Fine Mapping *Rag1* and *Rag2* and the Evaluation of New Aphid Resistance Sources

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Acknowledgements

- Research was supported by:
  - United Soybean Board.
  - Illinois Soybean Association.

Outline

- Mapping of aphid resistance genes *Rag1* and *Rag2*.
- Efforts in fine mapping cloning *Rag1* and *Rag2*.
- Screening germplasm to identify new aphid resistance genes.
- SNP haplotypes near *Rag2*.

Antibiosis Resistance in Dowling, Jackson and PI 200538

Li, Y., C.B. Hill, and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology 97: 1106-1111.

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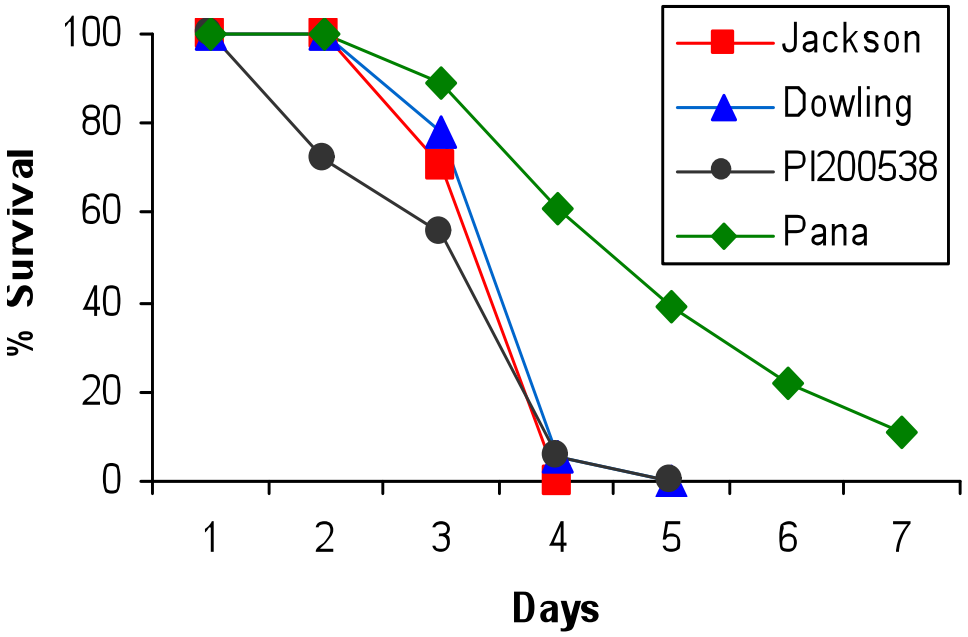
Aphid Mortality



