RosBREED

Enabling marker-assisted breeding in Rosaceae

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

September 30, 2013

Volume 4 Issue 3

v.rosbreed.org

Rosaceous crop breeding efficiency has been increased!

New apple, peach, cherry and strawberry cultivars with improved fruit quality will lead to increased consumer enjoyment and consumption, and will contribute to the profitability and sustainability of U.S. fruit production industries. However, breeding new fruit crop cultivars is a slow and inefficient process. It is hard to predict which selections will be the best parents; therefore, thousands of seedlings must be grown and evaluated in the field and fruit labs to identify the few with commercial potential.

DNA tests have been developed for traits of value for producers and consumers. For example, these DNA tests predict apple crispness, peach maturity date, cherry fruit size, and strawberry flowering time. These new genetic tests enable crop breeders to determine the best parents to combine and the best seedlings to advance, reducing the need to grow out and sort through thousands of seedlings with unacceptable fruit quality or harvest maturity. Using this strategy, called Marker-Assisted Breeding, the development of rosaceous crop cultivars that meet the desires of both consumers and producers is more efficient, accurate, and creative.

The series of articles in this Newsletter showcase some of the genetic tests developed, the science behind these genetic tests, their application in breeding programs, and the new cohort of young scientists trained to develop and put these DNA tests into application.

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Funding for this project is supported by:



Two major Rosaceae milestones have been accomplished!

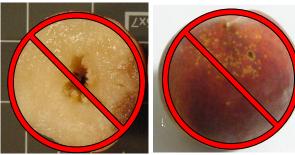


First peach SNP mini-arrays developed and tested

By Ksenija Gasic and Cameron Peace

Genetic tests were developed to predict peach fruit quality traits including fruit texture, flavor, size, and skin color. These new genetic tests enable peach breeders to determine the best parents to combine and the best seedlings to advance, thereby reducing the need to grow out and sort through thousands of seedlings with unacceptable fruit quality and other undesirable attributes. However, to implement these genetic tests, a cost-effective technology platform had to be designed and available service providers had to be involved to do the DNA tests.

The technology platforms developed in a RosBREED effort led by Cameron Peace, Marker-Assisted Breeding Team leader, were two SNP mini-arrays. These mini-arrays provide a DNA testing system for routinely predicting performance levels for certain traits of breeding program germplasm. Unlike the high-resolution 9K SNP array that scans the whole genome, the mini-arrays contain only the most informative sets of SNPs, the ones we have recently determined to be predictive for nine chosen traits. The two 24-SNP mini-arrays target the traits of maturity date, skin blush, nectarine vs. peach (*G* locus), fruit texture type (*F-M* locus), flesh color (Y locus), sweetness, acidity (*D* locus and another), mealiness, and fruit bacterial spot resistance. Our Fruit-Breedomics partners provided us with some of the SNPs; we shared some of ours.



Say good-bye to mealy peaches!

Say good-bye to bacterial spot!

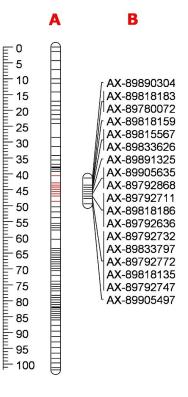
The service providers were two U.S.-based genotyping companies. The two mini-arrays were tested upon duplicated sets of 240 peach cultivars and selections from RosBREED's four demonstration peach breeding programs. We've received successful confirmation for one of the mini-arrays and are waiting for the second (so far so good). Examination of the raw results led to the conclusion that (1) the trait-targeted mini-arrays provide correct and easy-to-resolve trait predictions for most samples, but (2) due to wide haplotype diversity of U.S. peach breeding germplasm, a small proportion of germplasm requires special attention to assign haplotypes to correctly predict phenotype. Advantages and disadvantages and appropriate uses of the mini-array approach were previously described in the <u>November 2012 Community Breeders' Page</u> article.



First high density strawberry SNP linkage map constructed By Nahla Bassil and Eric van de Weg

SNP markers generated from the soon-to-be commercially available IStraw90® (for "International Strawberry 90K") strawberry SNP array were used by Eric van de Weg, Pedigree-Based Analysis Team Leader, to construct an initial high density map of the octoploid strawberry population 'Holiday' x 'Korona'.

The figure on the right is the first SNP-based genetic map for chromosome 5"A". This chromosome is resolved with 168 markers spanning 102 cM. Section A presents a schematic overview, of which the red marked region is detailed in section B to show that many markers map tightly together. Such strong co-localization underlines the robust scoring of these markers and the high resolution at which we can now track recombination events and dissect genetic variation for valuable traits. This robust genotype scoring was possible due to the development of a scoring mechanism for polyploid crops that was optimized through interactions between Eric van de Weg, the RosBREED strawberry team, and Affymetrix (click here for more details). Once this optimized genotyping mechanism is implemented, IStraw90® will be applied to the discovery of valuable marker-locus-trait associations in strawberry, their validation across breeding-relevant germplasm, and set the stage for marker-assisted breeding for accelerated release of superior new cultivars.



ASHS 2013 – RosBREED fruit quality QTLs: from publications to applications By Cholani Weebadde

Like many other graduate students interested in DNA-informed breeding, my training was in QTL discovery for breeder-focused traits (in strawberry), and at a time when easy-to-use marker types such as SSRs and SNPs were not available for the crop. At the time, I assumed that discovering a QTL is all it takes to then be able to conduct marker-assisted breeding (MAB). When I got involved with the RosBREED project in 2009, I started realizing that discovering a QTL is just the first step towards MAB. Over the past four years with RosBREED, I have learned to appreciate the amount of work after QTL discovery that is required to develop a genetic test that can be used for routine MAB applications.

Often the criticism about MAB is that there are not many success stories out there to justify the cost of the technology. I hear this A LOT especially from agencies funding work in developing countries, who often shy away from funding MAB research because QTL discoveries tend to end with publications (and no practical impacts!). Perhaps the issue is that not many researchers take the initiative to carry QTL discoveries all the way through to actual MAB to deliver impacts at the breeding end. Perhaps there are many researchers out there who still assume that QTL discovery is MAB. While we are very glad that USDA-NIFA supported RosBREED and other individual projects to take QTL discoveries all the way to MAB, something HAD TO BE DONE to give MAB its due respect at a critical time like this when successful plant breeding will impact food security.

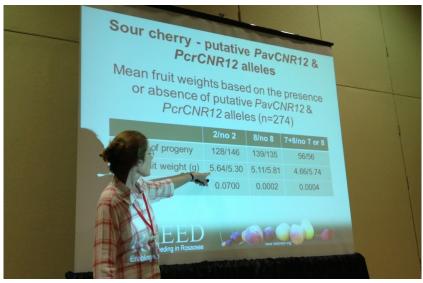
With this in mind, RosBREED organized a twohour workshop at the 2013 annual conference of the American Society for Horticultural Science (ASHS) in Palm Desert, CA to share MAB successes which take QTL discoveries from publication to actual application in breeding programs. The workshop addressed the steps needed for making a QTL discovery ("a bump on a chromosome") into a predictive genetic test for a given breeding program; from converting the marker type from the discovery research into a marker type amenable to routine use by the breeding program, validating



Cholani Weebadde, moderator for the presentation series, explains how RosBREED focused on bridging the chasm between genomics research and breeding programs.



