

Dedicated to the genetic improvement of U.S. rosaceous crops

February 28, 2013

Volume 4 Issue 1

RosBREED's Fourth Annual Meeting with Advisory Panels, San Diego, CA

Once again, RosBREED participants met with their Industry, Scientific, and Extension Advisory Panel members in San Diego, California, on January 10, 2013. There were 23 Advisory Panel members and 19 RosBREED participants at this meeting which was held at the DoubleTree Hilton hotel in San Diego. As the last of the Advisory Panel meetings of the current project, the main focus was on project deliverables and impacts. In the afternoon session, the Socio-Economics Team conducted an "apple auction" (see page 3 for details) to demonstrate how consumers were engaged to evaluate apple fruit quality. Advisory Panels were complimented the progress achieved so far and made many clever recommendations for the final stages of the project, including dissemination of impact statements that quantify successes. During the roundtable wrap-up, Kim Hummer (USDA-ARS, OR) stated, "The interaction among geneticists, breeders, socio-economics scientists, and overall logistics are a model for SCRI. I am impressed with the organization and branding of the project."

Participants also discussed the best strategies for continuing to create impacts beyond the life of the project.



Attendees of RosBREED's fourth annual meeting with Advisory Panels.



Advisory Panel members experience how consumer values of fruit quality attributes are determined using sensory tasting and an experimental auction of apples.

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United States Department of Agriculture
National Institute of Food and Agriculture



Advisory Panel Member Profile

The success of RosBREED will be determined in large part by the people behind it. Because of this, we wanted to give you some insight into these individuals - whether they represent university extension, the scientific community, or industry - who are at the core of our efforts. Panel members were asked about their background and what they want to accomplish as part of the RosBREED project. Here is what they had to say.

Industry



John Lott

President of Bear Mountain Orchards, Inc. Bear Mountain, PA,
john@bearmountaininc.net

What work do you do? Bear Mountain is in its 75th year as a family-run business located in Pennsylvania. We are growers and packers of apples, peaches, nectarines, cherries and plums. I oversee the production of our farming operations and also our packing and storage facilities. Also, I am a director of Knouse Foods Cooperative and on the Adams County Industrial Development Board.

Why are you interested in RosBREED? To stay competitive in today's worldwide marketplace requires investment in research. Collaboration is extremely important in order to be as efficient and effective as possible. RosBREED is dedicated to cutting-edge forward-looking research with a collaborative approach. RosBREED has the opportunity to take fruit growing to new levels. I want to be a part of that opportunity. We need better fruit for our customers, it may be flavor, storability, color or firmness. Growers need horticulture-friendly cultivars. Fruit is grown in micro-climates. I want to find the right fit of fruit for our growing regions.

How do you feel you can contribute to RosBREED? Communication is vital in any project. With my experience in growing and marketing, I hope to bring that knowledge to the researchers. At the same time I wish to learn of their concerns and problems. In the end I hope to get a better product for the consumer.

RosBREED by the number

336,099

Number of strawberry phenotypic data points analyzed for genotype x environment interactions and combining ability for 69 traits. Data was collected by Lise Mahoney (University of New Hampshire), Sonali Mookerjee and Kazim Gunduz (Michigan State University), Vance Whitaker (University of Florida), Megan Mathey (Oregon State University) and Phil Stewart (Driscoll's, CA). Data was analyzed by the Strawberry Team with assistance by Umesh Rosyara.



Photo courtesy of Sonali Mookerjee.

Socio-Economic Team's Apple Auction

By: Cholani Weebadde, Extension Team Leader

The RosBREED Socio-Economics Team has conducted several consumer studies to understand consumer preferences, quantified as marginal values of specific fruit quality traits. Given that breeders have a long list of traits to focus on in their breeding programs, such data can provide useful information to assist breeders better establish priorities among traits and trait levels. At the fourth annual meeting with Advisory Panels on January 10, 2013, the Socio-Economics Team showcased how one such study was conducted with consumers using an approach of sensory evaluation with a "second-price" auction.

Five promising apple varieties (one cultivar and four selections) that differed in appearance, sweetness, firmness, crispness, juiciness, and tartness were included in the demonstration. The apples were kindly provided by the Washington Tree Fruit Commission, the University of Minnesota Apple Breeding Program, and The Washington State University Apple Breeding Program. RosBREED Advisory Panel members and project participants served as the panelists for the demonstration.

