

Molecular interactions between soybean aphid and resistant soybean



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Our soybean checkoff.
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Soybean Aphid Biotype Genetics, Genomics, Adaptation

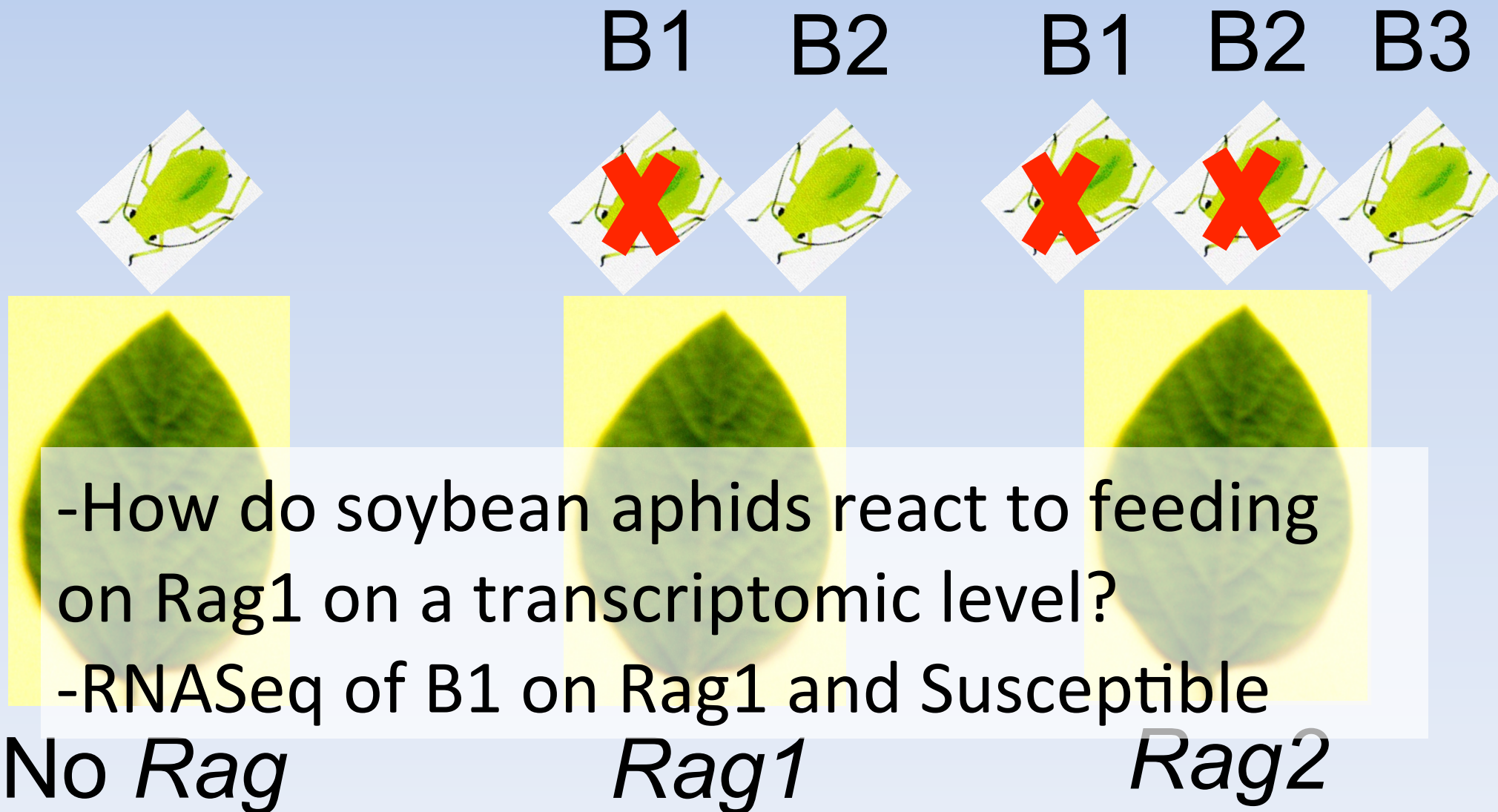
- How do they arise? What are changes in gene function or expression for biotype adaptation?
 - Genomic and transcriptomic approaches
- Where do biotypes exist, in what proportions and how far can they spread?
 - Population genetic studies, molecular markers and diagnostics

