FINAL PROJECT REPORT

Project Title: Identifying sweet cherry fruit size genes and molecular markers

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Other funding Sources

Agency Name: USDA/CSREES/NRI Plant Genome
Amount requested/awarded: $400,000 requested on 2/14/08 and awarded 8/15/08
Notes: This WTFRC/OSSC project provided funds for us to get preliminary data that helped us successfully compete for this federal grant.

Total Project Funding: $ 24,830

Budget History:

Item	**Year 1:**	**Year 2:**	**Year 3:**
Salaries	$ 7,158		
Benefits	$ 3,342		
Wages	$ 2,500		
Benefits			
Equipment			
Supplies	$ 4,830		
Travel			
Consulting fee: M. Bink	$ 2,000		
Other: DNA sequencing &	$ 5,000		
primer synthesis			
Miscellaneous			
Total	**$ 24,830**		

OBJECTIVES

1. Identify the genomic regions in sweet cherry that control fruit size.

- Regions of DNA that are associated with variation for a particular trait are termed quantitative trait loci (QTL).

2. Develop a conserved ortholog set (COS) of markers for cherry suitable for comparative mapping within the Rosaceae (partial funding is requested).

- COS markers are "state of the art" sequence based molecular markers this can be used for cherry mapping and for connecting maps between cherry and other rosaceous species.

SIGNIFICANT FINDINGS BY OBJECTIVE

1. Identify the genomic regions in sweet cherry that control fruit size.

- Four genomic regions (e.g. QTLs) were identified in sweet cherry that control fruit size. The QTL identified on cherry linkage group two appears to increase fruit size by increasing fruit mesocarp cell number. In contrast, the QTL identified on cherry linkage group six appears to increase fruit size by increasing pit size. Two more QTL were newly identified on linkage groups four and eight. The morphological basis of the fruit size increases associated with these newly identified QTLs has not yet been determined.

- The molecular markers flanking the QTLs on linkage groups two and six have been identified and will be used to examine the affect of these fruit size QTL in different genetic backgrounds.

- The newly identified fruit size QTLs on linkage groups four and eight will be validated in other germplasm used in the sweet cherry breeding program.

- A genetic database was generated to support the PNW sweet cherry breeding program. It includes marker genotyping data (e.g. genetic barcodes) for over 39 parents used in the breeding program plus progeny from the cross between PMR × Rainier. This database will serve as the foundation for future QTL discovery and marker assisted breeding through the use of pedigree based analysis.

2. Develop a conserved ortholog set (COS) of markers for cherry suitable for comparative mapping within the Rosaceae (partial funding is requested).

- From an existing set of 1041 Rosaceae COS, PCR primers were designed that successfully amplified 739 (86%) COS in *Prunus*. To date, 319 COS have been placed on the eight *Prunus* linkage groups with a density ranging from 1.19 to 2.10 markers per centimorgan (cM). The COS markers are also well distributed across the *Prunus* genome as COS markers were mapped in 53 of the 67 *Prunus* peach × almond (T×E) reference map bin positions (*Howad et al. 2005. Genetics 171:1305-1309*). A subset of these COS markers will also be mapped in sweet cherry, apple and strawberry, providing a lasting resource of high throughput markers.

- A survey of COS marker diversity was undertaken using seven sweet cherry selections (Bing, Van, Regina, Emperor Francis, NY54, Cristobalina, and Windsor). The majority of COS markers exhibited only two allelic variants per marker.