At the start of the auction, Karina Gallardo from the Socio-Economics Team explained how the "second-price" auction works. Four "RosBREED dollars" specially designed for the auction were given to each panelist, who were asked to first look at the apples on offer and then place a bid for one of the displayed varieties. After bidding, the highest and the second highest bids for each apple sample were identified and displayed. The highest bidder won the auction but paid the price of the second-highest bid. A similar bidding process was conducted after each panelist tasted the apple varieties. One lucky winner took home a pound of scrumptious apples genetically superior in many ways thanks to the efforts of our Demonstration Breeders!

Overall, the Advisory Panel members enjoyed taking part in the auction and sensory evaluations. After the variety identities were unveiled, Jim Luby (Breeding Team Leader and Minnesota Apple Breeding Program breeder) concluded the session by displaying the pedigree relationships among the related varieties, describing their alleles for a certain genomic region (the *Ma* locus associated with tartness, crispness, and juiciness), and explaining how marker-assisted breeding is making a difference in supporting breeding decisions. Dr. Luby mentioned how he is using a DNA test for the *Ma* locus to make crosses expected to produce better tasting apples for the market in the coming years.



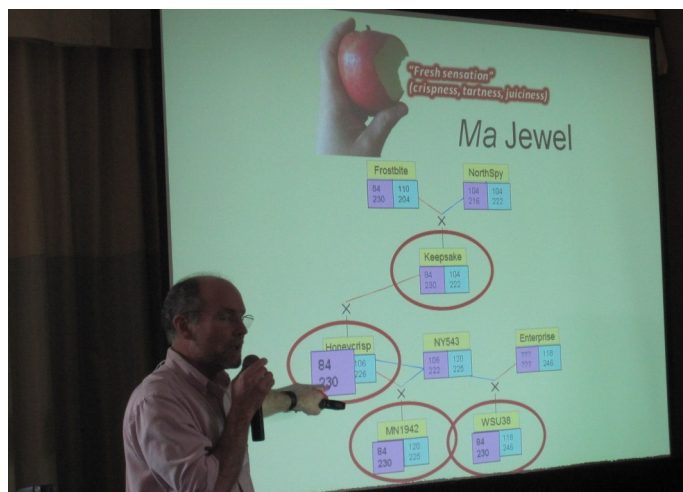
Karina Gallardo (Socio-Economics Team Leader, Washington State Univ.) explains the fun of a second-price auction.



Advisory Panel members inspect apples for visual attractiveness prior to placing their bids.



Kate Evans (apple Demonstration Breeder, Washington State Univ.) reveals the identities of the auction's apple varieties.



Jim Luby (Breeding Team Leader, Univ. of Minnesota) explains pedigree relationships and inheritance of a particular jewel in the genome.

Apple Breeder Workshop

By: Cholani Weebadde, Extension Team Leader

The *MAB in Action!* Apple Breeder Workshop took place in San Diego, CA on January 11, 2013 a day prior to the Plant & Animal Genome Conference. The 21 participants were apple breeders and students from U.S., Europe, New Zealand, and South Africa.

Following a brief introduction by Amy Iezzoni (RosBREED's Project Director), a recap was made by Cameron Peace (MAB Pipeline Team Leader) of the foundation laid in the *MAB in Action!* workshop held in Miami, FL in August 2012. RosBREED's three Demonstration Breeders, Jim Luby (Univ. of Minnesota), Kate Evans (Washington State Univ.), and Susan Brown (Cornell Univ.) then explained how each of them are using marker-assisted breeding to increase their breeding efficiency, accuracy, and creativity. Their presentations included descriptions of DNA tests currently used to select parents that would produce the highest proportion of high-performing seedlings for traits and combinations such as "fresh sensation," fruit storability, resistance to apple scab disease, and skin color. These DNA tests were developed by the RosBREED project and are currently available for the use of all apple breeders around the world.