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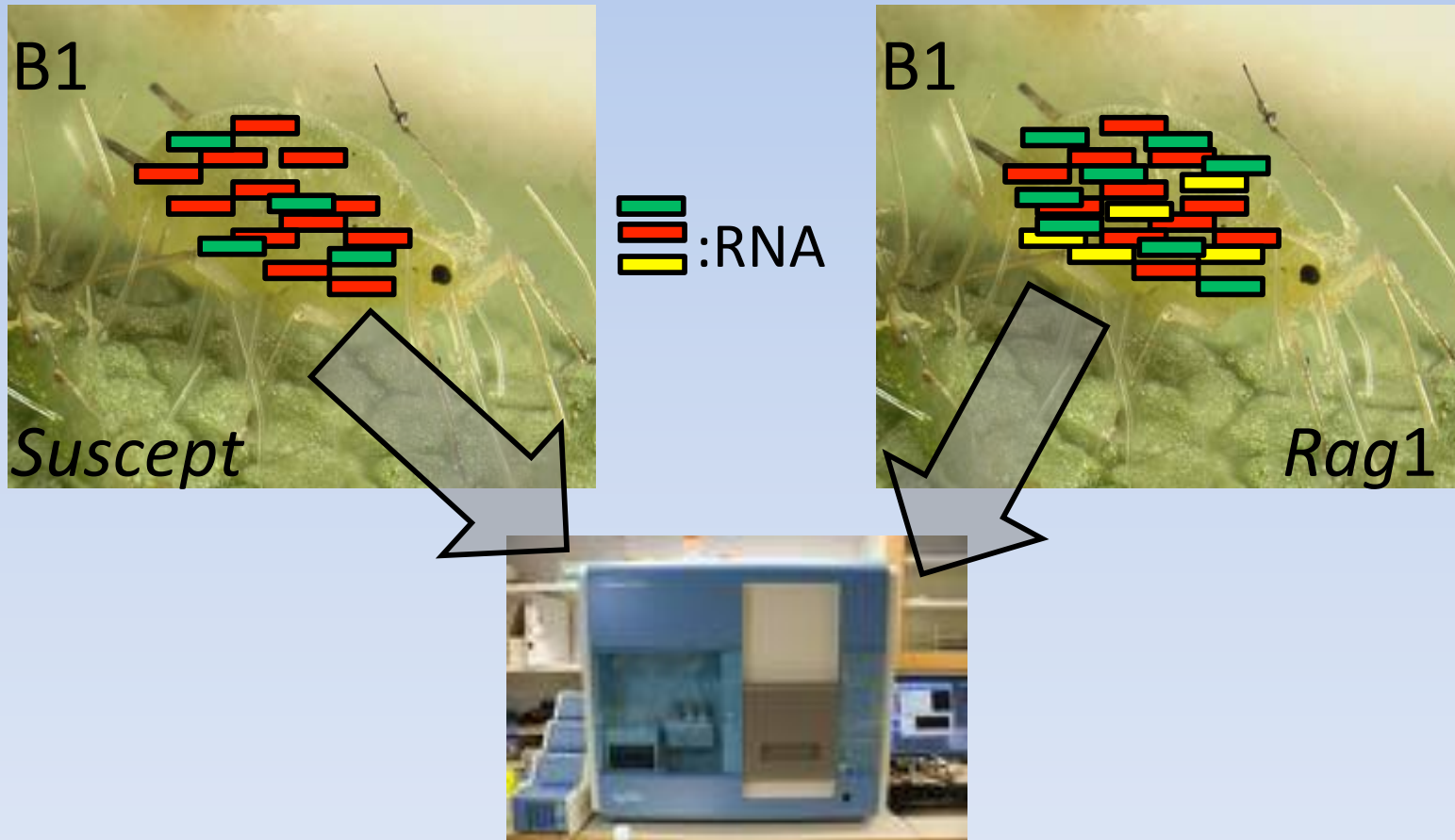


Host plant resistance & aphid biotypes

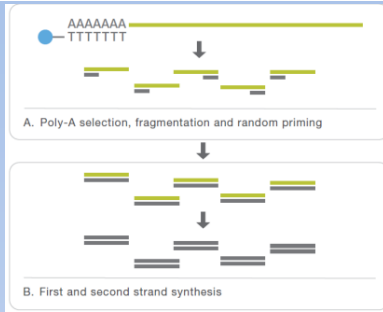


RNA-Seq

- Sequence all RNA in samples and compare



	A	B	C	D	E
1	Locus_Name	Sequence	Description	Count-B1	Count-B2
2	GMAGBIIRR000472	TATGTGGGCAAGA	PREDICTED: similar to GH03753p [Acyrtosiphon pisum]	100	100
3	GMAGBIIRR000058	CGTTTAAAAAAGT	PREDICTED: similar to predicted protein [Acyrtosiphon pisum]	50	200
4	GMAGBIIRR000875	AATGCAATACGGG	PREDICTED: similar to reverse transcriptase (put.); putative [Acyrtosiphon pisum]	0	200
5					

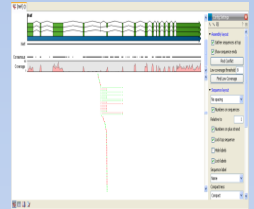


Paired RNA-Seq using Illumina's Genome Analyzer-II



RNA extraction and cDNA libraries preparation

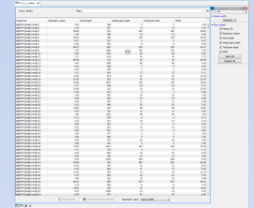
Individual reads mapped to reference transcriptome* using CLC Genomics Workbench



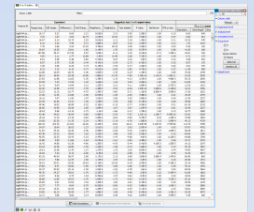
B1



RPKM (expression value) of each gene calculated in all 6 libraries



Differentially expressed genes in two treatments identified using Baggerley's test ($P < 0.01$)



New born nymphs fed for 12 hrs (3 Reps for each)

Sequences for differentially expressed genes annotated using blast2go



NILs



Susceptible:
No *Rag1*

Resistant:
Rag1

*Reference transcriptome contained 76883 high quality ESTs from soybean aphid assembled using adult and nymph cDNA libraries

