FINAL PROJECT REPORT

Project Title: Consulting for the Pacific Northwest sweet cherry breeding program

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Cooperators: Nnadozie Oraguzie and other members of the cherry team (Matt Whiting, Cameron Peace, Amit Dhingra and Fred Bliss)

Other funding sources

Agency Name: USDA-CSREES NRI Plant Genome
Amount awarded: $400K, Aug 2009 – Aug 2013
Notes: "The development of COS markers for comparative mapping in the Rosaceae and their application for understanding variation in fruit size". PI: Iezzoni. Develop and validate fruit size genetic markers for sweet cherry and new state-of-the-art marker development for cherry. Leveraged with WTFRC/OSCC funding.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace and Oraguzie. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding.

Total Project Funding: $ 13,000

Budget History:

Item	**Year 1: 2013**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	$ 3,000		
Consulting fee	$ 10,000[a]		
Miscellaneous			
Total	$ 13,000		

[a]These activities, which began in 2004, have historically been funded as a consulting arrangement. This was done so that Michigan State University would not be a shared "inventor" of the forthcoming sweet cherry cultivars. I then waived my personal "inventor" rights to any cultivars in exchange for a consulting fee that I donate to MSU to help support the MSU tart cherry breeding program.

OBJECTIVES:

1. Assisted in generating breeding populations. This included developing the crossing plan, sourcing germplasm, and making crosses along with the breeding team.
2. Provided horticultural guidance. This was provided by site visits, phone consultations, and sharing results from my cherry research at MSU.
3. Provided genetic expertise. My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the development of DNA markers and genotyping of many of the parents used in the program. This work is funded by USDA grants.

SIGNIFICANT FINDINGS/ACCOMPLISHMENTS:

- Visited Prosser in March and discussed recent genetic findings and spring crossing plans. Specific parents that are likely to transfer early maturity, self-fertility and fruit firmness to their progeny were identified.
- The recommendation was made to re-evaluate the use of precocious abundantly flowering rootstocks for some of the seedlings predicted to have the most potential as parents. In certain cases, this benefit may be cost effective as it would reduce the number of years per generation.
- Continued to provide new specific information to C. Peace on new markers that can be used to refine performance predictions based on the region on cherry linkage group 2 that controls variation for fruit size.
- Three other regions on the cherry chromosomes were identified that contain loci associated with fruit size. Other traits associated with these same genomic regions were also summarized. Use of this new knowledge was shared with N. Oraguzie and C. Peace with the goal of them using this information to identify parents and seedlings that transfer large fruit size to their offspring along with other desired characteristics.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In March 2013 I traveled to Prosser to meet with N. Oraguzie, C. Peace and members of their teams to discuss progress in the genetic understanding of important traits and to plan for spring activities, specifically spring crossing. One of the topics of utmost importance was which parents to use for spring 2013 crosses to transfer early maturity along with self-fertility from the cultivar 'Cristobalina'. Results from the Peace lab had identified 16 offspring from the cross of 'Rainier' × 'Cristobalina' that were predicted to be self-fertile and have large fruit size alleles. Of these individuals, 5 also had very early maturity dates. Upon visiting these own-rooted seedlings in the field, it was determined that the flower numbers were very low, thus the pollen available for spring crosses was limited. This illustrates the benefit of using a precocious rootstock to increase flower number on potential parents. It was recommended that if DNA diagnostics can be used in the early stages of seedling growth, to identify a seedling as a potential parent, this seedling could be grafted on a precocious rootstock as soon as possible to ensure an early and abundant pollen supply.

A second topic of discussion was fruit firmness. The Spanish cultivar 'Ambrunes' has very firm fruit and many of its offspring have inherited this trait. However, none of these immediate 'Ambrunes' descendants are suitable as cultivar candidates due to small fruit size. Therefore, it is very important to continue the breeding with these 'Ambrunes' offspring used as parents for the next generation so that this firm-fruited characteristic can be transferred into future cultivar candidates.