Apple breeding trainees of the RosBREED project described their studies and breakthroughs, and received guidance on continued success. Allied scientists Kenong Xu (Cornell University) and David Chagne (Plant and Food Research, New Zealand) described their experiences with other MAB applications, DNA test development, and genomic studies in apple. Towards the end of the workshop, Cameron Peace led a discussion on application of the available DNA tests for apple and on polishing of DNA tests that are still "in the pipeline".

Overall, a flexible agenda engaged workshop participants in productive discussions about apple MAB application. Participating apple breeders, allied scientists, and students from around the world left with practical information about available DNA tests currently in use for supporting apple breeding decisions in crossing and seedling evaluation.



Photo courtesy of Jim McFerson, Washington Tree Fruit Commission.



Attendees of RosBREED's Apple Breeder Workshop, San Diego, CA.

Strawberry Breeder Workshop

By Jill Bushakra, Research Associate, Molecular Biology, USDA-ARS-NCGR, Corvallis, OR

RosBREED's *MAB in Action!* Strawberry Breeder Workshop was held at the USDA-ARS National Clonal Germplasm Repository and Horticultural Crop Research Unit in Corvallis, OR on January 31 and February 1, 2013. The workshop was attended by public and private strawberry breeders and allied scientists from the U.S., Canada, and Europe, as well as representatives from the strawberry industry, for a total of 33 attendees. The workshop focused on the progress that RosBREED researchers have made toward developing DNA tests for strawberry and the issues inherent in working with an octoploid crop.

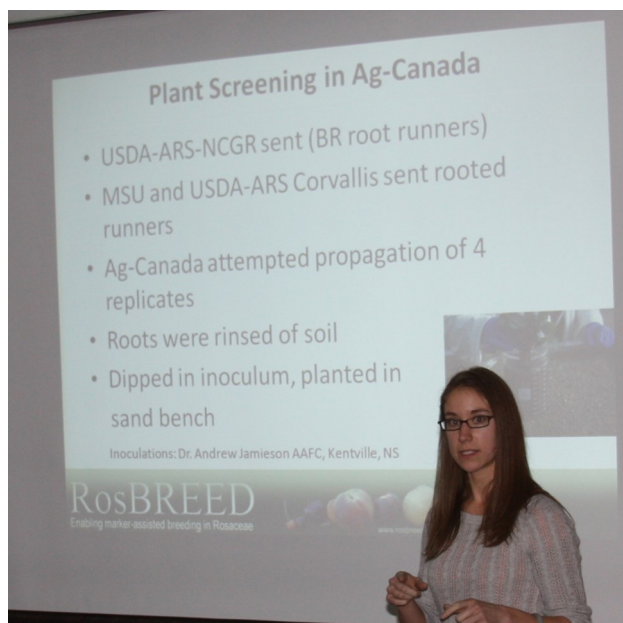
One of the goals of RosBREED is to accelerate the process from parent selection to cultivar release by combining marker and trait information. Currently for strawberry, parent selection to cultivar release can take from 6-12 years, including nursery propagation time. A diverse set of strawberry germplasm representing a broad array of genetics is being evaluated in multiple field locations across the country. This Strawberry Crop Reference Set includes 165 cultivars chosen from around the world and representing selections from the late 1700s to the present; 70 wild plants from North and South America (including the *Fragaria* supercore*); 39 elite breeding parents from six North American (U.S. and Canada) breeding programs; 340 seedlings of the Michigan State University (MSU) and Oregon State University (OSU) strawberry breeding programs; and 251 seedlings of mapping populations from The Netherlands, the U.K., Spain, France, and the U.S. The plan is to identify and validate jewels in the genome from this widely representative breeding germplasm to provide practical tools and knowledge for strawberry breeders. The phenotypic and genotypic data from this large study will also be collated and made accessible to all researchers. Data from the 2011 and 2012 seasons is already available in the Breeding Information Management System hosted on the Genome Database for Rosaceae (www.rosaceae.org/breeders_toolbox).

