A fruit firmness QTL identified on linkage group 4 in sweet cherry (Prunus avium L.) is associated with domesticated and bred germplasm

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Fruit firmness is an important market driven trait in sweet cherry (Prunus avium L.) where the desirable increase in fruit firmness is associated with landrace and bred cultivars. The aim of this work was to investigate the genetic basis of fruit firmness using plant materials that include wild cherry (syn. mazzard), landrace and bred sweet cherry germplasm. A major QTL for fruit firmness, named qP-FF4.1, that had not previously been reported, was identified in three sweet cherry populations. Thirteen haplotypes (alleles) associated with either soft or firm fruit were identified for qP-FF4.1 in the sweet cherry germplasm, and the “soft” alleles were dominant over the “firm” alleles. The finding that sweet cherry individuals that are homozygous for the “soft” alleles for qP-FF4.1 are exclusively mazzards and that the vast majority of the bred cultivars are homozygous for “firm” alleles suggests that this locus is a signature of selection. Candidate genes related to plant cell wall modification and various plant hormone signaling pathways were identified, with an expansin gene being the most promising candidate. These results advance our understanding of the genetic basis of fruit firmness and will help to enable the use of DNA informed breeding for this trait in sweet cherry breeding programs.

Sweet cherry (Prunus avium L.) is an important fruit crop in temperate regions and fresh fruit is highly valued. Sweet cherries for the fresh market are hand harvested, often mechanically sorted and frequently in transit for several weeks to distant markets. Because of the harvesting, handling and marketing practices, fresh market sweet cherries need to be firm when harvested. Consumers also prefer fresh market sweet cherries that are firm1. Sour cherry (Prunus cerasus L.), a tetraploid relative of the diploid sweet cherry, is primarily used for processed products such as jam, juice and pie filling, and therefore neither the supply chain nor the consumer requires the level of firmness necessary for sweet cherry. In addition, the softer texture of sour cherry is a positive attribute for their use in cooked products and beverages. However, very soft sour cherries are rejected by the processors as mechanical pit removal is problematic.

The sweet cherries grown for fruit production were domesticated from wild cherry (syn. mazzard), which is believed to have originated around the Caspian and Black Seas and subsequently spread throughout Europe and south to Iran2. Mazzards have extremely small fruit and are grown for their high value lumber. The three mazzards used in this study also have very soft fruit. Increases in fruit size and firmness are the main fruit traits associated with the domestication of sweet cherry from its wild relatives. Sour cherry was formed from the hybridization between sweet cherry and the tetraploid ground cherry (P. fruticosa Pall.), a wild bush species native to Eastern Europe2. Sour and ground cherry have fruit that is significantly softer than that of the commercial sweet cherry.

The importance of fruit firmness for sweet cherry can be traced back to early records of cherry cultivation. For instance, sweet cherry cultivation in the Jerte Valley, Extremadura, Spain, reported for the first time in 1352, was based on farmer’s selection of local cultivars with improved quality attributes, including firmer fruit2. Cherry cultivation in this region grew significantly during the XIXth century, and was mainly based on four very firm cultivars harvested stemless, which were traditionally known as ‘Picotas’ including ‘Ambrunés’, ‘Pico Negro’, ‘Pico
The progeny in the ‘Fercer’ × ‘X’ population exhibited a wide range of firmness and variation in these populations is genetically controlled (Table 1). The highest heritability for fruit firmness (0.97) was observed among years compared to the genetic variation as suggested by the bimodal phenotypic distribution. In the ‘Fercer’ × ‘X’ population, the range of firmness mean of ~67 g/mm² (Min 56, Max 82) which is aligned with the firm-fruited progeny group. A wide range of variation for fruit firmness was also observed for individuals from the INRA sweet cherry germplasm collection (Table 1). In this case, a previously undetected QTL was identified on LG 5 along with a previously identified QTL on LG 6. No QTL for fruit firmness have yet to be identified in sour cherry.

Limon Negro’ and ‘Pico Colorado’. At a period when no modern transportation systems existed in Spain, cherries were transported with mules from this valley to the country’s capital, Madrid, a several day journey, and consumer demand suggested that the fruit still kept acceptable quality. Another example of a relatively old firm cultivar is ‘Bing’, which was selected from a seedling of ‘Black Republican’ in 1875 in Oregon, USA. Today, ‘Bing’ still remains the most important cultivar of the Pacific Northwest, USA, and has also been fundamental in the extremely fast development of sweet cherry cultivation in Chile, which is a country that directs most of its production to long-distance export markets. As an example, the INRA breeding program released during the 1980’s the cultivar ‘Ferdouce’, ‘Fertille’ or ‘Ferdiva’ which was one of the first cultivars producing very large fruit (up to 15 g) with a high level of firmness as well. Subsequently, this cultivar was heavily used as a parent in the INRA breeding program, leading to many new cultivars, such as ‘Folfer’, ‘Ferdouze’, ‘Fertille’ or ‘Ferdiva’.

Little is known about the genetic control of fruit firmness in either sweet or sour cherry. In a study of two F₁ populations derived from three sweet cherry cultivars (‘Regina’ × ‘Lapins’, ‘Regina’ × ‘Garnet’), the phenotypic data of the progeny fit normal distributions suggesting that the trait was quantitatively inherited. Multiple quantitative trait loci (QTLs) for firmness were identified, the largest one on linkage group (LG) 5, but none of the QTL explained more than 24.1% of the phenotypic variance. A second QTL study was done using the sweet cherry population ‘Ambrunés’ × ‘Sweetheart’ that also exhibited a continuous distribution for firmness. In this case, a previously undetected QTL was identified on LG 1 along with a previously identified QTL on LG 6. No QTL for fruit firmness have yet to be identified in sour cherry.

Genes controlling fruit firmness have been well investigated in many species including tomato, peach and apple. In these species, the physiological modifications of the cell wall organization were considered important components of tissue firmness. Many enzymes, which are capable of altering cell wall texture, have been proposed. For example, endopolygalacturonase (endoPG), encoded by a multiple-gene family, is well established as one of the major enzymes involved in pectin disassembly in tomato and kiwifruit. Also, endoPG was also shown to be involved in fruit softening process and its regulation was found to be ethylene dependent. Copy number variation of a gene cluster encoding endoPG was also found to moderate flesh texture in peach. In sweet cherry, genome-wide transcriptional dynamics from developing fruit between flowering and maturity at 14 time points were investigated and the results suggested tight developmental regulation of genes functioning in diverse processes such as sugar transport, lipid metabolism and cell wall rearrangement related to changes in fruit firmness. To date, no genes have been identified that control the variation for fruit firmness in sour cherry, but candidate genes underlying the firmness QTL identified on LG 5 in sweet cherry have been proposed. In sour cherry, expansin genes were found to be upregulated during ripening (also the period of fruit softening).

The objectives of this study were to (1) identify and characterize the QTLs for fruit firmness segregating in an F₁ sweet cherry population, (2) explore whether the QTL identified is associated with the firmness that accompanied sweet cherry domestication and breeding, and the presence of softer fruit exhibited by sour cherry, and (3) identify candidate genes for fruit firmness within the QTL region.

