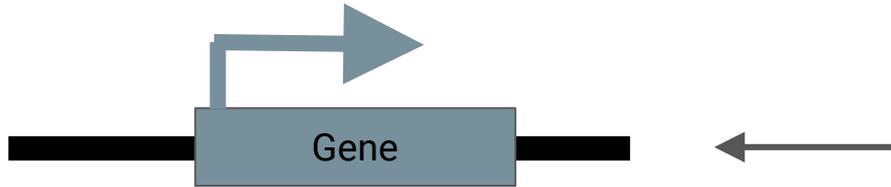


Multiple Approaches to Dissecting Fusiform Rust Disease

Daniel Ence
SFTIC 2017
June 20, 2017

The Big Question:

How has artificial selection shaped the genetics of important traits?



Artificial Selection/Domestication Leads to Phenotypic Changes

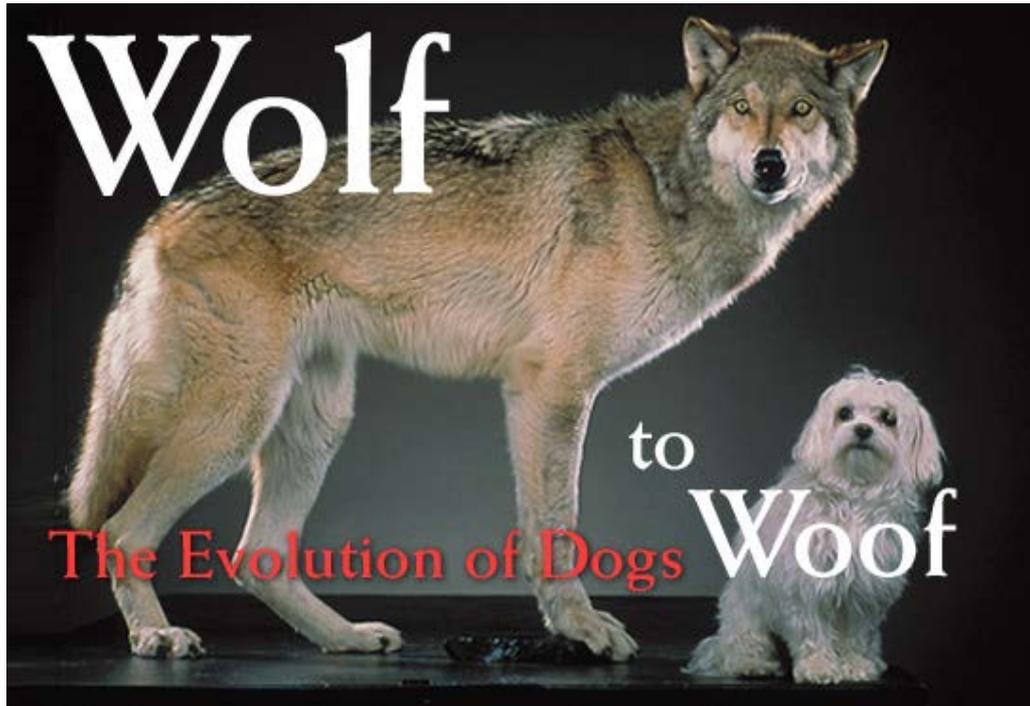


Older

More recent

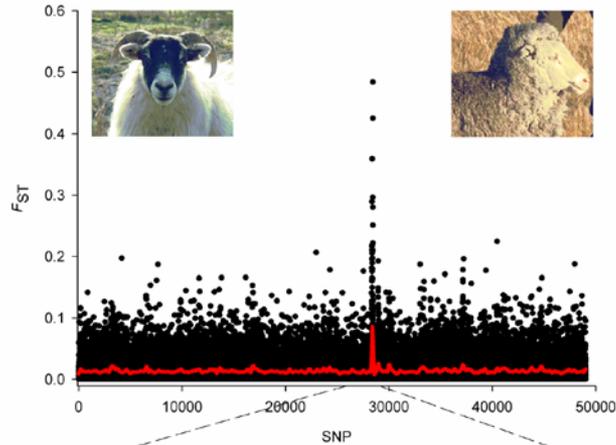
Adapted from:
learn.genetics.utah.edu
©Robert S. Peabody
Museum of Arch.

Artificial Selection/Domestication Leads to Phenotypic Changes

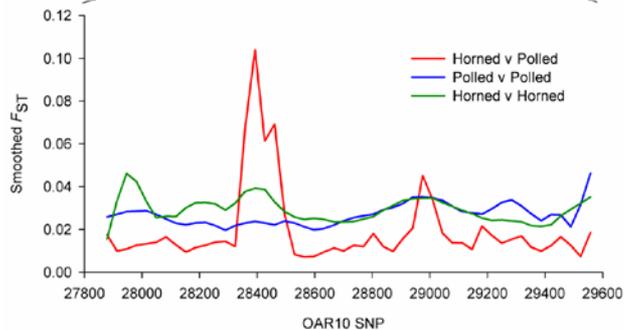


Photos by: Robert Clark
National Geographic
Magazine, Jan. 2002;
Karen Arnold,
publicdomainpictures.net

Population Genetics Identifies Genes w/ Strong Effect in Segregating Populations



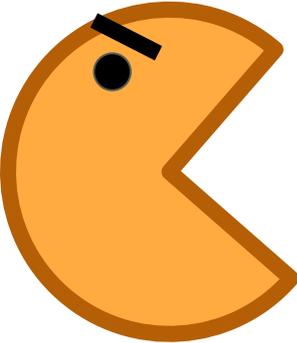
F_{ST} identifies SNPs responsible for polled vs horned phenotype in domestic sheep.



Kijas et al. 2012, PLOS Biology, vol. 10, 2012

Fusiform Rust:Loblolly Pine Pathosystem

Fusiform Rust Fungus
(CQF)



