A fruit quality gene map of *Prunus*

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Abstract

**Background:** *Prunus* fruit development, growth, ripening, and senescence includes major biochemical and sensory changes in texture, color, and flavor. The genetic dissection of these complex processes has important applications in crop improvement, to facilitate maximizing and maintaining stone fruit quality from production and processing through to marketing and consumption. Here we present an integrated fruit quality gene map of *Prunus* containing 133 genes putatively involved in the determination of fruit texture, pigmentation, flavor, and chilling injury resistance.

**Results:** A genetic linkage map of 211 markers was constructed for an intraspecific peach (*Prunus persica*) progeny population, Pop-DG, derived from a canning peach cultivar 'Dr. Davis' and a fresh market cultivar 'Georgia Belle'. The Pop-DG map covered 818 cM of the peach genome and included three morphological markers, 11 ripening candidate genes, 13 cold-responsive genes, 21 novel EST-SSRs from the ChillPeach database, 58 previously reported SSRs, 40 RAfs, 23 SRAPs, 14 IMAs, and 28 accessory markers from candidate gene amplification. The Pop-DG map was co-linear with the *Prunus* reference T × E map, with 39 SSR markers in common to align the maps. A further 158 markers were bin-mapped to the reference map: 59 ripening candidate genes, 50 cold-responsive genes, and 50 novel EST-SSRs from ChillPeach, with deduced locations in Pop-DG via comparative mapping. Several candidate genes and EST-SSRs co-located with previously reported major trait loci and quantitative trait loci for chilling injury symptoms in Pop-DG.

**Conclusion:** The candidate gene approach combined with bin-mapping and availability of a community-recognized reference genetic map provides an efficient means of locating genes of interest in a target genome. We highlight the co-localization of fruit quality candidate genes with previously reported fruit quality QTLs. The fruit quality gene map developed here is a valuable tool for dissecting the genetic architecture of fruit quality traits in *Prunus* crops.

**Background**

Molecular genetic linkage maps have become a major tool in genetics, genomics and breeding of plant and animal species. Linkage maps provide opportunities for unlocking the complex genetics of quantitatively inherited traits through the localization of quantitative trait loci (QTL),
The concept of fruit quality of *Prunus* fruit crops includes both its attainment, such changes in color, flavor, and texture as fruit develop, grow, and ripen, and its maintenance following harvest from the tree as the perishable tissues senesce. *Prunus* fruit development, growth, ripening, and senescence includes major biochemical and sensory changes in texture, color, and flavor. The genetic dissection of these complex processes has important applications in crop improvement, to facilitate maximizing and maintaining stone fruit quality from production and processing through to marketing and consumption.

The goal of the present study was to develop a genomic resource to facilitate the genetic dissection of *Prunus* fruit quality traits. This paper reports the genetic mapping in the *Prunus* genome of candidate genes for fruit texture, pigmentation, flavor, and cold-responsiveness of peach, using both an intraspecific peach population to create a linkage map for genetic analyses of fruit quality and chilling injury (CI), and the interspecific *Prunus* reference map. The utility of the "fruit quality gene map" developed here for *Prunus* is demonstrated by highlighting co-localization of fruit quality QTLs with mapped fruit quality candidate genes.

**Results**

**Morphological markers**

Peach blossom petals can be large and showy or small and curved on margins (non-showy). Non-showy is dominant to showy [30]. 'Dr. Davis' and 'Georgia Belle' are both heterozygous and therefore non-showy for this locus. Pop-DG progeny segregated as 115 non-showy: 37 showy, fitting the expected Mendelian ratio of 3:1 ([2] = 0.04; *P* = 0.98). This trait was designated *Sh* and mapped to the middle of linkage group G8 of Pop-DG and flanked by SSR marker CPPC1006 at 5 cM above and CI resistance CG marker Utk5 at 11.2 cM below. Segregation and mapping of the peach mesocarp color (Y) and the freestone melting flesh (F-M) loci have been reported previously for Pop-DG [31,32].

