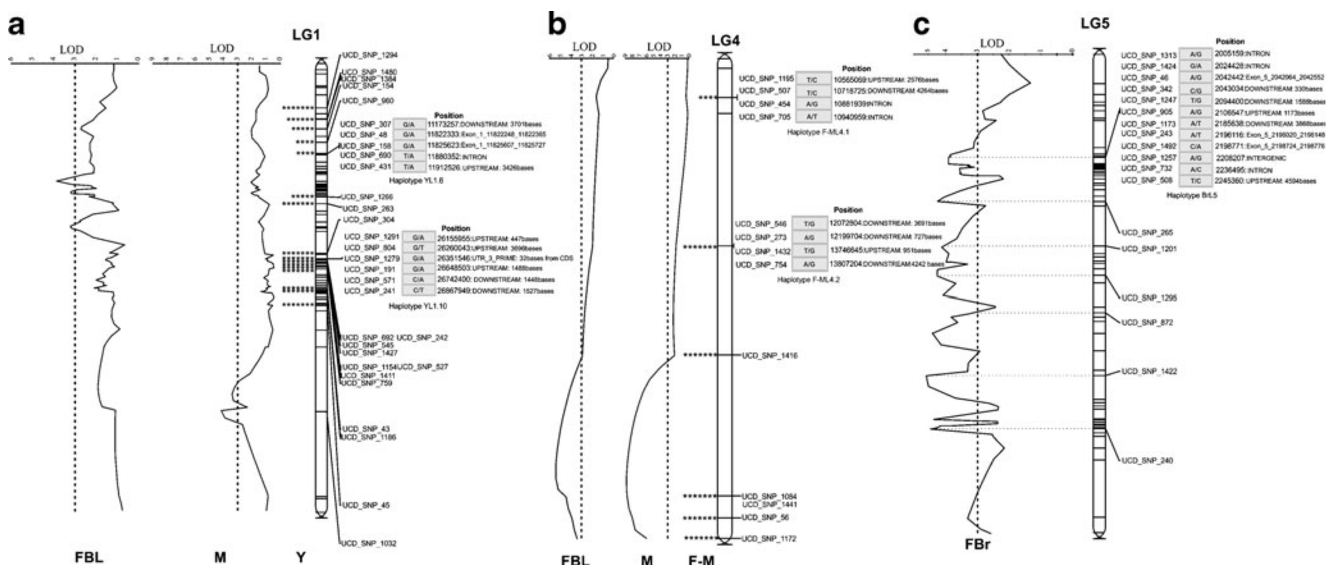

Fig. 7 QTL and haplotype blocks obtained for fruit quality and chilling injury symptoms in Pop-DG. **a** QTL for yellow flesh (Y), mealiness (M), and flesh bleeding (FBL) in linkage group 1 (LG1). **b** QTL for freestone-melting flesh (F-M), mealiness (M), and flesh bleeding (FBL) in linkage group 4 (LG4). **c** QTL for flesh browning (FBr) in linkage group 5 (LG5), *horizontal lines* highlight the positions with high percentage of variance explained

QTL detection

The results obtained from the nonparametric Kruskal–Wallis and χ^2 contingency table tests for yellow flesh (Y) revealed that 30 SNP markers from linkage group 1 (LG1) were strongly associated with this trait (Fig. 7, Table 2); all of these markers were very highly significant ($P<0.005$). The significant markers associated with Y were located in 19 different regions from qYL1.1 to Y 1.19, distributed through LG1. UCD_SNP_759, UCD_SNP_242 and UCD_SNP_692 showed the greatest K–W values; 28.15 for the first one and 23.4 for the last two. The SNP marker UCD_SNP_759 was located in qYL1.16 region (30.169 cM) and UCD_SNP_242 and UCD_SNP_692 in qYL1.11 region (28.93 cM).

Thirteen SNPs were significantly associated with freestone-melting flesh (F-M) on linkage group 4 (LG4). These markers were located in six different regions from qF-ML4.1 to qF-ML4.6 on LG4 (Fig. 7, Table 3). This QTL appeared to be spread across several markers starting from UCD_SNP_454 to UCD_SNP_1172, spanning about 60 % of the length of LG4. The regions qF-ML4.4 (35.98 cM), qF-ML4.5 (37.73 cM), and qF-ML4.6 (39.39 cM) contain four different SNPs markers UCD_SNP_ 1084, UCD_SNP_1441, UCD_SNP_56, UCD_SNP_1172, which had high K–W values for F-M. The SNP markers UCD_SNP_ 1084 and UCD_SNP_1441, located in qF-ML4.4, had the highest K–W values.

The genome-wide LOD significance thresholds for the CI variables were obtained using permutation tests. The values were 5.0, 7.0, and 4.5 for mealiness (M), flesh bleeding (FBL), and flesh browning (FBr), respectively, while the linkage-wide-group LOD significance threshold varied from 2.6 to 2.7.

One highly significant ($P<0.01$ for genome-wide significance) and four suggestive (linkage-group-wide significant) QTLs were detected by the whole genome scan. One highly significant QTL (qML4) was detected for M on LG4. The qML4 was detected near markers UCD_SNP_1084 and UCD_SNP_1441. The phenotype variance explained by these loci was 58.8 %. The suggestive QTL for M in LG1, qML1, was detected on the region near marker UCD_SNP_1032; this locus explained 80.9 % of the observed phenotypic variance of this trait (Table 4). The SNP marker UCD_SNP_297 was also close to qML1 and explained 80.9 % (LOD 4.16) of the observed phenotypic variance (data not shown).

