



Tools for Polyploids 2024

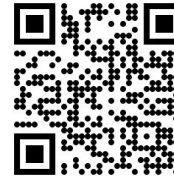
Genomic Prediction in an autotetraploid and outcrossing crop: lessons from blueberry

Felipe Ferrão

Research Scientist

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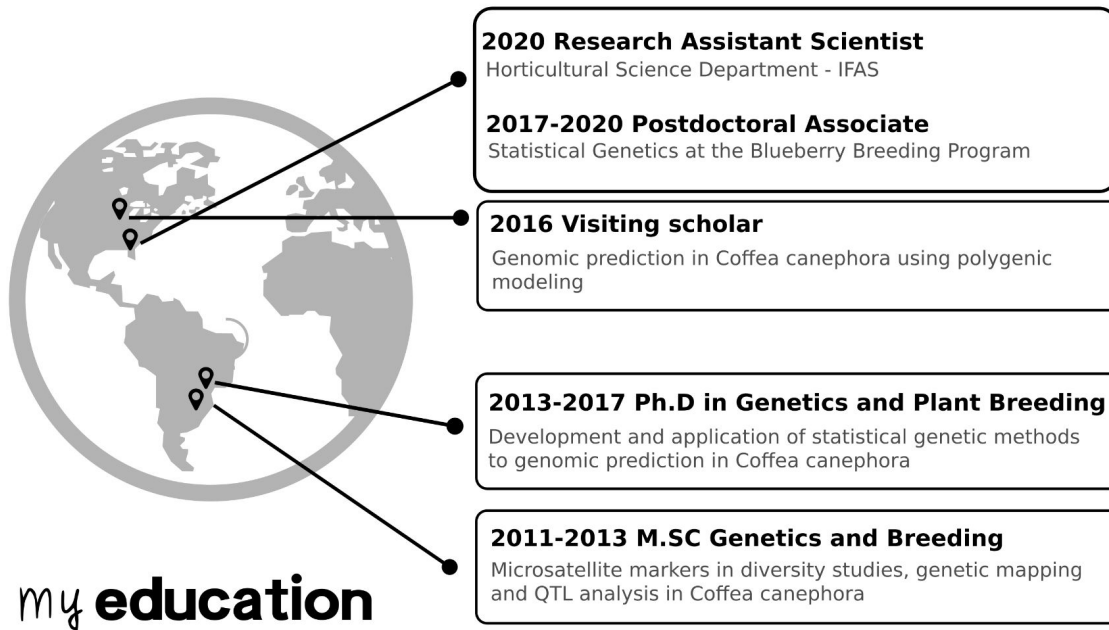
🐦 lfelipeferrao



San Diego, CA, USA
January, 2024

Background

Who am I?

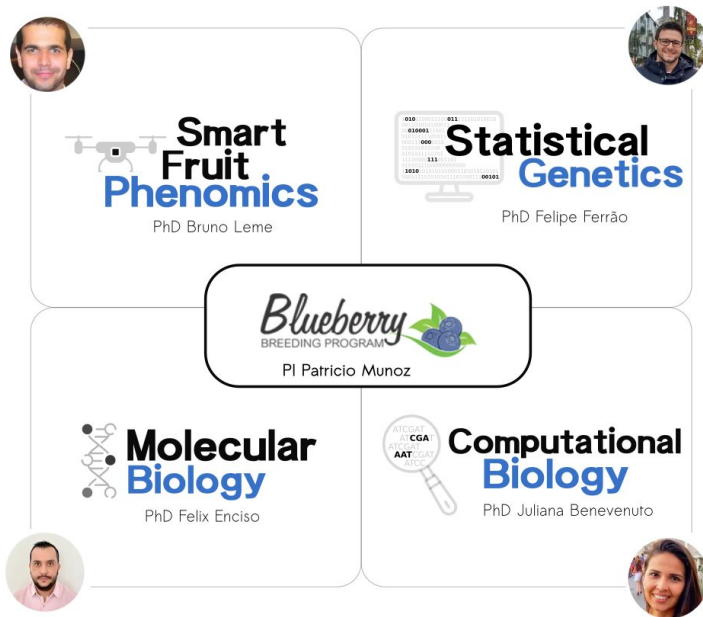


Background

The Blueberry Breeding Program

Blueberry Breeder

Associate Professor
Horticultural Science Department
University of Florida



Background

The Blueberry Breeding Program

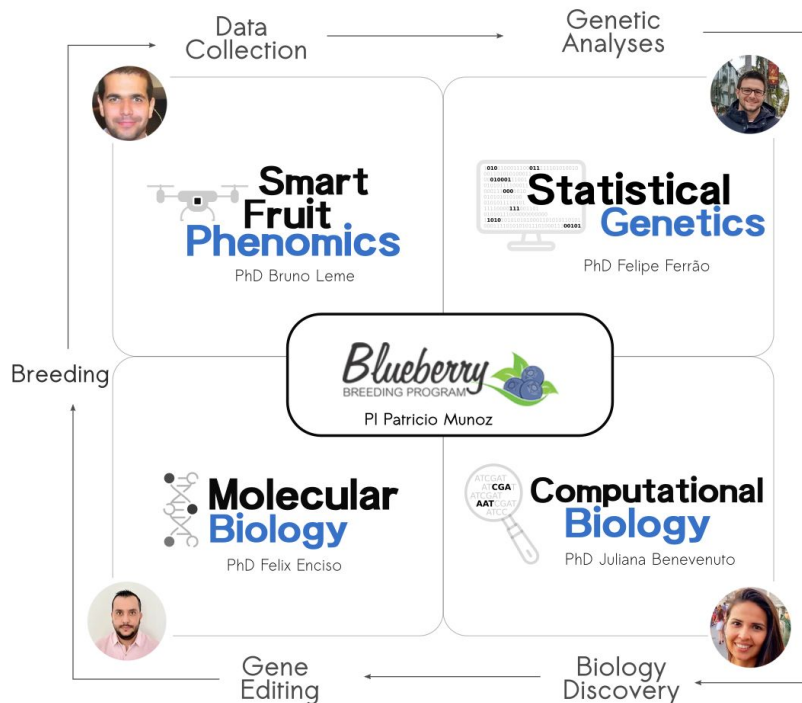
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Phenomics

- Computer vision
- Robots
- Mobile technologies



Statistical Genetics

- Genomic Selection
- Genome-wide association
- Statistical Learning

Molecular Biology

- Transformation
- CRISPR
- Tissue culture

Computational Biology

- Variant discovery
- Genome assembly
- Pan-genome
- RNAseq



Background

My research line

Goal

Explore problems at the interface of Statistics and Genetics.

Developing novel methods and software, or learn something new compared with existing approaches.



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Motivation

Why genomic selection in blueberry?

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Importance

- Blueberry is the second most important soft fruit
- Per capita consumption has increased 97% in the past 10 years
- Reason:
 - Delicious !!
 - Health benefits



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University of Florida

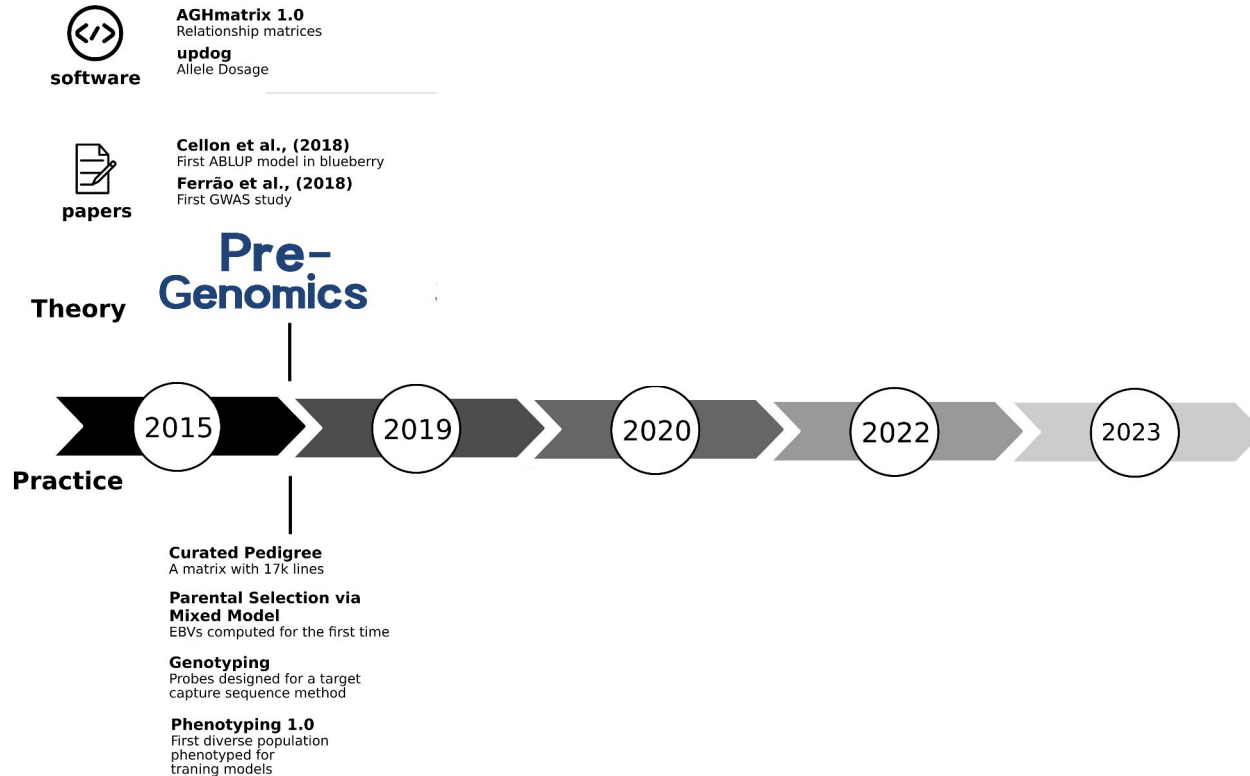
- UF plays an important role on cultivar development
- Released more than 60 cultivars (from 1970 to 2023)
- PLUS ~45 releases in collaboration or directly from our crosses
- Physolosofy: **breeding** & **research** running side-by-side



www.blueberrybreeding.com

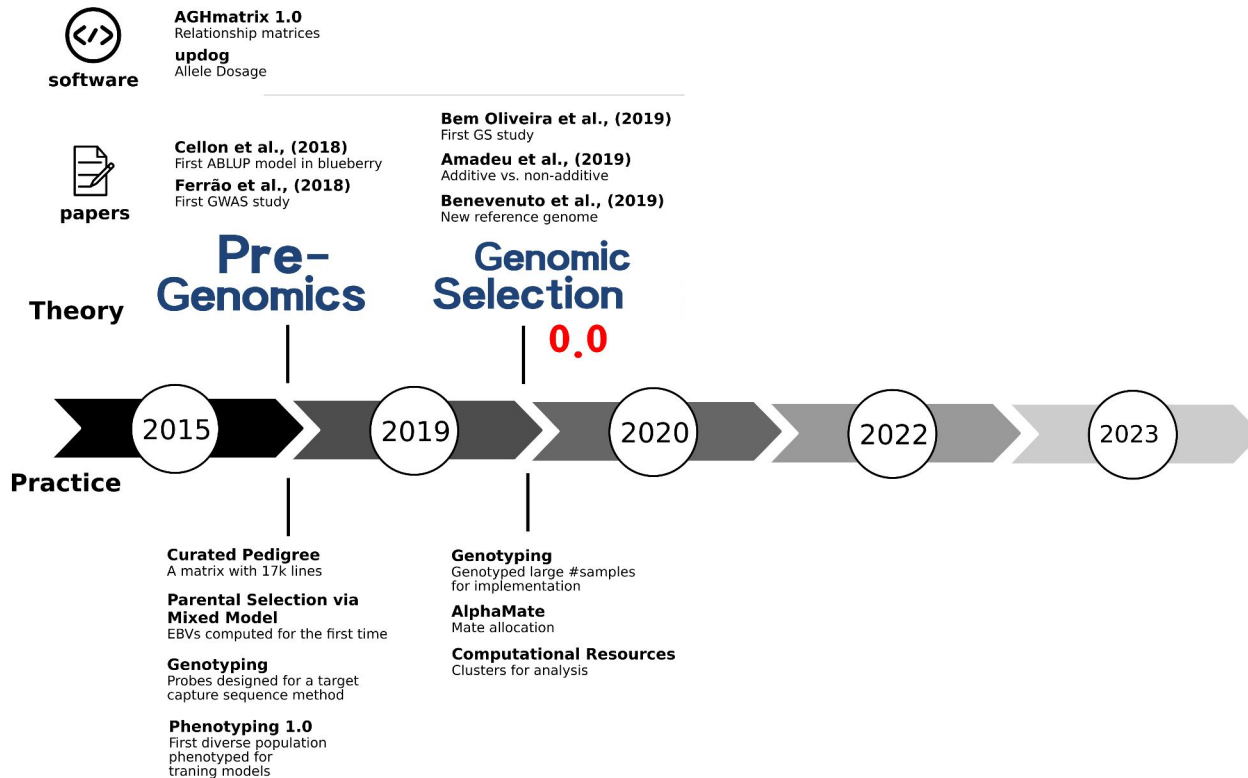
Timeline

How and when do we start implementing GS?



