FINAL PROJECT REPORT

Project Title: Adapting available genomics tools to enhance PNW sweet cherry breeding

PI:	Cameron Peace	**Co-PI(2)**:	Jim Olmstead
Organization:	WSU-Pullman	**Organization**:	WSU-Yakima Extension
Telephone:	509-335-6899	**Telephone**:	509-574-1600
Email:	cpeace@wsu.edu	**Email**:	jwolmstead@wsu.edu
Address:	Wash. St. Univ.	**Address**:	104 N. 1st St.
Address 2:	Dept. of Hort and LA	**Address 2**:	Suite 204
City:	Pullman	**City**:	Yakima
State/Zip:	WA 99164	**State/Zip**:	WA 98901
Co-PI(3):	Amy Iezzoni	**Co-PI(4)**:	
Organization:	Mich. St. Univ.	**Organization**:	
Telephone:	**517-355-5191 x391**	**Telephone**:	
Email:	Iezzoni@msu.edu	**Email**:	
Address:	Dept. of Horticulture	**Address**:	
Address 2:	A288 PSSB	**Address 2**:	
City:	East Lansing	**City**:	
State/Zip:	MI 48824	**State/Zip**:	

Cooperators: Matt Whiting (WSU-IAREC), Wayne Loescher (MSU), Fred Bliss (Davis, CA), Jim McFerson (WTFRC)

Other funding Sources

Agency Name: Prunus Crop Germplasm Committee
Amount requested/awarded: $8,833
Notes: Metabolite profiling of the National Clonal Germplasm Repository cherry collection

Total Project Funding: $67,900

Budget 1 History: WSU

Item	Year 1:	Year 2:	Year 3:
Salaries – postdoc* for 9 months	22,500		
Benefits	9,900		
Wages – for activity 2	3,587		
Benefits	413		
Equipment			
Supplies – activities 1,3,5	5,000		
Travel – in-state	4,000		
Travel - interstate**	7,500		
Miscellaneous – database software	5,000		
Total	57,900		

Budget 2 History: MSU

Item	Year 1:	Year 2:	Year 3:
Salaries	4,772		
Benefits	2,228		
Wages	2,000		
Benefits			
Equipment			
Supplies	1,000		
Travel			
Miscellaneous			
Total	10,000		

Footnotes:

*We hired an Agricultural Project Assistant (grad student level) from May 2008, which leveraged support from the Department of Horticulture and LA for a Teaching Assistantship starting in the Fall 2008 semester for this prospective PhD student. Summer salary for this person was instead paid by an existing federally funded project of the PI focusing on stone fruit texture genetics. A full-time technical assistant for this WTFRC/OSCC cherry project from August 2008.

**$1500 for one trip to CA (Davis repository), $6000 for PIs (and local collaborators) to meet once in Prosser and once in East Lansing

ORIGINAL OBJECTIVES

The main goal is to assess opportunities for genetic marker assistance in the Pacific Northwest sweet cherry breeding program (PNWSCBP) and develop the technical infrastructure for ready translation of genomics information and tools into practical breeding benefit.
Specific objectives are to:

1) Develop protocols for high-throughput genetic screening in the PNWSCBP.
2) Conduct flavor and texture phenotypic analysis of key breeding germplasm.
3) Collate, validate, and package available markers of value to the PNWSCBP through coordination with other sweet cherry projects on gene/marker identification.
4) Establish a database for the PNWSCBP that meets traditional breeding needs and is compatible with ongoing genomic analyses.
5) Assess internationally-renowned cherry germplasm collections for genetic diversity of potential value to the PNW.

