

18TH ANNUAL STRAWBERRY PRODUCTION MEETING, VENTURA COUNTY, 2019

UNIVERSITY OF CALIFORNIA, DAVIS STRAWBERRY BREEDING PROGRAM



TRADITIONAL & GENOME-INFORMED BREEDING FOR DISEASE RESISTANCE

FUSARIUM WILT, VERTICILLIUM WILT, MACROPHOMINA, & PHYTOPTHORA



USDA NIFA Specialty Crops Research Initiative: Next-Generation Disease Resistance Breeding and Management Solutions for Strawberry

September 1, 2017 - August 31, 2021

- ▶ UC Davis: Thomas Gordon, & Rachael Goodhue
- ▶ University of Florida: Vance Whitaker, Seonghee Lee, & Natalia Peres
- ▶ UC Riverside: Alex Putman
- ▶ Cal Poly: Kelly Ivors & Gerald Holmes
- ▶ UC-ANR: Oleg Daugovich
- ▶ UC Santa Cruz: Julie Guthman



UC SANTA CRUZ

UC RIVERSIDE
UNIVERSITY OF CALIFORNIA

CAL POLY
SAN LUIS OBISPO



United States Department of Agriculture
National Institute of Food and Agriculture



Genetics of Resistance to Fusarium Wilt is Simple, Whereas the Genetics of Resistance to the Other Three Pathogens is Complex (Quantitative)

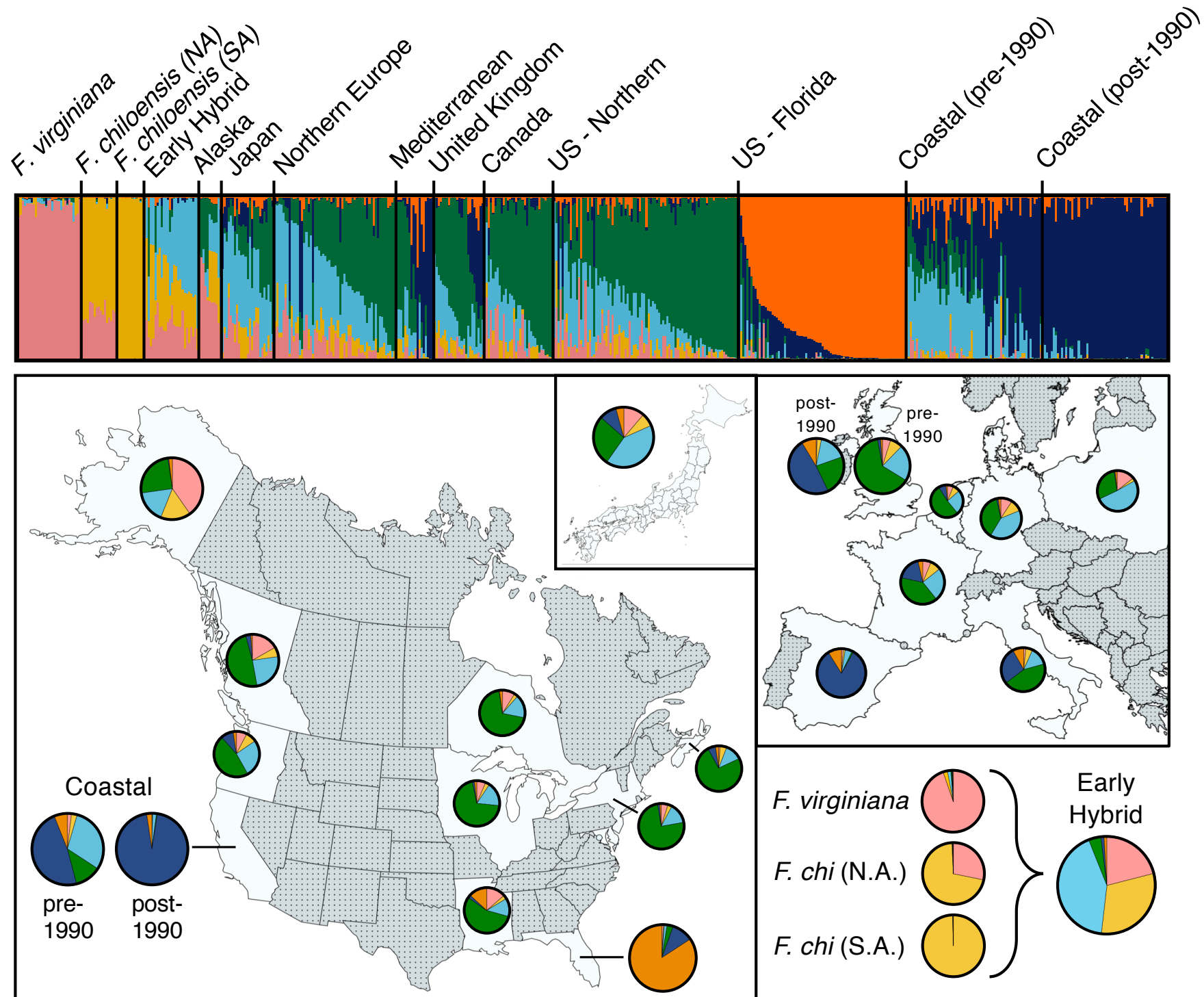
Highly Resistant = 1, Highly Susceptible = 5

Cultivar	Fusarium Wilt	Verticillium Wilt	Phytophthora Crown Rot	Macrophomina
Royal Royce	4.5	3.1	3.3	5.0
Valiant	4.5	3.3	3.0	5.0
Moxie	1.3	2.1	3.5	5.0
Warrior	1.0	2.9	2.9	3.0
Victor	1.0	2.4	4.8	5.0
Cabrillo	4.4	2.3	3.6	5.0
Monterey	4.5	2.7	3.8	5.0
Albion	5.0	2.7	4.9	4.8
Fronteras	1.1	2.9	2.9	4.3
Portola	1.7	3.0	2.3	5.0
Wiltguard	1.1	1.8	2.5	3.0
Shasta	1.6	2.1	2.3	5.0
Camarosa	4.7	3.6	2.7	5.0
Benicia	4.3	4.5	4.3	5.0

Population Structure of Germplasm Worldwide

DNA Profiling of 2,400 Elite and Exotic Germplasm Accessions

Screened 1,000 Horticulturally and Genetically Diverse Cultivars and Other Genotypes for Resistance to the Four Soil-Born Diseases



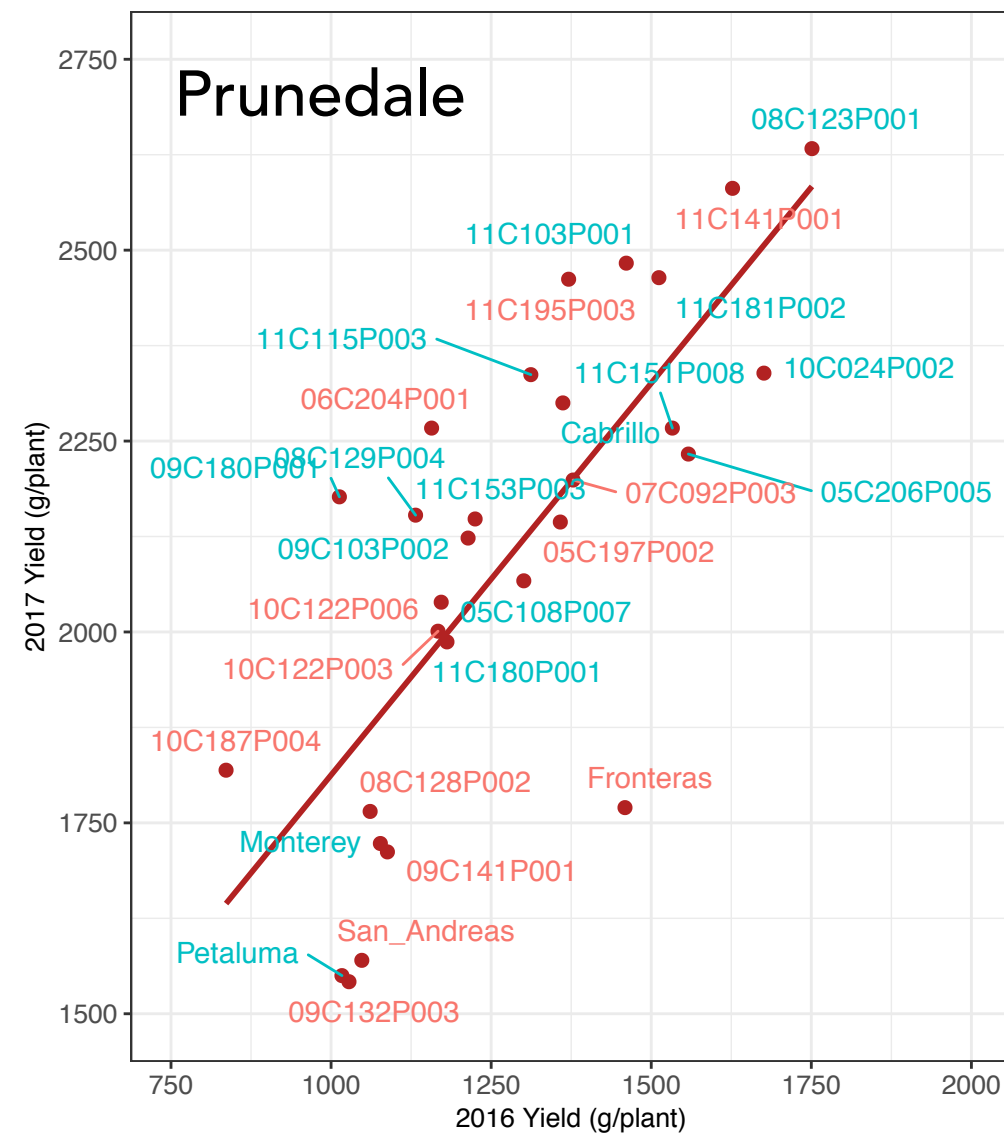
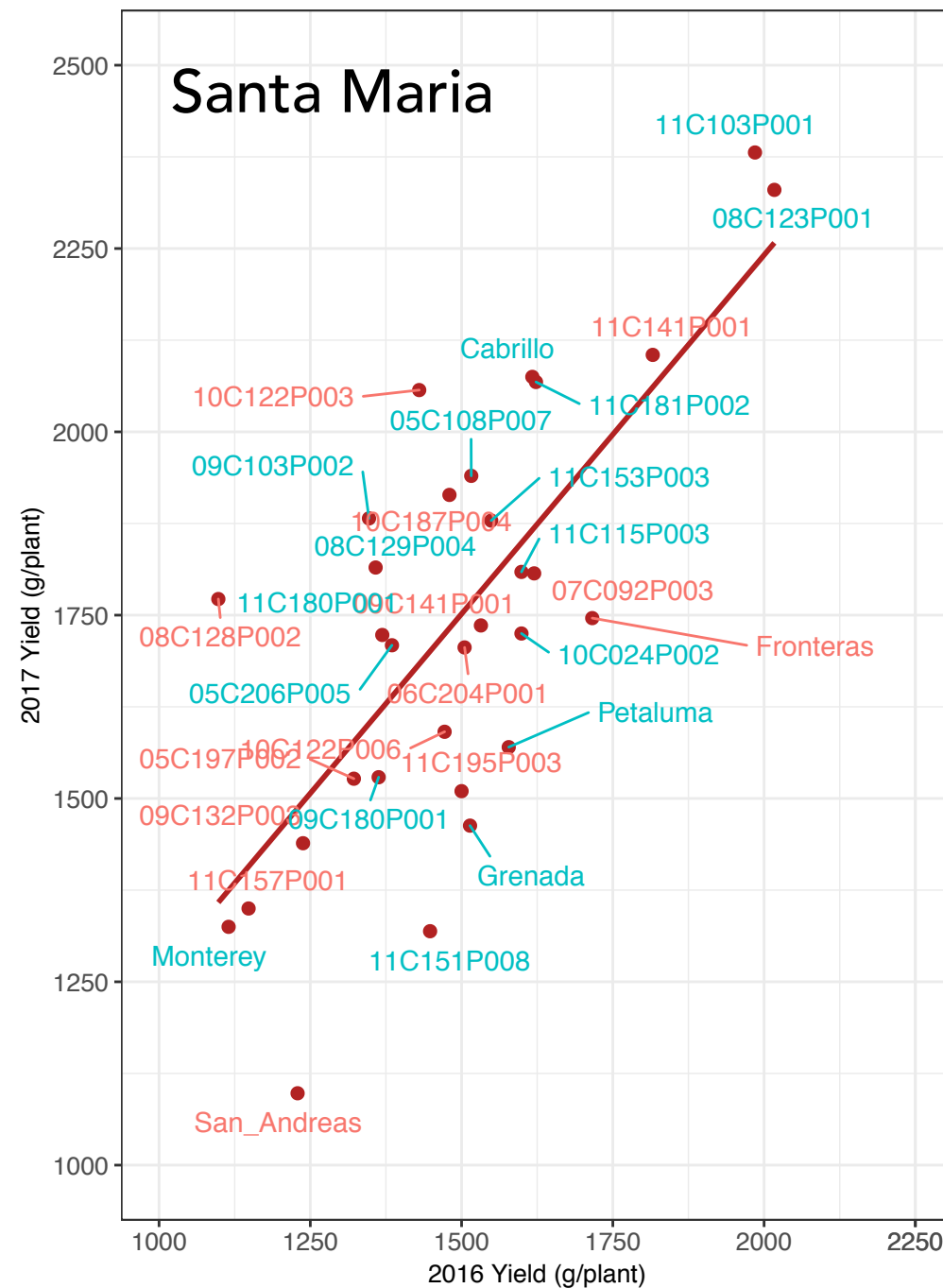
FW STARTING POINT IN 2015

- Disease First Discovered in California 2006 (Koike et al. 2009)
- Few Previous Genetic Studies, Limited Genetic Insights, Significant Confusion & Ambiguity
- Race-Specific Resistance Genes Had Not Been Discovered
- Genetics of Resistance Unclear
- Working Hypothesis: Resistance is Genetically Complex (Quantitative)



Several Cultivars Carry *Fw1*, a Dominant Gene Conferring Resistance to Fusarium Wilt

Red = Resistant (*Fw1fw1*) & Turquoise = Susceptible (*fw1fw1*)

