



# Happy Earth Day

April 22, 2020

A stylized illustration of a tree trunk and grass. The tree trunk is on the right side, rendered in a light green color with white horizontal lines representing bark texture. The grass is at the bottom, also in a light green color, with individual blades pointing upwards. The background is a dark green horizontal band.

# Trees, Fungi, Insects: How Host Plant Genetics Builds a Community

PhD Dissertation Defense

April 22, 2020

Sandra Simon





## Insects and fungi cause millions in damage

**Agricultural:** 16-18% of crop losses

**Forestry:** 6 million acres of trees lost (2015)

Estimated **\$13 billion** in management of herbivores alone in the United States (2014)

## But are also beneficial!

Provide over **\$57 billion** in ecological services

**Human recreation:** education, aesthetic enjoyment, and gardening

**Plant benefits:** pest control, nutrient cycling, pollination, and seed dispersal



Can ecosystems be managed to balance the harmful interactions and beneficial services?

# Community genetics: determining how shifts in genes contribute to the biotic-plant relationship





# The Salicaceae family contains many species of ecological and agricultural importance

Poplars, Cottonwoods, Aspens (*Populus*); willows (*Salix*)

**Agricultural:** important in biofuel production

**Ecological:** dominant species in riparian environments

**Genetic resource:** full genome sequencing of *P. trichocarpa* and *P. deltoides*

**Hybrid zones:** tree genetic composition can both directly and indirectly influence community interactions

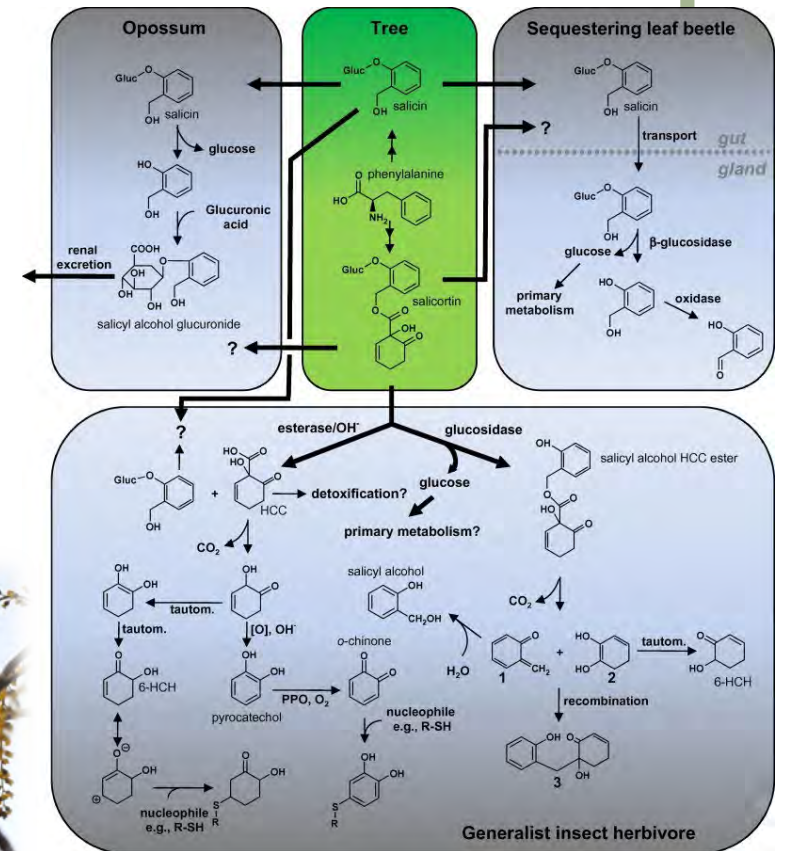


# There are many physiological and morphological traits under genetic control in *Populus* and *Salix*

**Secondary metabolites:** primarily act as a feeding deterrent to herbivores/animals and are highly variable among species

**Morphological traits:** leaf and petiole shape may affect host plant recognition

**Dioecious:** separate male and female individuals in the population affecting resource allocations



Kelly Moody







## Research objectives

**Objective 1:** Identify and understand the genetic mechanisms of fungal and insect association in a hybrid *Populus* backcross

**Objective 2:** Identify genes underlying arthropod community composition in a pure species population of *P. trichocarpa*

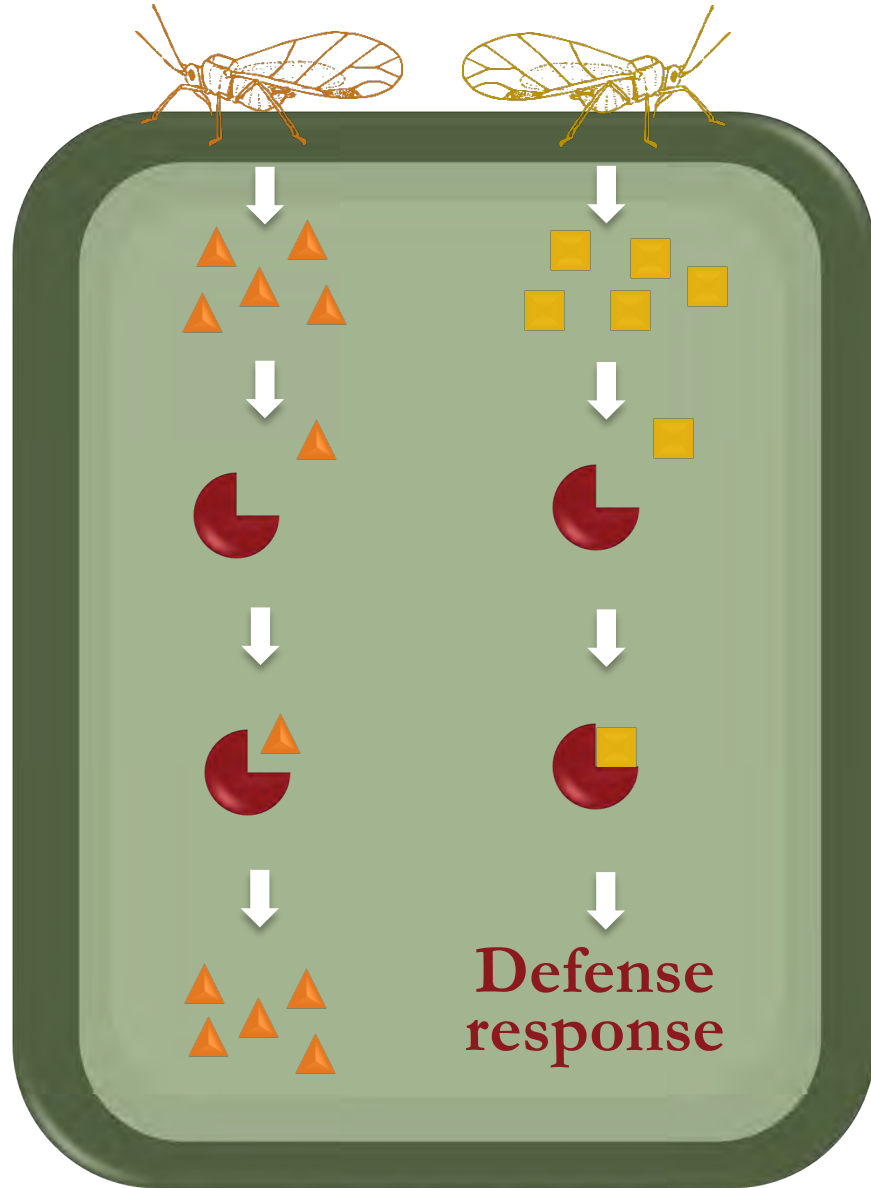
**Objective 3:** Determine the impact of dioecy on assembly of floral insect communities in a widespread species *S. nigra*

**Objective 1:** Identify and understand the genetic mechanisms of fungal and insect association in a hybrid *Populus* backcross