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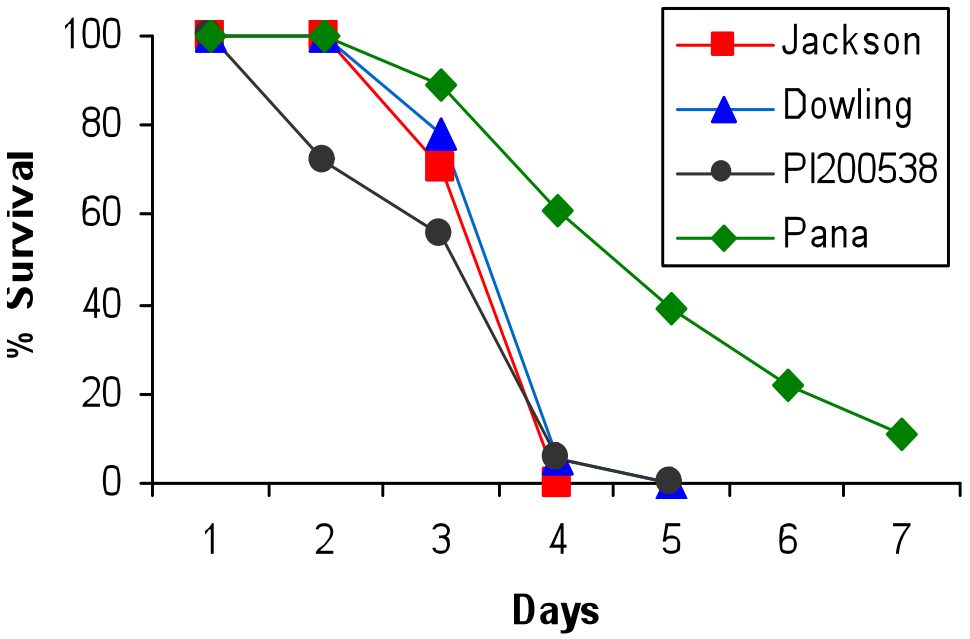
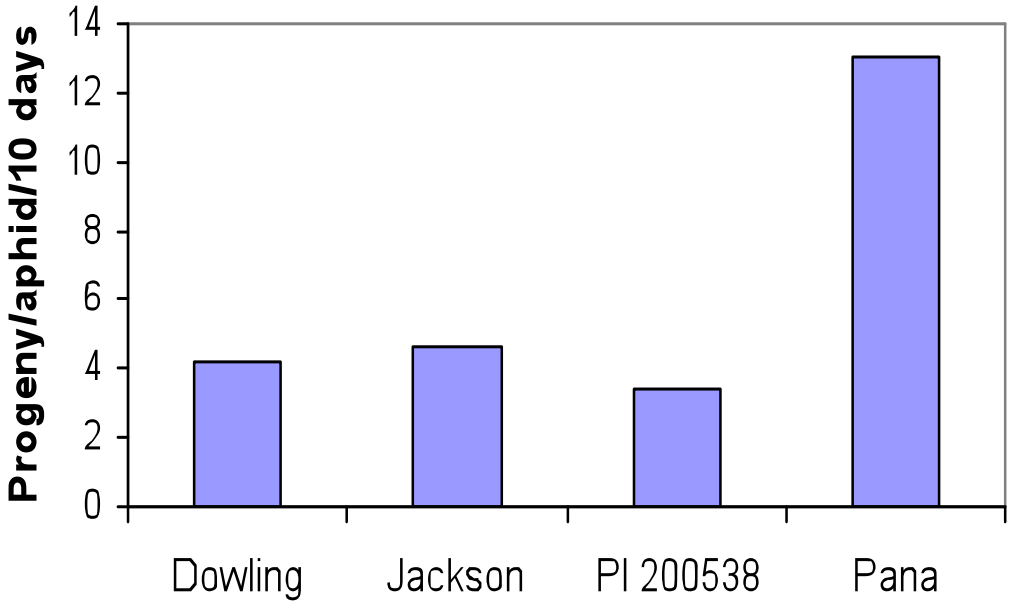