Two suggestive QTLs for FBL and one for FBr (spread over a large part of LG5) were located on LG1, LG4, and LG5 (Fig. 7), respectively. The two QTLs (qBL1 and qBL4) for FBL explained around 30 % and 34 % of the phenotypic variance; several markers were significantly associated with the QTLs. For qBL1, the markers UCD_SNP_821, UCD_SNP_1507, UCD_SNP_641, UCD_SNP_ 1347, UCD_SNP_1067 explained between 25.5 % and 30.9 % of the phenotype variance observed. For qBL4, SNP markers UCD_SNP_1084 and UCD_SNP_1441 explained 33.6 % of the phenotype variance observed. These loci were also significantly associated with freestone-melting flesh (qF-ML4.4) and M (qML4) on LG4 (Table 4).

The QTL for FBr on LG5 (qBrL5) was associated with several markers. The most significant markers explained between 29.2 % and 35.8 % of the phenotype

Table 2 SNP markers and positions associated with yellow flesh (Y)

Markers	Name	Kruskal–Wallis[a]					Contingency table[a]	
		LG	Position	K[b]	Df[c]	Significance	χ^2	Significance
UCD_SNP_1294	qYL1.1	1	6.488	15.391	1	*******	15.71	*****
UCD_SNP_1480	qYL1.2	1	8.163	13.25	1	******	13.52	*****
UCD_SNP_1384	qYL1.3	1	8.226	12.485	1	******	12.74	*****
UCD_SNP_154	qYL1.4	1	9.636	11.308	1	*****	11.54	*****
UCD_SNP_960	qYL1.5	1	11.639	9.486	1	****	9.68	***
UCD_SNP_307	qYL1.6	1	13.197	9.486	1	****	9.68	***
UCD_SNP_48	qYL1.6	1	13.197	9.486	1	****	9.68	***
UCD_SNP_158	qYL1.6	1	13.197	9.486	1	****	9.68	***
UCD_SNP_431	qYL1.6	1	13.197	9.486	1	****	9.68	***
UCD_SNP_690	qYL1.6	1	13.197	9.486	1	****	9.68	***
UCD_SNP_1266	qYL1.7	1	19.567	14.052	2	*****	14.33	*****
UCD_SNP_263	qYL1.8	1	20.609	22.658	1	*******	23.12	*****
UCD_SNP_304	qYL1.9	1	27.88	22.82	2	*******	23.29	*****
UCD_SNP_191	qL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_241	qL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_571	qYL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_804	qYL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_1291	qYL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_1279	qYL1.10	1	28.687	22.12	2	*******	22.57	*****
UCD_SNP_692	qYL1.11	1	28.931	23.447	2	*******	23.93	*****
UCD_SNP_242	qYL1.11	1	28.931	23.447	2	*******	23.93	*****
UCD_SNP_545	qYL1.12	1	29.184	21.12	2	*******	21.57	*****
UCD_SNP_1427	qYL1.13	1	29.349	22.447	2	*******	22.93	*****
UCD_SNP_1154	qYL1.14	1	29.693	22.82	2	*******	23.28	*****
UCD_SNP_527	qYL1.14	1	29.693	22.82	2	*******	23.28	*****
UCD_SNP_1411	qYL1.15	1	29.932	21.425	2	*******	21.87	*****
UCD_SNP_759	qYL1.16	1	30.169	28.153	2	*******	28.72	*****
UCD_SNP_43	qYL1.17	1	33.001	15.167	2	*****	15.47	*****
UCD_SNP_1186	qYL1.18	1	33.097	18.329	2	******	18.7	*****
UCD_SNP_45	qYL1.19	1	35.303	15.124	2	*****	15.43	*****

[a]**: 0.05; ***: 0.01; ****: 0.005; *****: 0.001; ******: 0.0005; *******: 0.0001

[b]*Df* degree of freedom

[c]*K* Kruskal–Wallis test statistic (Lehmann 1975)

variance for this trait (Table 4). Four haplotype blocks were associated with fruit quality (two in LG1 for yellow flesh and two in LG4 for F-M) and one haplotype block was associated with flesh browning in LG5. The description of each haplotype block, name of SNPs, base and positions on the scaffolds are shown in Fig. 7.

Discussion

The 6,654 high quality SNPs selected from two genetically distinct cultivars of peach ("Dr. Davis" and "Georgia Belle") and one interspecific breeding line ("F8,1-42") of the peach breeding program at UC Davis (Ahmad et al. 2011) were validated by selection of 1,536 SNPs (from Pop-DF parents) and generation of two separate linkage maps, both of which showed a high level of homology with the draft "peach v1.0" physical map (Fig. 6).

The high-throughput Illumina GoldenGate protocol was used to evaluate 1,536 SNPs in peach for map generation. Of the 1,536 SNP loci, 1,400 (91.14 %) were successfully validated for genotyping Pop-DF and 962 (62.63 %) for Pop-DG genotyping. The large number of mapped SNP markers generated from this oligonucleotide assay pool demonstrates the efficacy of identifying SNPs from parent genome sequencing in peach. The 1,407 true SNPs from both populations, combined, represent 92 % of the SNPs evaluated, with only an 8 % error rate. This percentage was lower than the previously observed (36 %) error rate in rhesus macaque [Zimmermann] (Malhi et al. 2007). As observed in other crops such as barley (Rostoks et al.