**Results**

**Phenotypic variation for fruit firmness.** The progeny in the ‘Fercer’ × ‘X’ population exhibited a wide range of fruit firmness; however, the distribution was bimodal with more individuals exhibiting soft fruit (Table 1, Figs 1a and S1). ‘X’ was assigned as the paternal parent of this population since the recorded paternal parent was found incorrect based on genotype data and the correct parent is unknown. ‘Fercer’ had a multi-year fruit firmness mean of ~67 g/mm² (Min 56, Max 82) which is aligned with the firm-fruited progeny group. A wide range of variation for fruit firmness was also observed for individuals from the INRA sweet cherry germplasm collection and RosBREED germplasm (Fig. 1b,c). In the INRA sweet cherry germplasm collection, the majority of soft-fruited individuals were characterized as landraces as opposed to bred cultivars. In the RosBREED germplasm, the majority of soft-fruited individuals were either mazzards or hybrids with mazzards. When the two INRA populations were compared for firmness for the one year that they were both phenotyped, the sweet cherry germplasm collection exhibited a wider phenotypic distribution compared to the F₁ population (Fig. 1). Within the three populations, the ANOVA analysis revealed highly significant effects for the different genotypes (Table 1). In all three populations, the broad-sense heritabilities were high (0.73–0.97), indicating that much of the phenotypic variation in these populations is genetically controlled (Table 1). The highest heritability for fruit firmness (0.97) was obtained from the multi-year data for the ‘Fercer’ × ‘X’ population, likely because the environmental variation was low among years compared to the genetic variation as suggested by the bimodal phenotypic distribution.

The firmness of the RosBREED sweet cherry materials and sour cherry materials could be directly compared as they were phenotyped using the same instrumentation. Fruit from the sour cherry individuals exhibited a

| Population* | Size | Mean ± SD | Range | $\sigma^2$ | $\sigma_1^2$ | Heritability |
|-------------|------|-----------|-------|-----------|-------------|-------------|
| INRA ‘Fercer’ × ‘X’ F₁ population | 67   | 55 ± 12   | 32–80 | 139       | 30          | 0.97        |
| INRA germplasm collection | 193  | 65 ± 11   | 55–86 | 95        | 72          | 0.73        |
| RosBREED pedigreed population | 528  | 243 ± 47  | 137–351 | 2535 | 244 | 0.91 |

Table 1. Summary of fruit firmness data for three sweet cherry populations. Note: *Different methods for phenotyping were used for the INRA and RosBREED populations. Phenotypic data is from seven years (2009–2013, 2015–2016). Phenotypic data is from two years (2014–2015). Phenotypic data is from two years (2011–2012).
smaller range of firmness (98–197) compared to sweet cherry (137–397) (Figs 1 and S2). The fruit firmness of 95% of the sour cherry individuals was less than 170, indicating that almost all the sour cherries were softer than the sweet cherry landraces, bred cultivars, and their offspring.

QTL analysis. ‘Fercer’ × ‘X’. The two parental maps constructed consisted of 110 SNPs for ‘Fercer’ and 87 SNPs for ‘X’ with an average coverage of one marker every 6.7 and 7.5 cM, respectively. QTL analysis from the ‘Fercer’ × ‘X’ population identified four QTL in the multi-year analysis (LGs 4, 5, 6 and 8); however, the major stable QTL was located on LG 4 (Table 2, Fig. 2 and Table S1). This QTL segregating from the ‘X’ parent was significant in all seven years evaluated and across years, and the percentage of variation explained by the QTL ranged from 54.0% to 84.6%. The QTL confidence interval based on multiple years’ analysis was small (33.1–36.0 cM).

Figure 1. Frequency distributions of fruit firmness for three sweet cherry panels. (a) INRA ‘Fercer’ × ‘X’ F1 population; (b) INRA germplasm collection; (c) RosBREED pedigreed germplasm. Data from 2015 was used for (a,b) since it is the only common year in which firmness data was collected for the two INRA sweet cherry populations. Data summaries and frequency distributions using multi-year data are presented in Table 1 and Fig. S1, respectively.

Table 2. Summary of QTLs for fruit firmness identified on linkage group (LG) 4 in the ‘Fercer’ × ‘X’ F1 population. Note: ‘Linkage group (LG), LG of ‘X’ (X), LG of ‘Fercer’ (F); Logarithm of odds ratio (LOD); Confidence interval (CI); Difference between the two homozygotes at the marker loci (d); Percentage of variation explained by the QTL (PVE). Significant QTLs are presented for each year as well as significant QTL identified when data was combined over multiple years (MY).

| Year | LGa | LODa | CI (cM)a | Peak (cM) | D− | PVE (%)a |
|------|-----|------|----------|----------|----|----------|
| 2009 | X4  | 10.7 | 28.4–38.0| 33.2      | −17.6 | 62.8     |
| 2010 | X4  | 23.3 | 30.2–38.0| 34.1      | −26.3 | 82.4     |
| 2011 | X4  | 18.1 | 30.0–40.1| 35.0      | −22.5 | 79.2     |
| 2012 | X4  | 22.4 | 31.4–39.1| 35.2      | −22.9 | 78.3     |
| 2013 | X4  | 11.3 | 24.9–42.8| 33.9      | −18.7 | 54.0     |
| 2015 | X4  | 25.9 | 30.5–38.0| 34.2      | −25.7 | 84.6     |
| 2016 | X4  | 17.6 | 30.8–39.0| 34.9      | −21.7 | 70.8     |
| MYb | X4  | 20.6 | 10.3–67.7| 39.0      | −12.1 | 20.1     |
| MYb | P4  | 125.3| 33.1–36.0| 34.5      | −21.8 | 70.2     |

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and estimated to only cover about one Mbp (10,335,393–11,216,807 bp) based on the peach physical map v.2. The peak position of this QTL was located at 34.5 cM, which was estimated to be approximately 10.76 Mbp on the peach physical map v.2 (Table 2). One QTL was identified segregating from 'Fercer', but this QTL was only significant over multiple years, explaining 20.1% of the phenotypic variance. As the QTL confidence intervals overlapped, the QTLs derived from 'X' and 'Fercer' were considered to be the same and this QTL was named qP-FF4.1.

**INRA Sweet Cherry Germplasm Collection:** In the INRA sweet cherry germplasm collection, variation in fruit firmness significantly associated with SNPs located on chromosome four (Fig. 3a). The SNP most significantly associated with fruit firmness, ss490552928, has a peach physical map position (v.2) of 11,472,398 bp. Fruit firmness was significantly different among the three SNP genotypes, as illustrated for ss490552928 and the second most significant SNP on chromosome 4, ss490552906, located at 10,880,163 bp (Fig. 3b,c). For ss490552928, mean fruit firmness for the SNP genotype AB, was intermediate to that of BB and AA, with increased firmness associated with BB (Fig. 3b). For ss490552906, mean fruit firmness for the SNP genotype AB was significantly less than that of the most firm genotypic class (AA) and not significantly different from the softest class (BB) (Fig. 3c). In addition to the SNPs on chromosome 4, an additional SNP on chromosome 1 (ss490546759, 23,455,434 bp) was significantly associated with fruit firmness (Fig. 3a). This SNP is within a region where a fruit firmness QTL was previously identified in four of six years in a 'Regina' × 'Lapins' population.

**RosBREED Sour cherry germplasm:** The sour cherry germplasm was also segregating for ss490552928; however, no sour cherry individuals had the BBBB genotype (Fig. S3a). The majority of the individuals were AAAB, followed by AAAA, AABB and then ABBB. The association of SNP genotype and fruit firmness was investigated within two segregating sour cherry populations and found to be not significant (Fig. S3b,c). This is in contrast to the INRA sweet cherry germplasm collection where BB was the most prevalent genotype for this SNP and BB individuals had significantly firmer fruit than AB or AA individuals (Fig. S3b).

**RosBREED sweet cherry germplasm:** Two fruit firmness QTLs were identified in both years for the RosBREED pedigree germplasm, one small effect QTL on LG 2 and one large effect QTL on LG 4 (Table 3, Fig. 4 and Table S1). The QTL on LG 4 explained 16.4% and 83.5% of the variation for fruit firmness in 2011 and 2012, respectively. 