Ksenija Gasic describes how socio-economics research results were used to guide choices of the peach QTLs on which to focus attention.



Amy lezzoni shows how QTL alleles were validated and a diagnostic DNA test developed for cherry fruit size.

the QTL in breeding germplasm, calculating the specific effects of available alleles, and finally to deploying the diagnostic genetic test that differentiates the functional alleles and ensuring a routine DNA testing service is available.

The presentation series, called "RosBREED Fruit Quality QTLs: from Publications to Applications," was sponsored by the ASHS Genetics and Germplasm Working Group. The Series included five talks and ended with a discussion session. About 75 Rosaceae breeders, allied scientists, academics, students, and industry leaders attended.

I opened the workshop with a presentation that emphasized the fact that discovering a QTL for a trait is just a stepping-stone into practical MAB, not a major milestone. This presentation explained RosBREED's "MAB Pipeline," the process we use to convert reported or newly discovered QTL information into routine genetic tests that can be used for selection in breeding. It also mentioned the collaborative efforts needed between genomicists, geneticists, and breeders.

Amy lezzoni (RosBREED's Project Director, at Michigan State University) then took the floor to describe what QTLs and QTL alleles are and how to validate them in breeding populations using cherry for her examples. Both Sujeet Verma (PhD student under Cameron Peace at Washington State University) and Ksenija Gasic (peach demonstration breeder, at Clem-

| QTL Validation Results Validated position of LG16Ma for acidity! |
|---|
| Posterior Intensity Posterior Intensity Di V W 4 10 10 0 0 0 0 Expected CG6Ma locus position Sensory acidity ather 10 wks storage |
| Evidence for bitter pit QTL there too Some, but less, evidence for crispness/juiciness |

Sujeet Verma points out a jewel for apple fruit acidity that he helped polish.

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Jim Luby highlights how DNA markers can be used to identify and correct parentage errors in breeding programs, in addition to their more commonly touted applications for trait performance prediction.

son University) followed Amy, describing QTL and QTL allele validation examples and principles from apple and peach, respectively. In her talk, Ksenija mentioned the development of two peach mini-arrays developed for breeders (see page 2 for further details) – great tools that will support decision-making for peach breeders using them.

Jim Luby (RosBREED's Breeding Team Leader and apple demonstration breeder, at the University of Minnesota) rounded out the talks by describing MAB application successes and lessons learned. His examples were from the University of Minnesota apple breeding program and from the Washington State University breeding program directed by Kate Evans (apple demonstration breeder). He showcased the estimated cost savings achieved over the last several years by implementing diagnostic DNA tests for valuable traits to increase the efficiency of breeding – which simply wowed the crowd.

Looking at the results achieved by RosBREED over the past four years, it is encouraging to see how the project has actually bridged the chasm from genomics discoveries to practical breeding application – a massive task undertaken by a multi-disciplinary team. It is also comforting to know that the MAB Pipeline approach is a model that can easily be adopted by any breeding program involving perennial and clonally propagated crops.

Volume 4 Issue 3 Page 5 International meetings

International Society of Horticultural Science (ISHS) Peach Symposium, June 17-20, 2013 Matera, Italy

Tom Gradziel and Ksenija Gasic (peach demonstration breeders at UC Davis and Clemson University, respectively), Greg Reighard (Extension Team member), and Jonathan Fresnedo (breeding trainee in the UC Davis program) attended the VIII annual ISHS Peach Symposium in Matera, Italy, June 17-20, 2013. Ksenija's presentation was titled, "Bacterial spot resistance in peach: Functional allele distribution in breeding germplasm". Jonathan's presentation was titled, "Pedigree correction and estimation of breeding values for peach genetic improvement." Both presentations were well received and increased understanding in the international research community of RosBREED's mission, approaches, and achievements.



Jonathan Fresnedo, Tom Gradziel, and Ksenija Gasic enjoy the beautiful weather in Italy during a break in presentations.

International Symposium of Fruit Tree Genomics, June 13, 2013, South Korea

Nahla Bassil was invited to the "International Symposium of Fruit Tree Genomics" by The National Institute of Horticultural & Herbal Science and The Agricultural Genome Center of Next Biogreen 21 Project, Rural Development Administration (RDA), in South Korea to present results of her research on molecular characterization of temperate fruit and nuts. Dr. Daeil Kim, a visiting scientist in her lab at the USDA-ARS National Clonal Germplasm Repository in 2012, organized Nahla's first visit to South Korea. Nahla visited Jeju Island where she toured the Agricultural Research Center for Climate Change and the Citrus Research Station and met with RDA and university researchers and presented her work on "Molecular characterization at the Corvallis genebank". Nahla also visited Chungbuk National University in Chongju and met with students and researchers and gave an overview of her work. In Suwon, she toured the National Institute of Horticultural and Herbal Sciences' National Agrobiodiversity Center and the floriculture breeding program.

The visit culminated at the International Symposium of Fruit Tree Genomics in Korea where she was one of four international keynote speakers presenting current status of tree fruit genomics and applications to breeding. Nahla thanked the organizers for hosting her and presented "RosBREED: How we are implementing MAB in Rosaceous crops" describing her work and that of collaborators on the RosBREED project.

The participants expressed strong interest in enhancing breeding efficiency using markers mentioned in the presentation and desire to interact with the RosBREED group.



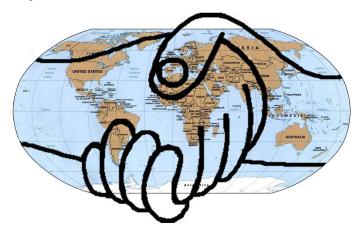
Nahla Bassil's explains, "RosBREED: How we are implementing MAB in Rosaceous crops."



International speakers, the Rural Development Administration (RDA), center directors, researchers, and organizers of the Symposium.

FruitBreedomics, Plant & Food Research, and RosBREED: collaboration, continuation, community By Cameron Peace

While RosBREED funding is coming to an end, the science behind marker-assisted breeding continues vigorously. <u>Fruit-Breedomics</u>, our sister project across the Atlantic, is powering along. That project, cited in the <u>Community Breeders</u> Page article of the February 2012 Newsletter, has just entered the second half of its 4.5-year course. And there is every reason to expect that the critical mass long since assembled at <u>Plant & Food Research</u> in New Zealand will sustain its significant contributions at all levels.