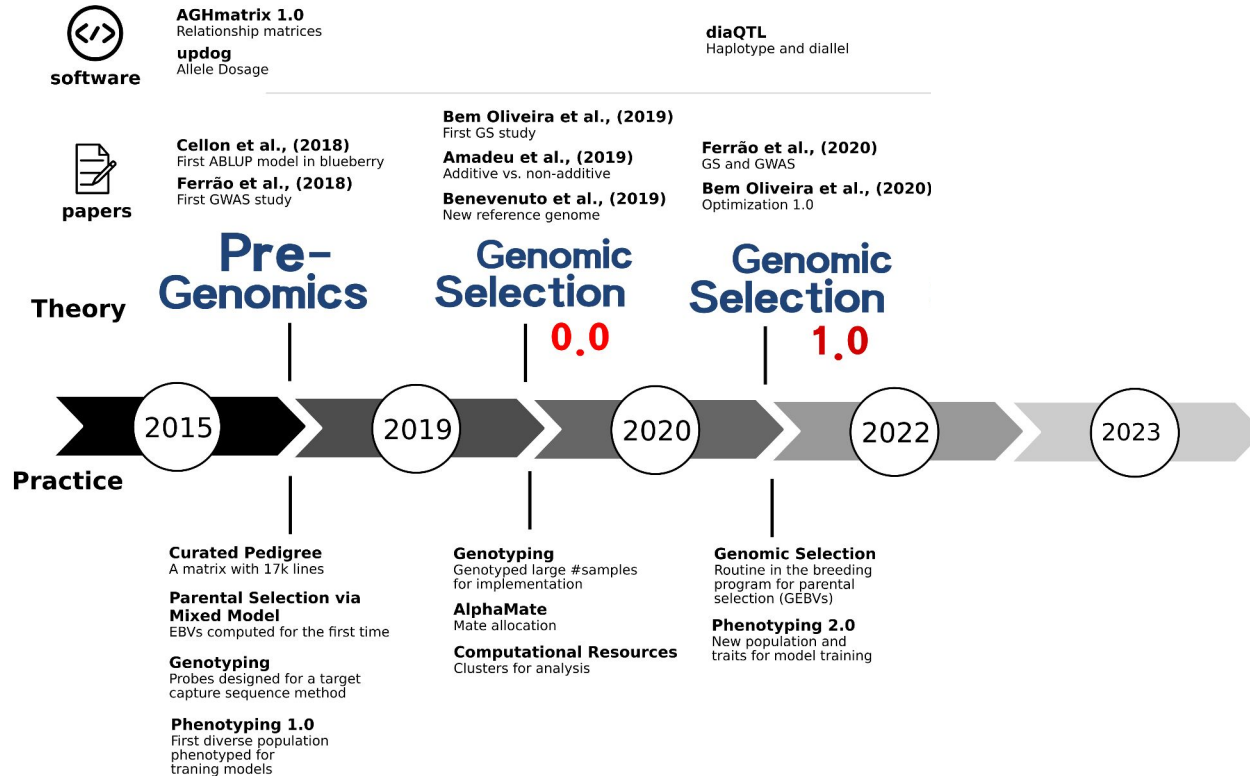
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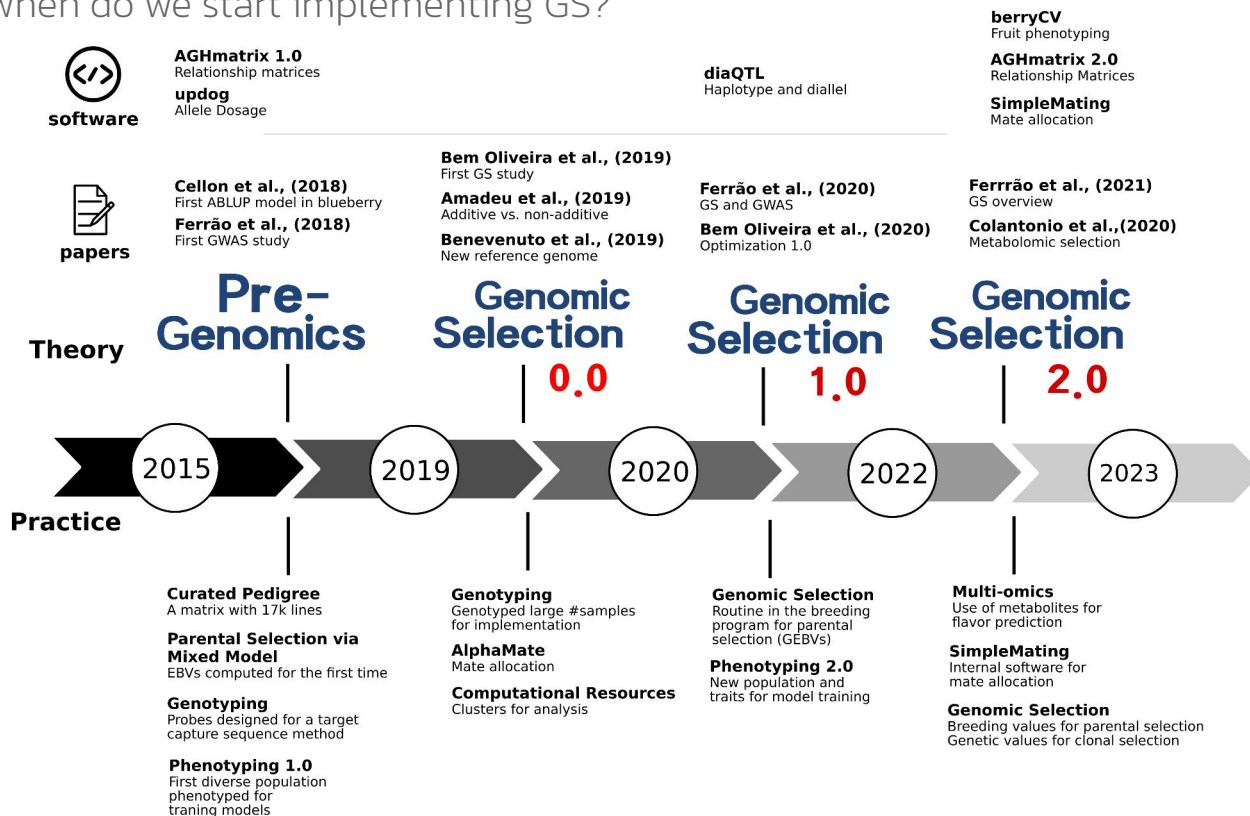
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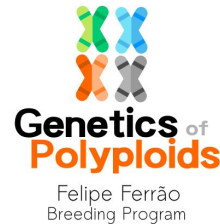
Genomic Selection 1.0

Past and present in blueberry

Genomic Selection 1.0

Questions:

- Is genomic selection better than phenotypic selection?
- What are the best predictive models?
- What is the importance of better genomic resources?
- Can we unify prediction and discovery in a single framework?



Genomic Selection 1.0

Questions:

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Genomic Selection 1.0

Is genomic selection better than phenotypic selection?

Background

Field year	Stage	# Plants	Goal
0	0	~150 crosses	Crossing + Seedlings
1	I	20.000	High-density nursery– Single Plant Selection
2–4	II	2.000	Single Plant Selection
5–9	III	200	Farm Condition – Experimental design
10–15	IV	20	Regional Yield Trial – Experimental design
16	V	1-2	Cultivar Release

Genomic Selection 1.0

Is genomic selection better than phenotypic selection?

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Challenges

- Time-consuming (15 years → cultivar)
- High level of inbreeding depression
- Autotetraploid ($2n=4x$)

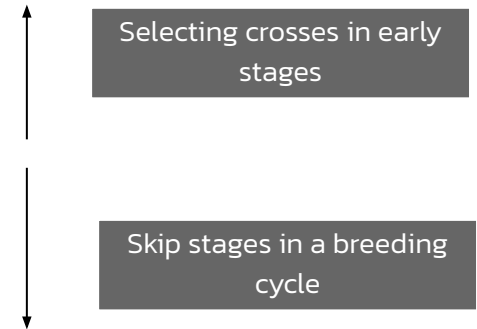
Genomic Selection 1.0

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Genomic Selection



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Genomic Selection 1.0

Is genomic selection better than phenotypic selection?



Genomic Selection in an Outcrossing Autotetraploid Fruit Crop: Lessons From Blueberry Breeding

Luis Felipe V. Ferrão¹, Rodrigo R. Amadeu¹, Juliana Benevenuto¹, Ivone de Bem Oliveira^{1,2} and Patricio R. Munoz^{1*}

¹Blueberry Breeding and Genomics Lab, Horticultural Sciences Department, University of Florida, Gainesville, FL, United States, ²Hortifrut North America, Inc., Estero, FL, United States

Blueberry (*Vaccinium corymbosum* and hybrids) is a specialty crop with expanding production and consumption worldwide. The blueberry breeding program at the University of Florida (UF) has greatly contributed to expanding production areas by developing low-chilling cultivars better adapted to subtropical and Mediterranean climates of the globe. The breeding program has historically focused on recurrent phenotypic selection. As an autopolyploid, outcrossing, perennial, long juvenile phase crop, blueberry breeding cycles are costly and time consuming, which results in low genetic gains per unit of time. Motivated by applying molecular markers for a more accurate selection in the early stages of breeding, we performed pioneering genomic selection studies and optimization for its implementation in the blueberry breeding program. We have also addressed some complexities of sequence-based genotyping and model parametrization for an autopolyploid crop, providing empirical contributions

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Goda Mordalo,
Virginia Tech, United States
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GENOMIC PREDICTION

Genomic Prediction of Autotetraploids; Influence of Relationship Matrices, Allele Dosage, and Continuous Genotyping Calls in Phenotype Prediction

Ivone de Bem Oliveira,^{*,1} Marcio F. R. Resende, Jr.,[†] Luis Felipe V. Ferrão,^{*} Rodrigo R. Amadeu,^{*} Jeffrey B. Endelman,[§] Matias Kirst,^{**} Alexandre S. G. Coelho,[†] and Patricio R. Munoz^{*,1}

^{*}Blueberry Breeding and Genomics Lab, and [†]Sweet Corn Genomics and Breeding, Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, [‡]Plant Genetics and Genomics Lab, Agronomy College, Federal University of Goiás, GO, Brazil, 74690-900, [§]Department of Horticulture, University of Wisconsin, Madison, WI 53706, and ^{**}Forest Genomics Lab, School of Forestry Resources and Conservation, University of Florida, Gainesville, FL 32610

ORCID IDs: 0000-0003-3723-9747 (I.d.B.O.); 0000-0002-2367-0766 (M.F.R.R.); 0000-0002-9655-4838 (L.F.V.F.); 0000-0001-5127-4448 (R.R.A.); 0000-0003-0957-4337 (J.B.E.); 0000-0001-8973-9351 (P.R.M.)



Genomic Selection 1.0

Is genomic selection better than phenotypic selection?

Challenges on Quantitative Genetics analyses applied to polyploid analyses

- More genotypic classes -> allele dosage
- Multisomic segregation -> relationship matrix
- More complex gene actions -> multiple levels of non-additive effects

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updog R package

- ~20000 downloads
- Inference on allele dosage,
- Accounting by common features of NGS data

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AGHmatrix R package

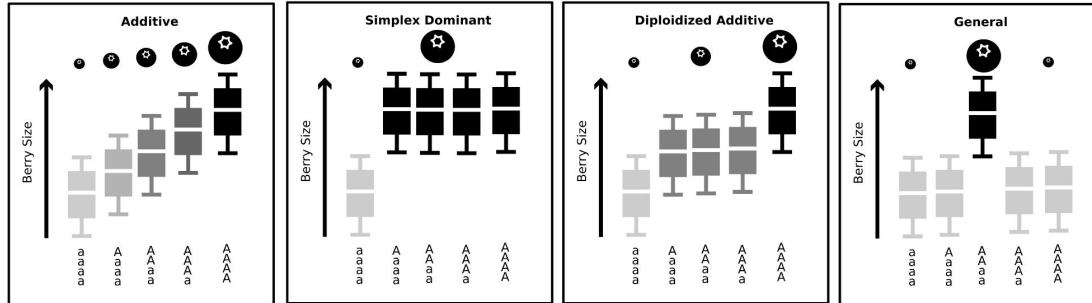
- Pedigree (**A**), genomic (**G**) and hybrid matrices (**H**)
- Any ploidy level
- Fast and user friendly

Genomic Selection 1.0

Is genomic selection better than phenotypic selection?

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Additive vs. non-additive

- Multiple gene actions
- Different dominance levels

Genomic Selection 1.0

Is genomic selection better than phenotypic selection?

Back to 2017 ...

- **Population:** large population (~2000 ind) representing our breeding collection
- **Phenotype:** fruit quality traits (firmness, size, brix, acidity and weight)
- **Models:** ABLUP (pedigree), G2 (GBLUP assuming diploid markers), G4 (GBLUP accounting for allele dosage)

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Trait	Matrix	Accuracy	Method
Firmness	A	0.375	Pedigree
Firmness	G2	0.415	GBLUP (2n=2x)
Firmness	G4	0.426	GBLUP (2n=4x)
Size	A	0.386	Pedigree
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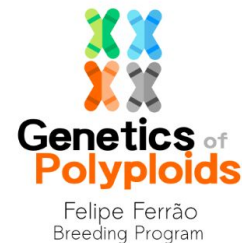
Take-home message

- (i) Genomic > Pedigree
- (ii) Use polyploid methods

Genomic Selection 1.0

Questions:

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Genomic Selection 1.0

What are the best predictive models?



Felipe Ferrão
Breeding Program

RESEARCH

Impact of Dominance Effects on Autotetraploid Genomic Prediction

Rodrigo R. Amadeu, Luis Felipe V. Ferrão, Ivone de Bem Oliveira, Juliana Benevenuto, Jeffrey B. Endelman, and Patricio R. Munoz*

ABSTRACT

Many commercially important plants are autopolyploid. As a result of the multiple chromosome sets in their genomes, higher orders of allele interactions can occur, implying

R.R. Amadeu, L.F.V. Ferrão, I.D.B. Oliveira, J. Benevenuto, and P.R. Munoz, Blueberry Breeding and Genomics Lab, Horticultural Sciences Dep., Univ. of Florida, Gainesville, FL 32611; J.B. Endelman, Dep. of Horticulture, Univ. of Wisconsin, Madison, WI 53706. Received 28 Feb. 2019. Accepted 22 May 2019. *Corresponding author (p.munoz@ufl.edu). Assigned to Associate Editor Carlos Messina.

frontiers
in Plant Science

ORIGINAL RESEARCH
published: 06 February 2020
doi: 10.3389/fpls.2020.00025



Exploring Deep Learning for Complex Trait Genomic Prediction in Polyploid Outcrossing Species

Laura M. Zingaretti^{1*}, Salvador Alejandro Gezan², Luis Felipe V. Ferrão³, Luis F. Osorio⁴, Amparo Monfort^{1,5}, Patricio R. Muñoz³, Vance M. Whitaker⁴ and Miguel Pérez-Enciso^{1,6*}

¹ Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-LIB, Campus UAB, Barcelona, Spain, ² School of Forest Resources and Conservation, University of Florida, Gainesville, FL, United States, ³ Blueberry Breeding and Genomics Lab, Horticultural Sciences Department, University of Florida, Gainesville, FL, United States, ⁴ IFAS Gulf Coast Research and Education Center, University of Florida, Wimauma, FL, United States, ⁵ Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Barcelona, Spain, ⁶ ICREA, Passatge de Lluís Companys 23, Barcelona, Spain



Genomic Selection 1.0

What are the best predictive models?

Background

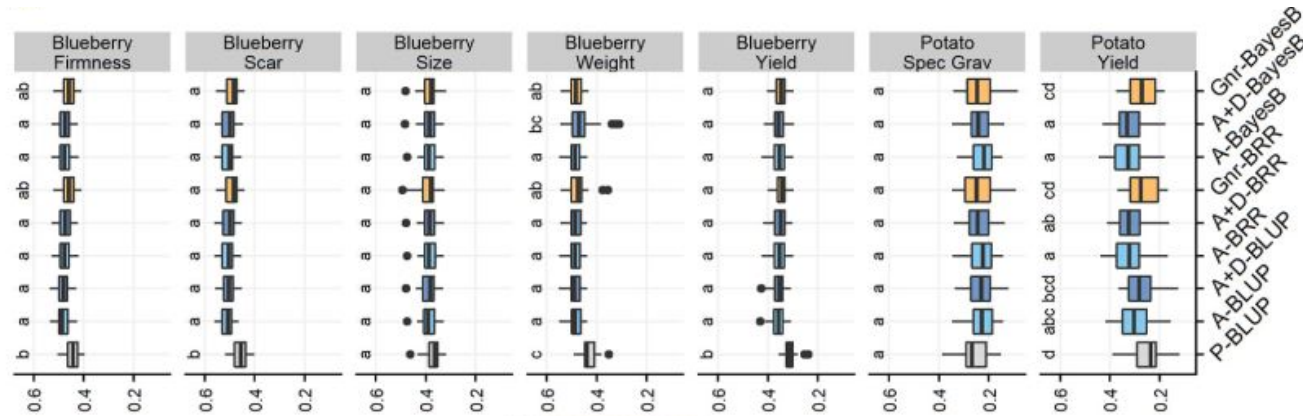
- Polyploid is complex !
- Most of prediction models are based on linear methods and additive gene actions
- Can we use more elaborate models?
 - Bayesian alphabet + mixed models
 - Different gene actions
 - Deep learning methods that can incorporate non-linearity

Genomic Selection 1.0

What are the best predictive models?