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**Figure 2.** Summary of QTLs for fruit firmness (FF) identified in the INRA 'Fercer' × 'X' F1 population. QTLs are shown for each year when detection was significant as well as by combining multiple years of data (MY) using the 'multi-environment' option of MultiQTL software. Explained percentage of variance is given for each QTL.
This difference is probably due to the high number of missing values in 2011 compared to 2012 (219 vs. 126, respectively). The peak genetic map position of this QTL was 33 cM and the peak physical map position was estimated to be ~10.8 Mbp. As this is similar to the peak position of the QTL identified in the ‘Fercer’ × ‘X’ population, this QTL was considered to be qP-FF4.1. Predictions for the genotypes of qP-FF4.1 for the RosBREED germplasm were calculated by FlexQTL, as QQ, Qq and qq, where Q and q represent the “firm” and “soft” alleles, respectively. FlexQTL used a bi-allelic model denoted by Q and q to estimate the QTL genotypes. The only individuals in this germplasm set predicted to be qq (and therefore soft) were three mazzard accessions, MIM 17, MIM 23, and NY 54 (Table S2). Likewise the only individuals in this germplasm set predicted to be Qq were offspring from these three mazzard accessions plus ‘Moreau’ and ‘Cristobalina’, old landrace cultivars, and their offspring. All other individuals were predicted to be QQ. This suggests that homozygosity for the firm Q allele at qP-FF4.1 is a signature of selection exhibited by domesticated and bred sweet cherries.

Haplotype analysis. To further trace and evaluate the allele effects of qP-FF4.1, five SNPs that span the peak physical map QTL location were chosen for haplotype (allele) construction (Fig. 5). These five SNPs spanned a ~1.23 Mbp and ~0.3 cM region of LG 4. Using these five SNPs, 13 haplotypes (H1 to H13) were identified in the RosBREED sweet cherry germplasm and an additional three haplotypes were identified in sour cherry (H14 to H16) (Tables S3 and S4). As the QTL haplotypes were based on SNP marker composition spanning the QTL region and not variation in underlying genes, it is possible that the haplotypes identified over-represent the

| Year | Interval (cM) | Peak (cM) | BF | Peak (cM) | Effect | PVE (%) |
|------|--------------|-----------|----|-----------|--------|---------|
| 2011 | 31–47        | 33        | 4.8| 33        | 48     | 16.4    |
| 2012 | 31–37        | 33        | 32.1|33        | 83     | 83.5    |

Table 3. Summary of the fruit firmness QTL identified on linkage group (LG) 4 in the RosBREED germplasm. Note: *Significance was presented by Bayes Factor (BF); Additive effect (Effect); Percentage of variation explained by the QTL (PVE).
number of functional alleles. Of the thirteen haplotypes exhibited by sweet cherry, four were only identified in the mazzards (H8, H11, H12 and H13) (Tables S2, S3). The haplotypes most frequent in the RosBREED sweet cherry germplasm and also not present in any mazzards were H4 (49.1%) and H1 (28.2%), suggesting that these haplotypes are associated with firm fruit possibly due to the influence of human selection and breeding. However, as only three mazzards were used in this study, it is possible that other mazzards might also possess a “firm” allele. Two of the commercially dominant cultivars notable for their fruit firmness, ‘Bing’ and ‘Ambrunés’, are H1H1 and H4H4, respectively. The haplotypes most frequent in the sour cherry selections were H3 (46.9%) followed by H11 (18.8%) and H10 (12.8%).

The haplotypes for the fruit firmness QTL, qP-FF4.1, and their physical positions on the peach Genome v2.0. In sweet cherry, haplotypes only identified in mazzard are marked as blue. Haplotypes only identified in sour cherry are marked as green. The full list of plant materials exhibiting the 16 haplotypes are listed in Table S3. The haplotypes for eight sour cherry parents are presented in Table S4. The haplotypes were deduced to be associated with soft or firm fruit based on diplotype analysis in sweet cherry (Fig. 6).

Figure 5. Haplotypes for the fruit firmness QTL, qP-FF4.1, and their physical positions on the peach Genome v2.0. In sweet cherry, haplotypes only identified in mazzard are marked as blue. Haplotypes only identified in sour cherry are marked as green. The full list of plant materials exhibiting the 16 haplotypes are listed in Table S3. The haplotypes for eight sour cherry parents are presented in Table S4. The haplotypes were deduced to be associated with soft or firm fruit based on diplotype analysis in sweet cherry (Fig. 6).

Figure 6. Comparison of fruit firmness (g/mm²) for progeny based on their diplotypes for qP-FF4.1 from (a) the ‘Fercer’ × ‘X’ F₁ population; and (b) the RosBREED pedigreed population. Only diplotypes represented by six or more individuals were included. Phenotypic means that were significantly different (P < 0.05) are identified by different letters.
individuals. The inheritance of H3, uniquely present in 'Early Burlat' and not present in any other RosBREED germplasm, was further followed through breeding using this germplasm. Five U.S. cultivars have 'Early Burlat' in their ancestry and all five inherited the 'Early Burlat' H6, and not H3 (Fig. S4).

The effects on fruit firmness associated with 14 qP-FF4.1 diplotypes were compared for the RosBREED germplasm. These 14 diplotypes, representing 10 haplotypes (H1, H2, H4 to H6, H8, H10 to H13), each consisted of firmness data from six to 104 individuals (Fig. 6b). Fruit firmness ranged from a mean of 275 down to 162 for the softest fruit. The four diplotypes that had the firmest fruit all had one or two copies of H1, paired either with itself or with H4, H5 or H6. This suggests that in addition to H1 and H4, H5 and H6 can also be considered “firm” alleles. However, when H1 or H4 were paired with H2, the mean fruit firmness was reduced significantly. This is consistent with the dominant ‘soft’ effect of H2 observed in the ‘Fercer’ × ‘X’ progeny where H1H2 progeny had significantly softer fruit than H1H1 progeny. Progeny with H8 and H10-13, only present in the mazzards, had significantly softer mean fruit firmness than the majority of progeny homozygous for the firm diplotypes. These effects were based on pairings with the “firm” haplotypes H1 or H4, indicating that these “soft” haplotypes present in wild cherry are dominant to the “firm” haplotypes that are found in bred cultivars. It was not possible to determine if the three haplotypes identified in sour cherry (H14-16) were associated with firm or soft fruit due to the dominance of soft compared to firm fruit (Table S4).

In silico candidate genes. The qP-FF4.1 interval identified from both the INRA F1 and RosBREED pedigreed populations was used for candidate gene identification. This ~ 1.8 Mbp interval was between SNPs located at 10,156,468 and 11,956,655 bp on chromosome 4 of the peach genome v2.0 and the same SNPs located between 12,928,603 and 14,860,789 bp on the sweet cherry genome (Fig. 7). In this region, 241 genes were predicted in the sweet cherry genome (Table S5). From these genes, 14 genes were selected based on their potential to be involved in the control of fruit firmness (Table 4, Fig. 7). The most promising candidate gene identified was Pav_sc0002828.1_g410.1.mkr which encodes an expansin protein related to plant cell wall metabolism. This gene is very close to the QTL peak and an expansin gene with homology to Pav_sc0002828.1_g410.1.mkr was found to be expressed in sour cherry fruit and associated with tissue softening16. Of the three expansin genes identified that were upregulated during softening in sour cherry fruit, the expansin gene PcEXP4 had the highest similarity to the expansin gene in the sweet cherry genome as evidenced by their placement on a distal lineage, relative to the other sour cherry expansins (Fig. S5a,b). The expansin gene gene contains two functional domains (Expansin EG45 and Expansin CBD) and three encoded signal peptide regions (H, N, and C) located on the N-terminus region (Fig. S5c). Nine other candidate genes were predicted to encode plant cell wall modifying enzymes which have been found to be potentially involved in regulating fruit firmness in peach and apple17,18. Fourteen candidate genes were included as they are potentially involved in various plant hormone signaling pathways well known to be involved in fruit maturation and ripening in non-climacteric and climacteric fruits and in sweet cherry firmness19,20. Among these candidate genes, two are predicted to be NAC (NAM/ATAF1, 2/CUC2) transcription factors involved in the ethylene signaling pathway. Of these two genes, Pav_sc0000029.1_g070.1.mkr, is a homolog of the peach NAC gene ppa008301m that has been predicted to control maturity date21,22. The final candidate gene, Pav_sc0000975.1_g210.1.mkr, was predicted to be a Squamosa promoter-Binding Protein which has been found to be associated with fruit ripening in tomato23.