Channels of collaboration run deep. Cross-Atlantic and cross-Pacific partnerships in Rosaceae genomics, genetics, and breeding research have flourished for decades. Numerous projects leading up to as well as concurrent with RosBREED and FruitBreedomics have involved scientists from the three regions. Not coincidentally, scientists from all three regions helped craft the original two funded proposals. Both projects take advantage of Pedigree-Based Analysis that unites rosaceous genomics and breeding like never before. In fact, RosBREED and FruitBreedomics share the co-PIs of Eric van de Weg and Marco Bink. Similarly, Plant & Food Research scientists such as Sue Gardiner, David Chagné, and Satish Kumar are major contributors to fundamental advances in both projects.

Other tangible collaborations have included:

- Developing and testing of the apple, peach, and cherry SNP arrays for genome-scanning
- Exchanging trait-diagnostic SNPs: RosBREED particularly focuses on fruit quality while FruitBreedomics efforts also target disease resistance and Plant & Food Research has expertise in both; by sharing information we expand our impact

As RosBREED's research winds down and focus shifts fully to implementing DNA information in individual breeding programs, our long-time collaborators continue the necessary upstream science. We anticipate breakthroughs and subsequent downstream benefits for RosBREED stakeholders as FruitBreedomics and Plant & Food Research continue their efforts in such areas as development of higher resolution genome-scan arrays, discovery and characterization of more QTLs, physiological and genetic dissection of complex but valuable traits, trials of new technologies in DNA testing for trait-predictive markers, consideration of increasingly advanced approaches for DNA-informed breeding like genomic selection, and integration of DNA information with quantitative genetics principles.

A heartfelt thanks to all involved – for who you are and what you do individually as well as for the collective inspiring community spirit that arises and infuses Rosaceae genomics, genetics, and breeding science.



Participants of the FruitBreedomics 2nd Annual Meeting, Lleida, Spain, 18-21 February 2013. The next scientific advances in marker-assisted breeding lie in the actions of these great people.

RosBREED by the numbers

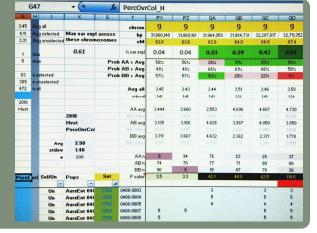
1 billion

Individual genotype-trait association effects being examined for statistical significance and breeding relevance by RosBREED's apple team, using spreadsheet tools to complement FlexQTL[™] analyses. The dataset consists of phenotypic data over three years for 40-55 fruit quality traits for each of three storage evaluation times. Performance levels are compared among AA, AB, and BB genotypes for >1600 trustworthy polymorphic SNPs for each of >900 individuals in the Apple Crop Reference and Breeding Pedigree Sets. All involved are getting square eyes.

27,930

Number of high quality polymorphic SNPs resulting from the IStraw90® strawberry SNP array that were used to construct the first high density linkage map of octoploid strawberry. This effort is a continuation of the cost-effective, high-throughput marker technologies developed in international RosBREED-led efforts for apple, peach, and cherry. These technologies were commercialized and made available to the scientific community in the U.S. and worldwide.

Number of apple QTLs populated in the <u>Trait Locus Warehouse</u>, which is an online tool on GDR (<u>www.rosaceae.org</u>) to facilitate access to public QTL information for your crop. Trait Locus Warehouse is the most "upstream" (reaching into genomics data) data-interfacing module for breeders developed by RosBREED's Breeding Information Management System Team to make routine marker-assisted breeding a reality for U.S. rosaceous crops.



H-02339

Korona

SNP/Probeset

2 AX-89778337 3 AX-89778338

4 AX-89778339

5 AX-89778340

6 AX-89778341

7 AX-89778342

8 AX-89778343

9 AX-89778344

10 AX-89778345

11 AX-89778346

12 AX-89778347

13 AX-89778348

14 AX-89778349

15 AX-89778350

18 0 indicates AA 19 1 indicates AB

20 -1 indicates missing

17 2 indicates BB Holiday

H-02379 H-02425 H-02476

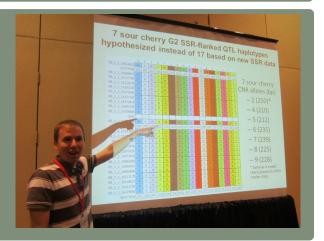
-1

-1

H-02519

| (112.4 eV) (112.4 eV) |
|--------------------------|
|--------------------------|

Number of talks and posters presented in several sessions at ASHS, July 22-25 in Palm Desert, CA. These included the five presentations of the RosBREED-hosted workshop of "RosBREED Fruit Quality QTLs: From Publications to Applications". Travis Stegmeir, right, explains his success in dissecting complex haplotypes in his presentation titled, "Enabling marker-assisted breeding in heterozygous polyploid species: The strategy used in sour cherry (Prunus cerasus)"



Raised by a village: the RosBREED breeding trainee experience By Jim Luby

The engine of RosBREED has been a cohort of 21 breeding trainees pursuing graduate degrees while embedded in demonstration breeding programs at universities around the U.S. The breeding trainees not only collected, analyzed and interpreted large data sets to advance marker-assisted breeding in Rosaceae fruit breeding programs (see page 13 in this issue), they were afforded unique graduate training opportunities as a national plant breeding student cohort with a common goal and a diversity of backgrounds and talents. Through four years of training sessions, "Facebooking", and communal strategizing on FlexQTL[™] software, the "RosBREEDlings", as they christened themselves, developed into a graduate community transcending their individual universities.

Recently, several breeding trainees who have been with the project from the beginning reflected on their RosBREED experiences and the opportunities that RosBREED afforded them beyond those available to other graduate students at their universities.

Networking: Every trainee recognized network building as perhaps the most important advantage of RosBREED, especially the chance to network with each other. Matt Clark, who is completing his PhD dissertation at the University of Minnesota, noted that "...the (RosBREED) graduate students developed a professional and personal network that was necessary for executing the project objectives from experimental design, through data collection, and ultimately in data analysis with developing software".



RosBREEDlings enjoying dinner after a day of software training.

Cari Schmitz, a recent Masters of Science (MS) grad-

uate from the University of Minnesota, appreciated the chance to network with RosBREED co-project directors from across the U.S. and several other countries. She felt "...the support of the other RosBREEDlings was especially useful because we had similar tools, projects and frustrations—this also meant it was easy to find friends and roommates at conferences and there was no shortage of people to rehearse talks with last minute".

Jonathan Fresnedo, a PhD student from Mexico embedded in the peach breeding program at the University of California-Davis, reflected that "...in RosBREED we are a group of people working in the same or similar species, and the diversity of experiences that we share is invaluable for the development of skills that trainees aim to acquire to master the art of breeding".

Travis Stegmeir, who recently completed his PhD in the tart cherry breeding program at Michigan State University, reflected that "...these are going to be many of the same people we will go on to be colleagues with for the rest of our careers, and we all have a similar background now with this experience where we can all relate to each other".

Support and Training Opportunities: Trainees recognized that RosBREED offered them some unique support and travel and training opportunities relative to their graduate students.

