# Population structure and marker-trait associations for pomological traits in peach and nectarine cultivars

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**Abstract** Marker-trait associations based on populations from controlled crosses have been established in peach using markers mapped on the peach consensus map. In this study, we explored the utility of unstructured populations for association mapping to determine useful marker-trait associations in peach/nectarine cultivars. We used 94 peach cultivars representing local Spanish and modern cultivars from international breeding programs that are maintained at the Experimental Station of Aula Dei, Spain. This collection was characterized for pomological traits and was screened with 40 SSR markers that span the peach genome. Population structure analysis using STRUCTURE software identified two subpopulations, the local and modern cultivars, with admixture within both groups. The local Spanish cultivars were somewhat less diverse than modern cultivars. Marker-trait associations were determined in TASSEL with and without modelling coefficient of membership (Q) values as covariates. The results showed significant associations with pomological traits. We chose three markers on LG4 because of their proximity to the endoPG locus (freestone-melting flesh) that strongly affects pomological traits. Two genotypes of BPPCT015 marker showed significant associations with harvest date, flavonoids and sorbitol. Also, two genotypes of CPPCT028 showed associations with harvest date, total phenolics, RAC and total sugars. Finally, two genotypes of endoPG1 showed associations with flesh firmness and total sugars. The analysis of linkage disequilibrium (LD) revealed a high level of LD up to 20 cM, and decay at farther distances. Therefore, association mapping could be a powerful tool for identifying marker-trait associations and would be useful for marker-assisted selection (MAS) in peach breeding.

***Keywords****: Prunus persica, germplasm, population genetics, linkage disequilibrium, simple sequence repeats*

## Introduction

Peach (*Prunus persica* L.) is the third most important temperate fruit crop worldwide, after apple and pear. The main producer countries are China, Italy, Spain, and the United States (FAOSTAT, 2011; http://faostat.fao.org). Peach is native to China and spread to the Mediterranean through Persia (Hedrick 1917). Later, peaches were brought by Spanish explorers to America and disseminated among the Aztecs in Mexico. From Mexico, peaches spread to New Mexico, Arizona, and California (Hedrick 1917). Early peach culture was based on seed propagation and for centuries, peach has been cultivated and selected for different agronomic characters, leading to locally adapted populations (Hedrick 1917). Modern peach cultivars have a narrow genetic base due to the limited number of genotypes used as parents in breeding programs (Myles et al. 2009). Consequently, peach diversity has been drastically reduced by the use of modern cultivars that share a few common ancestors (Aranzana et al. 2003). The Spanish peach industry was based on yellow, non-melting fleshed and clingstone types, but the replacement of the Spanish traditional varieties by introduced ones, mostly from North America, has induced the domain of the melting flesh cultivars (Badenes et al. 1998). The local germplasm collection at the Experimental Station of Aula Dei (Zaragoza, Spain) have been previously evaluated, regarding harvest season from June to October and horticultural traits like flesh and skin color (yellow/orange/white), depth of stalk cavity (deep/shallow), stone adherence (clingstone/freestone), and size and shape of fruit (small/large and round/ovate) (Bouhadida et al. 2011).

One of the most practical applications of DNA-based markers in breeding is the ability to select phenotypic traits using markers tightly linked to genes controlling these traits. Economically valuable fruit traits cannot be evaluated until the trees mature and produce ripe fruit. Once markers have been identified, marker assisted selection (MAS) can increase economic returns, as the larger selection gains compensate for the higher costs of MAS (Bus et al. 2009) since higher selection gains compared with phenotypic selection (Moreau et al. 2000) will accelerate the breeding process (Yousef and Juvik 2001). The MAS application during the juvenile phase has been proposed to speed selection or reduce progeny sizes and the cost of carrying individuals to maturity in the field. The endoPG marker plays a vital role in fruit texture and cell wall degradation in peach. It has been used in peach breeding programs to distinguish between freestone and clingstone melting flesh and clingstone non-melting flesh progeny at the seedling stage (Peace et al. 2005). Potential benefits of MAS for fruit breeding programs in *Prunus* are many, including estimation of haplotype frequencies and haplotype-phenotype associations (Bielenberg et al. 2009; Pozzi and Vecchietti 2009). Peach is one of the best genetically characterized *Prunus* species, with known genes controlling important traits that display Mendelian inheritance patterns such as flesh color, flesh adherence to the stone, or acidity (Dirlewanger and Arús 2004; Monet et al. 1996). The conventional approach for analysis of marker-trait association in *Prunus* uses mapping populations which segregate for the characters of interest. In peach, several candidate genes and quantitative trait loci (QTLs) controlling important traits, such as blooming and harvest date, soluble solids content, titratable acidity, sugars, and other fruit quality traits, have previously been mapped and many have been located on the *Prunus* reference map (Arús et al. 2012 and references therein; Illa et al. 2011; Ogundiwin et al. 2009). To our knowledge, few of these molecular markers associated with fruit traits are being used in practical peach breeding programs.

Association mapping, also known as linkage disequilibrium (LD) mapping, is an approach that detects and locates genes relative to an existing map of genetic markers (Mackay and Powell 2007). In plants, it can be done using a case-control design or unstructured populations (i.e., populations without progenies that are also non-pedigree linked) (Oraguzie et al. 2007). A few studies have been carried out in the Rosaceae family members, including apple (Cevik et al. 2010) and pear (Oraguzie et al. 2010). These studies demonstrated that association mapping is a valuable tool for determining marker-trait association, detecting novel genes for important agronomic traits, and developing tools for genome-wide variability surveys. The complex breeding history of many important crops and the limited gene flow in most wild

plant species have created complex stratification within the germplasm, which could complicate association studies (Sharbel et al. 2000). Analysis of population structure and accounting for admixture or subgroups within unrelated germplasm (Ganopoulos et al. 2011; Mariette et al. 2010) increases confidence in association studies.

Our study was designed 1) to analyze population structure within the peach/nectarine germplasm located at the Experimental Station of Aula Dei [Consejo Superior de Investigaciones Científicas (CSIC)], Spain, and 2) to explore the utility of association mapping for detecting marker-trait association in fruit quality traits for potential application in breeding programs.

## Materials and methods

### Plant material

A collection of 94 peach and nectarine [*Prunus persica* (L.) Batsch] cultivars encompassing a wide range of geographic origins were used in this study (Table 1). This set included 43 native local Spanish cultivars and 51 modern cultivars mostly from the U.S., but also from France, Italy, New Zealand, and South Africa. The presumed parentage of most of these cultivars is also included. The genotypes were grown under Mediterranean soil conditions at the Experimental Station of Aula Dei (CSIC) located at Zaragoza in the Ebro Valley (northern Spain).

### Fruit Sampling

Twenty fruits were randomly harvested from each cultivar at commercial maturity. Fruits were peeled and cut longitudinally into two halves and a portion of the mesocarp was removed from each half and cut into small pieces. A composite sample of 5 g was built by mixing all pieces from the selected fruits. This was frozen in liquid nitrogen and kept at -20ºC until analyses. Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% $HPO_3$) until analysis for preservation of oxidation. For analysis of sugars content, samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water. For analysis of antioxidant compounds, samples were homogenized with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v) and, to determine vitamin C, samples were homogenized with 5% $HPO_3$. Samples were homogenized using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington) and extracts were centrifuged at 20,000 g for 20 min at 4ºC, and the supernatant was collected and stored at -20ºC.

**Evaluation of pomological traits**

The germplasm was evaluated for morphology of flowers, leaves, and fruits. Bloom and harvest dates were recorded in Julian days. Flower and leaf traits were measured directly in the field while some of the fruit traits were measured in the laboratory immediately after harvest. Phenotypic evaluations were made in 2008, 2009, and 2010. The eleven pomological traits of flowers and leaves evaluated include anther color (red-brown, red-yellow), bloom type (showy, non-showy), flower density (high, medium, few), flower size (small, big), flesh color (yellow, white), flesh type (melting, non-melting), fruit type (peach, nectarine), gland type (globose, reniform), petal color (pink-salmon, pink), shape type (round, ovate), and stone type (clingstone, freestone). Moreover, other fifteen parameters were analyzed including fruit weight (g), flesh firmness (N), soluble solids content (SSC) (ºBrix), titratable acidity (TA) (g malic acid/100 g FW), ripening index (RI) (SSC/TA), and concentrations of vitamin C (mg AsA/100 g FW), anthocyanins (mg C3GE/kg FW), total phenolics (mg GAE/100 g FW), flavonoids (mg CE/100 g FW), relative antioxidant capacity (µg TE/g FW), and sugars (g/kg FW). Soluble solids content (SSC) measures total juice dissolved solids, including sugars (sucrose, glucose, fructose, and sorbitol), salts, proteins, and acids, while total sugars is the sum of sucrose, glucose, fructose, and sorbitol after fixation and separation by HPLC.

The fruit weight was calculated considering the total number of fruits and the total yield per tree, as previously reported (Font i Forcada et al. 2012). Flesh firmness was measured using a penetrometer (Model FT-327) on both sides of each fruit after removing a 1 mm thick disk of skin. Soluble solids content (SSC) was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). Titratable acidity and pH were determined using an automatic titration system with NaOH titrated to pH end-point of 8.1 (Metrohm Ion analysis, 807 Dosing Unit, Switzerland). Ripening index was calculated based on SSC/TA ratio. Details for all methods were described by Abidi et al. (2011) and Cantín et al. (2009a).

Phytochemical analyses were performed as described by Cantín et al. (2009b) with minor modifications based on Abidi et al. (2011) using a spectrophotometer (Beckman Coulter DU 800). Spectrophotometric determination of vitamin C (ascorbic acid) was as described in Zaharieva and Abadía (2003). Total phenolics were determined by the Folin-Ciocalteau method as described in Singleton and Rossi (1965), while measurement of total flavonoids was according to Zhishen et al. (1999). The determination of total anthocyanins was based on Fuleki and Francis (1968) while determination of antioxidant capacity was according to Brand-Williams et al. (1995). Total sugars were purified and analyzed by HPLC (Waters 515, Milford, MA, USA) using a 300 x 7.8 mm column (Aminex® HPX-

87C, CA, USA) and manual injection (20 µL injection volume) interfaced with a PC Millenium[32] software.