# Biotic interactions are complex as they involve two evolving systems



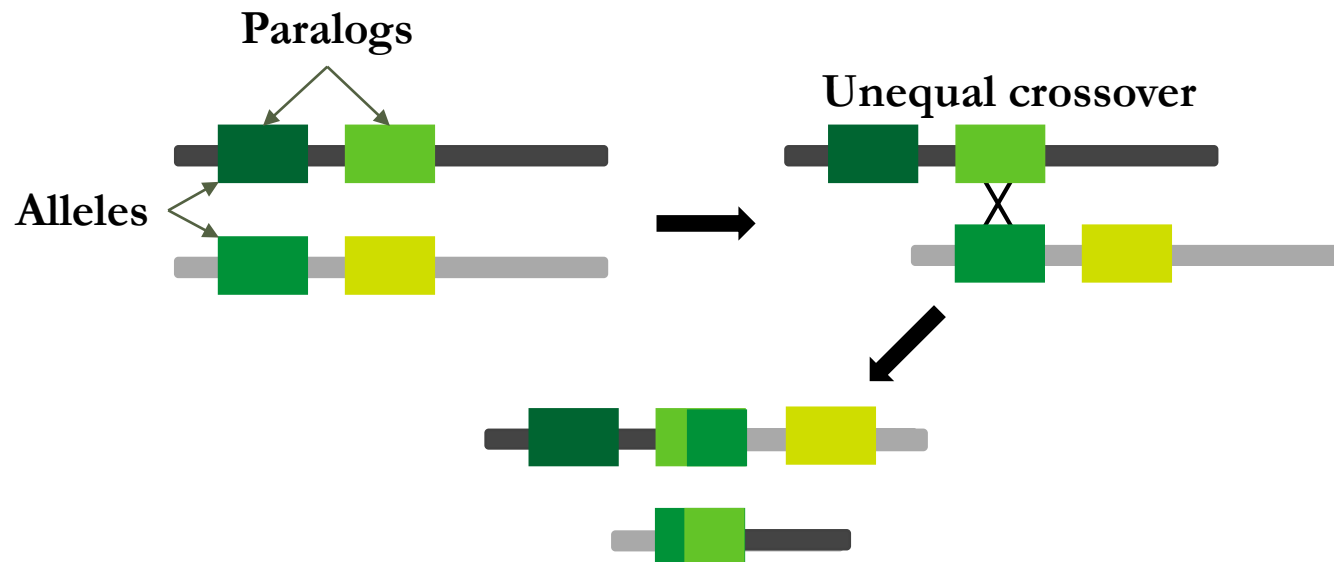
1. Insect stylus/pathogen enters plant cell via wound
2. Elicitor molecules are released from pest
3. Plants produce resistance proteins (R-genes)
4. If resistance protein binds elicitor molecule a defense response against pest can be stimulated
5. When resistance protein does not bind elicitor no defense is activated and plant succumbs to pest

**Gene-for-gene hypothesis**

# Diversifying selection pressure on hosts can have lasting impacts on the plant genome

## Tandem-duplications

- Accumulate mutations leading to new function
- Increasing genetic diversity for selection



## Insect-plant relationships

- Kunitz trypsin inhibitors (KTIs)
- Disrupt insect digestion

## Pathogen-plant relationships

- Nucleotide-binding site leucine-rich repeat resistance genes (NBS-LRR R-genes)
- Signal for hypersensitive response



# Genetic underpinnings of biotic relationships can be difficult to study

*Populus* hybrids are a useful research tool

- More susceptible to insect/fungal attack
- Result of production of novel traits
  - *Populus* secondary metabolites
  - Affect host plant recognition
  - Defense responses

Utilize backcross hybrid progeny

- Identify genetic regions of interest

Compare parental genomes

- Study structure, look for unique genes and genetic patterns





# Objective 1: Questions

1. What genetic regions associate with each insect and fungal species?
2. Which genes are potential candidates for mediating biotic interactions?
3. Is tandem duplication characteristic of candidate genes present the genetic regions?



# Plantation surveys and methods

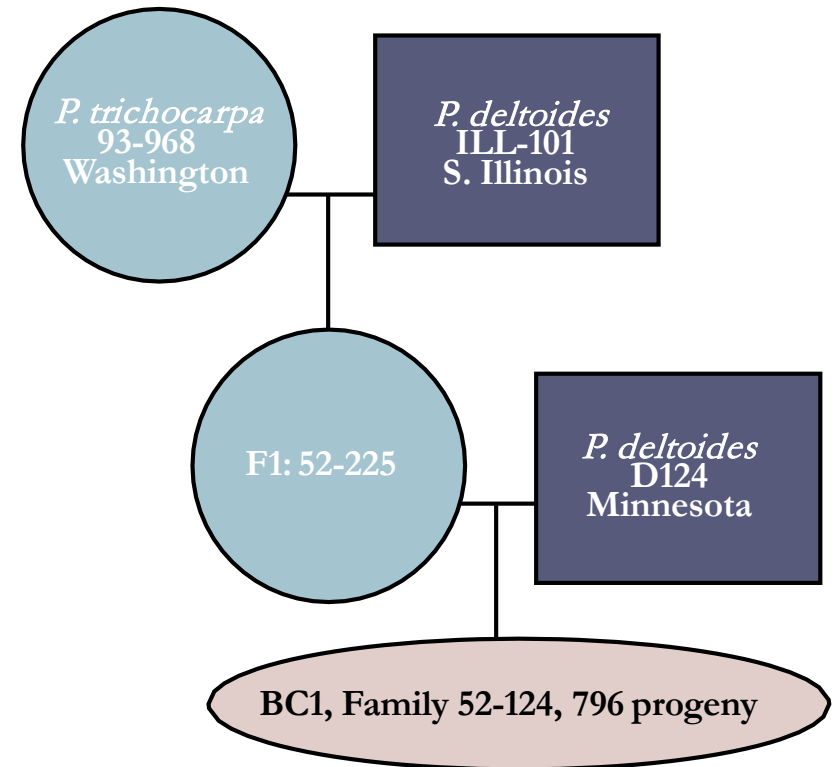
Surveyed tree canopies for a variety of insects and fungal pathogen symptoms

Quantitative Trait Loci (QTL) Mapping

- ~3,500 segregating single nucleotide polymorphic (SNP) markers

Comparative genomics of significant genetic regions to look for patterns and candidate genes mediating interactions

## Pseudo-backcross pedigree





### Leaf-folding sawfly (*Phyllocolpa* sp.)

- Female sawflies sting leaf edge and lay one egg

### Vagabond aphid (*Mordwilkoja vagabunda*)

- Aphids feed on buds forming bladder-like galls



### Poplar petiole galling aphid (*Pemphigus populitransversus*)

- Gall formation on petiole
- Reproduce clonally on *Populus*; sexually on Brassica plants





## *Melampsora* sp. leaf rust

- Biotrophic fungus
- Uses poplar and willow tissue to perpetuate spores, intensifying the infection
- Requires poplar and conifers to complete life-cycle

## *Sphaerulina musiva* leaf spot

- Necrotrophic fungus
- Dead lesions on leaves
- After infection obtains nutrients from and reproduces in dead host tissue