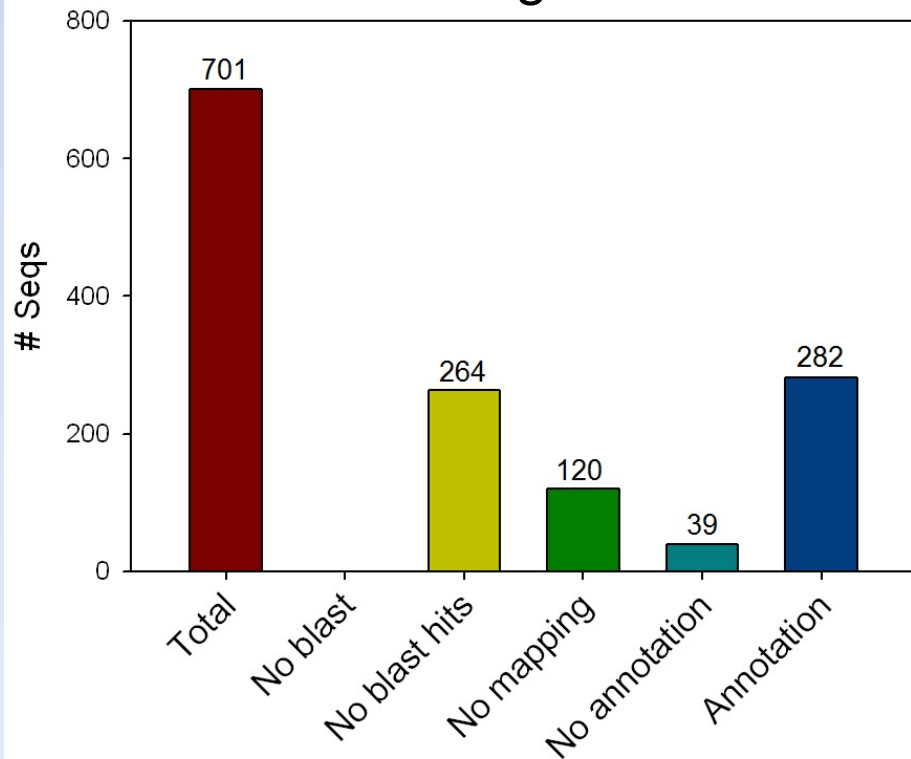
Mapping statistics

Treatment*	Replicates	Total reads	Mapped reads	Uniquely mapped reads
S	R1	19,043,918	15,277,859	14,567,880
	R2	28,579,810	24,070,793	22,979,265
	R3	15,795,962	13,352,987	12,773,858
R	R1	21,354,822	17,928,145	17,015,329
	R2	11,599,306	9,866,821	9,339,220
	R3	35,176,984	29,809,274	28,296,387

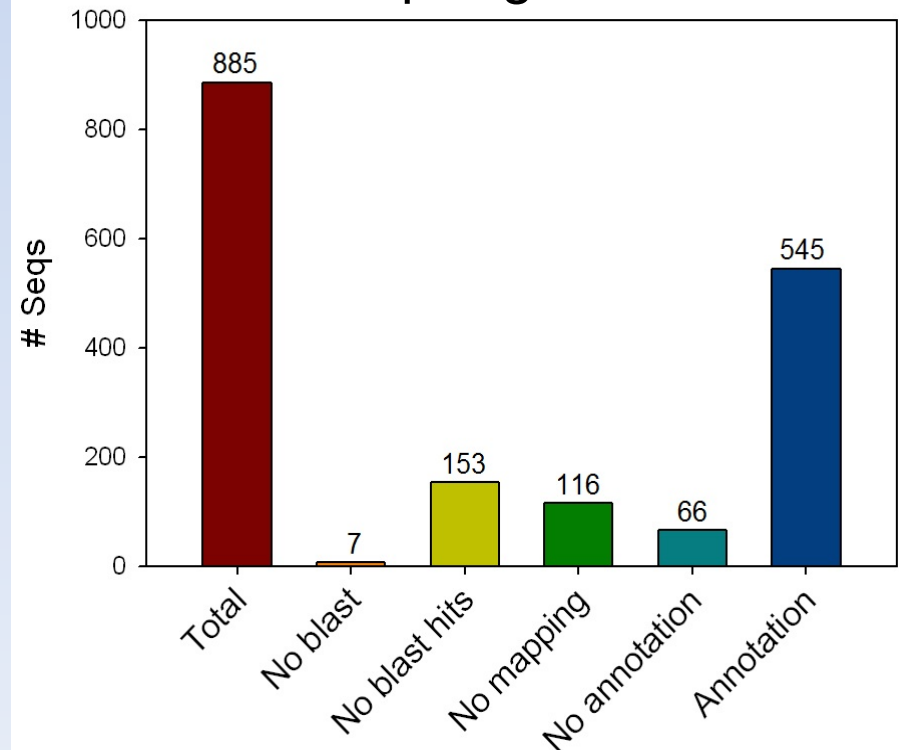
*Fed on susceptible (S) and resistant (R) plants