**Microsatellite loci analysis and genotyping**

For DNA extraction, one young leaf was collected from each tree, frozen immediately in liquid nitrogen, and stored at -20ºC. DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Dusseldorf, Germany) following the manufacturer's instructions. Forty-two markers previously described in *Prunus* were tested in our population (Table 2). These markers were selected for their polymorphism in peach (Bouhadida et al. 2011) (dinucleotide or complex repeats) and their location on the *Prunus* reference map of 'Texas' x 'Earlygold' (Dirlewanger et al. 2004, http://www.rosaceae.org). Twenty-nine SSRs were separated using polyacrylamide gels, eleven markers were separated using an ABI PRISM 3130 Genetic Analyzer and two were analyzed using an ABI PRISM 310 Genetic Analyzer as it is shown in Table 2. Forward SSR primers were labelled with 5'-fluorescence dyes including PET, NED, VIC, and 6-FAM and the size standard was Gene Scan™ 500 Liz® (Applied Biosystems) for the ABI PRISM 3130 and ROX (Applied Biosystems) for the ABI PRISM 310. For primers that were separated by polyacrylamide gels, the polymerase chain reaction (PCR) was performed in a 15 µL volume (Bouhadida et al. 2011) and the reaction mixture contained 1x PCR buffer (Biotools, Madrid, Spain), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.15 µM of each primer, 0.5 units Taq DNA Polymerase (Biotools, Madrid, Spain), and 10 ng genomic DNA. PCR was performed in a 16 µL volume when using genetic analyzer, and the reaction mixture contained 1x PCR buffer (Biotools, Madrid, Spain), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 0.5 units Taq DNA Polymerase (Biotools, Madrid, Spain), and 30 ng genomic DNA. Both amplifications were conducted in a Gene Amp 2700 (Applied Biosystems) programmed as follows: one cycle of 3 min at 95° C, followed by 35 cycles of 1 min at 94° C, 45 s at the annealing temperature indicated in Table 2 for each primer, and 1 min at 72° C, followed by a final incubation of 7 min at 72° C and an infinite hold at 4° C. The gels were silver-stained as described in Bassam et al. (1983). Fragment sizes were estimated with the 30-330 bp AFLP ladder DNA sizing markers (Invitrogen, Carlsbad, CA) and analyzed using the Quantity One program (Bio Rad, Hercules, CA).

For automatic sequencing analysis, PCR products were multiplexed according to their size and primer labelling and separated on the platform of PCTAD (Parque Científico y Tecnológico de Aula Dei, Zaragoza, Spain, in an ABI PRISM 3130 Genetic Analyzer). Amplified fragments were sized using GeneMapper and PeakScanner software (Applied Biosystems). Additionally, fragment analyses for

multiplexed primers in an ABI PRISM 310 Genetic Analyzer were performed following published protocols (Peace et al. 2005) at the Washington State University Irrigated Agriculture Research and Extension Center (WSU-IAREC), Prosser, USA.

**Data analysis**

*Genetic variability*

Several genetic parameters were calculated for all 40 SSRs and between local and modern cultivars (Table 2). Two multilocus markers (CPDCT013 and CPPCT004) were not included in this analysis because they are multiloci. The number of observed alleles per locus (*A*), effective number of alleles per locus (Kimura and Crow 1964) ($A_e$), observed heterozygosity ($H_o$ = number of heterozygous individuals/ number of individuals scored), expected heterozygosity ($H_e = 1-\sum \rho i^2$, where ρi is the frequency of the $i^{th}$ allele) (Nei 1973), Wright's fixation index ($F_{is} = 1-H_o/H_e$), Shannon's information index (*I*) (Lewontin 1972) and power of discrimination (*PD*) (Kloosterman et al. 1993) were calculated using PopGene 1.31 software (Yeh et al. 1997, http://www.ualberta.ca). The marker data was used to generate a 0/1 matrix (presence/absence of allele in heterozygosity or homozygosity at the marker locus) that was used to estimate the genetic distance between cultivars. Genetic similarities (GS) were calculated using the Dice coefficient (Nei and Li 1979) and a dendrogram depicting relationships of the germplasm was built from the GD matrix based on the un-weighted pair group method average (UPGMA) cluster analysis in NTSYS-pc version 2.1 (Rohlf 2000).

*Analysis of population structure*

STRUCTURE analysis was performed on the whole dataset to test whether peach local cultivars and modern cultivars can be separated. The program STRUCTURE (version 2.3) implements a model-based clustering criterion for inferring population structure using genotypic data from unlinked markers (Pritchard et al. 2000). We fitted all kinds of models including both 'ancestry' and 'allele frequency' models with the option of admixture/no admixture and allele frequency correlated/allele frequency independent, respectively. We used the statistic, Δ*K*, (where K specifies the number of subpopulations or clusters) based on the rate of change in the log probability of the data (Evanno et al. 2005) to select the number of K (in our case, varying from two to six under the admixture model). We also performed 10 independent runs per K value starting with 10,000 burn-in period and 100,000 MCMC replications. A burn-in of 20,000 and 250,000 Markov Chain Monte Carlo (MCMC) replications seemed to be the best fit

for our data at K=3. This cluster showed a very clear peak with the highest height which gave us an indication of the strength of the signal detected by STRUCTURE.

*Linkage disequilibrium*

The analysis of LD was calculated using the TASSEL (Trait Analysis by Association, Evolution and Linkage) version 3 software (http://www.maizegenetics.net). Alleles with frequency below 5% (MAF) were removed. LD between pairs of multiallelic loci was calculated using the $r^2$ coefficient, separately for loci on the same or on different linkage group (LG). We chose the statistical $r^2$ as a measure of linkage disequilibrium instead of "D'' which measures only recombination whereas $r^2$ gives an indication of both recombination and mutation (Flint-Garcia et al. 2003). The significance level of LD between loci was examined using a permutation test implemented in TASSEL software for multiallelic loci, using the ''rapid permutation'' option.

*Association mapping*

We used TASSEL with the General Linear Model (GLM) option (Yu and Buckler 2006) to examine association between the phenotypic traits and DNA markers. We focused the association mapping on LG4 on the *Prunus* reference map of 'Texas' x 'Earlygold' because the *endoPG* gene, involved in softening of peach fruit, is located on this linkage group (Peace et al. 2005), as well as BPPCT015 and CPPCT028. Moreover, these markers showed the highest discrimination power estimation in our study. It is believed (Yu and Buckler 2006) that a structured association approach could correct for false associations using a Q-matrix of population membership estimates. Therefore, the population membership estimates obtained from STRUCTURE analyses were fitted as a covariate in a GLM where, phenotype=population structure + marker effect + residual. A standard correction for multiple testing, such as Bonferroni procedure (Schulze and McMahon 2002), was applied. Significant markers were declared using the Bonferroni procedure at the $p<0.00125$ experimental-wide threshold. Alleles with minor frequency (MAF) lower than 5% were removed (Wilson et al. 2004). A minimal number of individuals (<10%) were excluded in the less frequent class of pomological traits.

## Results

### Phenotypic evaluation and correlations

A broad phenotypic variation was found for most of the parameters studied in the 94 peach/nectarine cultivars. Range and means for the pomological traits, bioactive compounds content and total antioxidant activity are shown in Table 3. Harvest time was earlier almost one week every year. The earliest cultivars to be harvested 185 Julian days (late June) belonged to 'Maria Serena' and 'Super Crimson Gold' whereas the 'Alcañiz 1' and 'Calanda Tardío' latest were harvested with 275 Julian days (late October). Mean values of flesh firmness, vitamin C, phenolics, flavonoids, RAC and total sugars were 38 N, 13 mg AsA/100 g FW, 44 mg GAE/100 g FW, 24 mg CE/100 g FW, 842 µg TE/g FW and 110 g/kg FW, respectively.

The Pearson's correlation coefficients between pairs of traits are shown in Table 4. High and significant correlations were found between harvest date, fruit weight, and concentrations of soluble solids, antioxidants, and sugars. These results show that when fruits are harvested late, they are sweeter, larger, and have high total phenolics, flavonoids, RAC, sucrose, sorbitol, and total sugars concentrations. A significant negative correlation was found between harvest date and flesh firmness and between ripening index, flesh firmness, and concentrations of flavonoids, total phenolics, sucrose, glucose, fructose, sorbitol, and total sugars. This suggests that softer fruit is linked to late harvest date and higher concentrations of sugars and health-benefiting compounds.

High and significant correlations were found between total sugars and sucrose, glucose, fructose, and sorbitol, and between SSC and flavonoids, total phenolics, RAC, and sorbitol (Table 4). Other important positive and significant correlations were found between RAC and fruit weight, SSC, vitamin C, flavonoids, and total phenolics and between total phenolics and fruit weight, SSC, and flavonoids. Flavonoids also correlated with fruit weight, SSC, and TA.

### Allelic variation, fixation index and heterozygosity measures

Forty-two SSR markers amplified successfully in the 94 peach/nectarine accessions. To avoid potential error in estimating genetic parameters, markers CPPCT004 and CPDCT13, which amplified more than one locus, were excluded from the analysis. The average estimates of allelic variation, heterozygosity measures, Wright's fixation index, Shannon's information index, and power of discrimination for the remaining 40 SSRs are shown (see supplementary file 1). All primers pairs but two produced a maximum

of two bands per genotype in accordance with the diploid level of this species. The mean value found in this study was of 5.10 alleles per locus. Microsatellite BPPCT025 detected the highest number of alleles (11) among the 94 genotypes analyzed, followed by BPPCT015 with 10 different alleles. BPPCT014, CPPCT023, CPPCT033, CPSCT005, pchgms4, pchgms5, UDP96-005, and UDP97-401 detected the lowest number of alleles, only two. Amplification with the others 30 SSRs were variable, ranging between 3 and 9 (see supplementary file 1). $H_o$ values ranged from 0.06 (BPPCT014) to 0.98 (BPPCT033, UDP98-025 and UDP98-409), and the values for $H_e$ ranged between 0.06 (BPPCT014) to 0.81 (BPPCT015), with an average of 0.48 and 0.49, respectively. $F_{is}$ values were positive in 23 primers, zero in BPPCT014, and negative in the remaining sixteen SSRs, indicating a high level of heterozygosis in the genotypes analyzed. Regarding power of discrimination, the BPPCT015 and CPPCT028 were the best at discriminating between two random cultivars (PD=0.73 and 0.72, respectively), whereas the less informative was BPPCT014 (PD=0.06). Generally, genetic parameters were higher in modern than in local cultivars. The total number of alleles across all 40 SSR loci was higher in local cultivars (172) than in modern cultivars (159) (see supplementary file 1).

**Population structure**

The peach collection, including local cultivars and modern cultivars, was evaluated for population stratification or admixture using STRUCTURE software. Bar plots were obtained with different values of K, the assumed number of subpopulations. The maximum rate of change in the log probability of the data occurred at K=3. In general, there were two populations with subpopulation one comprising modern cultivars and subpopulation two representing local cultivars. However, there was a little bit of admixture in each subpopulation suggesting allele sharing (Fig. 1). For comparison, at K=3 (Fig. 1b), the results were congruent, suggesting a more complex structure that with K=2 (Fig. 1a). When increasing K, the subpopulations became almost inseparable (Fig. not shown).

Clusters obtained by STRUCTURE for population stratification were compared with the UPGMA analysis. The pattern of diversity in morphological characteristics within the germplasm is shown in Fig. 2. A tree constructed from the SSR data divided the cluster into sub-clusters characterized by correspondence with fruit characteristics and local or modern cultivars. For example, nectarines, modern cultivars, and melting flesh varieties such as ‘Big Top’, ‘Fantasia’, ‘Flamekist’, ‘Flavortop’, ‘Queen Giant’, and ‘Venus’ are grouped in the same cluster. However, melting peaches ‘Benasque’, ‘Lovell’, and ‘Redhaven’ group according to their origin. ‘Lovell’ grouped close to ‘Halford’, ‘Gomes’,

and ‘Starn’, all USA cultivars, and ‘Redhaven’ grouped close to ‘Babygold 6’, ‘Babygold 7’, and ‘Babygold 8’, also all from the USA. Furthermore, some of the cultivars are clustered together following the reported parentage (Table 1). Thus, ‘Andora’ and ‘Carolyn’ are clustered together as they came from the same cross (‘Libee’ x ‘Lovell’). This was also the case with ‘Starn’ and ‘Shasta’, ‘Suncling’ and ‘Babygold 9’, ‘Andross’ and ‘Everts’ or ‘Fantasia’ and ‘Flamekist’, that share a common parent (‘Paloro’, ‘PI35201’, ‘Dix 5A-1’ and ‘Gold King’, respectively).