Aphid fecundity



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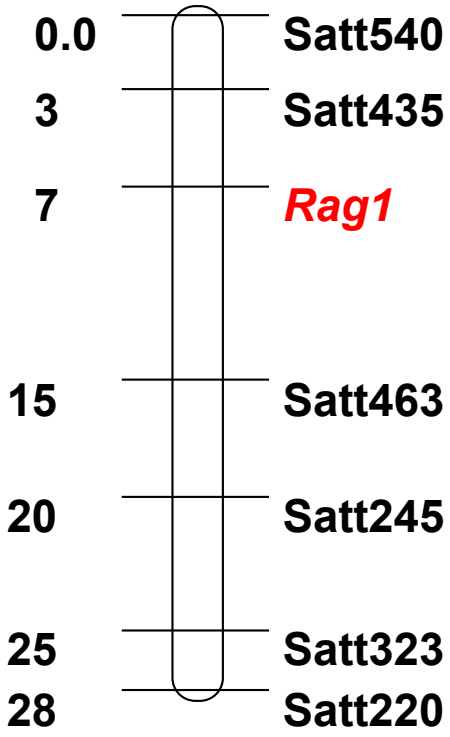
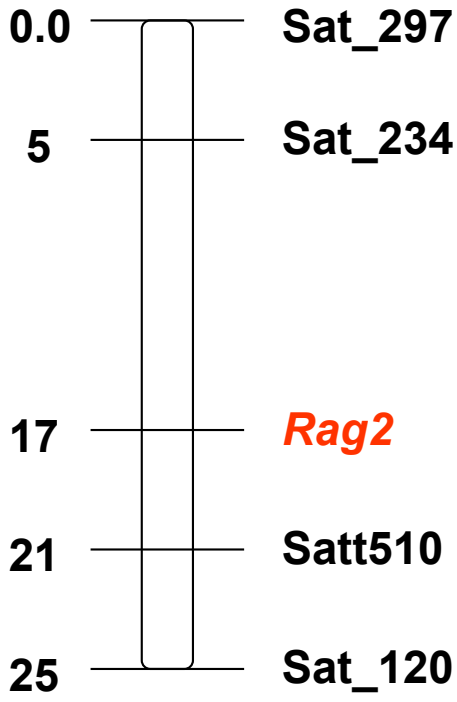
Map Location of the Aphid Resistance Genes *Rag1* and *Rag2*

Rag1 from Dowling Chromosome 7 (LG M)



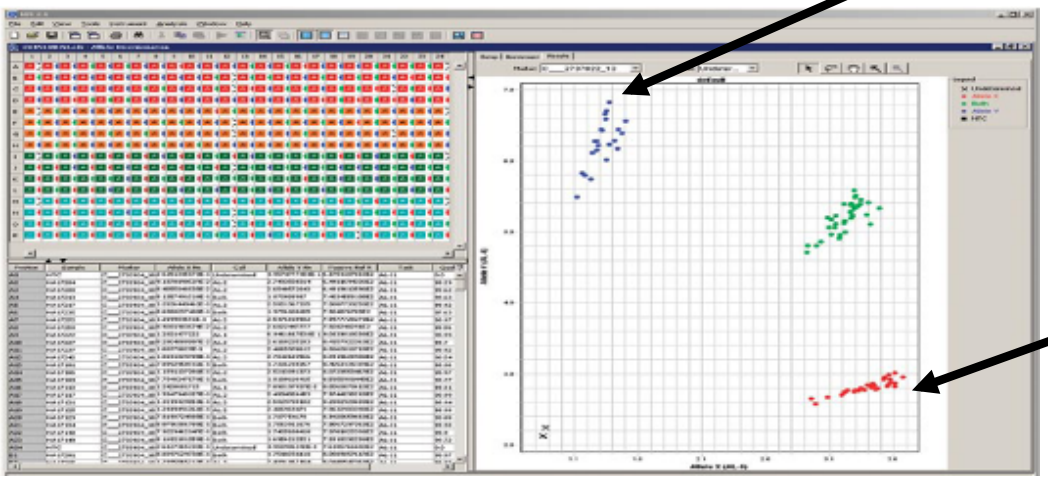
Rag2 from PI 200538 Chromosome 13 (LG F)



Rouf Mian mapped *Rag2* from PI 243540 (Mian et al. 2008).

Breeding for Aphid Resistance

- MAS with SNP markers has been successfully used to develop germplasm with *Rag1* and *Rag2*.