Paul Sandefur is a "double-degree" RosBREED trainee. Having completed his MS with the University of Arkansas peach breeding program, he is now pursuing a PhD with RosBREED Project Co-Director Cameron Peace at Washington State University. "I really appreciated that RosBREED provided me with data," Sandefur explained. "When working with perennial fruit crops, it can be hard to acquire enough good data to do the studies we want to do. But, thanks to all the universities/scientists working together, large, powerful data sets were 'ripe for the picking'".

Cari Schmitz felt that RosBREED offered her "...far more travel opportunities for collaboration and training exposure and access to a greater variety of software tools, and training..." compared to her graduate student peers at the University of Minnesota.

Sujeet Verma, PhD candidate at Washington State University, valued that RosBREED "...provided an open opportunity to communicate and discuss my research problems with any of our world wide project collaborators. I could go to sleep after sending my questions via email to a relevant group of scientists and wake up with several possible solutions".

Jonathan Fresnedo appreciated that the RosBREED training workshops offered some of the "few times that a graduate student has the opportunity to interact with students and breeders pursuing and collaborating for similar goals".

Several trainees highlighted particularly valuable experiences and internships that they had with private sector Rosaceae community breeders and other collaborators. Fresnedo appreciated "...the opportunity to attend the meetings with private breeders and get to know the perspective of people breeding out of academia, which sometimes is difficult to obtain".

Terrence Frett, who has worked with two demonstration peach breeding programs while earning his MS at Clemson University and is currently pursuing a PhD at the University of Arkansas, felt the "...highlights of my graduate career were training opportunities I obtained because of RosBREED, both as an intern in the breeding program at Sun World International and as an intern for Dr. Rodrigo Infante's stone fruit breeding program in Chile".

Megan Mathey, trainee with the USDA-ARS strawberry breeding program in Corvallis, OR, had the opportunity to collaborate with private sector breeders in California and Europe. Mathey recently completed her MS from Oregon State University and is now a private sector breeder in Michigan. Cari Schmitz is also interning in Europe with a private sector sugar beet breeding company on a Congress-Bundestag Fellowship.

Perspective: RosBREED trainees cited the broader perspectives they gained from participating in a large, multifaceted project. Terrence Frett explained that he realized he "...was an integral part of a large project from start to finish which has allowed me to first hand understand how a project of this size is undertaken in terms of all the steps involved from start to finish. We have learned how to take high quality standardized phenotypic data, how to work with the genotypic data, how to work with Pedimap and FlexQTL[™] programs, and perform important data analysis steps such as haplotyping after QTLs have been located to enable MAB for Rosaceae species. When we pursue our future careers we will bring the experience of already being a part of a large project, all the contacts we have made, and the molecular and traditional breeding knowledge and techniques we have learned throughout the RosBREED project".

Sujeet Verma and Travis Stegmeir commented on the value of being exposed to more than just the crop they were working with, so they gained an understanding of what is important and going on in several different crops.

Jonathan Fresnedo explained, "A trainee is not just aware about what is happening in and around the breeding program in which he is developing skills and pursuing research. The breeding trainee in RosBREED also needs to know and contrast what is occurring in



Networking



Support and training opportunities



the other breeding programs for the same species that she or he is working with, or even for different species. This is pursued through the interaction with the breeding trainees first, and eventually with the demonstration breeders."

Beyond the other breeding programs, he recognized that "...the interactions with the RosBREED socio-economics group and the informatics core are very useful to introduce to the trainees the front-end tools that are being developed for the breeding activities".

In addition to learning about other crops, Cari Schmitz "...valued the chance to feel out how the graduate programs are conducted at other universities, of interest to those of us who plan to start a doctorate later or will look for a postdoctoral position at another school". Indeed, Paul Sandefur explained, "Without RosBREED, I would not have met Cameron (Peace) and had the opportunity to finish my graduate work at WSU. I also would not have met the other breeding trainees who I now work with on projects and will likely work with later in my career".

Schmitz tidily summarized the RosBREED breeder trainee experienced as "...far more opportunities for collaboration and training... and the feeling of being raised by a village!"

Breeder trainees – maybe the biggest impact of RosBREED?

By John Clark

Some years back as I contemplated being a part of the RosBREED project, an attractive part of the proposal was for funding of graduate students. My first thought was about like anyone's – program support in any form is good, a graduate student, well that would be a great bonus. Program support plus the graduate student doing a substantial amount of project work appeared to be a good idea. The term "breeder trainee" caught my eye, sounded good. Let me tell you, as we complete the RosBREED project, this aspect of the project has been more than good, it has been outstanding. I can only comment at this point on the impact of the breeder trainees on the project, but really this is all about an investment in the future, and this investment will pay tremendous returns to plant breeding in the future as the careers of the trainees unfold and their impacts are made. Although not fully funded by RosBREED, I have had the honor of advising and working with three trainees, Paul Sandefur (MS), Alejandra Salgado (PhD) and Terrence Frett (PhD) (pictured on page 11). Advising these students has been fun and fulfilling. And, the trainees have been key components of applying marker -assisted breeding in the Arkansas peach breeding program. In fact, without their energy, knowledge, enthusiasm, and commitment, the program would not be using MAS actively. That sounds like success to me, an idea manifested into results with long-term dividends. And, I cannot describe the excitement and fulfillment of working with these three scholars. This experience changed their lives, and changed mine too. That sounds like impact, all the way around. When one talks about "letting the good times roll", this sure appears to be a good example of it.

A breeding trainee's journey

By Terrence Frett

I have been blessed as a graduate student to be a part of RosBREED. This group supported my MS under Dr. Ksenija Gasic at Clemson University. During my time with the group, I was able to meet the most important fruit breeders in the USA and beyond as well as several fellow fruit breeding graduate students (future breeding colleagues!). I have been grateful to have had the chance to visit with each of the RosBREED peach breeders and see their material first hand.

I know I can speak for all the RosBREEDlings in saying that the opportunities RosBREED and all its members have given to us have been extraordinary. We all would not be where we are as professionals and life in general if it wasn't for RosBREED. It has been amazing to be a part of this group from day one and help in its growth. We 100% support the funding for RosBREED II so this group can continue to bloom and fruit.

Here are some pictures from my time as a part of RosBREED since 2009. Currently I am working for Dr. John Clark at the University of Arkansas in Fayetteville, AR.



graduated from Clemson in May 2012. MS Thesis: Frett, T.J. 2012. Marker-Assisted Breeding (MAB) for blush in peach (Prunus persica (L.) Batsch.



I visited the breeding pragmas of Drs. Byrne (left) and Gradziel, RosBREED peach demonstration breeders.



My internship experience with Sun World Intl. surpassed my expectations; they helped me understand the differences between public and private fruit breeding and production. Many thanks to Terry Bacon, who was an excellent mentor, for making this experience possible.



Thanks goes out to Dr. Greg Reighard for putting me into contact with Dr. Rodrigo Infante for my University of Chile and private industry consortium stone fruit breeding internship. I was exposed to the dynamic South American stone fruit industry. Dr. Rodrigo Infante was a very exciting professor to work with and a passionate breeder. I took a lot away from my time with him in the field and laboratory.