1 & 2. This WTFRC & OSSC grant provided seed money that helped us successfully compete for the following USDA-CSREES-NRI Award (Appendix I):

Title: The Development of COS Markers for Comparative Mapping in the Rosaceae and Their Application for Understanding Variation in Fruit Size.
PD: Amy Iezzoni
Co-PDs: Esther van der Knaap (Ohio State University) & Dechun Wang (Mich. State Univ.)
Award Amount: $400,000
Award Period: 08/15/08 through 08/14/11

RESULTS and DISCUSSION

Fruit Size: Findings

Cherry fruit size is not only a critical trait for market profitability, but it is the key trait altered during the domestication of sweet cherry from it wild forest tree relative. Our initial approach to elucidate the genetic control of fruit size in sweet cherry was to determine the genetic changes that accompanied domestication. With prior USDA-CSREES-NRI funding we conducted an analysis of the genetic control of fruit size in 2006 and 2007 using progeny from a cross between the domesticated founder cultivar 'Emperor Francis' (EF) and the forest sweet cherry 'NY 54' (NY). EF is in the ancestry of all the self-fertile sweet cherry cultivars as it was the material parent in the cross from which the self-fertile mutant was identified. In 2008, we repeated our fruit size analysis of the EF × NY population, and broadened our analyses to include progeny from the cross PMR × Rainier, commercial sweet cherry cultivars, and other parental cultivars used as parents in the PNW sweet cherry breeding program. Statistical analysis of the pedigree linked populations was possible with the implementation of pedigree based analysis software.

- *Segregation for fruit size*: Fruit weight was measured for the progeny from the two segregating populations, EF × NY (Fig. 1A) and PMR × Rainier (Fig. 1B). Progeny from both populations exhibited fruit weight values well below that of the large fruited parent. This tendency for the vast majority of the progeny to be small fruited has been observed repeatedly in studies in cherry and peach. This result suggests that large fruited progeny are rarely obtained in breeding populations. Therefore, it is critical that we put strategies in place to increase the percentage of large fruited seedlings that are planted in the evaluation orchards.

- *QTL discovery in EF × NY*: In 2006 and 2007, analysis of progeny from a population generated from the cross of EF × NY resulted in the identification of fruit weight QTLs on two of the eight *Prunus* linkage groups (Fig. 2). This analysis of the EF and NY progeny was repeated in 2008, and confirmed our previous findings. Fruit length and diameter QTLs were identified at similar genomic regions as the fruit weight QTLs, as would be expected due to the trait correlations (Fig. 2). A QTL for mesocarp cell number was also detected on EF 2 which suggests that the morphological basis of this QTL is to increase cell number by increasing cell division in the mesocarp. The fruit weight QTL identified on NY linkage group 6, co-located with pit size (linear measures) QTL. This suggests that the morphological basis underlying this QTL is an increase in pit diameter and length and not an increase in cell number (as measured on a radial section) or cell size.

- *QTL discovery in a PMR × Rainier progeny population*: In 2008, we extended this analysis to include the parental cultivars and progeny from the cross PMR × Rainier using pedigree based analysis. The 39 cultivars and 103 progeny individuals were genotyped for 61 and 49 marker loci, respectively (12 fewer markers were scored in the PMR × Rainier population as

these markers did not segregate in this population.). Fruit weight was also measured. When this data set was combined with our data from the EF × NY population, fruit weight QTLs were also identified on cherry linkage groups four and eight (Table 1). Validating and fine mapping these newly discovered QTLs will be a major thrust undertaken with our newly acquired USDA-CSREES-NRI funding.

- *Determination of the fruit weight/cell number QTL allele*: Determining the favorable fruit size QTL allele(s), identified by its flanking markers, is critical for further QTL validation and eventually marker-assisted selection (MAS). For the fruit weight/cell number QTL on EF linkage group two, the unique PR96 allele present in EF is in coupling with the large fruit effect of the predicted fruit weight QTL. Future efforts will involve experiments to validate the presence and direction of this QTL in different genetic backgrounds using PR96 and other co-dominant markers linked in coupling with PR96.

- *Genome scans of 39 parental cultivars*: A description of the genetic make-up of 39 sweet cherry varieties and parental cultivars used in the breeding program was generated with past NRI funding and WTFRC/OSCC funding. This genetic data set (~ 5,000 genotypic data points) provides a critical foundation of genetic knowledge for the sweet cherry breeding program. This information will assist in our interpretation of genetic variation through the use of Flex QTL® and will inform future crosses and eventually strategies to pre-select superior seedlings prior to field planting. An example of how our genetic information is used to describe inheritance of genomic regions in cherry is illustrated in Fig. 3.