Resistant Line

Susceptible Line

Fine Mapping *Rag1* and *Rag2*

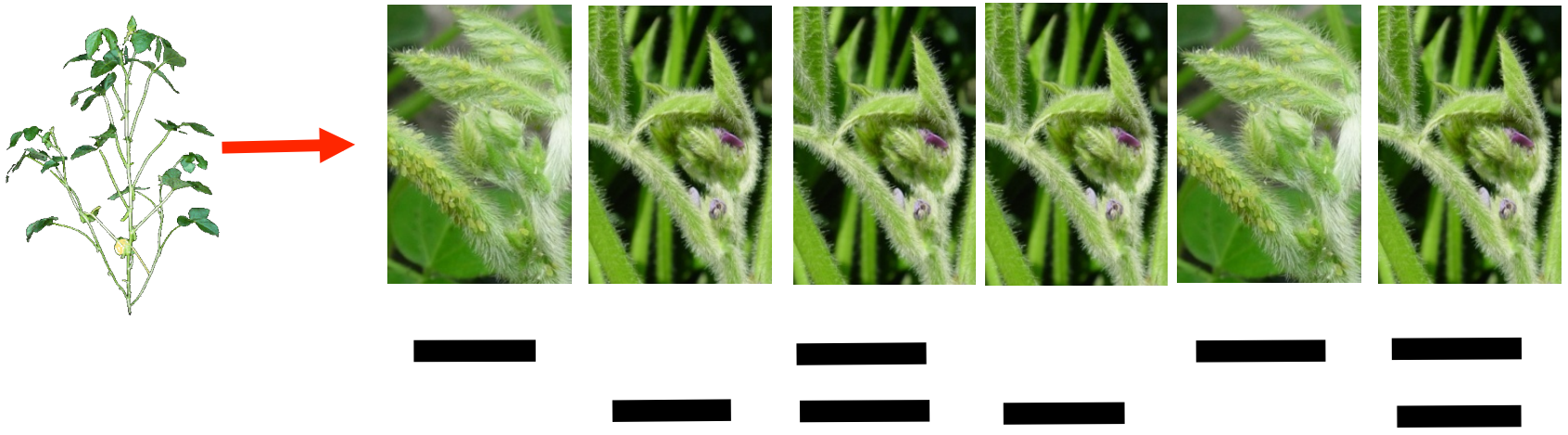
- Fine map *Rag1* and *Rag2* to provide better markers for MAS, evaluate germplasm, and to build resources for cloning.
- Identified recombinant plants from populations with markers flanking the genes.
- Tested 1,824 plants for *Rag1* and 5,783 plant for *Rag2*.

Fine Mapping *Rag1*

Physical position (Mb)	5.47	5.48	5.49	5.55	5.58	5.61	5.64		Aphid number				
	83A	25A	21A	56B	65906.2	46169.7	27A						
Plant								Pheno	R	H	S	*P > F*	R^2
100	R	R	R	H	H	H	H	Seg	71	62	657	<0.0001	0.97

Recombinant plant

Progeny



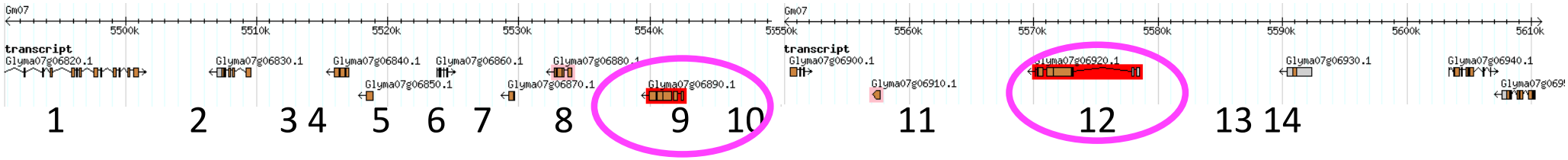
Rag1 Fine Mapping

Physical position (Mb)	5.47	5.48	5.49	5.55	5.58	5.61	5.64						
	83A	25A	21A	56B	65906.2	46169.7	27A		Aphid number				
Plant								Pheno	R	H	S	*P > F*	R^2
4	R	R	R	R	R	R	H	Res	62	47	57	0.2043	0.08
82	H	H	H	R	R	R	R	Res	49	58	55	0.2071	0.08
100	R	R	R	H	H	H	H	Seg	71	62	657	<0.0001	0.97
6	H	H	H	H	H	H	H	Seg	47	60	628	<0.0001	0.91
48	NT	NT	NT	NT	S	S	NT	Sus	941	953	984	0.2080	0.17
K39	S	S	S	S	S	H	H	Sus	774	761	770	0.9010	0.01