**Molecular marker polymorphism in Pop-DG**

Three types of reproducible marker polymorphism were observed on the PAGE profiles of the CG PCR products: fragment size polymorphism of the targeted gene fragments, additional markers that were designated as "CG accessory markers" generated elsewhere in the profile than the main CG fragments, and single strand conformation polymorphism (SSCP). Under the conditions used for PAGE, some reproducible sharp or shadowy banding patterns were observed in association with the target PCR product. We have proved this to be mobility shifts characteristic of SSCP resulting from SNPs within the amplicons [33]. The SSCP phenomenon also occurred for many SSRs (Figure 1).
Of all 229 SSR primer pairs screened for polymorphism in Pop-DG, 68 (~30%) were polymorphic and produced 79 SSR markers (Figure 2). Polymorphism was higher in the 133 genomic SSRs (37%) than in the 96 EST-SSRs (23%). Of the 76 novel ChillPeach EST-SSRs tested in Pop-DG, 17 (22%) were polymorphic, on par with the public EST-SSR polymorphism. The remaining 59 ChillPeach EST-SSRs were screened on the T × E bin set, out of which 51 (86%) were polymorphic. Approximately 13% and 18% polymorphism was obtained for CI resistance CGs and other CGs in Pop-DG, respectively, while 71% and 86% polymorphism was obtained in T × E, respectively (Figure 2). Marker polymorphism in Pop-DG was therefore at least four to five times less than in T × E for all classes of marker tested.

**Pop-DG linkage map**

The Pop-DG intraspecific peach linkage map contained a total of 211 markers (208 molecular and three morphological) distributed over eight linkage groups corresponding to the haploid chromosome number of peach (Figure 3). The map covered 818.2 cM of the peach genome with an average of 4.0 cM interval between markers. The markers on Pop-DG map consisted of three Mendelian trait loci, 24 CGs, 79 SSRs, 40 RAFs, 23 SRAPs, 14 IMAs, and 28 CG accessory markers associated with CGs. Of the 79 SSR markers on Pop-DG, 39 were shared with the published *Prunus* T × E reference map (Figure 3). These common markers enabled the determination of linkage group orientation and assignment of linkage group numbers for the Pop-DG map. Shared markers were co-linear between Pop-DG and T × E except in three cases. Marker positions for BPPCT024, BPPCT030, and pchgms1 were inverted at the lower end of linkage group G2 of Pop-DG compared to G2 of T × E, positions of BPPCT021 and UDP96-008 were inverted in the middle of G3 of Pop-DG compared to G3 of T × E, and positions of BPPCT026 and CPPCT004 were inverted towards the upper end of G5 of Pop-DG compared to G5 of T × E. One SSR marker (BPPCT036) that was originally placed on linkage group G4 of T × E [7] mapped to the distal end of G1 in Pop-DG. To resolve this discrepancy, BPPCT036 was tested on the T × E bin set which confirmed its true location in bin 1:73 (Figure 1), corresponding to its position on the Pop-DG linkage map.

The features of CGs mapped to Pop-DG are presented in Table 1. Eleven markers were derived from fruit quality-related CGs (Table 1), seven of which were texture CGs. One of these texture CGs, *endoPG* (endopolygalacturonase), mapped to linkage group G4 as previously reported (Peace et al. 2005a). The others were two pectin methyl-esterases (*PME1* & *PME5*) on G1 and G7, another polygalacturonase (*PG4*) on G8, pectate lyase (*PL2*) on G1, alpha-L-arabinofuranosidase (*Ara*) on G5, and a MADS
Box transcription factor similar to tomato ripening inhibitor (RIN) on G4. Three pigmentation CGs mapped to Pop-DG. These were beta-carotene hydroxylase (BCH) on G2, leucoanthocyanidin dioxygenase (PpLDOX) on G5 as previously reported by Ogundiwin et al. (2008), and zeaxanthin epoxidase (ZXE2) on G7. A flavor CG for sucrose phosphate synthase (SPS) mapped to G1. Thirteen mapped Pop-DG gene markers were obtained from CI resistance CGs. Seven of these have functional annotation: Aux/IAA protein (Aux-IAA) on G1, chloroplast nucleoid DNA binding (CND) on G1, thaumatin-like protein1 precursor (TP1) on G3, serine protease-like protein (SPP) on G7, tonoplast intrinsic protein (TIP) on G7, indole-3-acetic acid-induced protein ARG2 (Arg2) on G8, and sulfate transporter (ST) on G8. The other six had no functional annotation (labeled with the prefix "Unk" for "unknown") on G1, G2, G3, G8, and two on G6. Some dominant markers were generated from accessory fragments amplified alongside major amplicons of a few candidate genes (See Additional file 1 - Table S1). Seventeen of such markers were mapped to Pop-DG.

Features of the 18 ChillPeach ESTs that produced 21 EST-SSRs mapped to Pop-DG are provided (See Additional file 2 - Table S2). Eight of these have annotated functions: two zinc finger-RING type, thioredoxin domain 2, POZ/DBT containing protein, biotin synthase, lysine ketoglutarate reductase, transfactor-like protein, TRNA intron endonuclease, and farnesyltransferase beta subunit. Other ChillPeach EST-SSRs on Pop-DG have no known functional annotation.