Fruit Size: Significance

Achieving large fruit size is an essential component for profitable fresh market sweet cherry production. Therefore, new cultivars to be released from the WSU sweet cherry breeding program must exhibit the large fruit size demanded by the growers and marketplace. Our work in cherry and other reports in both cherry and peach clearly indicate that large fruited progeny individuals occur only rarely in segregating populations. Two factors are likely contributing to this prevalence of small fruited progeny individuals: dominance of alleles conferring small fruit size, and the rarity of those unique allelic combinations that result in large fruit size. Our ability to understand the genetic control of fruit size in sweet cherry and pre-select for those desirable allelic combinations will dramatically increase the efficiency of sweet cherry breeding.

This project, in combination with prior NRI funding, resulted in the identification of four genomic regions that contain desirable genes controlling fruit size. Future NRI funding will allow us to expand this to additional breeding populations with the goal of identifying and defining those QTL that contribute to large fruit size so that they can be effectively manipulated in the breeding program. The genetic data obtained for the parental selections in the breeding program will be used to dissect the genetic control of other target traits, plan future crosses, and accelerate the implementation of marker assisted selection.

Fig. 1. Progeny distributions for fruit weight from the crosses EF × NY (A) and PMR × Rainier (B). Data is from 2008.

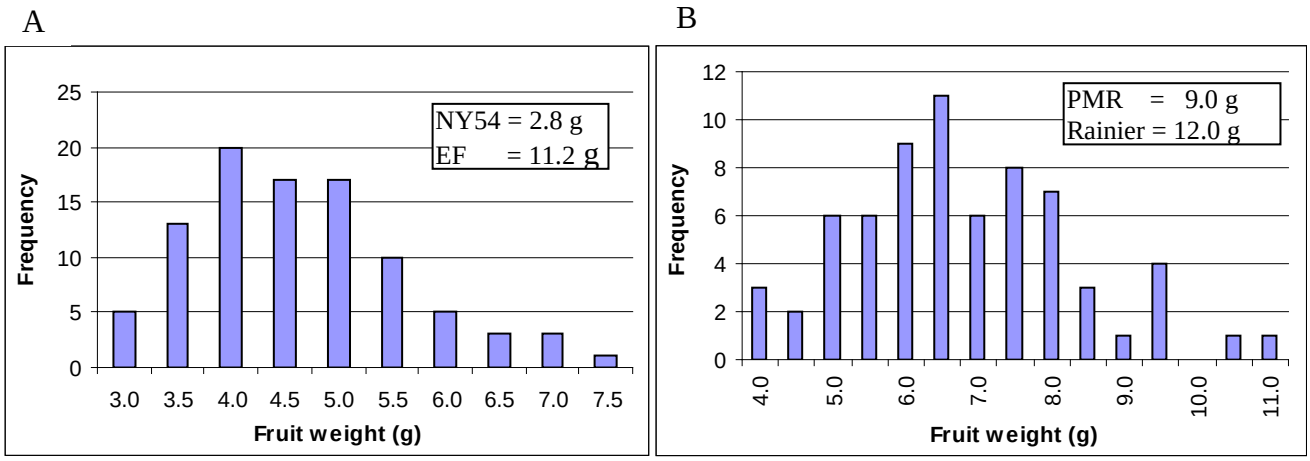


Table 1. QTLs identified using Flex QTL® and data from 41 parental cultivars and two mapping populations: EF × NY, PMR × Rainier.

Linkage group (parental map)	Bayes Factor[a]
2 (EF)[b]	6.4
2 (NY)	6.3
4 (EF)	3.1
8 (NY)	3.5

[a]QTL models interpreted based on the value of the 2ln Bayes Factor: 2.0-5.0 Positive, 5.0-10 Strong, > 10 Decisive.

[b]This QTL was also identified using fruit size and molecular marker data from progeny from the cross Regina × Lapins. The data from this population was generously provided by E. Dirlewanger and J. Queros, INRA, Bordeaux, France.