Selective Pressure
Between
Host and Pathogen

Loblolly Pine



Artificial Selection by
Breeders and Growers



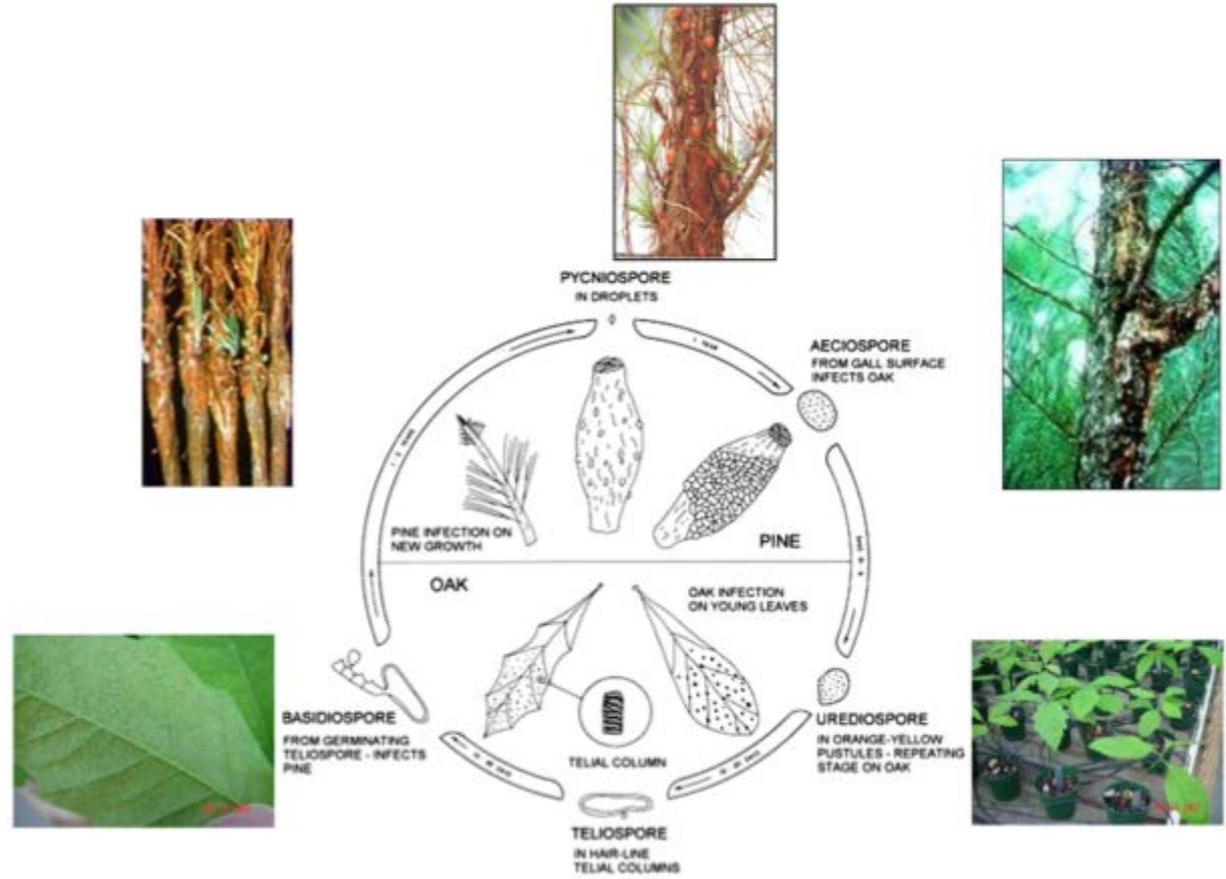
Fusiform Rust is an Important Problem for Growers



Kathy Smith

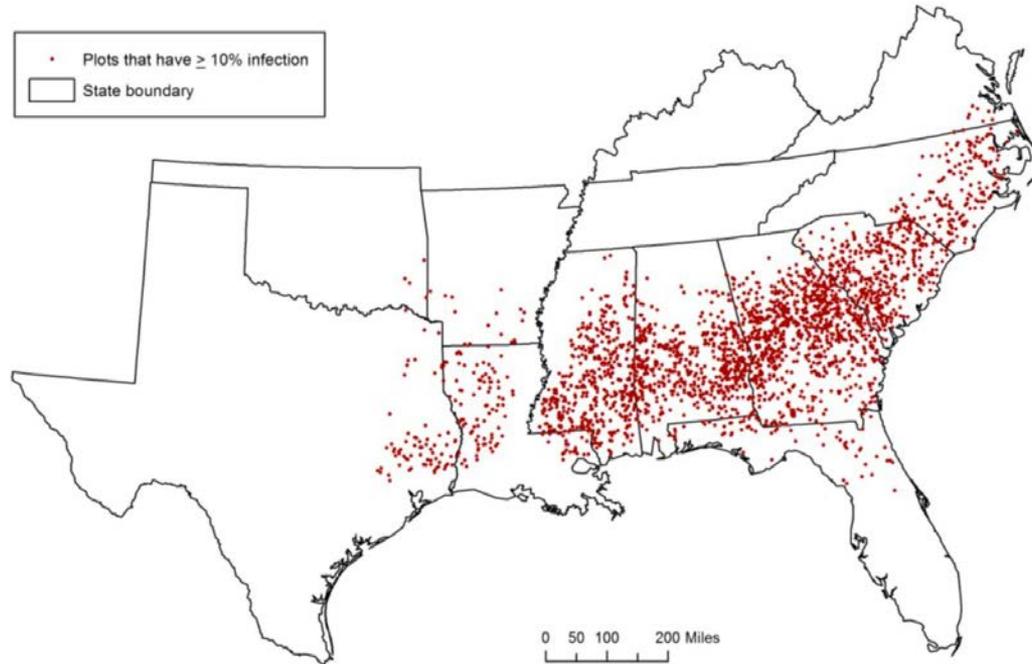


Fusiform Rust Life Cycle: Host Alternation





Fusiform Rust is an Important Problem for Growers



Cowling and Randolph, *Forests*,
vol. 4, 1220-1231, 2013



Rust Resistance in Pine is Controlled by Dominant Genes of Major Effect

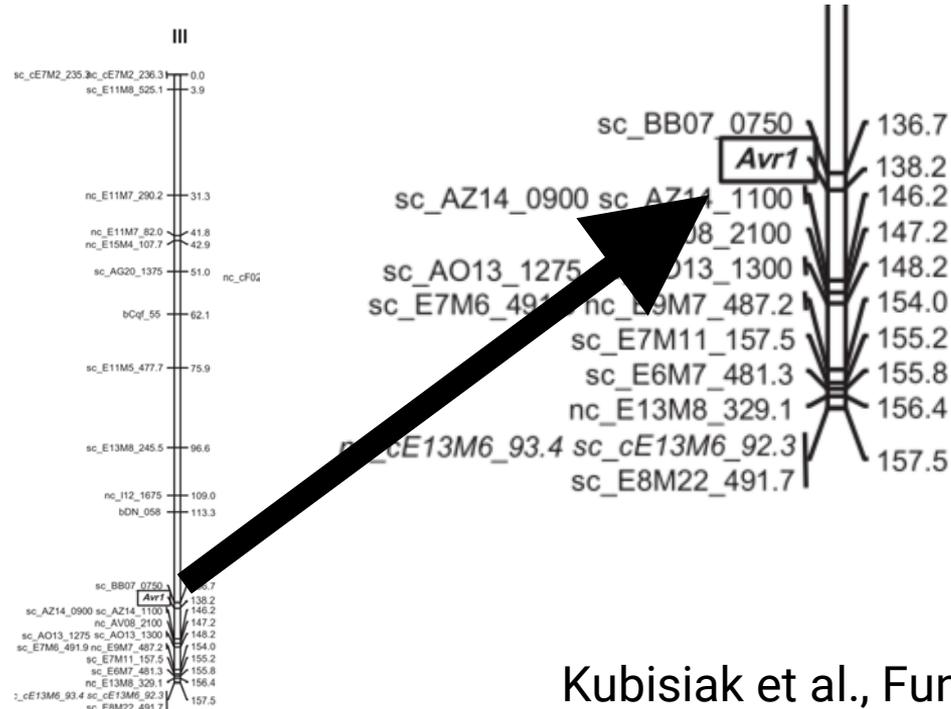
		Disease phenotype	
		No gall	Gall
Marker J7-485a	+	168	3
	-	52	163