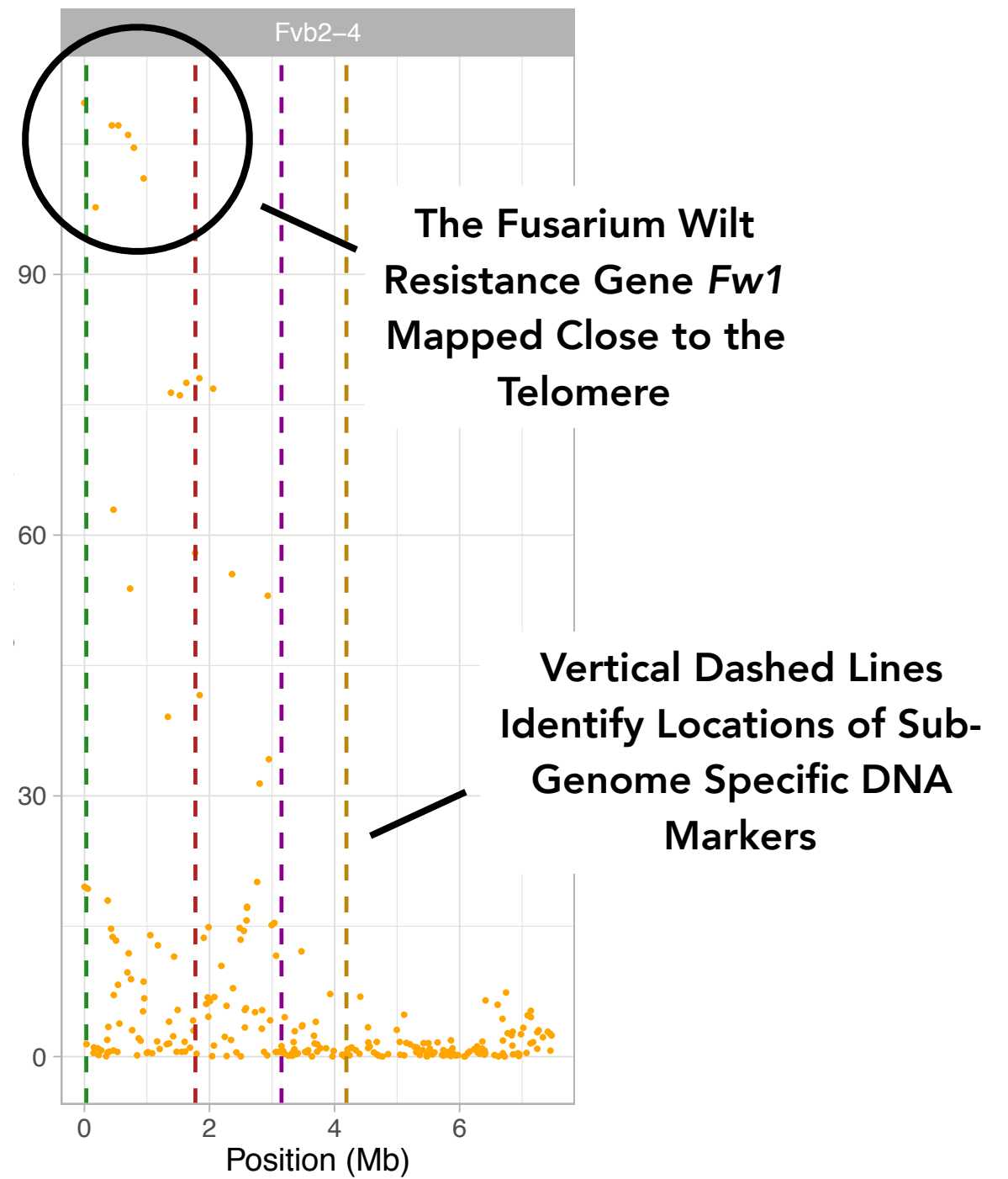
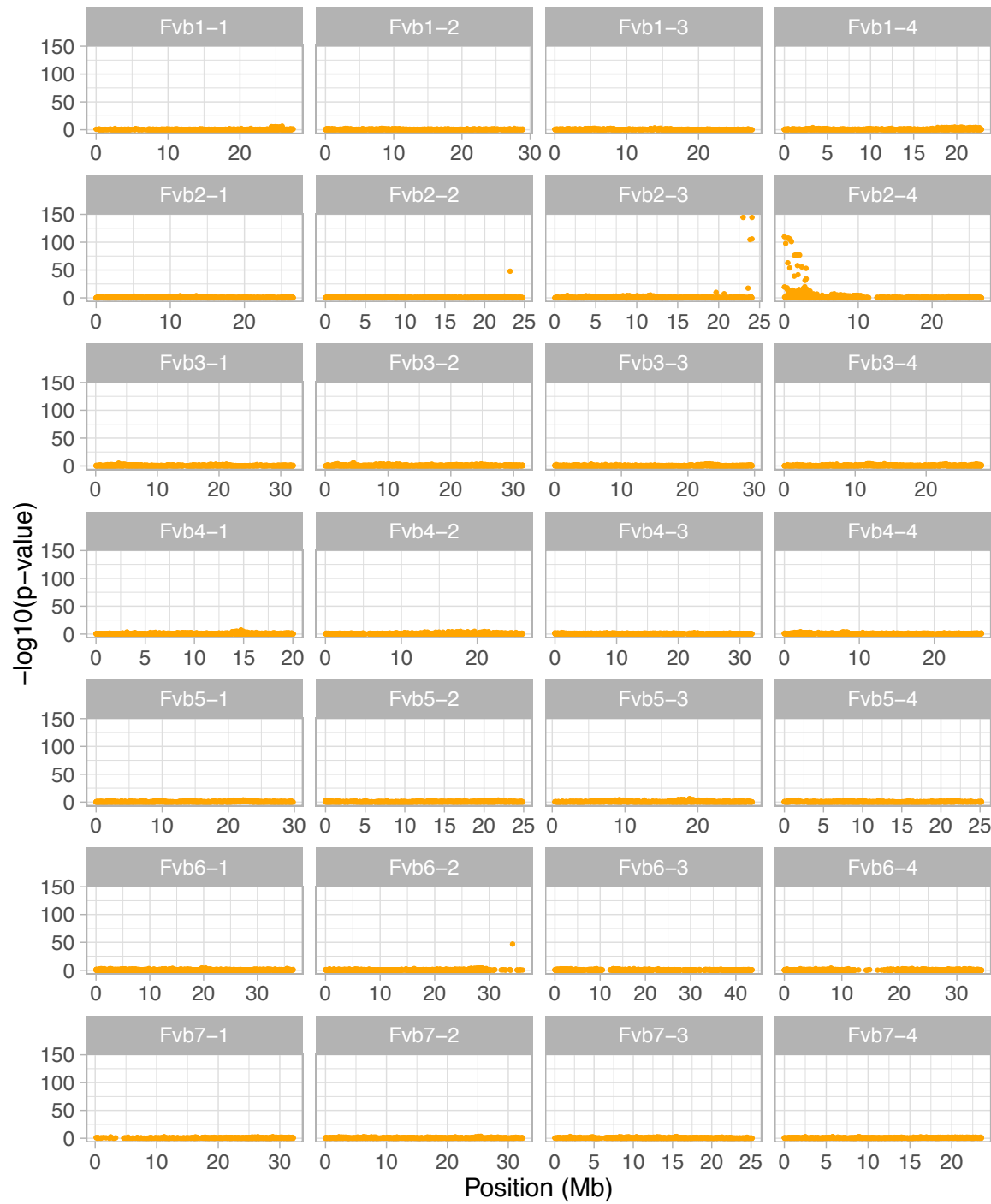


Pincot et al. 2018

Physical and Genetic Mapping of Fusarium Wilt Resistance Genes Genome-Wide Association Study (GWAS) Using the Octoploid Reference Genome

28 Chromosomes: 1-1, 1-2, ..., 7-4

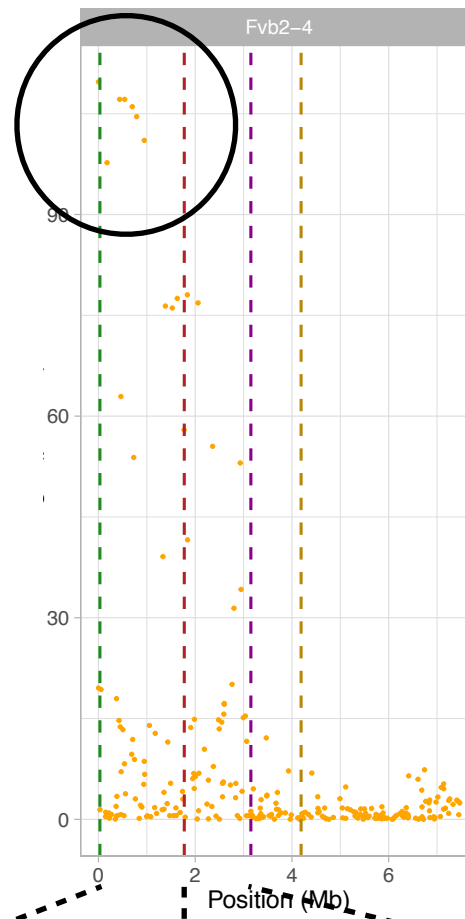
Chromosome 2-4



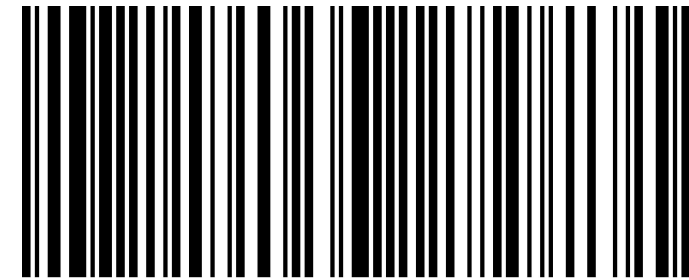
Cobo et al. (Unpublished Data)

Predictive Sub-Genome Specific KASP™ Genotyping Assays for the *Fw1* Locus in Strawberry

Physical Location of the *Fw1* Fusarium Wilt Resistance Gene

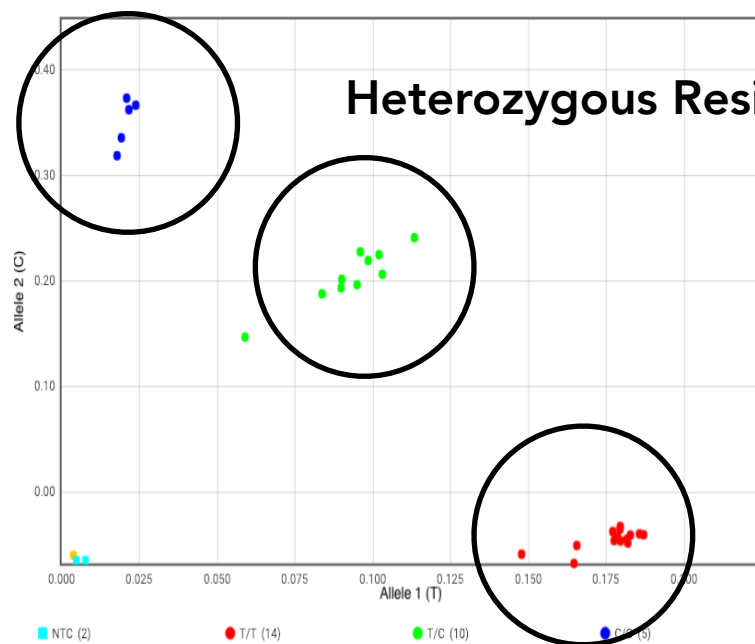


'Barcoding' Plants to Identify the Presence/Absence of Specific Genes

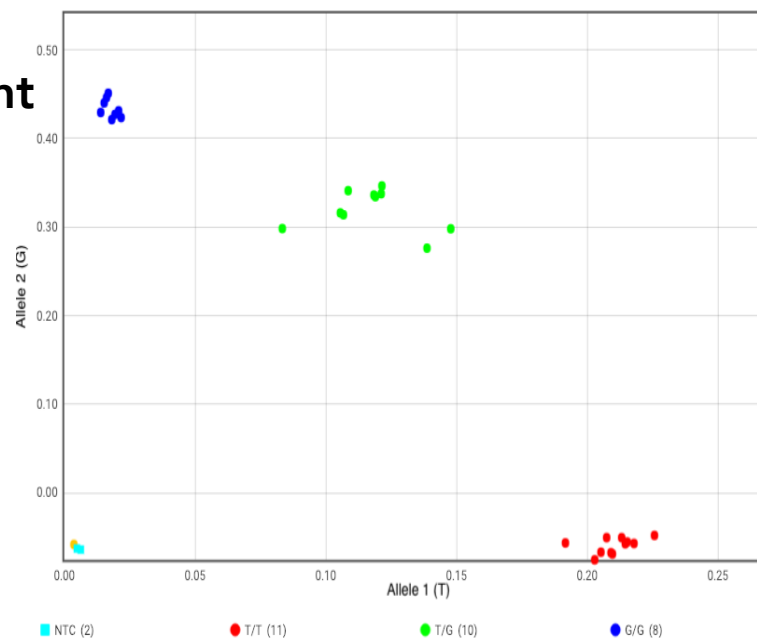


Manhattan Plot for the Upper Telomere of Chromosome 2-4

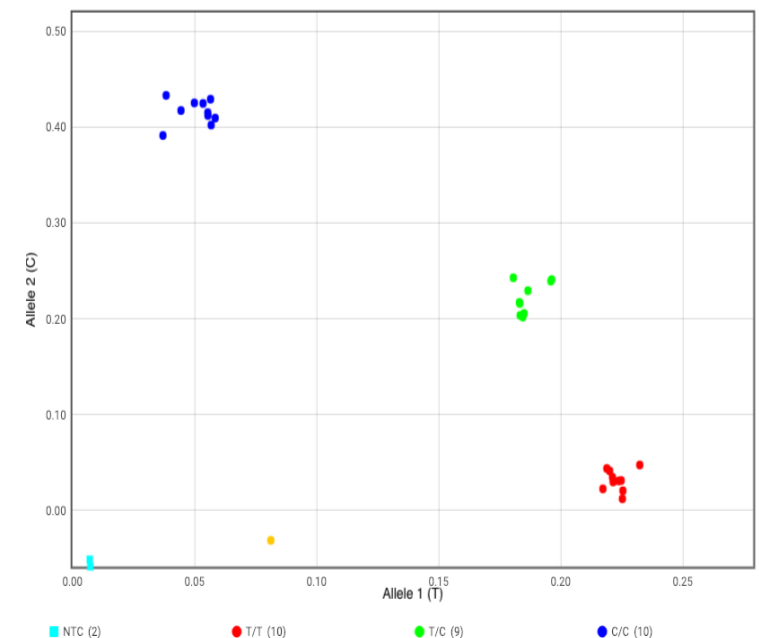
Homozygous Resistant



Heterozygous Resistant



Homozygous Susceptible



Cobo et al. (Unpublished Data)

Genome-Wide Association Mapping Uncovers *Fw1*, a Dominant Gene Conferring Resistance to Fusarium Wilt in Strawberry

Dominique D. A. Pincot,* Thomas J. Poorten,* Michael A. Hardigan,* Julia M. Harshman,* Charlotte B. Acharya,* Glenn S. Cole,* Thomas R. Gordon,† Michelle Stueven,† Patrick P. Edger,‡ and Steven J. Knapp*¹

*Department of Plant Sciences and †Department of Plant Pathology, University of California, Davis, California, 95616, and ‡Department of Horticulture, Michigan State University, East Lansing, Michigan 48824

ORCID IDs: 0000-0001-9768-0740 (T.J.P.); 0000-0002-5188-8084 (J.M.H.); 0000-0001-6498-5409 (S.J.K.)