SIGNIFICANT FINDINGS

Overall

- Numerous opportunities were identified for practical application of genomics tools and knowledge to enhancing sweet cherry breeding in the Pacific Northwest towards a tangible, and indeed revolutionary, effect on the sweet cherry industry. In 2008, we established some of the necessary technical infrastructure to translate genomics advances into industry impact. This project has helped leverage national efforts towards developing a nationwide" marker-assisted selection pipeline" approach, and helped maintain the strong collaboration between WSU and MSU for cherry genetic improvement.
- This project attracted and leveraged funding from the Department of Horticulture and Landscape Architecture for a PhD student Sanchita Haldar, under the direct supervision of PI Peace.
- Four large proposals for federal funding were submitted in 2008 that could help fulfill the goal of this current project. Only one of these proposals was funded, a 2-year USDA-NRI project beginning in 2009 focusing on the three major genes involved in Rosaceae tree fruit texture. In addition to a postdoc who will concentrate on apple, the PhD student mentioned above will focus on defining the role of these genes for practical application in the sweet cherry industry.

Objective 1: Technical infrastructure

- Objective in final stage of completion in October 2008.
- A method for high-throughput DNA extraction was tested for sweet cherry and is in the final stages of optimization. This method has a simple tissue sampling procedure, low start-up equipment costs, compatibility with our preferred high-throughput genotyping method, and is the same method to be used for the apple breeding program.
- A method for high-throughput genotyping was tested for sweet cherry and is in the final stages of optimization. We are currently seeking to purchase an ABI 3730 to service the PNW tree fruit breeding programs in collaboration with the USDA Small Grains Genotyping Laboratory on the WSU Pullman campus.

Objective 2: Phenotyping for flavor and texture

- Objective achieved for some germplasm, but spring freezes ruined the opportunity to obtain fruit quality data in Prosser, and reduced number of fruiting trees in Michigan.
- At Michigan State University (MSU), flavor phenotypic data (SSC, astringency, and GC measurement of major sugars and acids) were collected in the 2008 season for a key experimental population, NYxEF.
- Locating the genomic regions controlling these traits ("QTL analysis") will be performed in Nov-Dec 2008. Genetic markers are expected to be developed for flavor components, for utility in the PNWSCBP via marker-assisted selection (MAS) of seedlings and for describing the genetic predisposition of potential breeding parents and current cultivars of the PNW sweet cherry industry.
- Bird netting was used to cover the experimental orchard at MSU, ensuring sufficient fruit was available for multiple harvesting. Bird netting is recommended as a component of the Best Management Practices for the PNWSCBP.
- The 2008 spring freeze in Prosser destroyed our opportunity to collect texture and flavor phenotypic data on our pedigree-linked set of ~40 cultivars and selections growing at IAREC. We hope to gather this data in the 2009 season on an expanded set of cultivars, selections, and seedlings.
- An allied project conducted in 2008 by co-PI Olmstead and collaborator Dave Rudell, funded by USDA Prunus CGC funds, obtained interesting data on metabolic profiles (including sugars, acids, and aroma volatiles) on cherry germplasm from the Davis Repository and many parent cultivars used to date in the PNWSCBP. These results will feed into the PNWSCBP by identifying new sources of fruit flavor variation, developing protocols for flavor measurement, and enabling us to dissect the individual genetic components conditioning cherry fruit flavor.

Objective 3: Marker validation

- Objective achieved to the extent of known available markers. Collaborations and other projects identified provide an excellent source of new markers to be used in the PNWSCBP.
- Traits of highest priority for genetic testing in the PNWSCBP are: reduced tree juvenile period, self-fertility/cross-compatibility, fruit size, fruit firmness, sweetness, acidity, and rain cracking resistance. Recommendations for these traits are to develop and implement MAS as soon as possible. Of these, only one already has an available genetic test: self-fertility/cross-compatibility, previously developed for cherry in the programs of co-PI Iezzoni and researchers around the world. For the other traits, genetically variable plant material is available within the PNWSCBP for developing genetic markers, although improved phenotyping methods are required for each trait.
- The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. We are currently verifying or determining S-allele genotypes of PNWSCBP parents.
- Chloroplast markers are being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding.
- Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni, to be available for use on the PNWSCBP by the end of 2008.
- Other marker opportunities are arising from ongoing projects of the PIs and collaborators, with funding from various other sources. As marker-trait associations are discovered for high-priority traits, markers will be tested for validity and utility in the PNWSCBP.

