FOR2U-1, A DOMINANT GENE CONFERRING RESISTANCE TO FUSARIUM WILT IN STRAWBERRY

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Fusarium Wilt of Strawberry:

- Wilting disease caused by a soil-borne fungal pathogen : *Fusarium oxysporum* f.sp. *fragariae.*
- Originally discovered in Australia in 1965, but has since been identified in many major strawberry producing regions around the world.
  (Winks and William 1965; Koike and Gordon 2015.)
- Appearance in California in 2006 coincides with phase-out of Methyl Bromide and shifting cultural practices.
  (Koike et al 2009.)
- Few existing studies on the genetics behind Fusarium wilt resistance, with contradictory results. (Mori et al 2005; Paynter et al 2016.)

Fresno >

Camarosa >

< Fronteras

< Monterey

Outline

- Phenotypic Screen and Genome-Wide Association Study (GWAS)

- Forming a Working Hypothesis and Testing This Hypothesis using Mapping Populations

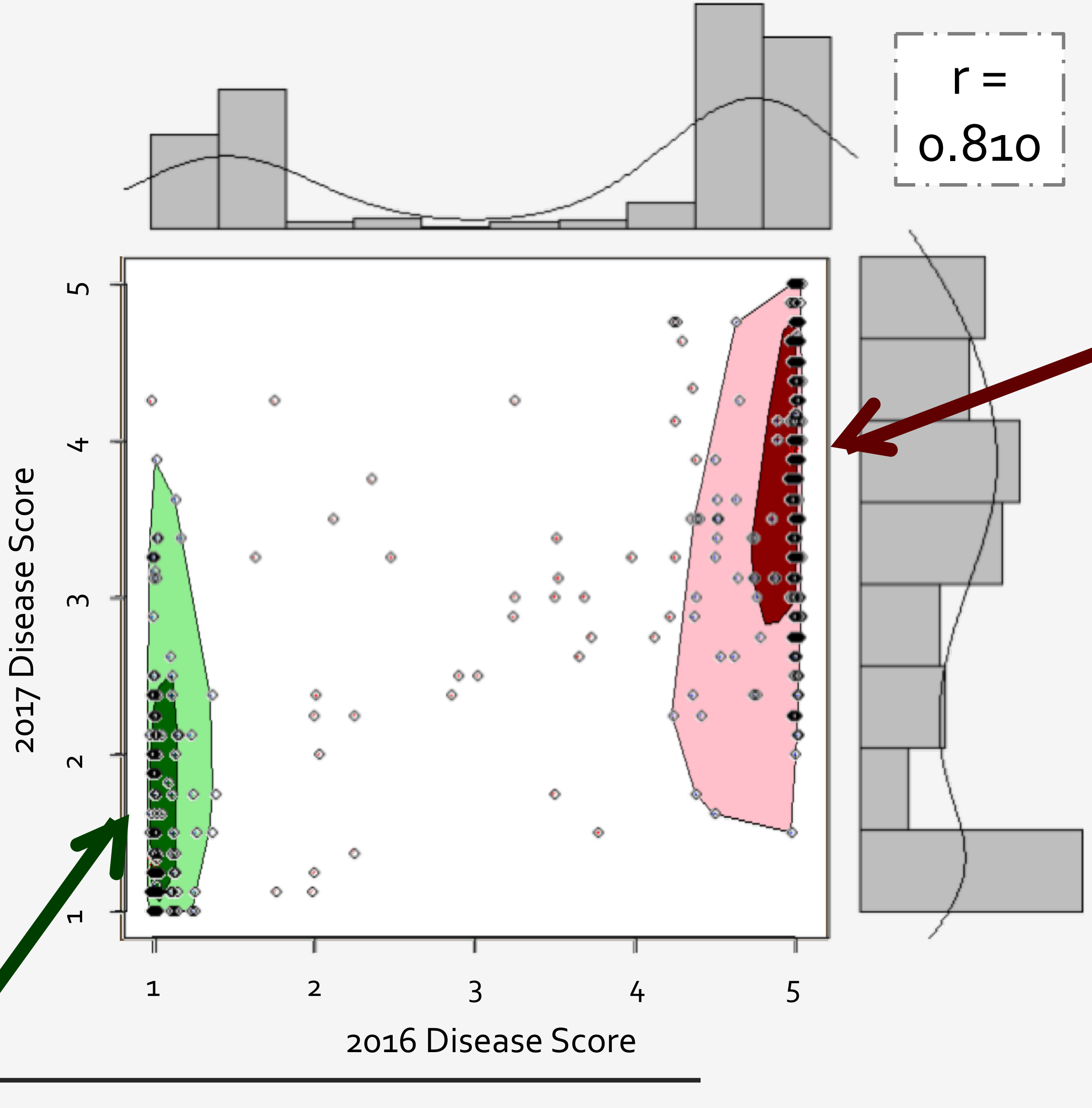
Phenotypic Screening and Genome Wide Association Study (GWAS):

GOAL

- Screen UC Davis germplasm collection to assess phenotypic diversity of Fusarium wilt resistance.
- Identify genetic bases of resistance.

