Subgenome expression of a fruit softening-associated expansin varies among cultivars of the tetraploid sour cherry (*Prunus cerasus* L.)

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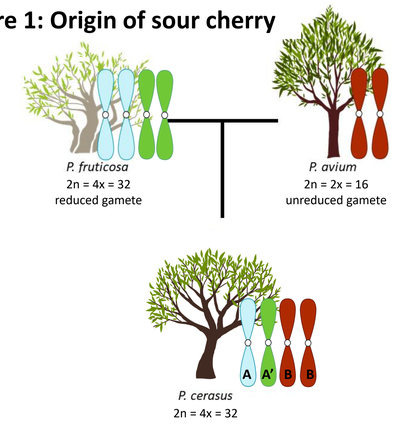
Introduction:

Sour cherry (*Prunus cerasus* L.) is a highly heterozygous allotetraploid tree whose characteristic acidic fruit is processed for use in pies, jams, and juices. Sour cherry came about from the interspecific cross of the diploid sweet cherry (*P. avium* L.) and the wild tetraploid ground cherry (*P. fruticosa* Pall.) **(Fig. 1)**. Evidence supports repeated progenitor introgressions into sour cherry followed by human selection. A new reference genome of the 'Montmorency' sour cherry (Goeckeritz *et al.*, in prep) provides us with an opportunity to investigate candidate genes at a level of detail not previously possible. A previously identified expansin associated with fruit softening (EXP2) in the sour cherry cultivar Montmorency (Yoo *et al.*, 2003) provided a valuable opportunity to investigate the subgenome origin, structural variation, and possible expression bias in a panel of sour cherry cultivars that exhibit a range of fruit firmness . Our results confirm that EXP2 expression increases over the course of fruit development and is most highly expressed during fruit softening in multiple accessions of sour cherry. In sour cherry, we also show that all homoeologs of EXP2 do not express at the same levels, and our findings suggest that structural variation is contributing to differential expression of EXP2 from the *P. fruticosa* subgenome in the sour cherry Erdi Jubileum. This work provides a template for further investigations of candidate genes in sour cherry.

Figure 1: Origin of sour cherry



Objectives:

Determine (1) if EXP2 is most highly expressed at the end of fruit development, (2) if all three subgenomes of sour cherry express EXP2 consistently across homoeologs and genotypes, and (3) if differential expression of EXP2 in sour cherry may be due to expression bias or structural variation in DNA.

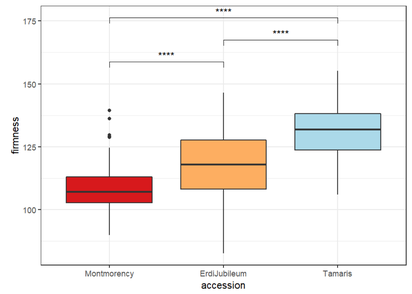
For full methods, references, and authors' contact info, scan this QR code or visit https://tinyurl.com/2jcvtydy

Materials/Methods:

Plant Material:
3 accessions of sour cherry:

- Montmorency (most common cultivar in US)
- Erdi Jubileum (Hungarian cultivar)
- Tamaris (Russian cultivar)

Figure 2: Fruit firmness in sour cherry

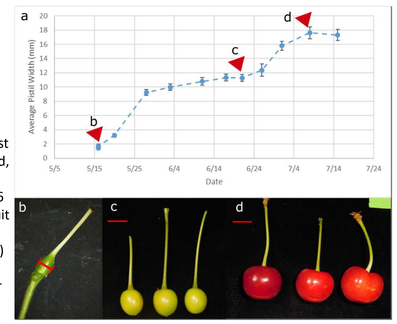


Phenotyping:
All fruit were picked at harvest ripeness and firmness was measured in grams per mm of compression using a Firmtech 2 (http://www.bio-works.us/). At least two and up to four years of firmness data were used to create **Fig. 2**.

Fruit Sampling for RNA:
Fruit was collected post-bloom to fruit maturity and the widest width of the fruit was measured at each collection point. Samples were selected for RNAseq based on their location in the fruit growth curve for each genotype so as to standardize developmental stage between genotypes with different bloom and maturity dates. The timepoints selected for RNA sequencing were the beginning of stage 1, when exponential growth and cell division are occurring **(Fig. 3b),** the end of growth stage 2, around the conclusion of pit hardening and just before the second exponential growth phase **(Fig. 3c),** and the end of growth stage 3, immediately after the second exponential growth phase **(Fig. 3d).**

Figure 3: fruit growth and collection for RNAseq

(a) Fruit growth curve of Montmorency in 2019 with arrows marking stages at which tissue was collected for RNA sequencing. (b) First fruit stage collected, red line indicates fruit width of 1.696 mm. (c) Second fruit stage collected, scale bar = 1cm. (d) Third fruit stage collected, scale bar = 1cm.