- The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was obtained from collaborator Marco Bink of Plant Research International in the Netherlands.

Objective 4: Breeding program database

- Objective not yet achieved – interrupted by start of a new breeder for the PNWSCBP, inability to secure access to an existing European-developed database package, and investment in developing a proposal to establish a US-wide common Breeders' Information Management System for Rosaceae ("RosBREED" proposal). We have pursued alternative options in the meantime for sweet cherry, with excellent progress and prospects.
- Working closely with the new breeder for the PNWSCBP, Nnadozie Oraguzie, we are creating a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. A Microsoft Excel-based database template was developed and is being filled with available data. This database template is compatible with Pedigree Based Analysis software.
- A genotyping database is being developed, designed to help efficiently process the genotyping of seedlings for the PNW tree fruit breeding programs.
- A breeders' Decision Support spreadsheet tool for MAS was developed in our program that determines the potential savings to be achieved by replacing phenotypic selection with marker selection, and determines the optimum stage for genotyping.

Objective 5: Allele mining in germplasm collections

- Objective half achieved thus far: US collections visited, but not yet genotyped with available high-priority markers and compared to PNWSCBP parents. To be completed later in 2008.
- Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the Davis collection, California. A visit by Peace and Olmstead to Michigan identified further potential sweet cherry individuals that could be used as parents from 2009 onward.
- Metabolite and aroma profiling of sweet cherry germplasm held in the Davis collection was funded through the Prunus CGC. All available cherries were sampled in June 2008, shipped to WA and processed in Dave Rudell's laboratory at USDA-TFRL in Wenatchee. Relative concentrations of primary and secondary metabolites have been analyzed and aroma profiling is underway.

RESULTS AND DISCUSSION

Objective 1: Technical infrastructure

A method for high-throughput DNA extraction, which we call the silica bead method (SBM), was tested for sweet cherry and is in the final stages of optimization. SBM was our method of choice for the apple breeding program, compared to two other high-throughput methods and presented at the 4th International Rosaceae Genomics Conference. We prefer SBM for the PNW sweet cherry breeding program because it requires the simplest tissue sampling procedure, has low start-up equipment costs, is compatible with our preferred high-throughput genotyping method, and is the same method to be used for the apple breeding program. The most common high-throughput DNA extraction method used in other programs is the metallic bead method (MBM), but MBM requires sampled tissue to be kept cold and moist, and freeze-dried as soon as possible – a complication that is not compatible with routine breeding operations. The ultra-high-throughput extraction provided by the Theonyx automated system (TAS) developed at HortResearch in New Zealand was also tested (for apple). This method is perhaps too efficient for our needs, with robotics replacing the need for technician time, but with relatively high supplies costs, equipment costs, and it requires prior freeze-drying of tissue

samples. A poster presenting our high-throughput DNA extraction method trialing was presented at the 4th International Rosaceae Genomics Conference in Chile (March 2008), which generated much interest.

A method for high-throughput genotyping, using DNA Analyzing equipment from Applied Biosystems (ABI), was tested for sweet cherry and is in the final stages of optimization. The ABI genotyping system was compared to a dozen other possible approaches for per-sample costs, number of samples that can be tested per week, versatility to handle the types of genetic markers that are useful for the PNW sweet cherry breeding program, technical skill required, and availability and affordability of equipment. We are currently seeking to purchase an ABI 3730 to service the PNW tree fruit breeding programs (and associated research programs) in collaboration with the USDA Small Grains Genotyping Laboratory on the WSU Pullman campus.

Objective 2: Phenotyping for flavor and texture

At Michigan State University (MSU), flavor phenotypic data were collected in the 2008 season for a key experimental population, NYxEF. This cross was originally created for genetic analysis of fruit quality traits, as it represents a cross between the small-fruited astringent wild forest cherry 'NY54' and the large-fruited elite cultivar 'Emperor Francis'. Usually three, and up to five harvests (2-4 days apart) were conducted for each tree to get a handle on maturity changes on fruit quality attributes in this population. Once fully analyzed, these multiple harvest data are expected to provide valuable information regarding changes in fruit quality traits as maturity progresses, enabling development of optimized sampling protocols to more accurately detect genetic potential.