Figure 2
Level of polymorphism of three molecular marker types in Pop-DG and T × E. The intraspecific peach population (Pop-DG) compared with the interspecific Prunus population (T × E); SSR = simple sequence repeat, CG = fruit ripening candidate genes, CIRG = CI resistance genes.

Discussion
We have developed a detailed fruit quality and ripening gene map for Prunus. The fruit quality gene map contains 133 candidate genes (CGs) implicated in fruit ripening, softening, flavor, and pigmentation, and chilling injury resistance. The Pop-DG peach map is almost entirely co-linear with the Prunus reference T × E map such that locations of markers and quantitative trait loci (QTLs) located on Pop-DG can be readily cross-referenced to T × E and other Prunus maps aligned to T × E. Similarly, markers and QTLs in other Prunus maps (and other Rosaceae crop maps as comparative genomics advances in this family) can be compared to the Prunus fruit quality gene map to identify genes controlling fruit ripening and sensory quality.

Co-linearity between Pop-DG and T × E maps
Using a reference genetic map with available bin-mapping resources to map genes and other DNA sequences not polymorphic within crop-specific maps is a powerful means of identifying marker-trait associations. The degree of resolution offered by bin-mapping in T × E (1 to 30 cM bins) is at the same scale as typical QTL mapping, such that detection of co-location (and equally useful, lack of co-location) of candidate genes with previously mapped QTLs is readily achievable. The remarkable conservation of co-linearity among the genomes of Prunus species [8] was exploited by bin-mapping to the T × E reference map many CGs that were monomorphic in Pop-DG. The higher rate of polymorphism observed in T × E compared to Pop-DG is not surprising because T × E is an F_2 population from an interspecific cross. Pop-DG's polymorphism results from a relatively high heterozygosity in 'Georgia Belle' compared to modern cultivars, low heterozygosity in 'Dr. Davis' arising from a pedigree of closely related yellow non-melting canning peaches, and divergent breeding histories of these two parent cultivars. We expect that intraspecific peach populations derived from modern cultivars within the same market type (fresh or canning) will display even less polymorphism than Pop-DG. Inversion of marker positions observed between Pop-DG and T × E...
in three locations (linkage groups G2, G3, and G5) were among marker pairs that were close together on the T × E map (< 10 cM), suggesting that they are more probably caused by errors in the assignment of marker order than to inversion of chromosome fragments [8].

**Simply inherited traits mapped to Pop-DG**

Markers on the Pop-DG linkage map included three simply-inherited Mendelian quality and morphological traits: freestone/clingstone and melting flesh/non-melting flesh (F-M), mesocarp color (Y), and non-showy flower petals (Sh). The Sh locus mapped to linkage group G8 of Pop-DG. This is the first report of its genomic location since its inheritance was elucidated in the early to mid 1940s. Confirmation of its location on G8 is emerging from some unpublished results (Shenhua Fan and Tatyana Zhebentayeva, personal communication). This is the only Mendelian trait mapped to G8 of Prunus to date. Linkage group G8 has proved difficult to map in some intraspecific peach mapping efforts [29,34]. In this report also, G8 was the linkage group with fewest number of markers. This could partly explain why it took so long to determine the genomic location of the Sh locus. The F-M (on linkage group G4) and Y (on linkage group G1) loci are among the list of 28 simply-inherited Mendelian traits mapped to Prunus genome in prior studies [8], and their locations are confirmed in Pop-DG.

**Novel Prunus EST-SSR markers**

Seventy new Prunus EST-SSR markers were mapped either directly to Pop-DG (21 markers) or bin-mapped to the T × E reference map (49 markers). The markers were obtained from the ChillPeach EST database [35]. The ChillPeach database is a specialized collection of ESTs from peach mesocarp tissue subjected to cold storage and ripening. The new set of mapped EST-SSR markers provides additional resources for molecular marker analyses in Prunus species.