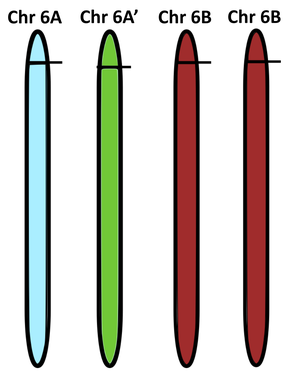
Sequencing and Analysis: Illumina short-read DNA sequencing (2x150bp) was performed on all accessions to a depth of ~50x. 3 biological replicates of each fruit growth stage for each accession underwent Illumina RNA sequencing (2x150bp) with a target of ~30 million reads per library. Reads were checked for quality using FastQC and trimmed with Trimmomatic. DNA reads were aligned to the respective species' reference genome (Goeckeritz *et al.*, in prep; Wang *et al.*, 2020) using BWA mem. RNA reads were aligned to the respective species' reference genome and transcript abundance was counted using kallisto. Differential gene expression was evaluated using the DESeq2 package in R.

Results:

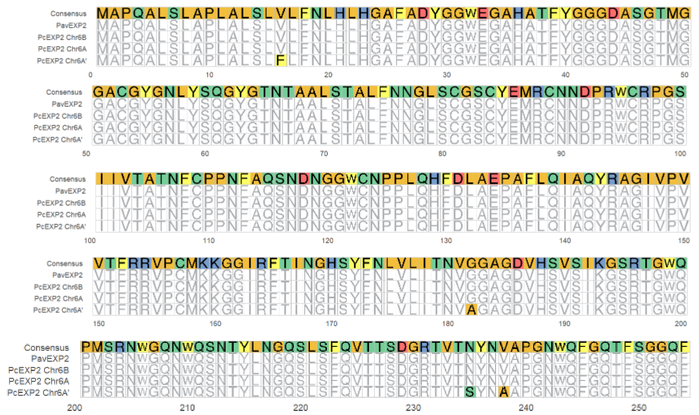
Each chromosome 6 homoeolog in sour cherry has a copy of EXP2.
Protein alignments show that the EXP2 copies are very similar to each other and to the sweet cherry EXP2. One of the ground cherry – derived homoeologs (chr 6A') EXP2 has some amino acid differences.

Figure 4: EXP2 in sour cherry

(4a) Locations

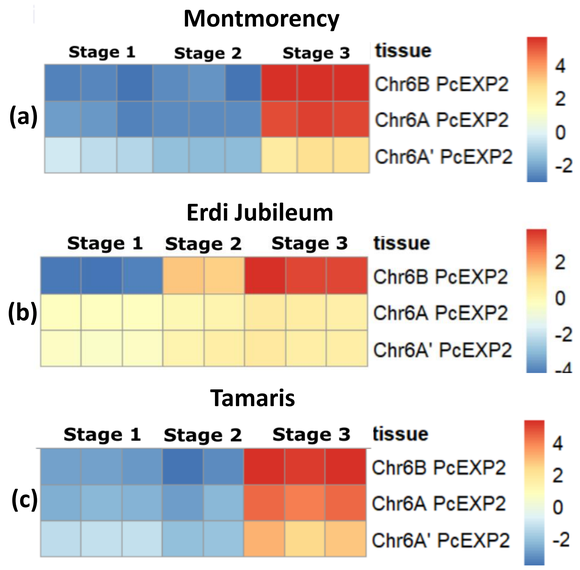


(4b) Montmorency protein alignment with sweet cherry EXP2



EXP2 is most highly expressed in ripe fruit (stage 3); each homoeolog shows different expression levels

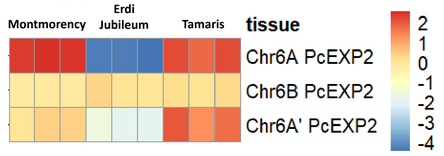
Figure 6: PcEXP2 expression over fruit development



Expression of the *P. fruticosa*-derived EXP2s at fruit stage 3 vary between genotypes

Figure 7: PcEXP2 expression at fruit stage 3 in three sour cherry genotypes

There is no significant difference in expression of the EXP2 on chr 6B among these three genotypes



Erdi Jubileum shows a deletion at the EXP2 locus on chr 6A (Fig. 8a); chr 6A' EXP2 locus does not show any significant structural difference among the sour cherry genotypes that could explain varying expression of the 6A' EXP2 homoeolog (Fig. 8b)

Figure 8a: Montmorency chr 6A

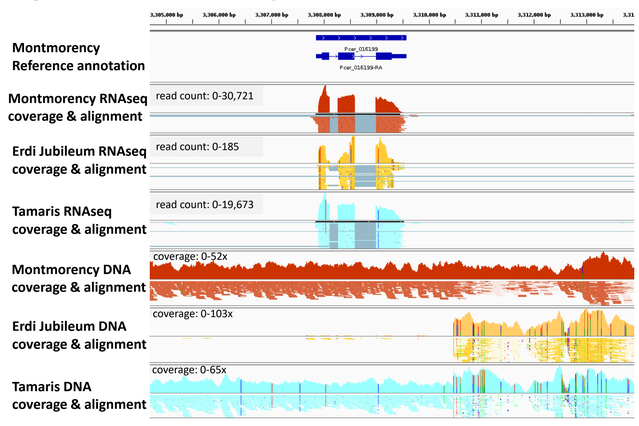


Figure 8b: Montmorency chr 6A'