In the orchard, astringency was scored on a 0-2 scale. There was insufficient sensory variation detected for sweetness and acidity to allow scoring on a similar scale as originally planned. In the lab, SSC was measured for all fruit. Juice samples were collected for Gas Chromatography (GC) analysis to describe the concentrations of the predominant sugars and acids in cherry fruit, which will be analyzed in the final months of 2008. In an associated project of co-PI Iezzoni, data were also collected for other fruit quality traits: fruit size (weight and diameter), skin color (ground and blush), flesh color, and maturity date. Joint analysis will allow us to detect physiological and genetic relationships between these traits.

From sensory analysis (tasting the fruit), 38 trees had an astringency score of 0 (no astringency), 28 had a score of 1 (mild astringency), and 24 had a score of 2 (highly astringent). Results were consistent with high astringency being inherited in a recessive manner, indicating that astringency in wild germplasm should not significantly hamper its use in cultivar development. Astringency scores did not change for a tree from harvest to harvest. Therefore, astringency was very stable and not affected by fruit maturity – improving our chances to identify controlling gene regions.

SSC ranged from 13 to 22 ^{o}Brix, averaging 19. Trees with highest astringency fruit tended to have a lower SSC (Figure 1), indicating a possible genetic correlation that a breeder could exploit. From other correlation analysis, we identified a significant positive correlation between SSC and fresh weight ($R=0.43$), a negative correlation with harvest date as described by growing degree days ($R=-0.37$), and no correlation with any measure of fruit color (skin blush, skin ground, or flesh). Trees in this population with smaller fruit tended to have highest astringency ($p<0.001$), while later maturing trees also tended to have more astringent fruit ($p=0.027$).

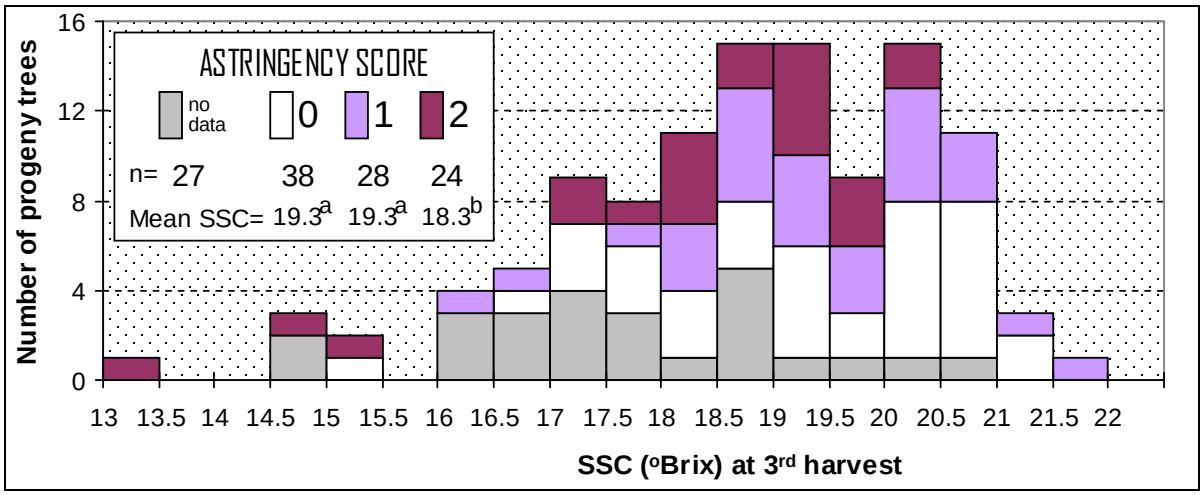

Figure 1: Genetic variation observed in flavor components of SSC and astringency for an experimental population, NYxEF. In the legend, numbers with different letters in superscript are significantly different ($p<0.05$).

