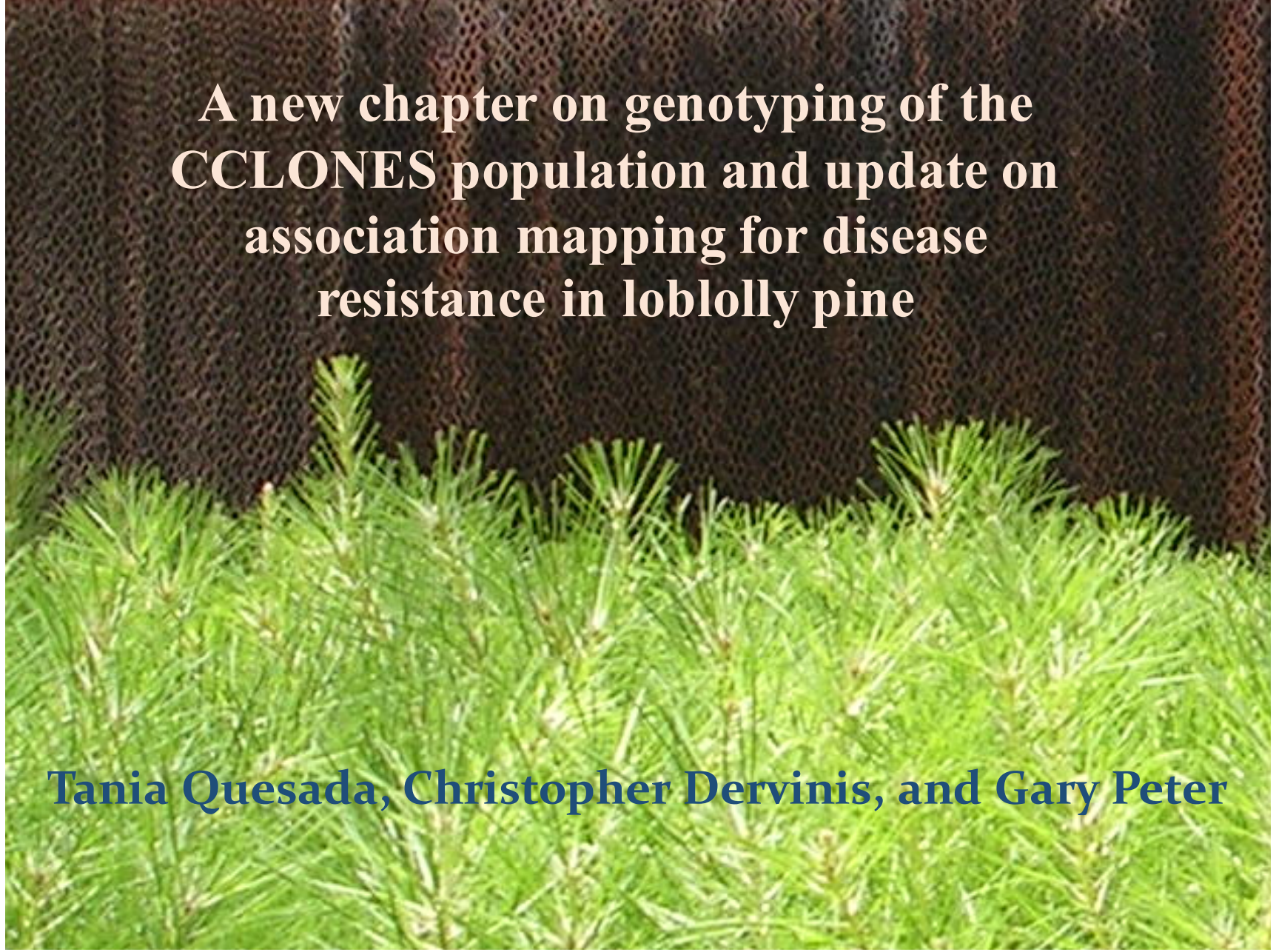
A new chapter on genotyping of the CCLONES population and update on association mapping for disease resistance in loblolly pine