Table 3 Number of SNP markers and positions associated with freestone-melting flesh (F-M)

Markers	Name	Kruskal–Wallis[a]					Contingency table[a]	
		LG	Position	K*	Df	Significance	χ^2	Significance
UCD_SNP_454	qF-ML4.1	4	3.157	7.955	1	****	8.12	**
UCD_SNP_507	qF-ML4.1	4	3.157	7.955	1	****	8.12	**
UCD_SNP_705	qF-ML4.1	4	3.157	7.955	1	****	8.12	**
UCD_SNP_1195	qF-ML4.1	4	3.157	7.955	1	****	8.12	**
UCD_SNP_273	qF-ML4.2	4	15.36	20.364	1	*******	18.26	*****
UCD_SNP_754	qF-ML4.2	4	15.36	20.364	1	*******	18.26	*****
UCD_SNP_1432	qF-ML4.2	4	15.36	20.364	1	*******	18.26	*****
UCD_SNP_546	qF-ML4.2	4	15.36	20.364	1	*******	18.26	*****
UCD_SNP_1416	qF-ML4.3	4	24.329	29.126	1	*******	26.69	*****
UCD_SNP_1084	qF-ML4.4	4	35.891	45.16	1	*******	42.25	*****
UCD_SNP_1441	qF-ML4.4	4	35.891	45.16	1	*******	42.25	*****
UCD_SNP_56	qF-ML4.5	4	37.729	41.368	1	*******	38.57	*****
UCD_SNP_1172	qF-ML4.6	4	39.389	36.962	1	*******	34.28	*****

[a] **: 0.05; ***: 0.01; ****: 0.005; *****: 0.001; ******: 0.0005; *******: 0.0001

[b] *K* Kruskal–Wallis test statistic (Lehmann 1975)

[c] *Df* degree of freedom

2006), tomato (Shirasawa et al. 2010), soybean (Hyten et al. 2008), and maize (Jones et al. 2009), these results confirm that the GoldenGate assay can be used in peach to evaluate SNPs and create a high-density genetic linkage map. While SNPs were selected for 1:1 segregation (filter 3 in Ahmad et al. 2011), some SNPs exhibited a 1:2:1 segregation ratio, especially in Pop-DG, showing that both Pop-DG parents were heterozygous for that group of SNPs. Therefore, the selection of SNPs with particular allelic composition in one cross cannot be used to guarantee the applicability of those

Table 4 Identification of QTLs controlling mealiness, bleeding, and browning in the F_1 population from "Dr Davis"×"Georgia Belle"

Traits	QTL name	Flanking markers	Most significant markers	LOD score (Max) and (position (cM))	LOD threshold		R^2
					Genome-wide	Linkage-group-wide	
Mealiness	qML1	UCD_SNP_866–592	UCD_SNP_1032	4.18 (51.06)	5.0	2.7	80.9
	qML4.1	UCD_SNP_1416–1172	UCD_SNP_1084 UCD_SNP_1441	8.74[a] (35.891)	5.0	2.7	58.8
Bleeding	qBL1a	UCD_SNP_779–973	UCD_SNP_821 UCD_SNP_1507	3.86 (17.28)	7.0	2.7	29.9
	qBL1b	UCD_SNP_1472–366	UCD_SNP_641 UCD_SNP_1347	3.19 (18.80)	7.0	2.7	25.5
	qBL1c	UCD_SNP_547–904	UCD_SNP_1067	3.22 (23.96)	7.0	2.7	30.9
	qBL4.1	UCD_SNP_1416–1172	UCD_SNP_1084 UCD_SNP_1441	4.45 (35.891)	7.0	2.7	33.6
Browning	qBrL5	UCD_SNP_438–140	UCD_SNP_46	3.72 (9.50)	4.5	2.6	29.2
			UCD_SNP_265	4.07 (13.59)	4.5	2.6	34.3
			UCD_SNP_1201	3.94 (17.73)	4.5	2.6	35.0
			UCD_SNP_1295	4.08 (20.41)	4.5	2.6	33.4
			UCD_SNP_872	3.09 (23.84)	4.5	2.6	32.1
			UCD_SNP_1422	4.42 (29.56)	4.5	2.6	35.8
			UCD_SNP_240	4.28 (34.46)	4.5	2.6	34.8

For each QTL detected, the linkage group maximum LOD score, and percentage of variance explained (R^2) are indicated. The genome-wide LOD significance threshold are 5.0 mealiness, 7.0 for bleeding, and 4.5 for browning

[a] Highly significant QTL

SNPs in other crosses for which sequence data is not available. However, the results of this study show that a large number of the selected SNPs are transferable, and while the same SNPs will not be useful in all crosses, it appears that a significant majority will be transferable to different crosses. Hyten et al. (2008) showed that soybean SNPs with a >0.1 minor allele frequency (MAF) could be useful for marker-assisted selection, QTL mapping and association analysis. Only 91 SNPs from the 1,400 SNP set of Pop-DF show values between 0.088 and 0.099, the rest of the SNPs in both population show values of MAF greater than 0.1.