a *c*
b *d*

Wilcox et al., PNAS,
vol. 93, 3859-3864, 1996



Virulence in CQF is Controlled by Dominant Genes of Major Effect

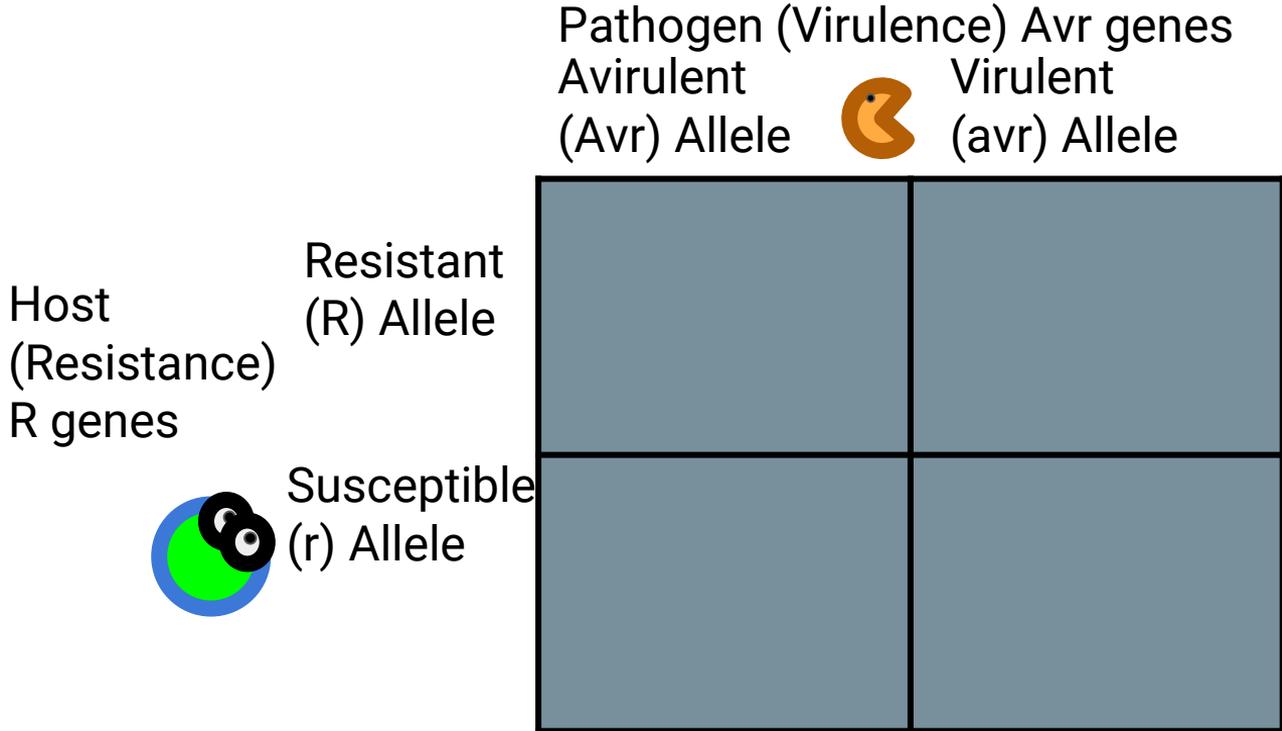


Kubisiak et al., Fungal Genetics and Biology, vol. 48, 266-274 2010

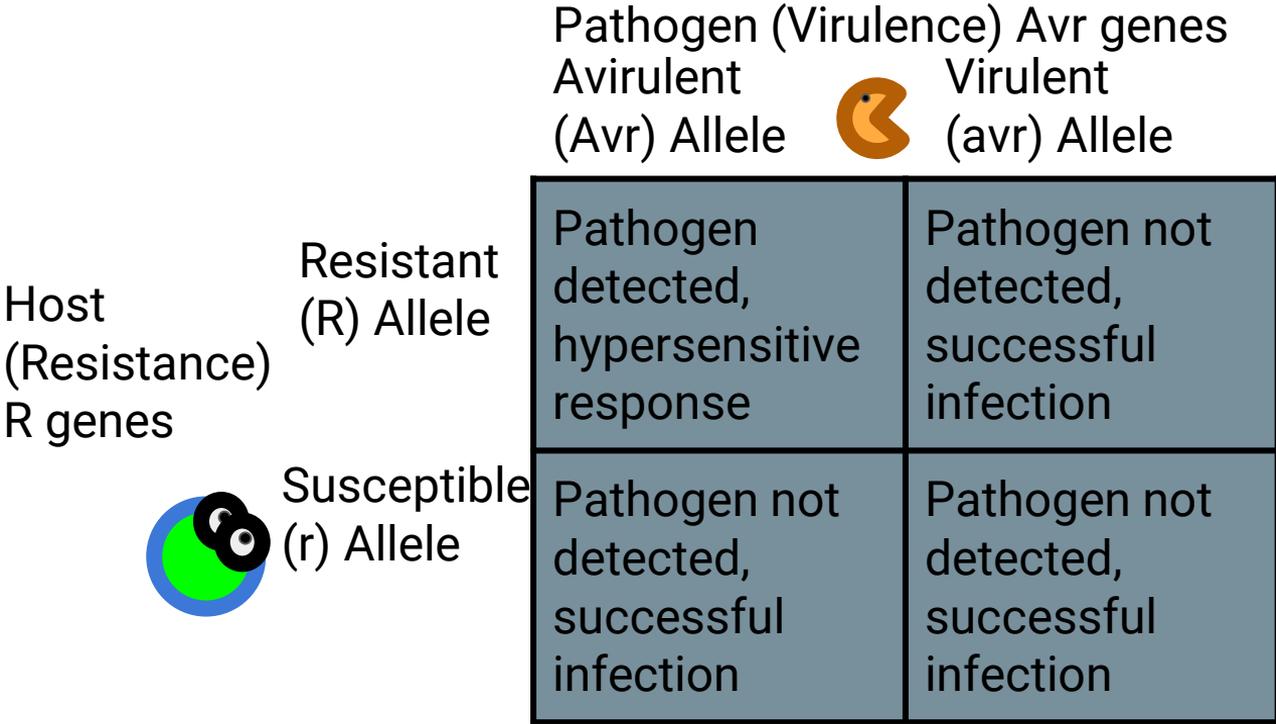
Gene-for-gene Hypothesis of Plant Pathosystems