SBA fed with *Rag1* plants

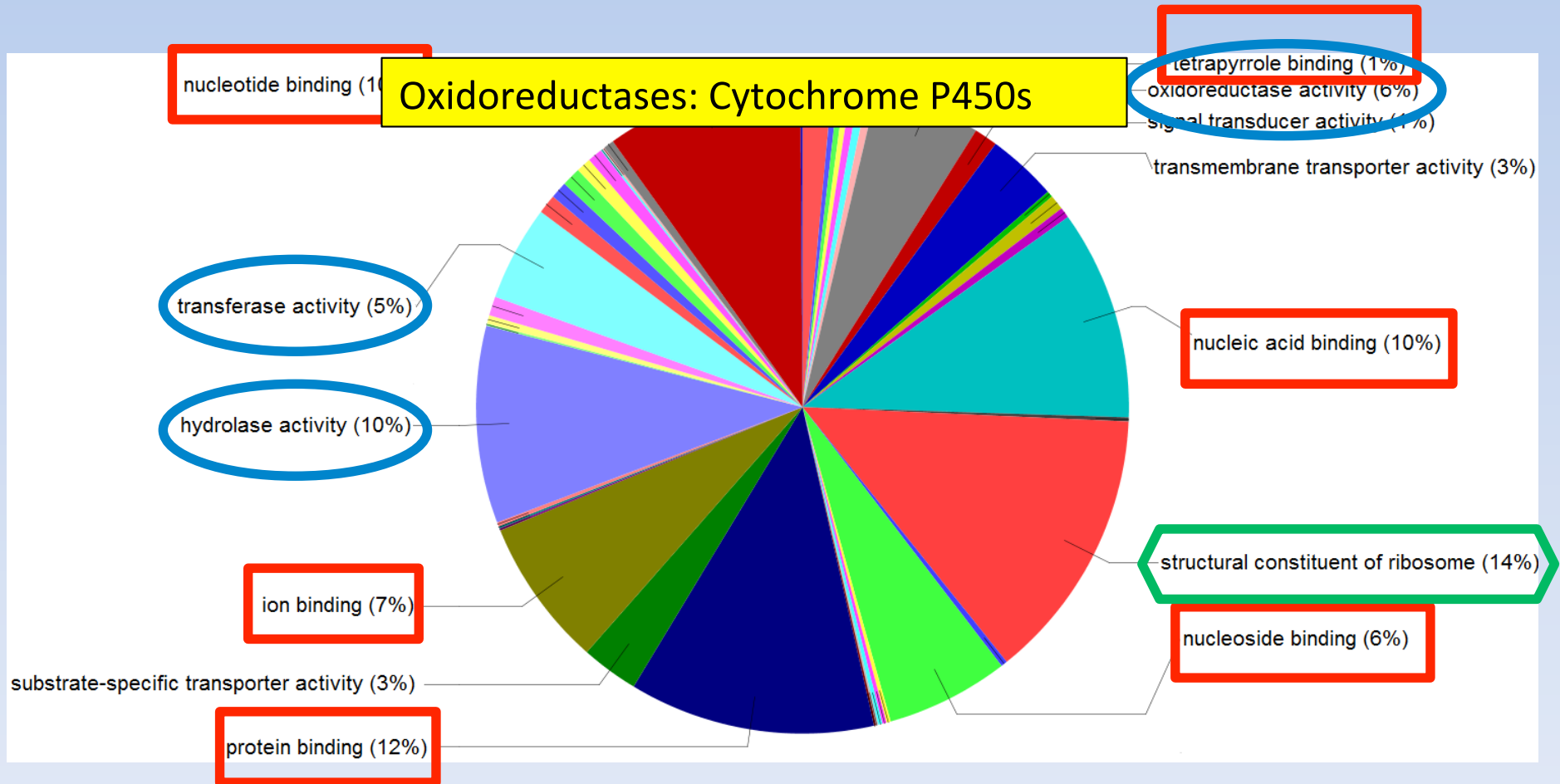
Down-regulated



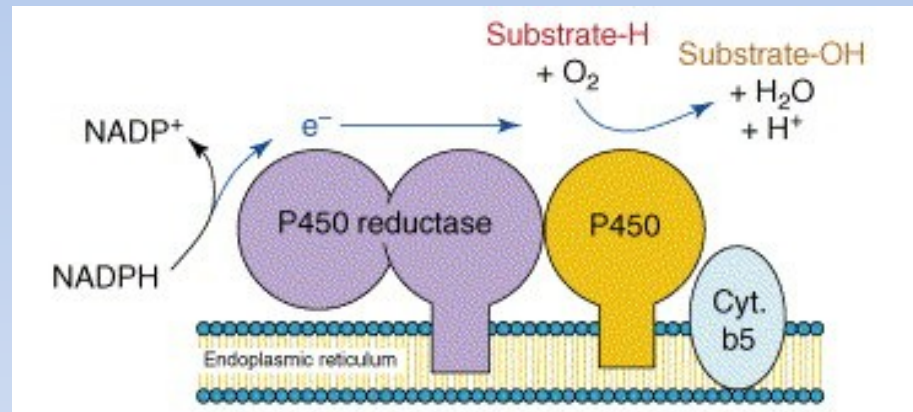
Up-regulated



Molecular function of up-regulated genes



Cytochrome P450



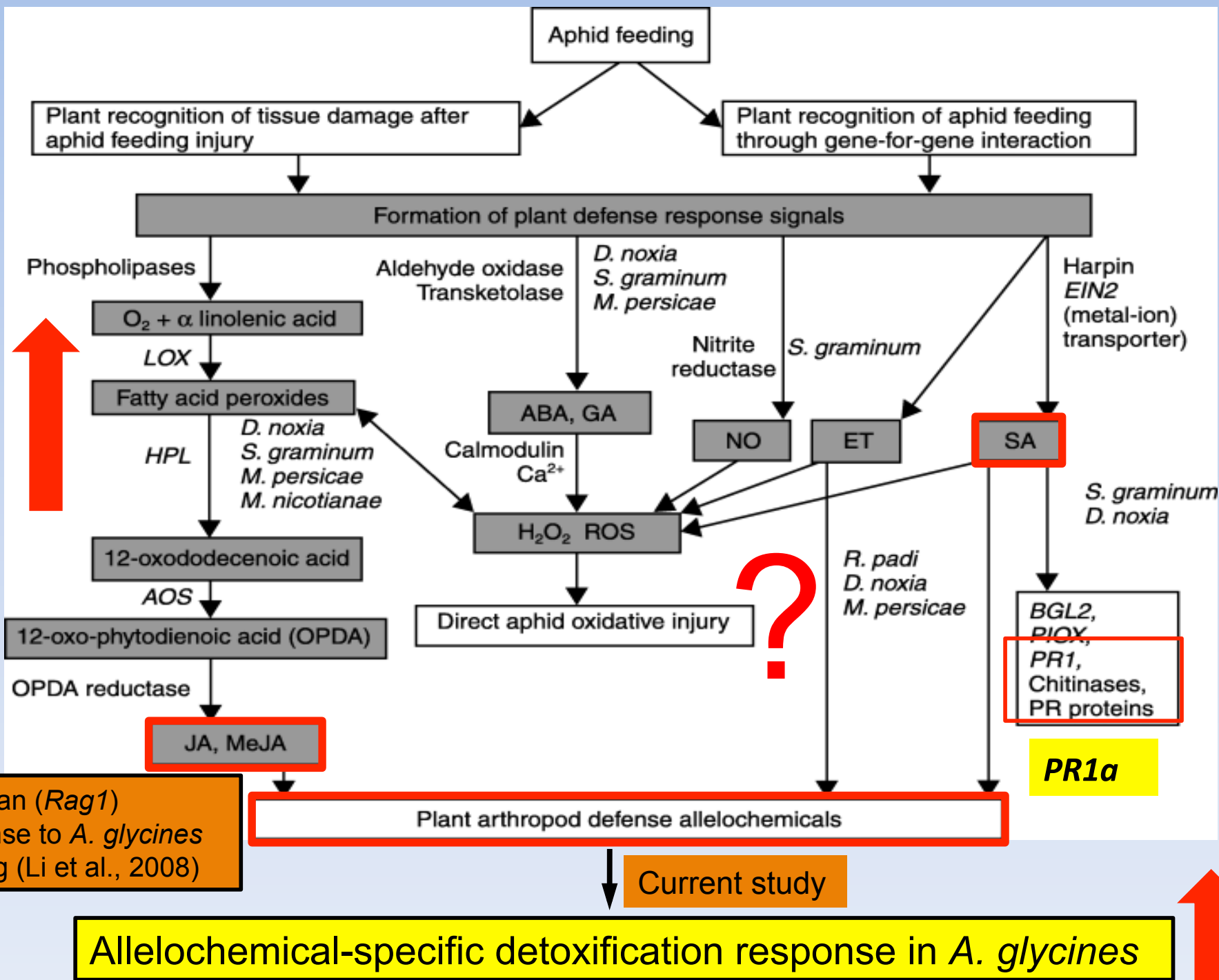
- Monooxygenase enzymes of 45-55 kDa found in all living organisms
- Specialized in the metabolism of endogenous substrates (hormones, pheromones etc.) and exogenous compounds (insecticides, allelochemicals etc.)