Low P-P-C heritability frequencies were found in 48 individuals of Pop-DF. They are probably due to relatively few SNPs that contributed most of the errors. Sources of these errors could be errors in OPA construction, priming, optical reading, or the location of SNPs in highly mutable regions of the genome. A morphological analysis for the presence of nectarines (Glabrous (*G*) locus (Bailey and French 1949)) was performed in Pop-DF; only two (DF-154 and DF-120) were found and were not included in this study. Since Glabrous is recessive and "Dr. Davis" is heterozygous for the *G* locus, with a segregation ratio of 1:2:1, six additional individuals in the original mapping population of 117 progeny were known to be selfs. These were eliminated during the removal of the 48 progeny with low quality scores. Low numbers of "Dr. Davis" selfed progeny were observed in a prior study (Peace et al. 2005) when "Dr. Davis" was used as a female parent. The authors of that paper used SSR locus BPPCT-018 to identify 18 "Dr. Davis" selfed progeny in a different mapping population. We used the same marker to screen the 48 Pop-DF individuals removed from the analysis due to low quality scores. Only two genotypes had the expected selfed genotypes (data not shown).

Three genetic maps were obtained from the final edited set of SNPs. The genetic distance of each was intermediate compared to previously published peach and almond maps: smaller than the 519-cM TxE map (*Prunus* reference map) (Dirlewanger et al. 2004). However, the consensus map was denser, at 0.81 cM/marker site (unique map position), than all previous maps. Despite the high number of SNP markers used, the map length was unchanged, principally because the number of progeny used from Pop-DF was reduced to 69. A large progeny set will be desirable for additional map resolution and funding is being sought for that effort.

The segregation distortion ratio computed by JoinMap®4.0 in Pop-DF and Pop-DG was lower (8.7 % and 0 %, respectively) than the 18.5 % observed in a cross of two closely related species, *P. persica* and *Prunus ferganensis* (Kostov and Rjabov) Kovalev and Kostov (Dettori et al. 2001). The Pop-DG values were very similar to the 2 % reported from an intraspecific cross of "Ferjalou Jalousia®"×"Fantasia" (Dirlewanger et al. 1998). The distortion in Pop-DF may be explained by the inter-specific nature of the peach×(almond×peach) progeny. In addition, the low distortion observed could be a consequence of the high selection pressure used to select the SNPs as recommended by the Infinium® Genotyping Data Analysis from Illumina® Technical Note (www.illumina.com).

Results from the comparison of genetic map and physical genome alignments confirm the efficiency of JoinMap®4.0 for mapping and further validate the results obtained for each population. More studies must be carried out to determine if the large apparent number of inversions observed in Pop-DF is a consequence of almond parentage in its background. This knowledge will be of increasing importance with increased use of exotic germplasm to overcome the limitations of a narrow germplasm base in crop breeding programs.

Chromosomal translocation events in linkage group G2, which had several SNPs from scaffold 4, were also identified by not using the "fixed order" option in JoinMap®4.0. These may be similar or identical to previously reported reciprocal translocations between almond and peach (Jauregui et al. 2001). In the Pop-DF population, a possible translocation event could be studied using anchor markers from other populations of the same or related species. Combining common anchor markers like SSRs with the high number of SNPs mapped in this study will thus provide an important new tool for the *Prunus* breeding community. The marker information from this study will be made publicly available through the Genome Database for Rosaceae (GDR) as well as by reference to the supplemental tables provided with this report.

The segregating QTLs obtained by K-W tests showed the typical gradient towards the markers with the closest linkage to the QTL. This nonparametric test is the best option to analyze traits that present a non-normal distribution or in the case of one trait present a clear segregation distortion. The simple Mendelian inheritance of freestone melting flesh (F-M) and yellow flesh (Y) have been assumed by several authors (Peace et al. 2004; Ogundiwin et al. 2009) based on phenotype scoring in several crosses. The statistically significant association obtained between several SNPs and both traits studied suggest that Y and F-M are not conditioned by single loci as had previously been reported (Peace et al. 2004). According to Peace and Norelli (2009), two copies of the same gene form the F-M locus and different effects such as additivity, dominance, pleiotropy, and perhaps epistasis between both influence the phenotype of each individual. However, our results suggest that multiple modifier genes (or duplicate loci) could be present and associated with the Y or F-M phenotypes.

Several explanations can be suggested for these results. One of the most likely explanations for the observed linkage

(statistically significant associations between different loci to each trait) is a cryptic linkage disequilibrium (LD) resulting from the population structure typical of this type of mapping population. Other explanations for this linkage can be that these loci are related to upstream/downstream elements in the pathway for the yellow flesh other than the simply inherited Y locus gene or because the presence of new alleles that may have been introduced from almond, and which were not scored previously. However, the most likely explanation for the observed cryptic LD is the limited number of samples evaluated, providing limited opportunities for recombination.