In the dendrogram, there is a clear agreement between clusters representing genetic diversity and population structure at K=2, particularly, the differentiation of local cultivars and modern cultivars (Fig. 2a). Most accessions grouped with either local cultivars (green) or modern cultivars (red). Also, there was clear separation between peaches and nectarines (Fig. 2b) and by leaf gland (Fig. 2e). At K=2, we observed a split between local cultivars and modern cultivars. At K=3, the clusters of local and modern cultivars split into two subpopulations and most cultivars fell into either a group with red-brown anthers (Fig. 2c) and pink-salmon petals (Fig. 2d) for local cultivars or a group with red-yellow anthers (Fig. 2c) and pink petals (Fig. 2d) for modern cultivars. For nectarines, there is a clear connection between red-yellow anthers, pink petals, and leaf reniform gland. For peaches, the results are mixed. Finally, the separation between showy and non-showy flowers was difficult because the clusters were mixed (Fig. 2f). With increasing K, the red subpopulation remained almost inseparable (at K=4 and K=5, Fig. 2), while the green subpopulation became divided into smaller subpopulations.

**Linkage disequilibrium**

Even though the density of coverage of the genome was low (the average distance between pairs of markers was 10 cM), we detected some trends of LD between pairs of markers (Table 5). For the whole set of varieties, overall LD was low, with some indication of higher LD up to 20 cM, and a decay at farther distances, to approximately the same level shown by unlinked markers. The same trend was observed for the local and modern cultivars. For the groups determined with the STRUCTURE analysis, LD relationship with distance was variable. Groups Q1 and Q3 showed higher LD overall, and it extended even to 30 cM at group Q1. For group Q2, LD was no different from background at any distance. Except for groups Q2 and Q3, intrachromosomal LD was slightly higher than interchromosomal LD. Attending to the distribution of LD across linkage groups, the markers of LG5 presented clearly higher scores than interchromosomal LD, or even intrachromosomal LD at the other linkage groups (Fig.

3 and supplementary file 2). LG7 also presented higher values than others at groups Q1 and Q3, but showed low values for the whole sample, or the local and modern cultivars (see supplementary file 2).

**Association mapping**

Analysis of marker-trait associations using 40 SSR markers with 26 pomological traits was done using TASSEL software. After the Bonferroni procedure the number of associations was reduced from 296 to 55 using a modelling coefficient of membership (Q) values estimates from STRUCTURE as co-variate and to 61 without co-variate. We will focus on significant associations obtained using Q values since they are more conservative (Table 6). Henceforth, our attention will be on associations identified based on endoPG1, marker involved in softening, BPPCT015, and CPPCT028, all located on LG4. The power of discrimination of these markers was higher than others located on the same LG (see supplementary file 1, 0.51, 0.73, and 0.72, respectively). BPPCT015 marker was significantly associated with harvest date ($p$=0.0000072), flavonoids ($p$=0.000081) and sorbitol contents ($p$=0.000013) (Table 6). CPPCT028 was associated with anther color ($p$=0.000011), flesh fruit color ($p$=0.0000001), harvest date ($p$=0.00037), phenolics ($p$=0.000019), RAC ($p$=0.00039) and total sugars ($p$=0.00016) contents, while endoPG1 was associated with flesh firmness ($p$=0.000070) and total sugars content ($p$=0.00061).

Table 7 shows the association between the genotype and haplotype with the pomological traits analysed. The 167_167 genotype of BPPCT015 was associated with low concentrations of flavonoids, and sorbitol content which are also linked to medium harvest date. In contrast, the 220_229 genotype was associated with late harvest, and high concentrations of flavonoids and sorbitol. Furthermore, the 136_136 genotype of CPPCT028 was strongly associated with low concentrations of total phenolics, relative antioxidant capacity and low to medium concentrations of total sugars, which are also linked to medium harvest date. The 136_138 genotype of CPPCT028 was associated with late harvest date and high concentrations of total phenolics, RAC and total sugars. The 192_196 genotype of endoPG1 was associated with high firmness, and low to medium concentrations of total sugars, while the 192_228 genotype was associated with high concentrations of total sugars, which are also negatively linked to firmness. Only two haplotypes were associated with one trait. In particular, the 169/136 haplotype from BPPCT015/CPPCT028 was linked to early to medium harvest date while the 209/134 haplotype was strongly associated with late harvest date.

## Discussion

### Phenotypic evaluation

A broad phenotypic variation was found for all the parameters studied in the 94 peaches and nectarines cultivars except for bloom date. Harvest date varied among cultivars with values in the range of 185-275 Julian days. This trait has been established as characteristic of each cultivar, and quantitatively inherited (Dirlewanger et al. 1999). Moreover, harvest date may change every year depending on the environmental conditions and/or cultivars but harvest season remains constant (Mounzer et al. 2008). All pomological traits evaluated were in the same range than those reported by other authors in other peach cultivars (Cantín et al. 2009a; 2009b; Cevallos-Casals et al. 2006; Gil et al. 2002; Tavarini et al. 2008; Tomás-Barberán et al. 2001).

### Allelic variation, fixation index, heterozygosity measures

The 42 SSR markers covering the peach genome used to screen the 94 peach/nectarine cultivars were previously used for cultivar identification and genetic mapping (Testolin et al. 2000) and for phylogenetic studies in peach and other *Prunus* species (Aranzana et al. 2003; Bouhadida et al. 2007; 2009; 2011). The successful amplification of these markers in peach and other *Prunus* species demonstrates the high synteny across this genus (Aranzana et al. 2003). Markers BPPCT001, BPPCT006, BPPCT008, CPPCT006, CPPCT022, CPPCT029, PceGA34, pchgms3, and UDP98-412 were also used to study genetic variation in peach (Bouhadida et al. 2007; 2011), with reported polymorphism similar to ours. The mean value found in this study was of 5.10 alleles per locus, which is slightly lower that the 6.36 observed by the Aranzana et al. (2010) and 6.73 by Bouhadida et al. (2011). The observed heterozygosity averaged (0.48) over the 40 SSR loci was slightly higher than reported values of 0.35 (Aranzana et al. 2003; 2010) and 0.23 (Bouhadida et al. 2011). High $F_{is}$ values in combination with homozygosity (or individuals showing only one band) in these primers suggest the presence of a null allele (Brookfield 1996). The presence of null alleles affecting heterozygosity could cause such differences. The fixation index and the power of discrimination was slightly lower than others reported (Aranzana et al. 2003; Bouhadida et al. 2011). The differences found in this study could be due either to the different plant material used or to the use of SSRs markers with lower PD. The modern cultivars in our collection were as genetically diverse as the local cultivars. These results are different to those found in a self-incompatible species such as cherry (Mariette et al. 2010), where local cultivars were more diverse than

modern cultivars. This is congruent with current understanding of the evolutionary history of clonally propagated domesticated plants (McKey et al. 2010). It is noteworthy that peach is the less polymorphic species within the *Prunus* because of its condition of self-compatibility.

**Population structure**

The analysis performed with the STRUCTURE software showed that using K=2 the results suggested that our peach germplasm comprises two main subpopulations with some degree of admixture within both subpopulations (modern and local cultivars). With K=3 and higher, the differentiation was not so apparent. Similar studies in peach reported three unstructured populations including 94 melting peaches, 39 non-melting peaches, and 91 nectarines, indicating a strong subpopulation structure (Aranzana et al. 2010). In our study, nectarines grouped in one cluster similar to what the authors above showed (see Fig. 2). Further, according to these authors, some non-melting peaches such as 'Jerónimo', 'Calabacero', 'San Lorenzo', and 'Maruja' grouped according to their Spanish origin while 'Babygold 7', 'Babygold 8', 'Andross', and 'Catherina' grouped according to their foreign origin; a finding similar to our results. The domestication of peach was likely a complex process with several origins resulting from clonal propagation of desirable genotypes and sexual reproduction with local wild peaches. Domestication and breeding generally cause diversity loss, resulting in bottleneck and genetic drift. Diversity after a bottleneck depends on the ratio of wild and cultivated population sizes and the duration of the bottleneck (Haudry et al. 2007). In many fruit species, domestication occurred relatively late, so the bottleneck was relatively recent and its duration short. Although the population genetic parameters obtained suggest that Spanish local cultivars are slightly less diverse than modern cultivars, we interpret these results with caution, since our sampling was limited to the material conserved in our collection. In particular, our local cultivars were selected from populations that have been seed-propagated, possibly over many generations, while the modern cultivars were obtained by crossing two individuals and selecting progeny. Other studies in peach addressing genetic variability of introduced and local Spanish cultivars showed differentiation of accessions according to adaptation to different environmental conditions (Bouhadida et al. 2011). In particular, Ebro Valley cultivars clustered with the USA releases, suggesting a common gene pool. These results agree, considering the active exchange of germplasm between both countries and the extensive use of Spanish cultivars in American peach breeding programs (Okie 1998).

**Linkage disequilibrium**

The overall level of LD detected was rather low, but this depends on the density of marker coverage, which was rather sparse in this study. The average interval was 10 cM, with a maximum of 16 cM at LG1, and a minimum of 8 cM at LG5, but the correlation of intrachromosomal LD with mean interval size across LG was low and non-significant (data not shown). Looking at trends of LD, it decreased with distance, fading away after 20 cM. This value is in the same range as the extent of LD found also in peach by Aranzana et al. (2010). The higher LD observed in LG5 was evident for all groups of varieties, except for Q2 (see supplementary files 2-7). This means that the haplotypes of markers at this LG tend to be more homogeneous within groups than at other LGs. This may have been caused by a selection event of a founder effect affecting specifically genes of this LG, and that did not affect the group of varieties in Q2. One possible cause was the presence of a distinct group of nectarines (7 individuals), which was included within the modern cultivars and the Q1 groups, respectively for the two classifications considered. This group is characterized by the presence of the allele that confers the non-hairy trait, at locus *G* in LG5. We can speculate that the varieties carrying this allele may have experienced linkage drag for the rest of LG5 during breeding, and this may have influenced the level of LD detected for this LG at the groups containing the nectarines. To test this hypothesis, we repeated the analyses of LD for the modern and Q1 groups excluding the nectarines, and the result was the same. Therefore, this higher level of LD at LG5 was not caused by the presence of the nectarine group.

**Marker-trait associations and phenotypic correlations**

Genome-wide analysis using a GLM procedure in TASSEL identified three loci, BPPCT015, CPPCT028, and endoPG1, which were previously mapped to chromosome 4 and associated with pomological traits in the peach/nectarine germplasm. We analyzed these markers separately because they are on LG4 and showed high polymorphism and power of discrimination.