As illustrated by these examples of early maturity and firmness, the most desirable parents to be used in crosses are now most likely seedlings that are determined to have the best breeding values (a quantitative genetics principle) and the most desirable trait locus alleles (a molecular genetics principle) for the traits of interest, rather than simply having the best performance (an antiquated pre-genetics principle). Therefore, a concerted effort is needed to evaluate the genetic potential of superior seedlings, not only for candidate cultivar status but as parents for crossing in spring 2014. Additionally, it would be important to re-evaluate the use of precocious, abundantly flowering rootstocks for some of the seedlings predicted to have the best potential as parents. In certain cases, this benefit will reduce the number of years per generation.

Provide genetic expertise

In 2012, a genome scan made up of thousands of anonymous markers was developed by the RosBREED genetics and genomics team. This genotyping array technology was made available through a commercial partner, Illumina Inc. These arrays were used to genome-scan 480 sweet cherry individuals. The arrays were run and scored by the team at Mich. State Univ., resulting in successful assessment of allelic states at ~1,900 positions along the eight cherry chromosomes of any individual scanned (Peace et al. 2012; Klagges et al. 2012). Collectively, these efforts provided the building blocks that allowed us to seek new practical knowledge of genetic diversity and trait inheritance in sweet cherry breeding germplasm. Examples of these outcomes are described below.

Using the available genetic marker data, 'Napoleon' was determined to be 'Bing's paternal parent (Rosyara et al. 201x). 'Napoleon' is also the grand parent of 'Stella', the self-fertile cultivar that is the ancestor of all the self-fertile cultivar released from the sweet cherry breeding program in British Columbia (BC) (Fig. 1). This indicates that the genepools used by both the BC program and the original WSU sweet cherry breeding program were very limited with extensive overlap. Our analysis also indicated that some of the parents that I used in 2004 to broaden the genetic base and introduce unique genetic diversity, such as 'Regina' and 'Ambrunes', are not related to 'Bing'. Understanding how to use genetic knowledge to transfer the superior attributes of these parents to elite cultivar candidates is a critical goal for which progress is being made.

Years ago, I initiated an effort to understand the genetic control of fruit size in cherry, because in my experience large-fruited progeny individuals were very rare. This suggested that marker-assisted selection could significantly increase the efficiency of achieving large fruit size. Fruit size data from the PNW sweet cherry breeding program supports this observation of the rarity of large-fruited seedlings. Therefore, using the available genetic data, we concentrated our efforts on the most important fruit size genomic region that is located on cherry linkage group 2. This genomic region is now used for marker-assisted selection in the PNW cherry breeding program as certain alleles for this trait locus are predictive of large versus small fruit size. However, this region also appears to be associated with other traits such as firmness, bloom time, and fruit cracking (Castede et al. 2012; Quero-Garcia 2012). Therefore we have increased marker density in this region with the goal of determining if the genetic control of firmness, for example, can be separated from that of fruit size (De Franceschi et al. 2013).

Although there is a major locus controlling fruit size on cherry linkage group 2, we identified other genomic regions also containing loci associated with fruit size. These are located on linkage groups 1, 3, and 6 (Fig. 2) in sweet cherry (Rosyara et al. 2013) plus linkage group 5 in tart cherry (Stegmeir 2013). The region on linkage group 3 that is associated with genetic variation for fruit size also contains the major locus for fruit skin and flesh color and the locus associated with 'Cristobalina' derived self-fertility (Sooriyapathirana et al. 2010; Fig. 2). The region on linkage group 6 containing the fruit size locus is linked to the self-incompatibility locus. Therefore, using DNA diagnostics to achieve desired phenotypes will require an understanding of segregation for multiple traits at a time and multiple genetic locations.