The Strawberry Crop Reference Set is being used to determine the genetics of and DNA test(s) for remontancy, also known as day-neutrality or perpetual or recurring flowering. Remontancy is a trait of value to strawberry breeders because expression of this trait can extend the strawberry growing season. One of the topics of discussion during the workshop was how to specifically define and measure remontancy, because expression of the trait can vary with temperature. Therefore, development of a robust DNA test for parental selection and seedling screening is of high priority. To date, one quantitative trait locus (QTL) has been identified for this trait. The next step to developing robust DNA markers is to gather data on the variation in expression in different individuals in different locations over various climatic conditions. Megan

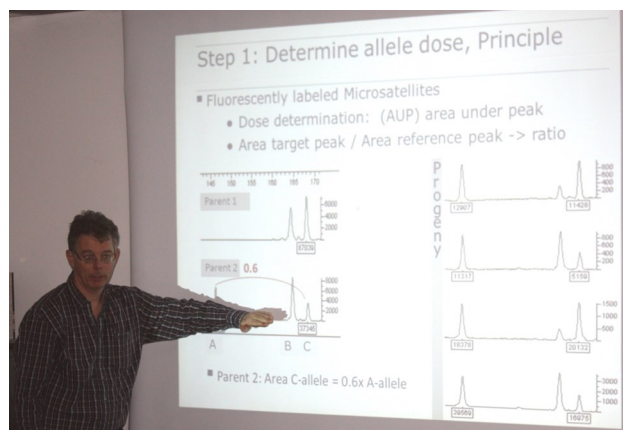
*The *Fragaria* Supercore, housed at NCGR, is composed of a diverse set of *F. virginiana* and *F. chiloensis* individuals that span their respective native geographic ranges.



Photo courtesy of Megan Mathey.



Megan Mathey of Oregon State Univ. presents her Master's thesis work on phenotyping the Strawberry Crop Reference Set.



Eric van de Weg of Plant Research International, The Netherlands, describes how to use the Microsatellite Allele Dosage Configuration Establishment approach to determine the source of an SSR allele.

Mathey (OSU), Master's student and "breeding trainee" with Chad Finn (USDA-ARS, Corvallis, OR) and Jim Hancock (MSU), presented the status of her work relating to this project.

Eric van de Weg of Plant Research International, The Netherlands, gave a very interesting presentation on how the octoploid genome of strawberry can be "diploidized" for marker evaluation. Strawberry is an allo-octoploid whose genome is divided into four sub-genomes that do not interact. However, the sub-genomes are homeologous, therefore markers can also be homeologous and occur on multiple sub-genomes within an individual. His group has developed a protocol, Microsatellite Allele Dosage Configuration Establishment (MADCE), that looks at SSR allele dosage to determine from which parent and from which sub-genome any allele has come. This method can make use of markers with skewed segregation ratios and has the potential to make use of many more markers and cover much more of the genome than using markers that just segregate 1:1. The octoploid complexity of cultivated strawberry must be resolved for marker-locus-trait associations to be made and DNA tests developed, and efforts so far have brought us a long way forward, coordinated across many institutions and experts.

Nahla Bassil (USDA-ARS, Corvallis, OR) discussed how the MADCE technique was used to fingerprint the Strawberry Crop Reference Set. During DNA fingerprinting, several inconsistencies were uncovered including finding that some clones used as parents for the breeding populations were not the same individuals at different locations. It was also discovered that some of the presumed parents for breeding populations were not correct. Therefore, MADCE can be used for paternity testing and identity confirmation and, in combination with pedigree analysis software, pedigree inconsistencies can be identified and fixed. Dr. Bassil also summarized a survey of companies providing DNA diagnostic services, describing types and costs of services. There is still much work to do in developing robust, reliable DNA markers especially for polyploid crops like strawberry.

The workshop ended with a discussion on the types of traits appropriate for marker development. Participants generally agreed that traits difficult to assess in the field or that take several generations to express would be most appropriate to target for marker development. In addition, a genome-wide genotyping approach was discussed as a possibly more cost-effective method for marker development because many traits, genome-wide, can theoretically be assessed at one time. Another point of discussion was the maintenance of the Strawberry Crop Reference Set, providing access to the plants and data collected from the plants, and creating a community resource. Jim Hancock suggested private companies form a collective to endow research on wild germplasm to maintain interest and improve introgression of new gene sources, especially disease resistances.



Nahla Bassil of USDA-ARS, Corvallis, OR is very excited about the use of Microsatellite Allele Dosage Configuration Establishment for fingerprinting and paternity testing in octoploid strawberry.



Chad Finn, USDA-ARS, Corvallis, OR, having fun with a great group of scientists.