**Identity of mapped CGs**

CG sequence identities were confirmed for endoPG [32], PpLOX [33], and RIN (data not shown), where fragment lengths and DNA sequence of amplicons corresponded to original EST sequences. The identity of remaining CGs was confirmed by fragment length, where the most intense amplicons were either the expected size (approximately two-thirds of CGs) or were 80-1300 bp larger presumably due to the inclusion of one or more introns (and confirmed for the aforementioned cases). Identity of CG amplicons was also supported in many cases by previously reported map locations (described below). Further validation via sequencing would be desirable for the remaining CGs, and is the next recommended step for pursuit of specific QTL candidates for fruit quality traits of interest.
Comparing locations of CGs bin-mapped on T × E to other Prunus maps

Several previously mapped fruit candidate genes in Prunus corresponded to their genome locations in the present study. The locations of three CGs bin-mapped to T × E in this study corresponded to a previous interspecific 'Padre' × 54P455 map [13]. Catalase was mapped as an isozyme locus (Cat1) to the top of linkage group G5 of the 'Padre' × 54P455 map. We bin-mapped this texture CG to T × E bin 5:04. In earlier studies, the peach fruit acidity locus (D) was mapped to the top of G5 [25] and a tight linkage between Cat1 and the D locus was also reported [36]. Another isozyme, isocitrate dehydrogenase, was mapped as IC DH to the top of G5 in 'Padre' × 54P455 [13] and as Icdh1 to T × E [37], and we bin-mapped this flavor CG as ICDH to T × E bin 3:06. Dehydrin was mapped as DHN1 and Dehy2 to the middle of group G7 of 'Padre' × 54P455 [13] and T × E (in this report), respectively.

The gene encoding polygalacturonase inhibiting protein (PGIP) mapped to different locations in the two studies; it mapped to G7 in 'Padre' × 54P455 and to G6 on T × E. Different members of the PGIP gene family may have been mapped in the two separate studies. Another independent study bin-mapped the genes endoPG (as EPPCU1775) to bin 4:63, ACO1 (as MD206a) to 3:36, ACO3 (as MD205a) to 4:18, and PG4 (as MD207a) to 8:63 to T × E [11]. PG4 was also mapped as isozyme PG to the same end of G8 in an almond linkage map [16]. PEPC was mapped as PEPc in a 'Ferjalou Jalousia' × 'Fantasia' map [37] in the same region as our PEPC (3:22). These authors [37] also reported the location of another ten candidate genes for sweetness and acidity, and a different expansin gene to those mapped here, to various locations on a peach map using the T × E population. Three further fruit quality CGs were mapped in a separate study [11]: NADP dependent sorbitol 6-phosphate dehydrogenase (as MD201a) for sweetness to bin 8:19, H+ ATPase (as

Table 1: Features of candidate and cold responsive genes mapped to Pop-DG

| LG | Marker Code | Functional Annotation | Clone/Accession # | EST Source | CG type |
|----|-------------|-----------------------|-------------------|------------|---------|
| 1  | PL2         | Pectate lyase          | BU041363          | GDR        | Texture |
|    | Unk23       | similar to F19P19.4 protein related cluster | PP1004A08-T7_c_s | ChillPeach | CIRG    |
|    | PME1        | pectinesterase, putative | BU043277          | GDR        | Texture |
|    | CND         | Chloroplast nucleoid DNA binding protein related cluster | PPN018D10-T7_c_s | ChillPeach | CIRG    |
|    | SPS         | Sucrose phosphate synthase | DY653691         | GDR        | Flavor   |
|    | Aux-I AA    | Aux/I AA protein related cluster | CL78Contig1       | ChillPeach | CIRG    |
| 2  | BCH         | Beta-carotene-hydroxylase | BU044761          | GDR        | Pigment  |
|    | Unk20       | OSJNb0039L24.13 protein | CL1095Contig1     | GDR        | CIRG    |
| 3  | Unk13       | highly similar to OSJNb0004A17.4 protein related cluster | CL32Contig2       | ChillPeach | CIRG    |
|    | TP1         | Thaumatin-like protein 1 precursor | PPN003H07-T7_c_s  | ChillPeach | CIRG    |
| 4  | RIN         | Similar to Solanum lycopersicum MADS-RIN MADS box transcription factor | BU045116          | GDR        | Texture  |
|    | endoPG      | endopolygalacturonase   | BU040689          | GDR        | Texture  |
| 5  | PpLDOX      | Leuconanthocyanidin dioxygenase | EU292217          | ChillPeach | Pigment  |
|    | Ara         | Alpha-L-arabinofuranosidase | DQ486870         | NCBI       | Texture  |
| 6  | Unk10       | No annotation available | PP1005B10-T7_c_s  | ChillPeach | CIRG    |
|    | Unk19       | No annotation available | PP1004C05-T7_c_s  | ChillPeach | CIRG    |
| 7  | SPP         | Serine protease-like protein related cluster | PPN007C09-T7_c_s  | ChillPeach | CIRG    |
|    | PME5        | pectin methylesterase - like protein | BU044844          | GDR        | Texture  |
|    | TIP         | Tonoplastic intrinsic protein related cluster | BU1003C07-T7_c_s  | ChillPeach | CIRG    |
|    | ZXE2        | Zeaxanthin epoxidase     | CL377Contig1      | ChillPeach | Pigment  |
| 8  | Unk5        | No annotation available | PP1004F11-T7_c_s  | ChillPeach | CIRG    |
|    | PG4         | P. persica PG gene       | X77231            | NCBI       | Texture  |
|    | ST          | Sulfate transporter 3.1  | PPN065F08-T7_c_s  | ChillPeach | CIRG    |
|    | Arg2        | Indole-3-acetic acid-induced protein ARG2 related cluster | CL704Contig1       | ChillPeach | CIRG    |