DESIGN

- 566 historically and commercially important cultivars and accessions planted in α – lattice designs in 2016 and 2017.
- 566 accessions x 2 years x minimum of 5 timepoints = ~23,000+ datapoints.
- Inoculation: Root-dip procedure using a virulent, local *Fusarium* isolate (Isolate: AMP132; Gordon et al 2016.)
- Genotyping: iSTRAW35 Affymetrix Axiom array: 38506 SNPs (Verma et al 2016.)
- GWAS was performed using the R package 'GENESIS' (via Bioconductor), using positions informed by a diploid reference genome.



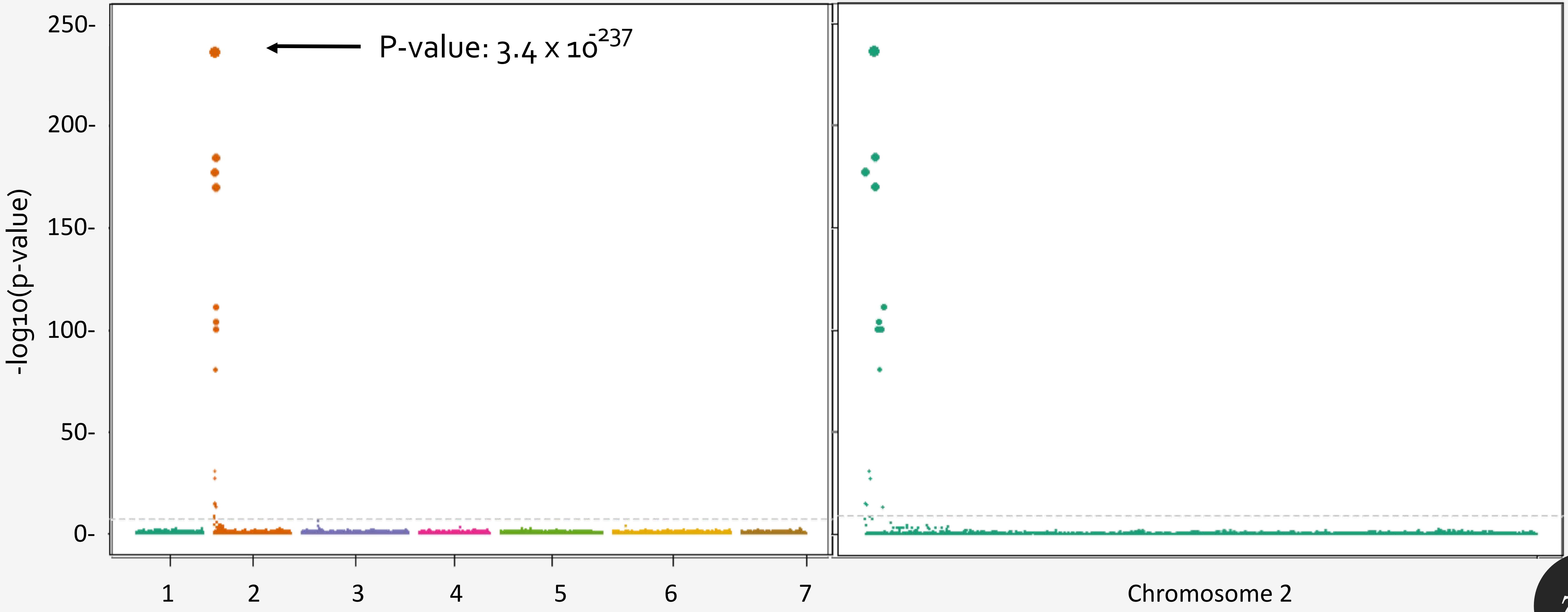
Ordinal Disease Scale:

1 = Healthy
5 = Dead

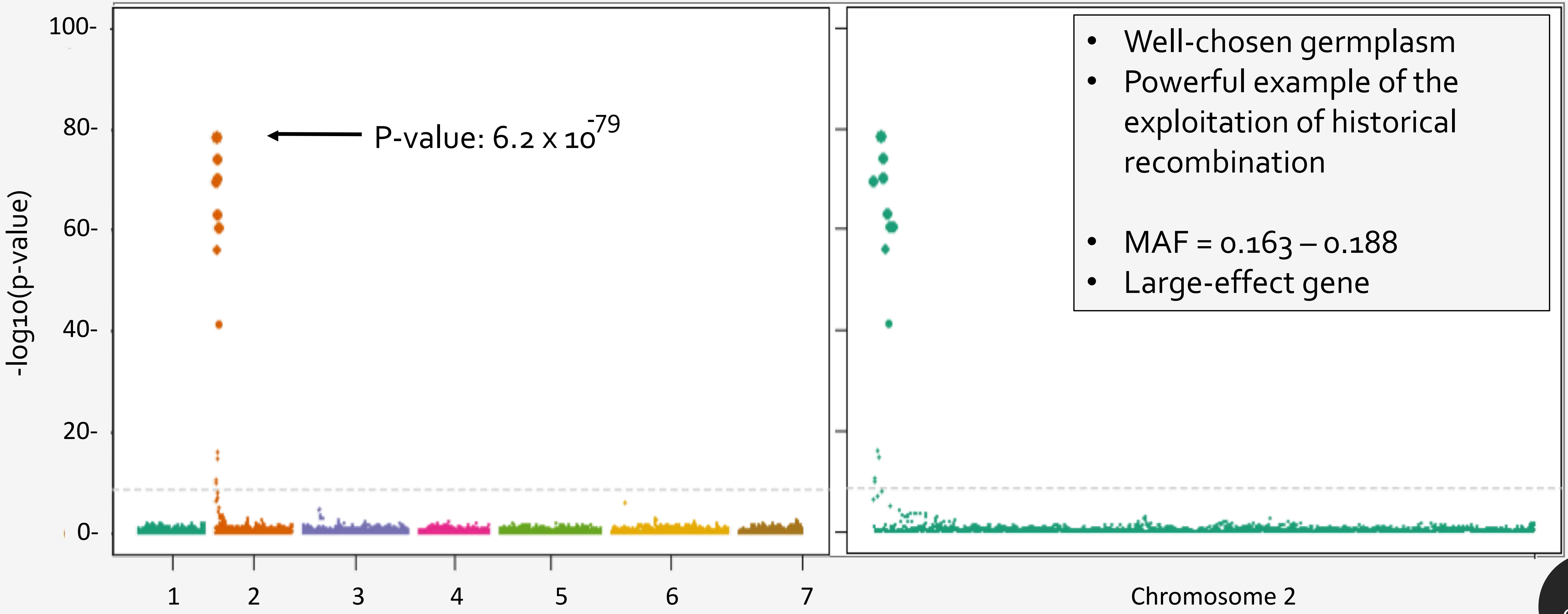
Repeatability:

2016 Data: 0.98
2017 Data: 0.90
Both Years Combined: 0.96

2016 GWAS


P-value: 3.4×10^{-237}
-log10(p-value)
250-
200-
150-
100-
50-
0-
1
2
3
4
5
6
7
Chromosome 2

2017 GWAS



P-value: 6.2 x 10^{-79}

- Well-chosen germplasm
- Powerful example of the exploitation of historical recombination