Genes for detoxification enzymes identified in soybean aphid transcriptome

Gene family	#Occurrence
Cytochrome P450	101
Glutathione-s-transferase	18
Esterase/carboxylesterase	45
Alkaline phosphatase	20
Aminopeptidase	56
Cadherin	16
γ -Glutamyl-transpeptidase	30
Thioredoxin	27
Glutaredoxin	8

Up-regulation of detoxification genes in soybean aphid feeding on resistant plants

Sequence Name	Length (bp)	Best hit accession no.	Family & clade	RNA-Seq fold change	qPCR fold change
contig19107	972	XP_001945100.2	6a14, CYP3	5.64	3.46
Contig20248	1086	XP_001952450.1	6a2, CYP3	4.31	2.19
Contig20782	1227	XP_003248187.1	6a2, CYP3	3.64	1.37
Contig71947	2570	XP_001947923.1	18a1, CYP3	10.26	2.91
Contig17427	2302	XP_001943923.2	4C1, CYP4	6.01	3.68
Contig72606	1779	XP_001946384.2	6a13, CYP3	2.90	2.51
Contig56787	185	XP_001951034.1	4C1, CYP4	2.68	1.41
Contig72642	1275	XP_974252.1	302a1, MiCYP	1.66	1.36
Contig17752	1231	XP_001946570.2	E4, Esterase	3.13	1.37
Contig43771	1669	NP_001156274.1	De, GST	2.58	2.27
Contig59162	1191	AAV31410.1	Sg, GST	1.43	1.36



Summary

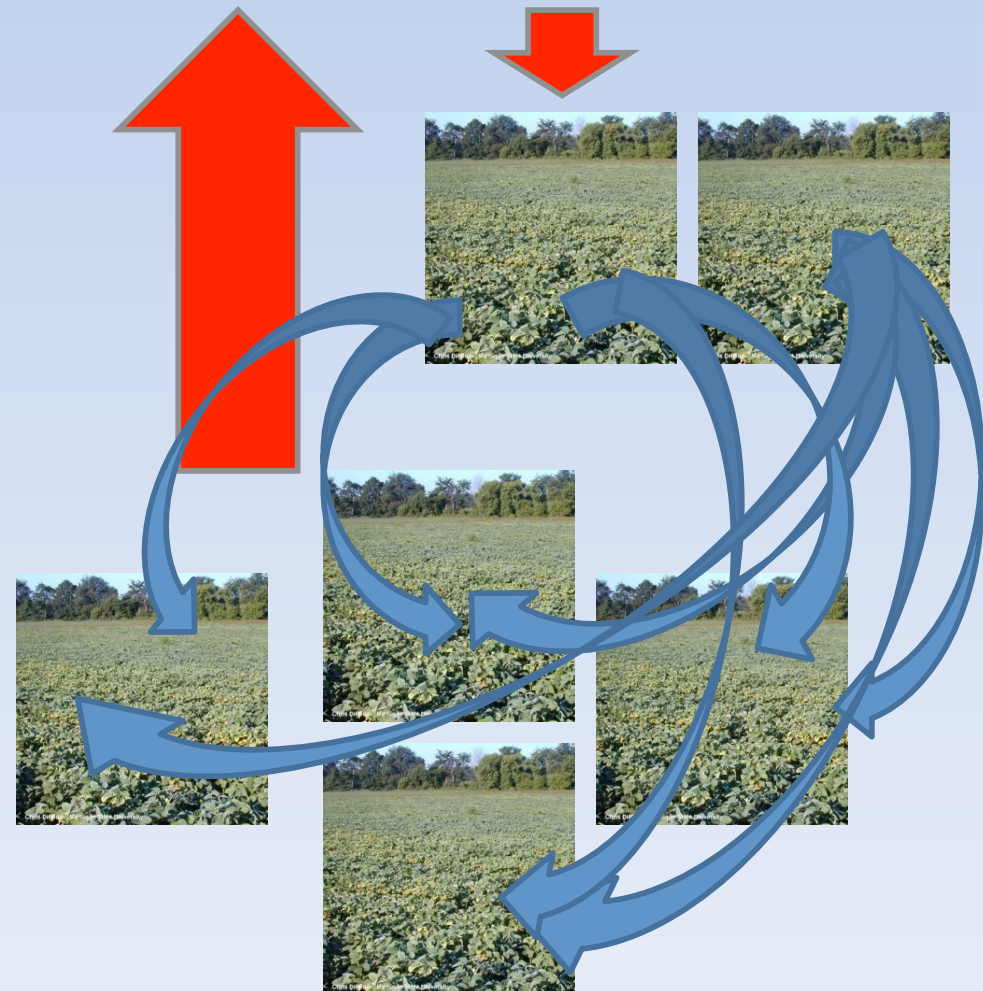
- Measured the gene expression in soybean aphid feeding on resistant and susceptible plants
- Majority of differentially expressed genes belong to binding and catalytic categories
- Selective regulation of detoxification genes in soybean aphid feeding on *Rag1* plants explains antibiosis mode of resistance (through allelochemicals) in these plants

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Movement of Soybean Aphids

- Dispersal/Migration Events:
 - 1) Spring colonization from buckthorn to soybeans
 - 2) Asexual reproduction, population increase
 - 3) Widespread dispersal among all fields
 - 4) Fall migration back to buckthorn—sexual reproduction