ABSTRACT *Fusarium* wilt, a soil-borne disease caused by the fungal pathogen *Fusarium oxysporum* f. sp. *fragariae*, threatens strawberry (*Fragaria × ananassa*) production worldwide. The spread of the pathogen, coupled with disruptive changes in soil fumigation practices, have greatly increased disease pressure and the importance of developing resistant cultivars. While resistant and susceptible cultivars have been reported, a limited number of germplasm accessions have been analyzed, and contradictory conclusions have been reached in earlier studies to elucidate the underlying genetic basis of resistance. Here, we report the discovery of *Fw1*, a dominant gene conferring resistance to *Fusarium* wilt in strawberry. The *Fw1* locus was uncovered in a genome-wide association study of 565 historically and commercially important strawberry accessions genotyped with 14,408 SNP markers. Fourteen SNPs in linkage disequilibrium with *Fw1* physically mapped to a 2.3 Mb segment on chromosome 2 in a diploid *F. vesca* reference genome. *Fw1* and 11 tightly linked GWAS-significant SNPs mapped to linkage group 2C in octoploid segregating populations. The most significant SNP explained 85% of the phenotypic variability and predicted resistance in 97% of the accessions tested—broad-sense heritability was 0.96. Several disease resistance and defense-related gene homologs, including a small cluster of genes encoding nucleotide-binding leucine-rich-repeat proteins, were identified in the 0.7 Mb genomic segment predicted to harbor *Fw1*. DNA variants and candidate genes identified in the present study should facilitate the development of high-throughput genotyping assays for accurately predicting *Fusarium* wilt phenotypes and applying marker-assisted selection.

KEYWORDS
Fragaria
Fusarium wilt
strawberry
innate immunity
polyploid

Cultivated strawberry (*Fragaria × ananassa* Duchesne ex Rozier) plant health and yield are adversely impacted by several soil-borne diseases (Maas 1998). One of the greatest threats to strawberry production

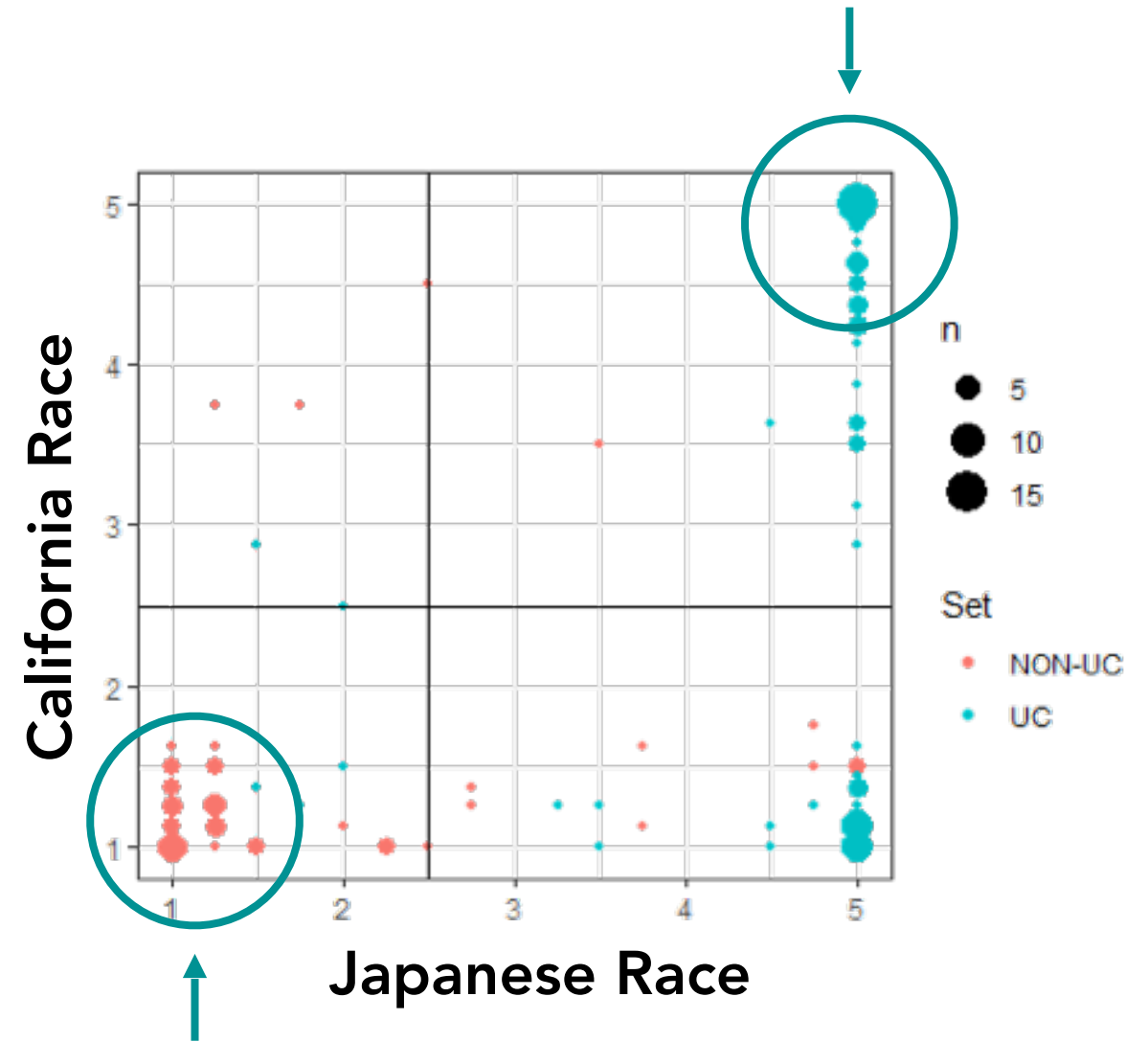
worldwide has been *Fusarium* wilt, a soil-borne disease caused by the fungal pathogen *Fusarium oxysporum* f. sp. *fragariae* (Winks and Williams 1965; Okamoto *et al.* 1970; Mena *et al.* 1975; Castro-Franco and Davalos-Gonzalez 1990; Huang *et al.* 2005; Abdet-Sattar *et al.* 2008; Arroyo *et al.* 2009; Koike *et al.* 2009; Gordon 2017; Henry *et al.* 2017). Historically, strawberry fruit and nursery stock growers have relied on powerful soil fumigants to suppress *F. oxysporum* f. sp. *fragariae* and other soil-borne pathogens, allowing for monocultures or very tight crop rotation cycles (Goodhue *et al.* 2005; Lloyd and Gordon 2016; Tourte *et al.* 2016). Because fruit and nursery production are typically concentrated in unique coastal and high-elevation environments in California, the availability of land for crop rotations is limited, often necessitating continuous cropping (Guthman 2016).

Until 2005, the most widely used soil fumigant in strawberry production was methyl bromide (MeBr), an ozone-layer depleting chemical

Copyright © 2018 Pincot *et al.*
doi: <https://doi.org/10.1534/g3.118.200129>
Manuscript received February 5, 2018; accepted for publication March 26, 2018; published Early Online March 30, 2018.
This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Supplemental material available at Figshare: <https://doi.org/10.25387/g3.6007715>.
¹Corresponding Author: 112 Robbins Hall, Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616, E-mail: sknapp@ucdavis.edu

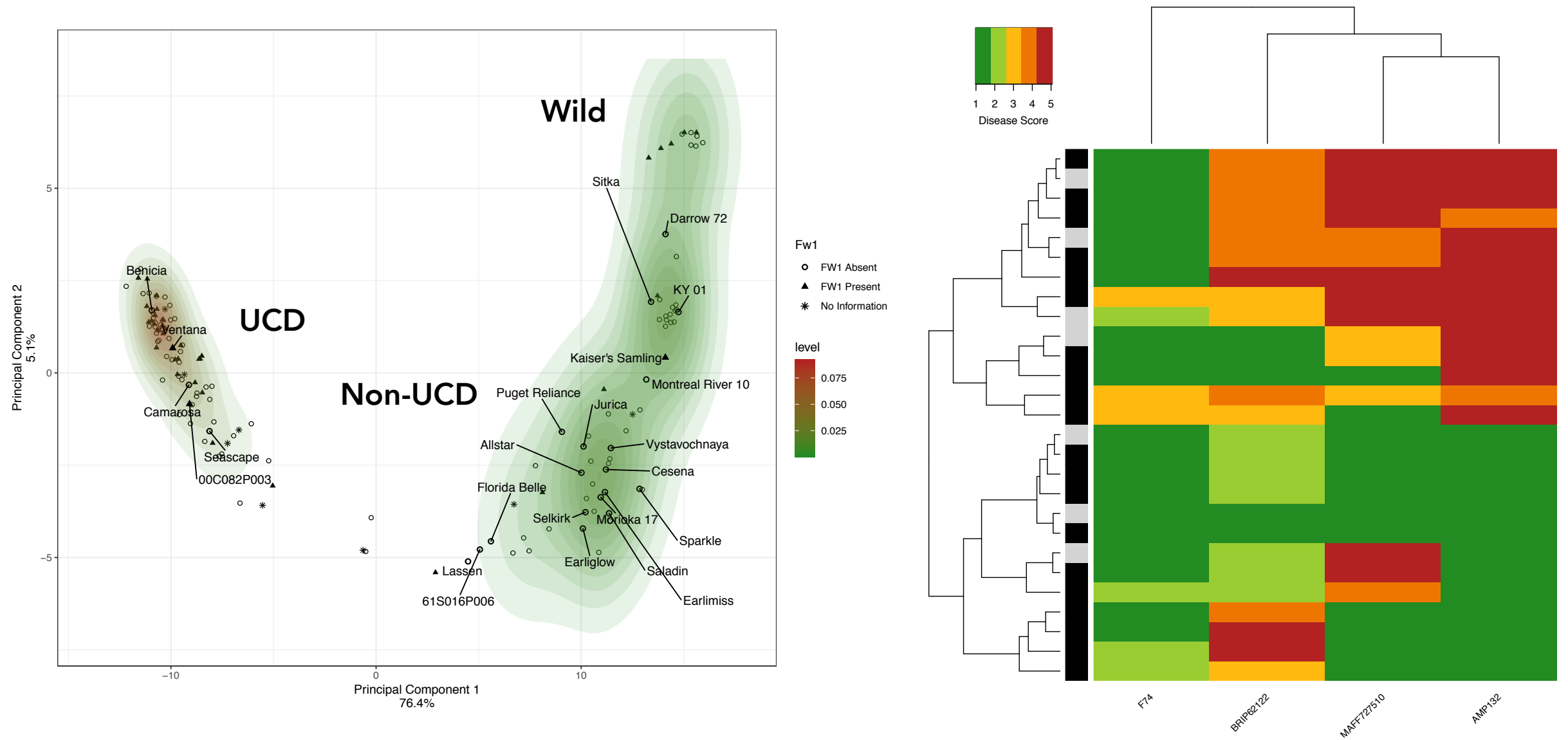
Since Discovering *Fw1*, Additional Dominant Genes Have Been Discovered That Confer Resistance to California and Non-California Races of *Fusarium* (Pincot, Rodriguez, Henry, & Gordon)

Susceptible to Both



Host Differentials Resistant to California, Japanese, Australian, and Spanish Races of *Fusarium oxysporum*

1,000 Genotypes Screened for Resistance to the California Race AMP132
128 Genotypes Selected for Host Differential Study



Henry et al. (Unpublished Data) & Pincot et al. (Unpublished Data)

VW STARTING POINT IN 2015

- Disease First Reported 1932
- 30+ Previous Studies, Limited Insights & Information, Significant Confusion & Ambiguity
- Race-Specific Resistance Genes Have Not Been Discovered
- Genetics of Resistance Unclear
- Working Hypothesis: Resistance is Genetically Complex (Quantitative)

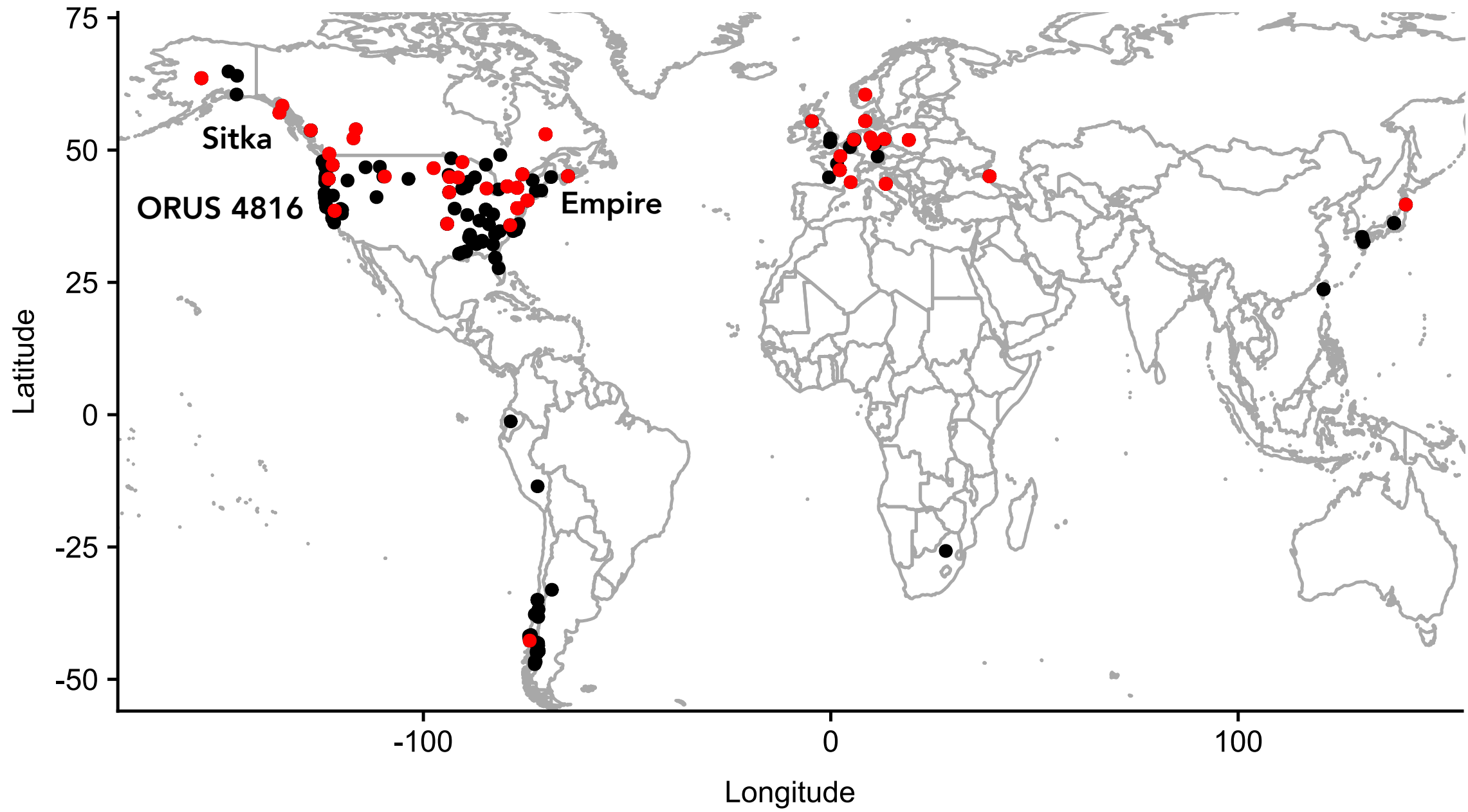


**Resistance to Verticillium Wilt is Genetically Complex
Categorical Classifications Only Safely Apply at the
Phenotypic Extremes**