“Rust resistance in flax is inherited as a dominant character although with some genes, dominance is not complete. Virulence in flax rust ... is inherited as a recessive character. “ H.H. Flor, *Advances in Genetics*, vol. 8, 1956

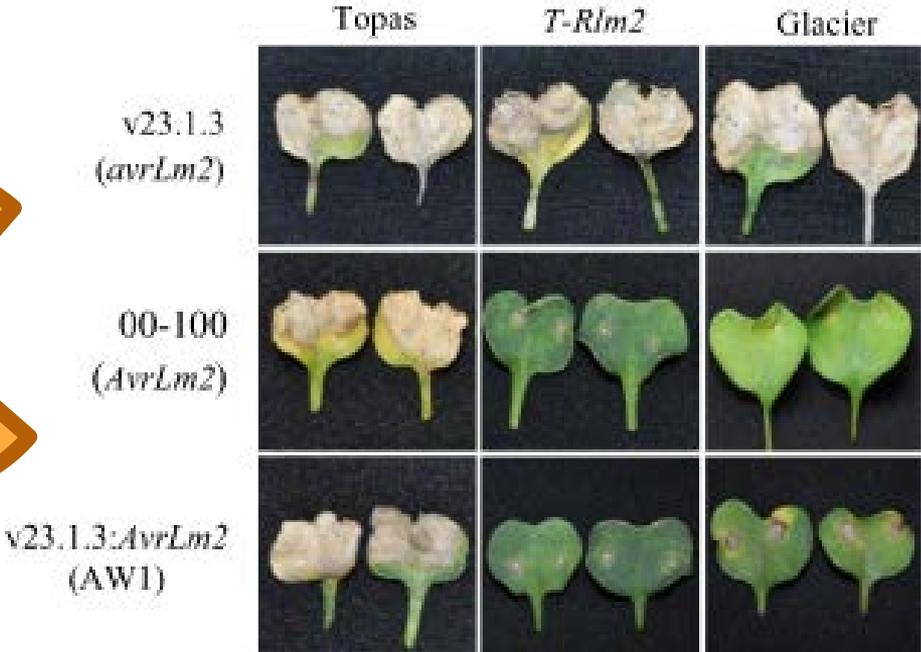
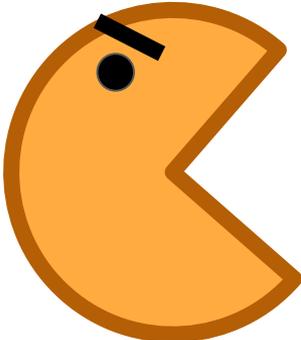
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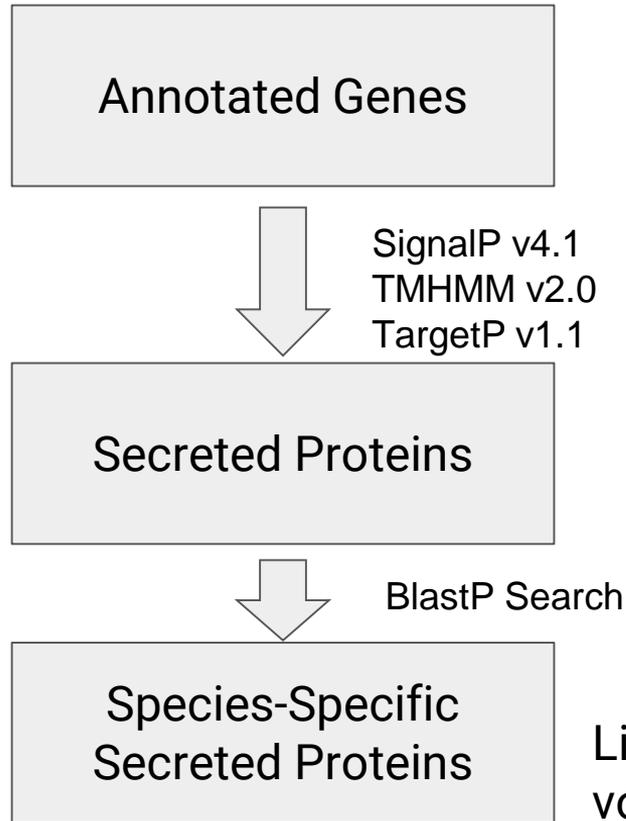
Gene-for-Gene Interactions in Blackleg of Canola



Ghanbarnia et al.
Molecular Plant
Pathology vol. 16, 2015



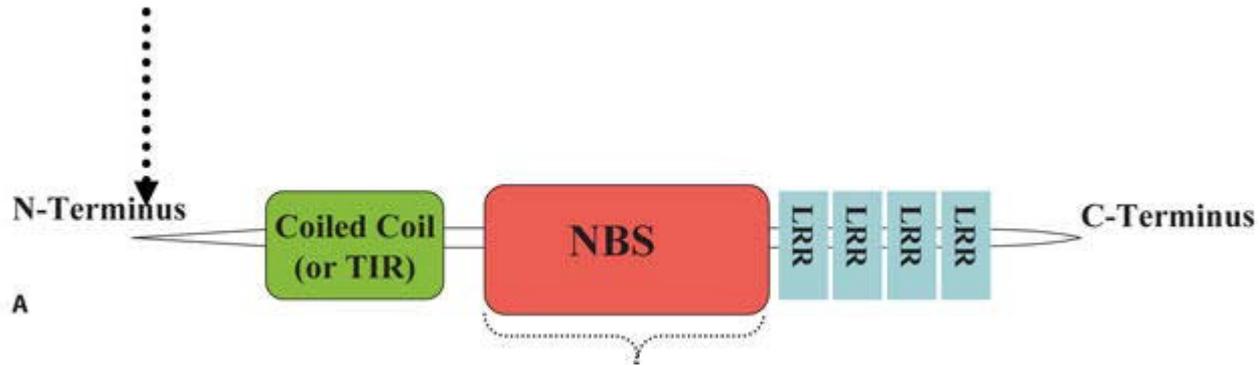
Bioinformatics Search for Putative Avr Genes



Liu et al., BMC Genomics,
vol 16, 2015



Domain-based Search for Putative R Genes



Azhar and Heslop-Harrison,
Cytogenetics and Genome Research,
vol 121, 59-66 2008

Questions in the Fusiform Rust: Loblolly Pine Pathosystem

Within segregating family:

What is the genic/allelic identity of *Avr1*?