Rag1
115 kb interval

Rag 1 and Rag2 Fine Mapping

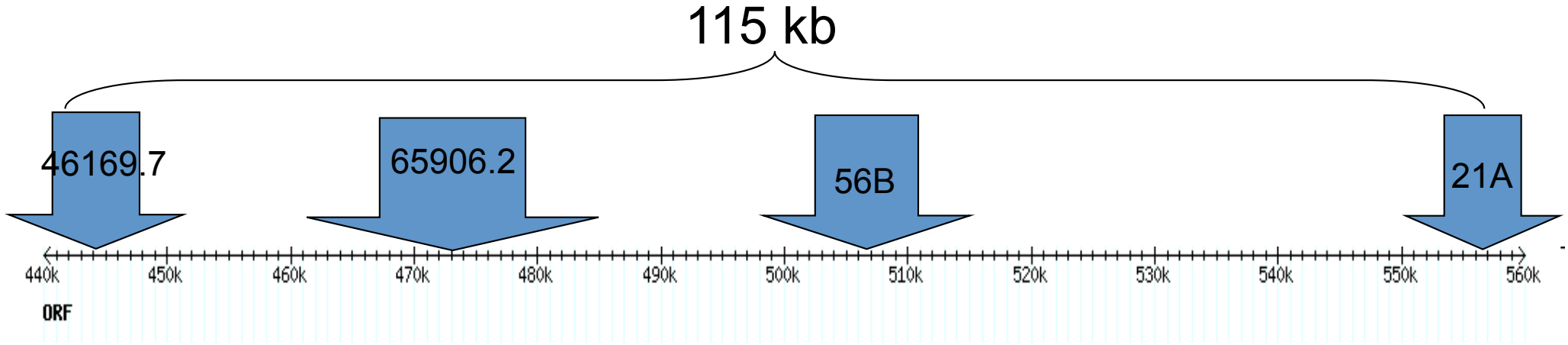
- *Rag1* mapped to a 115 kb interval.
  - Interval contains 14 genes in Williams 82 with predicted expression. Two are NBS-LRR genes.



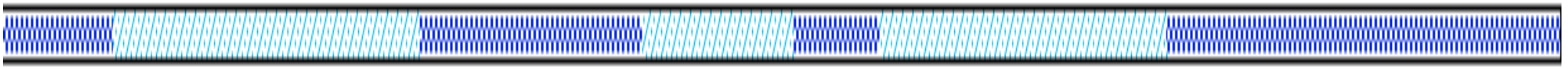
Rag 1 and Rag2 Fine Mapping

- *Rag2* mapped to a 54 kb interval.
  - Interval contains 7 genes in Williams 82 with predicted expression. One is a NBS-LRR.
- Are these genes present in the resistance sources? (Williams 82 susceptible)
- Need to clone these regions from resistant genotypes.
- Collaborating Matt Hudson in cloning.