Different combinations of genotypes/haplotypes associated with important pomological traits were obtained. For example, the 192_196 and 192_228 genotypes of endoPG1 associated significantly with low/high content of total sugars and high/low firmness. Both parameters are indirectly linked because when fruits are ripe, they have low firmness and high total sugars content. Also, the significant negative correlation obtained between them confirmed the associations found. On the contrary, we did not find significant associations between endoPG1 and flesh type and stone type. This lack of association is probably because melting and freestone peaches and nectarines are not well represented in our

germplasm. Only 10 cultivars out of 94 cultivars belong to the melting type and 5 cultivars out of 94 belong to freestone. The lack of melting flesh type material in our collection happened because historically, the Spanish peach industry was based on non-melting flesh peaches, primarily derived from native populations, both for fresh market and canning purposes (Badenes et al. 1998; Cambra 1988; Herrero 1953). Other important associations were found between the 167_167 and 220_229 genotypes of BPPCT015, the 136_136 and 136_138 genotypes of CPPCT028, with other pomological traits (i.e. different content in antioxidants and sugars). In addition, associations were found between the haplotypes 169/136 and 209/134 of BPPCT015/CPPCT028 with harvest date.

Furthermore, the correlations found in this work among several pomological traits confirm the associations discussed above. For example, high sorbitol was associated to high flavonoids and late harvest, and it exist significant positive correlations among harvest date, SSC, flavonoids, sorbitol and total sugars. Genotypes with high sorbitol are currently of interest for fruit breeders (Ledbetter et al. 2006) since this sugar can be alternatively used as sweetener for diabetics (Cantín et al. 2009a). Moreover, from a practical point of view, the significant positive correlations found between SSC and total sugars, and the fact that those characters were associated, suggest that high SSC can be used as an indirect measure to select genotypes for high total sugars and flavonoids content.

The results found in this study support the potential of the SSR association mapping for agronomical and biochemical important traits in peach. Besides several studies in identifying marker-trait association have been published in other plant species in the Rosaceae family (Cevik et al. 2010; Oraguzie et al. 2010), to our knowledge this is the first study concerning association mapping with pomological traits in peach.

Previously in peach, several QTLs affecting pomological and agronomic traits that have been on the *Prunus* reference map were reported on LG4 for SSC, TA and pH (Cantín et al. 2010a); SSC, glucose fructose, sorbitol, blooming and harvest date (Arús et al. 2012 and references therein). Other QTLs for fructose, sorbitol content and several organic acids were also located on LG4 on a region corresponding to bin 4:27 of T × E (Ogundiwin et al. 2009). In addition, it is remarkable to note that other authors found QTLs for glucose, fructose and sorbitol in peach linked to the BPPCT015 marker (Illa et al. 2011) and for ripening date in almond linked to the CPPCT028 (Sánchez-Pérez et al. 2007). Other QTL explaining maturity date was mapped near the EPPISF032 marker on LG4 (Eduardo et al. 2011) and others controlling antioxidant compounds content (Abidi, personal communication) were located on this linkage

group. Besides of these QTLs, several candidate genes linked to a potential role acidity, and phenolic content and fruit growth were mapped on other LGs 3, 5, and 7 (Le Dantec et al. 2010). Regarding bloom date we did not find any correlation or association in our study. However, Fan et al. (2010) found strong QTLs on LG1 during four years in a segregating family. These differences could be probably due to the different plant material used in both studies apart of the environmental effects on bloom date as it was already discussed by these authors. The range of blooming date in the population varied from 16 days (year 2006) to 53 days (year 2007) while our 94 genotypes showed only eight days of variation among genotypes. Likewise, some SSR markers linked to specific monogenic traits have been developed in peach although few practical examples have been described in MAS. The *endoPG* gene has been used in marker assisted selection for distinguishing between melting and non-melting at the seedling stage in peach breeding programs (Peace et al. 2005). Concerning the showy flower type (*Sh*), Fan et al. (2010) located the gene on LG8 1cM from CPPCT006 and Eduardo et al. (2011) described the character cosegregating with ssrCITA15 on the same LG. Another marker, MA014a, apparently was defined controlling flat fruit (*S*) and aborting fruit (*Af*) as single gene (Dirlewanger et al. 2006), however, some discrepancies were described for other authors (Cantín et al. 2010b).

Based on the significant marker-trait association highlighted above, marker-assisted breeding facilitate selection, including prediction of genotype of progeny, leaving only selections with favourable genotypes/alleles for desired pomological traits, and characterising parents used in peach breeding programs. Additionally, this work provided promising results concerning association mapping with pomological traits that could be applied in other *Prunus* species because of the complete synteny found inside the Rosaceae family.

The present study demonstrates for the first time evidence concerning the utility of association genetics and its potential to generate useful marker-trait associations for application in peach breeding. STRUCTURE analysis identified two main groups, local and modern cultivars, with some admixture within groups. The local cultivars were slightly less diverse than modern cultivars, probably because they were mainly non-melting peach types while the modern cultivars comprised both melting and non-melting peach and nectarine varieties. In addition, our results indicate a subpopulation structure and a relatively high level of linkage disequilibrium conservation. Furthermore, significant associations were observed between genotypes and haplotypes of markers BPPCT015, CPPCT028, and endoPG1 and pomological traits. In particular, two genotypes from BPPCT015 were associated with low and high

values of harvest date, flavonoids and sorbitol content. Also, two genotypes from CPPCT028 were associated with low and high values of harvest date, total phenolics, RAC and total sugars. Finally, two genotypes of endoPG1 were linked to flesh firmness and total sugars. As these traits are linked, using a marker to select for one trait would mean indirect selection for other traits, capturing correlated responses. The associations determined in this study would be very useful for deployment for marker-assisted selection (MAS) in peach breeding programs although further research is needed to validate these associations in other populations from a different genetic background. New studies are in progress mapping thousands of SNPs (*RosBREED_Peach* chip from Illumina® Infinium®) to facilitate genome-wide scans and validate marker-locus-trait associations for application in breeding.

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**Table 1** Cultivar name, classification, origin, main fruit characteristics and pedigree of the cultivars studied

| Cultivar | Classification | Origin | Flesh colour | Fruit type | Flesh type | Stone type | Reported parentage |
|---|---|---|---|---|---|---|---|
| Adriatica | Modern cultivars | Italy | Yellow | Peach | Non-melting | Clingstone | - |
| Alcañiz 1 | Local cultivars | Teruel, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Alcañiz 2 | Local cultivars | Teruel, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Alejandro Dumas (351 AD) | Local cultivars | La Rioja, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Amarillo Calanda (131 AD) | Local cultivars | Huesca, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Amarillo Calanda (2400 AD) | Local cultivars | Huesca, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Amarillo Gallur | Local cultivars | Zaragoza, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Andora | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Libbee x Lovell |
| Andross | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Dix 5A-1 x Fortuna |
| Baby Gold 5 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | PI35201 x NJ196 |
| Baby Gold 6 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | NJ13232 x NJ196 |
| Baby Gold 7 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | (Lemon Free x PI35201) x NJ196 |
| Baby Gold 8 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | PI35201 x Ambergem |
| Baby Gold 9 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | PI35201 x PI43137 |
| Baladin | Modern cultivars | France | Yellow/Orange | Peach | Non-melting | Clingstone | - |
| Benasque (3135 AD) | Local cultivars | Huesca, Spain | White | Peach | Melting | Freestone | op |
| Big Top | Modern cultivars | USA | Yellow | Nectarine | Melting | Clingstone | - |
| Bonet I | Local cultivars | Lérida, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Bonet II | Local cultivars | Lérida, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Bonet III | Local cultivars | Lérida, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Bonet IV | Local cultivars | Lérida, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Bonet V | Local cultivars | Lérida, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Borracho de Jarque | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Brasileño | Local cultivars | Murcia, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Calabacero (2247 AD) | Local cultivars | Murcia, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Calanda San Miguel | Local cultivars | Teruel, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Calanda Tardío | Local cultivars | Teruel, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Campiel (3139 AD) | Local cultivars | Huesca, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Campiel Rojo | Local cultivars | Huesca, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Carolyn | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Libbee x Lovell |
| Carson | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Maxine x Leader |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Catherina | Modern cultivars | USA | Yellow/Orange | Peach | Non-melting | Clingstone | NJC95 x D42-13W |
| Del Gorro | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | clingstone | op |
| Diamante Amarillo | Local cultivars | Teruel, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Dixon | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Orange Cling x Australian Muir |
| Everts | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Dix 5A-1 x Dix 22A-5 |
| Fantasia | Modern cultivars | USA | Yellow | Nectarine | Melting | Freestone | Gold King x P101-24 |
| Flamekist | Modern cultivars | USA | Yellow | Nectarine | Melting | Clingstone | Gold King self |
| Flavortop | Modern cultivars | USA | Yellow | Nectarine | Melting | Freestone | Fairtime op |
| Fortuna | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Leader sdlg x (Tuscan x Paloro) |
| GF3 | Modern cultivars | France | Yellow | Peach | Non-melting | Clingstone | - |
| Goiri | Local cultivars | Bilbao, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Golden Queen | Modern cultivars | New Zealand | Yellow | Peach | Non-melting | Clingstone | - |
| Gomes | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | unknown (originated in California) |
| Halford | Modern cultivars | USA | Yellow/Orange | Peach | Non-melting | Clingstone | chance sdlg in Phillips Cling orchard |
| Infanta Isabel (1068 AD) | Local cultivars | Castellón, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Jerónimo de Alfaro | Local cultivars | Murcia, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Jungerman | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Dix 22A-5 x Dixon 1 |
| Kakamas | Modern cultivars | South Africa | Yellow | Peach | Non-melting | Clingstone | St. Helena op |
| Keimoes | Modern cultivars | South Africa | Yellow/Orange | Peach | Non-melting | Clingstone | Transvaal op |
| Klamt | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Dixon 1 x Wiser |
| Loadel | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Lovell op? |
| Lovell | Modern cultivars | USA | Yellow | Peach | Melting | Freestone | chance sdlg |
| Maluenda | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Maria Serena | Modern cultivars | Italy | Yellow | Peach | Non-melting | Clingstone | - |
| Maruja | Local cultivars | Murcia, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Maruja Porvenir | Local cultivars | Murcia, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Miraflores (2844 AD) | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Mountaingold | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | PI35201 x NJ196 |
| Nectar del Jalón | Local cultivars | Zaragoza, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| NJC 97 | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | - |
| Nuevo (2803 AD) | Modern cultivars | France | Yellow | Peach | Non-melting | Clingstone | Includes PI32374, Peak, Elberta, Peen-To |
| Oropel | Local cultivars | Teruel, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Paloro A | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Paloro B | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | - |
| Queen Giant | Modern cultivars | USA | White | Nectarine | Melting | Clingstone | - |
| Redhaven | Modern cultivars | USA | Yellow | Peach | Melting | Semi-clingstone | Halehaven x Kalhaven |
| Rojo del Rito | Local cultivars | Lérida, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| San Jaime | Local cultivars | Lérida, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| San Lorenzo | Local cultivars | Huesca, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Sarell | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Selma | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | - |
| Shasta | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Leader sdlg x (Tuscan x Paloro) |
| Stanford | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Hauss x Phillips |
| Starn | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | chance sdlg in Paloro orchard |
| Sudanell 1 | Local cultivars | Lérida, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Sudanell 2 | Local cultivars | Lérida, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Sudanell 3 | Local cultivars | Lérida, Spain | Yellow/Orange | Peach | Non-melting | Clingstone | op |
| Sudanell Blanco | Local cultivars | Zaragoza, Spain | White | Peach | Non-melting | Clingstone | op |
| Sudanell GF (2804 AD) | Modern cultivars | France | Yellow/Orange | Peach | Non-melting | Clingstone | - |
| Sudanell GF (2972 AD) | Modern cultivars | France | Yellow/Orange | Peach | Non-melting | Clingstone | - |
| Suncling | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | PI35201 x NJ196 |
| Super Crimson Gold | Modern cultivars | USA | White | Nectarine | Melting | Clingstone | - |
| Tebana | Modern cultivars | Italy | Yellow | Peach | Non-melting | Clingstone | - |
| Tempranillo de Aytona | Local cultivars | Huesca, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Tipo Campiel (2921 AD) | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Venus | Modern cultivars | Italy | Yellow | Nectarine | Melting | Freestone | - |
| Vesuvio | Modern cultivars | Italy | Yellow | Peach | Non-melting | Clingstone | - |
| Vivian | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | (Marine x Leader) x [(Tuscan x Paloro) x (Paloro x Pratt Low)] |
| Walgant | Modern cultivars | South Africa | Yellow | Peach | Non-melting | Clingstone | Kakamas self |
| Wiser | Modern cultivars | USA | Yellow | Peach | Non-melting | Clingstone | Lovell x Sims |
| Zaragozano (553 AD) | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Zaragozano Amarillo (2857 AD) | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |
| Zaragozano Rojo (2858 AD) | Local cultivars | Zaragoza, Spain | Yellow | Peach | Non-melting | Clingstone | op |