What is the genic/allelic identity of *Fr1*?

Across elite pine families:

How many R genes does each family possess?

How many are shared between families?

How diverse are R genes in terms of nucleotide diversity?

Multiple Approaches to Dissecting Fusiform Rust Disease



Identify *Avr1* Gene

Bulk segregant analysis identified candidate *Avr1* gene.

Developing biological assay of candidate gene.



Identify *Fr1* Gene

Leveraged previously published *P. taeda* genome and linkage map.

Currently using sequence-capture on a set of half-sibs from resistant mother

R Gene Diversity

Transcriptome of 30 pools of 92 elite rust resistant pine families.

Preliminary results will be presented today.

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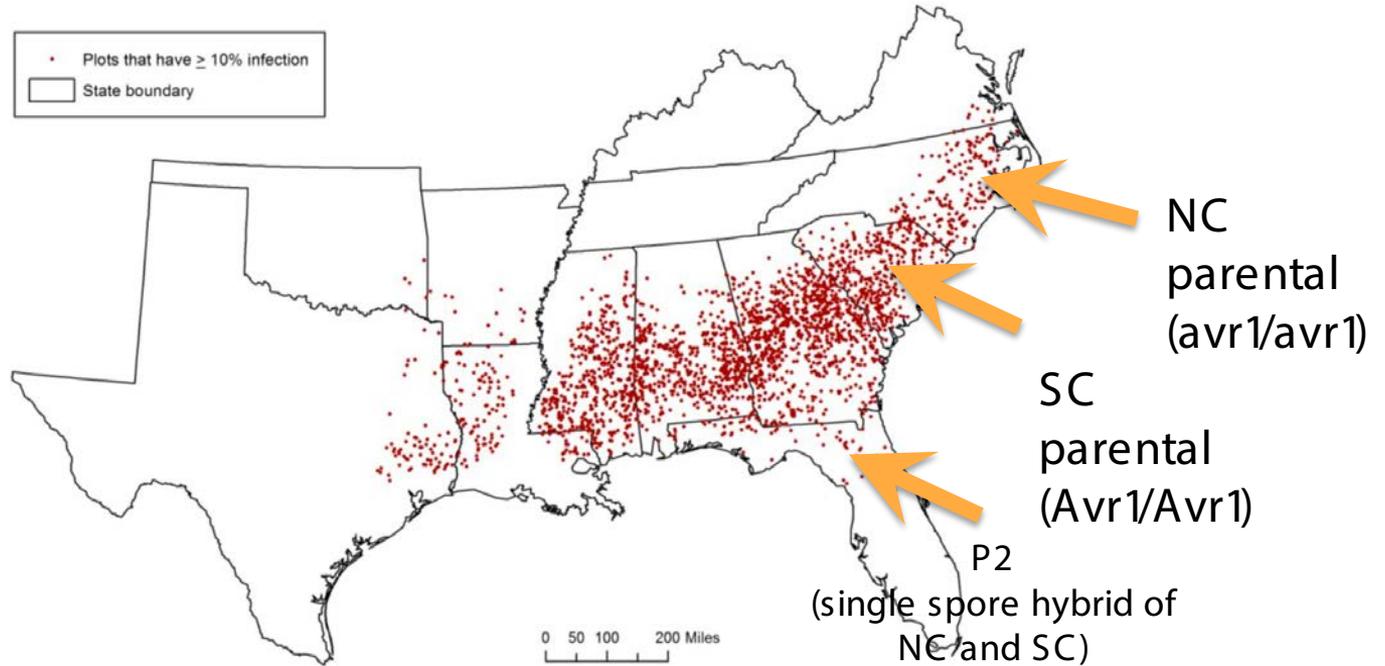
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Sequenced Pools of *Cqf* Genomes



Cowling and Randolph, Forests, vol. 4, 1220-1231, 2013

Bulk Segregant Analysis of P2 Pycnial Spore Pools

		Pine genotype	
		<i>Fr1</i> / - (resistant)	<i>fr1</i> / <i>fr1</i> (permissive)
<u>Fungal strain</u>	<i>Avr1</i> (avirulent)	No Gall	Gall
	<i>avr1</i> (virulent)	Gall 	Gall

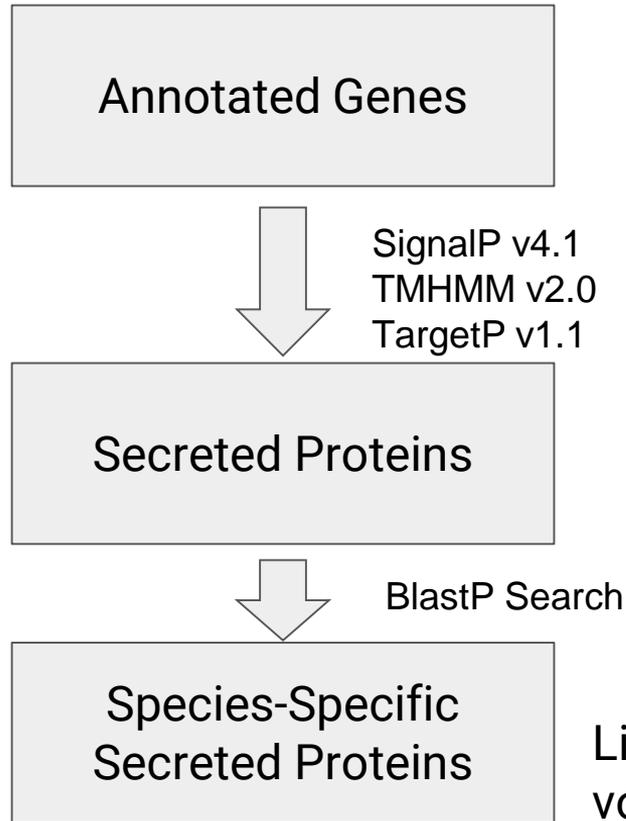


Courtesy of J.M Davis

Avr1 => Avirulent fungus, should grow only on *fr1/fr1* pines
avr1/Avr1 => Virulent fungus, should grow on *Fr1* and *fr1/fr1* pines