*: CIRG = chilling injury resistance genes
Candidate genes (CGs) and novel Prunus EST-SSR markers bin-mapped to the Prunus T × E reference map. CI resistance CGs are in bold fonts, texture CGs are underlined, CGs related to fruit pigmentation are italicized, other CGs are asterisked, and new Prunus EST-SSRs are in normal font.

[Figure 4]

Co-locations of candidate genes and chilling injury QTLs

The co-linearity between the Pop-DG and T × E maps has begun to yield benefits in dissecting the genetic control of fruit quality traits in peach. We are using the fruit quality genome during hybridization-based efforts to physically map peach ESTs [4].

MD203a) for sweetness/acidity to bin 1:73, and endo-beta-1,4-glucanase for texture to bin 5:41 (a different gene family member to our EGase of bin 3:22). Additional fruit quality candidate genes have been located in the Prunus genome during hybridization-based efforts to physically map peach ESTs [4].

endOEG on G4 with major QTLs for mealiness and bleeding [31,38,39] as well as to the Freestone-Melting flesh locus [31,32] and PpLDOX on G5 with cold storage-induced browning [33].

Mealiness

A peach homolog of tomato MADS-RIN ripening inhibitor gene (RIN), necessary for fruit ripening in tomato, mapped close (6 cm) to endoEG. Two RIN accessory markers (rin-a and rin-b) also mapped distally to endoEG. The functional role of RIN in peach fruit ripening, mealiness, or bleeding has not yet been established. Four additional minor QTLs were reported for mealiness in Pop-DG corresponding approximately to T × E bins 4:18, 4:22-4:27, 4:28, and 6:80-6:84 [39]. We mapped one texture CG (ACO3) to bin 4:18, one CI resistance CG (Unk17) to bin...
4:27, two CI resistance CGs (TAT and Unk28) to bin 4:28, and two CI resistance CGs (Unk15 and Unk24) to bins 6:80-6:84. ACO3 encodes a NADH dehydrogenase, a key enzyme in the electron transport chain.

**TAT** (tyrosine aminotransferase) is an inducible protein in the plant methyl jasmonate defense system [40]. Unk15, Unk17, Unk24, and Unk28 are genes of unknown function that were differentially regulated in cold-treated peach mesocarp tissue [35]. Each of these CGs are potential markers for mealiness resistance and warrant further investigation.

**Flesh browning**

Some CGs mapped to locations where QTLs have been reported for cold storage-induced flesh browning in peach fruit. A major QTL was located on linkage group G5 of Pop-DG corresponding to bin 5:21 of T × E [33,39]. *PpLDOX* (leucine aminopeptidase) is encoded in the plant methyl jasmonate defense system [40]. Unk15, Unk17, Unk24, and Unk28 are genes of unknown function that were differentially regulated in cold-treated peach mesocarp tissue [35]. These CGs will be further investigated for possible roles in the formation of bleeding and developing bleeding-free peach cultivars.

**Inferred co-locations of candidate genes and other published fruit quality traits**

Several CGs mapped to genomic regions corresponding to fruit quality QTLs reported in other studies. Examples are those reported by [34] and [46].

