# VacCAP

Student Spotlight:

Hector Lopez-Moreno



Exploring the New

Frontier of Flavonoid

Genetics in Blueberry



The Vaccinium Coordinated Agricultural Project (VacCAP) is a nationwide project aimed at developing new genetic tools to enhance breeding for improved fruit quality of cranberries and blueberries.

VacCAP is a nationwide coordinated transdisciplinary project focused on addressing major bottlenecks limiting the growth of the U.S. *Vaccinium* industry by developing and implementing marker assisted selection (MAS) capacity in breeding programs.

This will enable breeders to select and pyramid fruit characteristics that positively contribute to fruit quality and market value.

Long term, the scientific resources developed will increase production of fruit with improved characteristics that meet ever-changing industry, market, and consumer preferences.

# Understanding the W85 Blueberry Genome

Issue 6 | March 2023

Investigating chromosomal recombination—the exchanging of genetic material between chromosomes—behavior is crucial to transfer traits under genetic control from one plant to another one and for advancing genetic discoveries. Factors that can limit chromosome recombination include preferential pairing in the case of polyploid species and chromosome rearrangements (e.g., chromosome inversions or translocations). Understanding chromosome genetic behavior and structure of blueberry chromosomes is relevant for two reasons:

- 1. Highbush blueberry is a polyploid species carrying four copies of each homologous chromosomes.
- 2. The genome of blueberry cultivars is heterogeneous, harboring pieces of DNA from wild diploid species that are used in breeding programs to introgress favorable traits (e.g., low chilling requirement).

Due to these characteristics, the blueberry genome is complex and it remains unclear if it behaves as an autopolyploid where each of the four homologous chromosomes recombine randomly or if they recombine preferentially in pairs of two like in allopolyploid plants. It is also still unknown if its genome harbors any chromosome rearrangements that can affect recombination.

In this study, a high-quality reference genome and three linkage maps were developed along with three available genomes representing the cultivar Draper, and two wild species (V. *darrowii and V. mirtillus*). These resources were used to compare the structure of the genomes, and to assess chromosome recombination behavior. Several methods were integrated into this study.

### Plant material, sequencing, and genome assembly

The genome assembly was developed using a wild diploid species V. *caesariense* clone W85, also known as diploid blueberry (V. *corymbosum*) and a potential progenitor of tetraploid cultivated blueberry. Assembly of the W85 genome was



Chromosomal features of Vaccinium caesariense W85 genome (2n = 2x = 24) and representative V. corymbosum tetraploid blueberry cultivars (2n = 4x = 48). Figure from Mengist et al., (2022) Additional caption details available in the <u>full manuscript</u>.

performed using long reads sequencing technology (PacBio) along with integration of a linkage map, including 17,486 single-nucleotide polymorphism (SNP) markers (Qi et al., 2021), that was used to anchor the genome.

### Gene prediction and annotation

Gene prediction was performed using Maker v.3.01.03 (Cantareletal., 2008) by integrating ab initio gene prediction and evidence-based prediction and was performed independently on the two haplotypes.

### Repetitive sequence annotation and analysis

One million random read pairs of W85 (NCBI accession no. SRR837868) were analyzed using Tarean (Novák et al., 2017) to identify potential satellite DNA sequences—highly repetitive DNA consisting of short sequences repeated many times.

Selected satellites of W85 were analyzed by fluorescence in situ hybridization (FISH) using young flower buds of the W85, *V. darrowii* and of the tetraploids 'Draper' Selection-44392, 'Draper', and 'Jewel', according to published procedures (lovene et al., 2008). Oligonucleotide probes and PCR primers were designed using the consensus sequences of the repeats.

### Linkage map construction

Three F1 mapping populations named  $DS \times J$  (n = 196), R×A (n = 346) and D×B (n = 168), were used for linkage map construction. Abbreviations stand for 'Draper' (D) and Draper Selection-44392 (DS are northern highbush blueberry (V. *corymbosum*; NHB) selections/cultivars, whereas Arlen (A), Jewel (J), Biloxi (B) and Reveille (R) are southern highbush cultivars (V. *corymbosum* interspecific hybrids; SHB) cultivars with 10–60% of their genome represented by wild species.