Discussion
Genetic determinism and signature of selection for fruit firmness in sweet cherry. The bimodal segregation for fruit firmness in the ‘Fercer’ × ‘X’ population provided the opportunity to identify a major QTL for fruit firmness in sweet cherry that was also identified in a wide range of genetic backgrounds represented by the INRA sweet cherry germplasm collection and the RosBREED pedigreed population. This is the first report of a major QTL for fruit firmness identified on LG 4 in sweet cherry. However, due to the small size of the ‘Fercer’ × ‘X’ population, the QTL interval would be affected by potential errors in phenotyping and genotyping as well as the environmental conditions. Despite this population size limitation, the QTL region estimated for all seven years was stable and consistent, possibly due to the large effect of this QTL in this population as suggested by the bimodal phenotypic distribution. However, future fine mapping is needed to more precisely define the QTL interval. In a prior study of two sweet cherry populations between bred cultivars, ‘Regina’ × ‘Lapins’ and ‘Regina’ × ‘Garnet’, QTLs for fruit firmness detected in at least three of the six years of study, were identified on LG 1, 2 and 5. On LG 4, a QTL was detected in only two of the six years analyzed in one of the two populations (‘Regina’ × ‘Lapins’), and this QTL was located on the upper region of chromosome 4 in a region that does not overlap with that for qP-FF4.1. It is possible that the LG 4 QTL, qP-FF4.1, was not identified in these two populations, because all three parents only had “firm” alleles for this locus. Indeed, all three haplotypes present in ‘Regina’ (H4H5) and ‘Lapins’ (H1H4) were identified as “firm” alleles in this study. In contrast, the plant materials used in this study resulted in the identification of qP-FF4.1 because of the presence of “soft” alleles in the plant materials.

The finding that sweet cherry individuals that are homozygous for the “soft” alleles for qP-FF4.1 are exclusively mazzards and that the vast majority of bred cultivars are homozygous for “firm” alleles suggests that this locus was a signature of selection during domestication and modern breeding. In addition, three of the old cultivars included in this study, ‘Moreau’, ‘Cristolabina’ and ‘Early Burlat’, have relatively soft fruit and their qP-FF4.1 genotypes include one “soft” and one “firm” allele. In sour cherry, all of the germplasm are soft, as firmness comparable to sweet cherry has not been a critical trait. This QTL region is the second region in sweet cherry that has been shown to have been under selection, the first being a QTL region on LG 2 that contains a major QTL for fruit size14.

The results from the ‘Fercer’ × ‘X’ population and the RosBREED germplasm further indicate that the “soft” alleles present in the mazzard accessions are dominant, or at least partially dominant over the “firm” alleles present in bred cultivars. This is consistent with the findings in sour cherry where no individuals exhibited the firmness of bred sweet cherry cultivars. No sour cherry individual was homozygous for the “firm” allele at qP-FF4.1,
and therefore all the sour cherry individuals had at least one “soft” allele for \( qP-FF4.1 \). These results are also consistent with the results from progeny from a cross between a sweet cherry cultivar ‘Emperor Francis’ and the mazzard accession NY 54. A major QTL for fruit size associated with domestication was identified in this F1 population\(^2\); however, QTL for fruit firmness could not be identified from this population because all the progeny had soft fruit. Given that the \( qP-FF4.1 \) diplotypes for ‘Emperor Francis’ and NY 54 are H1H1 and H13H13, respectively, and the conclusion that soft fruit is dominant over firm fruit, this result would be expected.

In peach, a major QTL for fruit firmness was also identified on LG 4, first reported by Dettori et al.\(^2\). This locus, termed \( F-M \), controls both peach fruit firmness and flesh adhesion to the endocarp, with soft (melting) fruit dominant to firm (non-melting) fruit. Two genes encoding endopolygalacturonase (endoPG) are considered to be the causal genes at this locus. The peach physical map (v.2.0) positions of these two genes are as follows: ppa006839m 19046344–19049605 bp, and ppa006857m 19081325–19083984 bp. Using the peach genome as a proxy for cherry, this places the endoPGs and the \( F-M \) locus, ~8 Mbp distal to the \( qP-FF4.1 \). In contrast to peach, no studies in cherry have associated endoPG with flesh firmness, nor were any endoPG genes been identified in the \( qP-FF4.1 \) region. Peach and cherry also differ in their ethylene requirement for ripening. Peach is a climacteric fruit meaning that it has a strong requirement for ethylene to ripen, while cherry is a non-climacteric fruit. Taken together, these results suggest that the genetic control of fruit firmness in cherry evolved separately from that of peach. This conclusion is consistent with \( qP-FF4.1 \) being associated with domesticated and bred germplasm.
Table 4. Summary of 25 candidate genes for fruit firmness located in the qP-FF4.1 interval on chromosome 4 in sweet cherry. Note: *The cherry and peach genome sequences used were from Shirasawa et al. [34,35] and Verde et al. [36,37], respectively.* aNot available (NA) as no sequence match was found in the peach genome sequence. bThe description is the Blast2GO ontology term. cThe tomato annotation was included in italic when it differed from the cherry and/or peach annotation(s) and suggested a potential involvement with fruit firmness.