Bayesian vs. Mixed Models vs. Gene actions

- Blueberry (5 traits) vs. Potato (2 traits)

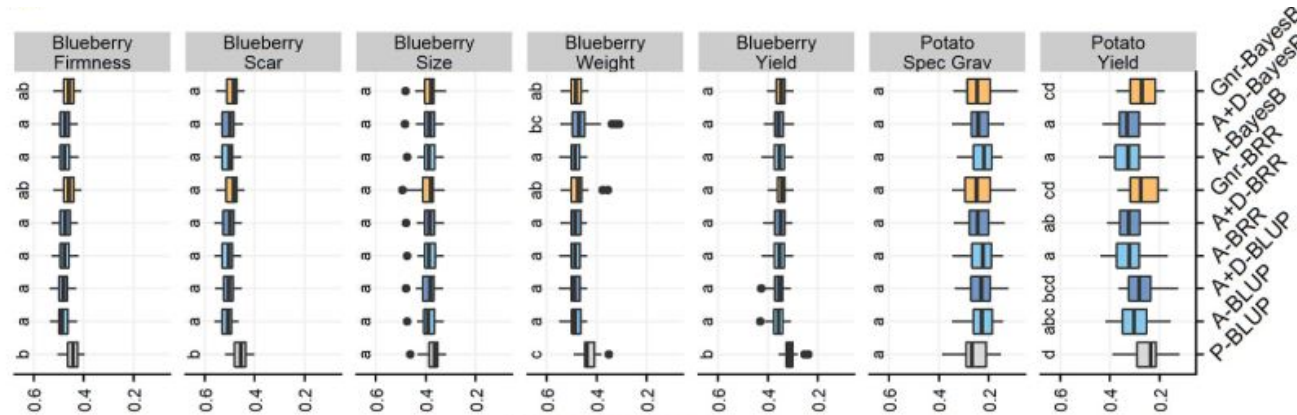


Genomic Selection 1.0

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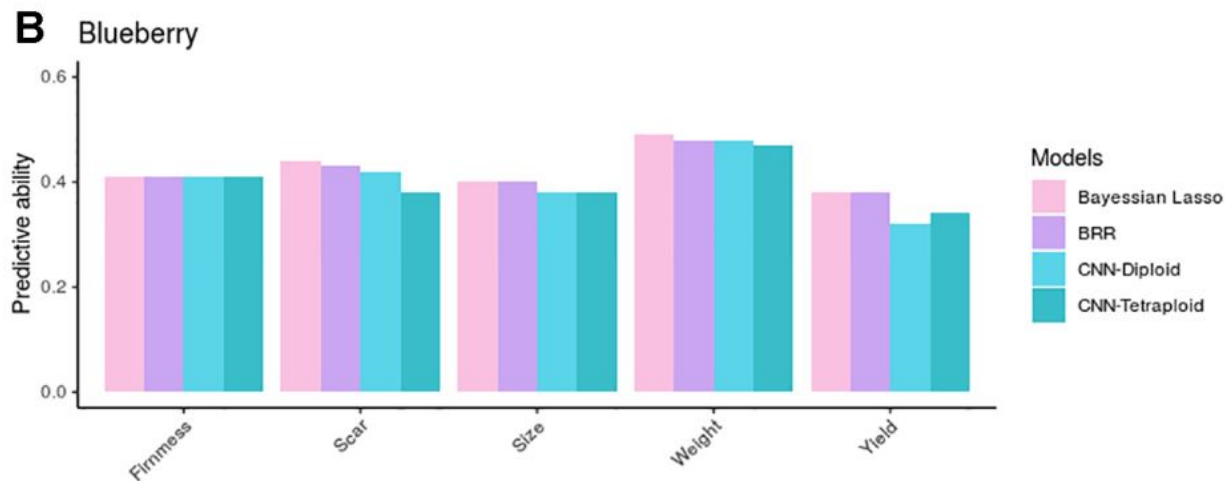
Take-home message

On the relevance of additive models

Genomic Selection 1.0

What are the best predictive models?

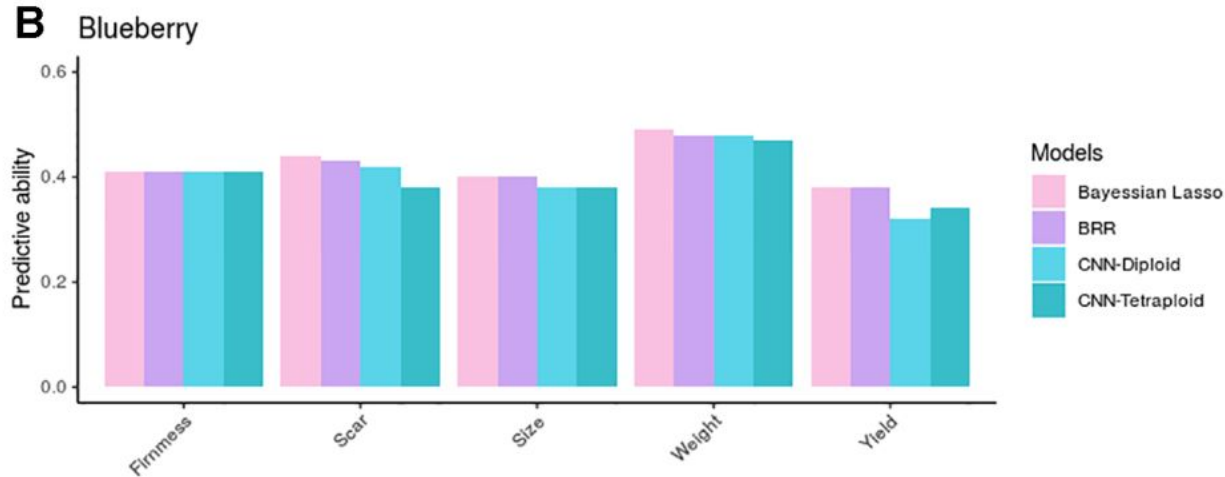
Bayesian vs. Mixed Models vs. Deep Learning



Genomic Selection 1.0

What are the best predictive models?

Bayesian vs. Mixed Models vs. Deep Learning



Take home message

On the relevance of additive models² !!!

Genomic Selection 1.0

What are the best predictive models?

More recently ...

- New paper using a transformer DL architecture
- Similar architecture used by ChatGPT
- The authors tested in multiple data set ...
- ... and made some strong claims !!
- Ok, let's test it !!

GPFN: Prior-Data Fitted Networks for Genomic Prediction

Jordan Ubbens^{1,2}, Ian Stavness^{1,2}, and Andrew G. Sharpe¹

¹Global Institute for Food Security (GIFS), University of Saskatchewan, Saskatoon, SK S7N 0W9, Canada
²Department of Computer Science, University of Saskatchewan, Saskatoon, SK S7N 0W9, Canada

Genomic Prediction (GP) methods predict the breeding value of unphenotyped individuals in order to select parental candidates in breeding populations. Among models for GP, classical linear models have remained consistently popular, while more complex nonlinear methods such as deep neural networks have shown comparable accuracy at best. In this work we propose the Genomic Prior-Data Fitted Network (GPFN), a new paradigm for GP. GPFNs perform amortized Bayesian inference by drawing hundreds of thousands or millions of synthetic breeding populations during the prior fitting phase. This allows GPFNs to be deployed without requiring any training or tuning, providing predictions in a single inference pass. On three populations of crop plants across two different crop species, GPFNs perform significantly better than the linear baseline on 13 out of 16 traits. On a challenging between-families NAM prediction task, the GPFN performs significantly better in 3 locations while only falling behind in one. GPFNs represent a completely new direction for the field of genomic prediction, and have the potential to unlock levels of selection accuracy not possible with existing methods.

least as well as newer, more modern methods while being simpler, faster, and requiring less tuning (Azodi et al., 2019; Abdollahi-Arpanahi et al., 2020; Zingaretti et al., 2020; Ubbens et al., 2021; John et al., 2022; Ray et al., 2023).

In this work, we develop a new approach to GP based on amortized Bayesian inference, which we term *Genomic Prior-Data Fitted Networks* (GPFNs). Unlike existing methods based on neural networks, a GPFN is not trained on the end user's dataset, does not require any tuning, and in fact is never exposed to any real data at all prior to inference.

As a proof of concept, we show that the GPFN is the first method which is able to consistently and significantly outperform classical methods in several datasets of crop plants.

An implementation as well as several trained GPFNs for various population types are provided at <https://github.com/jubbens/gpfn>. We propose the GPFN approach as a major new direction for genomic prediction.

Genomic Selection 1.0

What are the best predictive models?



Trait	#data points	GPFN	GBLUP (2x)	GBLUP (4x)
pH	~3000	0.31	0.32	0.35
Brix	~1000	0.28	0.27	0.28
Eucalyptol	~1000	0.42	0.37	0.40
Yield	~250	0.33	0.36	-
Maturation	~250	0.11	0.21	-
Vigor	~250	0.08	0.13	-

Camila Azevedo. Deep Learning for Genomic Prediction in Blueberry (work in progress). 2023

Genomic Selection 1.0

Questions:

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- **What is the importance of better genomic resources?**
- Can we unify prediction and discovery in a single framework?



Genomic Selection 1.0

What is the importance of better genomic resources?





(GIGA)ⁿ
SCIENCE

GigaScience, 8, 2019, 1–4

doi: [10.1093/gigascience/giz068](https://doi.org/10.1093/gigascience/giz068)
Commentary

COMMENTARY

How can a high-quality genome assembly help plant breeders?

Juliana Benevenuto , Luís Felipe V. Ferrão, Rodrigo R. Amadeu and Patricio Munoz 

Blueberry Breeding and Genomics Laboratory, Horticultural Sciences Department, University of Florida, Gainesville, 2550 Hull Road, FL, USA

Genomic Selection 1.0

What is the importance of better genomic resources?

Background

- Back to 2019, no genome reference for blueberry.
- Available only a poor draft
- Challenges:
 - Design our genotyping platforms
 - Poor gene annotation for gene mining
 - Complex to design markers for marker assisted selection (MAS)

Genomic Selection 1.0

What is the importance of better genomic resources?

Background

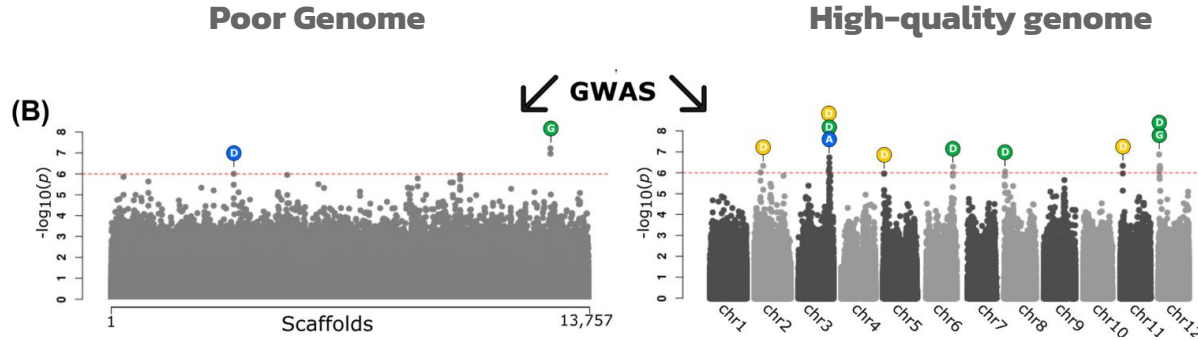
- Back to 2019, no genome reference for blueberry.
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Good news:

- Started a collaboration with the Michigan State (Patrick Edger)
- Access to a high-quality and haplotype-phased reference genome (Colle et al., 2019)

Genomic Selection 1.0

What is the importance of better genomic resources?



More associations

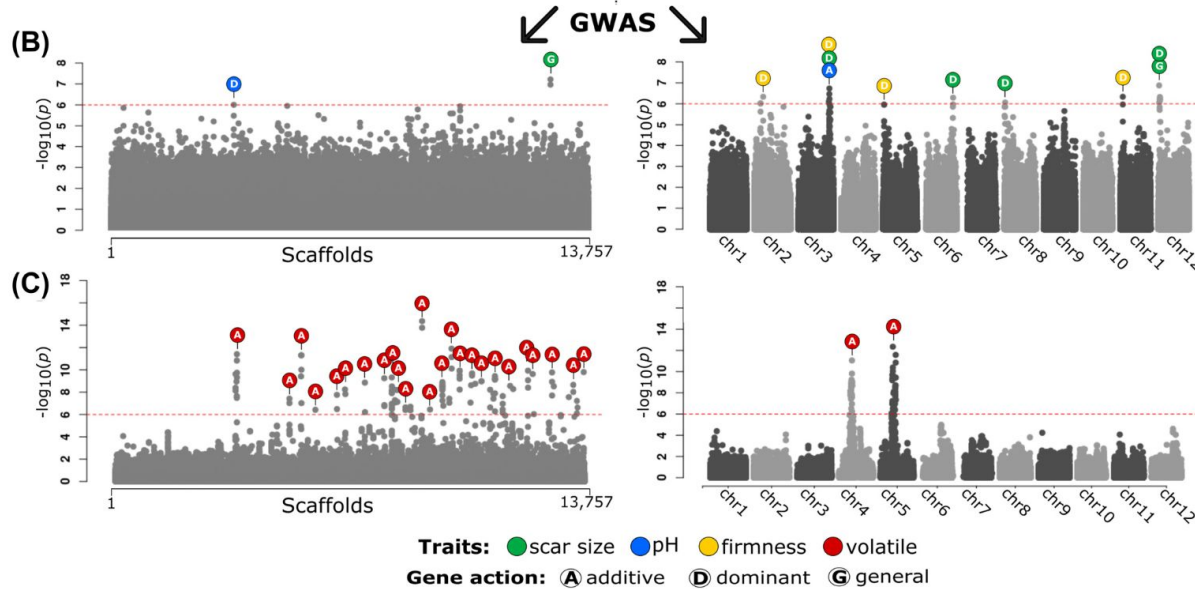
Traits: ● scar size ● pH ● firmness ● volatile
Gene action: ● A additive ● D dominant ● G general

Genomic Selection 1.0

What is the importance of better genomic resources?

Poor Genome

High-quality genome



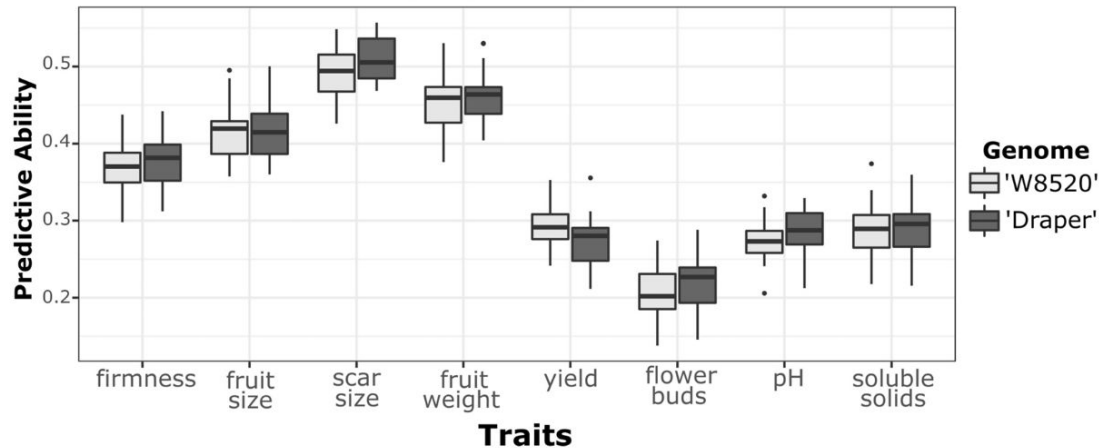
More associations

Better understanding of the genetic architecture

Genomic Selection 1.0

What is the importance of better genomic resources?

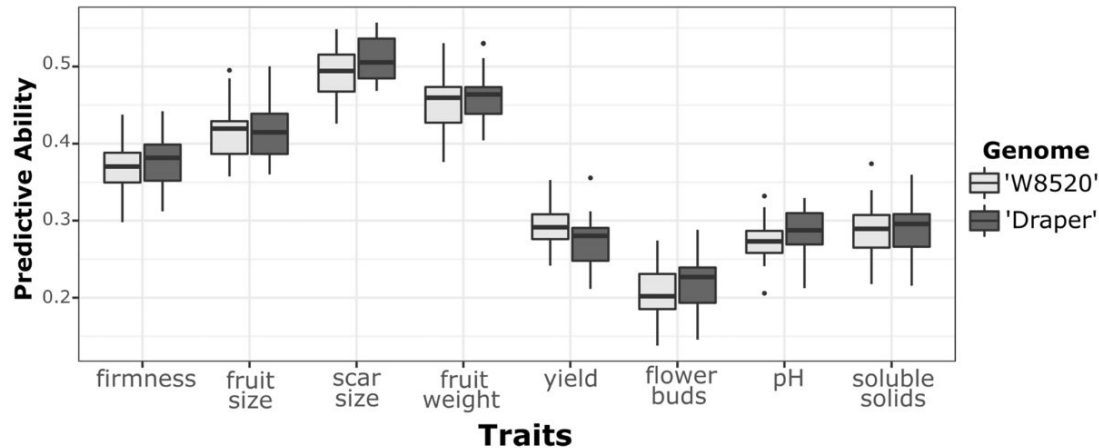
Reduced the number of probes (from 30k to 10k) only using bioinformatic



Genomic Selection 1.0

What is the importance of better genomic resources?

Reduced the number of probes (from 30k to 10k) only using bioinformatic



Take home message

Good genomic resources can help plant breeders

Genomic Selection 1.0

Questions:

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- **Can we unify prediction and discovery in a single framework?**

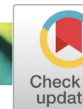


Genomic Selection 1.0







Can we unify prediction and discovery in a single framework?