**Putative candidate genes for fruit quality QTLs on P1908 × 'Summergrand' map**

On linkage group G1 QTLs were detected by [34] for fructose (Fru), sweetness (Swe), quinic acid (Qui), fruit cheek diameter (FCheekD), fruit mass (FMass), and fruit suture diameter (FSutureD) in the region corresponding to the T × E bin 1:26 on which we bin-mapped *Polyub* (Polyubiquitin) and Unk26. QTLs for citric acid (Cit) and total sugar (TSugar) in the region equivalent to bin 1:50 of T × E on which we bin-mapped *SAMDC* (S-adenosylmethionine decarboxylase), Unk29, 4CL (4-coumarate-CoA ligase-like protein), C-0472 (6-phosphogluconolactonase), C-PPN36E09, C-PPN73A08; QTLs for total sugar (TSugar) and quinic acid (Qui) in the region corresponding to bin 1:52-1:55 of T × E where we bin-mapped Chitib (Chitinase Ib), Unk9 and SREB (Sucrose-responsive element binding protein). Notable among these gene markers are those encoding sucrose-responsive element binding protein (SREB) and phosphogluconolactonase (C-0472) for their possible involvement in sugar biosynthesis [47] and inducible expression [48], respectively. In addition, [34] localized QTLs for sucrose (Suc), sucrose-glucose (Glu), and quinic acid (Qui) on linkage group G7 region comparable to bin 7:56 of T × E containing bin-mapped CG AspS (Asparagus synthetase). The expression of AspS has been shown to increase with a decrease in sucrose levels [49].

**Cold-induced bleeding**

In addition to the major QTL detected in peach fruit at the F-M/endoPG locus on G4 of Pop-DG, two minor QTLs were also reported for cold-induced bleeding on Pop-DG linkage group G1 [39], corresponding to T × E bins 1:14 and 1:34. A texture CG (PL2) and a CI resistance CG (Unk23) mapped close to the top of the cold-induced bleeding QTL peak at the top end of G1. Two texture CGs (PME3 and Ara2) and four CI resistance CGs (CIPK1, PDK2, C-CoAR, and Unk8) mapped to bin 1:14, while *PPCAO* (pigmentation CG) and Unk25 (CI resistance CG) mapped to bin 1:34. PL2 (pectate lyase), PME3 (pectin methylesterase), and Ara2 (alpha-L-arabinofuranosidase) are cell wall-degrading enzymes [41,43]. *PPCAO* (peroxisomal copper-contain-
lowing peach fruit quality QTLs on linkage group G5: glucose (Glu), fruit suture diameter (FSutureD), and fruit cheek diameter (FCheekD), as well as the major locus controlling fruit acidity, D, on a region corresponding approximately to bin 5:04 of T × E on which we bin-mapped PAE1, PAE2, and Cat1; red skin coloration (SRColor), dry flesh mass content (DFMC), soluble solid concentrate (SSC), fruit mass (FMass), fruit polar diameter (FPolarD), and fruit suture diameter (FSutureD) on bin 5:46-5:49 of T × E on which PL, C-PP03C02, and C-PPN05E11 (Sulf-like protein) were bin-mapped. Sulf-related proteins have been implicated in Fe-S metabolism and export [50].

Putative candidate genes for fruit quality QTLs on 'Ferjalou Jalousia' × 'Fantasia' map [46]

A number of fruit quality QTLs (including fresh weight, sucrose, and SSC) were detected by [46] on linkage group G6 of 'Ferjalou Jalousia' × 'Fantasia' map, on the region corresponding to bin 6:74-6:84 of the T × E linkage map. To this bin, we mapped AGAT (Alanine--glyoxylate aminotransferase), CDTTPL (C-terminal domain phosphatase-like), Sod4a (Superoxide dismutase), Unk5, Unk24, C-1182 (BZIP transcription factor bZIP105), C-1290, C-PPN12C07, C-PPN19B01, and C-PPN31E06 (Glutamine-fructose-6-phosphate transaminase). Glutamine-fructose-6-phosphate transaminase is an important enzyme in biosynthesis of amino sugar-containing macromolecules [51]. Also on linkage group G4, Dirlewanger et al [1999] localized QTLs for SSC and fructose to a region equivalent to the T × E bin 4:46. The candidate genes mapped to this bin were SeCy (Sesquiterpene cyclase), SAMM (S-adenosylmethionine:2-demethylmenaquinone methyltransferase), and GPPDE (glycerophosphoryl diester phosphodiesterase).

Conclusion

We have developed a fruit quality gene map for Prunus by determining the genomic locations of 133 fruit quality candidate genes with an intraspecific peach population, Pop-DG, and the Prunus reference map, T × E. Sufficient SSR marker anchoring between both maps allowed easy cross-referencing of marker and trait locus positions. We demonstrate here the use of this gene map for dissecting the molecular genetics of CI in peach. Using the results of microarray experiments that studied gene expression in cold-treated peach mesocarp tissue, 63 cold-responsive genes were located on the fruit quality gene map, allowing the detection of co-locations of these CI resistance CGs with QTLs for CI symptoms. We also highlight new CGs for previously reported Prunus QTLs of other fruit quality traits. The fruit quality gene map presented here is expected to be a valuable resource for the genetic analysis of fruit ripening and related fruit quality traits in Prunus species.