Bioinformatics Search for Putative Avr Genes

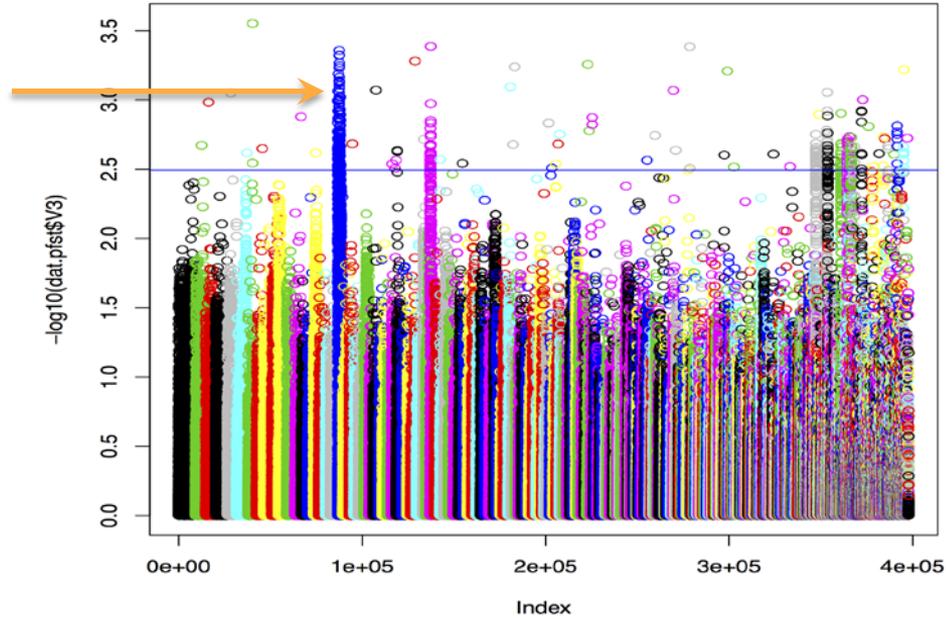


Liu et al., BMC Genomics,
vol 16, 2015



pFst Gives A Strong Genome-Wide Signal

scaffold 20



Criteria for candidate Avr1 genes

Possess properly segregating SNPs

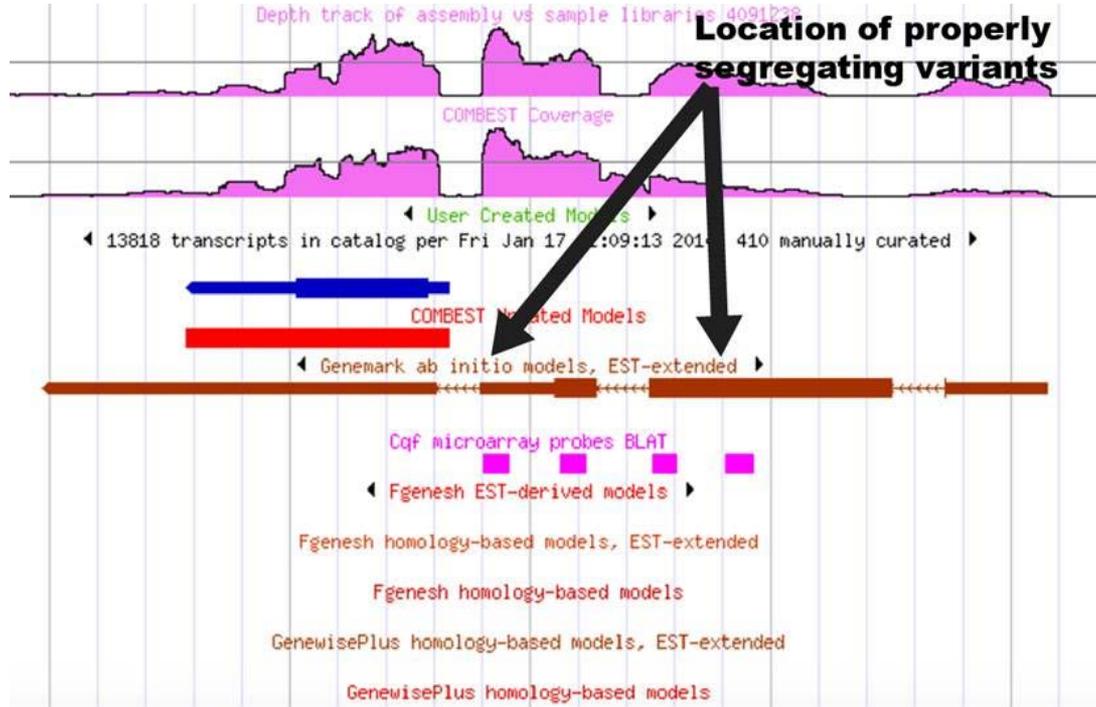
SNPs must be nonsynonymous

Genes must be within a genomic interval defined by linked-markers

Genes must have been identified by the secretome effector protein search



Candidate secreted effector Avr1 protein w/ SNPs



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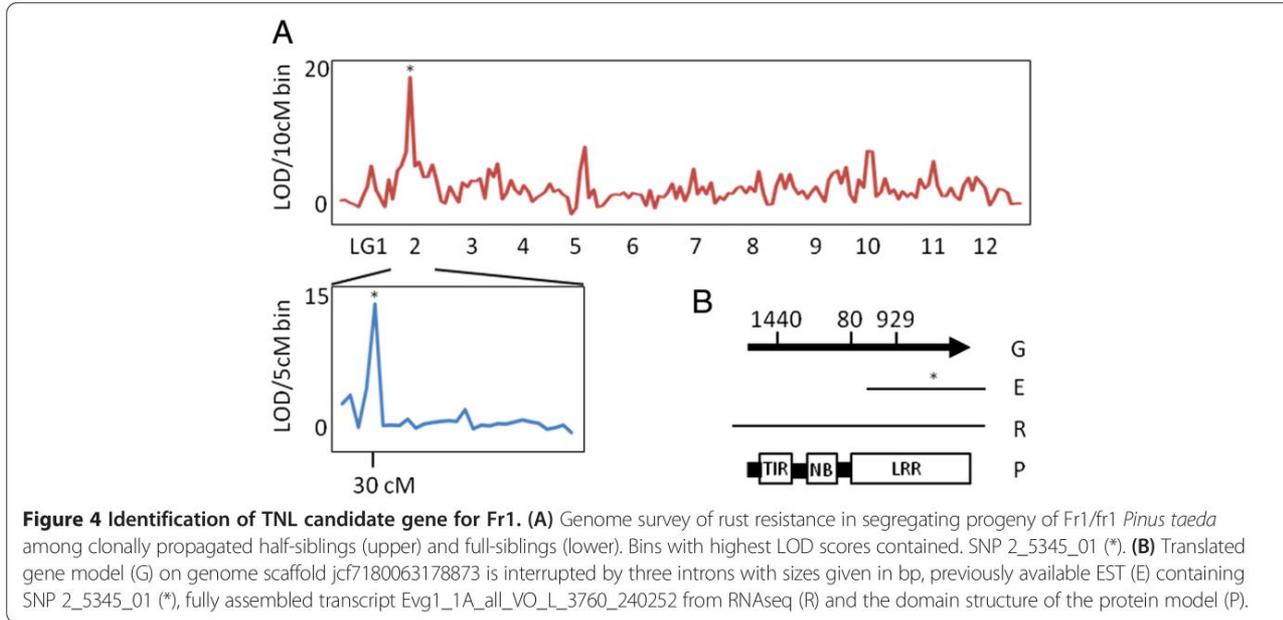
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SNP LOD Scores Map *Fr1* to a Candidate Gene