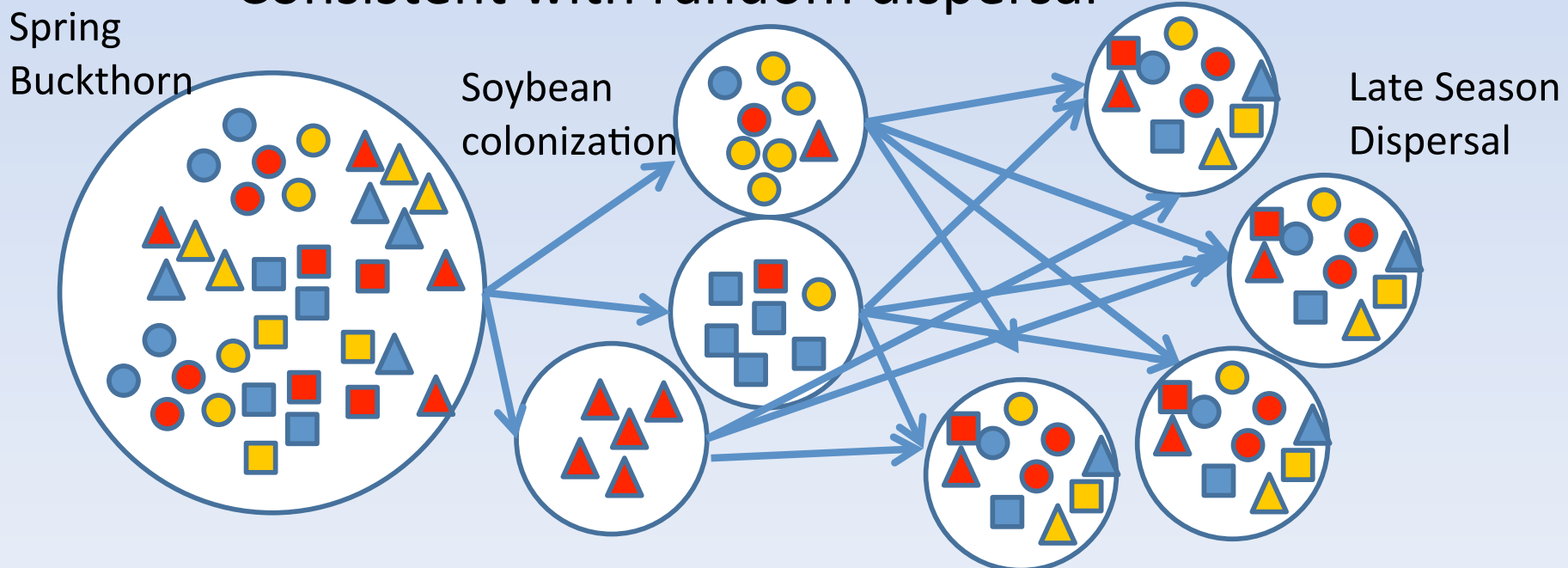


Genetic Approach to Dispersal and Migration

- Hypothesis: Founder effect occurs in spring soybean colonization. Predict:
 - Low genetic diversity (ie. Low number of clones or distinct genotypes)
 - High relatedness among aphids from a population
 - High genetic differences among populations
 - Restricted dispersal related to proximity to buckthorn

Genetic Approach to Dispersal and Migration

- Hypothesis: Late season dispersal spreads genetic variation. Predict:
 - High genotypic diversity (number of clones)
 - Low relatedness among aphids within a population
 - Slight differences among populations
 - Consistent with random dispersal

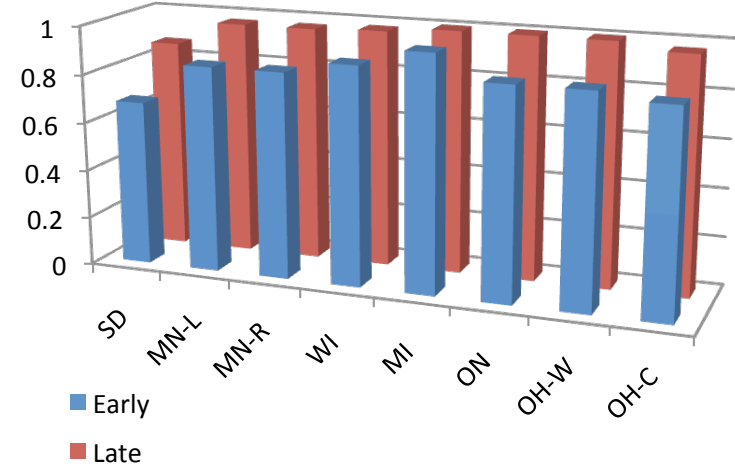


Genetic Approach to Dispersal and Migration

- Use 6 microsatellite markers and 24 single nucleotide polymorphisms
- 8 populations collected twice (early and late) from the same field