Tania Quesada, Christopher Dervinis, and Gary Peter

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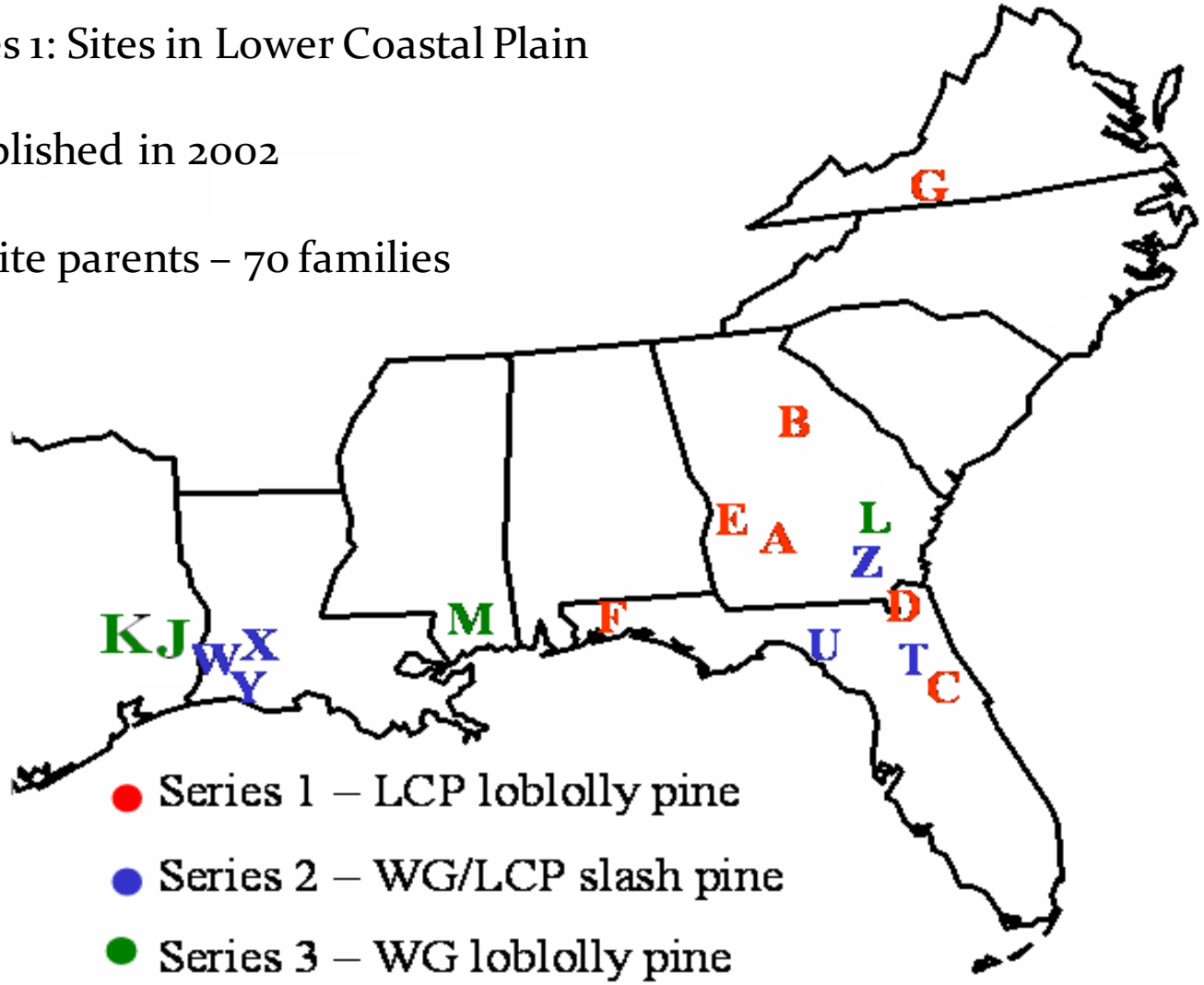
CCLONES: Comparing Clonal Lines ON Experimental Sites