There is a high correlation (R=0.70) between mealiness (M) and flesh bleeding (FBL) for Freestone Melting Flesh (F-M) progeny in Pop-DG (Martínez-García et al. 2012). Samples susceptible to M also tended to be more susceptible to FBL for all seedlings and mealy fruit in general tended to have more flesh bleeding. FBL is associated with mealy fruit of many cultivars (Crisosto et al. 1999, 2008). Although the physiological basis for this connection is unclear, it is suspected to be related to tissue senescence and decreased membrane permeability (Lurie and Crisosto 2005). Ogundiwin et al. (2007) found that mealiness and flesh bleeding were controlled by the same major QTL on linkage group G4, explaining 43 % and 61 % of the phenotype variance observed for each trait, respectively, the peak of this QTL falls on a gene for *endoPG* and the co-located morphological marker, *F-M*. Our results confirm the co-location of both QTLs of each trait in LG4 and confirm the association between the genomic region in LG4 and F-M flesh and chilling injury symptoms such as M and FBL.

The SNP marker UCD_SNP_1084, a significant marker for freestone-melting flesh, mealiness, and flesh bleeding, revealed a Guanine to Adenine transition. The change represents a synonymous substitution for lysine, and is found in an intronic region, so is not functionally responsible for the phenotypic difference. The female cultivar "Dr. Davis" showed a genotype (GG) and "Georgia Belle" presented a genotype (GA) for this locus. The frequency of both genotypes (GG and GA) in the entire population was 42 % and 58 %, respectively. "Dr. Davis" (GG) is resistant to M and "Georgia Belle" (GA) has a high susceptibility to M. The progenies with the GG genotype showed resistance to M and progenies with GA genotype presented a medium-low susceptibility to M (data not shown). The SNP marker UCD_SNP_1084 could be an important biomarker for fruit quality and chilling injury symptoms such as M and FBL in peach. More research is needed to determine the potential of this marker for selection in peach breeding programs.

QTLs for flesh browning (FBr) appear to be similar to those found, previously, by Ogundiwin et al. (2007) on LG5 of Pop-DG where three significant QTLs (LOD >3) were observed. As might be expected, the results showed no common QTLs between FBr and the other two CI symptoms despite significant phenotypic correlations among them. This result indicates that either environmental factors or different genetic components than those identified for M and FBL underlie the physiological expression of fruit FBr following cold storage.

Previously, high levels of linkage disequilibrium conservation have been observed in peach cultivars from Europe and North America and were explained by cultivar development through self-pollination and by a bottleneck in modern breeding programs due to the limited germplasm pools used for crossing (Aranzana et al. 2010). Our results identified a total of five different haplotype blocks, sites with multiple markers, associated with the fruit quality traits analyzed here that can be explained by the presence of cryptic LD or low frequency of recombination. In addition, the majority of markers from each haplotype block are located in non-coding regions such as intron, intergenic regions, or downstream and upstream of genes. These are regions with reduced selection pressure (Wong and Nielsen 2004) and are more likely to contain regulatory sequences (Colinas et al. 2002). However, at present there is insufficient information about the functions of non-coding regions in peach to determine if our results represent functional associations with quality traits or are historical artifacts. Their use in haplotype-assisted selection (HAS) in peach will require additional study.

Conclusions

The results presented in this paper are part of a larger project that began with the sequencing of specific peach cultivars, followed by development of SNP markers in a breeding population segregating for important quality traits, construction of high-density SNP maps, and analysis of QTLs and determination of genome regions controlling fruit quality characteristics.

A large number of good quality SNPs have been successfully genotyped and used to map two peach breeding populations. Two saturated peach SNP linkage maps were generated in this study and a third high-density SNP marker consensus map was obtained. In addition to the specific SNP markers that have been associated with fruit quality traits and chilling injury symptoms, several haplotype blocks have been associated to those traits, more studies must be needed to dissect these blocks and to determine their potential use in haplotype-assisted selection in peach breeding programs. The SNP maps, locations of SNPs on the physical map, and QTL maps will be available through GDR for anchoring and annotating the peach genome.

Acknowledgements We gratefully acknowledge the assistance of DNA Technologies Core at the University of California, Davis as well as the support of the National Research Initiative of USDA's National Institute of Food and Agriculture (NIFA) grant # 2008-35300-04432 and US-Israel Bi-national Agriculture Research and Development Fund (BARD) Grant no. US-4027-07 for provided financial support to this project, as well as UC Davis, UC Agricultural Experiment Station and USDA-CREES (Hatch Experiment Station funding). We would especially like to thank Maria J. Truco from UC Davis Genome Center for helpful comments on JoinMap®4.0, Jonathan Fresnedo-Ramírez, PhD student at UC Davis, for his collaboration in data processing and we gratefully, acknowledge Dr. Cameron Peace for his contributions to our earlier discussion of potential traits for peach marker assistance selection.