Design of sequence capture probes for Fr1 mapping

Sequence-capture w/ three subsets of probes:

1. Probes near candidate Fr1 gene

1. Probes targeting putative R genes:
 - a. Annotation of gene models in genome
 - b. Annotation of putative R genes in transcriptomes

3. Probes evenly spaced throughout genome

4. About 10K probes in total

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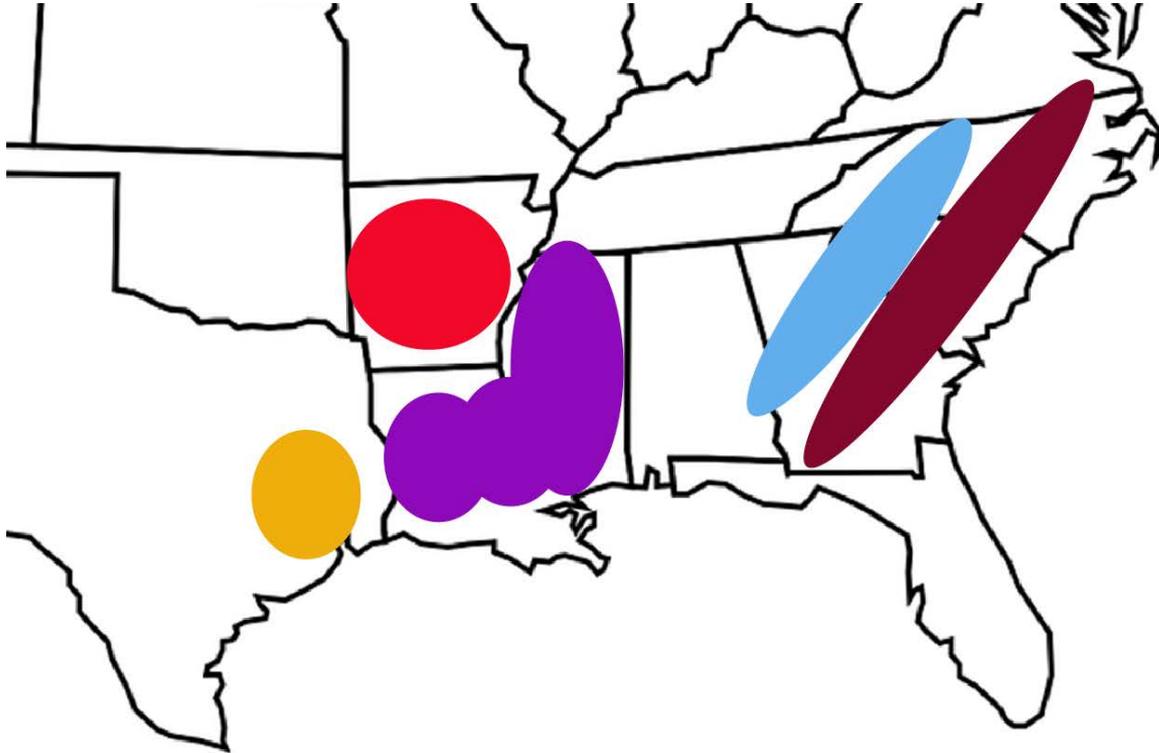
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Surveying R Gene

Diversity in Elite Rust Resistant Pine Families

RNA extracted from stem tissue of 92 elite rust resistant families of *P. taeda* from 5 regions across the southeast US.



Annotation of Putative R Genes

Robin Temple and Prof.
Jill Wegrzyn at U.Conn

ILLUMINA RNAseq from
pooled
elite rust resistant families

Transcripts assembled and
annotated with Trinity and
Transdecoder

Mined transcripts for R gene
domains

Analyzing Diversity of R Genes:

Method: Analyze R Gene Transcriptomic Diversity Using the Loblolly Pine Reference Genome

Hypotheses:

1. Most of the transcripts from each library would align to the reference genome
2. Most of the putative R genes from each library would align to the reference genome
3. Variants in mapped putative R genes local to each region
4. R genes that don't align to the reference are novel resistance genes
5. Novel putative resistance genes could map to novel Fr loci

Analyzing Diversity of R Genes: Results

1. Most of the transcripts from each library would align to the reference genome
 - a. Range from 30% - 70% of transcripts aligned. Median 45%.

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1. Most of the transcripts from each library would align to the reference genome
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2. Most of the putative R genes from each library would align to the reference genome
 - a. Between 8% - 92% of putative R genes don't align to the reference.

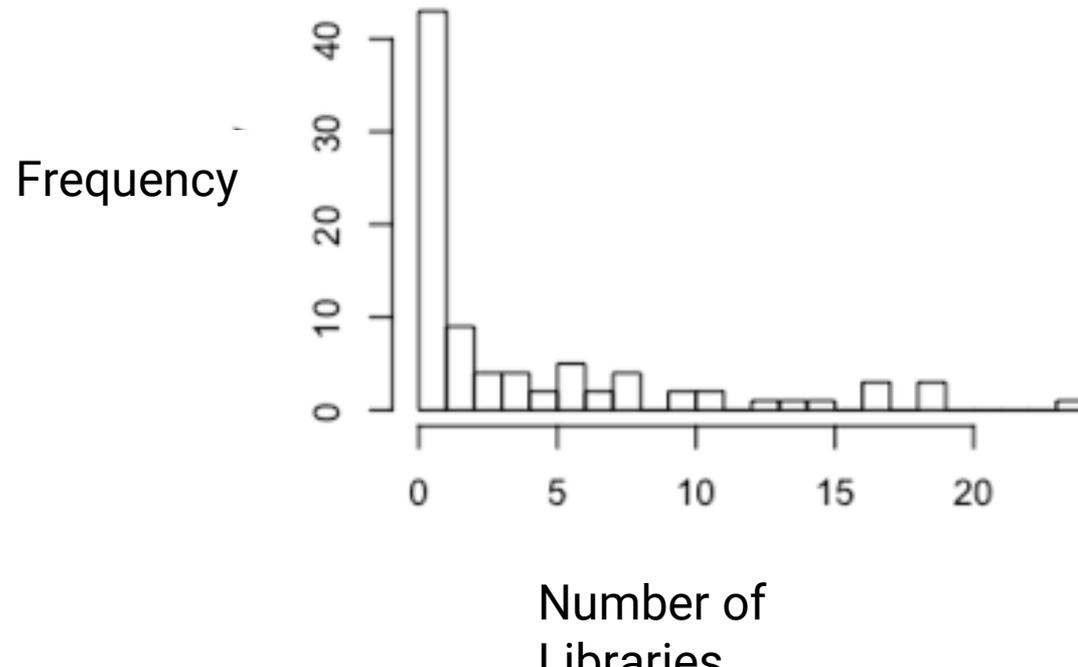
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3. R genes that don't align to the reference are novel resistance genes
 - a. Yet to be seen
4. Novel putative resistance genes could map to novel Fr loci
 - a. Yet to be seen