After additional statistical analyses of the NYxEF phenotypic data later in 2008, we will develop recommendations for the PNWSCBP to genetically improve sweetness and TA while avoiding astringency. We will determine whether SSC is suitable enough as a quick laboratory screening of the sweetness trait rather than the detailed information that the GC acquires (although with much greater effort and expense). Already we have found that field tasting for sweetness is not sufficient, as this was deemed to not be scorable in 2008 on these trees. For breeding population in the PNWSCBP, field tasting may be worthwhile to assess genetic expression of sweetness only in crosses that vary widely for sweetness.

Locating the genomic regions controlling these traits ("QTL analysis") will be performed in Nov-Dec 2008, taking advantage of the powerful genetic resource previously developed by co-PIs Iezzoni and Olmstead and collaborator Audrey Sebolt: a genetic map of the genome of sweet cherry based on this NYxEF population. From this QTL analysis, we expect to develop genetic markers for flavor components that can enter the practical MAS pipeline and be tested for utility in the PNWSCBP seedling selection and for describing the genetic predisposition of potential breeding parents and current cultivars of the PNW sweet cherry industry.

A spring freeze in Michigan left about half the trees of the experimental population fruitless. Bird netting was used to cover the experimental orchard, ensuring a large crop set on those trees that had received effective pollination. Sufficient fruit was then available for multiple harvesting, and a solid data set was obtained that we can mine for useful information for many months to come. Bird netting is recommended as a component of the Best Management Practices for the PNWSCBP.

The 2008 spring freeze in Prosser destroyed our opportunity to collect texture and flavor phenotypic data on the "Pedigree Genotyping set" of approximately 40 cultivars and selections growing at IAREC. We hope to gather this data in the 2009 season on an expanded set of cultivars, selections, and seedlings, chosen for obtainment maximum genetic knowledge using the Pedigree Based Analysis approach.

An allied project conducted in 2008 by co-PI Olmstead and collaborator Dave Rudell, funded by USDA Prunus CGC funds, obtained interesting data on metabolic profiles (including sugars, acids,

and aroma volatiles) on cherry germplasm from the Davis Repository and many parent cultivars used to date in the PNWSCBP. These results will feed into the PNWSCBP by identifying new sources of fruit flavor variation, developing protocols for flavor measurement, and enabling us to dissect the individual genetic components conditioning cherry fruit flavor.

Objective 3: Marker validation

In collaboration with Dr. Fred Bliss and his WTFRC-funded projects in 2007 and 2008, we determined that the traits of highest priority for genetic testing in the PNWSCBP are reduced tree juvenile period, self-fertility/cross-compatibility, fruit size, fruit firmness, sweetness, acidity, and rain cracking resistance, for which the recommendations are to develop and implement MAS as soon as possible. Of these, only one already has an available genetic test: self-fertility/cross-compatibility, previously developed for cherry in the programs of co-PI Iezzoni and other researchers around the world. For the other traits, genetically variable plant material is available within the PNWSCBP for developing genetic markers, although improved phenotyping methods are required for each trait.

The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. For most parents used thus far in the PNWSCBP, S-allele scores are available in published reports. We are currently verifying these genotypes for all PNWSCBP parents, and collecting new S-allele data for those parents and selections with unknown S-alleles. The S-allele genetic test was adopted as the primary test for verifying parentage of seedlings in the breeding program, which we have recently begun. We are using the S-allele genetic test to verify the ability of the ABI high-throughput genotyping system to efficiently provide genetic data for thousands of breeding program seedlings.

Sweet cherry chloroplast markers are also being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Published reports on such markers describe only three maternal lineages within cultivated sweet cherry, although another 13 were reported in wild populations of Europe. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding. Thus far we have replicated the reported genetic tests, determined which of the three maternal lineages each member of the PNWSCBP parent cultivar belongs to, and verified that they will be an effective tool for genetic descriptions of cherry material.

Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni. In that program, previously identified fruit size markers in the NYxEF population are currently being validated in sweet cherry germplasm that fully covers the breadth of the PNWSCBP. We expect that these markers will be available for use in the PNWSCBP by the end of 2008, at which time we will test them on parents and 2004 cross seedlings using the high-throughput ABI genotyping system.