Workshop attendees enjoy a brief break in the sun.

Community Breeders' Page

Deliverables of the RosBREED project for U.S Rosaceae breeders: Part one

By Cameron Peace, Kate Evans, Michael Coe, and Fred Bliss



deliverable [dih-liv-er-uh-buh l]:

A tangible or intangible object produced as a result of a project that is intended to be delivered to a customer

– www.dictionary.com, www.wikipedia.com

We're into the final stretch now for the RosBREED project, so it's time to deliver! Breeders are our primary stakeholders, and RosBREED's efforts are designed for your benefit. Although our ongoing progress is reported to our federal agency (National Institute of Food and Agriculture), to our enthusiastic Advisory Panels, to the scientific community through technical journal manuscripts, and to rosaceous crop industries, RosBREED will succeed only if breeders find our work useful enough to engage and apply in their own programs.

In recent decades, genomics and molecular genetics have promised to influence, **impact**, **REVOLUTIONIZE** horticultural breeding. RosBREED is the latest and largest research endeavor to attempt to enable marker-assisted breeding capacity for U.S. Rosaceae, taking advantage of huge advances in fundamental genomics resources that preceded it and that continue apace. Now, we must deliver.

This article begins a two-part focus on deliverables to U.S. Rosaceae breeders. While just a few examples are provided below to set the scene, just you wait till Part Two in May! Finally, in August 2013's article, the focus will shift to *Impacts*.

Deliverables

We've placed the various types of MAB-enabling deliverables from RosBREED into the categories of Knowledge, Tools, and Germplasm.

Knowledge: This category of deliverable is the most intangible yet probably the most profound ("teach a man how to fish..."). RosBREED's knowledge-based deliverables include expanded **professional networks**, understanding of useful **concepts** and removal of action-limiting misconceptions, new breeding **strategies**, new **protocols**, information on **plant identity**, **socio-economic information** about the value of traits, information on **trait genetics**, **pedigree** information, information on **genetic potential** of breeding germplasm, and **experience** using DNA markers in breeding programs.

Tools: This category includes more tangible kinds of deliverables: **DNA tests** (what we like to call "polished jewels") and **software** (to assist with complex analytical processes, helping to sift out valuable nuggets from the ore). Both are designed to make routine breeding efforts more efficient, accurate, and predictable, as well as to support your creativity by providing unprecedented access to jewels in the genome and jewels in the germplasm.

Germplasm: This is the most tangible group of deliverables: the material that breeders deal with every day and the product outputs that ultimately determine the success of breeding efforts. RosBREED's germplasm deliverables include access to new **gene pools**, new **parents**, new **progenies**, promising **selections**, and **cultivar releases**.

Below and in the next Newsletter we will describe specific examples of these deliverables, made available to breeding programs like yours.

Deliverable #1: Knowledge – parentage refuted and deduced for WSU C7 [coded]

To Whom: Kate Evans, pome fruit breeder, Washington State University

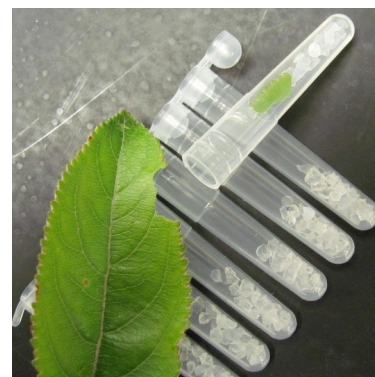
Story: The Washington Apple breeding Program (WABP), begun in 1994, uses scores of parents, has advanced more than a hundred numbered selections, and has generated many tens of thousands of seedlings. Controlled crosses are made, but not all pedigree records are correct, as contaminating pollen can sneak in and plant labels sometimes get mixed up. Selection WSU C7 was identified as having several excellent attributes after years of phenotypic evaluation and eventually advanced to "elite" status in 2010. But its parentage records of 'Hatsuaki' × 'Cripps Pink' were shown to be incorrect based on genotyping with two SSR markers at the Ma locus. In fact, the selection's allelic combination didn't match either recorded parent. But two alleles were a match with a specific rare haplotype (believed to partially contribute to excellent crispness, juiciness, and tartness) found in only one commonly used WABP parent and its offspring: 'Honeycrisp'. The other haplotype is a common one with many possible donors. WSU C7's parentage was updated to 'Honeycrisp' open-pollinated.