| Cherry candidate genes | Transcript start and stop (bp) | Description | Biological process |
|------------------------|--------------------------------|-------------|--------------------|
| Pav_sc0003492.1_g360.1.br | 13090321~13090626 | putative protein PRUPE_7G080000/BHLH transcription factor | Ethylene signaling pathway |
| Pav_sc0003492.1_g260.1.mk | 13314328~133147715 | UDP-glycosyltransferase 7986-like | Plant cell wall metabolism |
| Pav_sc0003492.1_g250.1.mk | 13147752~13148159 | UDP-glycosyltransferase 7986-like | Plant cell wall metabolism |
| Pav_sc0003492.1_g640.1.br | 13251924~13253405 | Zinc finger MYB-type protein 1-like | Brassinosteroid signaling pathway |
| Pav_sc0000975.1_g110.1.mk | 13329847~13330536 | Ethylene-responsive transcription factor 4 | Ethylene signaling pathway |
| Pav_sc0000975.1_g200.1.mk | 13386372~13389094 | Probable protein phosphatase 2C29 | Plant cell wall metabolism |
| Pav_sc0002828.1_g670.1.mk | 13612734~13616541 | Beta-glucuronosyltransferase GlcAT14B | Plant cell wall metabolism |
| Pav_sc0002828.1_g650.1.mk | 13627727~13637959 | Probable serine/threonine-protein kinase | Plant cell wall metabolism |
| Pav_sc0002828.1_g640.1.mk | 13648670~13654523 | Inner membrane protein oxaA | Plant cell wall metabolism |
| Pav_sc0002828.1_g510.1.mk | 13741324~13741986 | Putative RING-H2 finger protein ATL19 | Brassinosteroid signaling pathway |
| Pav_sc0002828.1_g470.1.mk | 13776148~13776732 | Putative RING-H2 finger protein ATL19 | Brassinosteroid signaling pathway |
| Pav_sc0002828.1_g460.1.mk | 13778460~13779378 | Putative RING-H2 finger protein ATL19 | Brassinosteroid signaling pathway |
| Pav_sc0002828.1_g380.1.mk | 13831978~13833770 | F-box/FBD/LRR-repeat protein At4g26340-like | Gibberellin signaling pathway |
| Pav_sc0002828.1_g310.1.mk | 13868641~13869483 | Glycine-rich cell wall structural protein 2 | Plant cell wall metabolism |
| Pav_sc0002828.1_g280.1.mk | 13892641~13893365 | Fiber protein Fb34 | Plant cell wall metabolism |
| Pav_sc0002607.1_g160.1.mk | 14294224~14301322 | B3-domain-containing protein Os07g0679700-like | ABA signaling pathway |
| Pav_sc0000029.1_g020.1.mk | 14069597~14074899 | Beta-glucosidase 18-like isoform X1 | Plant cell wall metabolism |
| Pav_sc0000029.1_g040.1.br | 14079090~14079410 | Beta-glucosidase 18-like isoform X1 | Plant cell wall metabolism |
| Pav_sc0000029.1_g050.1.mk | 14080593~14083893 | Beta-glucosidase 18-like isoform X1 | Plant cell wall metabolism |
| Pav_sc0000029.1_g070.1.mk | 14097151~14098925 | NAC domain-containing protein 72 | Ethylene signaling pathway |
| Pav_sc0000029.1_g090.1.mk | 14118318~14120442 | NAC transcription factor 56 | Ethylene signaling pathway |
| Pav_sc0002607.1_g160.1.mk | 14294224~14301322 | B3-domain-containing protein Os07g0679700-like | ABA signaling pathway |
| Pav_sc0000124.1_g110.1.mk | 14575143~14618623 | Cellulose synthase-like protein G2 isoform X2 | Plant cell wall metabolism |

QTL hotspot of qP-FF4.1 region. The scope of the work presented herein is limited to fruit firmness; however, the qP-FF4.1 region is an important QTL “hotspot” for cherry breeders because major QTL for other traits map to this region. LG 4 loci for two phenology traits, bloom and maturity date, have been conserved across multiple Prunus species. For bloom time, the major locus named Lh, was first reported in almond by Ballester et al. and subsequently identified in multiple Prunus species. In sour cherry, the peak peach genome v2.0 position for the bloom time QTL on chromosome 4 was ~10.8 Mbp. For maturity date, a major QTL termed qMD4.1, was identified first in peach and subsequently in cherry. The most likely candidate gene for the peach QTL qMD4.1 is ppa008301m, which is believed to be an NAC transcription factor. It maps to ~11.106 Mb on the peach genome sequence v1.0 which is equivalent to ~11.117 Mb on the peach genome sequence v2.0. In a recent study, Isuzugawa et al. found that two sweet cherry candidate genes, homologous to the NAC transcription factors identified in peach, also mapped within the maturity date QTL on LG 4. In addition, QTL for fruit weight and soluble solids content have also been reported in this qP-FF4.1 region in peach. An analysis of maturity date for the ‘Fercer’ × ‘X’ population used in our study identified a QTL that explained on average 50% of the phenotypic variance for maturity time. This QTL was detected in the same region as the one for firmness; however, the peak detected with the multi-year analysis was at 36.1 cM, which is almost 2 cM downstream from the peak for the firmness QTL. The clustering of these QTLs is in agreement with the correlations observed in the populations studied among the two fruit traits: firmness and maturity date. Indeed, within the ‘Fercer’ × ‘X’ population, early maturing individuals bear smaller and softer cherries than the late maturing ones. In the RosBREED germplasm, a prior study of this germplasm identified a QTL for maturity date that overlapped with qP-FF4.1. In this prior study, ss490552928 was associated with maturity date with the ‘A’ and ‘B’ alleles associated with “early” and “late” maturity, respectively; however, one of the haplotypes that contained the ‘A’ allele was associated with late maturity. A similar result was obtained from the INRA sweet cherry germplasm collection population where the ‘A’ for ss490552928, was also associated with early maturity and soft fruit. The phenomenon of early maturing cultivars which tend to be softer than late maturing ones could probably result from a developmental impossibility of...
having a firm fruit in a short period of time between blooming and maturity. This would be in particular the case of cultivar 'Early Burlat', which has one of the shortest developmental periods between blooming and maturity. Hence, the recent method of developing sweet cherry cultivars which come to maturity at the same period as 'Early Burlat', but exhibit a significantly higher firmness, was to use genitors with an extra-early blooming time. In sour cherry, none of the individuals evaluated had the genotype BBBB for s490552928; therefore, the 'A' allele was always present. This is consistent with "soft" fruit alleles for this locus being dominant to "firm" fruit alleles. In sour cherry, bloom and maturity time are also correlated, however all individuals whether early or late maturing have soft fruit compared to the firm fruit associated with bred sweet cherries.

The multiple QTLs in the qP-FF4.1 region should be taken into consideration when performing breeding selection in both sour and sweet cherries. Hence, it is of utmost importance to disentangle the genetic determinism of the traits' variation within this QTL; in particular, it would be helpful for breeders to know whether maturity date and firmness are controlled by the same pleiotropic locus or by two closely linked genes. Fine mapping initiatives might be conducted in order to search for recombinants within this narrow genetic interval. More specifically, using cultivars such as 'Early Burlat' and 'Fercer' might be highly informative. Indeed, the predicted diplotype for both cultivars are H3H6 and H1H2, respectively; that is, each would have one "firm" and one "soft" haplotype. However, 'Fercer' is known to be a significantly firmer cultivar as compared to 'Early Burlat'. Multi-year data from INRA indicate a mean firmness, as measured by Durofel, of 67 and 49 for 'Fercer' and 'Early Burlat', respectively. This shows the complexity of the genetic determinism of fruit firmness, as already demonstrated by Campoy et al. and might suggest as well the existence of epistatic interactions. To test the hypothesis that maturity time and firmness are controlled by distinct genes in this LG 4 'hotspot', breeders will need to produce very large progeny populations when crossing 'Early Burlat' with other firmer but also later ripening cultivars in order to obtain recombinants between the two hypothesized closely linked genes. Finally, the fact that among the founders used in modern breeding, the haplotype H3 was only found in 'Early Burlat' agrees with the 'originality' of this cultivar in terms of developmental cycle; as already stated, 'Early Burlat' has a rather intermediate bloom time but is one of the earliest maturing cultivars.