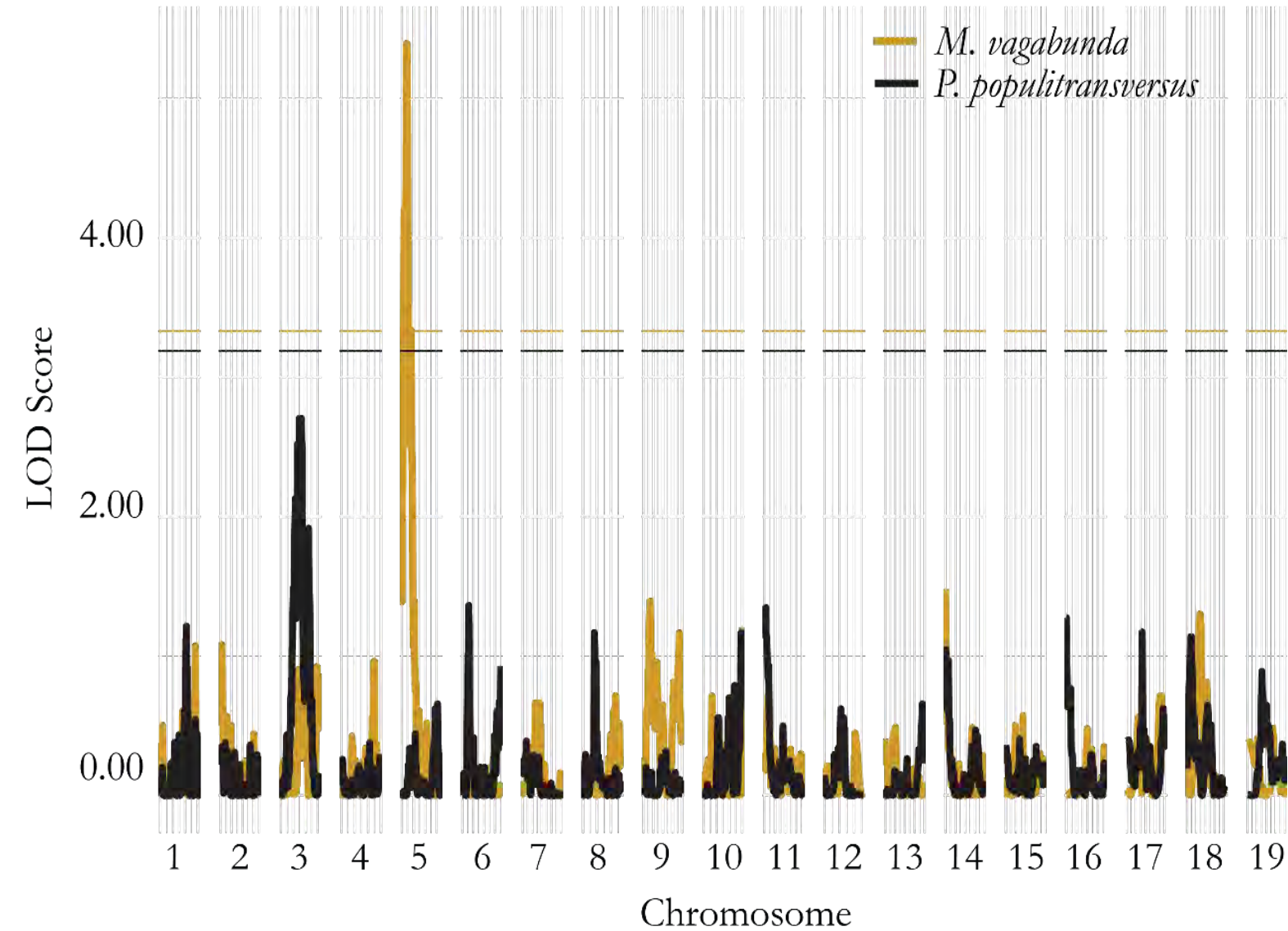


## *Sphaerulina musiva* stem canker

- Causes lesions on stems
- Lead to canopy breakage
- Tend to be more aggressive on hybrids



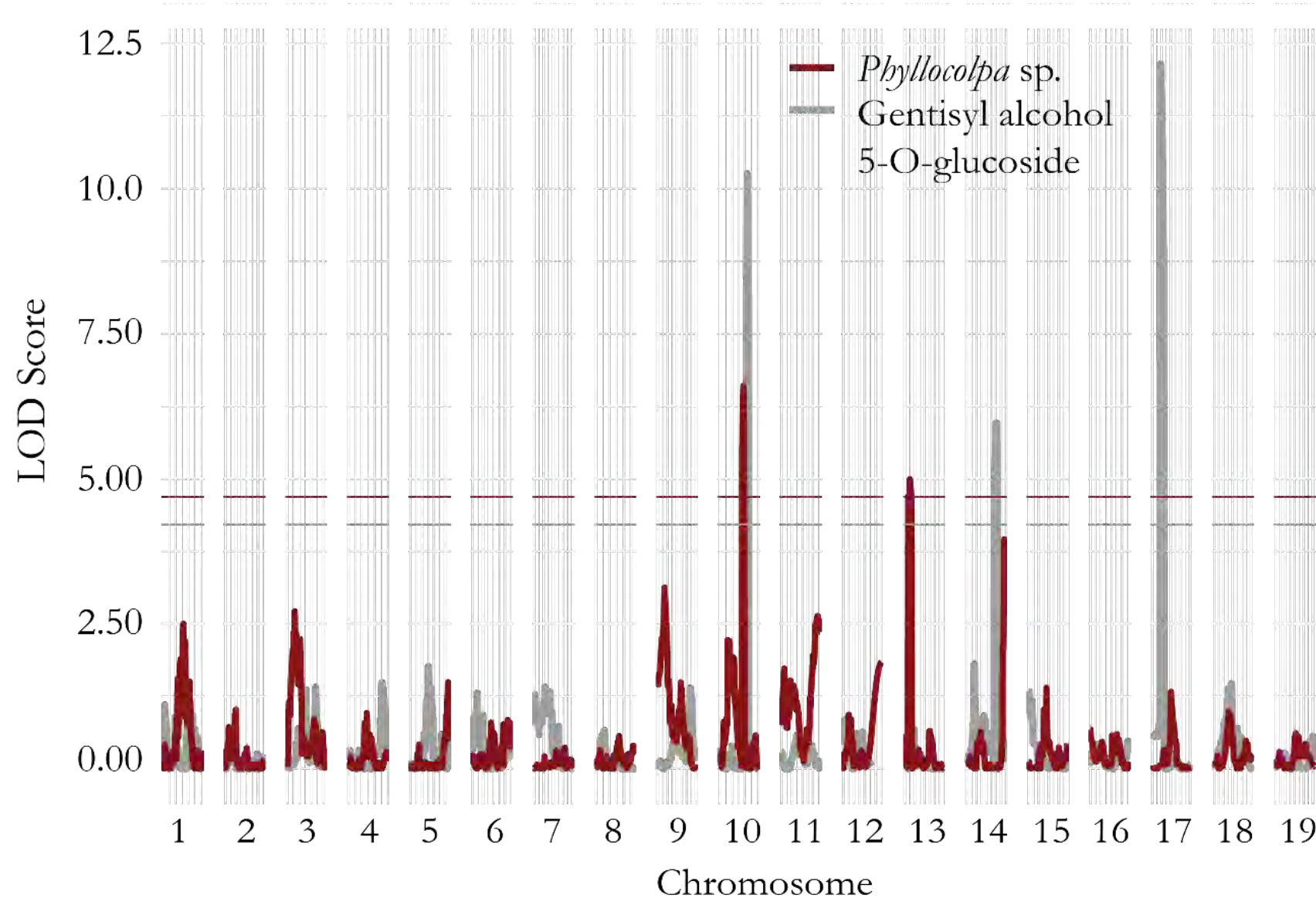
# A genetic region on Chr05 associated with *M. vagabunda*







# Genetic regions on Chr10 and Chr13 associated with *Phyllocolpa* sp.



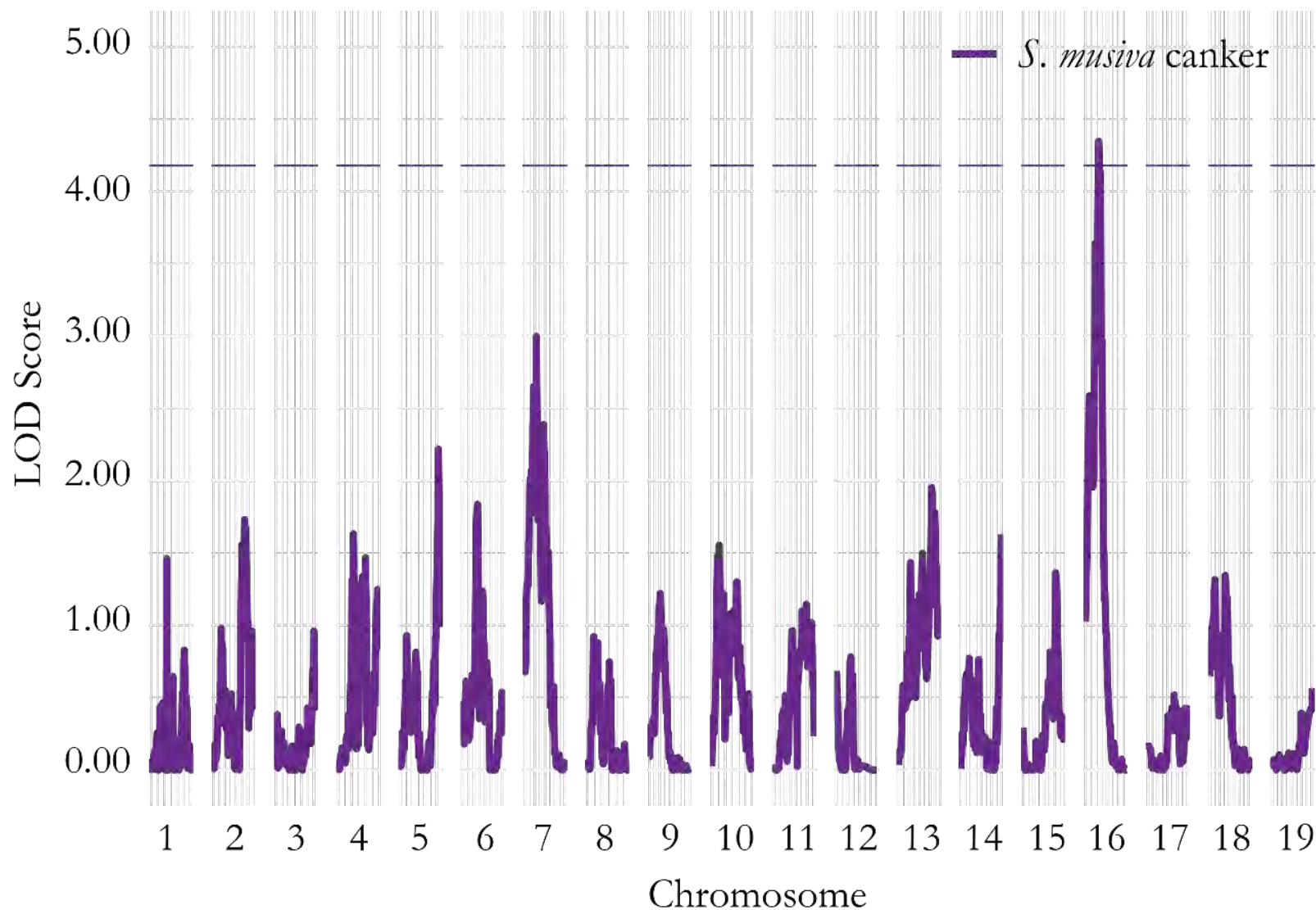
An adjoining QTL peak on Chr10 is known to be related to the production of *Populus* phenolic gentsyl alcohol 5-O-glucoside