*op* open-pollinated, *sdlg* seedlings, *NJ* New Jersey, *self* self-pollinated

**Table 2** Names and characteristics of the SSR markers used for genotyping the 94 peach/nectarine cultivars

| SSR | Species of origin | Position on LG in the 'TxE' reference map (cM from the top) | AT (ºC) | References | SSRs analysis |
|---|---|---|---|---|---|
| | | **Position on LG 1** | | | |
| UDP96-005 | Peach | 29.2 | 57 | Cipriani et al. 1999 | Polyacrylamide gels |
| pchgms3 | Peach | 37.5 | 60 | Sosinski et al. 2000 | Polyacrylamide gels |
| CPPCT029 | Peach | 65.1 | 55 | Aranzana et al. 2002 | Polyacrylamide gels |
| BPPCT028 | Peach | 77.4 | 57 | Dirlewanger et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| | | **Position on LG 2** | | | |
| CPPCT044 | Peach | 7.4 | 58 | not published (origin IRTA) | Polyacrylamide gels |
| UDP98-025 | Peach | 9.6 | 57 | Testolin et al. 2000 | Polyacrylamide gels |
| BPPCT001 | Peach | 20.9 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| UDP96-013 | Peach | 27.8 | 57 | Cipriani et al. 1999 | ABI PRISM 3130 Genetic Analyzer |
| BPPCT024 | Peach | 36.3 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| UDP98-410 | Peach | 38 | 57 | Testolin et al. 2000 | Polyacrylamide gels |
| PceGA34 | Sour cherry | 43.9 | 50 | Downey and Iezzoni 2000 | Polyacrylamide gels |
| | | **Position on LG 3** | | | |
| BPPCT007 | Peach | 11.2 | 57 | Dirlewanger et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| CPPCT002 | Peach | 31.9 | 52 | Aranzana et al. 2002 | Polyacrylamide gels |
| UDP96-008 | Peach | 36.4 | 57 | Cipriani et al. 1999 | Polyacrylamide gels |
| | | **Position on LG 4** | | | |
| BPPCT010 | Peach | 2.1 | 57 | Dirlewanger et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| CPPCT028 | Peach | 11 | 50 | Aranzana et al. 2002 | Polyacrylamide gels |
| pchgms5 | Peach | 24.1 | 55 | Sosinski et al. 2000 | Polyacrylamide gels |
| UDP96-003 | Peach | 28.3 | 55 | Cipriani et al. 1999 | ABI PRISM 3130 Genetic Analyzer |
| BPPCT015 | Peach | 44.0 | 62 | Dirlewanger et al. 2002 | ABI PRISM 310 Genetic Analyzer |
| endoPG1 | Peach | 47.8 | 60 | Peace et al. 2005 | ABI PRISM 310 Genetic Analyzer |
| CPSCT005 | Plum | 53.8 | 62 | Mnejja et al. 2004 | Polyacrylamide gels |
| | | **Position on LG 5** | | | |
| UDP97-401 | Peach | 11 | 57 | Cipriani et al. 1999 | ABI PRISM 3130 Genetic Analyzer |
| BPPCT017 | Peach | 20.1 | 57 | Dirlewanger et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| pchgms4 | Peach | 26.7 | 52 | Sosinski et al. 2000 | Polyacrylamide gels |

| | | | | | |
|---|---|---|---|---|---|
| BPPCT038 | Peach | 32.9 | 57 | Dirlewanger et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| BPPCT014 | Peach | 44 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| | | **Position on LG 6** | | | |
| UDP96-001 | Peach | 17.5 | 57 | Cipriani et al. 1999 | Polyacrylamide gels |
| BPPCT008 | Peach | 30.1 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| CPPCT023 | Peach | 41.5 | 55 | Aranzana et al. 2002 | Polyacrylamide gels |
| BPPCT025 | Peach | 56.4 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| UDP98-412 | Peach | 72 | 57 | Testolin et al. 2000 | Polyacrylamide gels |
| CPPCT030 | Peach | 80.2 | 50 | Aranzana et al. 2002 | Polyacrylamide gels |
| | | **Position on LG 7** | | | |
| CPPCT022 | Peach | 18.7 | 50 | Aranzana et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| UDP98-408 | Peach | 23.7 | 57 | Cipriani et al. 1999 | Polyacrylamide gels |
| CPPCT033 | Peach | 38.9 | 50 | Aranzana et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| CPPCT017 | Peach | 61.8 | 60 | Aranzana et al. 2002 | Polyacrylamide gels |
| | | **Position on LG 8** | | | |
| BPPCT006 | Peach | 14.1 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| BPPCT033 | Peach | 18.8 | 57 | Dirlewanger et al. 2002 | Polyacrylamide gels |
| CPPCT006 | Peach | 24.8 | 59 | Aranzana et al. 2002 | ABI PRISM 3130 Genetic Analyzer |
| UDP98-409 | Peach | 44.5 | 57 | Cipriani et al. 1999 | Polyacrylamide gels |
| CPDCT013 | Almond | **Multiloci: LG 3, 6 and 7** | 62 | Mnejja et al. 2005 | Polyacrylamide gels |
| CPPCT004 | Peach | **Multiloci: LG 1 and 5** | 52 | Aranzana et al. 2002 | Polyacrylamide gels |

*LG* linkage group location of the 42 SSR markers, *AT* annealing temperature used

**Table 3** Units, minimum, maximum and mean values for the pomological traits evaluated

| Trait | Units | Minimum | Maximum | Mean |
|---|---|---|---|---|
| Bloom date* | Julian days | 79 | 87 | 82 |
| Harvest date | Julian days | 185 | 275 | 224 |
| Fruit weight (FW)* | Grams | 64 | 315 | 178 |
| Soluble Solids Content (SSC)* | ºBrix | 12 | 18 | 15 |
| Flesh firmness (FF) | Newtons ($kg/cm^2$) | 9 | 61 | 38 |
| Titratable acidity (TA)* | g malic acid/100 g FW | 0.4 | 0.9 | 0.62 |
| Ripening index (RI) | SSC/TA | 15 | 67 | 25 |
| Vitamin C | mg AsA/100 g FW | 3 | 28 | 13 |
| Total phenolics | mg GAE/100 g FW | 18 | 62 | 44 |
| Flavonoids | mg CE/100 g FW | 3 | 63 | 24 |
| Anthocyanins | mg C3GE/kg FW | 0.7 | 12 | 3 |
| Relative Antioxidant Capacity (RAC) | µg TE/g FW | 186 | 1184 | 842 |
| Sucrose | g/kg FW | 35 | 97 | 75 |
| Glucose* | g/kg FW | 4 | 15 | 10 |
| Fructose* | g/kg FW | 2 | 14 | 10 |
| Sorbitol | g/kg FW | 2 | 35 | 13 |
| Total sugars (TS) | g/kg FW | 63 | 136 | 110 |

*AsA* ascorbic acid, *GAE* gallic acid equivalents, *CE* catechin equivalents, *C3GE* cyanidin-3-glucoside equivalents, *TE* trolox equivalents

* Association analysis was performed with these traits but no association was found

**Table 4** Pearson's correlation coefficients between pairs of pomological traits studied

| Trait | FW | SSC | FF | TA | RI | Vitamin C | Total phenolics | Flavonoids | RAC | Sucrose | Glucose | Fructose | Sorbitol | TS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Harvest date (Julian days) | 0.63** | 0.63** | -0.52** | ns | ns | ns | 0.65** | 0.79** | 0.72** | 0.62** | ns | 0.21* | 0.78** | 0.66** |
| Fruit weight (g) | ns | 0.56** | ns | 0.15* | ns | ns | 0.53** | 0.21* | 0.34* | ns | 0.36** | 0.39* | ns | 0.25* |
| SSC (ºBrix) | | - | 0.49** | 0.26** | ns | ns | 0.56** | 0.60** | 0.61** | 0.29** | 0.27** | 0.36* | 0.77** | 0.49** |
| Flesh firmness (N) | | | - | 0.40** | -0.57* | ns | -0.52** | -0.26* | ns | -0.50** | -0.64** | -0.49** | -0.42* | -0.59** |
| TA (g malic acid/100 g FW) | | | | ns | ns | 0.46** | ns | 0.35** | ns | ns | 0.41** | ns | 0.40** | ns |
| RI (SSC/TA) | | | | | ns | -0.21* | ns | ns | ns | 0.42** | 0.24* | 0.35* | 0.41** | 0.27** |
| Vitamin C (mg AsA/100 g FW) | | | | | | ns | ns | ns | 0.25* | ns | ns | ns | 0.37** | 0.42** |
| Total phenolics (mg GAE/100 g FW) | | | | | | | ns | 0.68** | 0.79** | 0.43** | 0.42** | ns | 0.52** | 0.58** |
| Flavonoids (mg CE/100 g FW) | | | | | | | | ns | 0.87** | 0.47** | 0.44** | 0.24* | 0.47** | 0.61** |
| RAC (µg TE/g FW) | | | | | | | | | ns | ns | 0.52** | ns | 0.64** | 0.64** |
| Sucrose (g/kg FW) | | | | | | | | | | ns | 0.57** | 0.63** | 0.48** | 0.95** |
| Glucose (g/kg FW) | | | | | | | | | | | ns | 0.83** | 0.44** | 0.81** |
| Fructose (g/kg FW) | | | | | | | | | | | | ns | 0.49** | 0.83** |
| Sorbitol (g/kg FW) | | | | | | | | | | | | | ns | 0.56** |
| Total sugars (g/kg FW) | | | | | | | | | | | | | | ns |

*ns* not significant, *FW* fruit weight, *SSC* soluble solids content, *FF* flesh firmness, *TA* titratable acidity, *RI* ripening index, *RAC* relative antioxidant capacity, *TS* total sugars, **p*≤0.05, ***p*≤0.01 represent significant values,