Candidate genes controlling fruit firmness. The available sweet cherry genome sequence provided the opportunity to identify agronomically important candidate genes for qP-FF4.1. In our study, the identification of candidate genes was employed across species including sweet cherry, peach and tomato. Peach and sweet cherry are closely related Prunus species and share a high level of synten[y. Therefore, prior to the publication of the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Tomato was included as fruit firmness has been extensively studied in this species and like cherry the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Among the candidate genes identified, an expansin gene was considered as the most promising candidate for several reasons. Firstly, expansin genes have been shown to contribute to fruit softening by weakening noncovalent interactions between cellulose microfibrils and hemicellulose components. In tomato, expansin genes have been shown to be associated with fruit ripening and firmness. Secondly, the candidate expansin gene in sweet cherry has sequence homology to the expansin gene in Prunus species. Therefore, prior to the publication of the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Tomato was included as fruit firmness has been extensively studied in this species and like cherry the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Among the candidate genes identified, an expansin gene was considered as the most promising candidate for several reasons. Firstly, expansin genes have been shown to contribute to fruit softening by weakening noncovalent interactions between cellulose microfibrils and hemicellulose components. In tomato, expansin genes have been shown to be associated with fruit ripening and firmness. Secondly, the candidate expansin gene in sweet cherry has sequence homology to the expansin gene in Prunus species. Therefore, prior to the publication of the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Tomato was included as fruit firmness has been extensively studied in this species and like cherry the sweet cherry genome sequence, the peach genome was used as a proxy for candidate gene prediction in sweet cherry. Among the candidate genes identified, an expansin gene was considered as the most promising candidate for several reasons. Firstly, expansin genes have been shown to contribute to fruit softening by weakening noncovalent interactions between cellulose microfibrils and hemicellulose components. In tomato, expansin genes have been shown to be associated with fruit ripening and firmness. Second
Methods

Plant materials. Three sweet cherry populations were used in this study. These were: (1) an INRA bi-parental F1 population, (2) the INRA sweet cherry germplasm collection, and (3) RosBREED (www.rosbreed.org) pedigreed population. The INRA bi-parental F1 population consisted of a progeny of 67 individuals derived from a cross between cultivar ‘Fercer’ and an unknown parent called ‘X’. The INRA sweet cherry germplasm collection, maintained by INRA’s Prunus Genetic Resources Center at Bourran, France, included 193 sweet cherry accessions collected from France and other 15 countries of America, Asia and Europe. RosBREED pedigreed populations consisted of a set of 65 elite sweet cherry and mazzard clones, and 463 unselected F1 seedlings from 86 crosses from the Washington State University sweet cherry breeding program (Table S2). The germplasm of RosBREED pedigreed population, spanning six generations, was considered representative of U.S. public breeding germplasm for this crop. For sour cherry, a total of 338 individuals including parents, ancestors and offspring from five bi-parental F1 populations, were used. These individuals were grown at the Michigan State University Clarksville Research Station, Clarksville, Michigan. A detailed description of these sour cherry plant materials is presented in Cai et al.

Phenotyping and phenotype modeling. Fruit were harvested from the field when ripe based on a subjective assessment of skin color, texture and taste, placed in coolers for transport back to the laboratory, and evaluated the same day. Fruit firmness (g/mm²) was evaluated using different methods for INRA’s populations and RosBREED’s sweet and sour cherry pedigreed populations. For the two INRA populations (F1 population and the sweet cherry germplasm collection), fruit firmness was measured using a Durofel (Setop Giraud Technologie, Cavaillon, France) texture analyzer on the day of harvest. A 3-mm probe was applied at two points on the fruit equator, the movement of the probe was recorded and the average of the two measures on ten fruits was used. Data were collected from seven years (2009–2013, 2015 and 2016) for the F1 population and two years for the germplasm collection (2014 and 2015). For the RosBREED sweet cherry and sour cherry individuals, fruit firmness was measured using a compression test of BioWorks FirmTech 2 (Wamego, KS, USA). Compression was performed from the fruit cheek and with the stems still on the fruit. The mean value of 25 measures were used. The data for the sweet and sour cherry individuals were collected for two years (sweet cherry, 2011 and 2012; sour cherry, 2011 and 2013). Since different methods were used to measure firmness in the RosBREED and INRA populations, the data for these populations were analyzed separately.

The phenotypic data were analyzed using SAS version 9.13 (SAS Institute Inc.) and the model PROC MIXED was used to obtain the variance components. PROC CORR was performed to calculate the correlation coefficients of fruit firmness among different years. Broad-sense heritability (HF) was calculated using estimates for the individuals based on the following random linear model:

\[ Y_{ij} = \mu + \gamma_i + g_{ij} + e_{ij} \]

where \( Y_{ij} \) is the phenotypic value of the \( j \)th individual in \( i \)th year; \( \mu \) is the mean value of fruit firmness; \( \gamma_i \) is the random effect of the \( i \)th year on the phenotype; \( g_{ij} \) is the random genotypic effect of \( j \)th individual; and \( e_{ij} \) is the model residual. HF was calculated using the following equation: \[ HF = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2 / n) \], where \( \sigma_g^2 \) is the genetic variance, \( \sigma_e^2 \) is the residual error, and \( n \) is the number of years.

Genotyping and genetic map. All individuals from the three sweet cherry populations were genotyped with the RosBREED Illumina Infinium cherry SNP array of 5,696 SNP markers and SNP genotypes were scored using the Genotyping Module of GenomeStudio Data Analysis software. For the ‘Fercer’ × ‘X’ F1 population, a total of 724 SNP markers were polymorphic and segregating in this population. A linkage map was constructed using JoinMap 4.0 and Kosambi’s mapping function was used to convert recombination frequency into map distance. The two resulting parental maps consisted of 110 and 87 SNP markers, polymorphic for ‘Fercer’ and ‘X’, respectively. For the INRA sweet cherry germplasm collection, marker data curation was described in Cai et al.

QTL analysis. QTL analyses were performed for all three sweet cherry populations using different mapping softwares. For ‘Fercer’ × ‘X’, QTL mapping was carried out using MultiQTL v2.6 software with the multiple interval mapping (MIM) approach used (MultiQTL Ltd, Haifa, Israel, 2005, www.multiQTL.com). Different types of analyses were performed for single year and multiple years, respectively. Each year was first analyzed independently in order to examine the stability of the QTLs. An analysis combining all years was performed using the multiple environment option with increase of the accuracy of the QTL detection. The detailed QTL mapping methodology was as described in Castède et al. The graphical presentation of QTL locations on the linkage groups was obtained using the MapChart software version 2.2.

A genome-wide association analysis was done for the INRA sweet cherry germplasm collection. This analysis tested the association between fruit firmness and the SNP markers on the chromosome where the firmness QTL was located. A total of 1215 SNP markers across the sweet cherry genome were used in the analysis and
the SNPs associated with fruit firmness were identified according to a mixed linear model (MLM) using the software TASSEL version 5.2.61.2. Corrections for population structure and kinship were adopted in the model. Population structure was described in the study of Campoy et al.3. The relative kinship matrix was calculated using SPA GeDi4. The genome-wide significance cutoff was set at $4 \times 10^{-5}$ (0.05/1215).

QTL analysis in RosBREED sweet cherry pedigreed population was done using FlexQTL software that is designed for use with multiple pedigree-connected families4. Markov Chain Monte Carlo (MCMC) simulation, implemented in FlexQTL software, was applied to obtain samples from the joint posterior distribution of the model. A total of 1,000 samples (500,000 iterations with thinning value of 500) were stored for each simulation and then used for statistical inference. The inference on the number of QTLs was based on a pairwise comparison of models differing from each other by one QTL. The Bayes factor parameters (2lnBF) were interpreted as non-significant (0–2), positive (2–5), strong (5–10) or decisive (>10) evidence for the presence of QTL. The inference on the QTL position was based on posterior intensity and the inference on the QTL contribution was based on the posterior mean estimates of the QTL effect size. Both additive and dominant genetic models were tested using a maximum number of 10 QTLs. Prior number of QTL was set to 1 or 3 and genome-wide analyses were performed twice for each prior using different seed numbers to test the robustness of the analysis.