The importance of considering all the chromosomal regions containing loci associated with fruit size together is illustrated using fruit size data from progeny from the cross 'Regina' × 'Lapins' provided by E. Dirlewanger and J. Quero-Garcia (Rosyara et al. 2013) (Fig. 3). 'Regina' is predicted to be heterozygous for the loci associated with fruit size variation on linkage groups 1, 2, 3 and 6, where each trait locus can have alleles that either confer large fruit or small fruit. 'Lapins' is predicted to have two copies of the alleles associated with large fruit size on linkage groups 1 and 3, but only one allele for large fruit size for the loci on linkage groups 2 and 6. Of the ~200 offspring from the cross 'Regina' × 'Lapins', the seedling with the largest fruit was predicted to be homozygous for the large fruit allele at three of the four fruit size loci (Fig. 3). In contrast, the offspring with the smallest fruit had two copies of the small fruit allele for the trait loci of linkage groups 2 and 6 and only one copy of the large fruit allele for the other two loci. The fruit size difference between these two offspring was more than two-fold (5.4 g versus 11.1 g). This example illustrates how genetic knowledge of these four trait loci can predict a large difference in fruit size. Putting genetic knowledge of these other fruit size loci into application in the PNW cherry breeding program is a current goal.

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Fig. 1. Pedigrees for sweet cherry cultivars bred in the PNW (modified from Rosyara et al. 201x). 'Napoleon' is the grandparent of 'Stella' and the newly identified paternal parent of 'Bing'. This shared ancestry illustrates the narrow gene pool used in breeding new sweet cherry cultivars for the PNW. The intensity of grey indicates the degree of relationship to 'Stella' according to pedigree records while a clear box indicates no known pedigree relationship to 'Stella'.