**Table 5** Linkage disequilibrium scores ($r^2$), averaged for distance classes and germplasm groups according to the analysis with software STRUCTURE (Q1-Q3) and previous knowledge of the varieties (local vs. modern)

| | | | Structure groups | | | Breeding history | |
|---|---|---|---|---|---|---|---|
| Range (cM) | N* | Total n=94 | Group Q1 n=20 | Group Q2 n=55 | Group Q3 n=19 | Local n=43 | Modern n=51 |
| 0-10 | 20 | 0.044 | 0.128 | 0.027 | 0.120 | 0.058 | 0.068 |
| 10-20 | 24 | 0.069 | 0.144 | 0.029 | 0.140 | 0.053 | 0.100 |
| 20-30 | 21 | 0.026 | 0.128 | 0.045 | 0.047 | 0.039 | 0.048 |
| >30 | 23 | 0.023 | 0.078 | 0.021 | 0.106 | 0.036 | 0.035 |
| Intrachromosomal | 88 | 0.041 | 0.120 | 0.030 | 0.105 | 0.046 | 0.063 |
| Interchromosomal | 692 | 0.028 | 0.098 | 0.033 | 0.105 | 0.037 | 0.045 |

*number of marker pairs included in each class

**Table 6** *p*-values for marker-locus-trait associations using the TASSEL program. For multiple test of genotypes was applied Bonferroni procedure (Schulze and McMahon 2002)

| | AC | FC | Harvest date | FF | RI | Phenolics | Flavonoids | Vitamin C | Anthocyanins | RAC | Sucrose | Sorbitol | TS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BPPCT001 | | * | | | | | | | | | ** | | |
| BPPCT006 | | | | | | * | | * | ** | | | | |
| BPPCT007 | | * | | | | * | | | | | | | |
| **BPPCT015** | | | **0.0000072** | | | | **0.000081** | | Δ | | | **0.000013** | |
| BPPCT017 | | | | | * | | | | | | | *** | |
| BPPCT025 | | * | | | | | | | * | | | | |
| BPPCT038 | | | | | ** | | | | * | | | | |
| **CPPCT028** | **0.000011** | **0.0000001** | **0.00037** | | | **0.000019** | Δ | | | **0.00039** | | | **0.00016** |
| CPPCT030 | | * | * | | | | | | | *** | | | |
| **endoPG1** | | | | **0.000070** | | | | | | | | | **0.00061** |
| PceGA34 | | * | | | | *** | | | | | * | | ** |
| pchgms5 | | ** | | | | | | | | | | | |
| UDP96-001 | | | ** | | *** | | Δ | | * | | | | |
| UDP96-003 | * | * | | | | * | | * | * | | | | |
| UDP96-008 | | | | | | | | | * | | | | |
| UDP96-013 | ** | * | | | | * | | | Δ | Δ | Δ | | |
| UDP98-025 | ** | | | | ** | | | | | | | | |
| UDP98-409 | * | * | | | | | | | | | * | | |
| UDP98-410 | | ** | | | | * | * | ** | * | | | | |
| UDP98-412 | | | | | | | | | | *** | | | |

The *p*-values for associations are considered when at least one allele is associated with the SSR
**p*<0.00001, ***p*=0.00001-0.0001, ****p*=0.0001-0.0012 (considering associations with co-variate), Δ considering associations without co-variate
*AC* anther color, *FC* fruit flesh color, see Table 3 for the rest of abbreviations

**Table 7** Characteristics and mean values of pomological traits for each genotype and haplotype of BPPCT015, CPPCT028 and endoPG1 markers

| | Genotypes | | | | | | | Haplotypes | |
|---|---|---|---|---|---|---|---|---|---|
| | BPPCT015 | | CPPCT028 | | | endoPG1 | | BPPCT015/ CPPCT028 | |
| | **167_167** | **220_229** | **134_136** | **136_136** | **136_138** | **192_196** | **192_228** | **169/136** | **209/134** |
| Anther color | - | - | Red-brown | - | - | - | - | - | - |
| Fruit flesh color | - | - | Yellow | - | - | - | - | - | - |
| Harvest date | 216 | 244 | - | 199 | 251 | - | - | 209 | 257 |
| Flesh firmness | - | - | - | - | - | 50 | 27 | - | - |
| Total phenolics | - | - | - | 21 | 56 | - | - | - | - |
| Flavonoids | 12 | 47 | - | - | - | - | - | - | - |
| Relative Antioxidant Capacity | - | - | - | 316 | 997 | - | - | - | - |
| Sorbitol | 7 | 27 | - | - | - | - | - | - | - |
| Total sugars | - | - | - | 97 | 135 | 90 | 127 | - | - |

(-): no associations between traits and genotypes and/or haplotypes
See Table 3 for units, maximum, minimum and mean values for the pomological traits evaluated

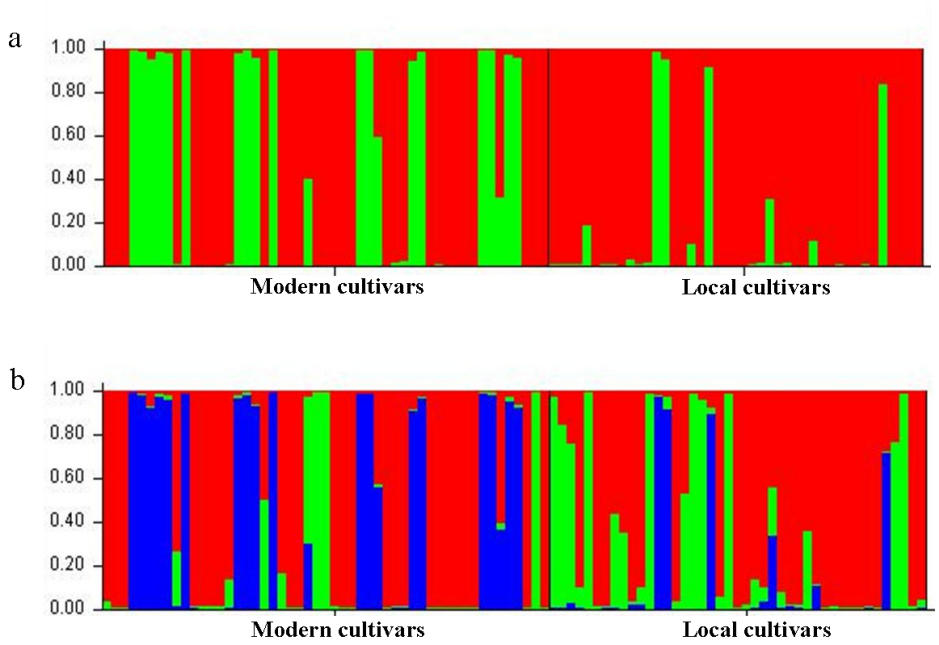



**Fig. 1** STRUCTURE bar plots based on 94 peach/nectarine cultivars at *K*=2 (a) and *K*=3 (b). *Green and blue* represent individuals within the subpopulations. Any *blue or green bar that is not completely filled* indicates admixture

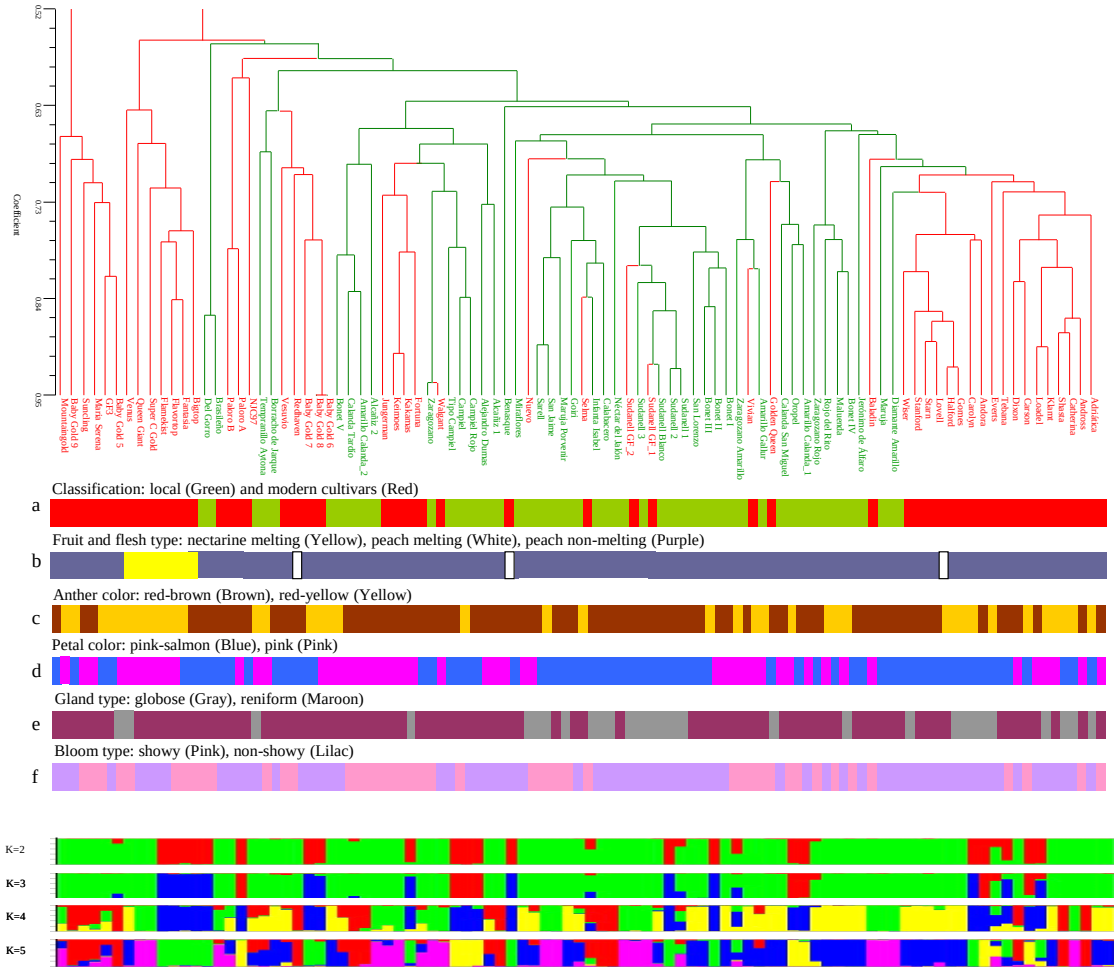



**Fig. 2** Dendrogram of 94 peach/nectarine cultivars based on pairwise genetic distances with 40 SSRs, and population structure based on different *K* values (*K*=2, 3, 4, and 5) separating individuals based on **a** local versus modern cultivars, **b** fruit characteristics, **c**, **d**, **f** flower, and **e** leaf characteristics