Research



Genome-wide association of volatiles reveals candidate loci for blueberry flavor

Luís Felipe V. Ferrão^{1*} , **Timothy S. Johnson^{2*}** , **Juliana Benevenuto¹** , **Patrick P. Edger³** ,
Thomas A. Colquhoun²  and **Patricio R. Munoz¹** 

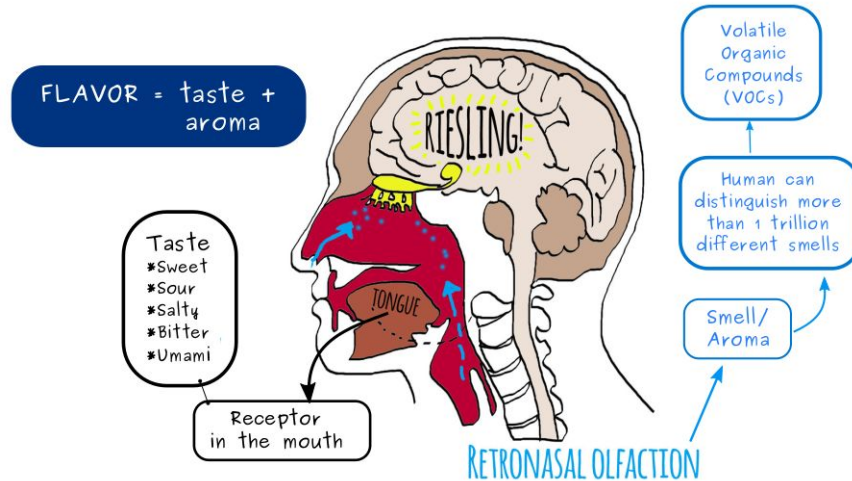
¹Blueberry Breeding and Genomics Lab, Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA; ²Environmental Horticulture Department, Plant Innovation Center, University of Florida, Gainesville, FL 32611, USA; ³Department of Horticulture, University of Michigan, Michigan State University, East Lansing, MI 48824, USA

Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?

What is Flavor?

- Flavor is the sum of inputs from multiple senses that inform our brain what we are eating



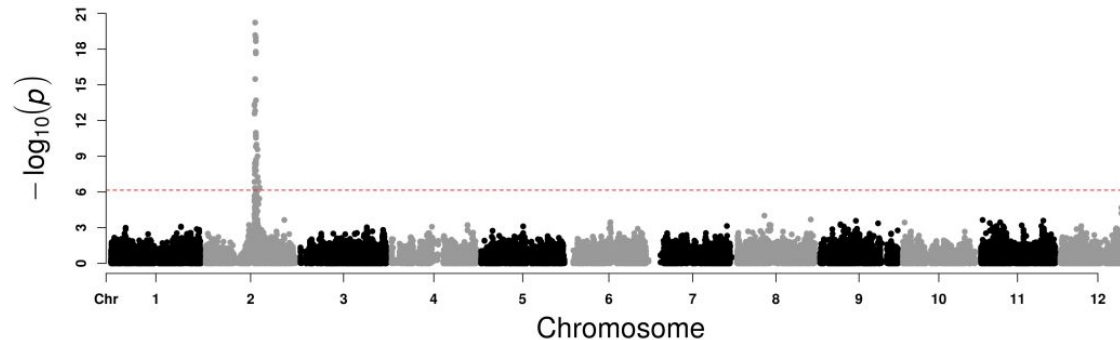
Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?

Evidences based on the GWAS analysis:

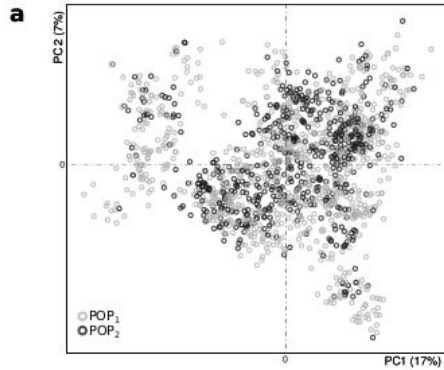
- Significant SNPs converging to a tower-like structure
- Single markers explaining large portions of the phenotypic variation
- Hypothesis: VOCs are traits with simple genetic architecture

2-undecanone



Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?

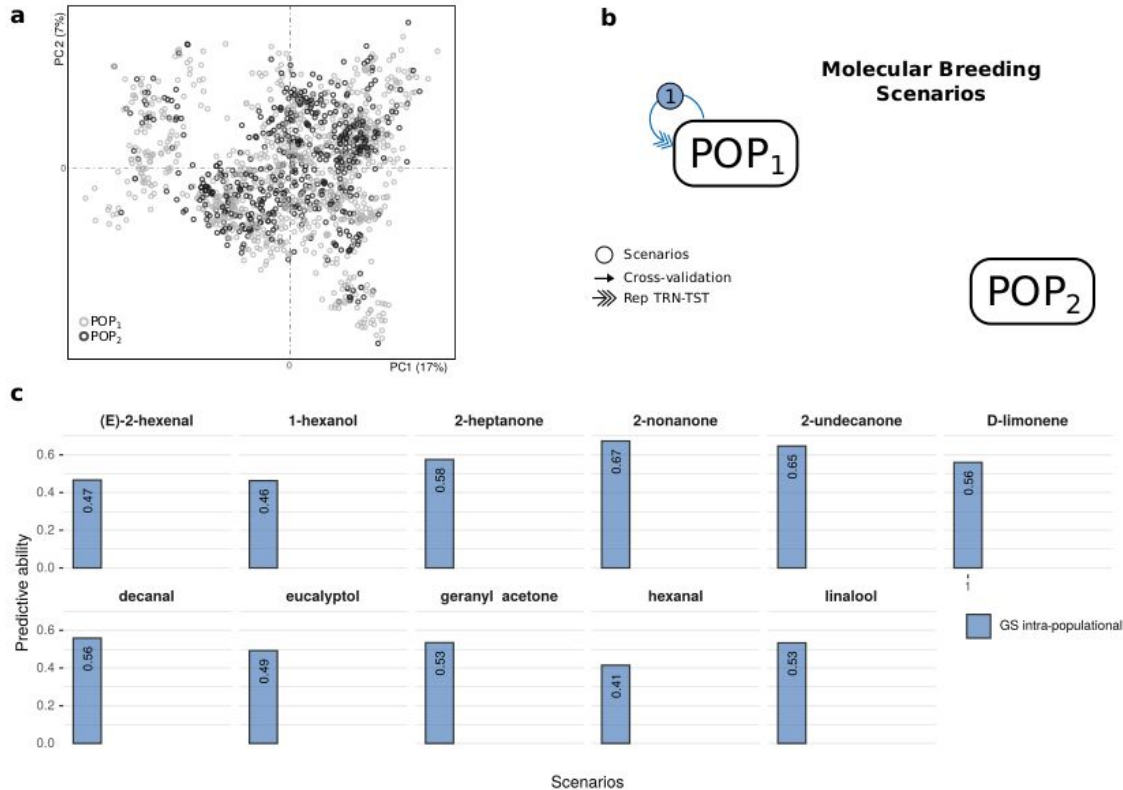


- *Original Population
- *GWAS
- *90 full-sib families

- *New Population
- *Validation
- *Genetic related to POP₁

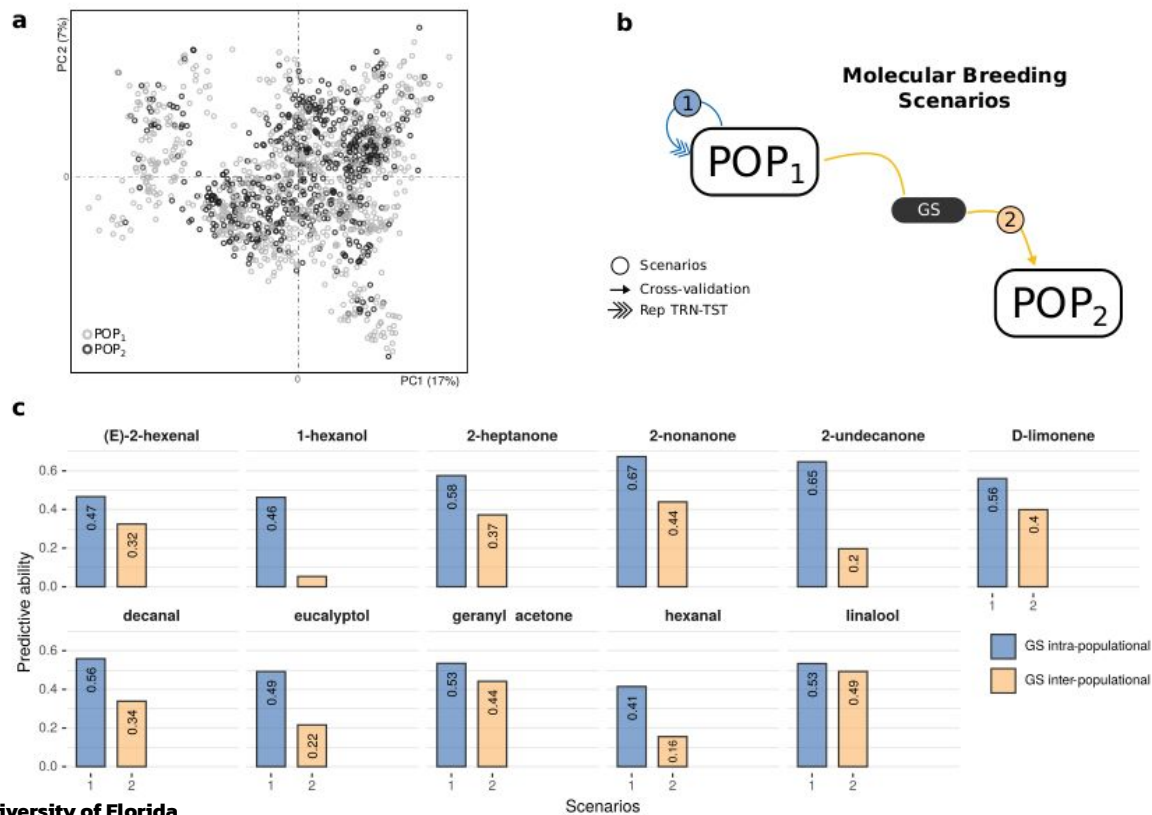
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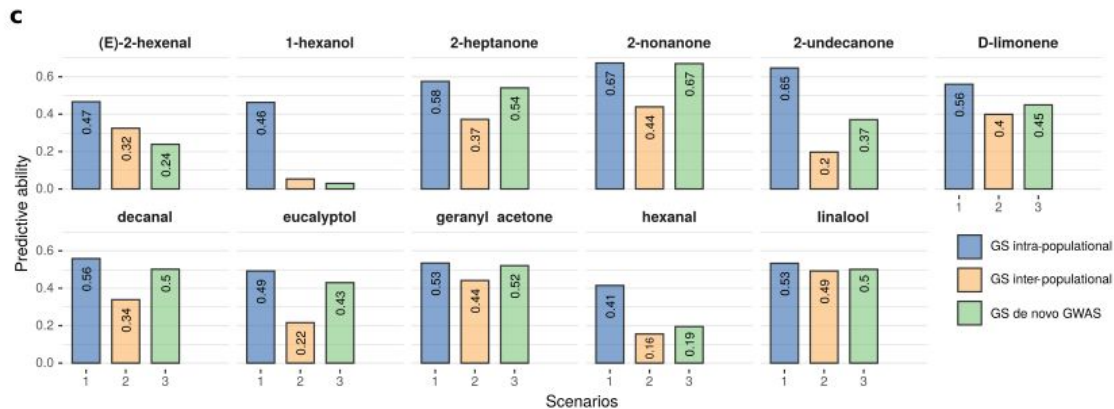
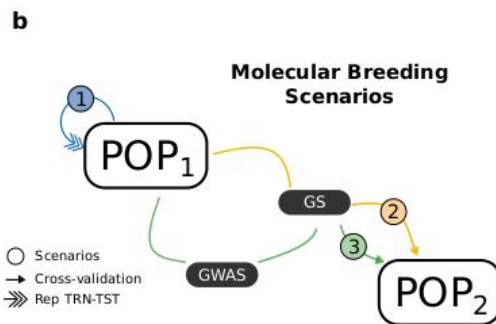
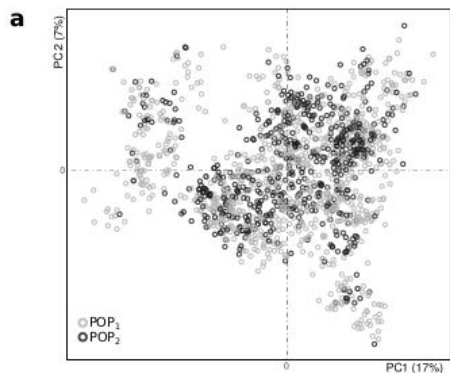
Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?



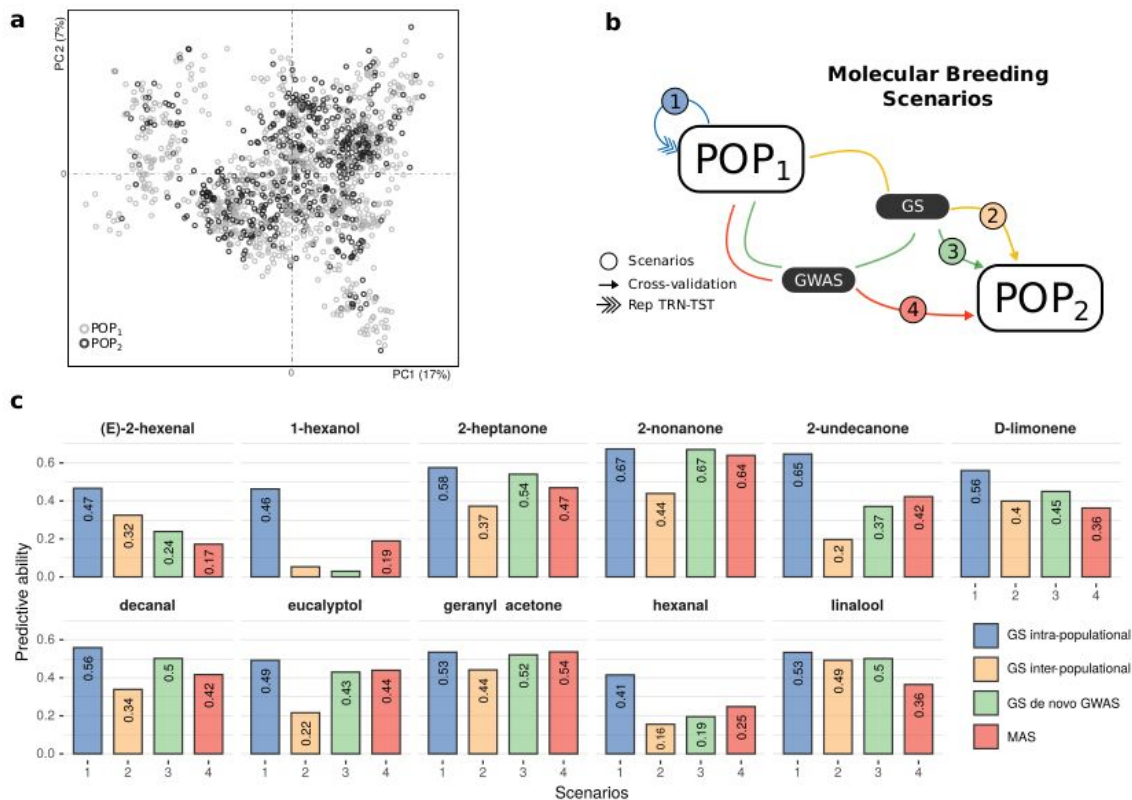
Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?



Genomic Selection 1.0

Can we unify prediction and discovery in a single framework?



Take home message

Prior biological information
can improve prediction
ability

Genomic Selection 2.0

Present and future in blueberry

What is the future?

New methods



New traits



Metabolomic Selection



Metabolomics for Flavor

Artificial Intelligence



Genomic Selection 2.0

Questions

- Can we reduce the number of markers and optimize our training set?
- Is multi-omic predictions a good alternative?
- What is the impact of phenomics on fruit quality prediction?
- How AI can shape the future of modern breeding programs?