**Haplotype (diplotype) analysis.** Haplotypes (i.e. alleles) for the fruit firmness QTL were identified for the INRA F1 population and the RosBREED sweet and sour cherry pedigreed germplasm. Five SNPs covering the consensus QTL region identified from the sets of plant materials were chosen for haplotype construction. Phased SNP marker information was obtained for each individual from the RosBREED sweet cherry germplasm from the FlexQTL output. Haplotypes were assigned using the PediHaplotyper software. The SNP phasing for the sour cherry haplotypes was described in Cai et al.3. Unique haplotypes were named from H1 to H16 randomly. Statistical analyses for the association between haplotype (diplotype) and fruit firmness were performed using the software R version 3.1.3. QTL genotypes (QQ, Qq, qq) predicted by FlexQTL were used to deduce if a haplotype was associated with soft or firm fruit. As ‘Q’ is assigned to the higher phenotypic value, in this case increased firmness, QQ, Qq, and qq genotypes correspond to two “firm” alleles, one “firm” and one “soft” allele, and two “soft” alleles, respectively. Haplotypes were also deduced to be associated with soft or firm fruit based on comparison of diplotypes.

**In silico Candidate Genes.** The list of genes within the QTL interval and their functional annotations in sweet cherry were obtained from the sweet cherry genome available on the Genome Database of Rosaceae website (GDR, https://www.rosaceae.org). The corresponding predicted cherry protein sequences obtained from GDR were blasted against the National Center for Biotechnology Information (NCBI) database to obtain gene ontology terms using BLASTP in the program Blast2GO with an E-value cutoff of 0.001. The sequences of the genes within the QTL interval were also blasted to the peach genome v2.0 available at GDR and the tomato genome ITAG 2.40 available at Sol genomics Network (https://solgenomics.net), and their best gene matches and annotations were extracted. Overall, three annotations (sweet cherry, peach and tomato) and gene ontology results from Blast2GO were considered for the identification of candidate genes for fruit firmness. Candidate genes considered were those genes predicted to be involved in plant cell wall metabolism or various hormone (Ethylene, Brassinosteroid, Auxin, Gibberellin, and ABA) signaling pathways associated with fruit ripening. A circular plot of the mapping and candidate gene data was prepared with the Circos plotting tool. Alignment and analysis of the expansin genes was performed with ClustalW v2.1 multiple sequences alignment tool. A dendrogram of the four genes was constructed using the Jukes–Cantor genetic distance model, and the neighbor-joining tree building method within the GeneiousV.11.1.4 GUI software.

**Data Availability**
Genotypic data for the INRA bi-parental population and RosBREED pedigreed populations is available at the Genome Database for Rosaceae (www.rosaceae.org/publication_datasets) under accession number tGDR1037. All other data generated or analyzed during this study are included within this article and its Supplementary Information Files.

**References**
1. Yue, C. et al. U.S. growers’ willingness to pay for improvements in rosaceous fruit traits. Agric. Resour. Econ. Rev. 46, 103–122 (2017).
2. Olden, E. J. & Nybom, N. On the origin of Prunus cerasus L. Hereditas 59, 327–345 (1968).
3. Flores del Manzano, F. La vida tradicional en el Valle del Jerte. Biblioteca Nacional, Sección de Manuscritos. Correspondencia de Tomás López, Párroco de Jerte, Mérida, Spain (1992).
4. Bargioni, G. Sweet cherry scions. Characteristics of the principal commercial cultivars, breeding objectives and methods in Cherries: Crop physiology, production and uses (eds Webster, A. D. & Looney, N. E.) 73–112 (CABI, 1996).
5. Quero-García, J. et al. Breeding sweet cherries at INRA-Bordeaux: from conventional techniques to marker-assisted selection. Acta Hortic. 1161, 1–14 (2017).
6. Campos, J. A., Le Dantec, L., Barrenchea, T., Dirlewanger, E. & Quero-García, J. New insights into fruit firmness and weight control in sweet cherry. Plant Mol. Bio. Rept. 33(4), 783–796 (2015).
7. Balas, F. et al. Firmness QTL mapping using an ‘Ambrunés’× ‘Sweetheart’ sweet cherry population. Acta Hortic. (In press).
8. Harker, F. R., Redgwell, R. J., Hallett, I. C., Murray, S. H. & Carter, G. Texture of fresh fruit. Hortic. Rev. 20, 121–224 (1997).
9. Brownleader, M. D. Texture of fresh fruit. Hortic. Rev. 20, 121–224 (1997).
10. Brummell, D. A., Dal Cin, V., Crisosto, C. H. & Labavitch, J. M. Cell wall metabolism during maturation, ripening and senescence of peach fruit. J. Exp. Bot. 55, 2029–2039 (2004).
11. Sitrit, Y. & Bennett, A. B. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a re-examination. Plant Physiol. 116, 1145–1150 (1998).
12. Atkinson, R. G., Schröder, R., Hallett, I. C., Cohen, D. & MacRae, E. A. Overexpression of polygalacturonase in transgenic apple trees leads to a range of novel phenotypes involving changes in cell adhesion. Plant Physiol. 129, 122–133 (2002).
13. Costa, E. et al. QTL dynamics for fruit firmness and softening around an ethylene-dependent polygalacturonase gene in apple (Malus × domestica Borkh.). J. Exp. Bot. 61(1), 3029–39 (2010).

14. Gu, C. et al. Copy number variation of a gene cluster encoding endopolygalacturonase mediates flesh texture and stone adhesion in peach. J. Exp. Bot. 67, 1993–2005 (2016).

15. Alkio, M., Jonas, U., Declercq, M., van Nocker, S. & Knoche, M. Transcriptional dynamics of the developing sweet cherry (Prunus avium L.) fruit: sequencing, annotation and expression profiling of escarp-associated genes. Hortic. Res. 1, 11 (2014).

16. Yoo, S. D., Gao, Z., Cantini, C., Loescher, W. H. & van Nocker, S. Fruit ripening: Changes in expression of genes encoding expansins and other cell-wall-modifying enzymes. J. Amer. Soc. Hort. Sci. 128, 16–22 (2003).

17. Cao, K. et al. Genome-wide association study of 12 agronomic traits in peach. Nat. Commun. 7, 13246 (2016).

18. Duan, N. et al. Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement. Nat. Commun. 8, 249 (2017).

19. McAtee, P., Karim, S., Schaffer, R. & David, K. A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. Front. Plant Sci. 4, 79 (2013).

20. Kondo, S. & Tomiyama, A. Changes of free and conjugated ABA in the fruit of 'Satanoshiki' sweet cherry and the ABA metabolism after application of (S)+ABA. J. Hortic. Sci. Biotech. 73(4), 467–472 (2015).

21. Piróa, L. et al. Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. BMC Plant Biol. 13, 166 (2013).

22. Nuñez-Lillo, G. et al. Identification of candidate genes associated with mealessness and maturity date in peach (Prunus persica (L.) Batch) using QTL analysis and deep sequencing. Tree Genet. Genomes 11, 86 (2015).

23. Manning, K. et al. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat. Genet. 38, 948–952 (2006).

24. Cai, L., Voorrips, R. E., van de Weg, E., Peace, C. & Lezzeno, A. Genetic structure of a QTL hotspot on chromosome 2 in sweet cherry indicates positive selection for favorable haplotypes. Mol. Breed. 37, 85 (2017).

25. Zhang, G. et al. Fruit size QTL analysis in an F1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genet. Genomes 6, 25–36 (2010).

26. Dettori, M. T., Quarta, R. & Verde, I. A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. Genome 44, 783–790 (2001).

27. Castède, S. et al. Genetic determinism of phenological traits highly affected by climate change in Prunus avium: flowering data dissected into chilling and heat requirements. New Phytol. 202(2), 703–715 (2014).