Other marker opportunities are arising from ongoing projects of the PIs and collaborators, with funding from various other sources. We are conducting the basic discovery research toward identifying marker-trait associations for sweet cherry for the other high-priority traits. As such associations are discovered, markers will be tested for validity and utility in the PNWSCBP. Currently we are testing cherry for genes believed to control fruit texture, arising from a federally funded project by PI Peace. Phenotypic data with which to compare promising markers to identify and validate marker-trait associations is still lacking in most cases, and we are pursuing many angles to obtain this necessary performance data.

The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was recently obtained from collaborator Dr. Marco Bink of Plant Research International in

the Netherlands. The free PediMap software was also obtained from another Dutch colleague, Dr. Eric van de Weg. PediMap was used to visualize the pedigree relationships among PNWSCBP parent cultivars, which was also overlain with information on maternal lineages (Figure 2).

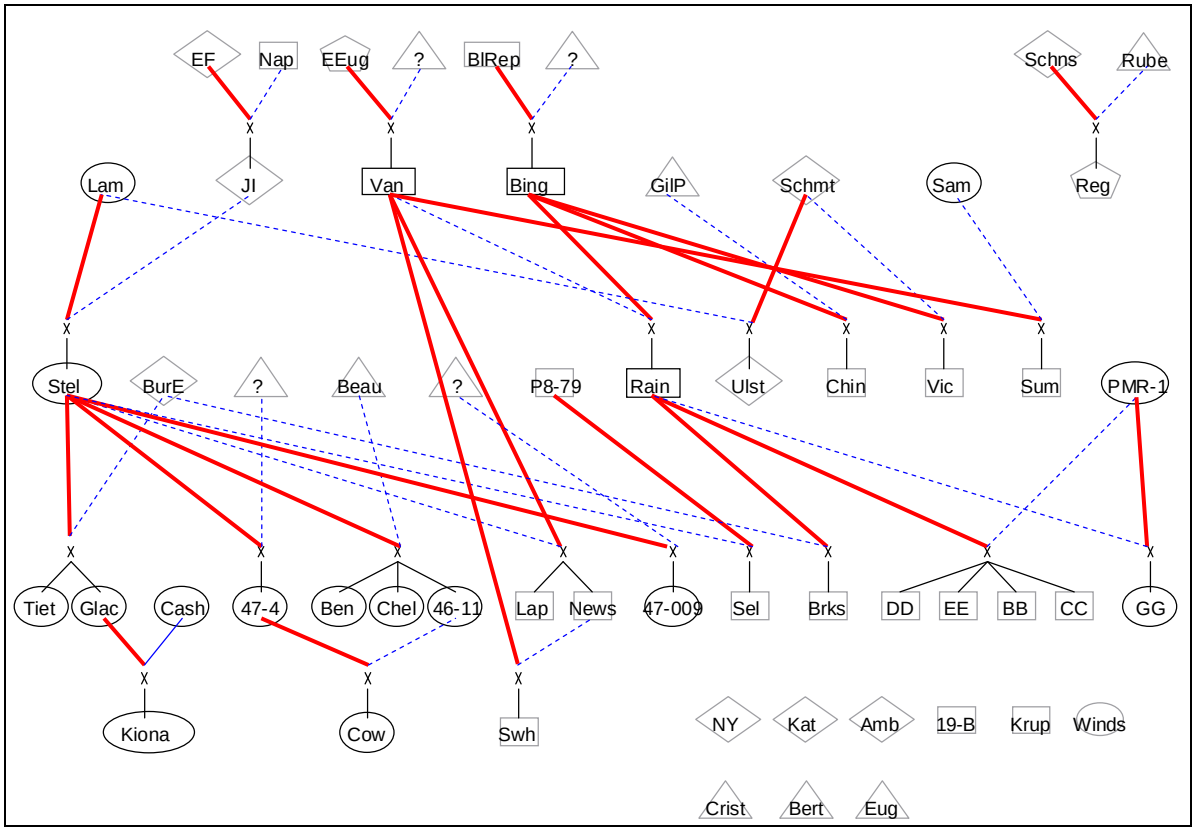

Figure 2: Pedigree structure of parent cultivars and selections of the PNWSCBP. Cultivar names are abbreviated. Solid lines represent maternal parentage, dotted lines pollen parentage. Cultivars in diamonds belong to the maternal lineage known as “H3”, rectangles are H4, and ovals are H5. Triangles are unknown at present. Two cultivars (Regina and Empress Eugenie, in pentagrams) were detected with a maternal lineage different to reported information in the literature. Nine cultivars (lower right) have no known pedigree relationships to any other cultivars in the program.

Objective 4: Breeding program database

Dr. Nnadozie Oraguzie was hired as the new breeder for the PNWSCBP in May 2008. This new beginning, combined with a recently established breeding program for which the first limited fruit quality data was obtained in 2008, provides an opportunity to install a breeding database that is able to efficiently take advantage of genomic advances. We believe that this database should be based on the Pedigree Based Analysis (PBA) approach. The breeder requires knowledge about previously used and potential future parents to determine breeding value, including their pedigrees, their performance for traits of industry value, and their genotypes for traits with available predictive genetic markers. The supporting researcher requires the same knowledge to use PBA to validate that promising genetic markers are relevant for the breeding program. Therefore the breeder and supporting researchers can use the same database format for breeding program germplasm.

A fully functional breeding program database is not yet established for the PNWSCBP, but in the meantime we have a suitable solution for collating appropriate data and identifying knowledge gaps. We are working closely with Dr. Oraguzie, to create a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. A Microsoft Excel-based database template was developed and is being filled with available data with the help of our PhD student. This database template is compatible with Pedigree Based Analysis software.