**R = Resistant
Symptomless (1)**

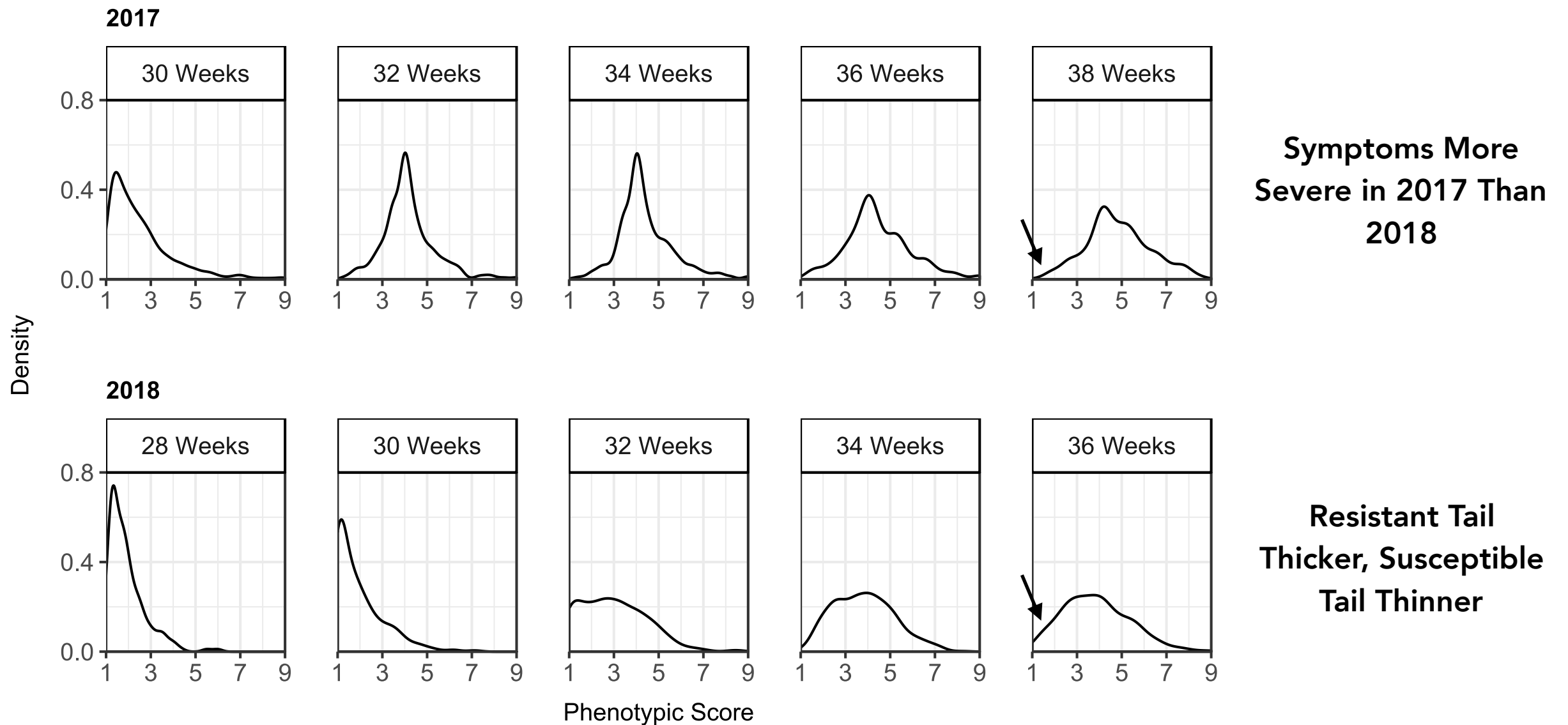
**S = Susceptible
Dead (9)**

Global Survey of Resistance to Verticillium Wilt



Verticillium Wilt Phenotypic Distributions

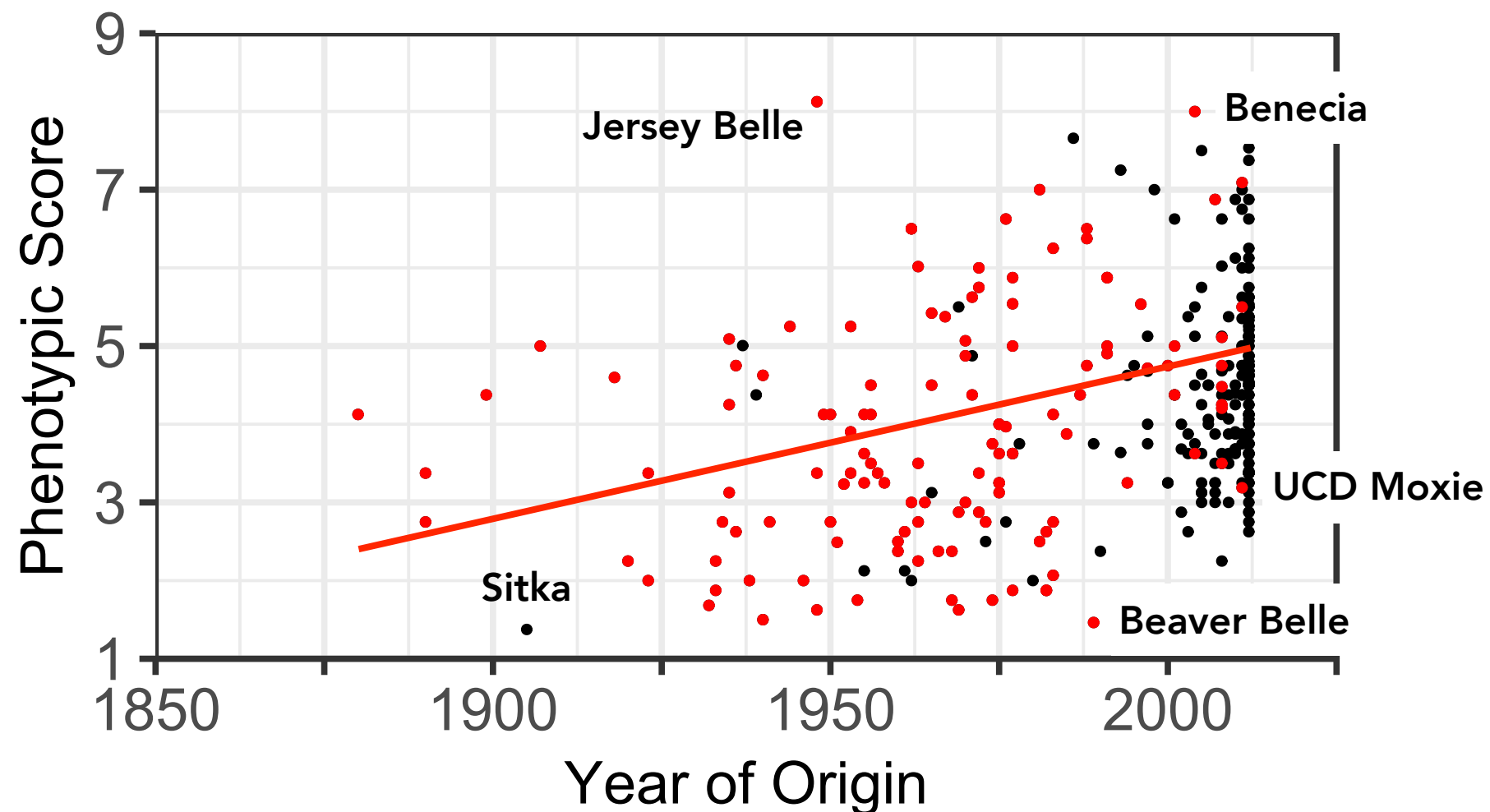
- Soil Fumigated Fields, Fall Planted, Plants Artificially Inoculated With a California Race of *Verticillium dahliae*
- Ordinal Phenotypic Scale: 1 = Highly Resistant (Symptomless), 9 = Highly Susceptible
- Genetic Analyses and Genomic Selection Experiments Focused on the Terminal Dates in July of Each Year (38 Weeks in 2017 and 36 Weeks in 2018)
- Symptoms More Severe in 2017 Than 2018
- Resistance Phenotypes Normally Distributed: Continuous Phenotypic Variation



Breeding and Genetics of Resistance to Verticillium Wilt: Screened a Diverse Collection of Cultivars and Germplasm Accessions ($n = 1,000$) to Understand the Global Situation and Identify the Strongest Sources of Resistance

- Genetic Gains Have Been NEGATIVE Worldwide
- MOST RESISTANT Germplasm Accessions: ORUS 4816 ($\bar{y} = 1.0$) and Sitka ($\bar{y} = 1.4$)
- MOST RESISTANT Cultivars: Beaver Belle (1989; $\bar{y} = 1.2$) and Empire (1952; $\bar{y} = 1.5$)
- MOST RESISTANT UCD Cultivars: Tahoe (1936; $\bar{y} = 2.6$), UCD Moxie (2011; $\bar{y} = 3.2$), Wiltguard (1952; $\bar{y} = 3.2$), & Camino Real (1994; $\bar{y} = 3.3$)

To Increase Resistance and Transgress the Range of Current UCD Cultivars, Favorable Alleles Must Be Introduced from Heirloom and Other Exotic Non-UCD Sources



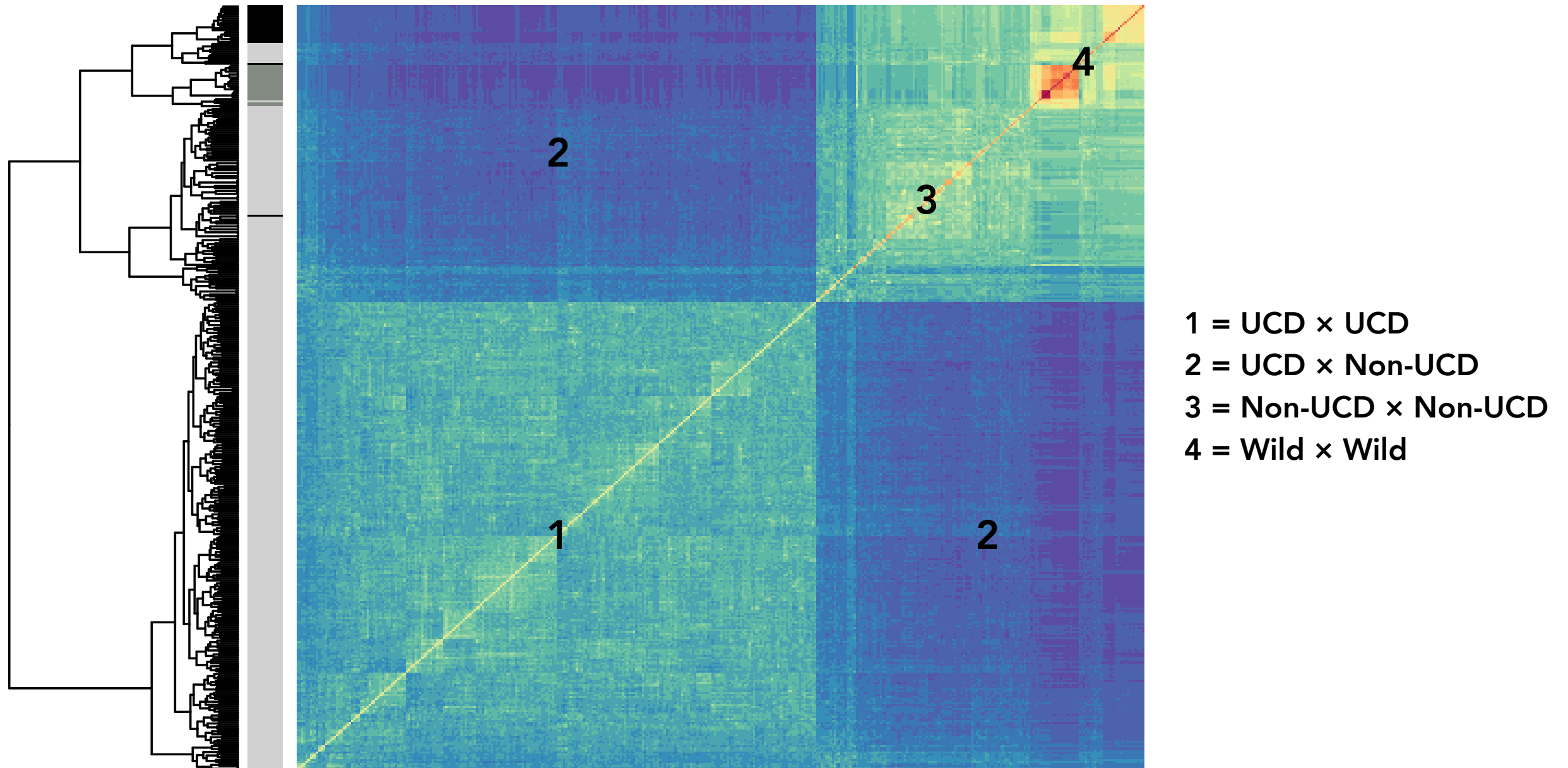
Pincot *et al.* (Unpublished Data) Accuracy of Genomic Selection in an Octoploid Strawberry Population Spanning 160 Years of Breeding

Additive Genetic Relationship Matrix

Training Population ($n = 388$)

Genotyped and Phenotyped: Four Replications/Year \times Two Years

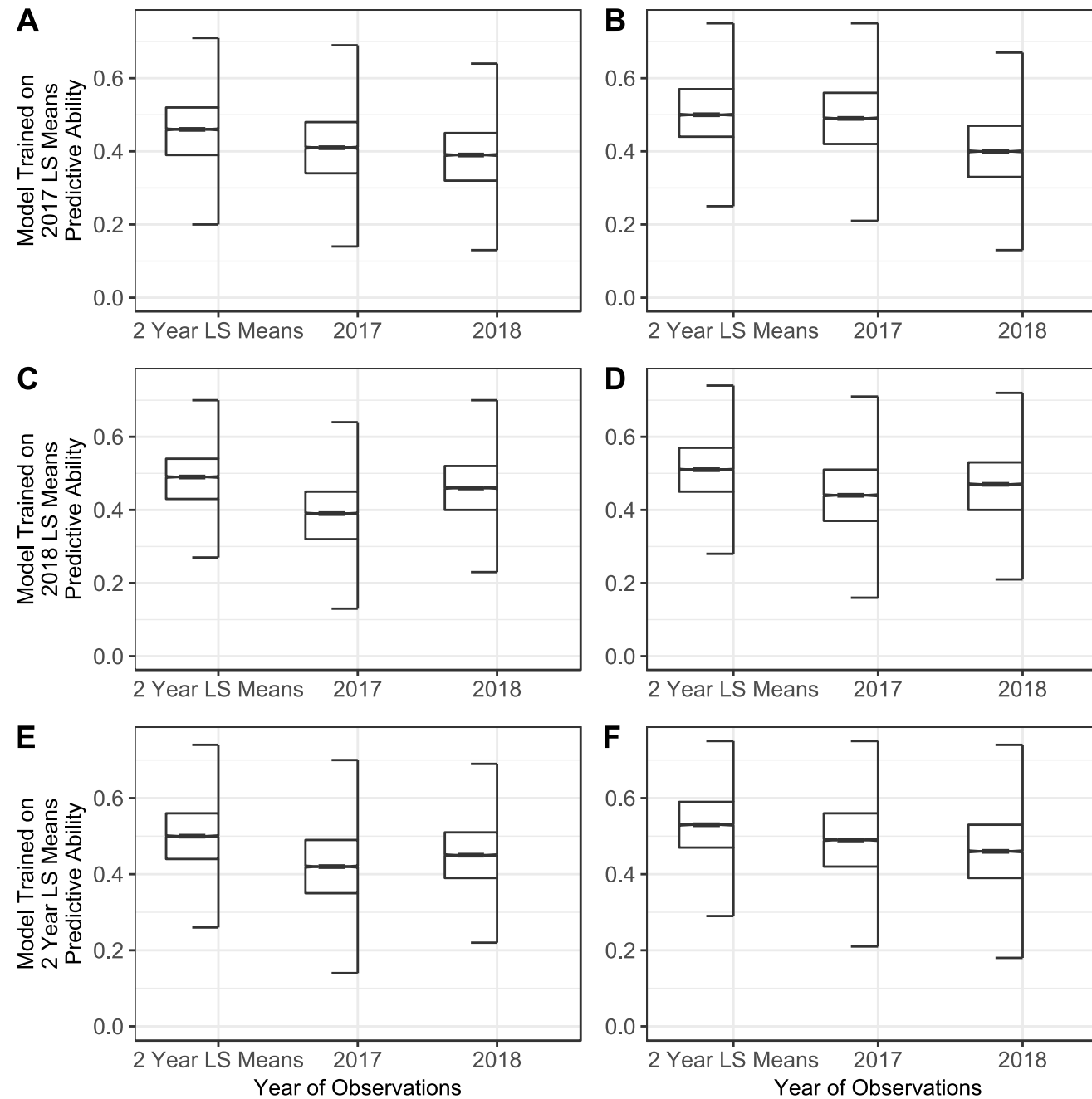
UCD Cultivars (1935-Present) + Non-UCD Cultivars (1862-Present) + Wild Ecotypes