Estimation of double reduction, quadrivalent, and preferential chromosome pairing

The rate of double reduction (DR) and quadrivalent chromosome pairing were estimated using TetraOrigin software (Zheng et al., 2016). For a given marker, the probability of DR rate was averaged over the number of offspring on parental meiosis. The quadrivalent chromosome pairing was calculated by dividing the number of offspring with the quadrivalent pairing by the total number of offspring in the mapping population.

The meiotic pairing behavior of the 4× genotypes 'Arlen', DS, 'Draper', and 'Jewel' was also evaluated cytologically, by examining at least 40 pollen mother cells at diakinesis-metaphase I in each variety.

### Key Results

### W85 high-quality genome assembly

The contiguity of the W85 genome assembly was relatively higher than currently available blueberry genome assemblies. In total, 34,895 genes were predicted and about 45% of the genome was annotated as repetitive sequence, which is comparable to the tetraploid 'Draper' genome representing similar gene and repeat fractions (32, 139 genes/haplotype, 44.3% repeats) (Colle et al., 2019).

# Centromeric repeat VacSat1 is conserved across diploid and tetraploid blueberry species

A putative centromeric repeat (named VacSat1) present in 10-11 chromosomes was identified. VacSat1 sequence and

distribution was conserved in tetraploid and diploid species, V. *darrowii*, and V. *mirtillus*.

A satellite repeat named VacSat169 was identified on chromosome 6, and was associated with ribosomal DNA, a region known to have low recombination frequency.

# Comparative analysis between W85 and tetraploid genomes highlights a reciprocal translocation in the 'Draper' genome

A major reciprocal inter-chromosomal translocation was identified between 'Draper' chromosome 6 and chromosome 10. The translocation spans the location of VacSat169 in chromosome 6. Reciprocal translocation is a type of chromosomal abnormality that occurs when two non-homologous chromosomes break at the same time, and the broken ends reattach to the opposite chromosome, resulting in a reciprocal exchange of genetic material between the two chromosomes.

The reciprocal translocation was not identified in the other five highbush cultivars and in the wild species.

Besides the chromosome 6/ chromosome 10 translocation the W85 genome was highly collinear with linkage maps, the 'Draper', and wild species genomes.

### The inter-chromosomal translocation alters chromosome 6 and chromosome 10 meiotic behavior in the 'Draper' genome

Analysis of chromosomal recombination in F1 progenies derived from crosses between 'Draper'; harboring the translocation, and 'Biloxi', not harboring the translocation, indicated that the translocation altered chromosome 6 and 10 pairing, recombination, and segregation behavior, and in turn, affected the linkage map construction.

# Blueberries behave as an autotetraploid with non-preferential chromosome pairing also called tetrasomic inheritance

DNA Maker analysis indicated that there was no preferential pairing among each of the four homologous chromosomes. Also, there was a substantial quadrivalent chromosome pairing in DS×J and R×A mapping populations, as also indicated by the cytological analysis of three parental varieties at diakinesismetaphase I ('Arlen', DS, and 'Jewel').

Overall, our results did not reveal strong enough evidence to demonstrate any preferential pairing in blueberry.

### Discussion

# High quality genomic resources provide novel insights into the structure of the blueberry genomes

In this study, a high-quality phased assembly of the blueberry genome was released, improving upon previous versions. The genome enables the localization of regions enriched by low complexity sequences (repeats) and highlighted that the structure of the genomes among *Vaccinium* species (section *Cyanococcus*) is highly conserved.

# Evidence and impact of a reciprocal translocation for blueberry genetic analysis and breeding

This study demonstrated the presence of reciprocal translocation, which formed two fused chromosomes,

chromosome 610 and chromosome 106. The co-localization between VacSat1 and VacSat169 that repeats on the same chromosome is specific to the translocation chromosome 106, making these ideal cytogenetic markers for future studies.