We are still pursuing a database format known as AppleBreed developed for the European HiDRAS project. To date the developers are unable to commercially release the software, but we have made personal connections and hope to take advantage of the time and expertise put into the development of AppleBreed.

In collaboration with Pullman-based USDA-ARS scientist and high-throughput genotyper for regional wheat/barley breeding programs, Dr. Deven See, a computer science student has recently been hired to develop a genotyping database. This database will be designed to help efficiently process the genotyping of seedlings for the PNW tree fruit breeding programs. As the thousands of seedlings each year are sampled, DNA-extracted, and genotyped, the information will be logged and sorted, and resulting genotypic information will be provided in a breeder-friendly format to recommend which seedlings to cull and which to keep prior to field planting. We expect this genotyping database to be ready by mid-2009.

As part of the decision support package we are establishing for genomics-assisted breeding, an Excel spreadsheet has been developed for calculating the cost efficiency of MAS using available markers. Using input parameters that describe aspects of a breeding program – such as the stages involved, costs of each routine operation with traditional phenotypic selection (i.e. without markers), and proportions of seedlings expected to be maintained through each stage – we can determine the potential savings of replacing phenotypic selection with marker selection and the optimum stage for genotyping. So far we have used this for the Washington apple breeding program, and identified that if only a single genetic marker is available that detects 50% of the seedlings as undesirable, approximately 40% of the total cost after eight years (from crossing to deciding on which seedlings to advance to replicated trials) can be saved by using that marker. Also, we unexpectedly discovered that the optimum stage for genotyping is not always as early as possible. With the availability of more good markers comes greater savings and efficiency, which could be reinvested into larger seedling numbers for genotyping. We expect similar predicted outcomes for the PNWSCBP, for which S-allele genotyping is already available. Parameters for the PNWSCBP need to be determined and used in this MAS Decision Support spreadsheet tool.

We hope to leverage efforts made on the part of the larger Rosaceae community for database management tools. As part of the recent proposal, "RosBREED: Enabling Marker Assisted Breeding in Rosaceae" submitted to the USDA-CSREES Specialty Crop Resarch Initiative, we proposed a U.S.-wide common Breeders' Information Management System (BIMS). The BIMS concept builds upon our efforts to develop database and decision support packages for PNW apple and cherry breeding programs.