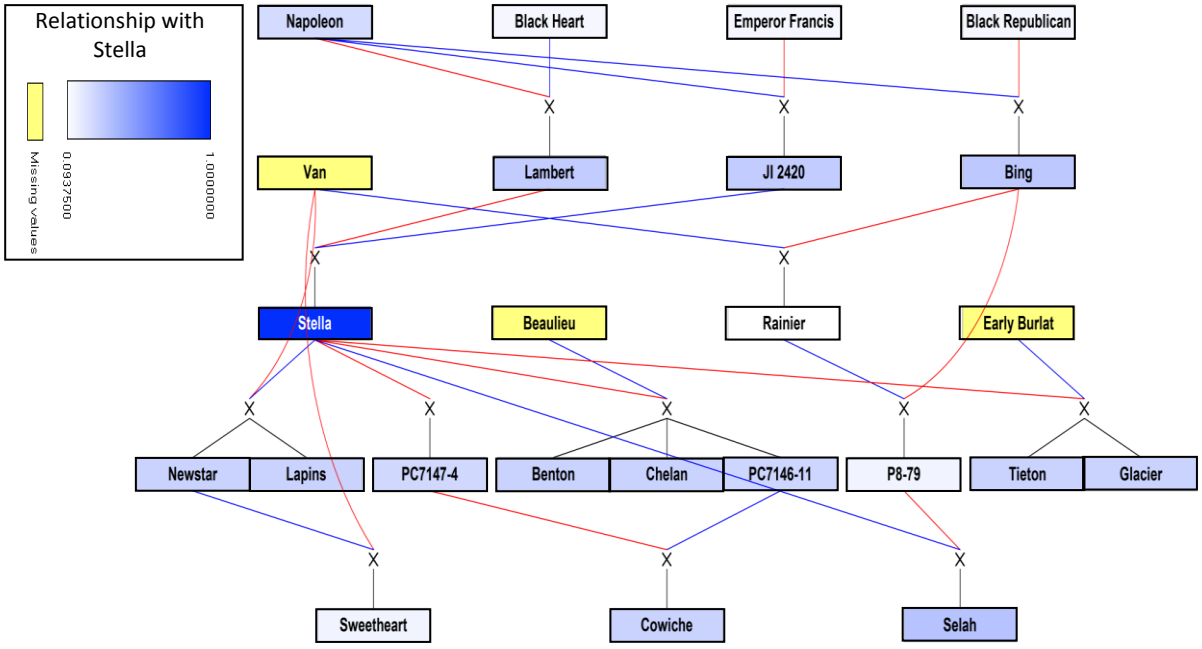


Fig. 2. Major loci associated with trait variation that have been identified on the eight cherry chromosomes (from 'Jewels in the genome: The Necklace, by A. Iezzoni in Weebadde et al. 2013). Fruit size trait loci have been identified on linkage groups 1, 2, 3, and 6 and on group 5 in tart cherry. The fruit size locus on group 3 co-locates with major loci associated with fruit skin and flesh color and 'Cristobalina'-derived self-fertility. The fruit size locus on group 6 co-locates with the locus controlling self-fertility and cross-compatibility. In tart cherry, a major locus associated with cherry leaf spot resistance was identified at the top of linkage group 4.

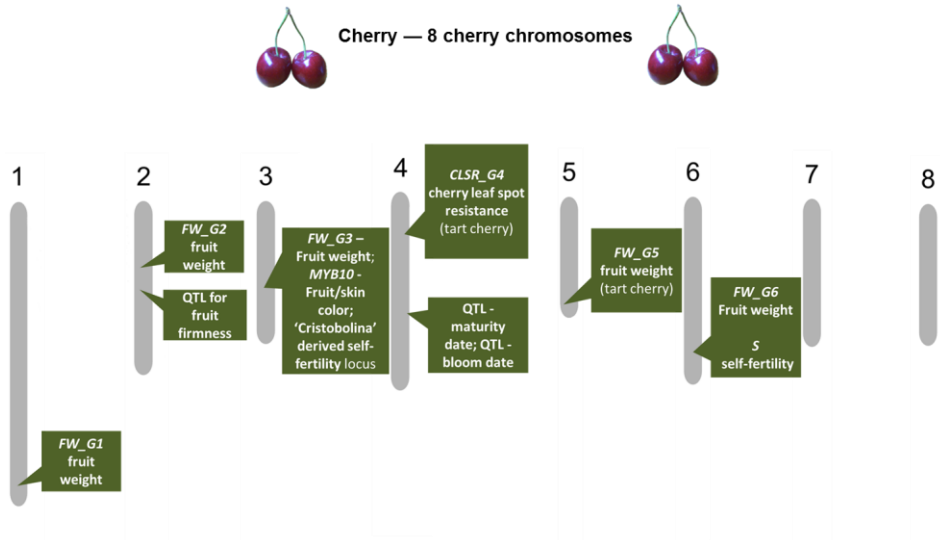
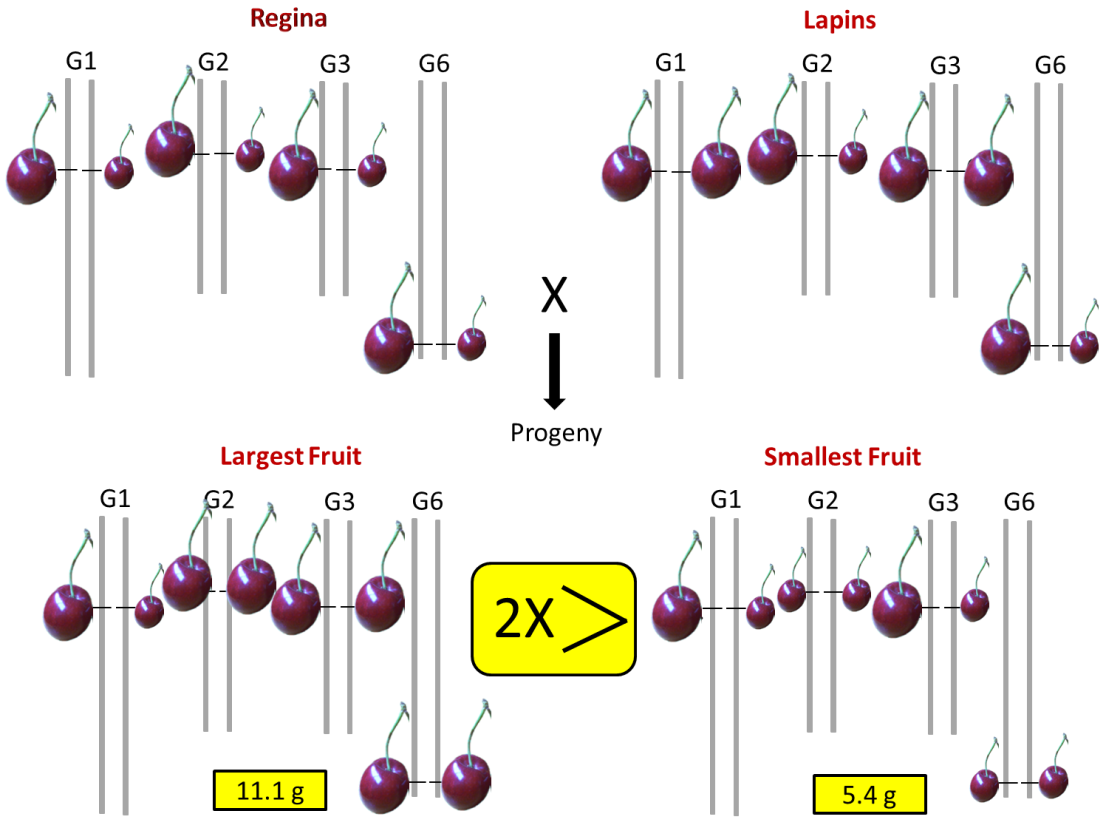