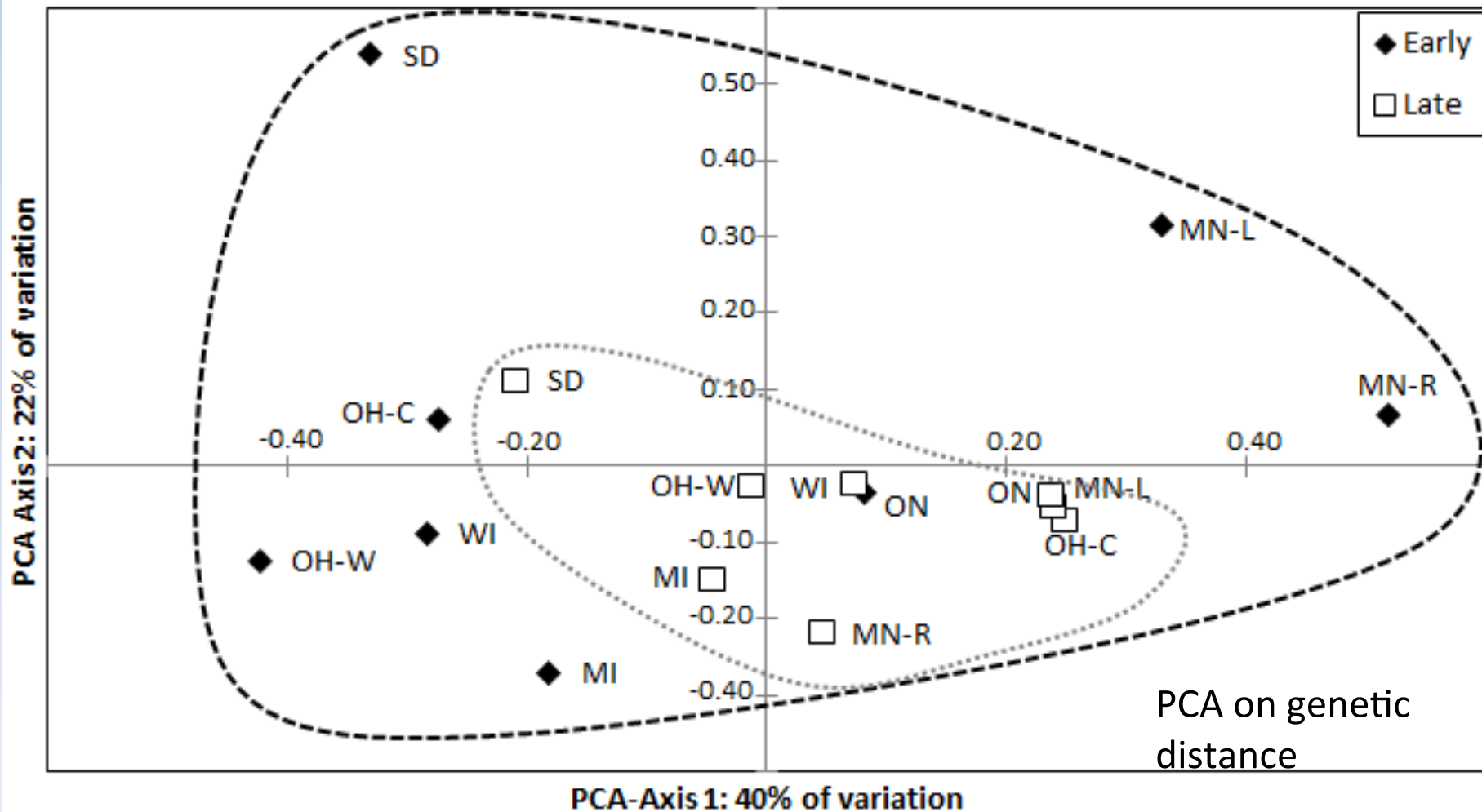
Population	Abbreviation	Early Collection Date	Late Collection Date
South Dakota	SD	June 13, 2009	July 30, 2009
Minnesota-Lamberton	MN-L	June 23, 2009	Sept 8, 2009
Minnesota-Rosemount	MN-R	June 3, 2009	Aug 26, 2009
Wisconsin	WI	June 22, 2009	Sept 8, 2009
Michigan	MI	June 10, 2009	Sept 14, 2009
Ontario	ON	July 6, 2009	Aug 25, 2009
Ohio-Wooster	OH-W	June 15, 2009	Aug 24, 2009
Ohio-Cortland	OH-C	July 8, 2009	Aug 20, 2009