The translocation was found to cause multiple abnormal chromosome pairing configurations, which affected recombination between chromosome 6 and chromosome 10. The reciprocal translocation can directly affect phenotypes and cell functionality, such as pollen viability. Also, it can directly affect inheritance of important traits, like cold hardiness, a phenotype that has been associated with a region of chromosome 10. Future work will need to assess the impact of the translocation and evaluate the frequency of the translocation in the blueberry germplasm.

### Evidence for autopolyploid genetic behaviors of blueberry

The overall results demonstrated that blueberry behaves as an autopolyploid during meiosis. This implies that the four homologous chromosomes can pair randomly, which increase recombination frequency. This result guides the selection of statistical models to use in genetic studies for blueberry. Models that account for random tetraploid recombination should be used and not those that account for preferential pairing.

Overall, the results of these study provide a framework for comparative genome analysis within *Vaccinium* spp. and can advance genomic-assisted breeding in blueberry.

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# Exploring the New Frontier of Flavonoid Genetics in Blueberry

A quick search of blueberry with the terms "anthocyanin and chlorogenic acid" bring up numerous articles extolling the virtues of blueberries as a healthy superfruit. Answering questions about what genetic mechanisms are controlling the accumulation of these bioactive compounds—and their relationship with fruit quality traits—is vital for the improvement of blueberry cultivars.

For instance, a recent study in blueberry indicated that through digestion, acylated anthocyanin (a form of anthocyanin that accumulates in blueberry fruits), are recovered at a higher rate. As a result, the adsorption of these health-related bioactive compounds in the human body could increase.

Recent developments and adoption of advanced genotyping platforms and genomic recourses have advanced our understanding of what controls accumulation of these bioactive compounds in *Vaccinium* crops. Two recent studies focused on dissecting the genetic mechanisms controlling chlorogenic acid (CHA) and anthocyanin content and their composition in blueberries (Mengist et al., 2022; Montanari et al., 2022).

Study 1. Dissecting the genetic basis of bioactive metabolites and fruit quality traits in blueberries (*Vaccinium corymbosum* L.)

Mengist et al. (2022) used a mapping population (DSxJ) representing highbush blueberry cultivars Draper and Jewel and

identified 180 quantitative trait loci (QTLs) for total and individual anthocyanin content, relative anthocyanin composition, and CHA.

A QTL influencing CHA concentration was identified on chromosome 2 that explained up to 21% of the phenotypic variation and was consistent over three consecutive years of the study. This is the only known QTL associated with CHS.

One cluster of stable QTLs influencing total anthocyanin content was mapped on chromosome 1 and explained up to 24% of the phenotypic variation. Four clusters of QTLs controlling the conjugations of anthocyanin with different sugar moieties and acylation were mapped on chromosomes 1, 2, 4 and 8 and were stable across three years.

 The cluster of QTLs that mapped on chromosome 4 explained up to 80% of the phenotypic variance, and was associated with an increase of glucoside-based anthocyanins relative to the arabinoside/galactoside-



Major-effect QTLs identified for anthocyanin glycosylation and acylation mapped on chromosomes 2 and 4. Figure from Mengist et al. (2022). Additional caption details available in the full manuscript.



based anthocyanins.

- A second cluster of QTLs mapped on chromosome 1, explained up to 35% of the phenotypic variance and was associated with reducing the concentration of arabinoside- and galactoside-containing anthocyanins.
- A third cluster of overlapping QTLs mapped on chromosome 8 associated with reducing cyanidingalactoside and peonidin-galactoside and increasing cyanidin-arabinoside concentration, and explained up to 44% of phenotypic variance.
- A cluster of stable QTLs mapped on chromosome 2 controlled content or proportion of acylated anthocyanin, explained up to 27% of the phenotypic variance and overlapped with the CHA QTL.

Haplotype analysis indicated that the QTLs for CHA and acylated anthocyanin were in different haplotypes, which implies they could be selected independently.