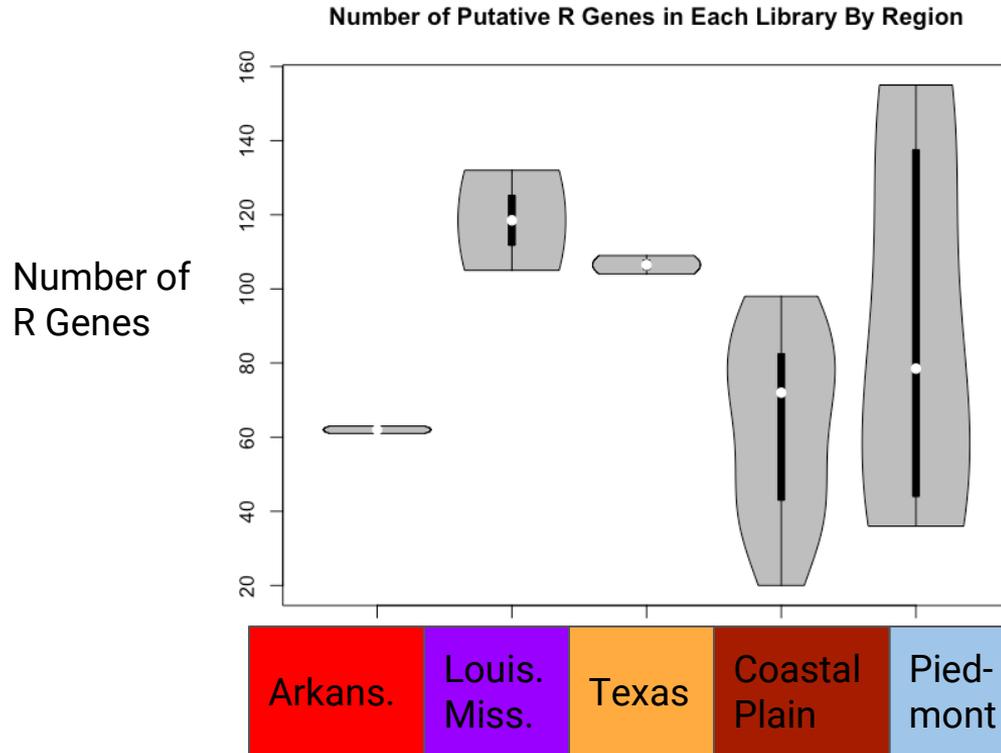
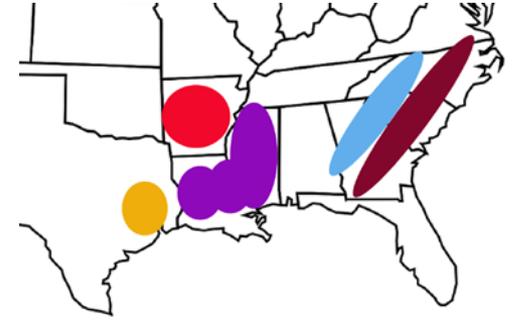
Analyzing Diversity of R Genes:

Elite Rust Resistant Families Have Different R Genes

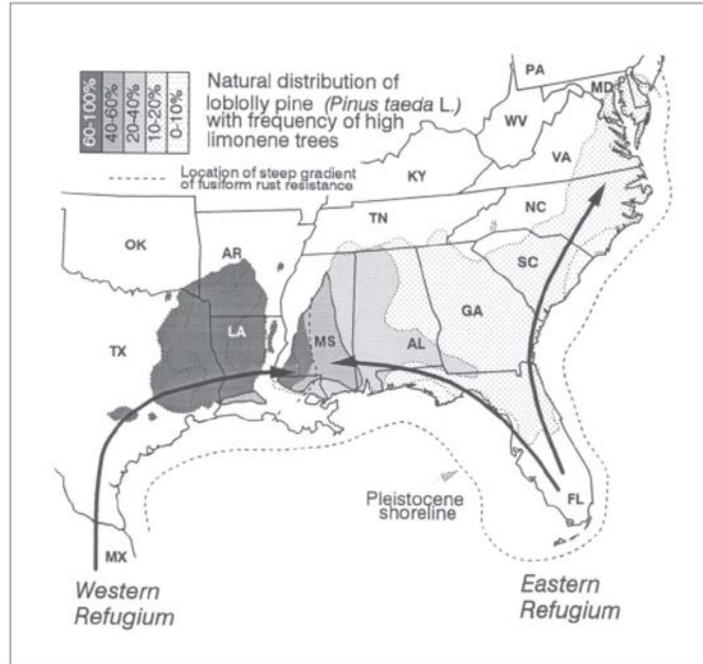
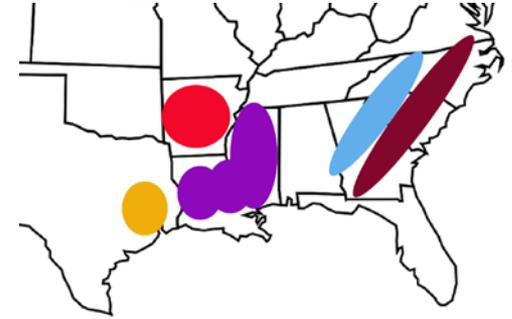
Number of Libraries Overlapping an R Gene



Analyzing Diversity of R Genes: Families from Different Regions Have Different Numbers of R Genes



Analyzing Diversity of R Genes: Possible evidence of proposed past refugia?



Adapted from Schmittling et al.
Silvae Genetica, vol. 48, 35-45, 1999

Summary and Future Directions

Summary

1. Ongoing work to identify Avr1 in fusiform rust
2. Ongoing work to identify Fr1 in loblolly pine
3. Survey of R Gene Diversity Across Loblolly Pine Families
 - a. Lots of diversity in number of putative R genes.
 - i. Diversity between and within geographic regions
 - b. Lots of transcripts can't be placed on the V1.01 ref. Assembly

Future Directions

1. Need to analyze diversity in non-ref. based methods
 - a. Sequence clustering

Acknowledgments



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CONNECTICUT
Jill Wegrzyn
Robin Temple



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United States Department of Agriculture
National Institute of Food and Agriculture

"Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture."

Questions

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