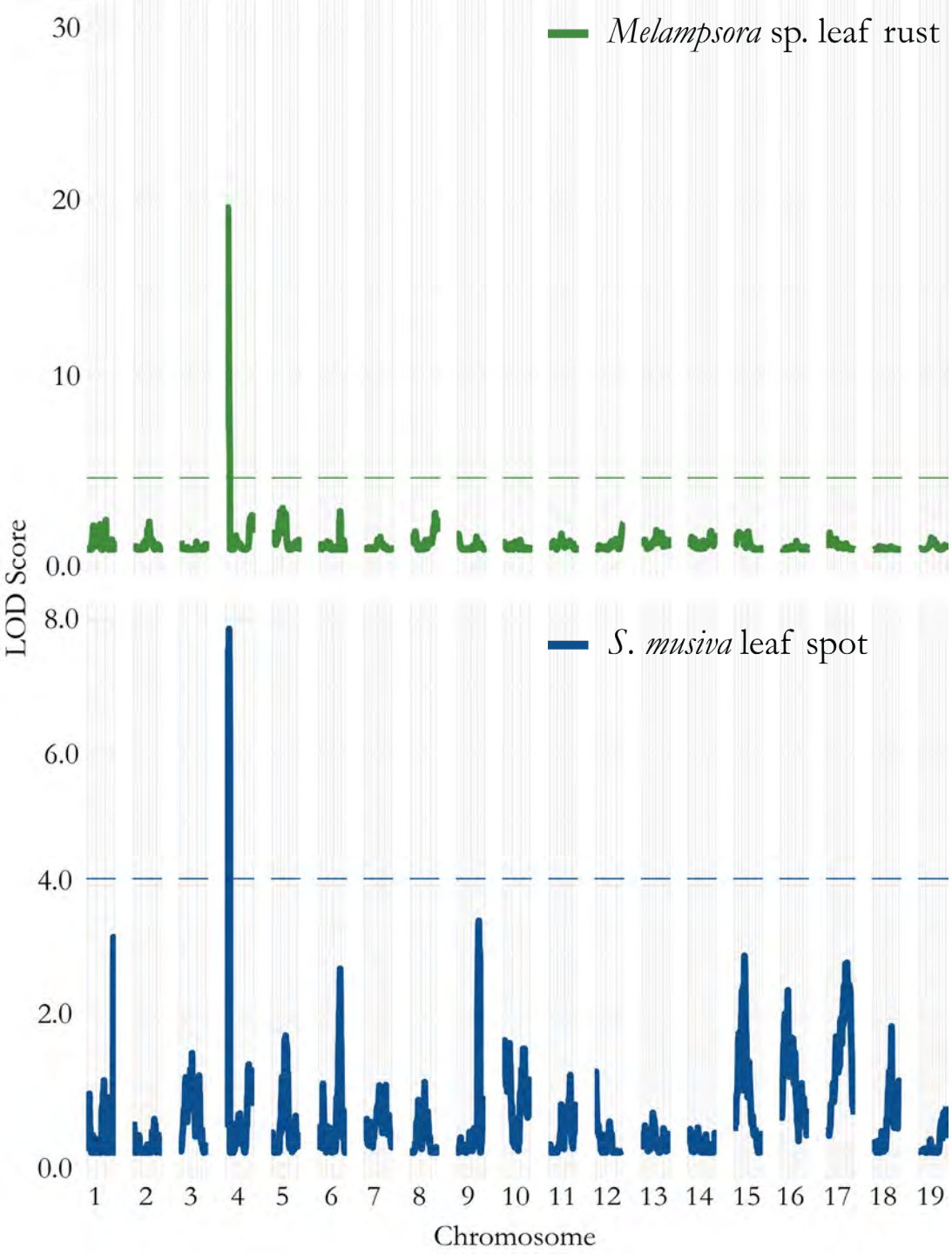
Possible ovipositional cue for *Phyllocolpa* sp. female sawflies