Genotypic Diversity: # of Clones

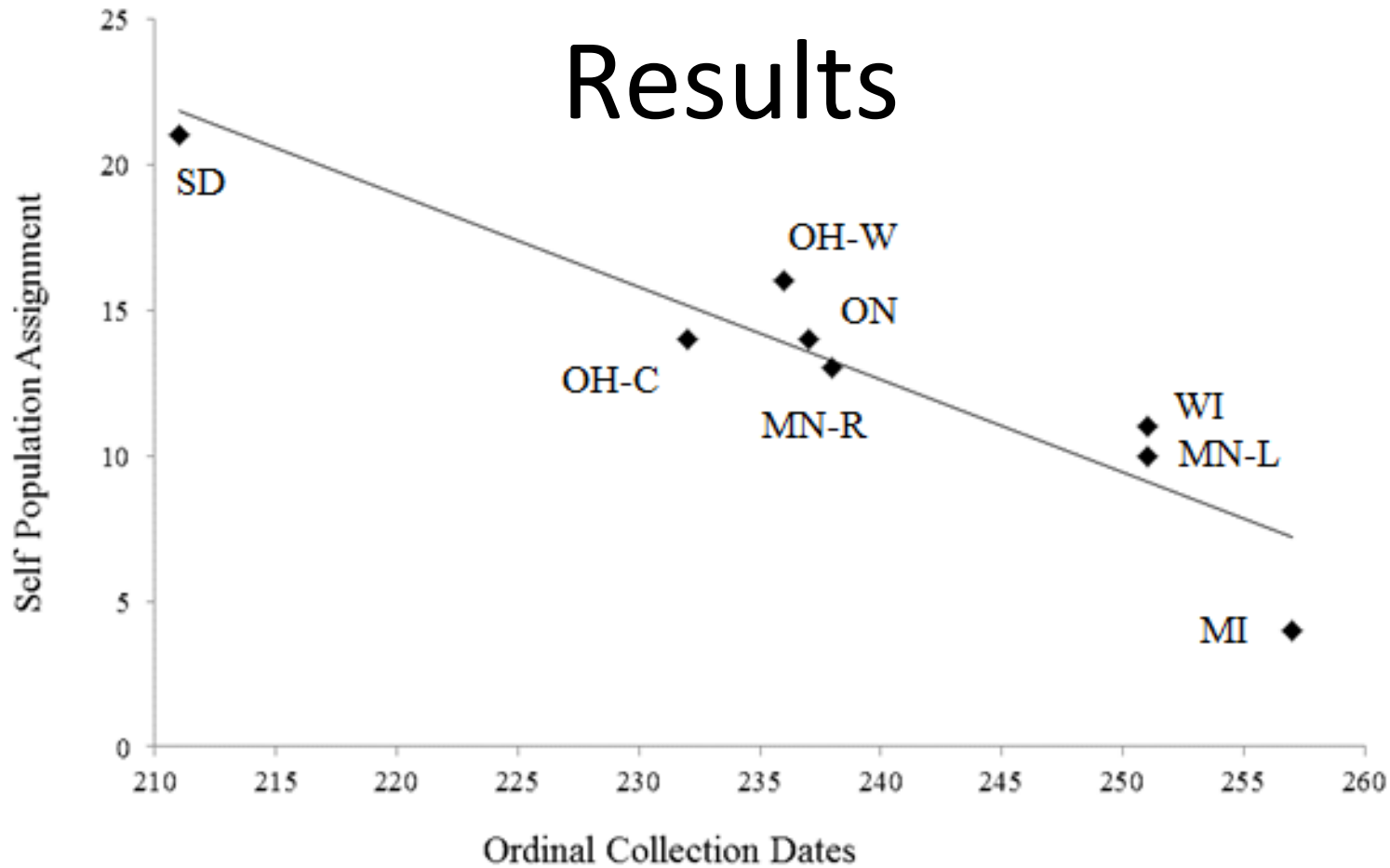


Results

- Genetic differentiation decreased in late population, less population structure



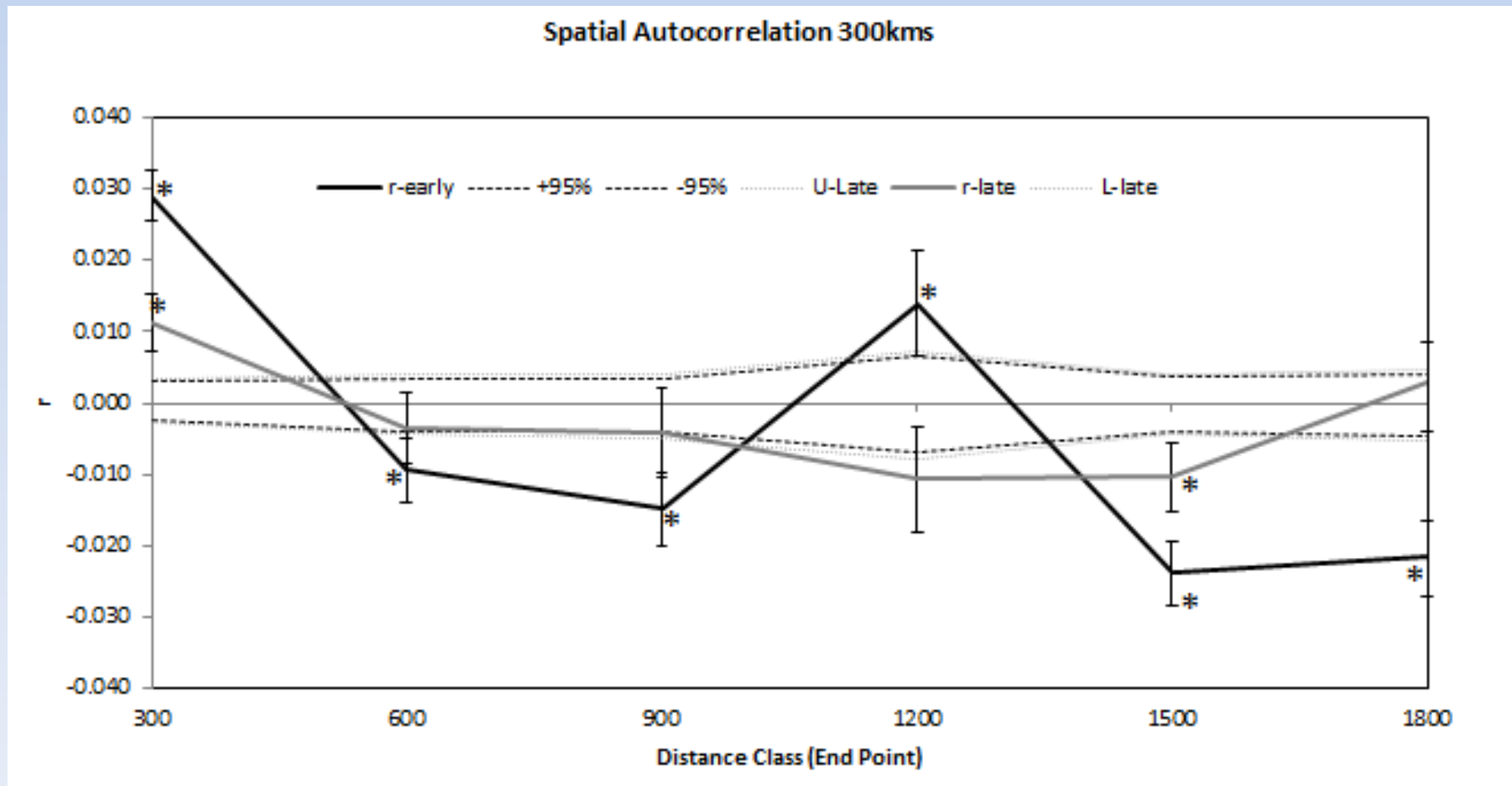
Results



- Self Assignment (# residents) values correlated with collection time in late populations
 - Later collected populations receive more migrants over time, leads to lower self-assignment

Results

- Spatial autocorrelation detects shift in dispersal pattern from spatially structured in early to random dispersal in late



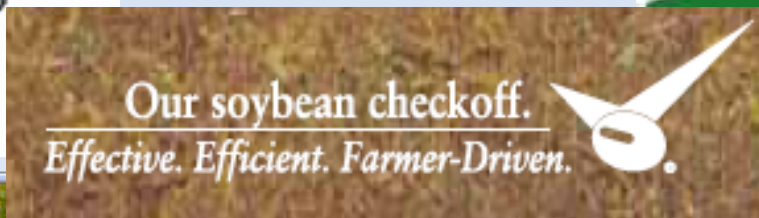
Conclusions

- Early soybean colonization by soybean aphid involves a founder effect and occurs locally
- Late season dispersal overcomes bottleneck by spreading genetic differentiation among fields
 - Would lead to population admixture, important for IRM?
- More research on mechanisms and markers

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