Fig. 3. Regions on cherry chromosomes 1, 2, 3, and 6 containing loci associated with fruit size in sweet cherry illustrated for 'Regina' and 'Lapins' and two of their offspring that contrast for fruit size (modified from Rosyara et al. 2013). The offspring that has the largest fruit size inherited the most large fruit alleles from its parents while the offspring that has the smallest fruit size inherited the most small fruit alleles from its parents.



Consulting for the Pacific Northwest Sweet Cherry Breeding Program

A. Iezzoni

EXECUTIVE SUMMARY:

The building blocks of a successful breeding program include the use of diverse germplasm, generation of large numbers of progeny populations for evaluation, appropriate horticultural management of the breeding materials, the ability to identify and commercialize superior cultivar candidates, and judicious use of genetics knowledge. The goal of my consultancy with the PNW sweet cherry breeding program was to assist in our ability to excel at all of these objectives so that we can deliver superior sweet cherry cultivars to the Oregon and Washington industries as quickly as possible. In 2013, parents were identified that confer early maturity, self-fertility, and firm fruit. These attributes were inherited from new germplasm introduced in the breeding program through crosses that began in 2004. This new germplasm and the novel attributes that this germplasm provides will increase the likelihood that the PNW program will identify elite cultivars that meet the maturity date and fruit quality targets. Major progress was made identifying genomic regions associated with fruit size. Knowledge for the linkage group 2 locus associated with fruit size is already being used routinely each year to select parents that are more likely to confer large fruit and eliminate seedlings predicted to have small fruit prior to field planting. These other fruit size trait loci are also targets for use in marker-assisted selection to increase the precision of fruit size predictions. Knowledge of what other traits are also associated with these loci has been summarized so that genetic improvement for multiple traits can occur simultaneously. Continued collaboration, whereby I contribute my time and knowledge of cherry germplasm, breeding, and genetics, will help us achieve our collective vision of a cost-effective aggressive and successful sweet cherry breeding program.

*RosBREED team members who have and continue to contribute substantially to this cherry genetics effort are Cameron Peace, Dorrie Main, Nahla Bassil, Umesh Rosyara and Audrey Sebolt.