References

Abbott AG, Rajapakse S, Sosinski B, Lu ZX, Sossey-Alaoui K, Gannavarapu M, Reighard G, Ballard RE, Baird WV, Scorza R, Callahan A (1998) Construction of saturated linkage maps of peach crosses segregating for characters controlling fruit quality, tree architecture and pest resistance. Acta Horticulturae 465:41–49

Abbott AG, Georgi L, Inigo M, Sosinski B, Yvergniaux D, Wang Y, Blenda A, Reighard G (2002) Peach: the model genome for Rosaceae. Acta Horticulturae 575:145–155

Abbott AG, Arús P, Scorza R (2007) Peach. In: Kole C (ed) Fruits and nuts, vol 4. Genome mapping and molecular breeding in Plants. Springer, Berlin, Heidelberg, pp 137–156. doi:10.1007/978-3-540-34533-6_5

Abbott AG, Arús P, Scorza R (2008) Genetic engineering and genomics. In: Desmond RL, Bassi D (eds) The peach: botany, production and uses. CABI, UK, pp 85–105

Ahmad R, Parfitt D, Fass J, Ogundiwin E, Dhingra A, Gradziel T, Lin D, Joshi N, Martínez-García PJ, Crisosto C (2011) Whole genome sequencing of peach (*Prunus persica* L.) for SNP identification and selection. BMC Genom 12:569. doi10.1186/1471-2164-12-569

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theor Appl Genet 119(3):507–517. doi:10.1007/s00122-009-1059-5

Aranzana MJ, Garcia-Mas J, Carbo J, Arús P (2002) Development and variability analysis of microsatellite markers in peach. Plant Breed 121(1):87–92

Aranzana MJ, Kim S, Zhao KY, Bakker E, Horton M, Jakob K, Lister C, Molitor J, Shindo C, Tang CL, Toomajian C, Traw B, Zheng HG, Bergelson J, Dean C, Marjoram P, Nordborg M (2005) Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. PLoS Genet 1(5):531–539. doi:10.1371/journal.pgen.0010060

Aranzana MJ, Abbassi EK, Howad W, Arús P (2010) Genetic variation, population structure and linkage disequilibrium in peach commercial varietes. BMC Genet 11:69

Bailey JS, French AP (1949) The inheritance of certain fruit and foliage characters in the peach. Mass Agr Exp Sta Bul 452

Ball A, Stapley J, Dawson D, Birkhead T, Burke T, Slate J (2010) A comparison of SNPs and microsatellites as linkage mapping markers: lessons from the zebra finch (*Taeniopygia guttata*). BMC Genom 11(1):218

Batley J, Edwards D (2009) Mining for SNPs and SSRs using SNPServer, dbSNP and SSR taxonomy tree. Meth Mol Biol 537:303–321. doi:10.1007/978-1-59745-251-9_15

Blenda AV, Verde I, Georgi LL, Reighard GL, Forrest SD, Munoz-Torres M, Baird WV, Abbott AG (2007) Construction of a genetic linkage map and identification of molecular markers in peach rootstocks for response to peach tree short life syndrome. Tree Genet Genomes 3(4):341–350. doi:10.1007/S11295-006-0074-9

Bliss FA, Arulsekar S, Foolad MR, Becerra V, Gillen AM, Warburton ML, Dandekar AM, Kocsisne GM, Mydin KK (2002) An expanded genetic linkage map of Prunus based on an interspecific cross between almond and peach. Genome 45(3):520–529. doi:10.1139/G02-011

Chaparro JX, Werner DJ, O'Malley D, Sederoff RR (1994) Targeted mapping and linkage analysis of morphological isozyme, and RAPD markers in peach. Theor Appl Genet 87(7):805–815. doi:10.1007/bf00221132

Colinas J, Birnbaum K, Benfey PN (2002) Using cauliflower to find conserved non-coding regions in *Arabidopsis*. Plant Physiol 129:451–454

Crisosto CH, Mitchell FG, Johnson S (1995) Factors in fresh market stone fruit quality. Postharvest News Inform 6(2):17–21

Crisosto CH, Mitchell FG, Ju ZG (1999) Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. HortSci 34(6):1116–1118

Crisosto CH, Crisosto GM, Day KR (2008) Market life update for peach, nectarine, and plum cultivars grown in California. Adv Hortic Sci 22(3):201–204

Dettori MT, Quarta R, Verde I (2001) A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. Genome 44 (5):783–790

Dirlewanger E, Pronier V, Parvery C, Rothan C, Guye A, Monet R (1998) Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. Theor Appl Genet 97(5–6):888–895

Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). Theor Appl Genet 98(1):18–31

Dirlewanger E, Graziano E, Joobeur T, Garriga-Caldere F, Cosson P, Howad W, Arús P (2004) Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc Natl Acad Sci USA 101(26):9891–9896. doi:10.1073/Pnas.0307937101

Dirlewanger E, Cosson P, Boudehri K, Renaud C, Capdeville G, Tauzin Y, Laigret F, Moing A (2007) Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. Tree Genet Genomes 3(1):1–13. doi:10.1007/S11295-006-0053-1

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bullet 19:11–15

Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, Hansen M, Steemers F, Butler SL, Deloukas P, Galver L, Hunt S, McBride C, Bibikova M, Rubano T, Chen J, Wickham E, Doucet D, Chang W, Campbell D, Zhang B, Kruglyak S, Bentley D, Haas J, Rigault P, Zhou L, Stuelpnagel J, Chee MS (2003) Highly parallel SNP genotyping. Cold Spring Harb Sym 68:69–78

Foolad MR, Arulsekar S, Becerra V, Bliss FA (1995) A genetic map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genet 91(2):262–269

Foulongne M, Pascal T, Arús P, Kervella J (2003) The potential of *Prunus davidiana* for introgression into peach [*Prunus persica* (L.) Batsch] assessed by comparative mapping. Theor Appl Genet 107(2):227–238. doi:10.1007/S00122-003-1238-8