- MAF = 0.163 – 0.188
- Large-effect gene

Outline

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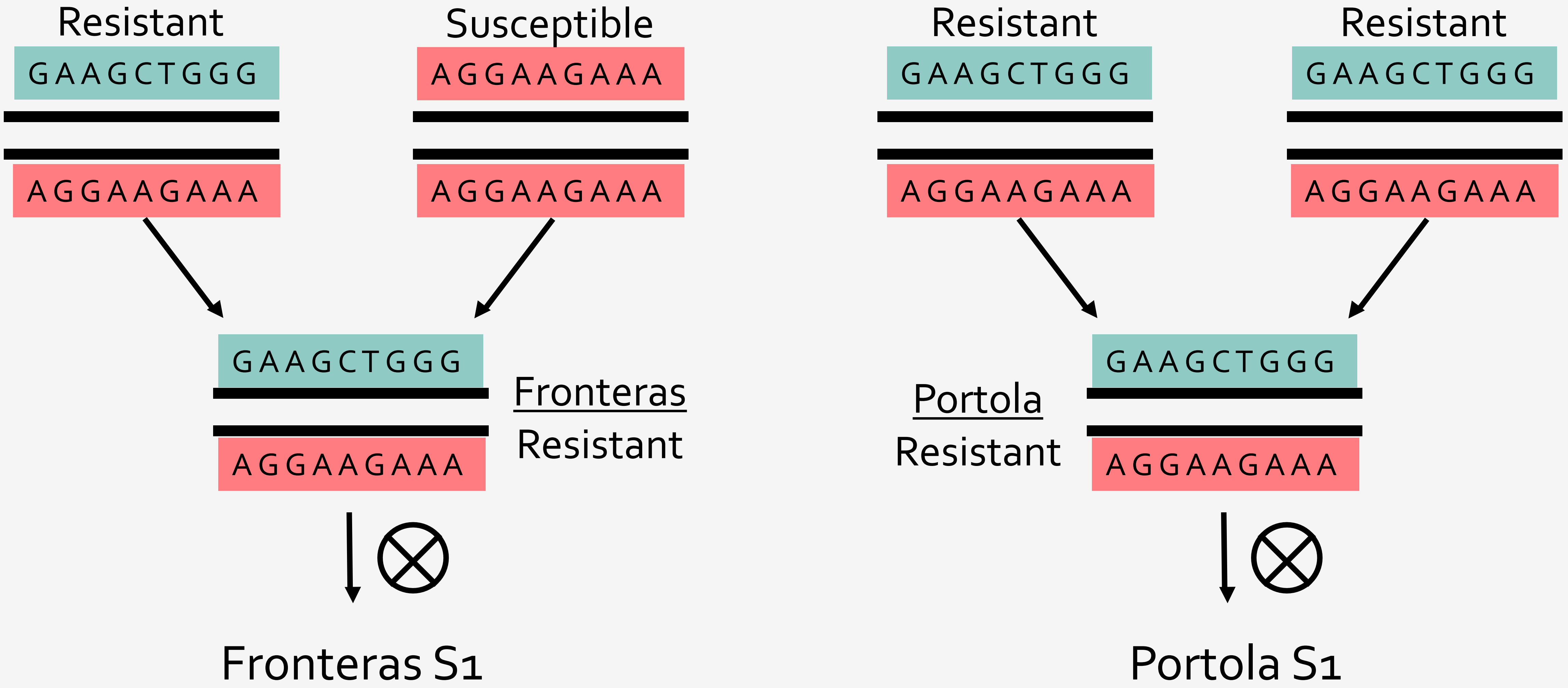
SNP ID’s	**R^2 (2016 / 2017)**	**δ/α (2016 / 2017)**
AX-574	0.888 / 0.575	0.997 / 0.869
AX-396	0.902 / 0.586	0.979 / 0.696
AX-947	0.895 / 0.581	1.001 / 0.829
AX-945	0.894 / 0.580	0.990 / 0.862
AX-058	0.786 / 0.523	1.007 / 0.875
AX-965	0.793 / 0.527	1.020 / 0.890
AX-940	0.774 / 0.522	0.962 / 0.854
AX-941	0.759 / 0.503	0.994 / 0.879
AX-640	0.767 / 0.509	1.017 / 1.000
Average	0.829 / 0.545	0.996 / 0.862



Resistant

GAAGCTGGG

AGGAAGAAA

Susceptible

AGGAAGAAA

AGGAAGAAA

GAAGCTGGG

AGGAAGAAA

Fronteras

Resistant

⊗

Fronteras S1

Resistant

GAAGCTGGG

AGGAAGAAA

Resistant

GAAGCTGGG

AGGAAGAAA

GAAGCTGGG

AGGAAGAAA

Portola

Resistant

⊗

Portola S1

Validating Hypothesis by Genetic Mapping:

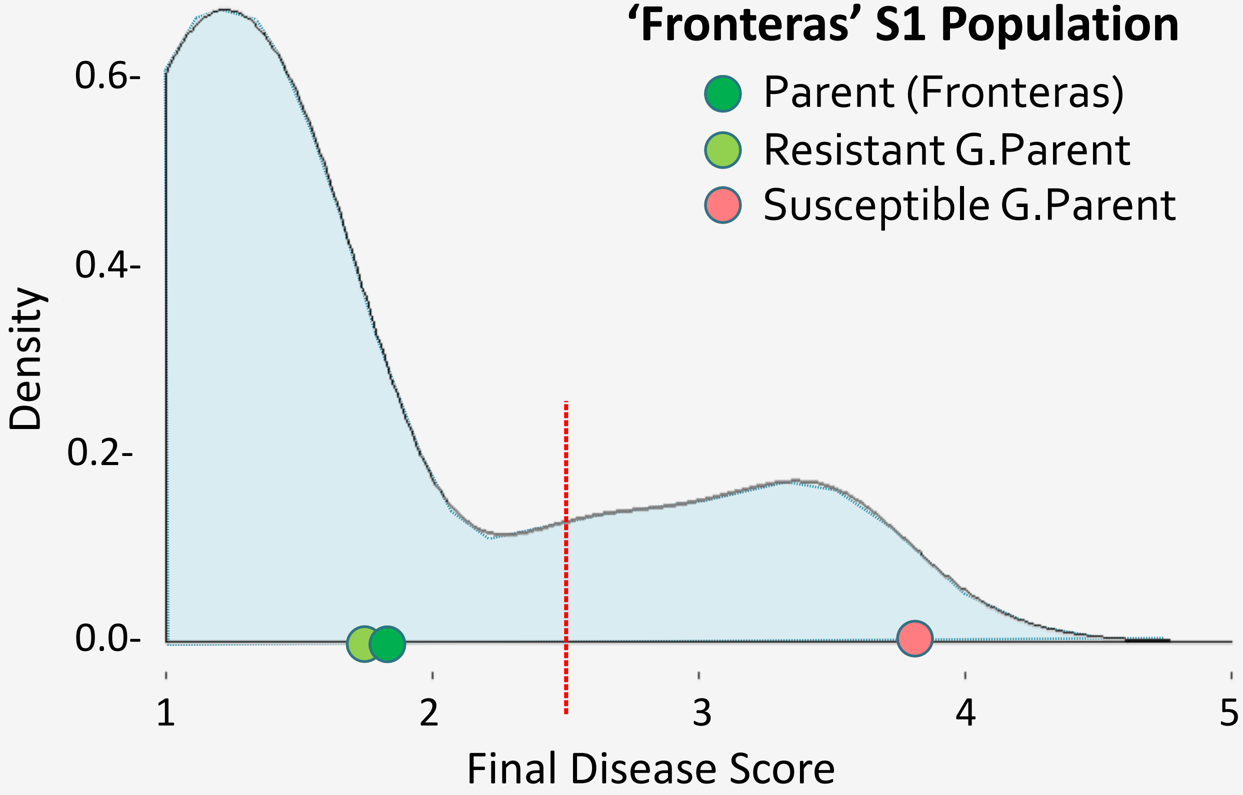
GOAL

- Test working hypothesis developed from GWAS results.

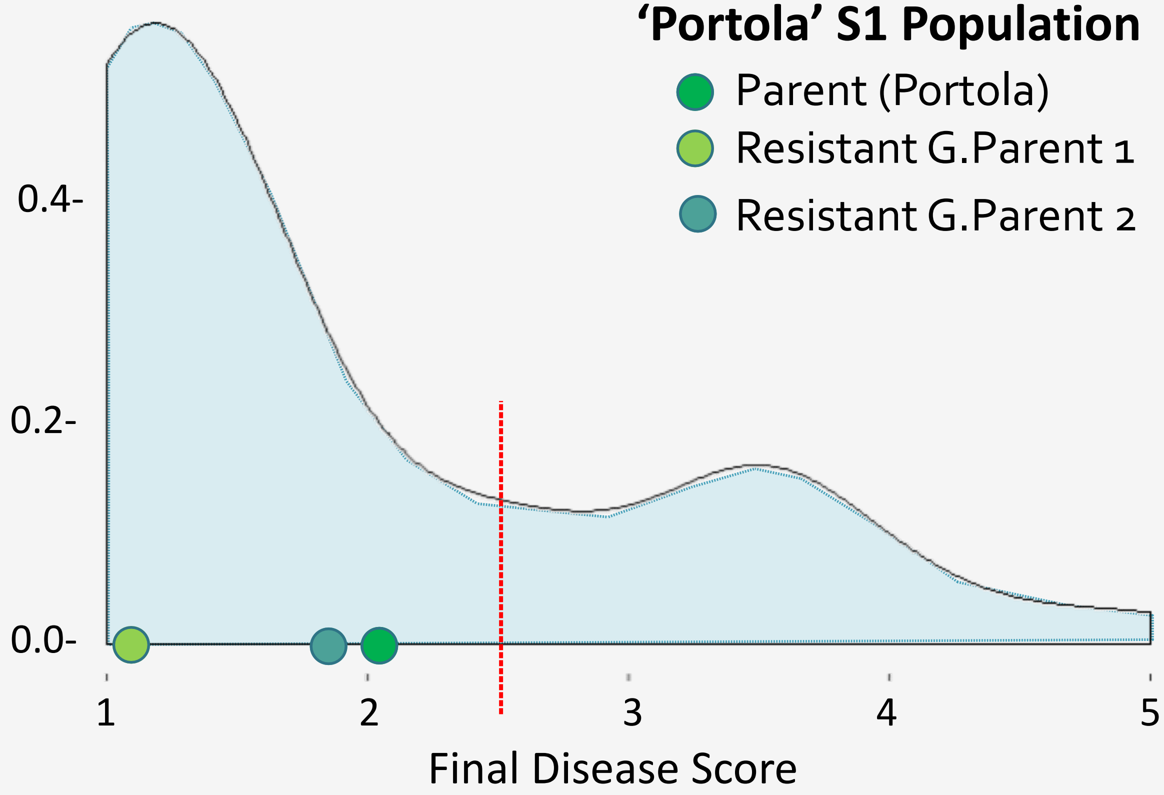
DESIGN

- 'Fronteras' and 'Portola' cultivars selfed to generate S1 mapping populations.
- Planted in augmented block design with parents/grandparents. Inoculation procedure the same as GWAS experiment.
- 93 random individuals from each population genotyped using the iSTRAW35 Affymetrix Axiom Array (38506 SNPs.)
- One *de novo* genetic map constructed for each mapping population using JOINMAP 4.0; QTL mapping performed using R/QTL in R.