Genomic Selection 2.0

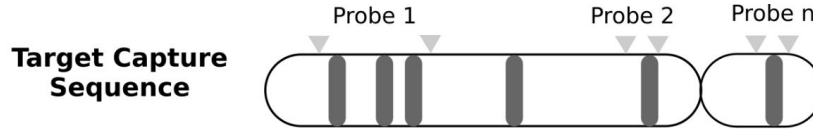
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Genomic Selection 2.0

How low can we go?

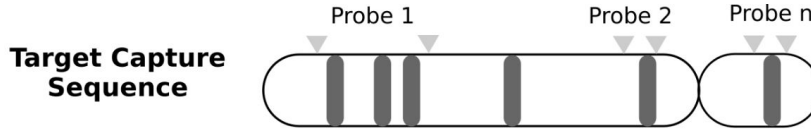


Target Capture Sequencing

- 30k probes originally designed in 2013 (Ferrão et al., 2018)
- 10k probes redefined after the reference genome (Benevenuto et al., 2019)
- Probes are targeting genomic regions aligning to the 4 homologous, and well distributed
- Different #SNPs per probe, and quality parameters

Genomic Selection 2.0

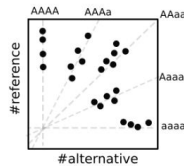
How low can we go?



Sequencing

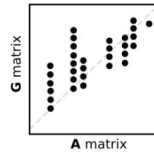
- 1 MAF
- 2 Call rate
- 3 #SNPs per probe

updog



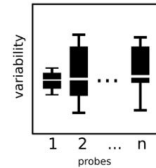
- 4 Modeling Bias
- 5 Overdispersion
- 6 Sequencing error

Pedigree



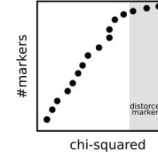
- 8 Correlation (r^2)

Repeatability



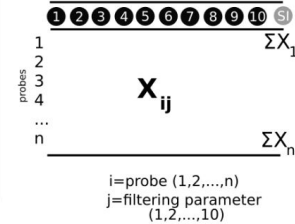
- 9 Repeatability

Segregation Distortion



- 10 Distortion

Ranking



- Data-driven methods to select a final number of probes
- It includes quality and genetic information
- We used a selection index to weight all these information

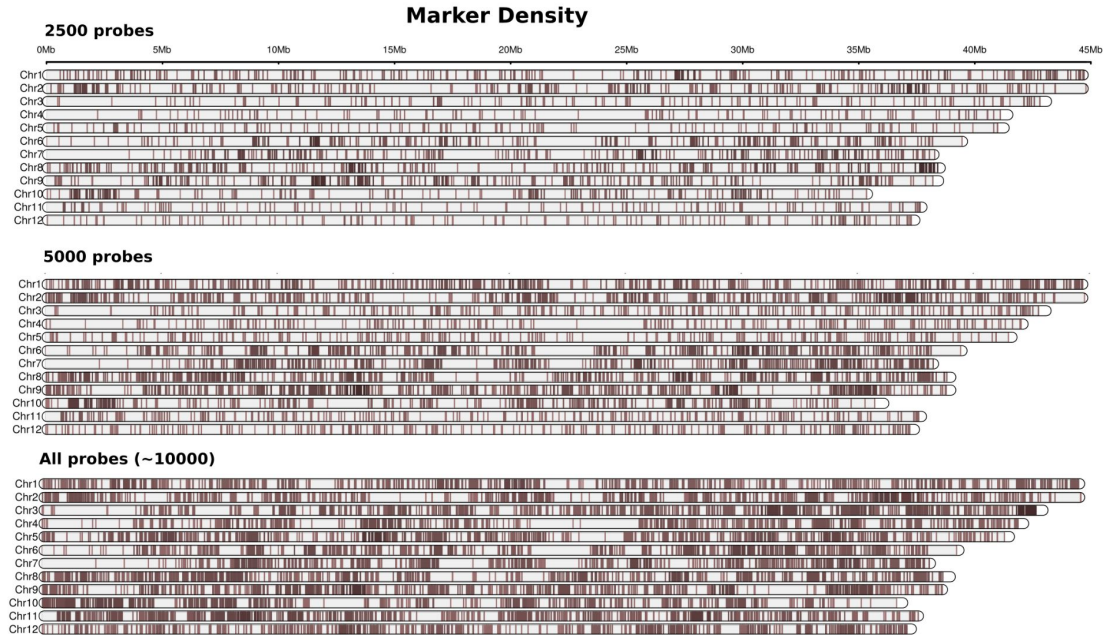
Genomic Selection 2.0

How low can we go?



Optimization
Simulation • Mate Allocation

Camila Azevedo
Breeding Program



Genomic Selection 2.0

How low can we go?

Can we keep reducing the number of probes?

# probes	Brix	Firmness	TTA
All (10k)	0.31	0.50	0.42
2500	0.32	0.48	0.40
5000	0.32	0.49	0.41

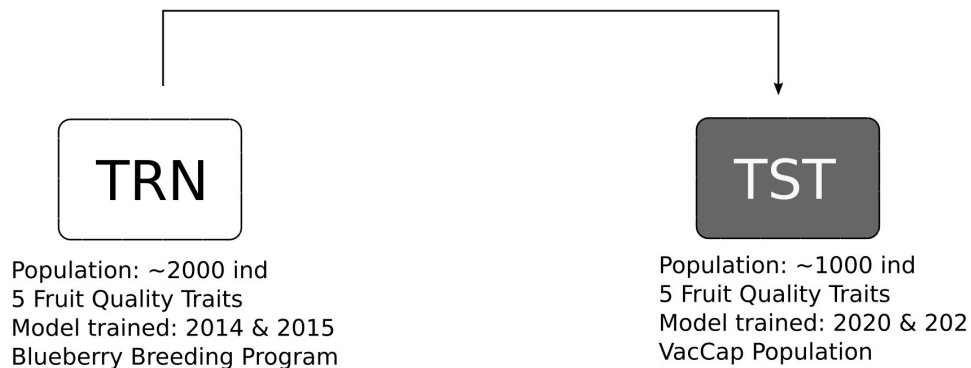
- All prediction abilities computed in cross-validation
- Probes are regions in the genome where SNPs will be mapped
- 2500 probes (~25% of the total costs) results in good predictive ability for multiple traits

Genomic Selection 2.0

How low can we go?

What about the TRN population ?

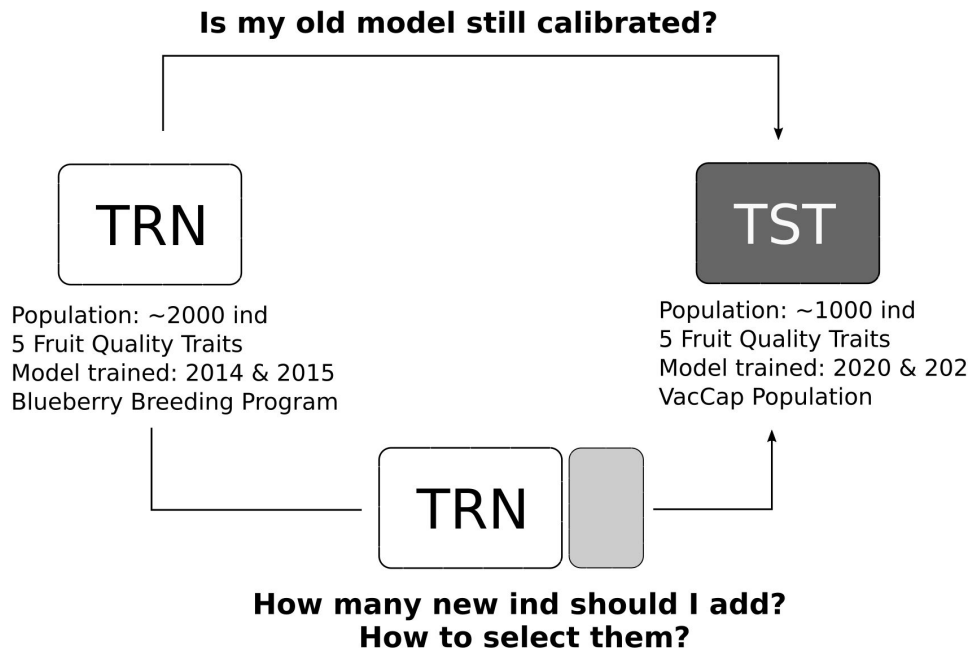
Is my old model still calibrated?



Genomic Selection 2.0

How low can we go?

What about the TRN population ?



Genomic Selection 2.0

How low can we go?



Optimization
Simulation • Mate Allocation

Camila Azevedo
Breeding Program

D)



Genomic Selection 2.0

How low can we go?

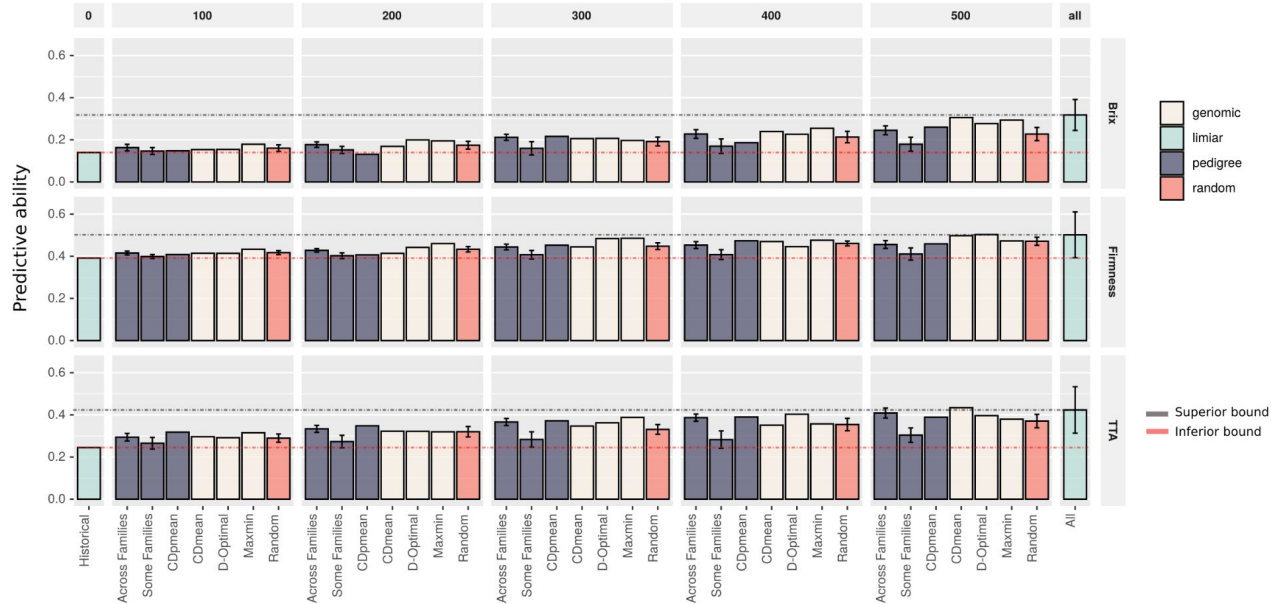


Optimization
Simulation • Mate Allocation

Camila Azevedo
Breeding Program

D)

Predictive ability optimized using different training population designs



Take home message

We can reduce number of markers and optimize our TRN population to maximize the accuracy



Genomic Selection 2.0

Questions

- Can we reduce the number of markers and optimize our training set
- **Is multi-omic predictions a good alternative?**
- What is the impact of phenomics on fruit quality prediction?
- How AI can shape the future of modern breeding programs?



Felipe Ferrão
Breeding Program



Genomic Selection 2.0

Metabolomic Selection



Metabolomic selection for enhanced fruit flavor

Vincent Colantonio^{a,1}, Luis Felipe V. Ferrão^{a,1}, Denise M. Tieman^a, Nikolay Bliznyuk^{b,c,d}, Charles Sims^e, Harry J. Klee^{a,2}, Patricio Munoz^{a,2}, and Marcio F. R. Resende Jr.^{a,2}

^aHorticultural Sciences Department, University of Florida, Gainesville, FL 32611; ^bDepartment of Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611; ^cDepartment of Biostatistics, University of Florida, Gainesville, FL 32611; ^dDepartment of Statistics, University of Florida, Gainesville, FL 32611; and ^eFood Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611

Contributed by Harry J. Klee; received August 27, 2021; accepted December 23, 2021; reviewed by Edward Buckler and Margaret Worthington

Although they are staple foods in cuisines globally, many commercial fruit varieties have become progressively less flavorful over time. Due to the cost and difficulty associated with flavor phenotyping, breeding programs have long been challenged in selecting for this complex trait. To address this issue, we leveraged targeted metabolomics of diverse tomato and blueberry accessions and their corresponding consumer panel ratings to create statistical and machine learning models that can predict sensory perceptions of fruit flavor. Using these models, a breeding program can assess flavor ratings for a large number of genotypes, previously limited by the low throughput of consumer sensory panels. The ability to

program. The difficulties associated with accurate flavor phenotyping have contributed to the lack of selection for fruit flavor and thereby contributed to the widespread consumer belief that commercial fruit flavor has declined (6, 7). Cheap and scalable flavor selection methods would greatly benefit the breeding process.

The main driver of fruit flavor perception is its chemical composition. Fruits contain a diverse array of sugars, acids, and volatiles whose concentrations are driven by genetic and environmental effects. Sugars and acids are largely perceived by taste receptors on the tongue and the volatiles by receptors

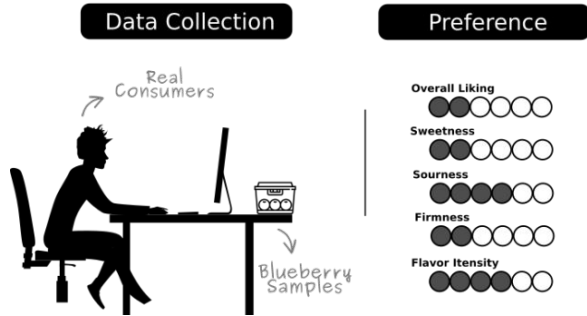


Genomic Selection 2.0

Metabolomic Selection

How to evaluate flavor?

1 Sensory Panels



- Most accurate way to evaluate flavor preference is by providing consumers with a sample set of diverse food and quantify their opinion
- Limitations:
 - Expensive
 - Time-consuming
 - Low throughput

Genomic Selection 2.0

Metabolomic Selection



Metabolomics
for **Flavor**

Felipe Ferrão
Breeding Program

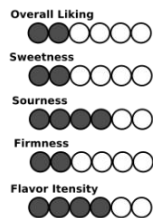
How to evaluate flavor?