Series 1: Sites in Lower Coastal Plain

Established in 2002

32 Elite parents – 70 families



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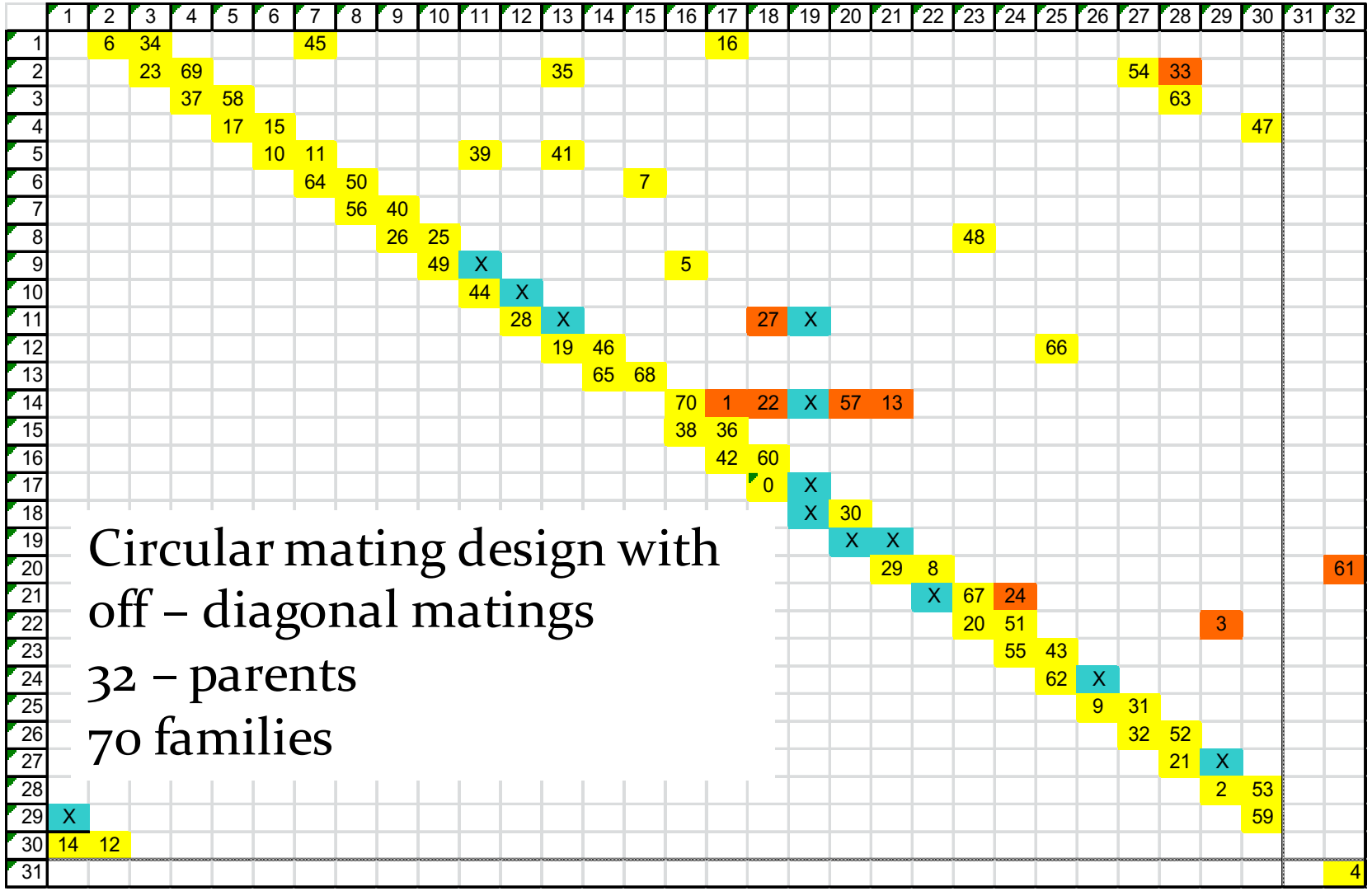
CCLONES mating design



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CCLONES phenotypic data

Category	Trait	Ages measured
Growth	Height, DBH, survival	1-6, 8, 10, 12
Crown	Crown width, height to live crown	2,6
Disease - greenhouse	Fusiform rust & pitch canker	1
Disease- field	Pitch canker lesion length, fusiform rust score	2-6
Branch	Diameter, angle	6
Shoot	# flushes, flush length, # parastichies	2
Phenology	Shoot initiation, cessation, duration	2
Root	Root number, dry weight	1
Water use	Carbon isotope	3
Wood properties	Wood density, stiffness, lignin, diterpenoid content	3-8
Resin production	Oleoresin flow, resin canal number	6,7