Pincot *et al.* (Unpublished Data) Accuracy of Genomic Selection in an Octoploid Strawberry Population Spanning 160 Years of Breeding

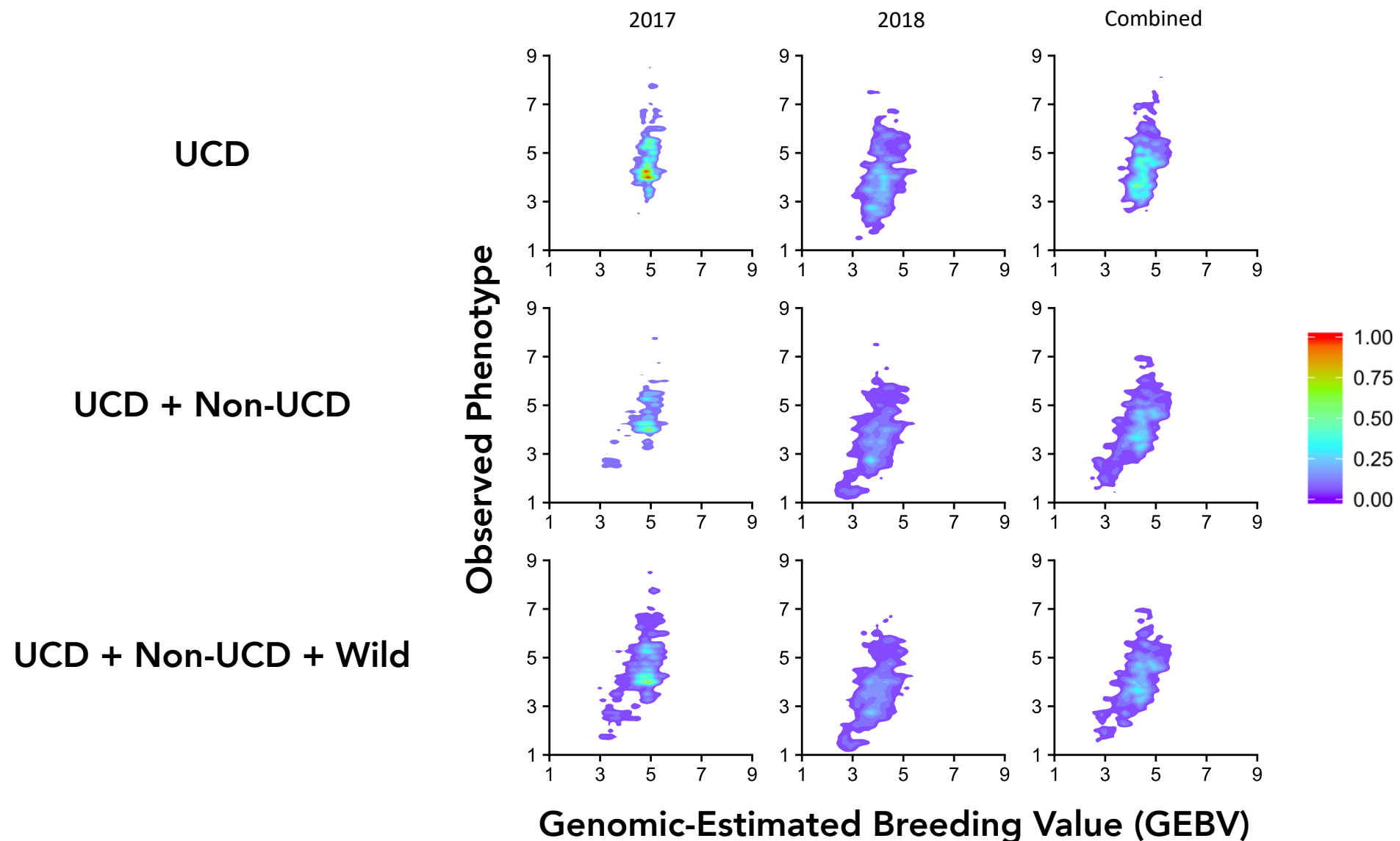
Breeding History Suggested that Genetic Gains Might be Increased Through the Application of Genomic Prediction Approaches

Broad-Sense Heritability = 0.71 in 2017, 0.76 in 2018, and 0.84 Across Years
Narrow-Sense Genomic Heritability = 0.36 in 2017, 0.44 in 2018, and 0.46 Across Years
Accuracy of Genomic Selection Was Excellent



Accuracy of Genomic Selection for Resistance to Verticillium Wilt

- Cross-Validation ($k = 10,000$ Replicates): Crescent-Shaped Densities Show That the Most Highly Resistant Individuals (Lower Horn of the Crescent) Were Predicted With the Greatest Accuracy
- Greatest Gains Can Be Achieved by Introducing Favorable Alleles That Were Previously Not Present in the UCD Breeding Program

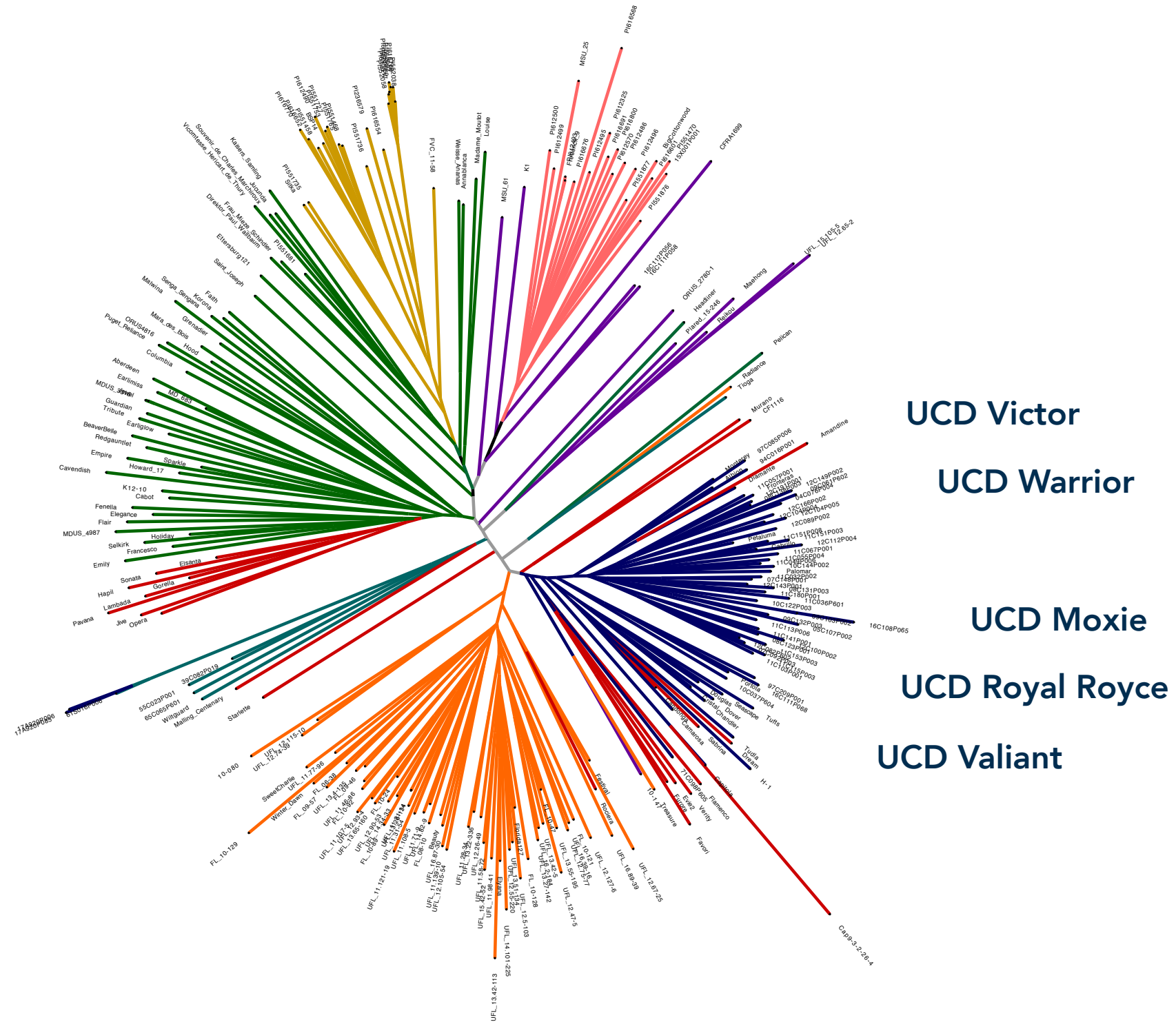




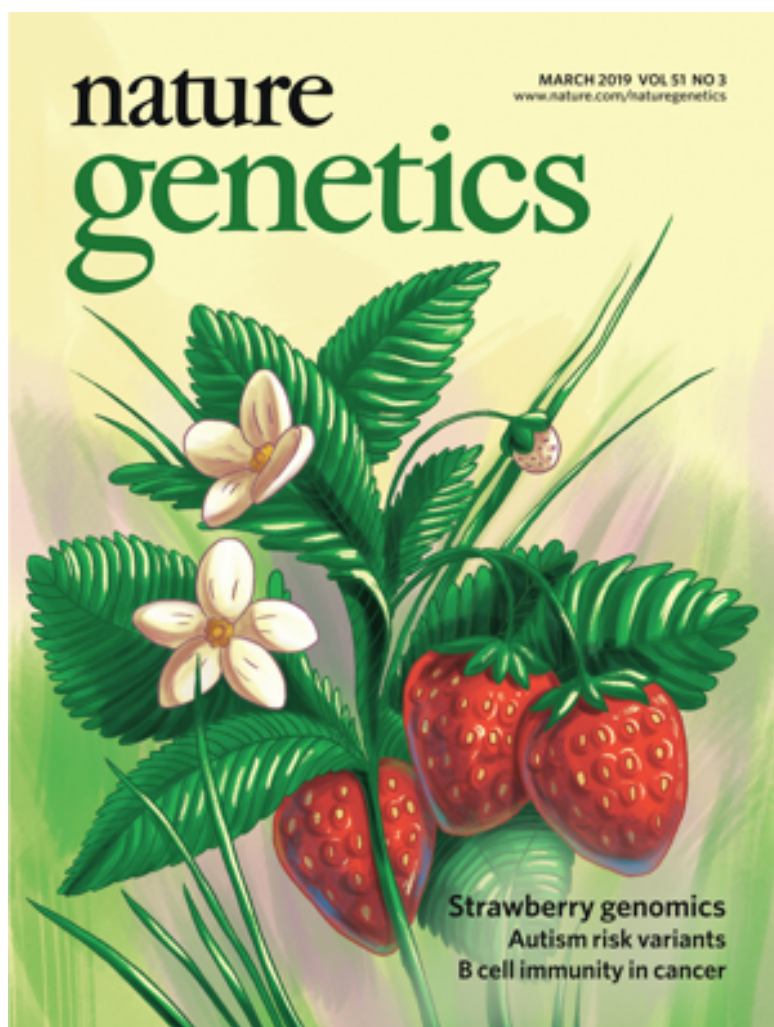
Macrophomina Phenotypic Selection
UCD Armstrong Farm
2019-6-30

89 Years of Strawberry Breeding @ the University of California, Davis

68 Cultivars Since 1935



Genome-Scale DNA Profiling → Global
'Family' Tree for Strawberry



UC DAVIS
UNIVERSITY OF CALIFORNIA

MICHIGAN STATE
UNIVERSITY

Origin and evolution of the octoploid strawberry genome

Patrick P. Edger^{1,2*}, Thomas J. Poorten³, Robert VanBuren^{1,4}, Michael A. Hardigan³, Marivi Colle¹, Michael R. McKain⁵, Ronald D. Smith⁶, Scott J. Teresi⁶, Andrew D. L. Nelson⁷, Ching Man Wai¹, Elizabeth I. Alger¹, Kevin A. Bird^{1,2}, Alan E. Yocca¹, Nathan Pumpplin³, Shujun Ou^{1,2}, Gil Ben-Zvi⁸, Avital Brodt⁸, Kobi Baruch⁸, Thomas Swale⁹, Lily Shiue⁹, Charlotte B. Acharya³, Glenn S. Cole³, Jeffrey P. Mower¹⁰, Kevin L. Childs^{11,12}, Ning Jiang^{1,2}, Eric Lyons⁷, Michael Freeling¹³, Joshua R. Puzey⁶ and Steven J. Knapp^{13*}

Cultivated strawberry emerged from the hybridization of two wild octoploid species, both descendants from the merger of four diploid progenitor species into a single nucleus more than 1 million years ago. Here we report a near-complete chromosome-scale assembly for cultivated octoploid strawberry (*Fragaria × ananassa*) and uncovered the origin and evolutionary processes that shaped this complex allopolyploid. We identified the extant relatives of each diploid progenitor species and provide support for the North American origin of octoploid strawberry. We examined the dynamics among the four subgenomes in octoploid strawberry and uncovered the presence of a single dominant subgenome with significantly greater gene content, gene expression abundance, and biased exchanges between homoeologous chromosomes, as compared with the other subgenomes. Pathway analysis showed that certain metabolomic and disease-resistance traits are largely controlled by the dominant subgenome. These findings and the reference genome should serve as a powerful platform for future evolutionary studies and enable molecular breeding in strawberry.

The cultivated garden strawberry (*Fragaria × ananassa*), an allo-octoploid ($2n = 8x = 56$), has a unique natural and domestication history, originating as an interspecific hybrid between wild octoploid progenitor species approximately 300 years before present¹. The genomes of the progenitor species, *Fragaria virginiana* and *Fragaria chiloensis*, are the products of polyploid evolution: they were formed by the fusion of and interactions among genomes from four diploid progenitor species (that is, subgenomes) approximately 1 million years before present². Whereas two of the diploid progenitor species have been identified³, the other two diploid progenitor species have remained unknown. Moreover, the history of events leading to the formation of the octoploid lineage and the evolutionary dynamics among the four subgenomes that restabilized cellular processes after 'genomic shock'⁴ in allopolyploids remain poorly understood. Here, we present what is, to our knowledge, the first chromosome-scale assembly of an octoploid strawberry genome, the identities of the extant diploid progenitor species of each subgenome, and novel insights into the collective evolutionary processes involved in establishing a dominant subgenome in this highly polyploid species.