1 Sensory Panels

Data Collection



Preference

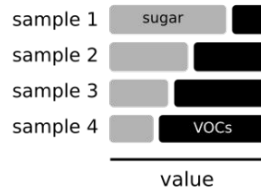


Chemical



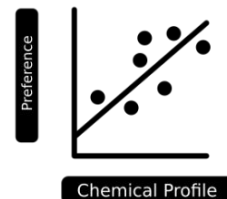
Sugar + acids
Volatiles

Chemical Profile

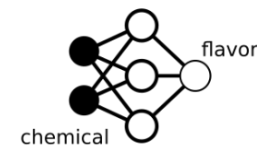


3 Statistical Analyses

Association



Prediction



Predict consumer preferences using chemical and genetics information








Genomic Selection 2.0

Metabolomic Selection



1) Organized Historical Data

Tomato and Blueberry UF Breeding Programs

	Tomato	Blueberry
	209 samples	244 samples
	34 Taste Panels (2010-2015)	53 Taste Panels (2012-2017)
	2844 panelists	3763 panelists
	68 VOCs + 6 Sugars/Acids	49 VOCs + 6 Sugars/Acids
	Liking, Texture, Sweetness, Sourness, Intensity, Salty, Bitter, Umami	Liking, Texture, Sweetness, Sourness, Intensity

$$\text{Sensory} \sim f(\text{metabolites})$$

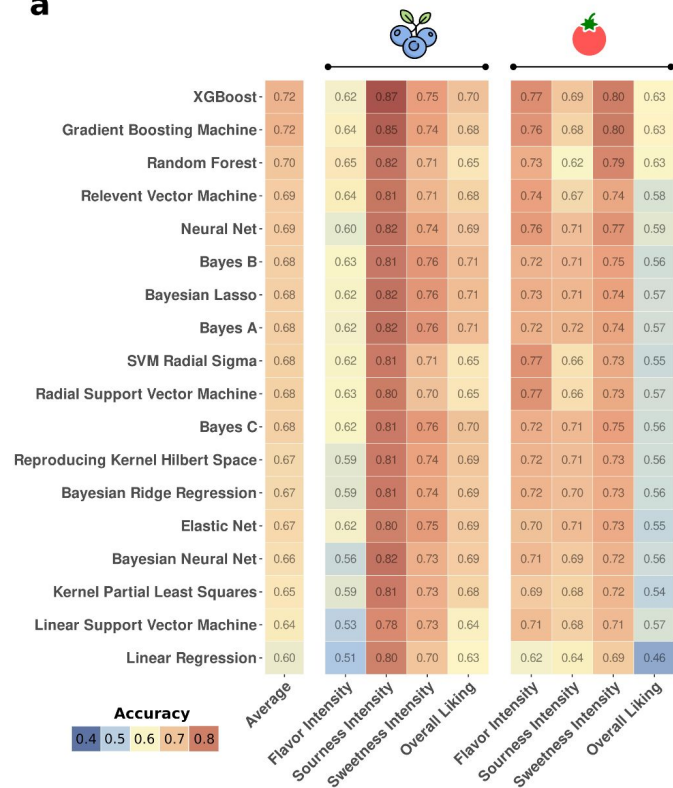
Can we predict flavor preference?

- We tested different machine learning and statistical approaches for prediction
- Historical sensory and chemical data
- Tomato and blueberry as our biological models



Genomic Selection 2.0

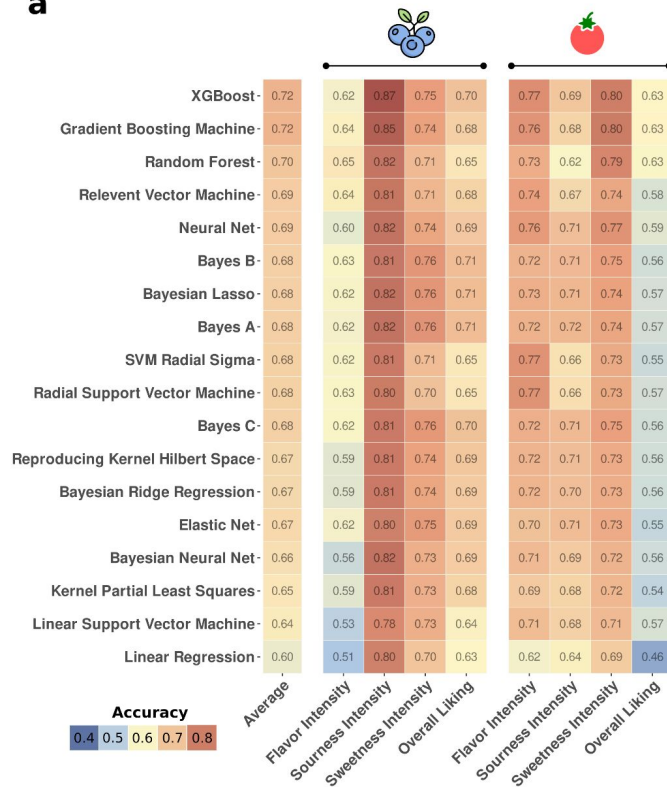
a



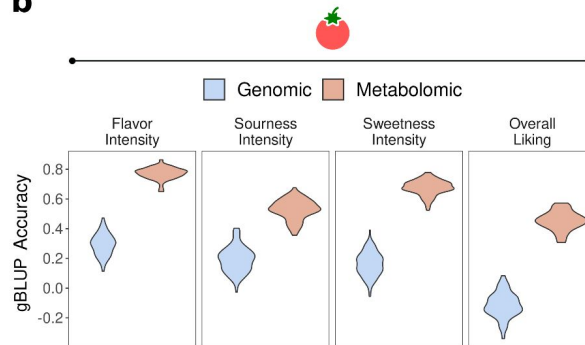
Felipe Ferrão
Breeding Program

Genomic Selection 2.0

a



b



Metabolomic selection >> Genomic Selection



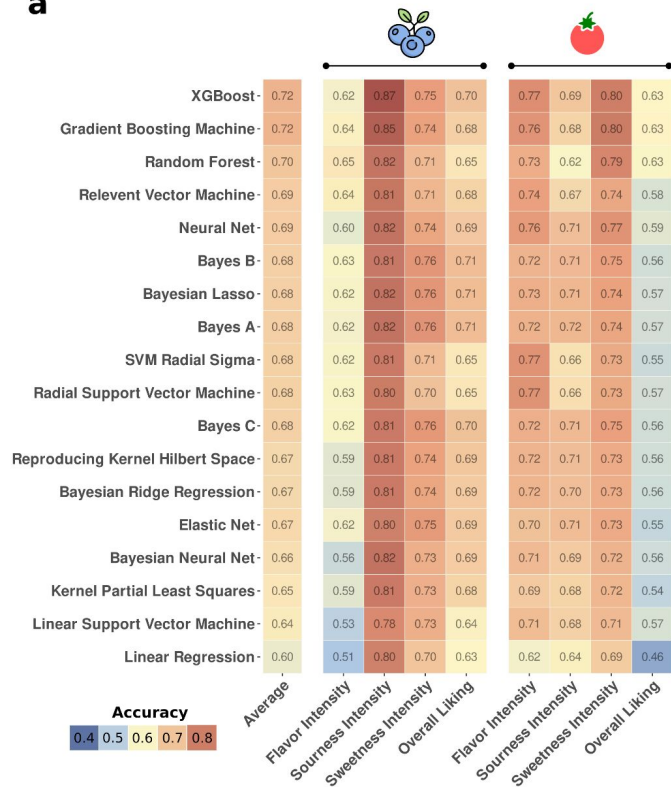
Metabolomics
for **Flavor**

Felipe Ferrão
Breeding Program

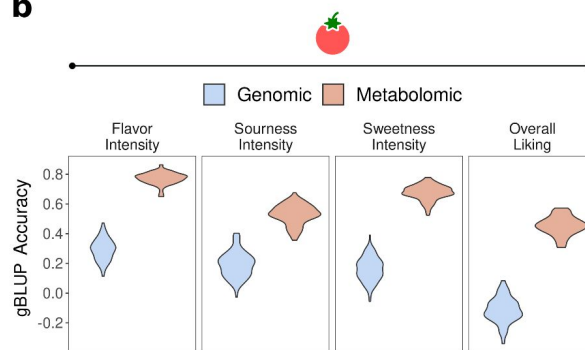


Genomic Selection 2.0

a



b



Metabolomic selection >> Genomic Selection



Metabolomics
for **Flavor**

Felipe Ferrão
Breeding Program



Genomic Selection 2.0

Metabolomic Selection



2022 Blueberry Example

- 1060 blueberry samples
- 60 volatiles, 5 FQ traits and ~50K snps
- Sensory: aroma (1-5) and liking (1-5)
- Two breeders evaluated sensory traits
- Multi-Kernel mixed model

Genomic Selection 2.0

Metabolomic Selection

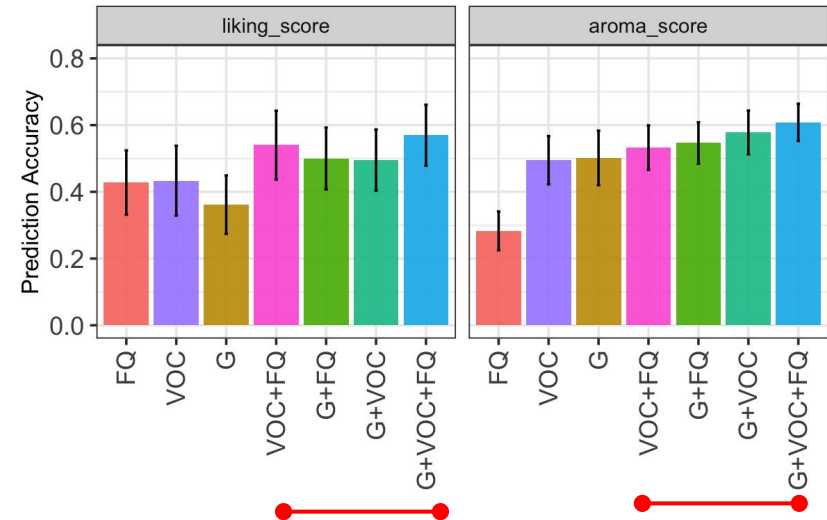


Metabolomics
for **Flavor**

Felipe Ferrão
Breeding Program

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Better predictive abilities when multiple source of information are combined



Genomic Selection 2.0

Metabolomic Selection



Metabolomics
for **Flavor**

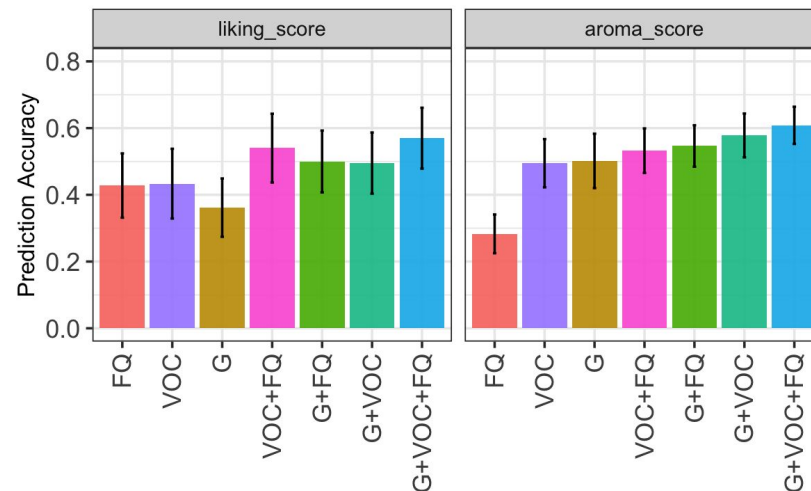
Felipe Ferrão
Breeding Program

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Take home message

Multi-omic data is a valid tool for predicting complex traits



Genomic Selection 2.0

Questions

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- Is multi-omic predictions a good alternative?
- **What is the impact of phenomics on fruit quality prediction?**
- How AI can shape the future of modern breeding programs?



Paul Adunola
PhD project



Genomic Selection 2.0

Phenomic Selection

Field year	Stage	# Plants	Goal
0	0	~150 crosses	Crossing + Seedlings
1	I	20.000	High-density nursery– Single Plant Selection
2-4	II	2.000	Single Plant Selection
5-9	III	200	Farm Condition – Experimental design
10-15	IV	20	Regional Yield Trial – Experimental design
16	V	1-2	Cultivar Release

Genomic Selection 2.0

Phenomic Selection

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Selection Criteria

Visual

GEBV

GEBV + metabolites +
phenotypic data

GEBV + metabolites +
phenotypic data + sensory

Genomic Selection 2.0

Phenomic Selection

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GEBV

GEBV + metabolites +
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GEBV + metabolites +
phenotypic data + sensory

Genomic Selection 2.0

Phenomic Selection

Motivation Phenomic Selection

- Near-infrared spectroscopy (NIRS) is a non-destructive high-throughput method
- It is based on the absorption of electromagnetic radiation in the near-infrared region
- While NIR has been used to predict target traits, recent studies suggested phenomic selection as a low-cost and high-throughput method

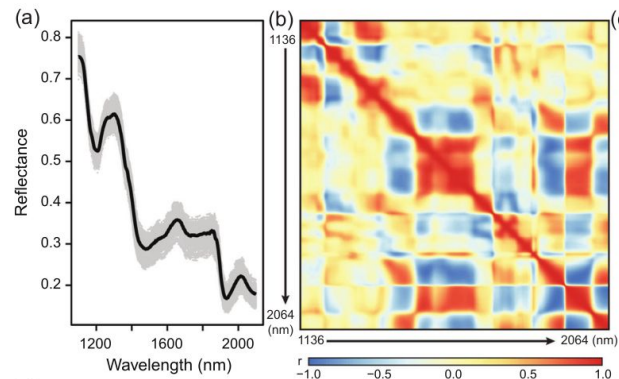
Genomic Selection 2.0

Phenomic Selection

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Example in soybean



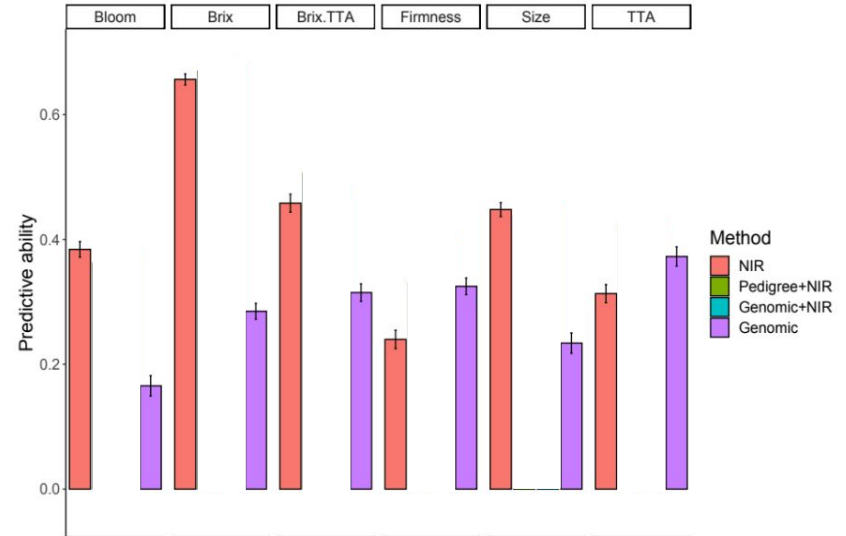
DOI: 10.1002/ppj2.20027

Genomic Selection 2.0

Phenomic Selection

Material and Methods

- Population Size: ~400 ind
- MicroNIR OnSite-W kit: 900–1700 wavelength
- Tissue: mature berries
- Model: P-GBLUP (multi kernel mixed model)



Genomic Selection 2.0

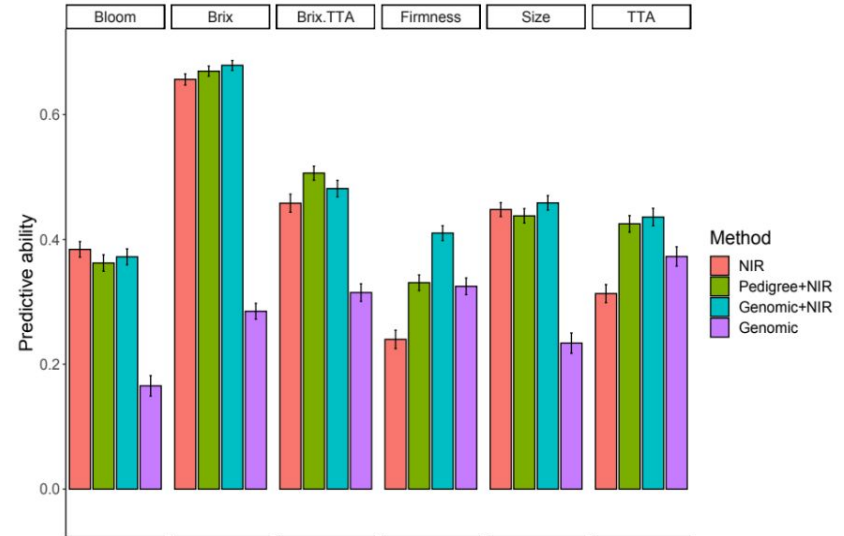
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Be careful...