28. Ballester, J., Socias, I., Company, R., Arus, P. & Vicente, M. C. Genetic mapping of a major gene delaying blooming time in almond. Plant Breed. 120(3), 268–270 (2001).

29. Quilot, B. et al. QTL analysis of quality traits in an advanced backcross between Prunus persica cultivars and the wild relative species P. davidiana. Theor. Appl. Genet. 109, 884–897 (2004).

30. Fan, S. et al. Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (Prunus persica). New Phytol. 185, 917–930 (2010).

31. Campoy, J. A. et al. Inheritance of flowering time in apricot (Prunus armeniaca L.) and analysis of linked quantitative trait loci (QTLs) using simple sequence repeat (SSR) markers. Plant Mol. Biol. Rep. 29, 404–410 (2011).

32. Dirlewaneg, E. et al. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three Prunus species: peach, apricot and sweet cherry. Heredity 109, 280–292 (2012).

33. Cai, L. et al. Identification of bloom date QTLs and haplotype analysis in tetraploid sour cherry (Prunus cerasus). Tree Genet. Genomes 14, 22 (2018).

34. Eduardo, I. et al. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. Tree Genet. Genomes 7, 323–335 (2011).

35. Hernández Mora, J. R. et al. Integrated QTL detection for key breeding traits in multiple peach progenies. BMC Genomics 18(1), 404 (2017).

36. Isuzugawa, K. et al. QTL analysis and candidate gene mapping for harvest day in sweet cherry (Prunus avium L.). Acta Hortic. (In press).

37. Quero-García, J. et al. Present and future of marker-assisted breeding in sweet and sour cherry. Acta Hortic. (In press).

38. Verde, I. et al. The high-quality draft genome of peach (Prunus persica) identifies unique patterns of genetic diversity, domestication and genome evolution. Nat. Genet. 45(5), 487–494 (2013).

39. Verde, I. et al. The Peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. BMC Genomics 18(1), 225 (2017).

40. Sandefur, P. Enhancing efficiency in tree-fruit breeding by developing trait-predictive DNA tests. PhD Thesis, Wash. State Univ. (2016).

41. Quero-García, J., Schuster, M., López-Ortega, G. & Charlot, G. Sweet Cherry Cultivars and Improvement in Cherries: Botany, Production and Uses (eds Quero-García, J., Lezzeno, A., Pulawaska, J. & Lang, G.) 60–94 (CAIB, 2017).

42. Shirasawa, K. et al. The genome sequence of sweet cherry (Prunus avium) for use in genetics-assisted breeding. DNA Res. 24(5), 499–508 (2017).

43. Dirlewaneg, E. et al. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc. Natl. Acad. Sci. USA 101, 9891–9896 (2004).

44. Chen, Y. et al. Ethylene receptors and related proteins in climacteric and non-climacteric fruits. Plant Sci. 276, 63–72 (2018).

45. Cruz, A. B. et al. Light, ethylene and auxin signaling interaction regulates carotenoid biosynthesis during tomato fruit ripening. Front. Plant Sci. 9, 1370 (2018).

46. Kumar, R., Khurana, A. & Sharma, A. K. Role of plant hormones and their interplay in development and ripening of fleshy fruits. J. Exp. Bot. 65(16), 4561–4575 (2014).

47. Fernández-Trujillo, J. P. et al. Climacteric and non-climacteric behavior inmelon fruit: 2. Linking climacteric pattern and main postharvest disorders and decay in a set of near-isogenic lines. Postharv. Biol. Technol. 50, 125–134 (2008).

48. McAtee, P. A. et al. The hybrid non-ethylene and ethylene ripening response in kiwifruit (Actinidia chinensis) is associated with differential regulation of MADS-box transcription factors. BMC Plant Biol. 15, 304 (2015).

49. Brummell, D. A. et al. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant cell 11(11), 2203–2216 (1999).

50. Cosgrove, D. J. Loosening of plant cell walls by expansins. Nature 407(6802), 321–326 (2000).

51. Rose, J. K., Lee, H. H. & Bennett, A. R. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proc. Natl. Acad. Sci. USA 94(11), 5955–60 (1997).

52. Perini, M. A. et al. Overexpression of the carbohydrate binding module from Solanum lycopersicum expansin 1 (SI-EXP1) modifies tomato fruit firmness and Botrytis cinerea susceptibility. Plant Physiol. Biochem. 113, 122–132 (2017).

53. Cosgrove, D. J., Bedinger, P. & Durachko, D. M. Some allergens of grass pollen as cell-wall-loosening agents. Proc. Natl. Acad. Sci. USA 94(12), 6559–6564 (1997).

54. Serra, O. et al. Genetic analysis of the slow-melting flesh character in peach. Tree Genet. Genomes 13, 77 (2017).

55. Campoy, J. A. et al. Genetic diversity, linkage disequilibrium, population structure and construction of a core collection of Prunus avium L. landraces and bred cultivars. BMC Plant Biol. 16, 49 (2016).
56. Chavoshi, M. et al. Phenotyping protocol of sweet cherry (Prunus avium L.) to facilitate an understanding of trait inheritance. J. Am. Pom. Soc. 68(3), 125–134 (2014).
57. Stegmeir, T., Seboli, A. & Iezzoni, A. Phenotyping protocol for sour cherry (Prunus cerasus L.) to enable a better understanding of trait inheritance. J. Am. Pom. Soc. 68(1), 40–47 (2017).
58. Peace, C. et al. Development and evaluation of a genome-wide 6K SNP array for diploid sweet cherry and tetraploid sour cherry. PLoS ONE 7(12), e48305 (2012).
59. Illumina Inc. GenomeStudio genotyping module v1.0, User Guide. Illumina Inc., Towne Centre Drive, San Diego, CA, USA (2010).
60. van Ooijen, J. W. JoinMap 4, Software for the calculation of genetic linkage maps in experimental populations, Wageningen, Netherlands: Kyazma B.V (2006).
61. Klagges, C. et al. Construction and comparative analyses of highly dense linkage maps of two sweet cherry intra-specific progenies of commercial cultivars. PLoS One 8(1), e54743 (2013).
62. Voorrips, R. E. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93(1), 77–78 (2002).
63. Bradbury, P. J. et al. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23, 2633–2635 (2007).
64. Hardy, O. J. & Vekemans, X. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620 (2002).
65. Bink, M. C. A. M. et al. Bayesian QTL analyses using pedigreed families of an outcrossing species, with application to fruit firmness in apple. Theor. Appl. Genet. 127(5), 1073–1090 (2014).
66. Voorrips, R. E., Bink, M. C. A. M., Kruisselbrink, J. W., Koehorst-van Putten, H. J. & van de Weg, W. E. PediHaplotyper: software for consistent assignment of marker haplotypes in pedigrees. Mol. Breed. 36, 119 (2016).
67. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org (2015).
68. Conesa, A. et al. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676 (2005).
69. Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485, 635–641 (2012).
70. Krywinski, M. et al. Circos: an information aesthetic for comparative genomics. Genome Res. 19, 1639–1645 (2009).
71. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948 (2007).
72. Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4), 406–25 (1987).

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Author Contributions
A.I. designed the experiments and provided financial support; L.C. and J.Q. analyzed the data; T.B. provided data for INRA sweet cherry germplasm collection; E.D. and C.S. provided candidate gene list; L.C. and J.Q. analyzed the data; T.B. provided data for INRA sweet cherry germplasm collection; E.D. and C.S. provided candidate gene list; L.C., J.Q. and A.I. wrote the manuscript. All authors reviewed and approved the final manuscript.

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