Fig. 2 Locations of QTLs for fruit weight (FW), fruit length (FL), fruit diameter (FD), mesocarp cell number (Cell), pit length (PL) and pit diameter (PD) in 2006, 2007, and 2008 using the multiple QTL mapping method. 1-LOD and 2-LOD support intervals of each QTL are marked by thick and thin bars, respectively. EF-2 and NY-2 represent EF and NY linkage groups 2, respectively, while NY-6 is NY linkage group 6.

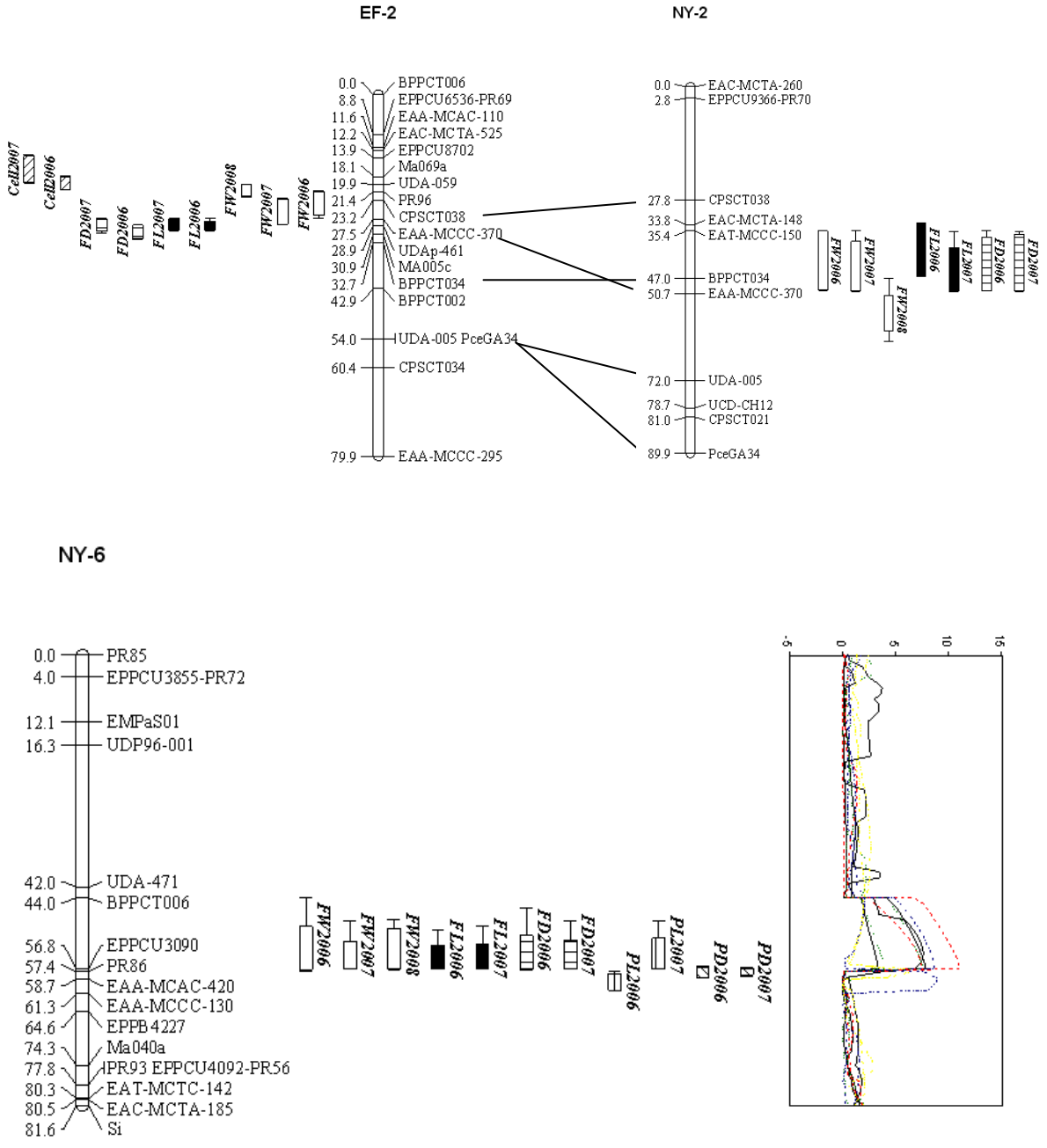
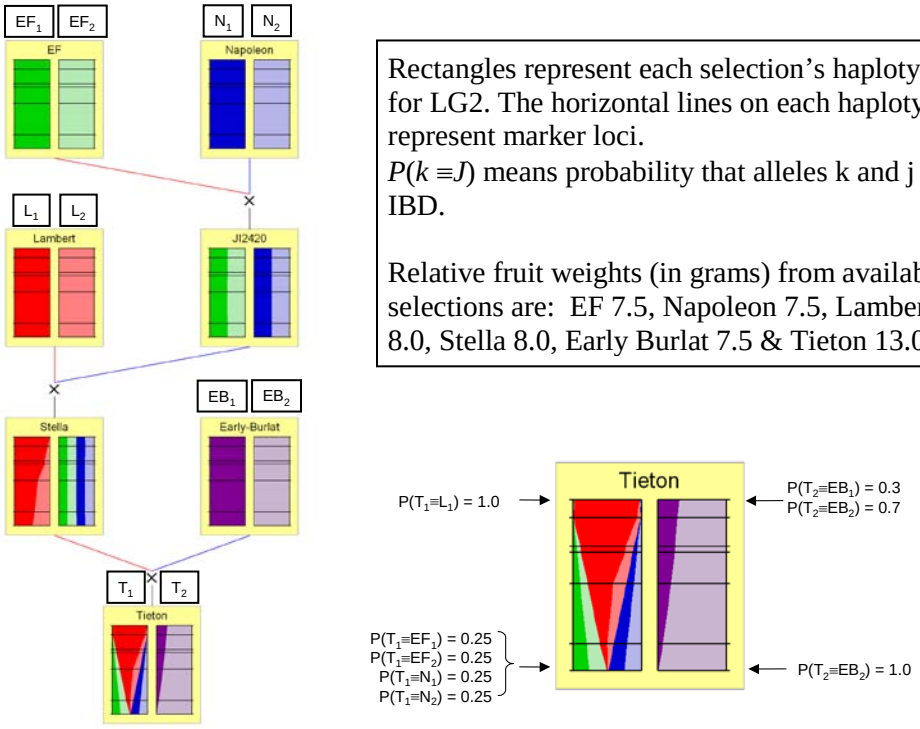