- Very sensitive to GxE
- Very sensitive to tissue (berry vs. fruit vs. juice)
- Working in progress



Genomic Selection 2.0

Phenomic Selection

Material and Methods

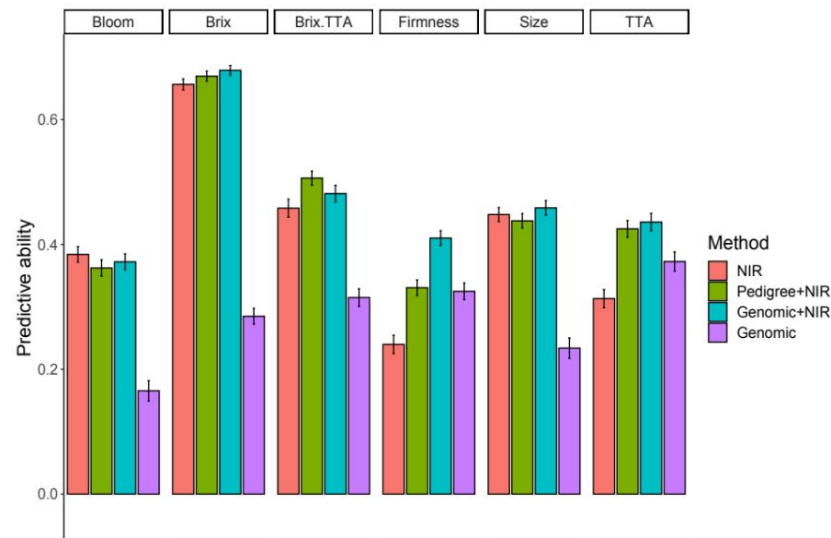
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Be careful...

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- Very sensitive to tissue (berry vs. fruit vs. juice)
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Take home message

An alternative to genomic selection



Genomic Selection 2.0

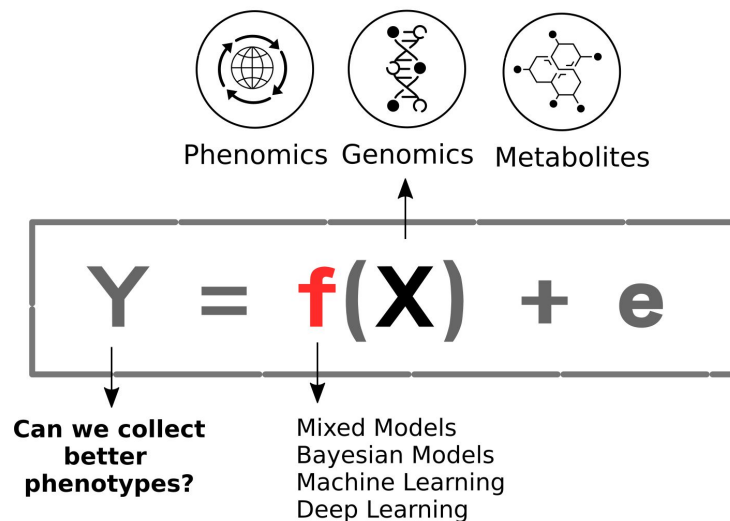
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Genomic Selection 2.0

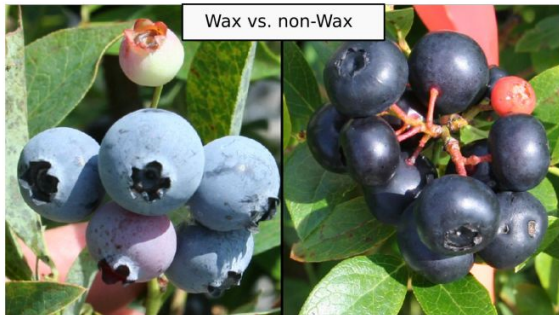
Artificial Intelligence



Genomic Selection 2.0

Artificial Intelligence

Motivation



Qi et al., 2019. doi.org/10.1186/s12870-019-2073-7

- The naturally occurring cuticular wax covering the fruit (a.k.a bloom) is what gives the blueberries their whitish looking
- Important for consumers, post-harvest and disease resistance
- Trait traditionally scored using visual scales

Genomic Selection 2.0

Artificial Intelligence

Motivation



Qi et al., 2019. doi.org/10.1186/s12870-019-2073-7

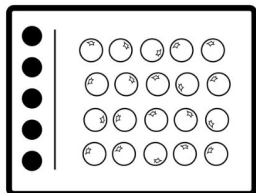
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Genomic Selection 2.0

Artificial Intelligence

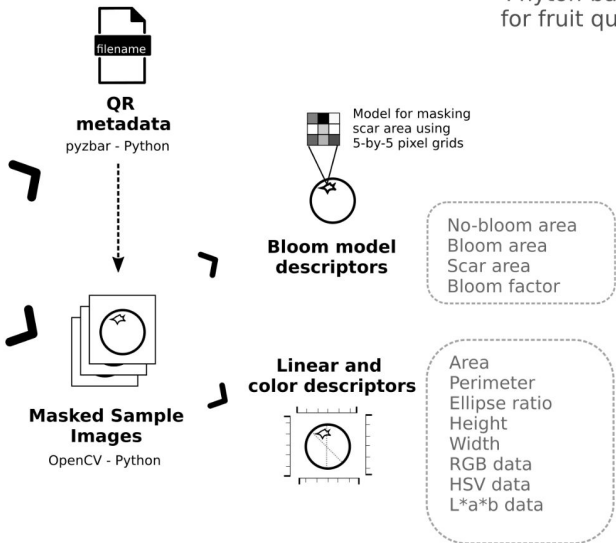
1 Data Collection



2 Raw input images

- individual berries
- reference

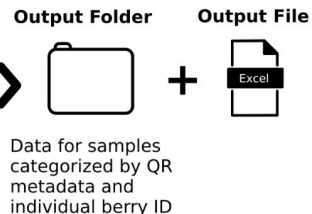
3 Feature Extraction



berryCV

Python-based computer vision workflow for fruit quality phenotyping in blueberry

4 Output results

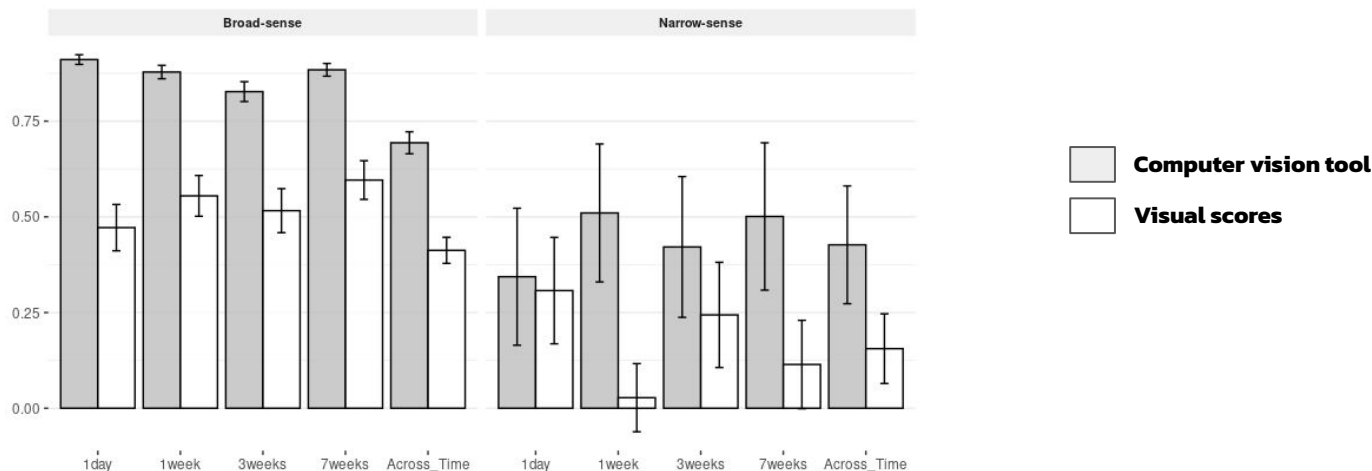


Genomic Selection 2.0

Artificial Intelligence

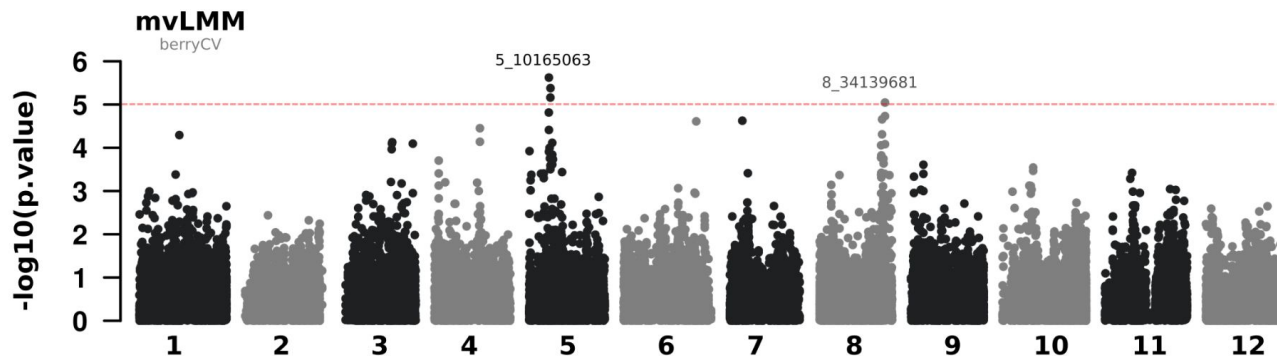
berryCV vs. Traditional Visual scores

- Across different time-points during the post-harvest
- Higher heritability values



Genomic Selection 2.0

Artificial Intelligence



- Hits identified only using computer vision

Genomic Selection 2.0

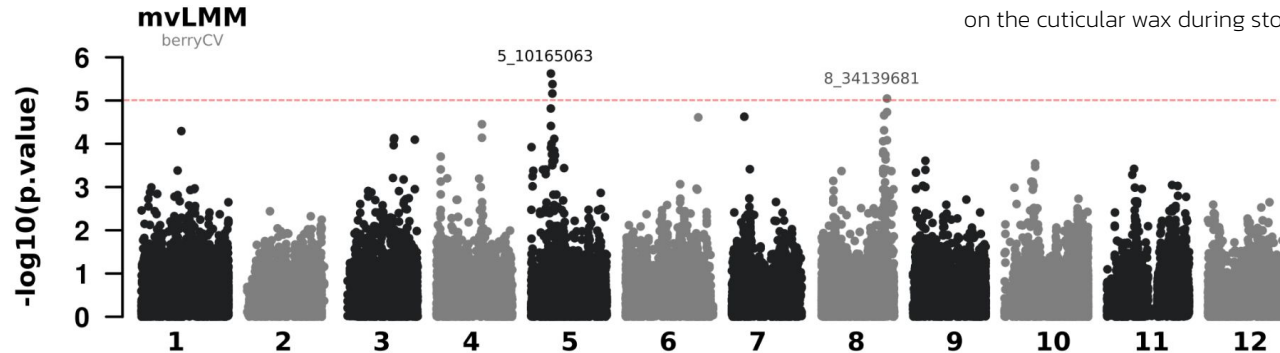
Artificial Intelligence

GDSL lipolytic enzyme family

Genes expressed in the epidermal cells ,
also reported for tomato and pepper

salicylic acid methyl transferase

recent studies in blueberry have
reported a direct effect of salicylic acid
on the cuticular wax during storage



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Genomic Selection 2.0

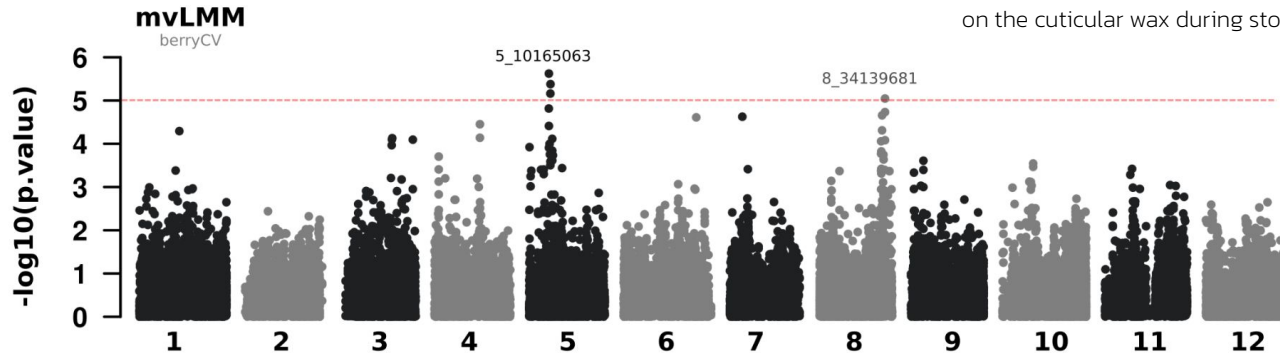
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- Hits identified only using computer vision

Take home message

AI can improve data collection and shed
new light on the genetic architecture

Conclusions

Final message

Conclusions

Final considerations



Other key tools used to support breeding decisions

- 1) To predict the future: stochastic **simulations**
- 2) To optimize the future: **mate allocation** for design crosses
- 3) To save time and money: Marker-Assisted Selection (**MAS**) for seedling selections

Conclusions

Final considerations



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- 1) To predict the future: stochastic **simulations**
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- 4) For biological validation: new protocols for **transformation** and **gene editing**
- 5) For an effective use of plant genetic resources: **pre-breeding** and **introgressions**
- 6) To speed up: testing a new generation of techniques for **speed-breeding**

Conclusions

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- 5) For an effective use of plant genetic resources: **pre-breeding** and **introgressions**
- 6) To speed up: testing a new generation of techniques for **speed-breeding**
- 7) **Artificial Intelligence** is everywhere:
 - a) Sensory Panels (**DeepFlavor**)
 - b) Field data collection (**Computer Vision**)
 - c) New methods for prediction

Conclusions

Final considerations



Seven main lessons from the last 7 years

- Genomic Selection works!
- Solid gains using data driven methods
- Prefer statistical genetics methods designed for polyploid systems
- "Simplicity is the Ultimate Sophistication"¹: on the Relevance of Additive GBLUP Models
- GS can be optimized, after having a good understanding about the breeding pipeline
- Knowledge is power: decisions based on multi-layer (or omics) on information
- AI has an important play to role on data collection and analyses

¹ Quote by Leonardo da Vinci



Conclusions

Final considerations



My personal opinion for practical implementation in polyploids:

- Genomic selection is a tool to assist breeding and not the other way around
- Implementation require a solid breeding program
- Genotyping might be not so easy and cheap
- Collect good phenotype is imperative
- Test new methods is valid. But don't forget to include an additive GBLUP as a benchmark
- Genomic Selection is multidisciplinary topic
- Biological discovery and prediction can run side-by-side



Acknowledges

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HOS departament

Marcio Resende Jr.
Denise Tieman
Harry Klee
Ali Sarkhosh
Lorenzo Rossi
Guilherme Locatelli
Jonathan Crane
Catalin Voiniciuc

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Charlie Sims
Raquel Dias
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Massimo Iorizzo (NCState)
Mary Ann Lila (NCState)
Antonio Augusto F. Garcia (USP)
Christophe Montagnon (R2D vision)
Incapert team (Brazil)
Embrapa team (Brazil)
Jeff Endelman (Wisconsin)
Rodrigo Amadeu (Bayer)
James Harynuk (University of Alberta)



Join us in PAG !



Abstract #53796

Genome-Wide Association Analyses Reveal Candidate Genes Associated with Health Components in Blueberry



Talk: Fruit and Nuts Workshop

Let's be Categorical: Different Genetic Statistical and Logistic Strategies to Increase Accuracy of Genomic Selection in an Apply Breeding Program



Talk: Plant Reproductive Genomics Workshop

Identification of markers and candidate genes for blueberry parthenocarpy through integration of GWAS and GS methodology



Abstract #53389

GWAS and Genomic Selection Strategies for Breeding Anthracnose Resistance in Southern Highbush Blueberries



Abstract #53417

Fruit Phenomic-Assisted Selection: Assessment of the Potential of Near-Infrared Spectroscopy for Blueberry Breeding



Abstract #52794

Chromosome-Level Assembly of Vaccinium stamineum: Unlocking the Genetic Basis of Anthocyanin Accumulation



Abstract #53463

Enhancing Blueberry Fruit Quality Traits Using Consumer-Centric Genomic Breeding



Talk: Coffee Workshop

Genomic-assisted breeding for climate-smart coffee cultivars

**Thank
you !!!**

Question ??