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RosBREED Graduate Student Community



Lilian Carrillo-Rodriguez PhD, WSU

Sonali Mookerjee

PhD, Mich. State Univ.



Terrence Frett MS, Clemson Univ. (graduated) PhD, Univ. of Ark. (current)





Richie Ragas MS, UC Davis





Matt Clark

PhD, Univ. of Minn.

Yingzi Li PhD, WSU



Natalia Salinas MS, Oregon State Univ.





Jonathan Fresnedo PhD, UC Davis



PhD, Univ. of NH



Sujeet Verma PhD, WSU



Ockert Greyvenstein

PhD. TAMU

Paul Sandefur MS, Univ. of Ark. (graduated) PhD, WSU (current)



Yingzhu Guan PhD, WSU



Ben Orcheski PhD, Cornell Univ.



Kazim Gunduz

PhD, Turkey

Cari Schmitz MS, Univ. of Minn.



Julia Harshman PhD, WSU



Alicia Rhin PhD. Univ. of Minn.



Yunyang Zhao PhD, WSU



Tim Hartmann MS, TAMU.



Travis Stegmeir PhD, Mich. State Univ.



Dinkar Kuchibhotla PhD. WSU



Sushan Ru PhD, WSU



Shuoli Zhao MS, Univ. of Minn.



Megan Mathey MS, Oregon State Univ.



Jong Choi PhD, Univ. of Minn.



Huixin Li PhD, Univ. of Minn.



Alejandra Salgado PhD, Univ. of Ark.



Xibei Zheng MS, Univ. of Minn.

To read more about these graduate students, including their advisors and thesis topics, click here

Congratulations! Four RosBREED trainees recently graduated

Lilian Carrillo-Rodriguez, Washington State University, received her PhD in August 2013. She was advised by Dr. Karina Gallardo.

Right after graduation I returned to my home country, Colombia. I have a position at Universidad Autónoma de Occidente as a professor of Economics and I am currently teaching Fundamentals of Economics.

Working for RosBREED was a blast for me. It was a great experience to be part of the Socio-Economics Team and also it was very interesting to collaborate with scientists from different fields. I would like to thank the RosBREED project and especially Karina Gallardo for giving me the opportunity to work on this wonderful project.



Ron Mittelhammer, Lilian Carrillo-Rodriguez, Jill McCluskey, and Karina Gallardo

Megan Mathey, Oregon State University, received her Masters in Science in June 2013. She was co-advised by Drs. Chad Finn and Nahla Bassil.

I recently became the ornamental plant breeder at Spring Meadow Nursery in Grand Haven, MI working with a multitude of flowering ornamental shrubs.

I believe that having had the opportunity to, through RosBREED, balance both lab and field work, develop phenotyping standards, and work with cutting edge technology for a plant breeding program set me apart from other job candidates. I would like to also thank RosBREED for giving me the opportunity to learn new techniques to aid in traditional breeding. I know the skills learned will help me throughout my career.



Nahla Bassil, Megan Mathey, and Chad Finn

Cari Schmitz, University of Minnesota, received her Masters in Science in July 2013. She was co-advised by Drs. Jim Luby and Jim Bradeen.

I am currently living in Germany as a participant in the Congress-Bundestag Youth Exchange for Young Professionals. I am nearing the end of the first of two months of language classes in Cologne and will attend Georg-August-Universität in Göttingen this fall. I'll cap the year off with a sugar beet and cereals breeding internship in Einbeck, about which I am very excited! When I return to the United States in July 2014, I hope to start working on a PhD in plant breeding and genetics.

I am incredibly grateful for the experiences I've had working with the RosBREED group and I wish you all the best.



Cindy Tong, Jim Luby, Cari Schmitz, and Jim Bradeen

Travis Stegmeir, Michigan State University, received his PhD August in 2013. He was advised by Dr. Amy lezzoni.

I am currently working for Amy lezzoni to assist with the 2013 phenotypic and genotypic data. I am applying for fruit breeding positions in the private sector.

Being a part of RosBREED was an experience I will never forget. It was great working with other students and breeders within the Rosaceae family, as by collaborating in this way, we were able to accomplish and learn much more than if we were only working within our respective crops.



Travis Stegmeir and Amy lezzoni

QTL discovery, validation, and application – efforts of demonstration breeders and breeding trainees By Jim Luby

For RosBREED demonstration breeders, routinely using iewels in the genome in our breeding programs for parent and seedling selection is the goal. Getting there is the grind. Long before you can wear the jewels, you have to identify promising rock formations and mine the ore. Breeders also needed the right tools developed by RosBREED's various teams, and, perhaps most importantly, you need some good miners and jewelers. Going back to 2009-10, RosBREED demonstration breeders had to identify the promising formations-what was their most important germplasm and which traits did they consider most important? These became the targets for three years of phenotyping. The RosBREED Socio-Economics Team gathered and compiled critical information by surveying not only the demonstration breeders, but also other breeders in the Rosaceae community concerning high value traits. For each RosBREED crop, these target traits were identified and phenotyping protocols were developed to efficiently collect the useful data-the high value ore! We had to have some miners to truck, sort, and polish this ore, so more than a dozen RosBREED trainees were recruited. These trainees dug the ore by harvesting tens of thousands of fruits and collecting hundreds of thousands of data points each season (you've seen it in RosBREED By the Numbers!). They also collected leaf samples for DNA extraction by the Genotyping Team to obtain millions of DNA data points using newly developed SNP arrays from the Genomics Team. Nevertheless, a big pile of ore still isn't worth much.

Now RosBREED trainees had to shuck their miner's helmets, put on their jewelers visors, and start sorting. First, quality checking of data: Each phenotype data point had to be reviewed for errors in measurement or data entry. Each genotype data point had to be reviewed for consistency. Each individual in the RosBREED Crop Reference germplasm sets had to be confirmed for identity and verified for ancestry. With the ore pile carefully examined, trainees could turn to their new facility with ore sorting tools, software such as FlexQTL[™], Pedimap, and R, gained through annual workshops and regular consultation with the Pedigree-Based Analysis Team. They sifted through all the data ore with these tools to find the sparkling rocks-markerlocus-trait associations-on various chromosomes of fruit crop genomes. Now the breeders and the Marker-Assisted Breeding Pipeline Team had some rough gems to polish! Simple, high-throughput markers were devised and tested by the breeders in their breeding programs in 2012 and 2013. Could the markers be reliably reproduced in the lab? Would they consistently relate to phenotype in the breeder's germplasm?