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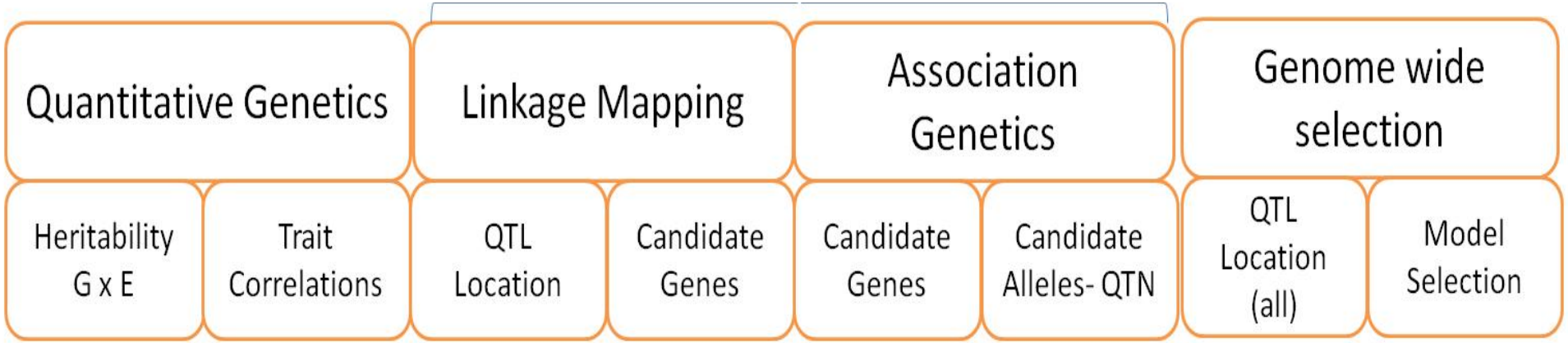
Genetic Architecture & Relating Genotype to Phenotype And applying it to breeding



- Infers genetic control from Phenotypic information
- Complex vs. simple inheritance
- Implicates common gene(s) pathways in genetically correlated traits

- Within family mapping of significant genomic regions
- Relatively small number of loci implicated
- Low resolution – large intervals

- Population level correlation/mapping
- High resolution – small intervals?

- Breeding Population level correlation/mapping
- All loci involved in trait variation
- Mediam resolution

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From probe design to SNP genotyping

Probe design	Probe selection	Probe testing / SNP genotyping
Unique for loblolly pine Genomic, EST unigenes and transcriptome 120 base pairs Exome sequences (target region of ~ 9.6 Mbp) High repeatability	Subset of population High-quality reads Good depth Adequate # SNPs Genic and intergenic	Total population SNP calling: • Polymorphic • Biallelic • Min. quality =10 • Minimum depth = 3 • Max. missing data = 0.4 • Minor allele freq. = 0.01
40K-80K probes	**20,000 probes**	**18,241 probes** **67,637 SNPs**

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Probe selection : Final Selection

Final Selection	N Probes
Selected: Genic polymorphic probes with high quality	15455
Selected: High-quality genic monomorphic probes with medium read number that align to genes. Excludes ADEPT2 contigs. Selected randomly from 5,663 probes.	1697
Selected: monomorphic genic probes with high quality and low read number	140
Selected: Randomly-selected intergenic high-quality probes with less than 4 SNPs.	2708
Total	20000

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Probe testing / SNP genotyping

Total population: **920 clones**

SNP calling:

- Polymorphic
- Biallelic
- Min. quality =10
- Minimum depth = 3
- Max. missing data = 0.4
- Minor allele freq. = 0.01

18,241 probes

67,637 SNPs

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Association mapping for disease resistance

Pitch canker

- **Caused by *Fusarium circinatum***
- **Episodic,Broad host range**
- **Causes resinous lesions**
- **Resistance quantitative and heritable**

Fusiform rust

- **Caused by *Cronartium quercuum* f.sp. *fusiforme***
- **Causes stem galls, high seedling mortality**
- **Resistance due to R genes**

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Results PINEMAP – Pitch canker