Methods

Mapping population and T × E bin set

Pop-DG is a peach intraspecific cross between 'Dr. Davis' (female parent) and 'Georgia Belle' (pollen parent). 'Dr. Davis' is a modern canning peach cultivar while 'Georgia Belle' is a century-old fresh market peach cultivar. These cultivars contrast for many fruit quality and other traits (Table 2, 3). Pop-DG, created and managed to study the genetics of fruit quality attainment and maintenance, particularly resistance to CI, in peach and nectarine, was established in two nearby orchards at Kearney Agricultural Center (Parlier, CA, USA). The first orchard was established in 1998 containing 51 verified hybrids [32]. Each progeny genotype was represented by two trees in the orchard; one tree planted on its own roots and the other tree on 'Nemaguard' rootstock. The second orchard was established in 2002, containing single trees of 277 true hybrid progeny on their own roots. All 51 progeny of the first orchard and 101 progeny of the second orchard (152 true hybrid progeny total) were used for Pop-DG linkage mapping. For bin-mapping in T × E, DNA samples of the bin set of the 'Texas' × 'Earlygold' population ('Earlygold' the F1 plant, and six F2 plants) [11], kindly provided by Dr. Werner Howad, were used. We also included the second parent - 'Texas' in each test.

Morphological markers

Pop-DG segregated for three Mendelian morphological traits: freestone melting flesh/clingstone non-melting flesh, white/yellow flesh color, and non-showy/showy flower petals. These traits were scored visually and included in linkage analysis alongside molecular markers.

Molecular markers

Various classes of molecular markers were evaluated. These marker classes consisted of genomic sequence-derived simple sequence repeats (SSR), expressed sequence tag [EST]-derived SSRs (EST-SSR), ethylene-related candidate genes (CG), texture CGs, pigmentation CGs, flavor CGs, CI resistance CGs, sequence-related amplified polymorphisms (SNP), randomly amplified DNA fingerprinting (RAF), and inter-microsatellite amplification (IMA).

Candidate genes were nominated from published works and review articles on physiology and biochemistry of fruit ripening, softening, color (pigmentation), and flavor [37,41,43,52-71]. EST sequences of most of the CGs (including those described below) were obtained from the GDR database [5]. Others were obtained from the ChillPeach database [35] and GenBank.

CGs assembled for texture included those putatively encoding ethylene-related enzymes (e.g. aminocyclopropane-1-carboxylate synthases, aminocyclopropane-1-car-
boxylate oxidases, ethylene receptors, ethylene responsive element binding proteins, s-adenosyl-l-methionine synthases, peptide methionine sulfoxide redutase, and ripening inhibitor protein), although ethylene-related genes are also relevant for other fruit ripening processes. Other texture CGs were those putatively encoding cell wall-degrading enzymes (e.g. polygalacturonases, pectinesterases, pectate lyases, glucanases, mannosidases, xylglucans, glycosylases and expansins). As indicated by [41], cell wall synthesis enzymes were also included (e.g. glycosyltransferases and fiber protein enzymes), and from the same study, several genes of unknown function but strongly up-regulated by ethylene in ripening peach fruit were also included.

For pigmentation (skin and flesh color, including browning and bruising), candidate genes were chosen from the carotenoid and anthocyanin biosynthesis pathways (e.g. neoxanthin cleavage enzyme, leucoanthocyanidin dioxygenase, anthocyanidin-3-glucoside rhamnosyltransferase, beta-carotene hydroxylase, lycopene beta cyclase, peroxidase, geranylgeranyl pyrophosphate synthase, zeta carotene desaturase and phytoene desaturase). Genes encoding diphenol oxidases and polyphenol oxidase genes were also included.

Flavor CGs included sugar and acid biosynthesis pathway genes (e.g. sucrose synthase, hydroxyl methylglutaryl CoA reductase, cell wall invertase, sorbitol dehydrogenase, phosphoenolpyruvate carboxylase, chalcone synthase, peroxisomal copper containing amine oxidase, zeanthin epoxidase, beta-carotene hydroxylase, lycopene beta cyclase, peroxidase, anthocyanidin-3-glucoside rhamnosyltransferase, beta-carotene hydroxylase, lycopene beta cyclase, peroxidase, geranylgeranyl pyrophosphate synthase, zeta carotene desaturase and phytoene desaturase). Genes encoding diphenol oxidases and polyphenol oxidase genes were also included.