Community Breeders' Page cont.

Deliverable #2: Knowledge – “fresh sensation” genetic potential for new parents

To Whom: Kate Evans, pome fruit breeder, Washington State University

Story: The WABP's advanced selections are not only candidates for new cultivar releases, but also represent possible parents to help aggregate valuable alleles into the next generation of superior cultivars. Two SSRs at the Ma locus associated with “fresh sensation” traits of tartness, crispness, and juiciness were used to obtain predictive functional genotypes of WABP parent cultivars and selections. This SSR genotyping has already been used to identify several elite selections with valuable alleles to use as parents.



Deliverable #3: Knowledge – “fresh sensation” genotype for new cultivar, ‘WA 38’

To Whom: Kate Evans, pome fruit breeder, Washington State University

Story: The young WABP has now officially released three cultivars. The most recent, ‘WA 38’, was released in early 2012 and is currently being mass-propagated to provide commercial-scale planting material. ‘WA 38’ is exceptionally crisp and juicy all year round and has spritely tartness – a fresh sensation with every bite! This crispness and juiciness are believed to be due at least in part to a ‘Honeycrisp’ haplotype that ‘WA 38’ carries at the Ma locus inherited from its confirmed father, ‘Honeycrisp’. The expected orchard performance of this new cultivar is therefore partially predicted by DNA-based information from RosBREED – and soon the public will be able to enjoy the great taste of ‘WA 38’ while benefiting from the nutritional goodness of apples!



WA 38, photo courtesy of Bruce Barritt.

Deliverable #4: Tool – DNA test of “fresh sensation” for routine seedling screening

To Whom: Kate Evans, pome fruit breeder, Washington State University

Story: An SSR-based DNA test targeting the Ma locus helps predict trait levels for tartness, crispness, and juiciness. Prior to RosBREED, this was a crude tool. The test has since been refined using large-scale multi-location phenotypic evaluations conducted across three breeding programs, now in their third season, combined with SNP-based genome-scanning. The DNA test originally used two SSRs, while now we use just one. This SSR marker is easy to run and score and very cost-effective – vital features for screening thousands of WABP seedlings each year.

Deliverable #5: Germplasm – new families in breeding nursery with superior genetics

To Whom: Kate Evans, pome fruit breeder, Washington State University

Story: Following crossing and obtaining seed in 2011, more than 5000 germinated seedlings from seven families had their DNA extracted and were screened in 2012 with the DNA test described in Deliverable #4. The DNA-based technicalities took place in the Washington Tree Fruit Genotyping Lab in Pullman, WA. Selecting only those seedlings with superior “fresh sensation” genetics, Kate reduced the family sizes by more than half. About 2000 seedlings were then moved to a nursery where they'll be bud grafted in 2013 and then planted in the breeding orchard in 2015. The creation and crafting of these families were therefore guided with RosBREED's polished Ma locus DNA test.



Jewels in the Genome

By Amy Iezzoni, Project Director

What is a "Jewel in the Genome?"

- An individual's genome is the full complement of genetic information inherited from its parents. Within this vast repertoire of genetic information, individual genes are being discovered that control critical production and fruit quality traits. As valuable rosaceous gene discoveries are made and put into breeding applications, we describe them as "Jewels in the Genome."