# Study 2. High-density linkage map construction in an autotetraploid blueberry population and detection of quantitative trait loci for anthocyanin content

Montanari et al. (2022) used recently released tools for genetic mapping to build a high-density linkage map and to detect QTLs for fruit anthocyanin content. The team used a different mapping population (NxHB) representing two different highbush cultivars, Nui and Hortblue Petite.

- They identified two clusters of stable QTLs on chromosomes 2 and 4, which explained up to 77.5% and 39.0% of the phenotypic variation for anthocyanin composition, respectively.
- QTLs mapped in chromosome 4 had a positive effect on the concentrations of most of the glucoside basedanthocyanins, and a negative effect on most of the arabinoside/galactoside-based anthocyanins.

### Integration of results from the two studies highlighted that:

- The two clusters of QTLs controlling acylation and glycosylation mapped on chromosomes 2 and 4 across the two studies were anchored to the W85 v2 genome (Mengist et al., 2022); this revealed that they overlap.
- The QTLs on chromosome 2 overlap in a 3.2 Mb region spanning position 8 to 11.2 Mb, and the QTLs in chromosome 4 overlap in a 4.8 Mb region spanning position 56.2 to 61Mb.
- Overlapping of these QTLs across different genetic backgrounds represents the first level of QTL validation and allowed for identification of possible genes controlling anthocyanin acylation and glycosylation.
- Across the two studies several minor-effect and non-stable QTLs were also identified, indicating that environmental factors affect anthocyanin accumulation.

Overall, these studies provide important insight and pave the way to understanding the genetic mechanisms that control CHA



Linkage map of 'Hortblue Petite' × 'Nui'. Figure from Montanari et al. (2022).

and anthocyanin content and composition in blueberries. Future studies can use these findings to further develop DNA markers to select blueberry cultivars with higher content of these bioactive compounds or for specific anthocyanin composition.

### Citations

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# Catch up on our VacCAP Webinars

In December, Dr. Massimo lorizzo, North Carolina State University, presented the webinar <u>"Autopolyploid Inheritance</u> <u>& Heterozygous Reciprocal Translocation Shape Chromosome</u> <u>Genetic Behavior</u>". This webinar provided an update on a new blueberry diploid reference genome (W85), and how it was leveraged to uncover recombination behavior and structural genome divergence/conservation across tetraploid and wild diploid species.

Dr. Nahla Bassil and Dr. Shaun Clare (USDA-ARS, NCGR) presented on <u>"Two New Flex-Seq-EX-L High Throughput</u> <u>Genotyping Platforms for Blueberry and Cranberry"</u> in January. They discussed the development and evaluation of these Flex Seq EX-L in their respective 192-member diversity set. These two high throughput platforms are valuable resources for genome-wide association and other genetic studies for the blueberry and cranberry communities.

Both webinar recordings are on our VacCAP Project YouTube channel, along with all our previous webinars throughout the project. Subscribe to the channel so you never miss another recording.



# **Student Spotlight: Hector Lopez-Moreno**

In our Student Spotlight Series, we want to introduce you to the students who help make VacCAP possible through their passion and hard work. In this segment, get to know **Hector Lopez-Moreno**, a PhD student at UW-Madison with advisor Dr. Juan Zalapa.



# What is the project you're working on for VacCAP about?

My project is focused on the study of genetics, genomics and phenomics of cranberry fruit quality. In my research, I analyze a wide variety of fruit quality traits related to external appearance, internal structure, and texture in order to generate relevant information and tools that can benefit research and the industry of this crop. I hope that the results of my research will contribute to the creation

of future cranberry varieties with improved fruit quality by identifying genomic regions of interest and developing new phenotyping methodologies.

# What is something you like or find most interesting about your work?

Because many areas of study in cranberry have been little explored, our research is pioneering in the field. This makes the

generation of knowledge in this crop challenging and exciting at the same time. In addition, one of the things I like the most about my research is that we work closely with stakeholders to generate highly applicable knowledge.