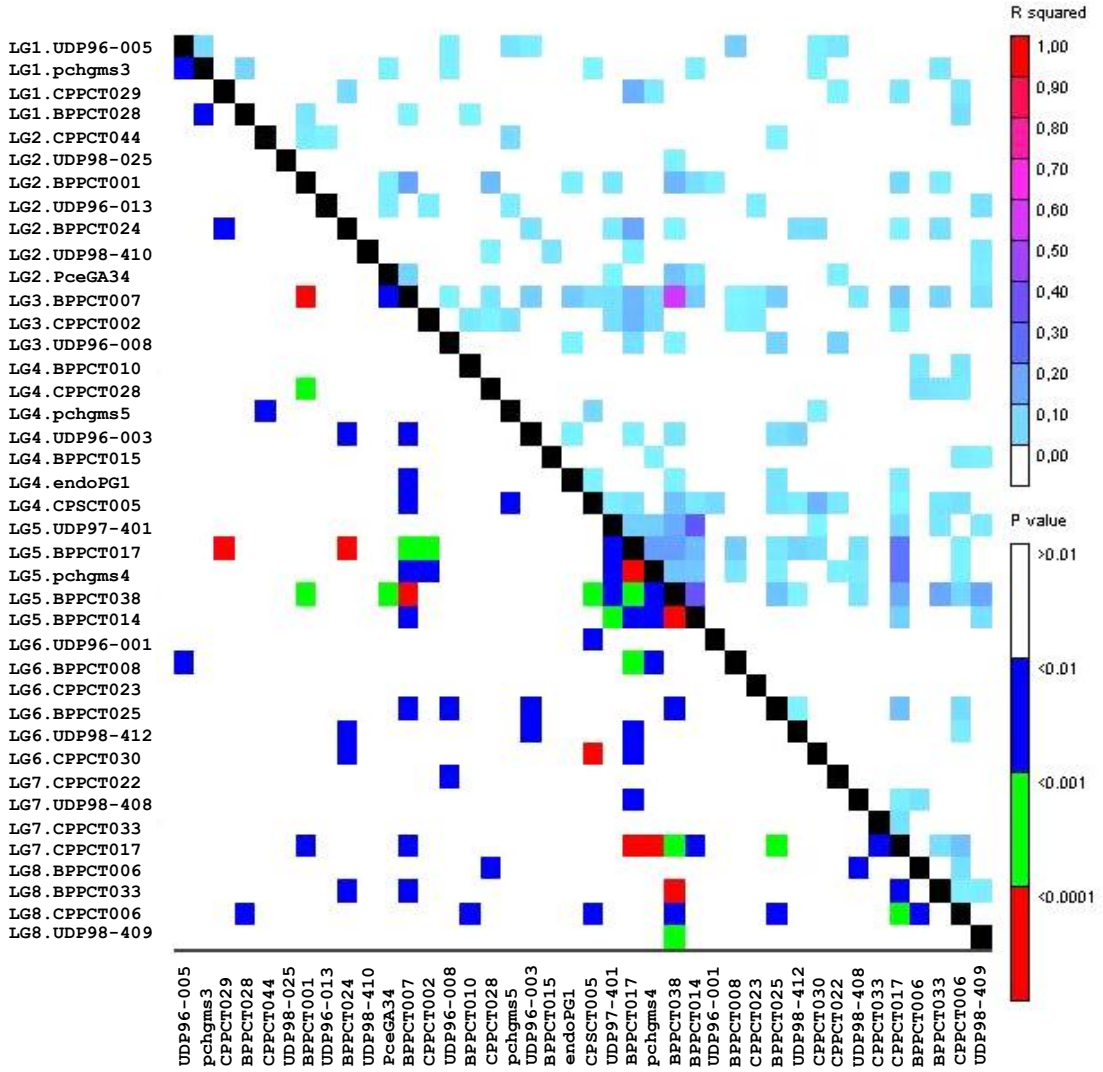



**Fig. 3** Linkage disequilibrium plot based on 40 SSR markers screened in 94 peach/nectarine cultivars. At the *right side* are represented the $r^2$ values and at the *left side* the *p*-values, according the colors of the legend

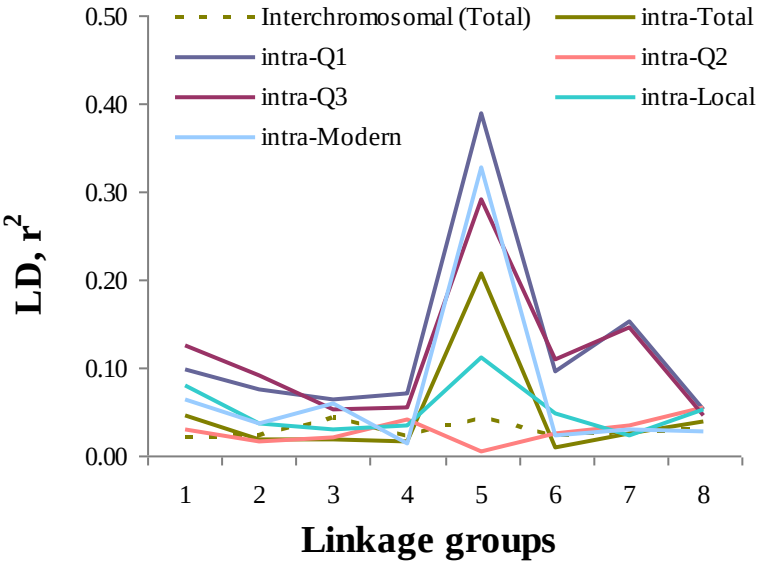
**Supplementary material**

**Supplementary file 1** Mean estimated values for different genetic parameters of the 94 peach/nectarine cultivars based on 40 SSR loci

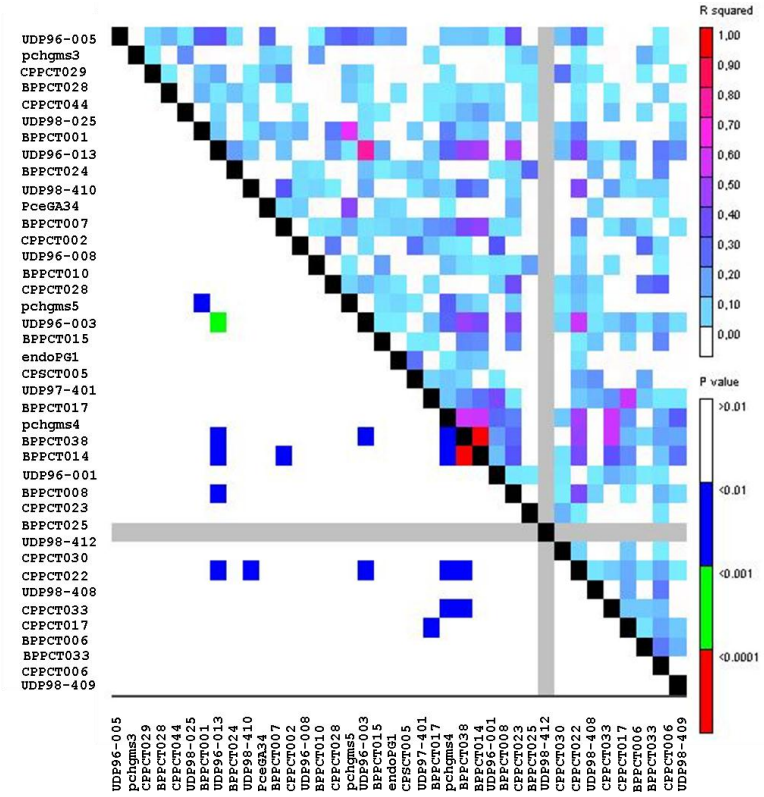
| SSR | A | Ae | Ho | He | Fis | I | PD |
|---|---|---|---|---|---|---|---|
| BPPCT001 | 7.00 | 3.77 | 0.30 | 0.74 | 0.59 | 1.40 | 0.69 |
| BPPCT006 | 7.00 | 3.61 | 0.43 | 0.73 | 0.41 | 1.55 | 0.70 |
| BPPCT007 | 5.00 | 1.41 | 0.18 | 0.29 | 0.38 | 0.64 | 0.29 |
| BPPCT008 | 4.00 | 2.56 | 0.32 | 0.61 | 0.47 | 1.78 | 0.61 |
| BPPCT010 | 3.00 | 1.14 | 0.13 | 0.12 | -0.08 | 0.26 | 0.12 |
| BPPCT014 | 2.00 | 1.06 | 0.06 | 0.06 | 0.00 | 0.13 | 0.06 |
| **BPPCT015** | 10.0 | 5.20 | 0.69 | 0.81 | 0.15 | 1.90 | 0.73 |
| BPPCT017 | 7.00 | 1.46 | 0.22 | 0.32 | 0.31 | 0.74 | 0.32 |
| BPPCT024 | 4.00 | 2.01 | 0.75 | 0.51 | -0.47 | 0.78 | 0.50 |
| BPPCT025 | 11.00 | 4.85 | 0.97 | 0.80 | -0.21 | 1.84 | 0.69 |
| BPPCT028 | 5.00 | 1.67 | 0.26 | 0.40 | 0.35 | 0.82 | 0.40 |
| BPPCT033 | 5.00 | 2.74 | 0.98 | 0.64 | -0.53 | 1.17 | 0.64 |
| BPPCT038 | 6.00 | 1.52 | 0.23 | 0.34 | 0.32 | 0.75 | 0.34 |
| CPPCT002 | 3.00 | 1.62 | 0.31 | 0.38 | 0.18 | 0.68 | 0.38 |
| CPPCT006 | 4.00 | 1.54 | 0.28 | 0.35 | 0.20 | 0.68 | 0.35 |
| CPPCT017 | 4.00 | 2.18 | 0.74 | 0.54 | -0.37 | 0.95 | 0.54 |
| CPPCT022 | 8.00 | 2.58 | 0.81 | 0.62 | -0.31 | 1.36 | 0.61 |
| CPPCT023 | 2.00 | 1.18 | 0.17 | 0.16 | -0.06 | 0.29 | 0.16 |
| **CPPCT028** | 7.00 | 3.49 | 0.68 | 0.72 | 0.06 | 1.45 | 0.72 |
| CPPCT029 | 6.00 | 3.21 | 0.92 | 0.69 | -0.33 | 1.29 | 0.69 |
| CPPCT030 | 5.00 | 1.35 | 0.28 | 0.27 | -0.04 | 0.60 | 0.26 |
| CPPCT033 | 2.00 | 1.37 | 0.17 | 0.27 | 0.37 | 0.44 | 0.27 |
| CPPCT044 | 8.00 | 3.93 | 0.97 | 0.75 | -0.29 | 1.52 | 0.69 |
| CPSCT005 | 2.00 | 1.69 | 0.26 | 0.41 | 0.37 | 0.60 | 0.41 |
| **endoPG1** | 5.00 | 2.01 | 0.76 | 0.51 | -0.49 | 0.82 | 0.51 |
| PceGA34 | 5.00 | 2.92 | 0.53 | 0.66 | 0.20 | 1.20 | 0.66 |
| pchgms3 | 5.00 | 1.95 | 0.17 | 0.49 | 0.65 | 0.88 | 0.49 |
| pchgms4 | 2.00 | 1.41 | 0.08 | 0.12 | 0.33 | 0.24 | 0.12 |
| pchgms5 | 2.00 | 1.16 | 0.13 | 0.14 | 0.07 | 0.27 | 0.14 |
| UDP96-001 | 9.00 | 3.19 | 0.95 | 0.69 | -0.38 | 1.39 | 0.69 |
| UDP96-003 | 7.00 | 2.01 | 0.38 | 0.51 | 0.25 | 1.09 | 0.50 |
| UDP96-005 | 2.00 | 1.99 | 0.29 | 0.50 | 0.42 | 0.69 | 0.50 |
| UDP96-008 | 5.00 | 3.14 | 0.66 | 0.69 | 0.04 | 1.30 | 0.68 |
| UDP96-013 | 6.00 | 1.87 | 0.31 | 0.47 | 0.34 | 0.89 | 0.47 |
| UDP97-401 | 2.00 | 1.35 | 0.12 | 0.26 | 0.54 | 0.42 | 0.26 |
| UDP98-025 | 4.00 | 3.59 | 0.98 | 0.73 | -0.34 | 1.32 | 0.68 |
| UDP98-408 | 3.00 | 1.24 | 0.15 | 0.20 | 0.25 | 0.37 | 0.19 |
| UDP98-409 | 6.00 | 2.32 | 0.98 | 0.57 | -0.72 | 0.99 | 0.57 |
| UDP98-410 | 5.00 | 3.51 | 0.97 | 0.74 | -0.31 | 1.40 | 0.70 |
| UDP98-412 | 8.00 | 4.78 | 0.94 | 0.80 | -0.18 | 1.70 | 0.69 |
| Mean | 5.10 | 2.39 | 0.48 | 0.49 | 0.05 | 0.96 | 0.47 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| All loci | 203 | | | | | | |
| Mean local cultivars | 4.41 | 2.26 | 0.54 | 0.45 | -0.20 | 0.85 | 0.45 |
| All loci for local cultivars | 172 | | | | | | |
| Mean modern cultivars | 4.50 | 2.34 | 0.59 | 0.50 | -0.18 | 0.94 | 0.49 |
| All loci modern cultivars | 159 | | | | | | |