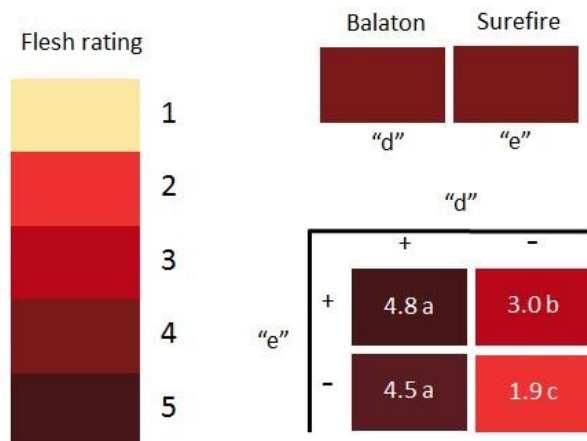
Tart cherry **skin and flesh color** varies widely from dark purple skin and flesh to orange skin and colorless flesh due to differences in anthocyanin pigmentation. Fruit flesh color variation is used to define market types with dark red-purple flesh types called "Morello" and clear fleshed types called "Amarello". As tart cherries are primarily used for processing, individual food manufacturers frequently have a color preference. The clear-fleshed 'Montmorency', the dominant cultivar in the U.S., is preferred for cherry pie, while dark-fleshed types are preferred in Europe for processed products such as juice, jam and compote. In apple and sweet cherry (and many other fruits), members of an anthocyanin-activating group of genes called MYB transcription factors control this variation for red skin and flesh color (Chagné et al., 2007; Espley et al., 2007; Sooriyapathirana et al., 2010). It was hypothesized that the same MYB gene may control fruit skin and flesh color in tart cherry because sweet cherry is a progenitor species of tart cherry. Addressing this hypothesis was complicated by the fact that tart cherry is genetically more complex than sweet cherry as it possesses twice as many chromosomes that pair somewhat erratically at meiosis. Therefore, unlike sweet cherry individuals where two alleles are present at the MYB locus, each tart cherry cultivar or seedling has four alleles at the MYB locus.



Clear fleshed cultivar, 'Montmorency', left, and a dark fleshed European cultivar, right.

Thirteen alleles of the MYB locus in tart cherry germplasm were defined based on 17 polymorphic DNA markers that span a ~7 cM region (T. Stegmeir, pers. comm.). Four of these alleles, observed in various combinations in breeding germplasm, were found to be predictive of increased pigmentation. In contrast, the clear-juiced 'Montmorency' was found not to possess any of these four dark color alleles. Most importantly, allele combinations associated with the fruit color desired by U.S. processors, i.e., bright red skin and flesh, were also identified.

Genetic knowledge of the MYB alleles allows the tart cherry breeder to discard seedlings predicted to have very dark fruit flesh color prior to incurring the expense of field planting. Therefore the seedling population in the breeding orchard will be enriched for individuals with the desired fruit flesh color.



Inheritance of red flesh color illustrated with progeny from the cross 'Balaton' × 'Surefire'. Left: Phenotypic scale used to rate flesh color. Top: 'Balaton' and 'Surefire' each have one copy of a MYB allele, termed allele "d" and "e", respectively, that contribute to dark red flesh color. Bottom right: Progeny mean flesh scores with the presence or absence of the "d" and "e" color alleles. Different letters following mean scores represent statistical differences ($P > 0.05$). Image courtesy of Travis Stegmeir.

This example illustrates how the common ancestry of rosaceous fruit crops can be used to accelerate discoveries across species boundaries. Furthermore, it demonstrates that the genetic tools developed in the RosBREED project (Peace et al., 2012) can be effectively used to dissect the genetic complexity of the MYB locus in this complicated polyploid. The duplicated MYB locus in tart cherry is chosen as the 13th "Jewel in the Genome" because knowledge of this region is leading to more efficient breeding of tart cherry.

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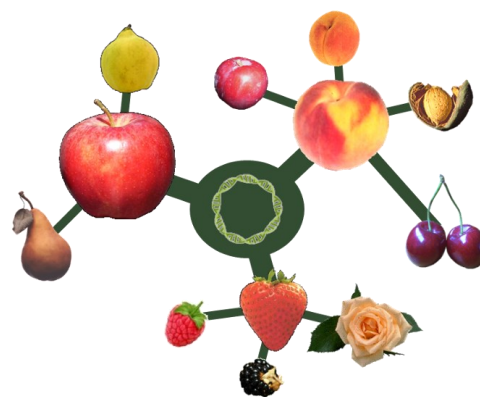
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RosBREED: Enabling marker-assisted breeding in Rosaceae

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Cameron Peace	Marker-assisted breeding pipeline	Washington State University
Eric van de Weg	Pedigree-based analysis	Plant Research International, The Netherlands
Cholani Weebadde	Extension	Michigan State University

Calendar of events

- **May 29-31, 2013. Fourth Annual RosBREED Project Participant Meeting, East Lansing, MI.**
- **July 22-25, 2013. American Society for Horticultural Science Conference, Palm Desert, CA.**