Phenotypic Distributions: 3 to 1 Ratios

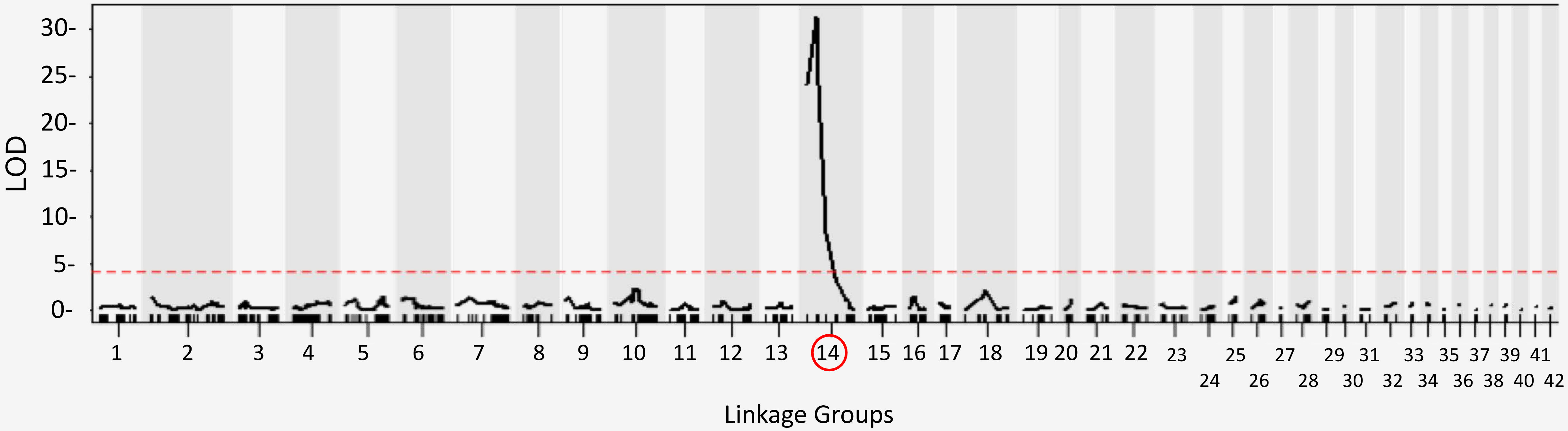


Fronteras S1: $\chi^2(1, N=92) = 0.058$, $p = 0.8097$

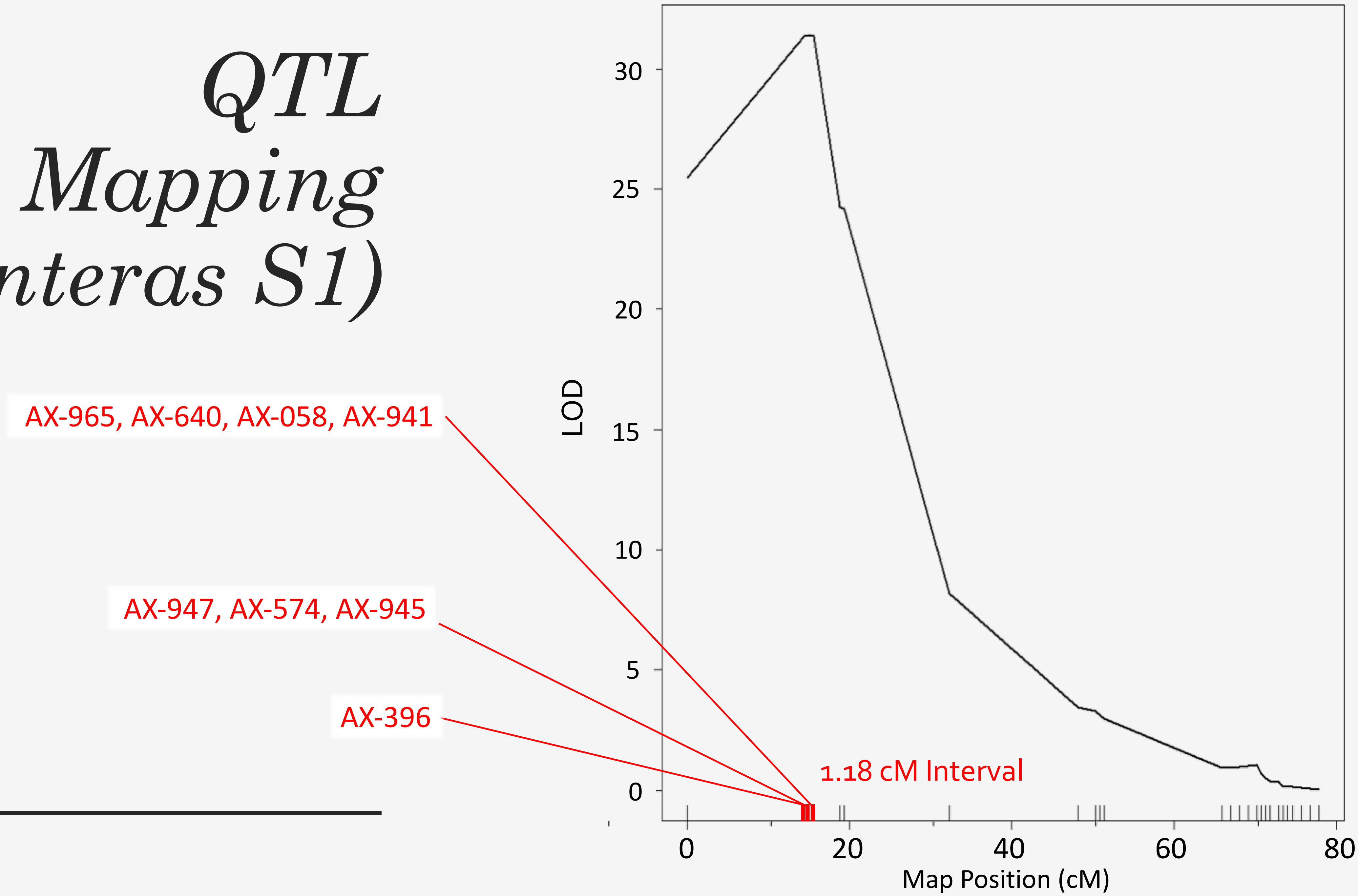


Portola S1: $\chi^2(1, N=92) = 1.449$, $p = 0.2286$

QTL Mapping (Fronteras S1)



QTL Mapping (Fronteras S1)