Objective 5: Allele mining in germplasm collections

Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the USDA's National Plant Germplasm System. A visit by Peace and Olmstead to MSU's Clarksville Horticultural Experiment Station in Clarksville, MI, and Northwest Michigan Horticulture Research Station in Traverse City, MI, identified further potential sweet cherry individuals that could be used as parents from 2009 onward. DNA samples were obtained for some of this germplasm. Samples

were also obtained for related species in sweet cherry's readily crossable "primary genepool", including *P. fruticosa*, *P. canescens*, and tart cherry (*P. cerasus*). In 2009, we plan to test available genetic markers on this wider germplasm using existing/remaining funds to identify further sources of genetic diversity for high-priority traits, targeting the genes underlying the traits rather than relying solely on phenotypic assessment. We also plan a germplasm acquisition trip to Europe in 2009 or 2010 to gain access to further diversity, guided by genetic analyses.

Additional allele mining of flavor components was possible through a Prunus CGC project (PIs Olmstead and Rudell) to profile primary and secondary metabolites and aromas from the USDA clonal cherry germplasm collection. Samples at harvest maturity were collected and shipped to WA for analysis. Standard fruit quality measurements were made (size, color, firmness, total soluble solids, and titratable acidity) prior to gas chromatography-mass spectrometry analysis at the USDA-TFRL. Cherry accessions in the collection will be grouped according to sugar and acid levels, and novel and/or unfavorable flavor and aroma profiles will be identified. The information will be used to identify potential parents for use directly in crosses or to increase the level of diversity for flavor traits in the PNWSCBP.

EXECUTIVE SUMMARY

The main goal of this project was to assess opportunities for genetic marker assistance in the Pacific Northwest sweet cherry breeding program (PNWSCBP) and develop the technical infrastructure for ready translation of genomics information and tools into practical breeding benefit.

Specific objectives were to:

1. Develop protocols for high-throughput genetic screening in the PNWSCBP.
2. Conduct flavor and texture phenotypic analysis of key breeding germplasm.
3. Collate, validate, and package available markers of value to the PNWSCBP through coordination with other sweet cherry projects on gene/marker identification.
4. Establish a database for the PNWSCBP that meets traditional breeding needs and is compatible with ongoing genomic analyses.
5. Assess internationally-renowned cherry germplasm collections for genetic diversity of potential value to the PNW.

A summary of significant accomplishments from 2008 includes:

- High-throughput DNA extraction and genotyping methods were tested and are being optimized. These methods are essential for routine application of MAB on the thousands of seedlings in the PNWSCBP.
- Flavor phenotypic data were collected at Michigan State University (SSC, astringency, GC measurement of primary sugars and acids) for the NY×EF experimental population. Development of genetic markers for these traits is currently underway.
- The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni, to be available for use on the PNWSCBP by the end of 2008. As marker-trait associations are discovered for other high-priority traits (for example, flavor phenotyping within this project) markers will be tested for validity and utility in the PNWSCBP.
- Chloroplast markers are being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding.
- The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was obtained from collaborator Marco Bink of Plant Research International in the Netherlands.
- Working closely with the new breeder for the PNWSCBP, we are creating a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. This database template is compatible with Pedigree Based Analysis software. Also included is a breeders' Decision Support spreadsheet tool for MAS was developed in our program that determines the potential savings to be achieved with marker selection, and determines the optimum stage for genotyping.
- Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the Davis collection, California. A visit by Peace and Olmstead to Michigan identified further potential sweet cherry individuals that could be used as parents from 2009 onward.
- This project attracted and leveraged funding from the Department of Horticulture and Landscape Architecture for a PhD student Sanchita Haldar, under the direct supervision of PI Peace.

Completion of this project has identified and put into practice several baseline steps for utilizing MAS in the PNWSCBP. Future efforts should focus on identifying useful germplasm variants for priority traits and validating the utility of existing and new markers in plant material from the PNWSCBP.