# Genetic region on Chr16 associated with stem canker symptoms of *S. musiva*

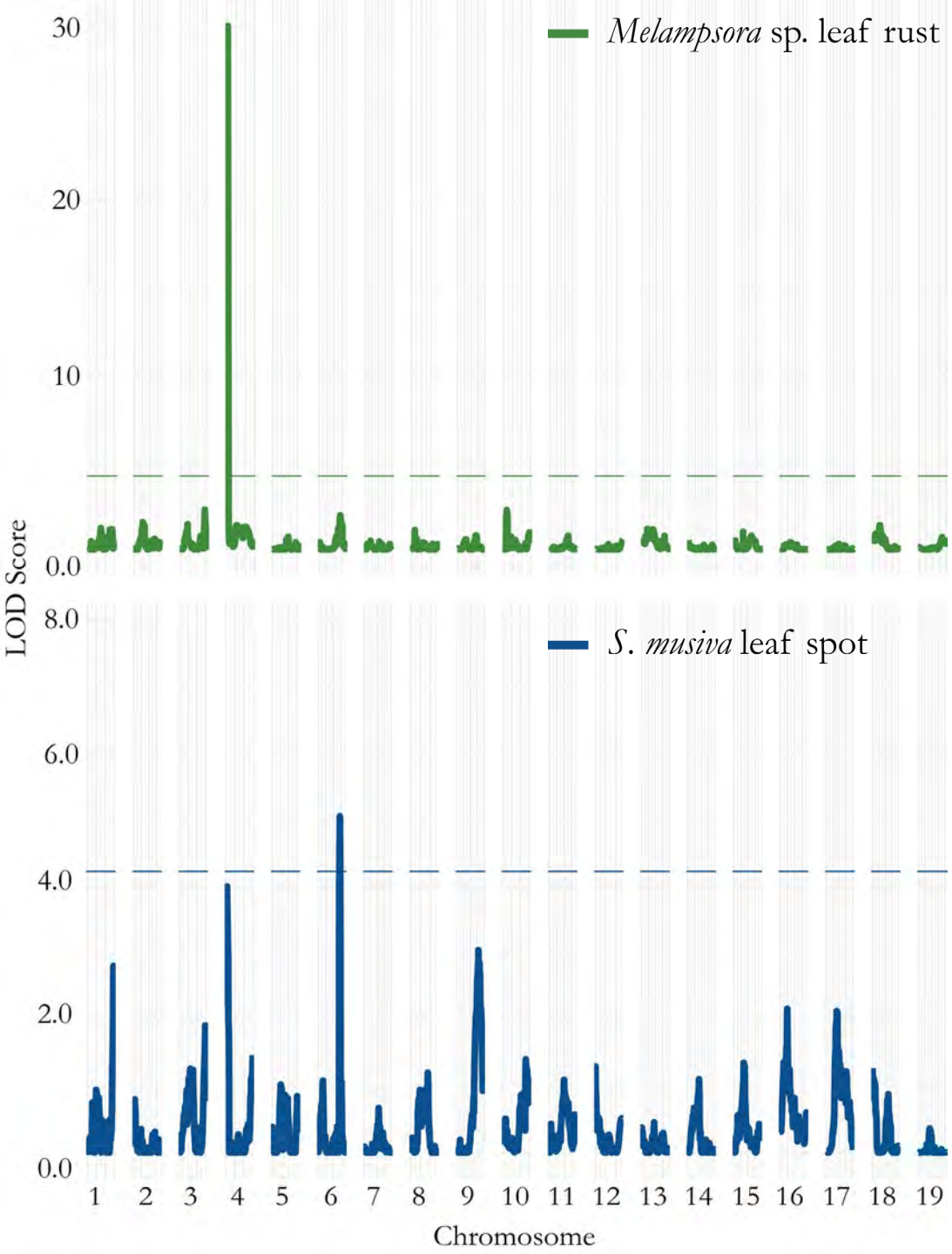




An overlapping peak on Chr04 was associated with two pathogenic fungi





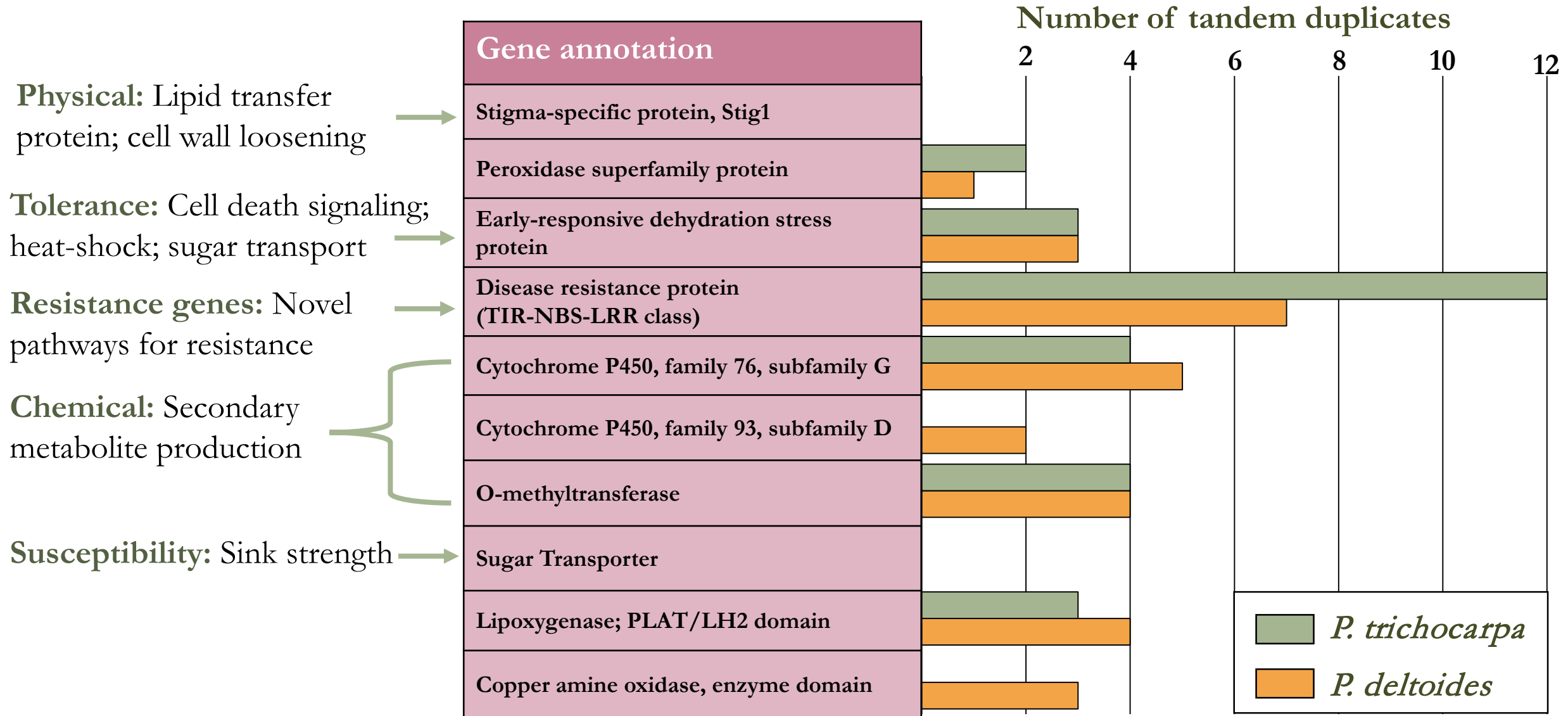


Removal of individuals infected by both pathogens changed Chr04 pattern



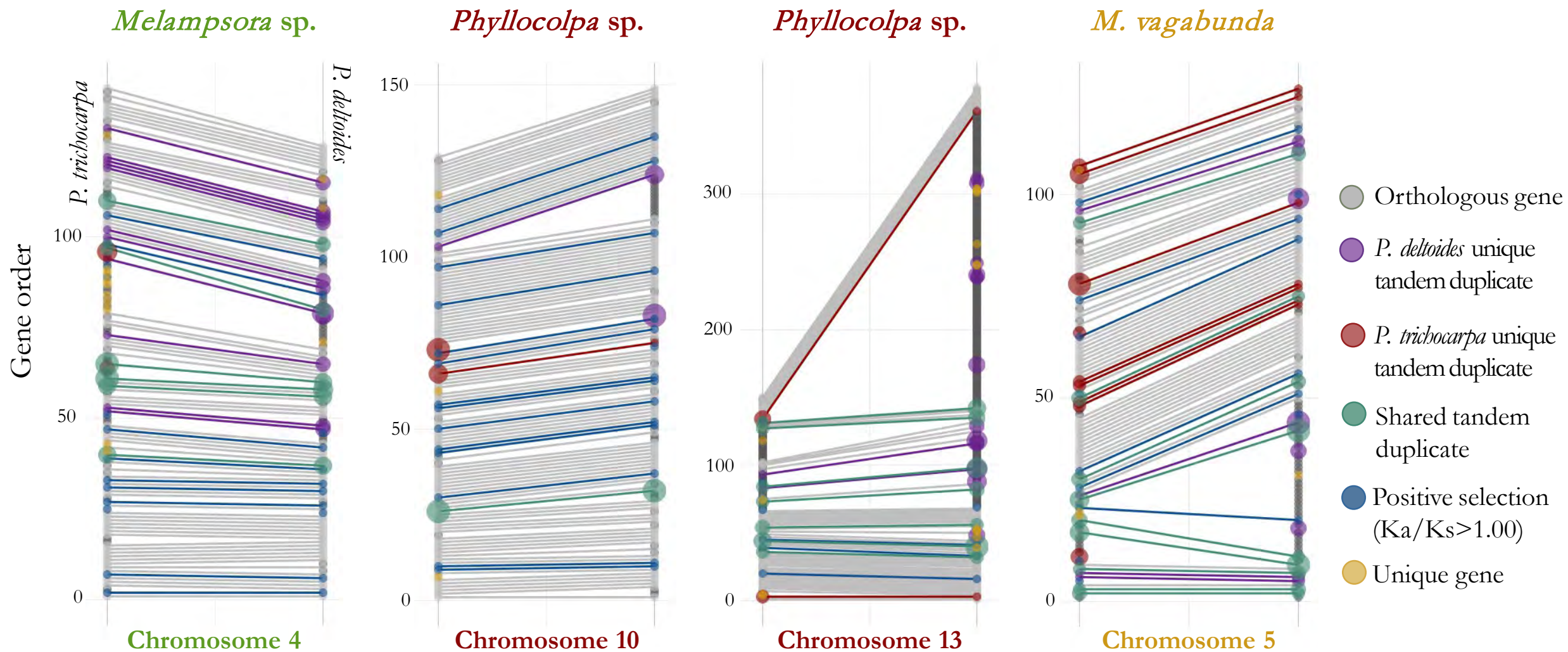


# Genes with functional enrichment had implications in disease resistance and many were tandemly duplicated





# Tandem duplications were enriched in biotic intervals in the *P. deltooides* parent





# Objective 1: Conclusions

1. What genetic regions associate with biotic species?
  - Six genetic regions; six chromosomes
  - Competitive interaction between pathogens (Chr04)
  - Phenolic production adjoined with *Phyllocolpa* sp. genetic region
2. Which candidate genes mediate biotic interactions?
  - Tissue toughening, transmembrane sensing/signaling (R-genes), secondary metabolite production, and resource allocation
3. Is tandem duplication characteristic of the genetic regions?
  - Tandem duplications were enriched in the *P. deltooides* parent
  - The native species to many of the pathogens and pests used in analysis



**Objective 2:** Identify genes underlying arthropod community composition in a pure species population of *P. trichocarpa*





## Hybrids show more significant responses to insect and fungal interactions

Capable of mediating unique associations

Not necessarily representative of most plant species

## Similar genetic patterns have been detected in single species populations

Communities structured by non-hybrid *Populus* species:

- arthropod-avian predator distributions
- microbes and lichens
- aquatic terrestrial linkages

**What are the underlying genes and functions?**





# Objective 2: Questions

Utilized an association population containing 1,100 genotypes of *P. trichocarpa* to answer:

1. How do different sites compare in their arthropod communities and does genotype influence the composition of arthropods observed on trees?
2. Which candidate genes underlie arthropod associations?
3. What possible biological functions could candidate genes confer based on co-expression networks?