Fig. 3. Graphical representation of the Identity by Descent (IBD) probabilities for the marker alleles on LG 2 using the cultivars in the 'Tieton' lineage and our genotyping data.



COS Markers: Findings

PCR primer pairs were designed based on available sequence data and used to amplify 730 COS markers in the *Prunus* reference bin mapping population (T×E). Allelic polymorphisms, defined as single nucleotide polymorphisms (SNPs) or insertions/deletions (InDels), were identified using available software. A total of 221 polymorphic COS markers were mapped on the 8 *Prunus* linkage groups (Table 2) with an average marker/centimorgan (cM) density of 1.19 to 2.10 COS markers per cM. These markers also mapped to 53 out of 67 bins on the T×E map (Fig. 4). With newly acquired USDA-CSREES-NRI funding, a subset of the markers mapped to each of the T×E bins will be placed on our sweet cherry map and available strawberry and apple bin maps, providing a genome wide set of comparable high-throughput markers.

COS sequence data from seven sweet cherry cultivars identified, on average, two allelic variants within this sweet cherry germplasm. Therefore, our sequencing strategy should result in the identification of the vast majority of the allelic variation for these COS in the parents used in the sweet cherry breeding program.

COS markers: Significance

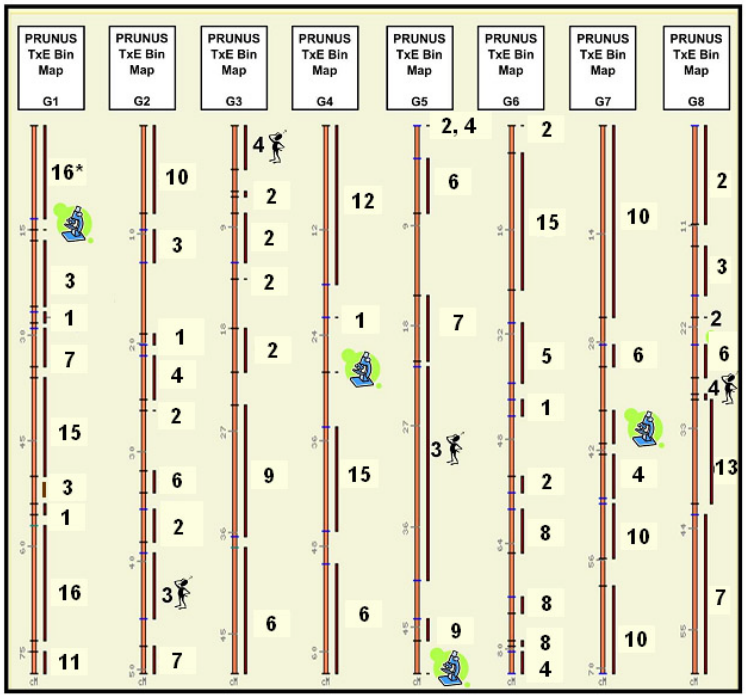
Dissection of the genetic basis of complex trait variation is accomplished by associating DNA variants with known linkage map location (e.g. markers) with phenotypic differences in the trait of interest. This strategy is used whether the target trait is in human genetics or cherry genetics. Unfortunately for sweet cherry, very few markers were available. To fill this void, the WTFRC/OSSC funded project provided us with seed money to obtain preliminary data that allowed us to successfully compete for NRI funds to generate and map additional markers in cherry and identify their comparative map locations in apple and strawberry. The COS markers that are being developed are state-of-the-art (identical marker type used in human genetics) and therefore amenable to high throughput phenotyping approaches (just ~ 10 cents per data point). With a detailed linkage map of COS markers, we will finally have the markers density that we need in sweet cherry to do

comprehensive genetic dissection for those traits so critical to future cultivars. In addition, as these markers will also be mapped in other rosaceous species, e.g. peach, almond, apple and strawberry, we will be able to immediately test any marker trait associations identified in these other crops for their relevance in cherry. This will speed up our ability to understand the genetic basis of important traits in the Rosaceae, and also provide a source of high-throughput markers for implementing MAS for desirable alleles.

Table 2. Number of COS markers per centimorgan (cM) placed on the 8 *Prunus* linkage groups utilizing the *Prunus* reference bin mapping population developed from a peach × almond cross (T×E).

Prunus Linkage Group	cM length from the T × E map	Number of COS markers mapped to this linkage group	Number. of COS markers mapped per cM
1	87.0	73	1.19
2	50.5	35	1.44
3	48.4	23	2.10
4	62.5	34	1.84
5	49.1	28	1.75
6	83.7	53	1.58
7	70.6	40	1.76
8	55.9	33	1.69

Fig 4. Locations of the COS markers on the *Prunus* reference (T×E) bin map. The eight vertical lines represent the 8 *Prunus* linkage groups while the lines to the right represent the *Prunus* bin locations. The numbers reflect the number of COS markers to date that have been mapped to each of the bins.