In each RosBREED crop, one or two handfuls of jewels with really shiny facets are available, and demonstration breed-

Seedling Selection: Genotype Data Report

Culling decision for each seedling combines all genetic tests



Seedling Selection: Culling



 Verbal repetition of seedling location and culling instruction to reduce errors

- Usually best with three people
- Culling requires 2-3 person/hours per 1000 seedlings



After DNA diagnostic tests were conducted, Ken Mullin and David Bedford (University of Minnesota) happily tossed out seedlings that do not have any shiny jewels!

ers can now routinely employ them to assist with selecting parents for crosses and culling seedlings. Yet certainly not all the jewels in these genomes have been picked from that massive ore pile. Verified phenotypic and genotypic data sets for the Crop Reference germplasm sets were delivered by the Breeding and Genotyping Teams to the Breeding Information Management System Team for uploading to the Genome Database for Rosaceae at <u>www.rosaceae.org</u>. There, the RosBREED ore pile and toolbox awaits breeders, allied scientists and a new generation of graduate student trainees in the community ready to hunt for and polish new jewels. Grab your rock hammers and start polishing stones!

IMPACTS of the RosBREED project for U.S. Rosaceae breeders

By Cameron Peace

"What has been the impact?" As I stepped off the plane at Northwest Regional Airport in Fayetteville, AR on 8 July, I reflected on the last time I came to visit Dr. John Clark's Arkansas peach (and nectarine) breeding program. On a similar day in 2008, five years earlier almost to the day, I had come to see John's fruit firsthand, having met John several years earlier when he had visited Dr. Carlos Crisosto's postharvest and peach genetics program at Kearney Agricultural Center, UC Davis, where I was a post-doctoral associate enjoying Carlos' mentoring. We had been in touch ever since that California meeting, including me getting up close and personal with the DNA of some of his material by running the endoPG DNA test for fruit texture type on some of his promising selections and one distinctly unusual family. On that Arkansas trip in 2008 I was accompanied by my good mate Dr. Jim Olmstead, blueberry breeder at the University of Florida since 2009 but at the time an Area Extension Educator at WSU and co-conspirator for the first incarnation of RosBREED. On the flight over, we came up with the project's short name, "RosBREED". For this planned project to truly have impact, we believed that breeding must be the crux.

"We need you to be part of this, John!" implored Jim and I as we gathered on the first night of our visit in July 2008 over pizza and beers. What became known as demonstration breeders were the nucleus of our plan. Recruiting John was critical. Back in Washington we were on the verge of delivering routine MAB to WSU's apple and sweet cherry breeding programs. There we had a critical mass of university resources and expertise (which included the breeders Nnadozie Oraguzie joining in May 2008 and Kate Evans in October 2008) and local industry support. The probable successes in enabling these two WSU programs made them obvious RosBREED inclusions. But John's protests about his lack of experience or expertise in MAB were our exact arguments for his inclusion. If MAB could be integrated into John's modest-sized traditional program, it could be done in any. And besides this bigger-picture consideration, John's participation was expected to lead to benefits flowing into the Arkansas peach breeding program.

"What unusual combinations!" was my reaction in 2008 to an Arkansas progeny of typical size (n=49) segregating for just about every Mendelian peach fruit trait known to man: yellow/white, peach/nectarine, low/normal acid, and round/flat shape, as well as the intriguing observation of slow-melting in the fruit of some seedlings. While I'd seen peach families with more diverse parents and DNA profiles because they were introgression lines seeking to incorporate alleles from other species, this Arkansas family was one from which new cultivars might directly arise. John's approach to peach breeding was encapsulated in this family where mixing alleles in all possible combinations might just lead to some exciting new fruit types for new markets. This family became the cornerstone of the contribution from John's program to RosBREED's peach germplasm set.

"Delicious!" Sampling (alright, gorging on) fruit from selections in John's breeding orchard in 2008 was a real treat - the kind of treat that every fruit consumer should experience every time they eat rosaceous and other specialty crop products. To my palate, the low-acid and exceptionally sweet delicacies offered up by several selections were the standouts. Evidently, the bees agreed (Figure 1). Very sweet - with that breeding-addressable fruit quality attribute in particular, I think fruit has a viable chance to increase its contribution to the healthy diets of our children. I applaud the strategic efforts of breeders like Mike Malone (Plant and Food Research [PFR], New Zealand, retiring) and Dave Byrne (Texas A&M and RosBREED demonstration breeder) in targeting the development of super-sweet peaches (SSC of well over 20 [°]Brix) and the dedication of breeding-support geneticists such as Emily Buck (PFR, New Zealand) and Yingzhu Guan (WSU, RosBREED apple breeding trainee) for tackling the recalcitrant trait of "sweetness". The U.S. breeding-production-marketing -consumption continuum allocates high socio-economic value to sweetness, and although at least a certain level of sweetness is ubiguitously targeted by peach breeders, its typically low heritability means that traditional selection only vaguely achieves target levels. This genetic aspect of sweetness is the very thing that would



Fig 1. *Very sweet* fruit selection from John Clark's University of Arkansas breeding program.



Fig 2. 'Bowden' nectarine. Photo credit University of Arkansas.

mean high impact for the breeding use of DNA information on it. The texture of some of John's selections was also memorable – smooth, buttery, surrendering sweet juice to each bite only as desired. My teeth felt like they were easing into a bed of silk sheets. I took home a tray of my favorites and one particular non-melting nectarine selection softened gradually to give my mouth a melting-like caress even after two weeks at room temperature – an excellent trait for the end-consumer! In 2013, John has released that selection as <u>'Bowden'</u> (Figure 2).

"You could transfer the MAB approaches to your other fruit breeding programs," Jim and I suggested to John, seeking to close the deal in 2008. Transferability of scientific advances among and beyond Rosaceae crops has long

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been a hallmark of genomics and genetics of this model plant family. The shared ancestry provides opportunities to transfer DNA information directly, such as the genomic region controlling fruit skin and flesh redness. The large industries and research funding support for these most economically important and diverse temperate crops makes them the most advanced in scientific understanding among perennial fruit crops. While it would be John's peach program under the RosBREED umbrella, his blackberry and grape breeding programs also stood to gain, beneficiaries of the "demonstration" concept. Coincidentally, I met JD Swanson, Rubus geneticist and in 2014 the Chair of <u>RosEXEC</u>, on this same 2008 trip, among John's seedling rows. JD is helping ensure RosBREED's advances are indeed being transferred to other Rosaceae crops.

"Y'all convinced me. I'm in! But I'll need help," John agreed, as we shook hands and wrapped up the 2008 visit, me with tray of peaches under arm. I assured John I'd stick with him and not only because of the sticky peach juice all over my outstretched hand. On the flight out, Jim and I devised RosBREED's byline. "Enabling" means we are not just touting the promise but providing the instructions for implementation (as mentioned in this column in May 2011), and one of the critical elements has proven to be provision of MAB expertise for individual programs. RosBREED's 12 demonstration breeding programs have received this support over the last few years in group settings as well as on individual bases to address program idiosyncrasies and facilitate MAB capability and execution. RosBREED's future impact will surely depend on how well such support is provided to any and all breeding programs.