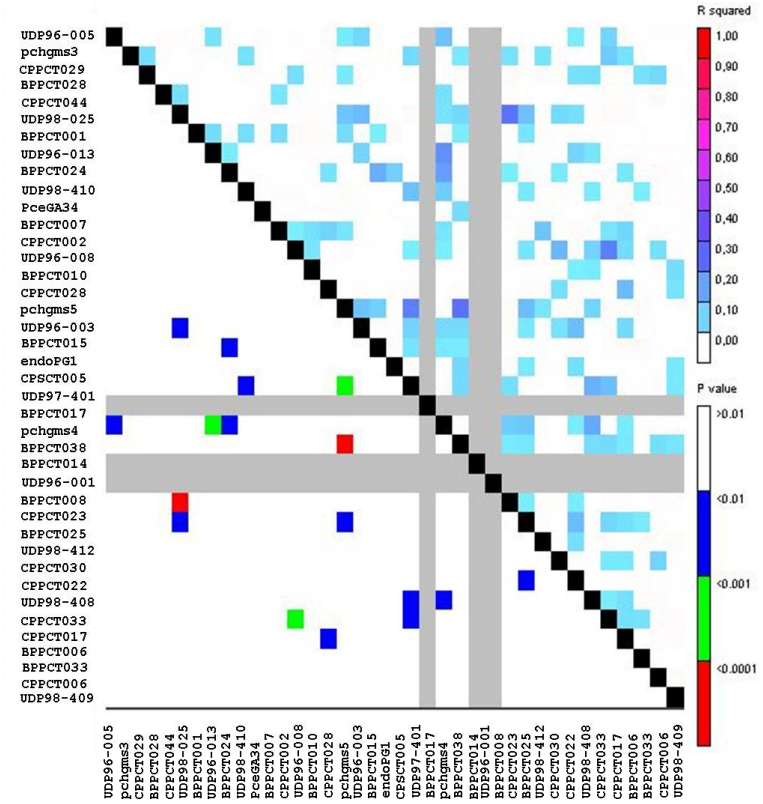
*A* observed number of alleles per locus, $A_e$ effective number of alleles per locus, $H_o$ observed heterozygosity, $H_e$ expected heterozygosity, $F_{is}$ Wright's fixation index, *I* Shannon's information index, *PD* power of discrimination



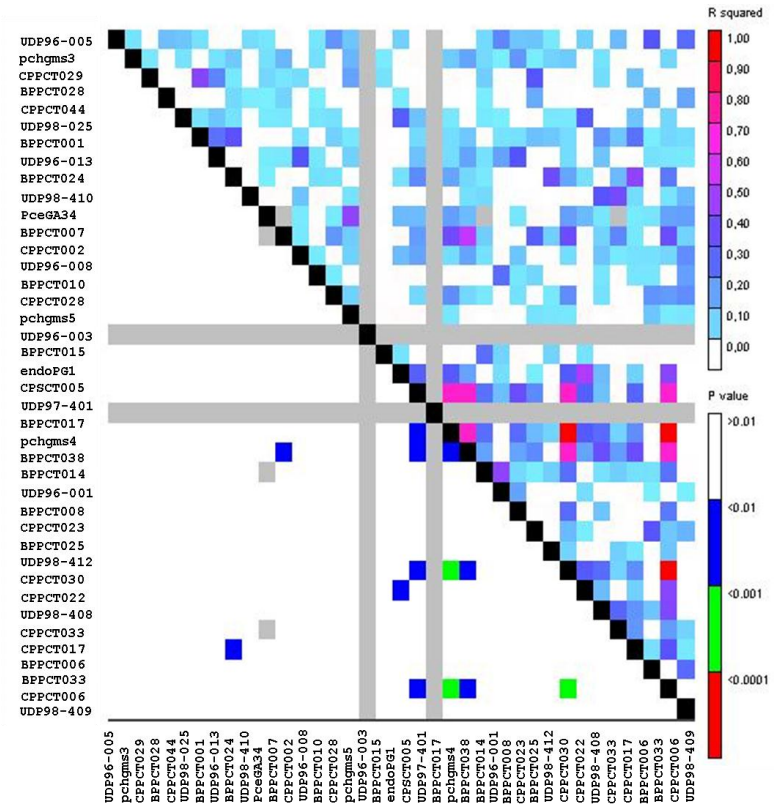

**Supplementary file 2** Linkage disequilibrium scores ($r^2$), averaged across chromosomes and germplasm groups, according to the analysis with software STRUCTURE (Q1-Q3), and to previous knowledge of the varieties (local and modern)



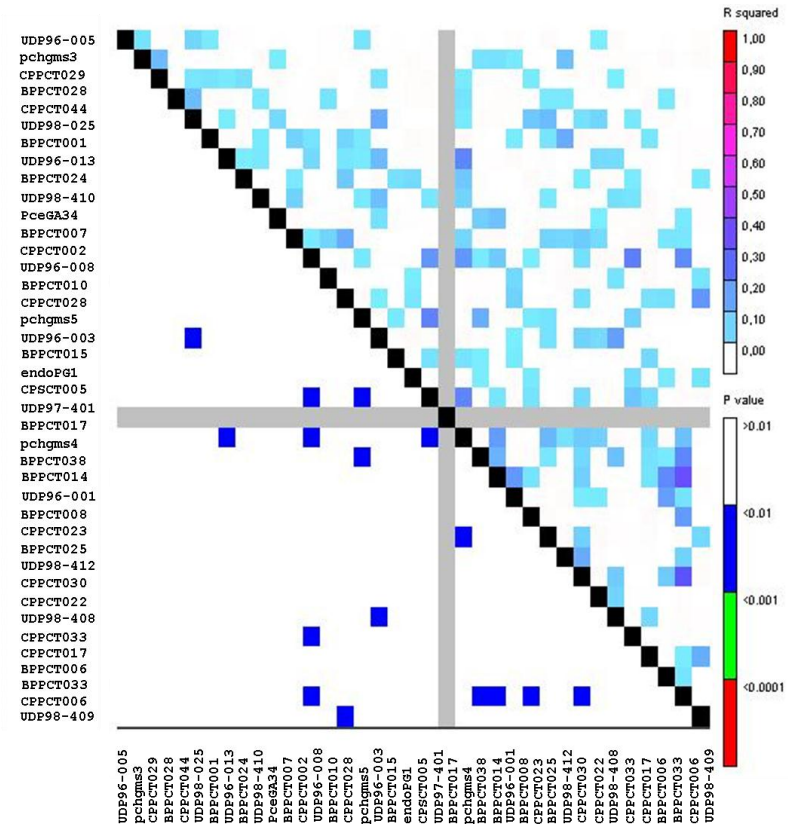

**Supplementary file 3** Linkage disequilibrium plot based on Q1 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the $r^2$ values and at the left side the *p*-values, according the colors of the legend



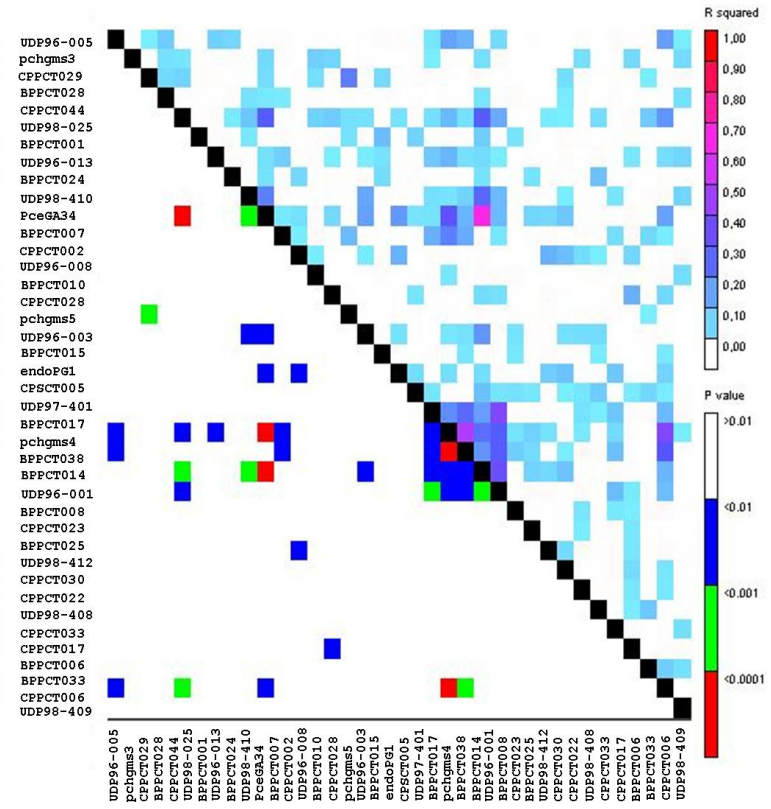

**Supplementary file 4** Linkage disequilibrium plot based on Q2 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the $r^2$ values and at the left side the *p*-values, according the colors of the legend




**Supplementary file 5** Linkage disequilibrium plot based on Q3 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the $r^2$ values and at the left side the *p*-values, according the colors of the legend




**Supplementary file 6** Linkage disequilibrium plot based on local cultivars screened in 94 peach/nectarine cultivars. At the right side are represented the $r^2$ values and at the left side the *p*-values, according the colors of the legend




**Supplementary file 7** Linkage disequilibrium plot based on modern cultivars screened in 94 peach/nectarine cultivars. At the right side are represented the $r^2$ values and at the left side the *p*-values, according the colors of the legend