# Plantation surveys and methods

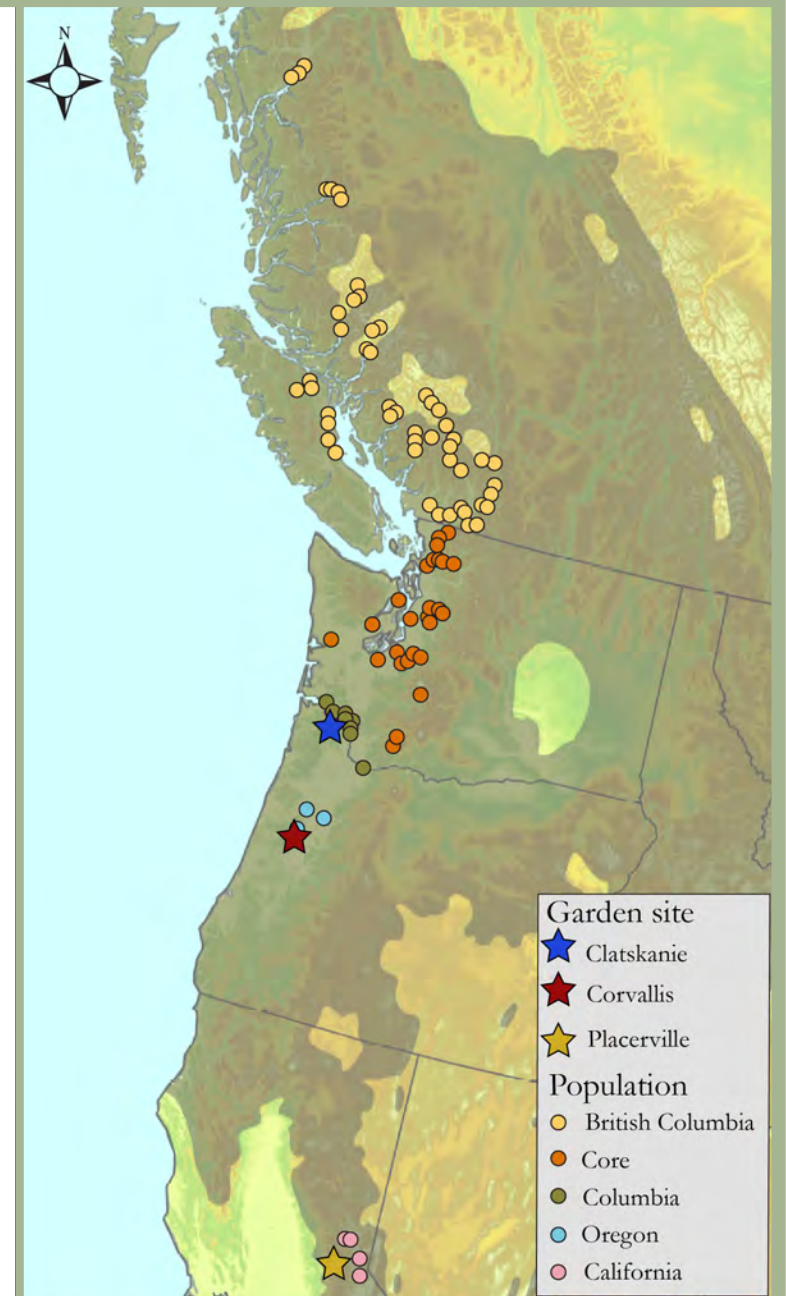
Surveyed *P. trichocarpa* canopies for arthropods and arthropod damage

- 2012: Clatskanie, Corvallis, Placerville
- 2015: Corvallis

Identify candidate genes mediating insect interactions and arthropod communities

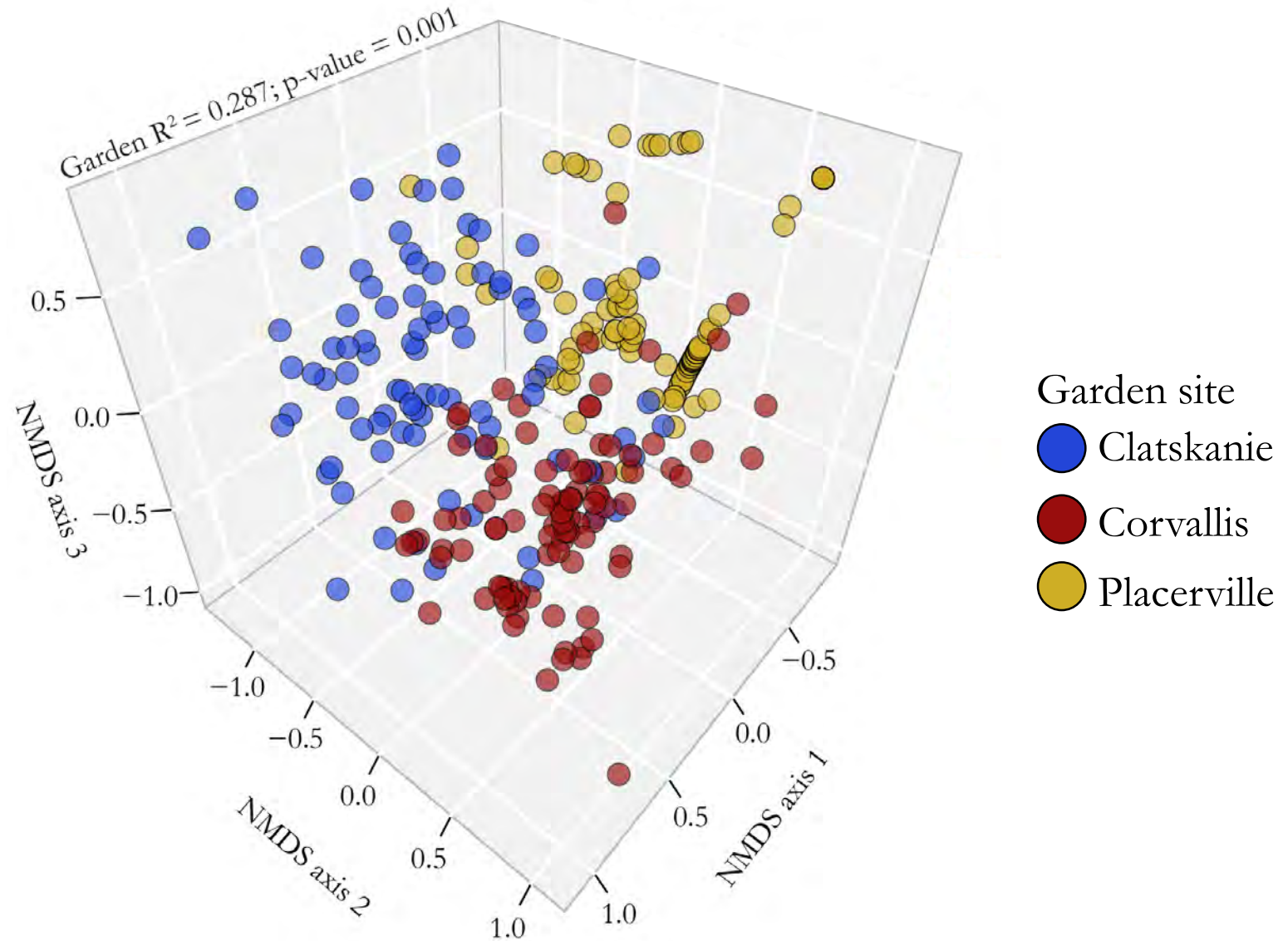
- Genome-wide association analysis (GWAS)
- 6 million single nucleotide polymorphisms (SNPs)