Effort to Sequence the *Rag1* Interval from Dowling

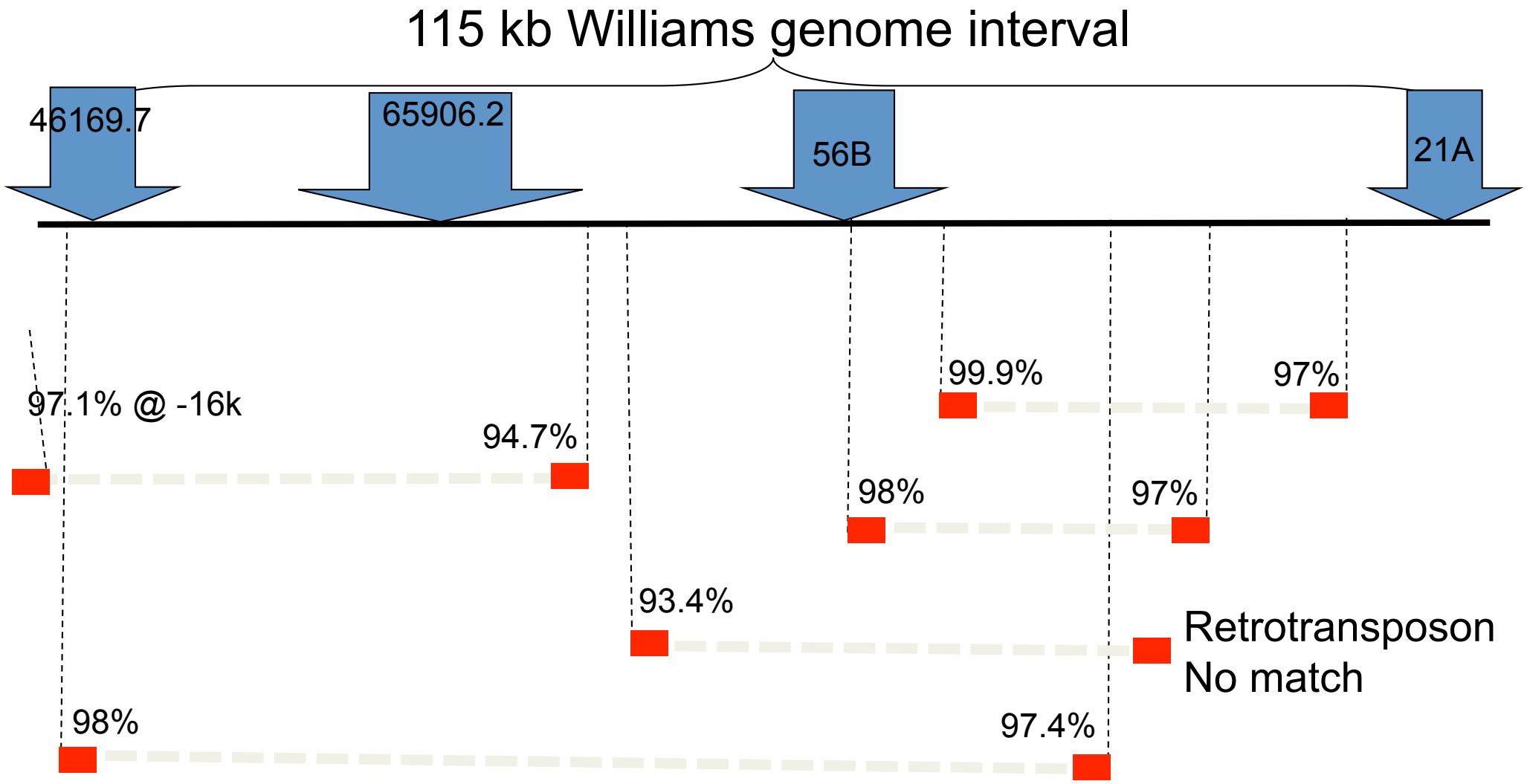


PCR coverage on Dowling



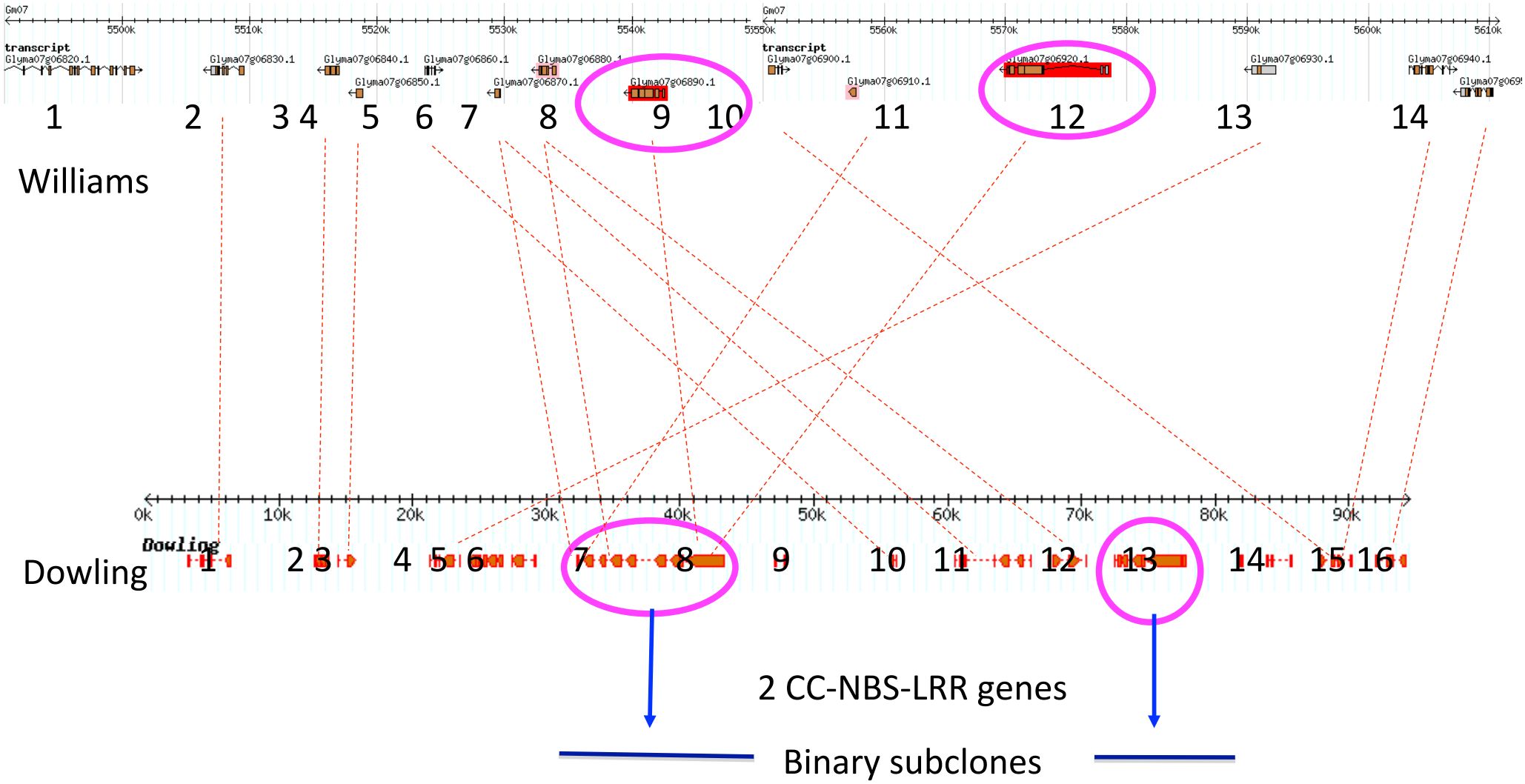
Gap Gap Gap

Dowling Fosmid Library Screened



Selected clones were sequenced on a 454 resulting in a single contig.

Comparison of Williams 82 and Dowling in *Rag1* Region



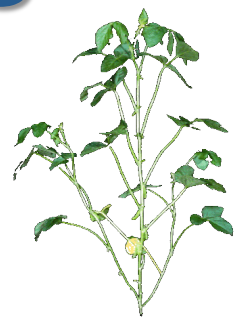
Rag1 Region in Dowling

- Williams 82 genome sequence was a critical resource for mapping.
- Candidate *Rag1* genes not in Williams 82.
- Rearrangements in the *Rag1* interval would make it difficult to assemble across the interval using next generation sequencing.
- Two *Rag1* candidates were cloned from Dowling and are being transformed into soybean by Tom Clemente.