The Rosaceae are a large eudicot family including a rich diversity of crops with major economic importance worldwide, such as nuts (for example, almonds), ornamentals (for example, roses), pome fruits (for example, apples), stone fruits (for example, peaches),

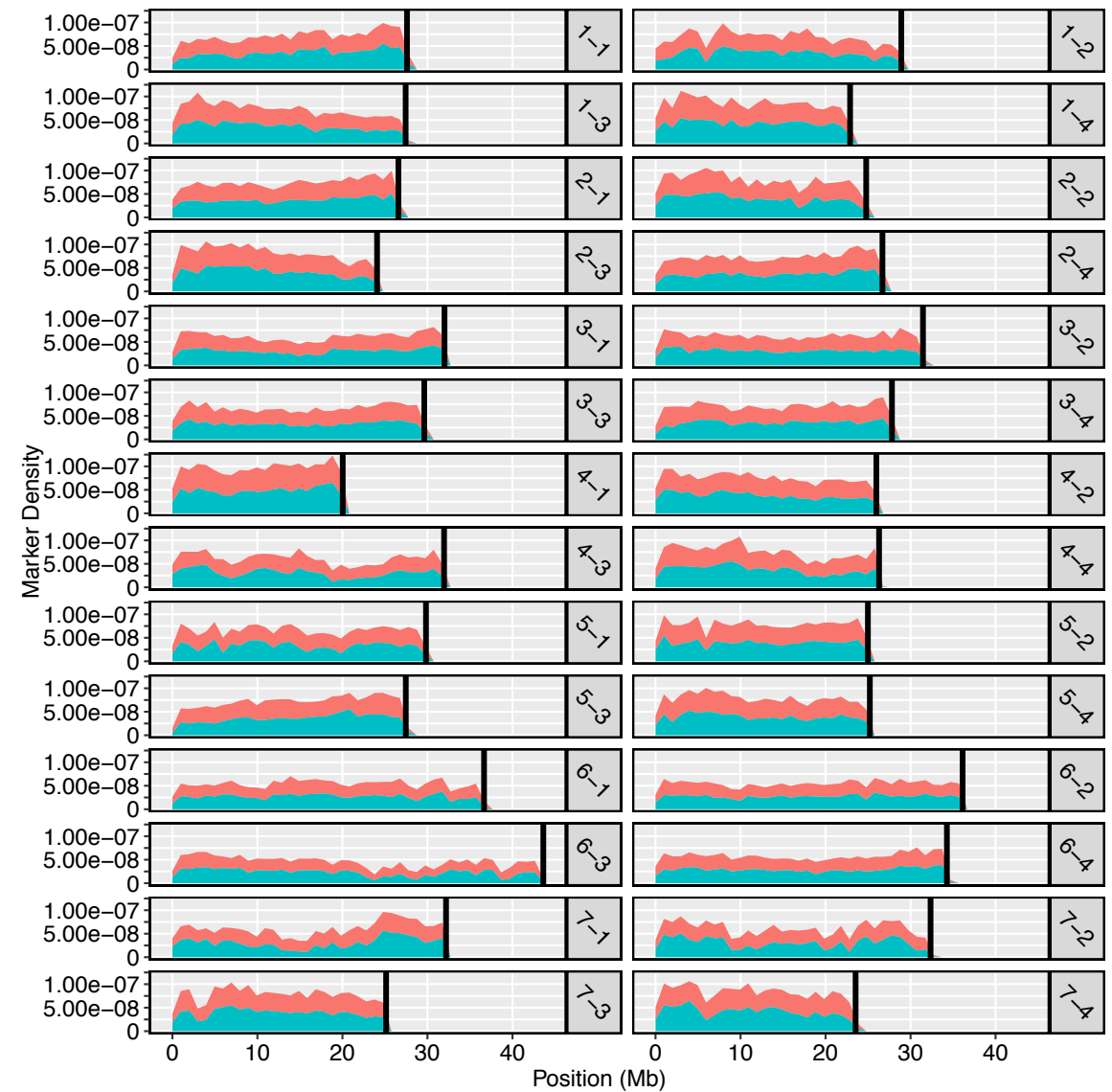
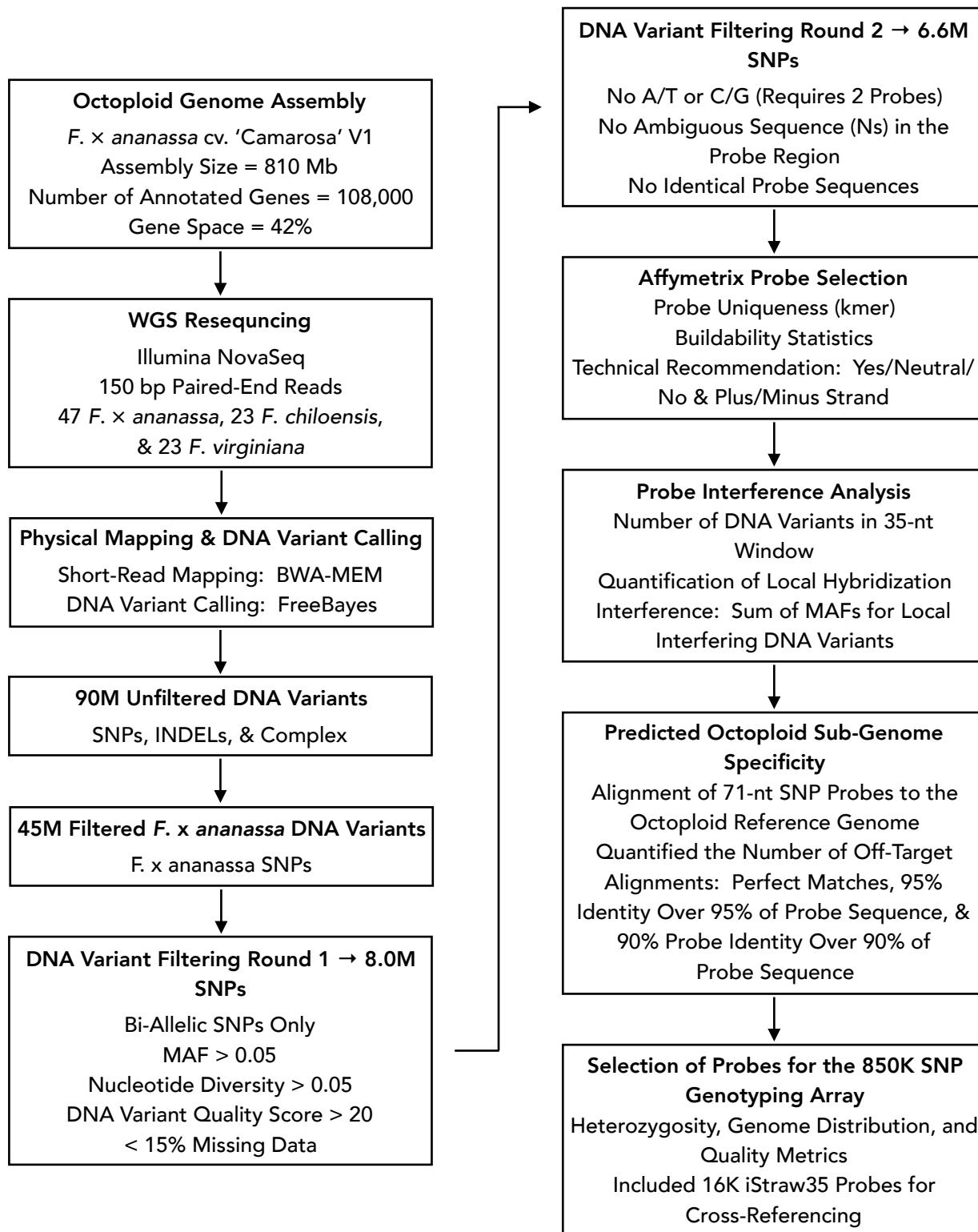
and berries (for example, strawberries)⁵. Strawberries are prized by consumers, largely because of their complex array of flavors and aromas. The genus *Fragaria* was named by the botanist Carl Linnaeus, on the basis of the Latin word 'fragrans', meaning 'sweet scented', describing its striking, highly aromatic fruit⁶. A total of 22 wild species of *Fragaria* have been described, ranging from diploid ($2n = 2x = 14$) to decaploid ($2n = 10x = 70$)⁷. The genus *Fragaria* is highly interfertile between and within ploidy levels, thus leading to the natural formation of higher-polyploid species⁸.

Polyploid events, also known as whole-genome duplications, have been an important recurrent process throughout the evolutionary history of eukaryotes and have probably contributed to novel and varied phenotypes^{9–13}. Polyploids are grouped into two main categories: autopolyploids and allopolyploids, involving either a single or multiple diploid progenitor species, respectively^{14,15}. Many crop species are allopolyploids¹⁶, thus contributing to the emergence of important agronomic traits such as spinnable fibers in cotton¹⁷, diversified morphotypes in *Brassica*¹⁸, and varied aroma and flavor profiles in strawberry¹⁹. Allopolyploids face the challenge of organizing distinct parental subgenomes—each with a unique genetic and epigenetic makeup shaped by independent evolutionary histories—residing within a single nucleus¹⁵. Previous studies have proposed, as part of the 'subgenome dominance' hypothesis²⁰, that the establishment of a single dominant subgenome may resolve

¹Department of Horticulture, Michigan State University, East Lansing, MI, USA. ²Ecology, Evolutionary Biology and Behavior, Michigan State University, East Lansing, MI, USA. ³Department of Plant Sciences, University of California–Davis, Davis, California, USA. ⁴Plant Resilience Institute, Michigan State University, East Lansing, MI, USA. ⁵Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, USA. ⁶Department of Biology, College of William and Mary, Williamsburg, VA, USA. ⁷School of Plant Sciences, University of Arizona, Tucson, AZ, USA. ⁸NRGene, Ness Ziona, Israel. ⁹Dovetail Genomics, Santa Cruz, CA, USA. ¹⁰Center for Plant Science Innovation, University of Nebraska, Lincoln, NE, USA. ¹¹Department of Plant Biology, Michigan State University, East Lansing, MI, USA. ¹²Center for Genomics Enabled Plant Science, Michigan State University, East Lansing, MI, USA. ¹³Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA. *e-mail: edgerpat@msu.edu; sjknapp@ucdavis.edu

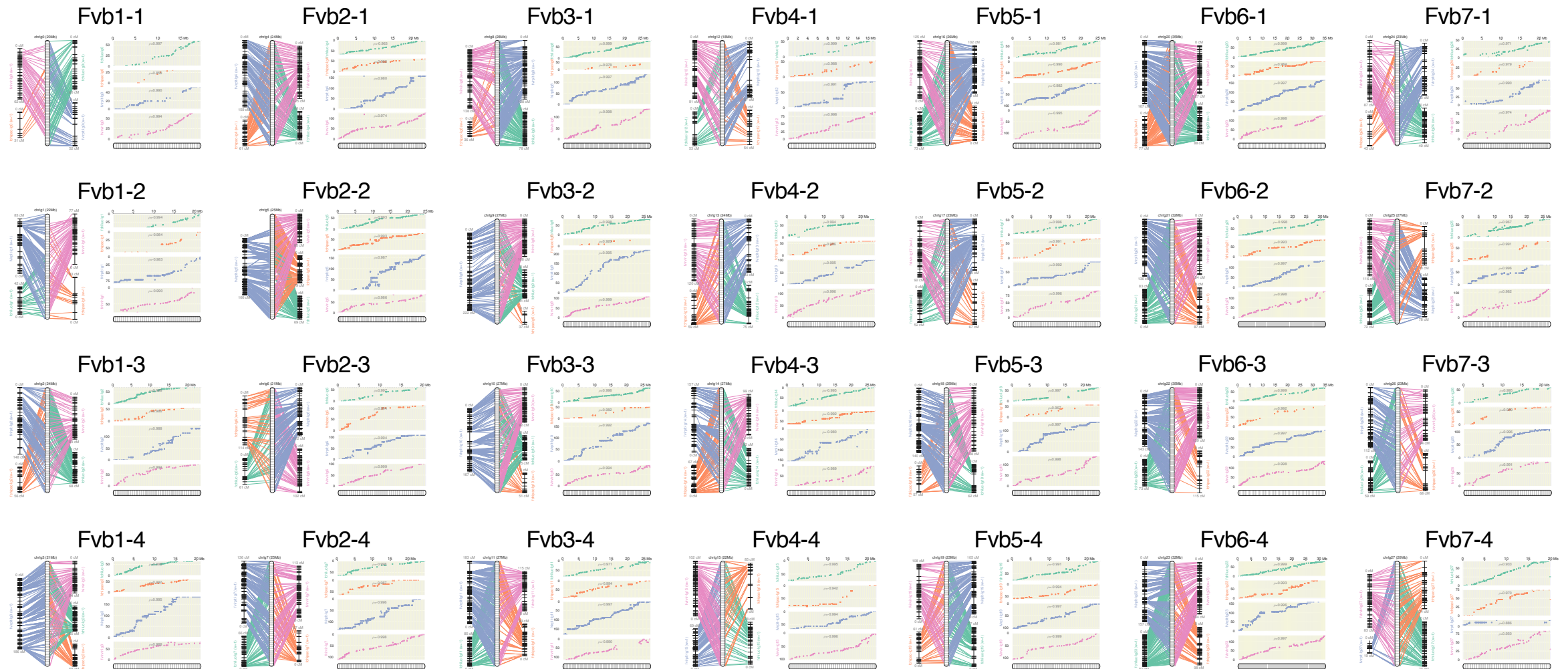
The Strawberry Genome Harbors Millions of Paralog- and Homolog-Specific DNA Variants—A Technically Critical and Important Discovery

Parsed 90M → 1M by Stringent Filtering and Selection to Maximize Heterozygosity Among Elite Genotypes, Minimize SNP and Sub-Genome Ascertainment Biases, and Eliminate Off-Target Signals



Hardigan et al. (Unpublished Data)

We Have a Complete Picture of the Genomic Landscape of Octoploid Strawberry—Can Pinpoint Genes