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

Grattapaglia D, Silva OB, Kirst M, de Lima BM, Faria DA, Pappas GJ (2011) High-throughput SNP genotyping in the highly heterozygous

genome of *Eucalyptus*: assay success, polymorphism and transferability across species. BMC Plant Biol 11:65. doi:10.1186/1471-2229-11-65

Hyten DL, Song Q, Choi IY, Yoon MS, Specht JE, Matukumalli LK, Nelson RL, Shoemaker RC, Young ND, Cregan PB (2008) High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. Theor Appl Genet 116(7):945–952. doi:10.1007/s00122-008-0726-2

International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, Willis TD, Yu FL, Yang HM, Zeng CQ, Gao Y, Hu HR, Hu WT, Li CH, Lin W, Liu SQ, Pan H, Tang XL, Wang J, Wang W, Yu J, Zhang B, Zhang QR, Zhao HB, Zhao H, Zhou J, Gabriel SB, Barry R, Blumenstiel B, Camargo A, Defelice M, Faggart M, Goyette M, Gupta S, Moore J, Nguyen H, Onofrio RC, Parkin M, Roy J, Stahl E, Winchester E, Ziaugra L, Altshuler D, Shen Y, Yao ZJ, Huang W, Chu X, He YG, Jin L, Liu YF, Shen YY, Sun WW, Wang HF, Wang Y, Wang Y, Xiong XY, Xu L, Waye MMY, Tsui SKW, Wong JTF, Galver LM, Fan JB, Gunderson K, Murray SS, Oliphant AR, Chee MS, Montpetit A, Chagnon F, Ferretti V, Leboeuf M, Olivier JF, Phillips MS, Roumy S, Sallee C, Verner A, Hudson TJ, Kwok PY, Cai DM, Koboldt DC, Miller RD, Pawlikowska L, Taillon-Miller P, Xiao M, Tsui LC, Mak W, Song YQ, Tam PKH, Nakamura Y, Kawaguchi T, Kitamoto T, Morizono T, Nagashima A, Ohnishi Y, Sekine A, Tanaka T, Tsunoda T, Deloukas P, Bird CP, Delgado M, Dermitzakis ET, Gwilliam R, Hunt S, Morrison J, Powell D, Stranger BE, Whittaker P, Bentley DR, Daly MJ, de Bakker PIW, Barrett J, Chretien YR, Maller J, McCarroll S, Patterson N, Pe'er I, Price A, Purcell S, Richter DJ, Sabeti P, Saxena R, Schaffner SF, Sham PC, Varilly P, Altshuler D, Stein LD, Krishnan L, Smith AV, Tello-Ruiz MK, Thorisson GA, Chakravarti A, Chen PE, Cutler DJ, Kashuk CS, Lin S, Abecasis GR, Guan WH, Li Y, Munro HM, Qin ZHS, Thomas DJ, McVean G, Auton A, Bottolo L, Cardin N, Eyheramendy S, Freeman C, Marchini J, Myers S, Spencer C, Stephens M, Donnelly P, Cardon LR, Clarke G, Evans DM, Morris AP, Weir BS, Tsunoda T, Johnson TA, Mullikin JC, Sherry ST, Feolo M, Skol A (2007) A second generation human haplotype map of over 3.1 million SNPs. Nature 449(7164):851–861. doi:10.1038/Nature06258

Jauregui B, de Vicente MC, Messeguer R, Felipe A, Bonnet A, Salesses G, Arús P (2001) A reciprocal translocation between 'Garfi' almond and 'Nemared' peach. Theor Appl Genet 102(8):1169–1176

Jones E, Chu W-C, Ayele M, Ho J, Bruggeman E, Yourstone K, Rafalski A, Smith O, McMullen M, Bezawada C, Warren J, Babayev J, Basu S, Smith S (2009) Development of single nucleotide polymorphism (SNP) markers for use in commercial maize (<i>Zea mays</i> L.) germplasm. Mol Breeding 24(2):165–176. doi:10.1007/s11032-009-9281-z

Joobeur T, Viruel MA, Vicente MCd, Jauregui B, Ballester J, Dettori MT, Verde I, Truco MJ, Messeguer R, Batlle I, Quarta R, Dirlewanger E, Arús P (1998) Construction of a saturated linkage map for *Prunus* using an almond×peach F2 progeny. Theor Appl Genet 97(7):1034–1041

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugenic 12:172–175

Lehman EL (1975) Nonparametrics. McGraw-Hill, New York

Lu ZX, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998) Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome 41(2):199–207

Lurie S, Crisosto CH (2005) Chilling injury in peach and nectarine. Postharvest Biol Technol 37(3):195–208. doi:10.1016/J.Postharvbio.2005.04.012

Malhi RS, Sickler B, Lin DW, Satkoski J, Tito RY, George D, Kanthaswamy S, Smith DG (2007) MamuSNP: a resource for rhesus macaque (*Macaca mulatta*) genomics. PLoS ONE 2(5):e438. doi:10.1371/journal.pone.0000438

Martínez-García PJ, Cameron P, Parfitt D, Ogundiwin E, Fresnedo-Ramírez J, Dandekar A, Gradziel T, Crisosto C (2012) Influence of year and genetic factors on chilling injury susceptibility in peach (*Prunus persica* (L.) Batsch). Euphytica 185(2):267–280