SNP	Conf. Int.	Scaffold	Position	Ref	Alt	Query acc.ver	Merge Marker ID	LG	SNP in Contig	Prot_desc
V18500	90	scaffold433234.2	19299	A	T	no match	N/A	N/A		
V22500	90	scaffold562427	135000	A	C	2_5516	N/A	N/A	1	unknown
V27650	90	scaffold739754	2698	T	C	no match	N/A	N/A		
V31916	90	scaffold857972	22985	G	C	no match	N/A	N/A		
V39665	90	tscaffold1240	157547	T	C	0_668	2_4205	4	0	unknown
						2_4205	2_4205		1	Ribosomal protein S27
V57839	90	tscaffold614	364426	C	T	0_10930	0_13765	5	0	ADR1-like 1
						0_13765	0_13765		1	
						CL1Contig163	0_13765		0	
						CL1Contig247	0_13765		0	
V19320	95	scaffold460829	31462	C	G	CL642Contig1	CL642Contig1	10	1	NA
						UMN_CL135Contig1	CL642Contig1		0	
V21018	95	scaffold513999	210140	A	G	no match	N/A			
V63202	95	tscaffold781	451675	A	G	0_11052	0_12447	8	0	ARM repeat superfamily protein
						0_12447	0_12447		0	
						0_16663	0_12447		0	
						0_1957	0_12447		0	
						2_4321	0_12447		0	
						CL2506Contig1	0_12447		0	
						CL465Contig1	0_12447		1	unknown
V57445	99	tscaffold602	32618	T	C	UMN_3547	N/A	N/A	0	

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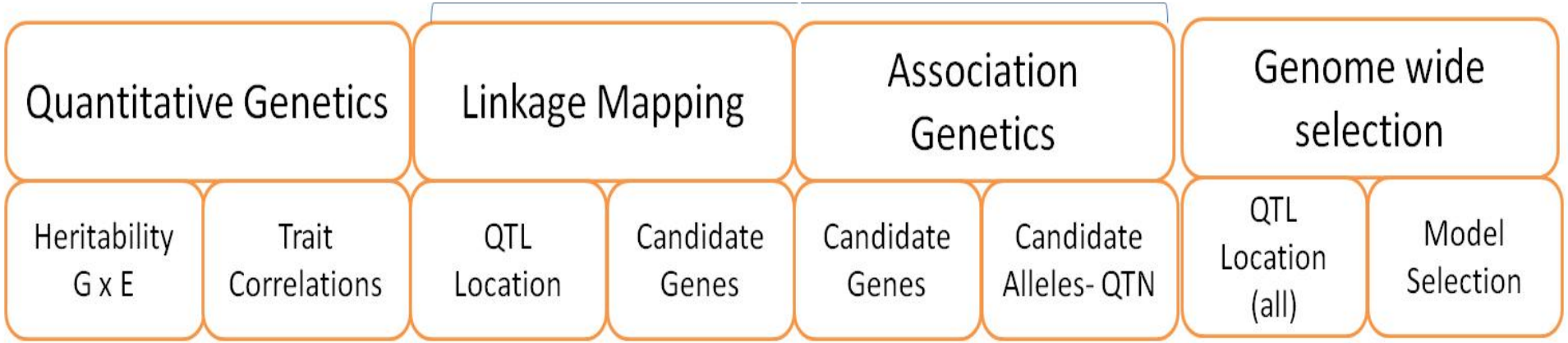
Genetic Architecture & Relating Genotype to Phenotype And applying it to breeding