EXECUTIVE SUMMARY

Key project goals were to identify favorable fruit size alleles segregating in sweet cherry breeding germplasm and determine the effects of these alleles in different genetic backgrounds. With the assistance of this project and prior USDA-CSREES-NRI funding we have identified and confirmed the presence of two fruit size QTLs on linkage groups 2 and 6. The QTL identified on cherry linkage group 2 appears to increase fruit size by increasing fruit mesocarp cell number. The molecular markers flanking this QTL have been identified and will be used to examine the affect of this fruit size QTL in different genetic backgrounds. The fruit size QTL identified on cherry linkage group 6 appears to increase fruit size by increasing pit size. Two more QTLs were newly identified on linkage groups 4 and 8. The morphological basis of the fruit size increases associated with these newly identified QTLs has not yet been determined.

A genetic database was developed to support the PNW sweet cherry breeding program. It includes marker genotyping data (e.g. genetic barcodes) for 39 parents used in the breeding program plus progeny from the cross between PMR × Rainier. This database of over 5,000 molecular marker data points, will serve as the foundation for future QTL discovery and marker assisted breeding through the use of pedigree based analysis

Whether one is a human geneticist or cherry geneticists, mapped markers (e.g. DNA variants with known genetic map locations) are a critical part of the investigator's "tool kit". It is this linked marker scaffold that is used to dissect the genetic basis of trait variation. With the genetic basis of the variation for a particular trait known, it is then possible to identify the desirable allelic variants and in cherry, select for those desirable allele variants prior to field planting. This has the potential to dramatically reduce the breeding cost and increase the chance of success, as those seedlings that are unlikely to have commercial potential can be discarded prior to field planting. With our prior NRI funding, bridging funds provided by the WTFRC/OSSC, and newly acquired NRI funding, we are building this scaffold for sweet cherry and linking it to peach, almond, apple and strawberry. With this toolkit in place, we can now move forward and use this resource for the genetic dissection of trait variation in sweet cherry, similar to our strategy for the genetic dissection of fruit size. To date, our accomplishments towards the construction of this marker scaffold for sweet cherry are as follows:

- Identification of a set of conserved othologous markers (COS) suitable for comparative mapping in the rosaceae.
- Generation of PCR primer pairs that successfully amplify 700 of these markers.
- Mapping of over 300 of these markers on the peach × almond bin map thereby setting the stage to map these in sweet cherry.
- Identification of allelic variants for these markers in sweet cherry thereby providing state-of-the art markers suitable for marker assisted selection in sweet cherry.

APPENDIX I

Title: The Development of COS Markers for Comparative Mapping in the Rosaceae and Their Application for Understanding Variation in Fruit Size.
PD: Amy Iezzoni
Co-PDs: Esther van der Knaap (Ohio State University) & Dechun Wang (Mich. State Univ.)
Award Amount: $400,000
Award Period: 08/15/08 through 08/14/11

The goals of this proposal are to accelerate Rosaceae comparative genomics through the development and mapping of a Rosaceae-Arabidopsis conserved ortholog set (COS) of markers. We will adopt these markers to enable our complementary long term goal to map and deploy beneficial alleles for increased fruit size in rosaceous crops. The COS markers developed will be a primary tool for integrating information across the family, and will provide additional markers for Rosaceae species for which current marker coverage is insufficient. For the first time, it will be possible to extensively align the linkage groups of the major fleshy fruited Rosaceae genera. The proposed research goals are to: (1) develop a Rosaceae-Arabidopsis COS marker set resource, (2) determine the comparative bin map locations of the COS markers in *Prunus*, *Malus* and *Fragaria*, (3) determine the sweet cherry linkage map locations for a set of COS markers with known bin map locations in *Prunus*, *Malus* and *Fragaria* to provide the basis for a genome-wide comparative gene-based linkage map, and (4) accelerate the discovery, quantification, validation, fine mapping and deployment of beneficial QTL alleles for fruit size in sweet cherry. This proposal addresses NRI priority 1: Use of genome-wide approaches for mapping and identification of important genes. The knowledge gained will enable comparative QTL mapping in Rosaceae species and significantly increase the efficiency of sweet cherry breeding in particular. Large fruit size, the focus of this proposal, is critical to the long term profitability of the U.S. Rosaceae fruit industries.