About Me:

SCAN ME



Extra Questions

Genomic Selection 2.0

Questions

- What is the impact of genomic selection in the long term?
- Can we reduce the number of markers and optimize our training set?
- Is multi-omic predictions a good alternative?
- What is the impact of phenomics on fruit quality prediction?
- **Is marker-assisted selection an alternative for seedlings selections?**
- How AI can shape the future of modern breeding programs?



Genomic Selection 2.0

Marker-assisted selection (MAS)

Motivation

- First breeding stages generates large volume of seedlings (~20.000)
- Moving all plants to the field is expensive and time-consuming for evaluation
- Genomic Selection is not an alternative at this level (\$\$\$\$)
- Can we use few markers, for key traits, to discard plants at the greenhouse level?

Genomic Selection 2.0

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Goals

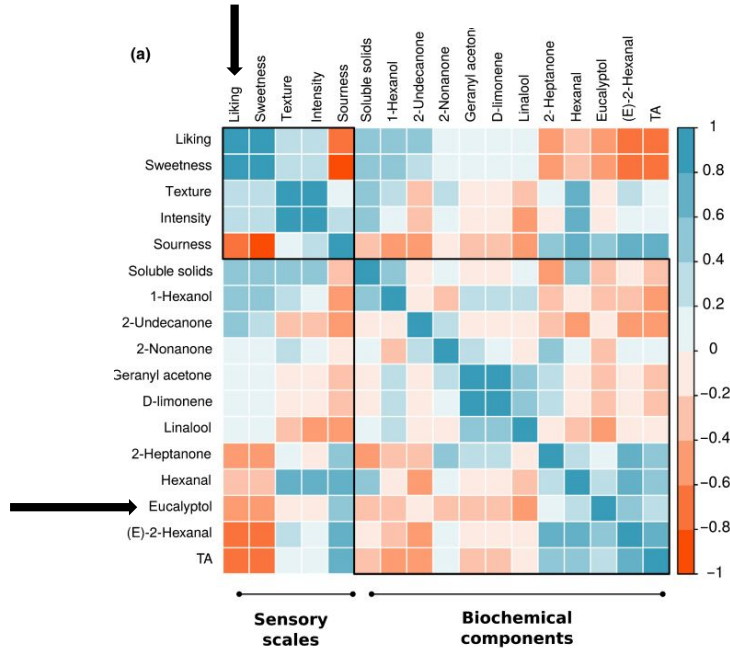
- Develop a rapid and cheap assay for DNA extraction
- Establish protocols for SNP genotyping using HRM and KASP
- Validate association between markers and phenotypes

Genomic Selection 2.0

Marker-assisted selection (MAS)

sensory~VOC

Eucalyptol negatively impact consumer preference



Ferrão et al., (2020)

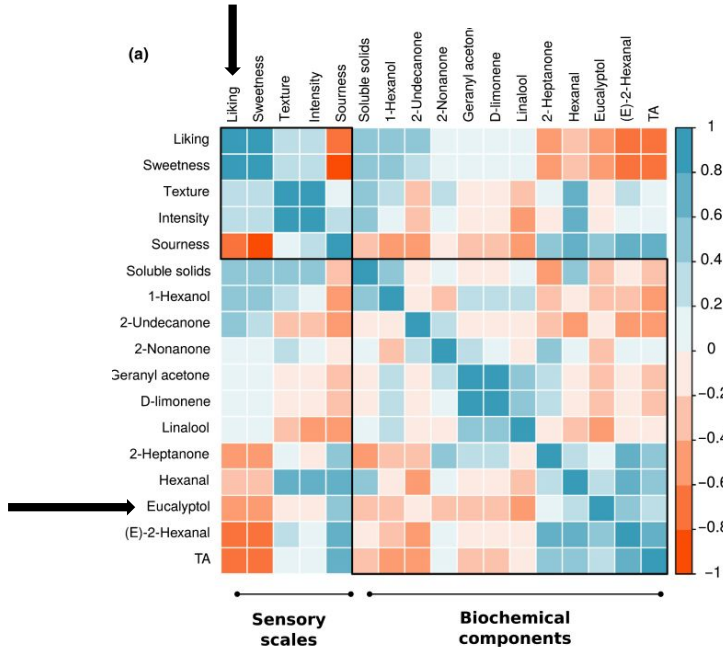
<https://doi.org/10.1111/nph.16459>

Genomic Selection 2.0

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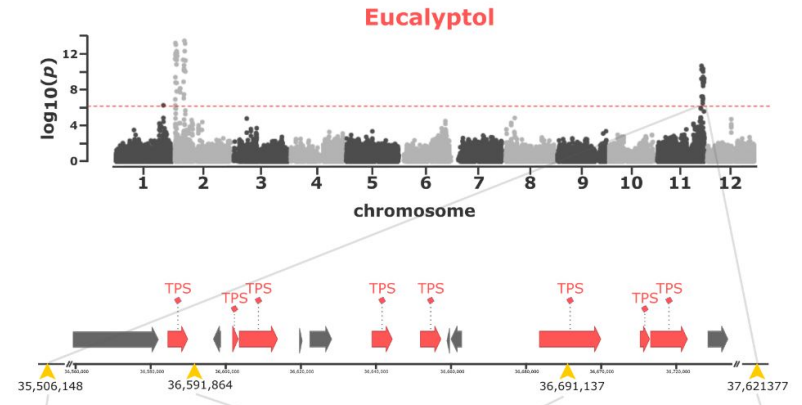
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Genome-wide association analyses

Eucalyptol has a simple genetic architecture



Ferrão et al. (2020)

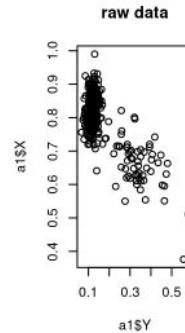
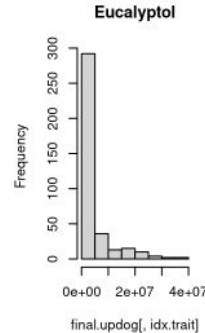
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Genomic Selection 2.0

Marker-assisted selection (MAS)

KASP markers

- Target: Eucalyptol
- Population: 384 individuals
- Single marker explaining > 50% phenotypic variance



Genomic Selection 2.0

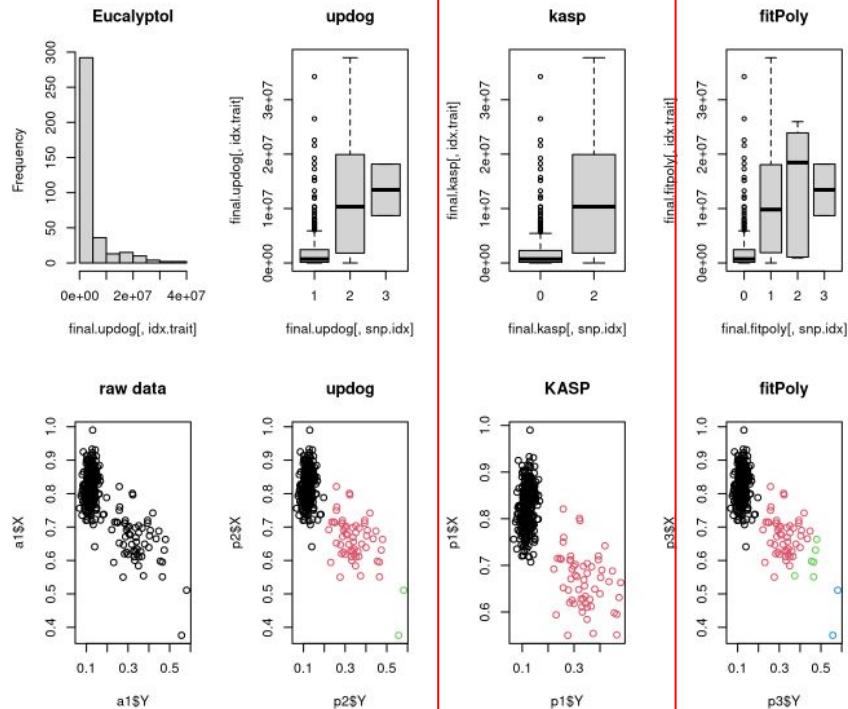
Marker-assisted selection (MAS)

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Multiplex vs. Duplex

Discriminate high vs. low eucalyptol

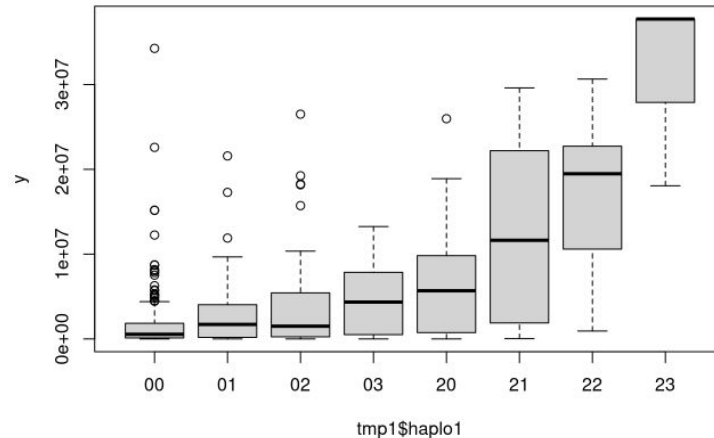


Genomic Selection 2.0

Marker-assisted selection (MAS)

Can we combine markers?

- Markers from two different chromosomes
- Marker1 + Marker2 = pseudo-haplotype
- Pseudo-haplotypes 22 (duplex-duplex) and 23 (duplex-triplex) high levels of eucalyptol

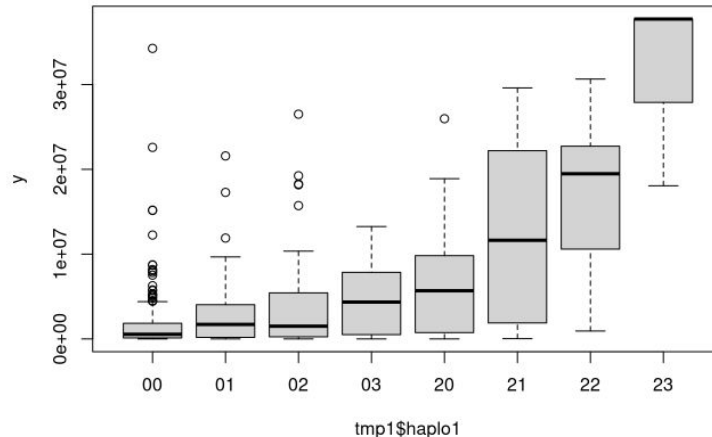


Genomic Selection 2.0

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Take home message

Cheap and low density marker platforms can be used to discard plants in the seedling stage

Genomic Selection 2.0

What is the impact of genomic selection in the long term?



Simulation

- We can use stochastic oriented simulation to project the future
- Oriented because we can use real information from the breeding program
- AlphaSim package, to simulate a complex trait ($h^2=0.30$, 100 QTL)



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PARENTAL SELECTION

Different criteria for selection and mate allocation in the UF/IFAS breeding program

Selection Criteria

1. Massal Selection
2. EBV (A-BLUP)
3. GEBV (G-BLUP)

Mate allocation for GS

1. GEBV based on parental means (GS)
2. GEBV + SimpleMatting (GS -Opt)

Genomic Selection 2.0

What is the impact of genomic selection in the long term?



Optimization
Simulation • Mate Allocation

Camila Azevedo
Breeding Program

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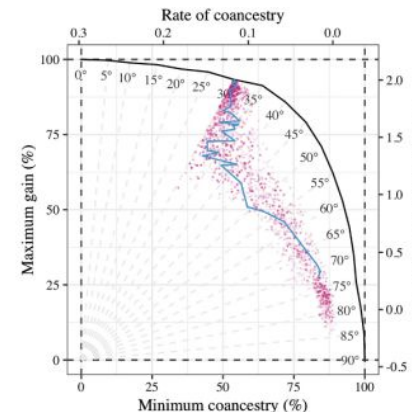
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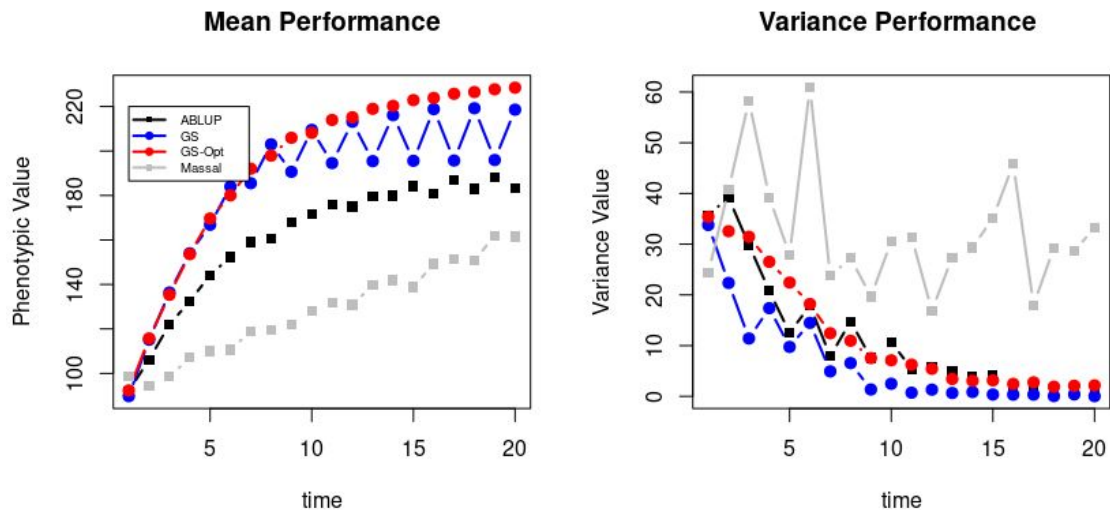
1. GEV based on parental means (GS)
2. GEV + SimpleMating (GS -Opt)

Example from AlphaMate



Genomic Selection 2.0

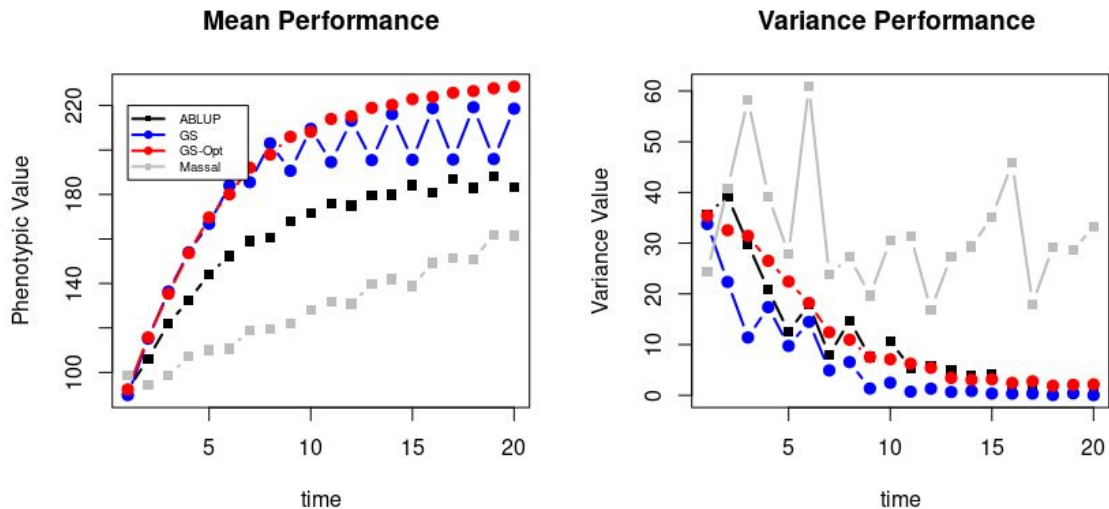
What is the impact of genomic selection in the long term?



Azevedo et al, 2023. Stochastic Simulation in Blueberry. [In preparation](#)

Genomic Selection 2.0

What is the impact of genomic selection in the long term?



Azevedo et al, 2023. Stochastic Simulation in Blueberry. [In preparation](#)

Take home message

GS and mate allocation can maximize the gains in the long term