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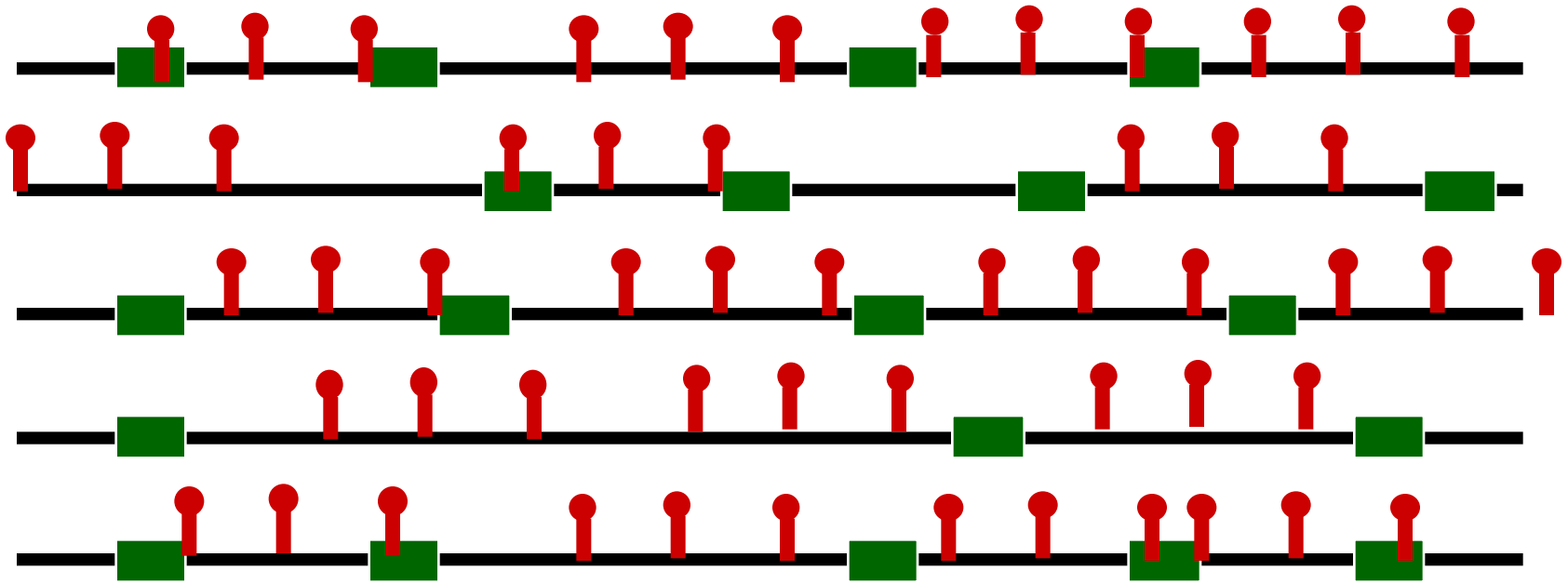
Genome Selection: Principles

1. Identify genetic markers covering most of the genome in the breeding population, so all alleles of interest are in LD with 1+ markers
2. Develop prediction models that capture most of the quantitative variation of interest in one breeding cycle.
3. Use prediction models to select superior genotypes in next cycle.



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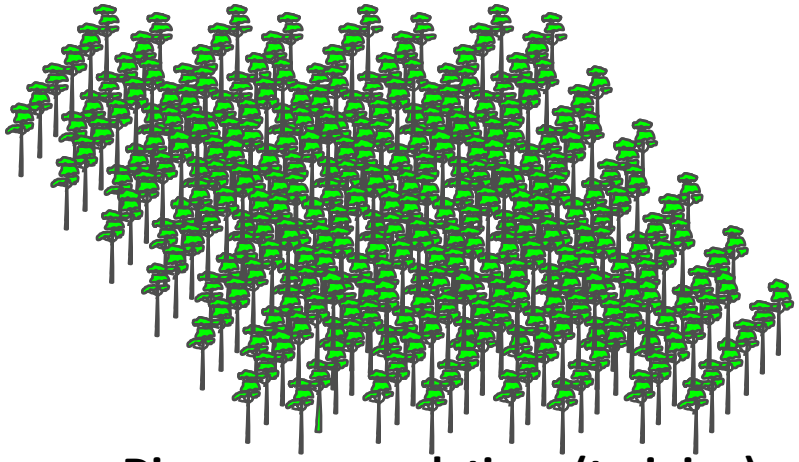
Gene/QTL involved in the target traits