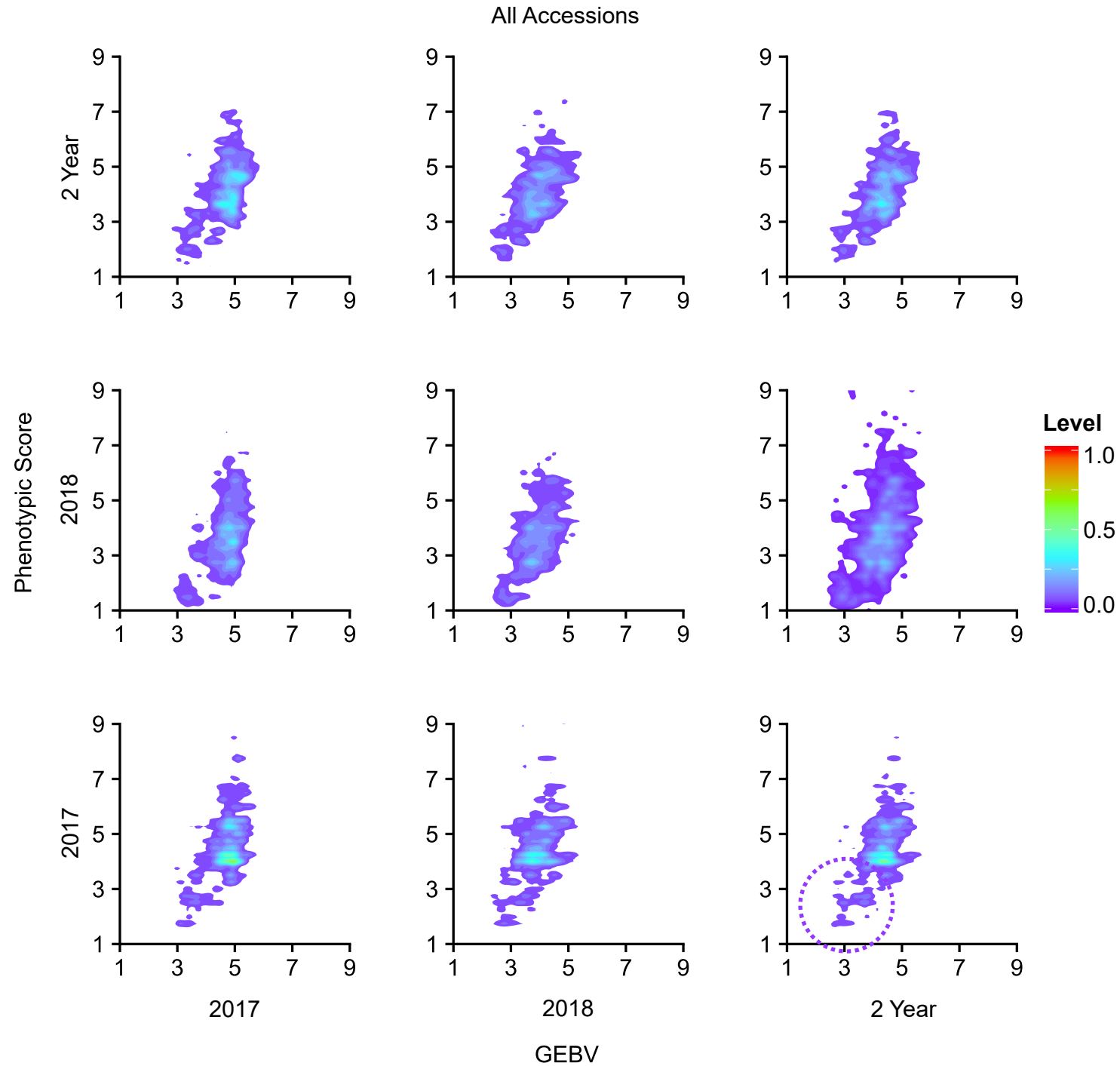
Hardigan *et al.* (Unpublished Data)

Genomic Prediction of Resistance to Verticillium Wilt Was Highly Accurate

x-Axis = Genomic-Estimated Breeding Value (GEBV)

y-Axis = Observed Phenotypic Value

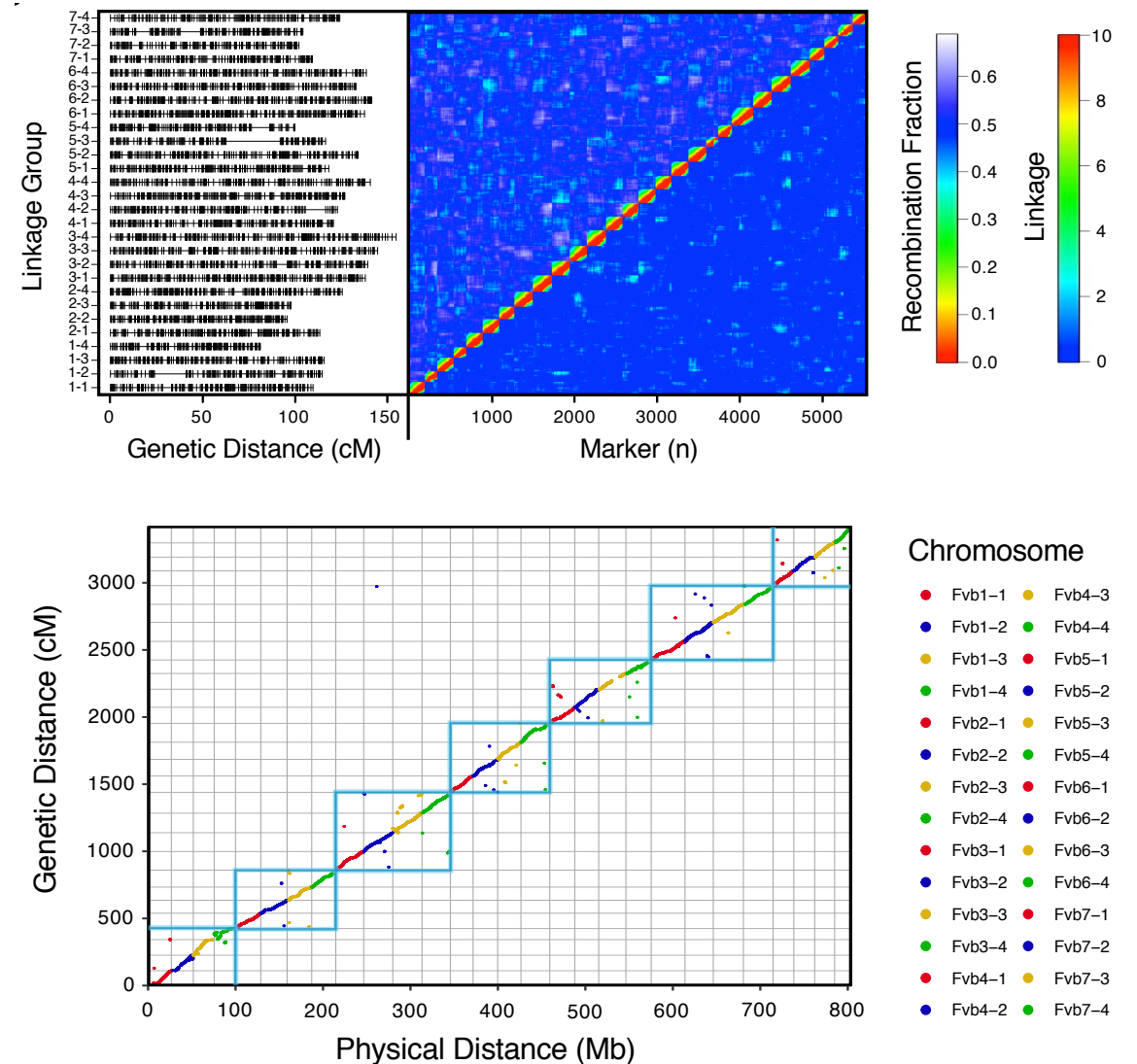
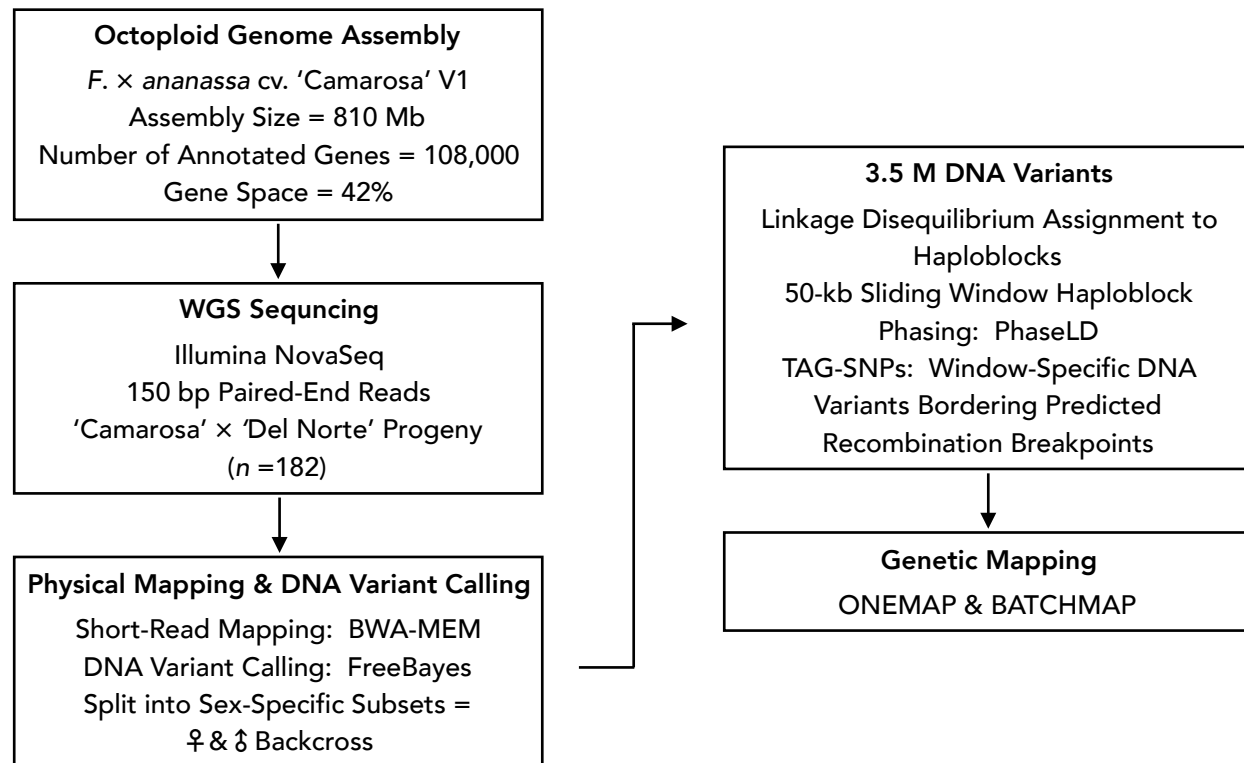
Cross-Validation ($k = 10,000$ Replicates): Crescent-Shaped Densities Show That the Most Highly Resistant Individuals (Lower Horn) Were Predicted With the Greatest Accuracy



Whole-Genome Shotgun Genotyping-by-Sequencing

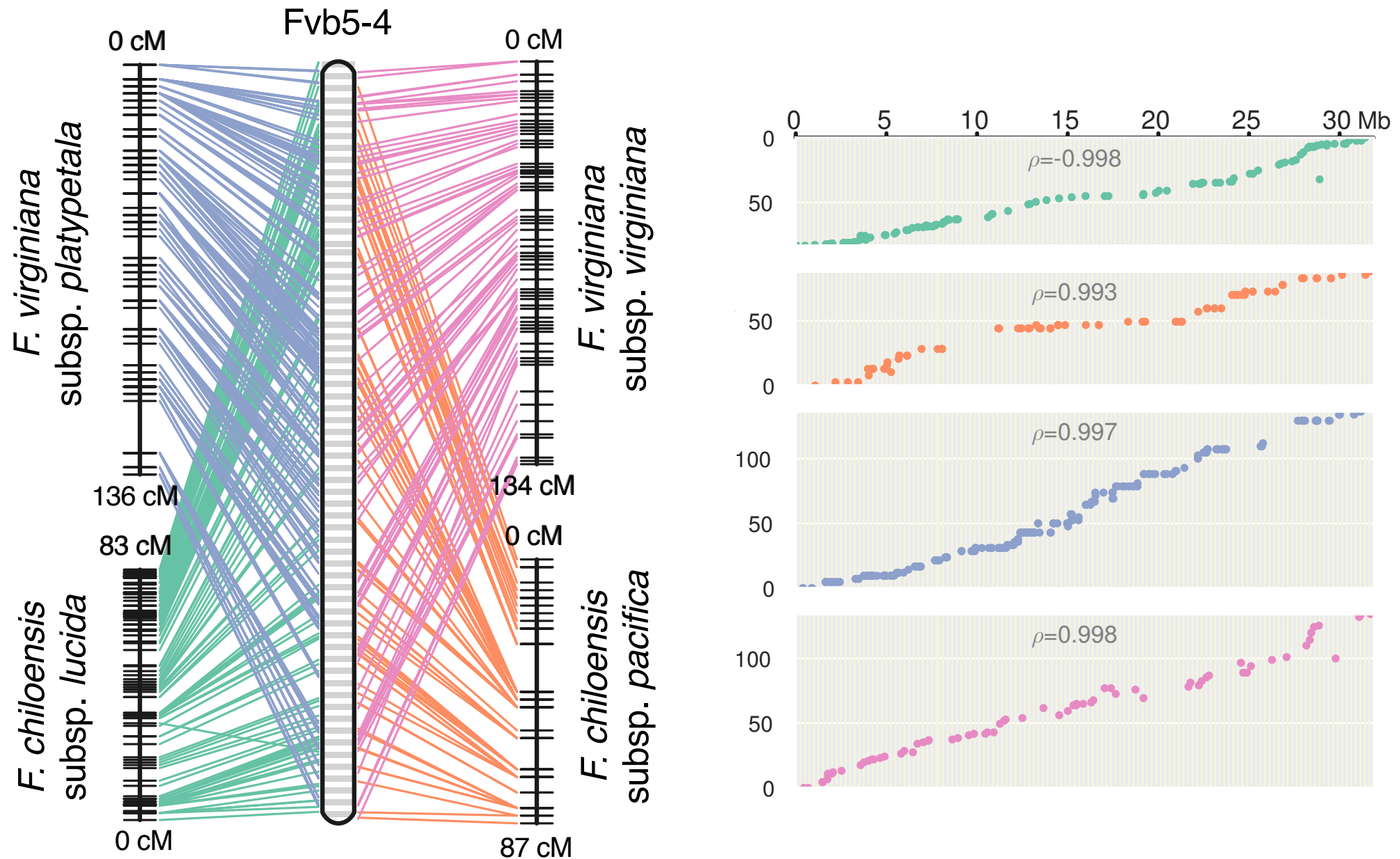
F. × ananassa 'Camarosa' × *F. chiloensis* subsp. *lucida* 'Del Norte' (n = 182)

- The Two Octoploid Genomes That We Sequenced*
- Predicted Number of Haploblocks = 5,096 = 28 Chromosomes x 182 Individuals
- 1.9 Million Genetically Mapped DNA Variants in 5,521 Haploblocks Spanning 3,394 cM in *F. chiloensis* subsp. *lucida* (Shown)
- 1.6 Million Genetically Mapped DNA Variants in 3,179 Haploblocks Spanning 2,017 cM in *F. × ananassa* (Not Shown)
- Genome-Wide Validation of Genotyping-by-Sequencing
- Complete Genetic-to-Physical Collinearity