Next Step in Aphid Resistance Research

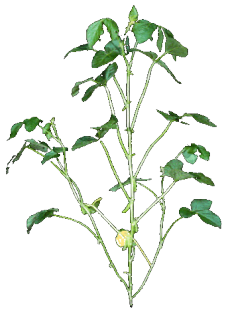
- Are there new genes in the germplasm collection?
- Over 40 new sources of aphid resistance have been identified from the germplasm collection by Hartman's group.
- Do these have new resistance genes?

Testing Known Resistance Genes



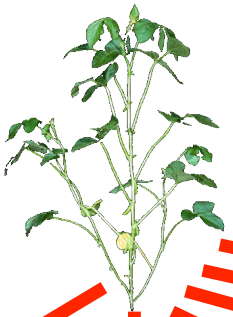
Source of resistance

X



Susceptible

F_1 Plant



F_2 Progeny

Marker linked to gene

Genetic Tests of Resistance Sources

Source	Biotype	Significant region	Significance
PI71506	*1*	*Rag1*, *Rag3*	0.004, 0.0131
PI88508	*1*	*Rag2*	<0.0001
PI437696	*1*	*Rag1*, *Rag2*	0.0045, <0.0001
PI507298	*1*	*Rag2*	<0.0001
PI548237	*1*	*Rag2*	<0.0001
PI567391	*1*	*Rag2*	<0.0001
PI587656	*1*	*Rag2*	<0.0001
PI587663	*1*	*Rag2*	<0.0001
PI587677	3	*Rag1*	<0.0001
PI587685	3	*Rag1*	0.006
PI587775	*1*	*Rag2*	<0.0001
PI587870	*1*	*Rag2*	<0.0001
PI587899	*1*	*Rag2*	<0.0001
PI587972	*1*	*Rag2*	<0.0001
PI588000	*1*	*Rag2*	<0.0001
PI594431	*1*	*Rag2*	<0.0001
PI594499	*1*	*Rag2*	<0.0001
PI594573	*1*	*Rag2*	<0.0001
PI594592	*3*	*Rag1*	<0.0001
PI594707	*1*	*Rag2*	<0.0001
PI594822	*1*	*Rag2*	<0.0001
PI594879	*1*	*Rag2*	<0.0001
PI599955	*1*	*Rag2*	<0.0001

Identify Diversity in the Germplasm Collection Efficiently

- Can we predict which accessions have resistance at *Rag1* or *Rag2*?
- Important use of genomic technology will be to identify useful diversity from germplasm collections.
- Germplasm collection being tested with 50,000 SNP markers.

Haplotypes Across *Rag2* Interval

Accession	Gene	SNPs	Haplotype
PI088508	*Rag2*	CCNGCAATAGG	1
PI243540	*Rag2*	TCCATGAGGGA	2
PI567391	*Rag2*	NNCATAGTAGN	3,4
PI587899	*Rag2*	NNCATNNTANN	3,4
PI587656	*Rag2*	TNCATAGTAGA	4
PI594822	*Rag2*	TNCATAGTAGG	3
PI507298	*Rag2*	TNCATGAGGGA	2
PI587775	*Rag2*	TNNATAGTAGG	3
PI587870	*Rag2*	TNNATAGTAGG	3
PI587972	*Rag2*	TNNATAGTAGG	3
PI588000	*Rag2*	TNNATAGTAGG	3
PI594707	*Rag2*	TNNATAGTAGG	3
PI594879	*Rag2*	TNNATAGTAGG	3

- None of the *Rag2* interval haplotypes are unique to only the aphid resistant genotypes.
- Suggests that greater marker density needed to predict the presence resistance at *Rag2*. Perfect marker would be the best.

Conclusions

- *Rag1* and *Rag2* mapped and fine mapped.
- Williams 82 sequence useful in fine mapping but did not lead to identifying gene candidates in the resistance source.
- Gene candidates for *Rag1* identified by cloning region from the resistance source.
- 23 new sources of resistance tested.
  - Aphid resistance gene in the *Rag2* interval found in 19 PIs.
- SNP haplotypes across the *Rag2* interval not predictive of aphid resistance in this region.