Marker

Genome Wide Selection: Principles



Discovery populations (training)
Current breeding cycles

Phenotyping (several traits) Progeny tests or clonal means

Genotyping at a high marker density

$$Y = Xb + Zh + e$$

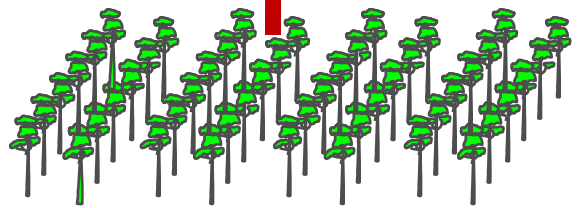
Fitting of predictive models

Evaluation of accuracy of predictive models

Application in early MAS Generation 3+

Application in early MAS Generation 2

Application in early MAS Generation 1



Validation population

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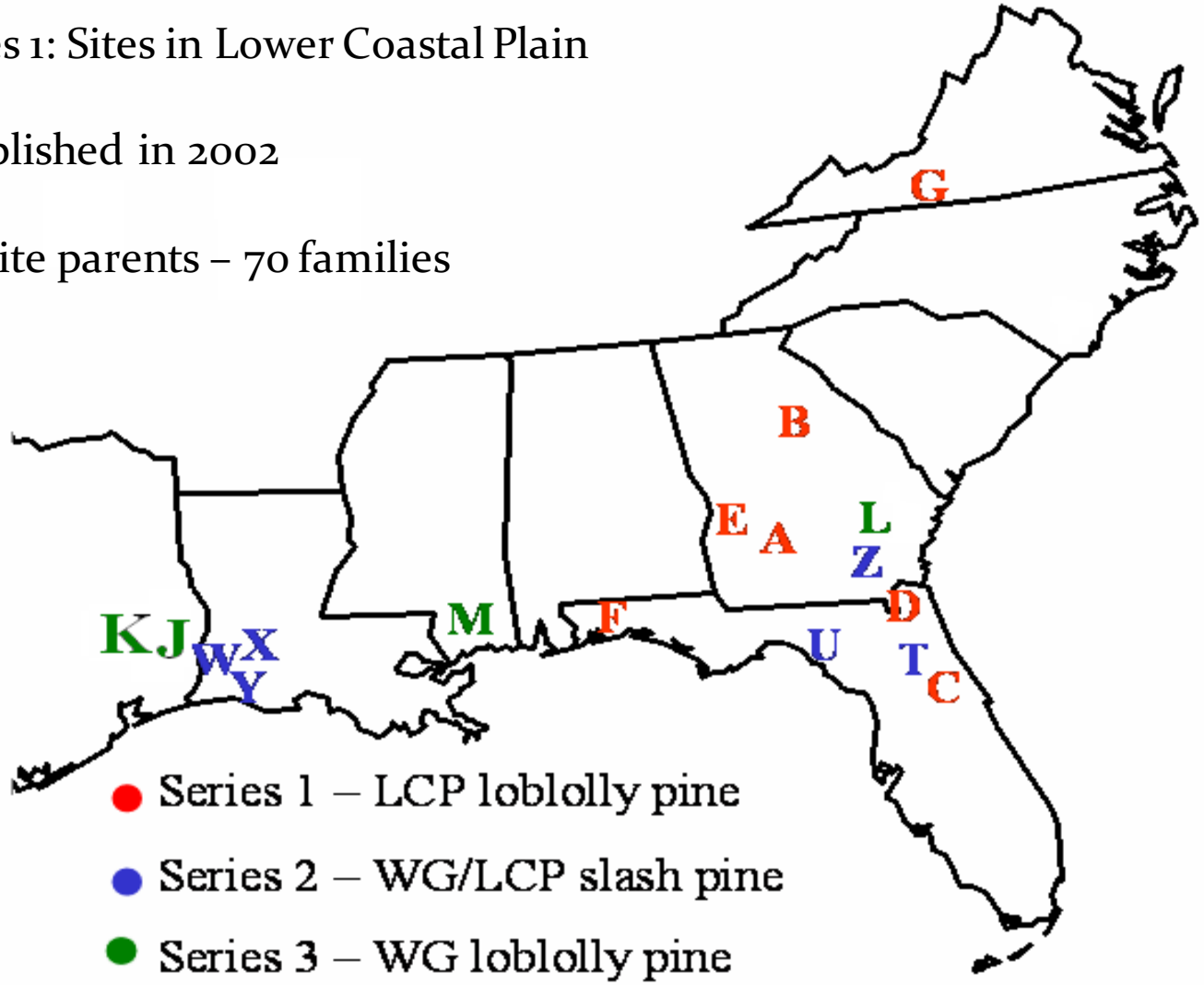

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Summary

- CCLONES → best characterized study in the FBRC
  - > 25 phenotypic traits
  - > 70,000 genotypic markers

- New genotyping process allows higher SNP density
- Association analyses with new SNP dataset produced equal or higher number of significant SNPs.
- When ADEPT2 SNPs and PINEMAP SNPs were analyzed together, some significant SNPs from ADEPT2 were also significant in the new dataset.
- Further characterization of significant SNPs is under way

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Acknowledgments

UNIVERSITY OF FLORIDA IFAS

SFRC

FOREST SERVICE U.S. DEPARTMENT OF AGRICULTURE

RAPiD GENOMICS

FBRC

USDA United States Department of Agriculture National Institute of Food and Agriculture

National Science Foundation WHERE DISCOVERIES BEGIN

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