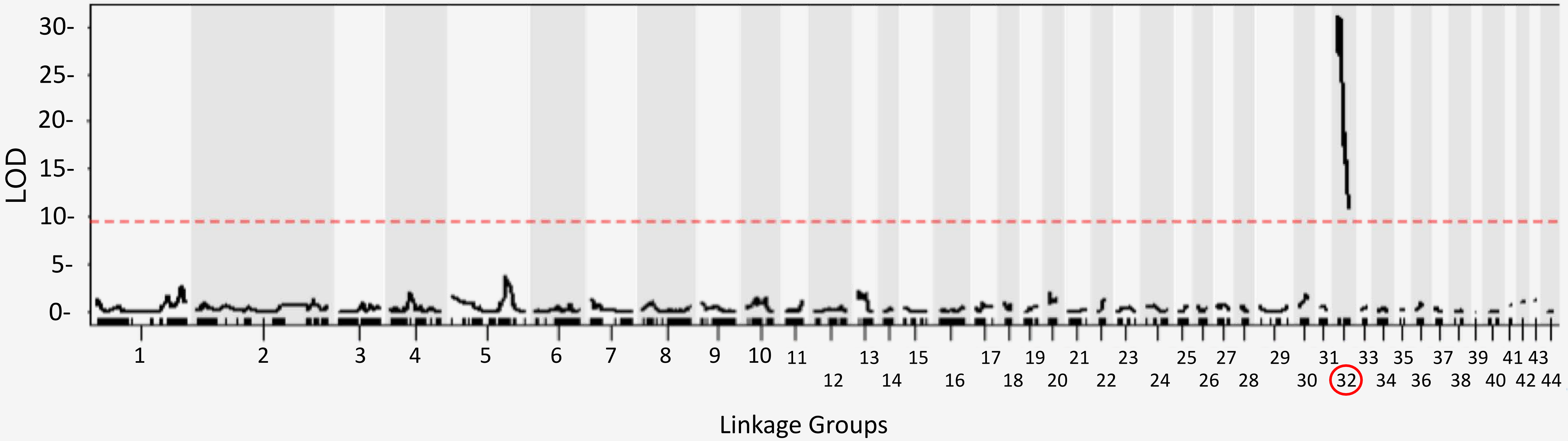
AX-965, AX-640, AX-058, AX-941

AX-947, AX-574, AX-945

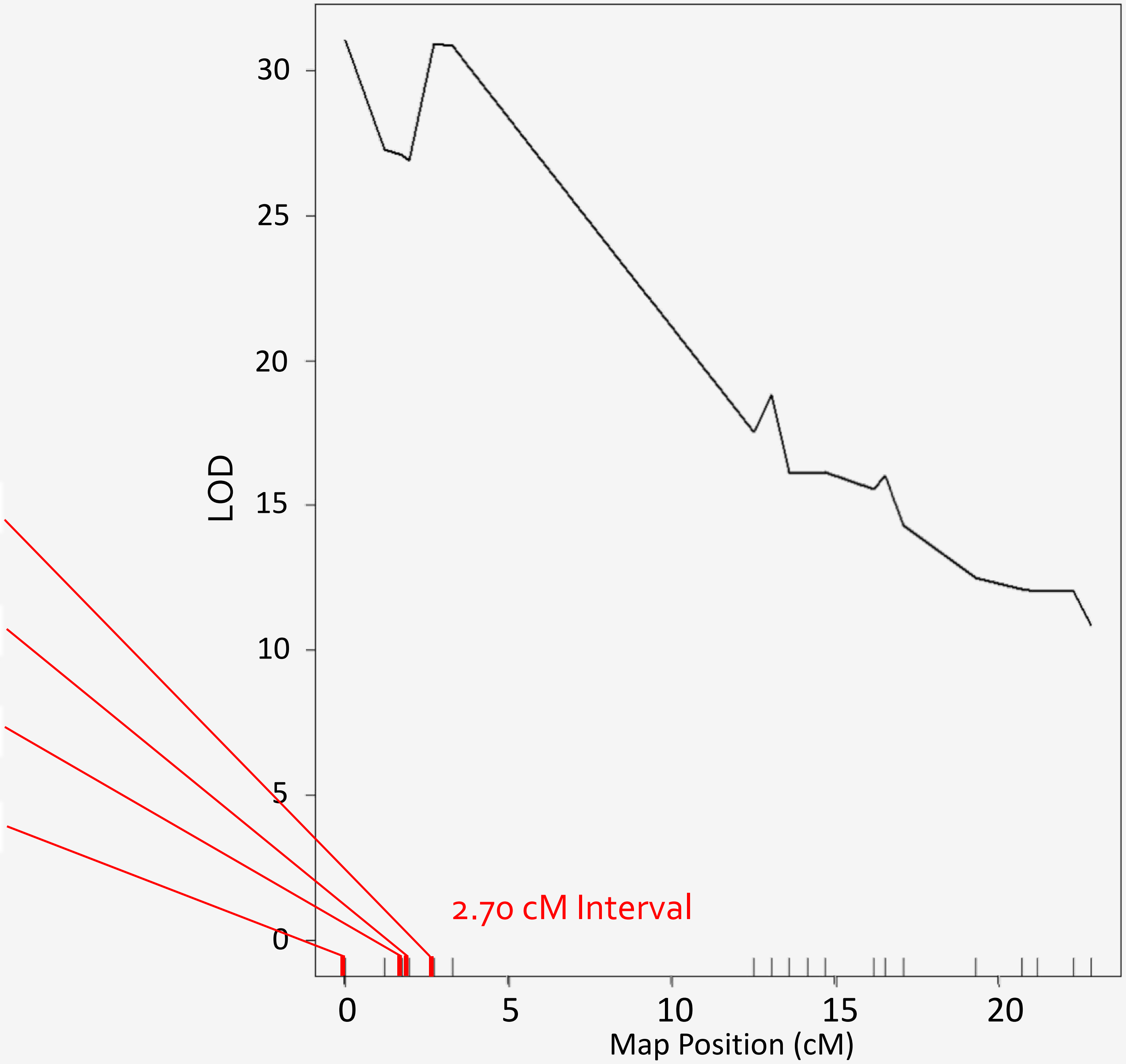
AX-396

1.18 cM Interval

QTL Mapping (Portola S1)



QTL Mapping (Portola S1)



AX-947, AX-574, AX-945

AX-058

AX-640, AX-965, AX-941

AX-396

2.70 cM Interval

Interpretation

- Fusarium wilt resistance trait strongly associated with 9 SNPs in 2 independent years of testing.
- Average dominance-additive ratios of these 9 SNPs close to 1.
- 3:1 segregation of Fusarium wilt resistance observed in two S1 mapping populations.
- QTL mapping in the S1 mapping populations indicates strong LOD in region containing the 9 SNPs.

Fusarium wilt resistance in strawberry appears to be conferred by a dominant-acting resistance gene ("*FoR2U-1*") that is linked to 9 predictive SNPs on diploid *F. vesca* chromosome 2.

What Remains to be Done

- Align significant SNPS against an octoploid reference. This would allow:
  - Subgenome identification.
  - Precise marker design.
- Identify and investigate possible alternate resistance loci:
  - GWA panel including USDA / wild material.
  - Promising material from 2016 developed into S1 mapping populations that will be planted in Fall 2017.
  - USDA/wild x Elite Crosses planned.
- Apply this new knowledge to the Breeding Program to develop a more resistant strawberry for the growers of California.

Acknowledgements

Steve Knapp

Thomas Gordon

Charlie Brummer

Strawberry
Breeding
Program

UNIVERSITY OF CALIFORNIA, DAVIS

Charlotte Acharya
Glenn Cole
Tom Poorten & Julia Harshman
Mitchell Feldmann & Stefan Petrasch
Bruce Campopiano & the Crew

.....

And all supporters of the UC Davis
Strawberry Breeding Program!

Citations

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QUESTIONS?

1

2.5

3

4

5

*Growth Chamber Plants