Ogundiwin EA, Peace CP, Gradziel TM, Dandekar AM, Bliss FA, Crisosto CH (2007) Molecular genetic dissection of chilling injury in peach fruit. Acta Horticulturae 738:633–638

Ogundiwin EA, Marti C, Forment J, Pons C, Granell A, Gradziel TM, Peace CP, Crisosto CH (2008) Development of ChillPeach genomic tools and identification of cold-responsive genes in peach fruit. Plant Mol Biol 68(4–5):379–397. doi:10.1007/s11103-008-9378-5

Ogundiwin EA, Peace CP, Gradziel TM, Parfitt DE, Bliss FA, Crisosto CH (2009) A fruit quality gene map of Prunus. BMC Genom 10:587. doi:10.1186/1471-2164-10-587

Peace C, Norelli JL (2009) Genomics approaches to crop improvement in the Rosaceae. In: Kevin M. Folta, Gardiner SE (eds) Genetics and genomics of Rosaceae. pp 19–53

Peace CP, Ahmad R, Gradziel TM, Dandekar AM, Crisosto CH (2004) The use of molecular genetics to improve peach and nectarine post-storage quality. Acta Horticulturae 682:403–415

Peace CP, Crisosto CH, Gradziel TM (2005) Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. Mol Breeding 16(1):21–31. doi:10.1007/S11032-005-0828-3

Quarta R, Dettori MT, Verde I, Gentile A, Broda Z (1998) Genetic analysis of agronomic traits and genetic linkage mapping in a BC1 peach population using RFLPs and RAPDs. Acta Horticulturae 465:51–59

Quarta R, Dettori MT, Sartori A, Verde I (2000) Genetic linkage map and QTL analysis in peach. Acta Horticulturae 521:233–241

Rajapakse S, Belthoff LE, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995) Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90(3–4):503–510

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF, Graner A, Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci USA 103(49):18656–18661

Shirasawa K, Isobe S, Hirakawa H, Asamizu E, Fukuoka H, Just D, Rothan C, Sasamoto S, Fujishiro T, Kishida Y, Kohara M, Tsuruoka H, Wada T, Nakamura Y, Sato S, Tabata S (2010) SNP discovery and linkage map construction in cultivated tomato. DNA Res 17(6):381–391. doi:10.1093/dnares/dsq024

Shimada T, Yamamoto T, Hayama H, Yamaguchi M, Hayashi T (2000) A genetic linkage map constructed by using an intraspecific cross between peach cultivars. J Japan Soc Hort Sci 69:536–542

Shulaev V, Korban SS, Sosinski B, Abbott AG, Aldwinckle HS, Folta KM, Iezzoni A, Main D, Arús P, Dandekar AM, Lewers K, Brown SK, Davis TM, Gardiner SE, Potter D, Veilleux RE (2008) Multiple models for Rosaceae genomics. Plant Physiol 147(3):985–1003. doi:10.1104/pp. 107.115618

Stam P (1993) Construction of integrated genetic-linkage maps by means of a new computer package—Joinmap. Plant J 3(5):739–744

Van Ooijen JW (2004) MapQTL® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma, B.V, Wageningen

Van Ooijen JW, Voorrips RE (2001) JoinMap® 4, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Verde I, Lauria M, Dettori MT, Vendramin E, Balconi C, Micali S, Wang Y, Marrazzo MT, Cipriani G, Hartings H, Testolin R,

Abbott AG, Motto M, Quarta R (2005) Microsatellite and AFLP markers in the *Prunus persica* [L. (Batsch)]×*P. ferganensis* BC1 linkage map: saturation and coverage improvement. Theor Appl Genet 111(6):1013–1021. doi:10.1007/s00122-005-0006-3

Verde I, Bassil N, Scalabrin S, Gilmore B, Lawley CT, Gasic K, Micheletti D, Rosyara UR, Cattonaro F, Vendramin E, Main D, Aramini V, Blas AL, Mocker TC, Bryan DW, Wilhelm L, Troggio M, Sosinski B, Aranzana MJ, Arus P, Iezzoni A, Morgante M, Peace C (2012) Development and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and validation in breeding germplasm. PLoS ONE 7(4):e35668. doi:10.1371/journal.pone.0035668

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

Wong WS, Nielsen R (2004) Detecting selection in noncoding regions of nucleotide sequences. Genetics 167(2):949–958

Yamamoto T, Shimada T, Imai T, Yaegaki H, Haji T, Matsuta N, Yamaguchi M, Hayashi T (2001) Characterization of morphological traits based on a genetic linkage map in peach. Breed Sci 51 (4):271–278. doi:10.1270/jsbbs.51.271

Yamamoto T, Yamaguchi M, Hayashi T (2005) An integrated genetic linkage map of peach by SSR, STS, AFLP and RAPD. J Japanese Soc Horticultural Sci 74(3):204–213

Yu H, Xie W, Wang J, Xing Y, Xu C, Li X, Xiao J, Zhang Q (2011) Gains in QTL detection using an ultra-high density snp map based on population sequencing relative to traditional RFLP/SSR markers. PLoS ONE 6(3):e17595. doi:10.1371/journal.pone.0017595

Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An *Arabidopsis* example of association mapping in structured samples. PLoS Genet 3(1):e4. doi:10.1371/journal.pgen.0030004