"I don't have the time to figure it all out myself." RosBREED's approach has always been to integrate new DNA tools and knowledge into ongoing breeding operations, not to distract breeders detrimentally from what they already must do and do well. One solution is to train the next generation of fruit breeders and allied scientists in the skills, experiences, knowledge, and specialties required for the new millennium (tip of the hat to Fred Bliss). RosBREED provided direct support to demonstration breeding programs in the form of graduate student assistantships - which supported breeding trainees (as the project called them) or RosBREEDlings (as they called themselves). Many programs, like John's, have leveraged that support by taking on further students to also join RosBREED. Or in the case of Tom Davis (University of New Hampshire, strawberry demonstration breeder), by completely funding their student and enjoying full participation in project activities and opportunities. The first Arkansas breeding trainee was Masters student Paul Sandefur, who graduated in December 2011. Paul then continued on perfecting peaches in Arkansas, working for John through 2012. There he implemented some ideas he'd developed in his thesis project, and gained further experience with blackberry and grape breeding. Paul has now begun his PhD studies with me at WSU from January 2013, where he'll get familiar with even more fruit breeding programs and of course with the crucial translation-to-practice of DNA information. In 2012, new PhD student Alejandra Salgado joined John's program, solidifying the connection with blackberry. Then came Terrence Frett, graduating with a Masters in 2012 as breeding trainee with Ksenija Gasic (Clemson University, peach demonstration breeder), now another PhD student of John's who's helping to bring new MAB knowledge and tools to application. All three are great examples of smart, enthusiastic, hard-working, and solutionminded soon-to-be professional breeders or breeding-support allied scientists who learned about both traditional and DNA-informed approaches under the RosBREED umbrella. In fact, I think that training of the next generation of fruit breeders will be RosBREED's most far-reaching impact.

Fast forward five years and I've again thoroughly enjoyed the fruits of John's continued innovative labors. What has changed for the Arkansas peach breeding program since John committed a portion of his time and joined the RosBREED family? I asked John straight out, "What has been the impact?"

"RosBREED provided me the incentive and pathway for doing marker-assisted breeding," says John. Being a demonstration breeder provided the activation energy to begin, to start along the path, and continued involvement showed him where to walk. RosBREED allowed John to come into close contact with knowledge and procedures for incorporating DNA information in breeding decisions. A key part, adds John, was the role of the breeding trainees – given that John himself didn't have the time to do all that needed to be done. And he took on the students in the first place because of the RosBREED participation – the project gave the connection to the DNA world that John believes "new students need these days."

Beyond his own program, John considers the positive impact that his program's involvement in RosBREED and conducting of MAB has had on his Department – expanding its scope, influencing new hires, and attracting more students. For his peach breeding efforts, John states that the DNA information is "throwing the curtain back" on his germplasm. For most selections he feels he knows about their genetic potential as new cultivar candidates or as new parents. "But I don't know them all, and I don't know future ones."

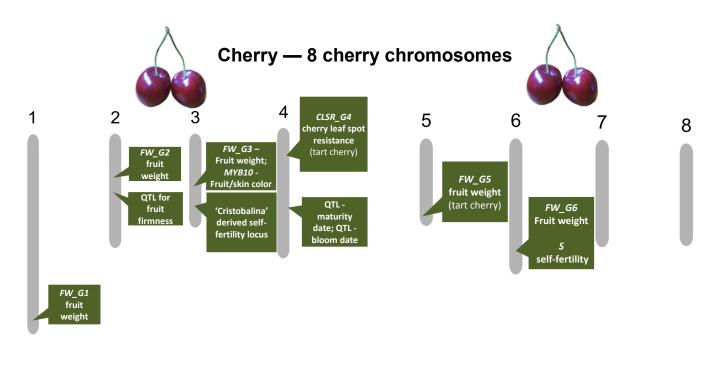
A cadre of current and future breeding professionals have been informed and equipped. Paths have been blazed and trodden. Jewels have been found and polished. More and more of RosBREED's <u>deliverables</u> will undoubtedly convert to impact for Rosaceae crop breeding and beyond. Of course, this is not the end of the MAB story – most of RosBREED's participants will continue to carry the torch and deliver positive change, and we hope and expect you community breeders will continue to benefit.

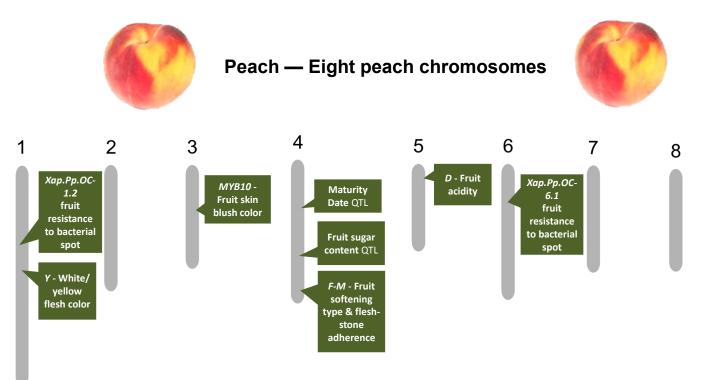
It's astounding how far we've come, and the future looks bright. But for now, John Clark revels in the present. "My inspiration is as high as it's ever been. I've hit my peak – but it's a high mountain. I think I'll sit here a while."

Jewels in the genome: The Necklace

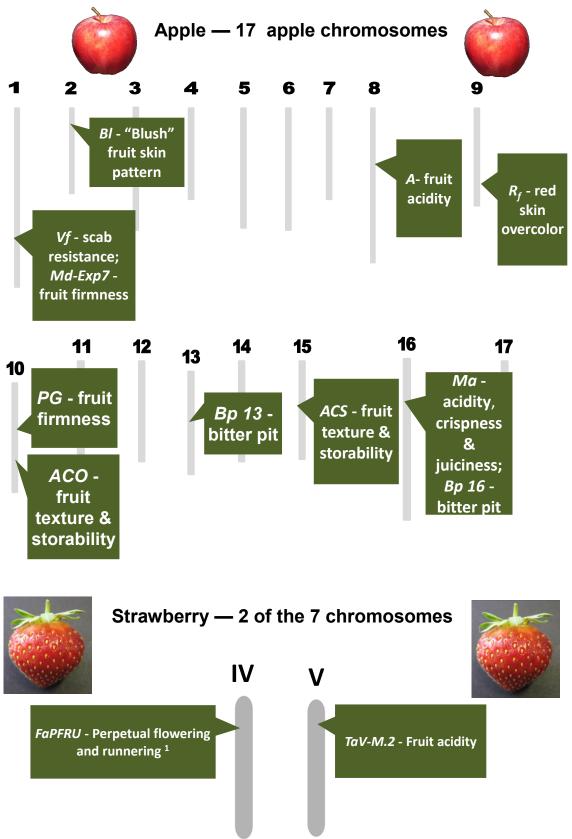
By Amy lezzoni, Project Director

"Jewel in the Genome" is a phrase used in RosBREED to identify valuable rosaceous genetic discoveries that have been put into breeding application. In this final article, the placement of these jewels is illustrated on the crop chromosomes collectively illustrating the growing genetic "necklace". Knowledge of these jewels and their locations relative to each other is being used to increase the efficiency of rosaceous crop breeding. Putting these genetic discoveries into application has been one of the hallmark outcomes of RosBREED.