CI resistance CGs were cold-responsive genes obtained from the results of microarray analysis of cold-treated peach mesocarp tissues versus untreated mesocarp tissues [35]. Selection of CI resistance CGs were made as follows: 25 top performing genes, 24 genes common to peach and 'Georgia Belle', and 39 genes unique to peach (i.e. not found in ColdArrayDB).

Published reports were the source of all genomic sequence SSRs (see below) and some EST-SSRs, while most EST-SSRs were newly obtained from the ChillPeach EST database [35]. A total of 153 published Prunus SSRs were screened for polymorphism between the Pop-DG mapping parents. These were P. persica SSRs with the prefix BPCT [72], CPPCT [73], EPPCU (GD database: [5]), Pchcms & Pchgms [74], PS [75], UCD-CH [76], and UDP [77,78], and P. dulcis SSRs with the prefix UDA [79]. The ChillPeach database provided 76 new EST-SSRs for screening, and the markers were labeled with the prefix 'C-' followed by the clone or contig number (e.g. C-PPN286P07 and C-1128).

RAF markers were obtained according to [80] protocols using Operon decamer primers AA18, B12, B15, D19, E02, E11, E16, P04, P07 and W06 after preliminary screening of many others via the bulked segregant analysis approach [81] with mealiness phenotypic extremes. SRAP marker analysis was conducted according to [82]. One ISA primer (IMA08: (GA)8GT; [25]) was also used to generate additional molecular markers for Pop-DG.

### PCR and PAGE

All PCR primers were designed using Primer3 software [83]. EST sequences of CGs were examined for microsatellite motifs, and whenever ESTs were part of a contig, the contig was examined for SNPs and indels. Primers were designed to exploit these polymorphic features. Where these features were not observable, sequences at the 3'-end of the ESTs were used for designing primers to avoid long introns and target less-conserved 3' UTRs. Generally, primers were designed to limit expected amplicon size to ≤300 bp such that a PCR product even with an intron as long as 1 kb would still be observable on the large (50x38 cm) PAGE plate and 1 bp indels of intron-less amplified fragments could be detected. PCR and PAGE conditions were as reported in Peace et al. (2005b). Primer sequences, annealing temperatures, expected and observed amplicon sizes, and type of polymorphisms are provided (See Additional files 3 and 4).

### Map construction

Linkage analysis was conducted with JoinMap® 4 [84]. Linkage parameters were set as 3.0 minimum LOD and 0.45 maximum recombination fraction. The Kosambi mapping function [85] was used to convert recombination fraction to map distances in centimorgans (cM). The marker data type was set as cross-pollination (CP). The bin-mapping procedure followed [11].

### Table 2: Fruit quality attributes of Pop-DG parent cultivars, 'Dr. Davis' and 'Georgia Belle'

| Trait                  | 'Dr. Davis' | 'Georgia Belle' |
|------------------------|-------------|-----------------|
| Ripening date          | Later       | Earlier         |
| Skin color             | Blush on orange ground | Green/yellow, no blush |
| Flesh color            | Yellow-orange | White-cream     |
| Stone adhesion         | Clingstone  | Freestone       |
| Flesh texture          | Firm, non-melting flesh | Soft, melting flesh |
| Aroma                  | Bland       | Sharp           |
| Sweetness (SSC)        | 11.5        | 13.0            |
| Acidity (TA)           | High        | Low             |
| Mealiness              | None        | High susceptibility |
| Browning               | Medium susceptibility | High susceptibility |
| Bleeding               | High susceptibility | Low susceptibility |

* TA = titratable acidity, SSC = soluble solids concentrate*
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Additional material

Additional file 1
Table S1. Features of dominant amplicons (accessory markers) generated alongside the target PCR products of candidate and cold responsive genes mapped to Pop-DG. The data provided represent information on accession number, map location, and fragment size information of dominant amplicons (accessory markers) generated alongside the target PCR products of candidate and cold responsive genes mapped to Pop-DG.

Additional file 2
Table S2. Characteristics of 71 new Prunus ChillPeach EST-SSR markers mapped to the peach/Prunus genome. The data provided represent information on unigene, map location, and functional annotation of 71 new Prunus ChillPeach EST-SSR markers mapped to the peach/Prunus genome.

Additional file 3
Table S3. Characteristics of candidate genes (CGs) bin-mapped to the T × E reference Prunus map. The data provided represent information on the genome location (bin name), marker location, clone/accession number, source of ESTs, and CG class of all CGs bin-mapped to the T × E reference Prunus map.

Additional file 4
Table S4. Primer details for candidate genes and EST-SSRs. The data provided represent information on the primer sequence, annealing temperature, amplicons size, and type of polymorphisms for candidate genes and EST-SSRs.
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