For example, a large part of my work is contributing to the development of the genetic improvement scheme to create varieties with better performance in the production of products with high added value in the industry such as sweetened and dried cranberries.

### What do you hope to do in the future after your work here?

In the future I plan to continue my career as a researcher/ breeder to give practical solutions to the different problems facing agricultural production. In particular, I would like to develop my own fruit quality research program by combining multi-omics tools, bioinformatics and AI.

### Anything else you would like to add?

I would like to thank my advisor Dr. Juan Zalapa for his guidance and mentoring during my doctoral studies and the VacCap team for their support and the opportunity to collaborate with a high-level team.

# Breeder Spotlight: Dr. Sara Montanari

In our Breeder Spotlight Series, we interview blueberry and cranberry breeders to learn more about their roles, challenges in their breeding programs, and have them highlight some of their favorite new cultivars. In this spotlight, we spoke to **Dr. Sara Montanari**, a molecular breeder at The New Zealand Institute for Plant and Food Research Limited.

### Please describe your role in the blueberry industry.

My expertise is in genetics and genomics and the application of genomics tools to speed up breeding. Blueberry is a crop that has been very recently domesticated and which is receiving an increasing commercial interest, mainly because of the beneficial health properties of its fruits. New Zealand has a large and growing blueberry industry, and The New Zealand Institute for Plant and Food Research Limited (PFR) carries out a well-established and successful breeding program.

My role at PFR is to enable the application of genomics tools into our breeding program, to speed up selection and help the breeders make more informed decisions. Since I joined PFR in mid-2019 I have been involved in collaborative projects aimed at developing reference genomes for our most important cultivars, designing high-throughput genotyping tools, evaluating the genetic diversity of our germplasm, and understanding the genetic control of traits of importance.

## Cultivar Highlight - Please tell us about some top cultivars you're excited about and why you chose them.

'Hortblue Petite' is one of my favorite blueberry cultivars. It was released in 2009 by the PFR breeder at the time, Jessica Scalzo. It is a cultivar developed for the home-garden and it provides tasty blueberries from spring to fall. It is also one of the parents of our main mapping population, which, thanks to 'Hortblue Petite''s particular and unique characteristics, segregates for several traits of interest. 'Hortblue Petite''s parentage is unknown, as it was generated from an open pollination event, and even its taxonomy remains unclear, and I am hoping to be able to answer these questions using genomics tools.

### What are some challenges in the breeding program?

One of the main questions we have at PFR is about the extent of genetic diversity included in our germplasm and the amount of inbreeding in our selections. While improved blueberry varieties were imported into New Zealand from the US in the 1970s, further introductions of new material have been very limited since then because of New Zealand's very strict quarantine requirements.

To answer this question, we are in the process of genotyping all our historical germplasm, as well as our parental pool and some of the early-stage selections. This will allow us to make more informed decisions when planning crosses, as well as to optimize our economic resources for the introduction of new material, with the objective to replenish and safeguard the genetic diversity in our program.



Where do you see the future of Vaccinium breeding going in the next 20 years?

The quick growth of blueberry production globally at the same time of the major challenge of the 21st century, climate change, is certainly driving a boost in the improvement of cultivars with increased yield, quality, and adaptability to different environments. The availability of a large and still almost unexplored diversity in *Vaccinium* is a precious resource that will help our breeders face these challenges. Additionally, research into new growing systems, such has controlled environment units, is likely to become more and more relevant for blueberry in the next 20 years, in my opinion.

# In what way have you used resources from VacCAP to facilitate your work?

I have been collaborating on the development of a highthroughput genotyping platform for blueberry, which I am now using to analyze the PFR germplasm collection. Additionally, the friendly and collaborative *Vaccinium* community fostered by VacCap has allowed me to share information about common research objectives with other breeders and to optimize our resources and efforts.

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# **Check Out These VacCAP Resources**



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United States Department of Agriculture National Institue of Food and Agriculture Award #: 2019-51181-30015

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