Jewels in the genome: The Necklace, cont.



¹ Gaston A, Perrotte J, Lerceteau-Köhler E, Rousseau-Gueutin M, Petit A, Hernould M, Rothan C, Denoyes B. 2013. PFRU, a single dominant locus regulate the balance between sexual and asexual plant reproduction in cultivated strawberry. J Expt. Bot. doi:10.1993/jxb/ert047.

Need statistical help? Just contact RosBREED's post-doctoral associate Umesh Rosyara By Amy lezzoni

How is a "jewel" (also called a marker-locus-trait association) identified and validated in RosBREED? This is accomplished using a statistical genetics approach called Pedigree-Base Analysis that uses three types of data. The first type is parentage data on multiple pedigree-linked populations of breeding germplasm. The second type is phenotypic data for traits of interest collected for these pedigree-linked plant materials. The third and final type is genome-wide marker data for all these plant materials. Using these datasets and the software package FlexQTLTM developed by the Pedigree -Based Analysis Team member Marco Bink, QTLs are discovered and validated simultaneously, providing information to the breeder about the value of marker-locus-trait associations in breeding populations. The end result is a polished "jewel" ready for application in breeding.

However, this rock polishing is not as easy as it sounds, as problems with any of these three datasets can derail an analysis. Frequently parentage is unknown, or in many cases just wrong in the breeder's records. These pedigree errors can bring a genetic analysis to a screeching halt. Phenotypic data also needs to be quality-checked to minimize biases that can occur in results due to errors in this dataset. An understanding of genotype x environmental interactions can help identify the appropriate statistical analysis strategy. The genotypic data need to be free of "technical" errors resulting from miss-scoring of marker genotypes. Finally, the order of the genetic markers along the chromosomes needs to be correct or else the chromosomal locations of the "jewels" cannot be identified with the desired precision.

Problems with your dataset or with your statistical analysis? Within RosBREED the go-to person is Umesh Rosyara, the statistical post-doctoral associate based at Michigan State University. Frequently, your solution would be use of an R-script written by Umesh. Parentage errors? Umesh wrote an R-script to not only identify the errors but to deduce the true parents. Marker quality problems? Umesh wrote an R-script to sort out the markers with technical and genetic problems. Problems with marker order? Umesh can help you construct your linkage maps. Problems with your FlexQTL™ analysis? Send Umesh your output files and he'll help you troubleshoot.

As the person at the data analysis day-to-day hub of RosBREED, Umesh has "handled" (quality checked and analyzed) more than a billion data points. Countless projects have benefited from Umesh's computational support, without which these projects would have taken much longer to accomplish or may simply have gone undone. The results of Umesh's computational support are and will be documented in the many current and forthcoming publications and felt by breeders as they benefit from the use of DNA information in their crop improvement efforts. Below are illustrated some of the "behind the scenes" calls for help fielded by Umesh. Thank you Umesh for the great support you have provided!

Questions for Umesh:

- "Please help me with FlexQTL™ error messages!"
- "Please help me with GenomeStudio manual clustering"
- "What does this FlexQTL™ output actually mean?"
- ".....I need software.....
- "How will the GxE effect influence our strawberry QTL analyses?"
- "What is 'Bing's' paternal parent?"
- "I think some of the pedigrees are wrong! Can you write an R script to help sort this out?"
- "Can you do an R script to format the data?"
- "Can you do an R script to sort the data?"
- "Help me make sense of this massive strawberry data set?"
- "Help! I need to do a QTL analysis for a tetraploid!"
- "Can you teach me how to do GenomeStudio?"
- "What parameter settings should I use for my FlexQTL™ file?"
- "Can you help me construct a SNP map with JoinMap software?"
- "Can you graph the positions of the cherry SNPs on the peach scaffolds?"
- "Can you tell us with your pedigree tool whether any of these mystery selections are related to Honeycrisp?"



Our final newsletter

By Amy lezzoni, Project Director

This issue is the final one for the RosBREED project Newsletter, as our four-year project ended on 31 August 2013. On behalf of the RosBREED team, we wish to thank our partners, collaborators, and Advisory Panel members for their dedication and contributions to our shared goals. The success of RosBREED was achieved due to these partnerships, the tireless collective energy put towards furthering our scientific goals, and our laser focus on our target outcome: making the breeding of rosaceous crops more efficient for traits of value for producers and consumers. Through RosBREED we have made unprecedented progress in scientific deliverables, contributed to a more vibrant research community, and enhanced stakeholder understanding and support for applying modern DNA tools to crop improvement. Ultimately, we believe our collective effort will delight consumers with our wonderful fruit crops, driving demand and improving industry profitability. Over the next few months, we will distill the outcomes and impacts of



RosBREED into a portfolio of "impact statements" that highlight the positive changes that have resulted from our project. I will send these to our Newsletter mailing list as our final formal communication. The science and databases behind these advances made in RosBREED can be found in current and upcoming <u>scientific journal publications</u>, as online content in <u>eXtension</u>, and in databases on the <u>Genome Database for Rosaceae</u>.

I wish to acknowledge the USDA's Specialty Crop Research Initiative program for this unprecedented opportunity to compete for such significant and meaningful funding. I also wish to express my sincere appreciation to those dedicated NIFA staff who created the SCRI and have made it such an exciting and consequential program. Finally, a big thank you to all those industry stakeholders who have worked so hard over so many years to raise the profile of specialty crops and engage the rosaceous scientific community, not to mention produce and deliver amazing rosaceous products to the world. I am certain this partnership will not end with RosBREED! Stay tuned for RosBREED 2!

Thank you Brian and David

By Cholani Weebadde

As we salute our partners, collaborators and Advisory Panel members who have been an integral part of RosBREED's success, noteworthy to mention are two of our AP members who have provided excellent editorial support "behind the scenes", making the RosBREED quarterly newsletter a success!

Our two AP members, Brian Sparks (Editor, American/Western Fruit Grower) and David Karp (Associate, Agricultural Experiment Station, UC Riverside), have extensive editorial experience and were ever willing to help us with our communications with the Rosaceae community. Every quarter, we would get their input on the newsletter, and often at very short notice. We even talked over the phone in the wee hours in the morning or late at night to go over the edits. They've both been very supportive and encouraging, assuring us we are making good progress, leaving fewer and fewer edits for them over the years.

A BIG THANK YOU to Brian and David for helping our articles shine while rest of the group was busy making the jewels shine.



David Karp



Brian Sparks

RosBREED: Enabling marker-assisted breeding in Rosaceae

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Visit us at www.rosbreed.org

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| | Dorrie Main | Genomics | Washington State University |
| | 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 |

Thank you for all of your efforts: Cho Weebadde, Joan Schneider, and Audrey Sebolt!