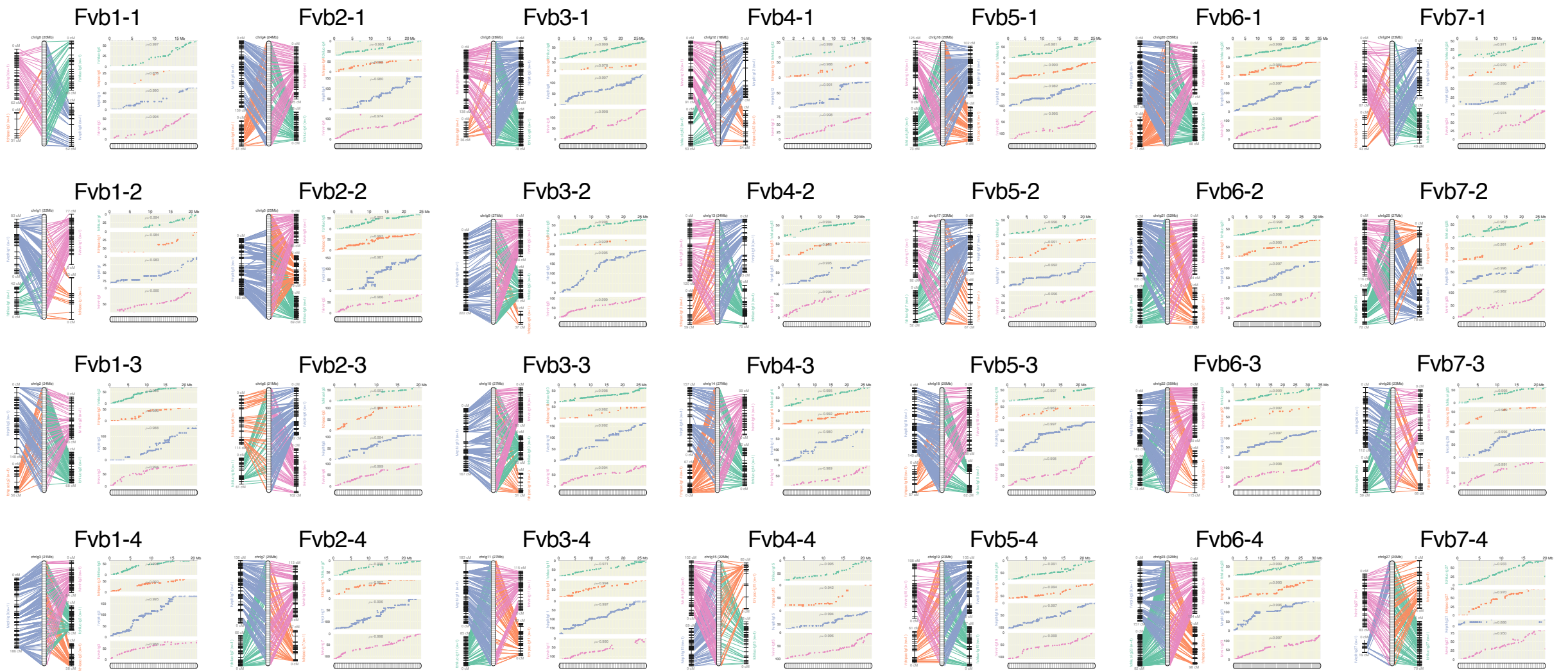
*Camarosa V1 (Edger et al. 2019); Camarosa V3 and Del Norte V2 (Unpublished Data)

High-Density Comparative Genetic Mapping in the Octoploid Progenitors of *F. x ananassa*—50K SNP Array

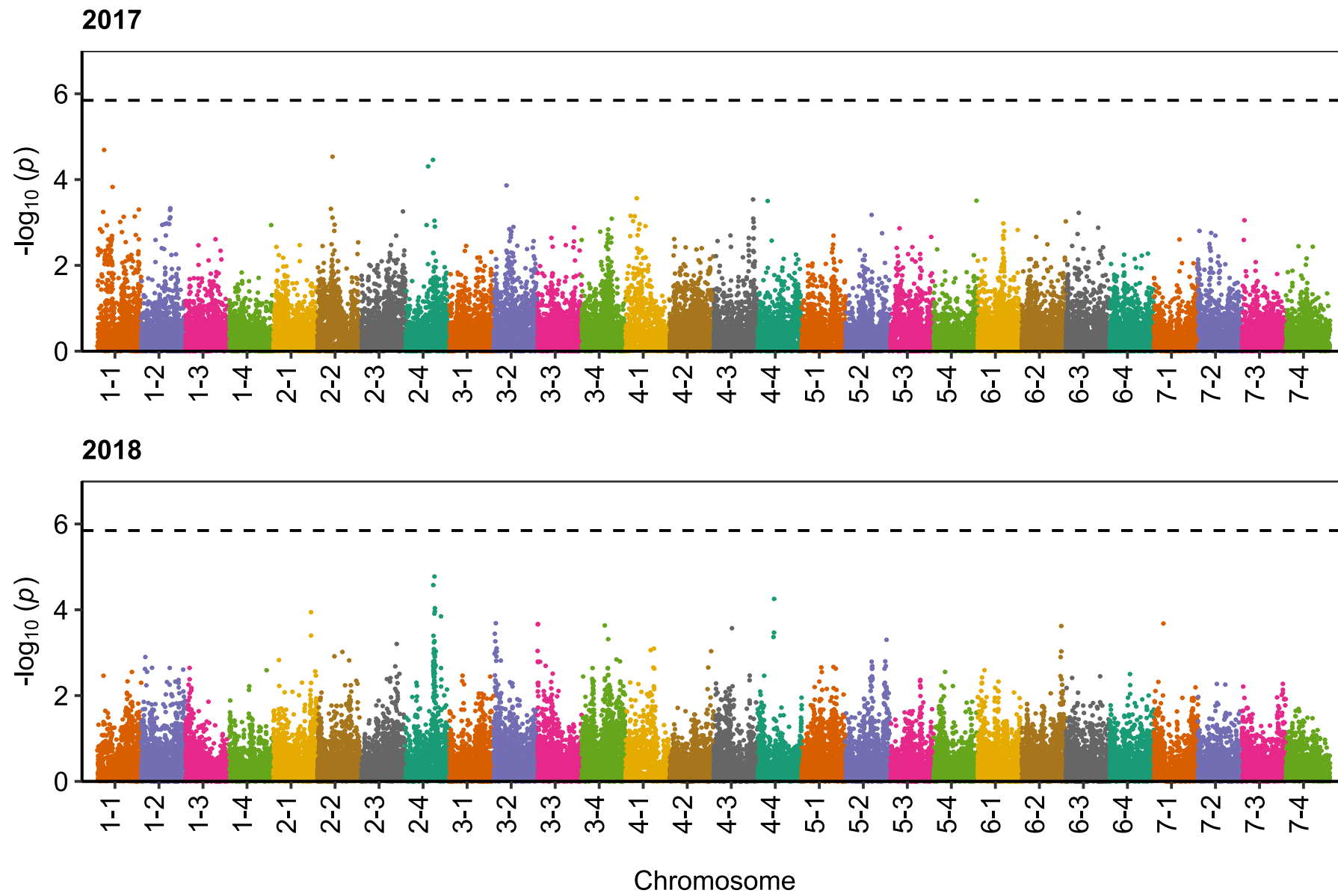


Hardigan, Feldmann, et al. (Unpublished Data)

High-Density Genetic Mapping in the Wild Octoploid Progenitors

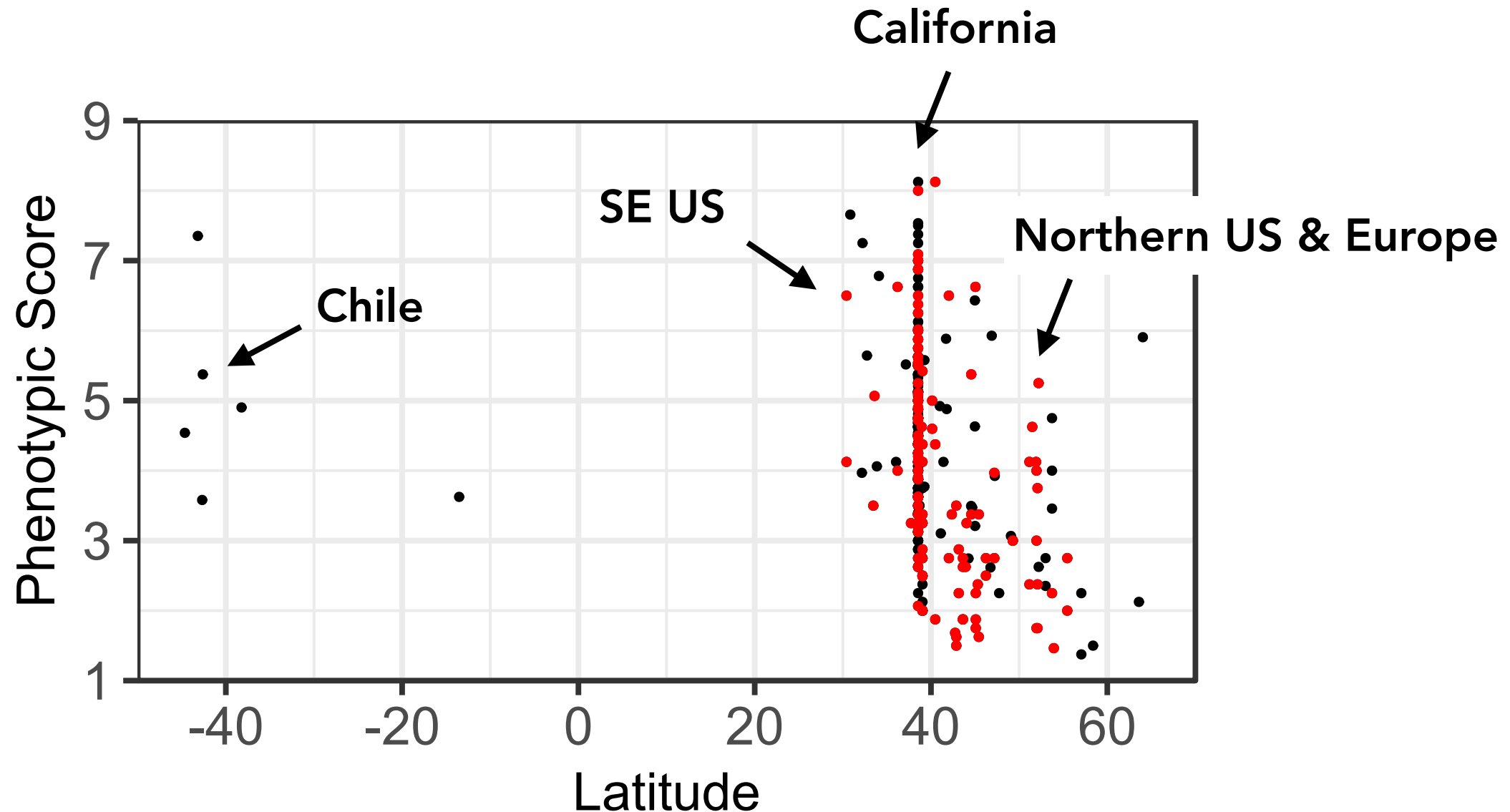


**Resistance to Verticillium Wilt is Genetically Complex (Quantitative):
Race-Specific Resistance Genes Have Not Been Discovered**
**Genome-Wide Association Study (GWAS) Using a Mixed Model to
Correct for Population Structure**



Verticillium Wilt Resistance Latitude Trends

- **MOST RESISTANT** Cultivars Originated in Northern Latitude ($> 45^{\circ}\text{N}$)
Breeding Programs: $1.4 < \bar{y} < 5.2$
- **California**: Moderately Resistant ($\bar{y} = 3$) to Highly Susceptible ($\bar{y} = 8$)



1 = Highly Resistant (Symptomless)

9 = Highly Susceptible (Dead)

Macrophomina Phenotypic Selection
UCD Armstrong Farm
2019-6-30



2019-6-19



2019-6-30







R

R

S

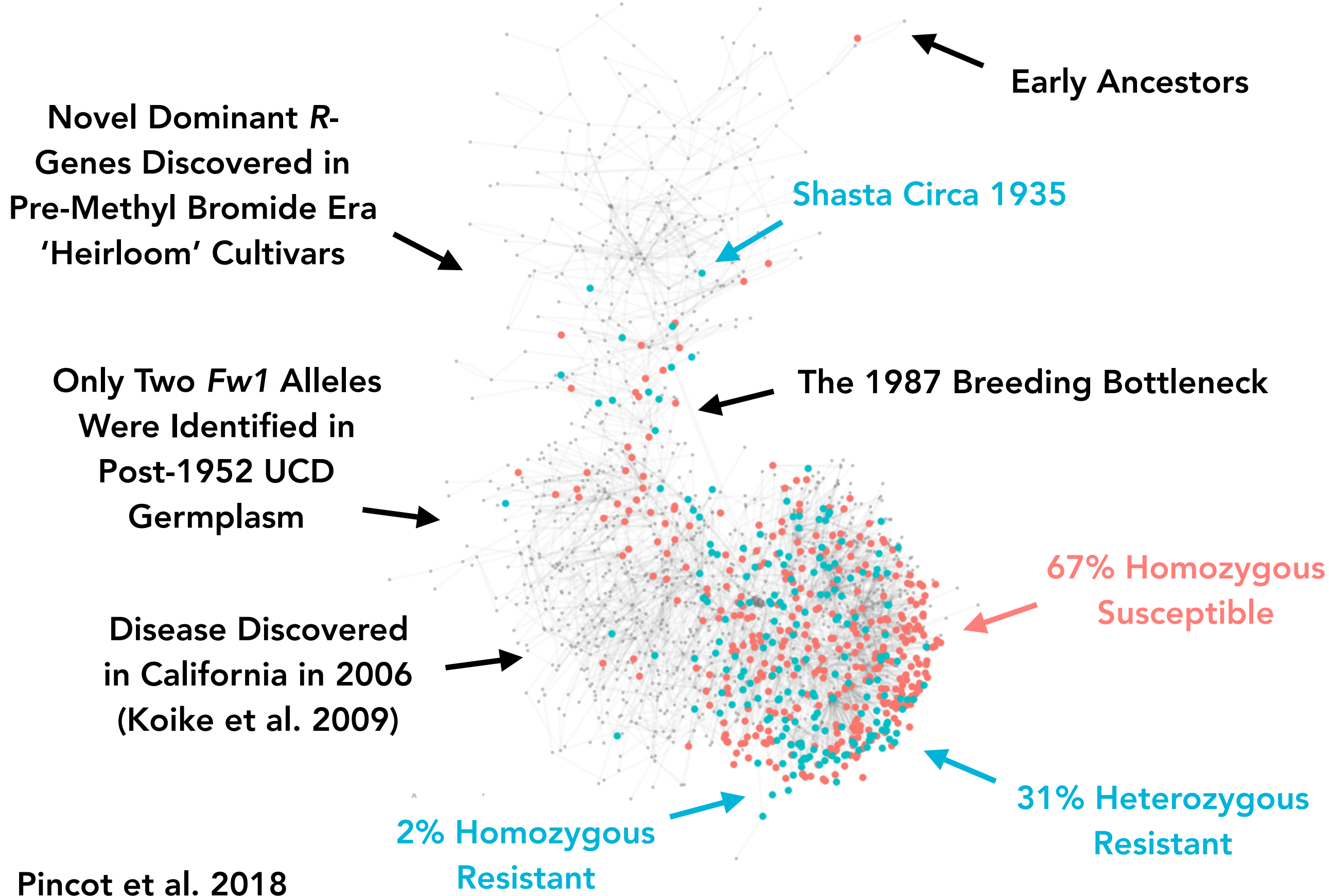
S

R

S

S

1,000 Genotypes Fusarium Wilt Resistance Study: Frequency of the *Fw1* Resistance Gene Was 0.16



Early Ancestors

Novel Dominant *R*-Genes Discovered in Pre-Methyl Bromide Era 'Heirloom' Cultivars

Shasta Circa 1935

Only Two *Fw1* Alleles Were Identified in Post-1952 UCD Germplasm

The 1987 Breeding Bottleneck

67% Homozygous Susceptible

Disease Discovered in California in 2006 (Koike et al. 2009)

31% Heterozygous Resistant

2% Homozygous Resistant