Built functional networks for candidate genes





# Each garden had a unique assemblage of arthropods





# Tree genotype had a strong influence over its arthropod community

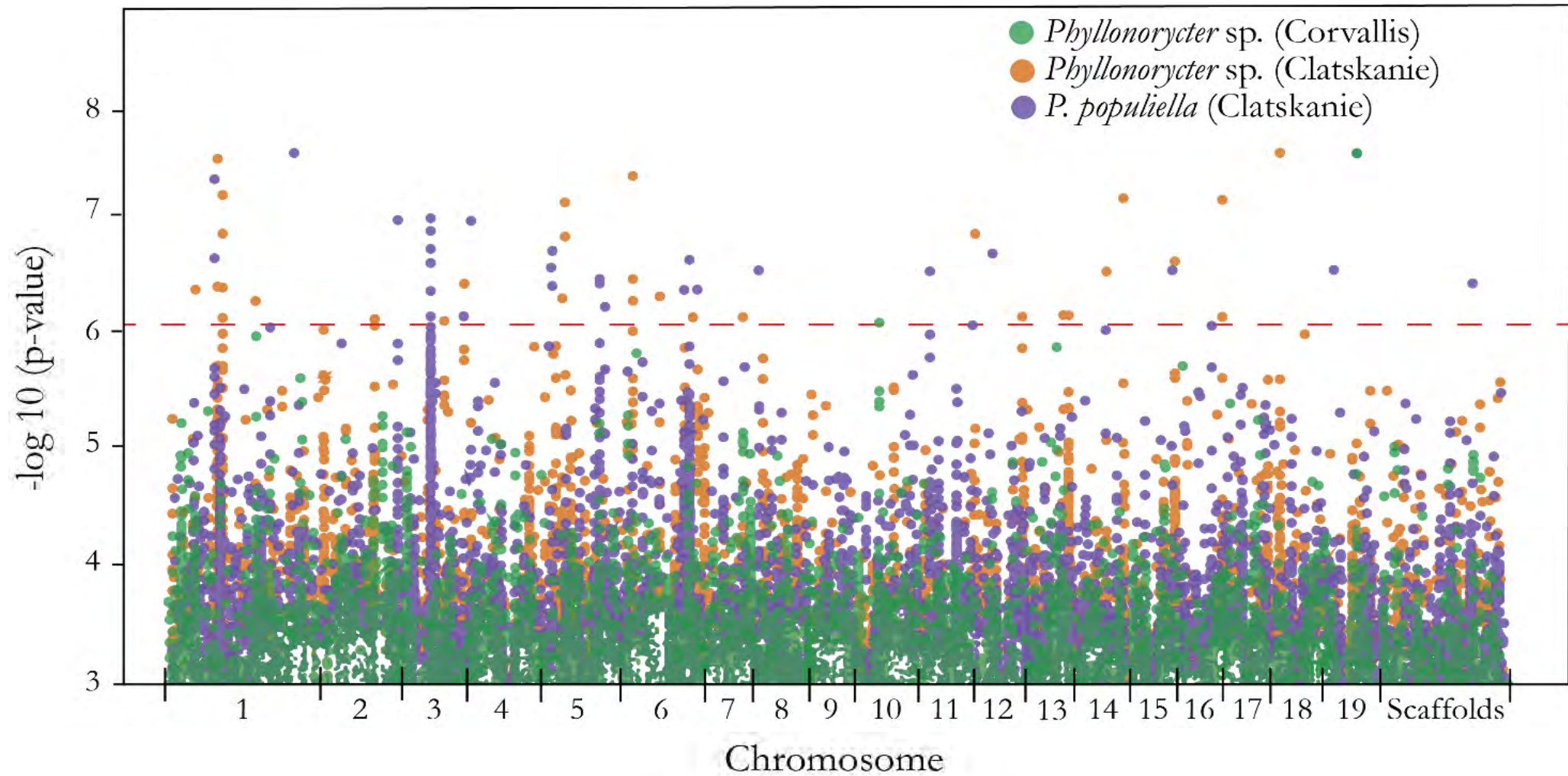
| Garden           | Factor   | R <sup>2</sup> | P-value |
|------------------|----------|----------------|---------|
| Clatskanie 2012  | Genotype | 0.435          | 0.029   |
| Placerville 2012 | Genotype | 0.416          | 0.036   |
| Corvallis 2012   | Genotype | 0.549          | 0.001   |
| Corvallis 2015   | Genotype | 0.550          | 0.032   |







There were 71 total loci that associated with arthropod traits





## *Phyllonorycter* sp. blotch miner

- Specialist pest
- Larvae develop in the leaf mesophyll
- Instar 1-3 feed on sap; 4-5 feed on leaf tissue

## Arthropod richness

- Number of unique species
- Generalist herbivores
- Specialist herbivores



## Arthropod richness

- Herbivore predators
- Spiders
- Ladybugs



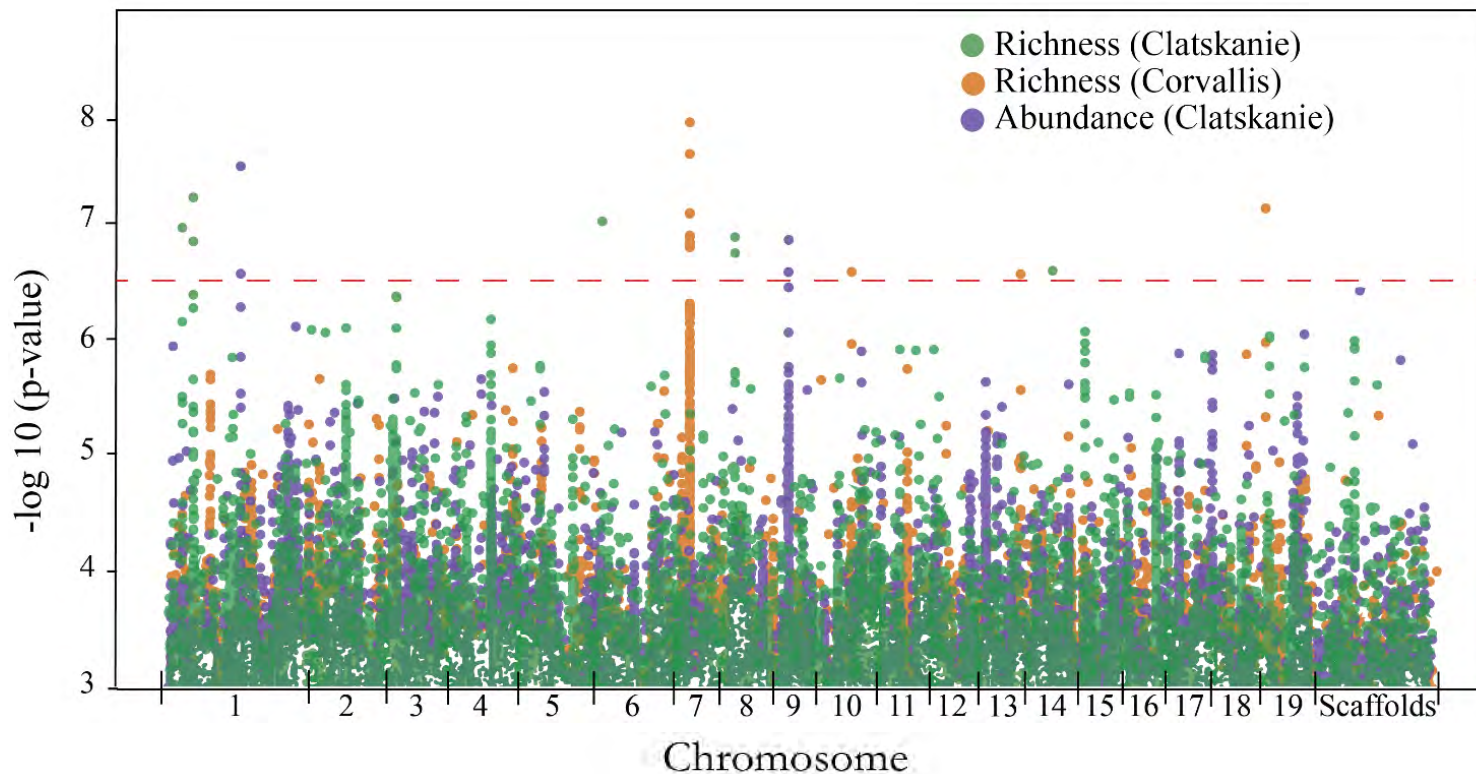
# Selection of candidate genes is difficult

## GWAS limitations

- Multiple independent testing
- Lacks power
- **How can we filter for the most meaningful genes?**

## Networks

1. Co-expression
2. Co-methylation
3. SNP-SNP correlations
4. GWAS for pyMBMS
5. GWAS for metabolites



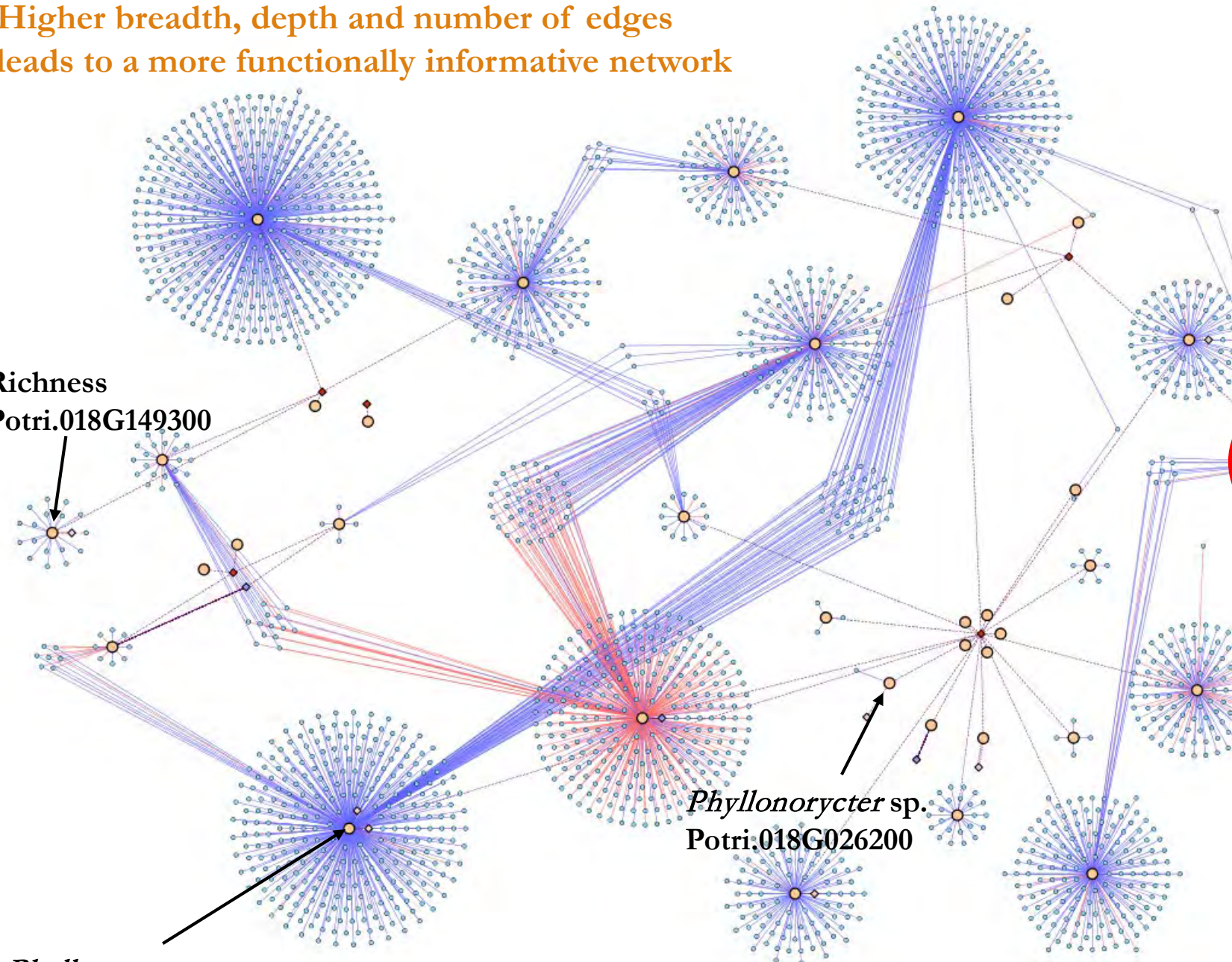
**Establish function based on connections made with candidate genes**



Higher breadth, depth and number of edges leads to a more functionally informative network

| Gene             | Breadth | Depth | Edges |
|------------------|---------|-------|-------|
| Potri.001G272000 | 4       | 1,196 | 1,750 |
| Potri.018G026200 | 4       | 615   | 757   |
| Potri.018G149300 | 4       | 1,610 | 6,074 |

Richness  
Potri.018G149300



Breadth- number of layers that network spanned (max = 5)

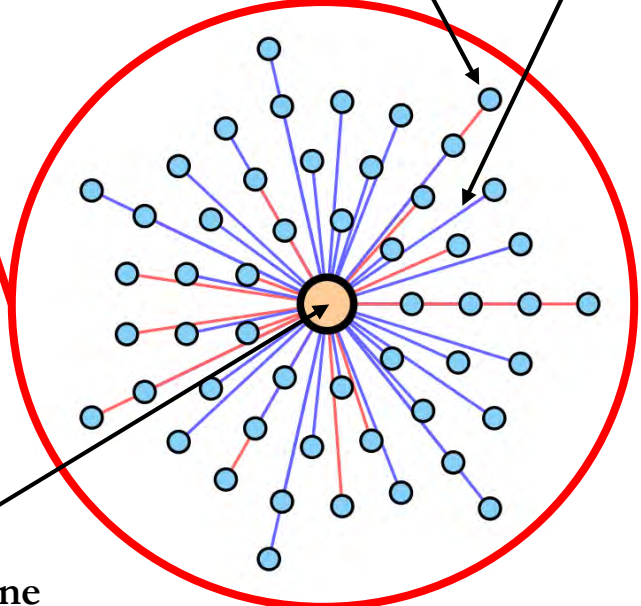
Depth: # nodes  
Edge

*Phyllonorycter* sp.  
Potri.001G272000

*Phyllonorycter* sp.  
Potri.018G026200

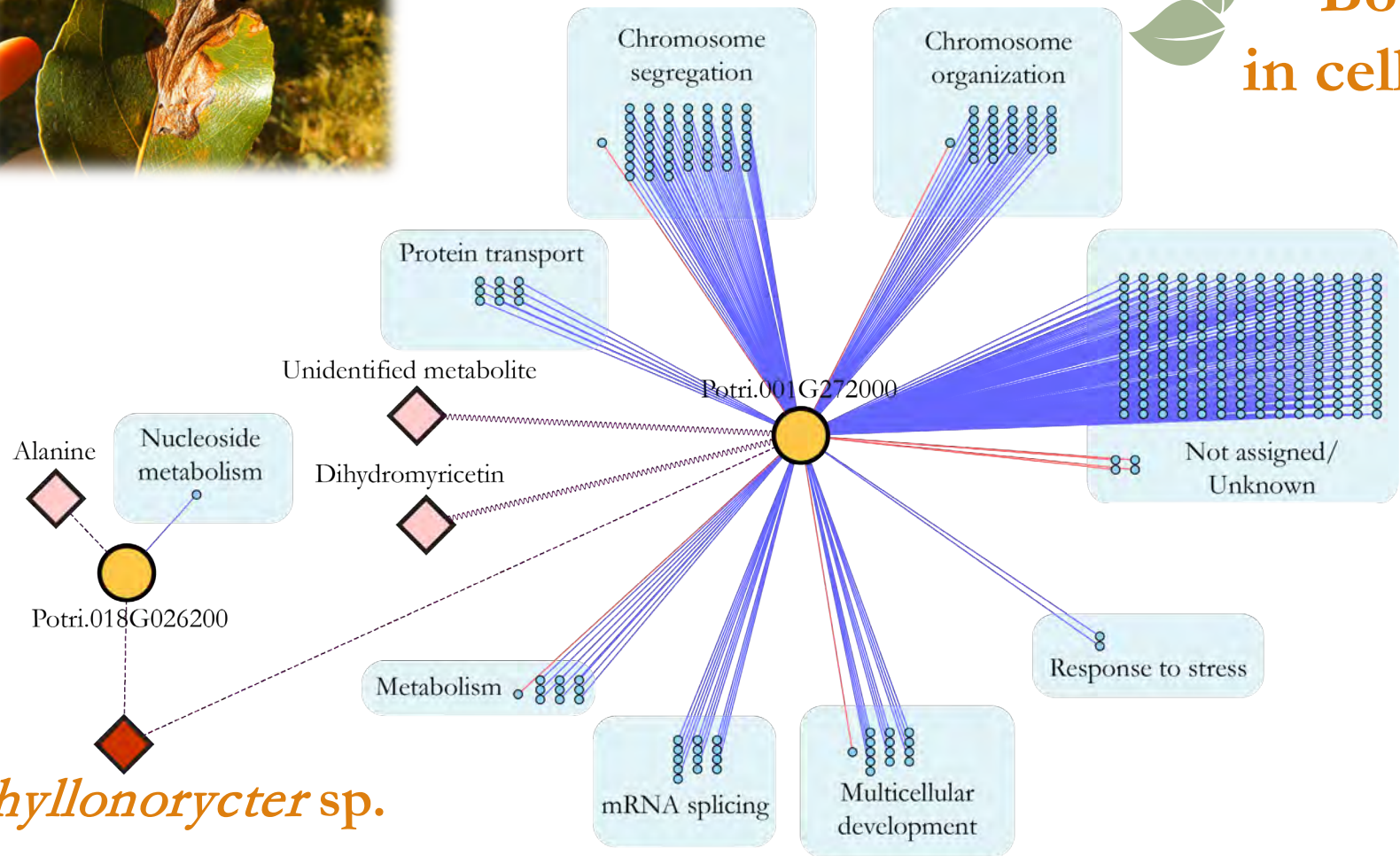
Candidate gene from GWAS

Objective 2: Question 3





# Both genes are important in cell proliferation and growth



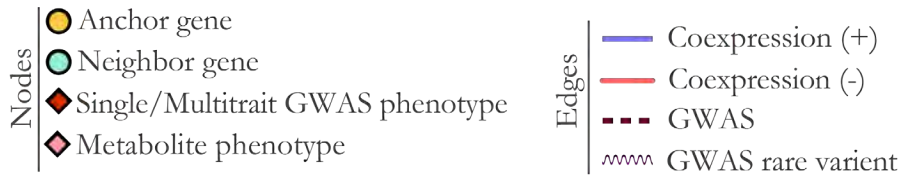
## Potri.001G272000- cyclin B1;4

- Cell cycle regulator
- *Phyllonorycter* manipulate cytokinin phytohormones
- Stimulates cell growth and prevent tissue senescence

## Potri.018G026200- adenine phosphoribosyl transferases

- Utilize alanine to inactivate cytokinin signaling

*Phyllonorycter* sp.





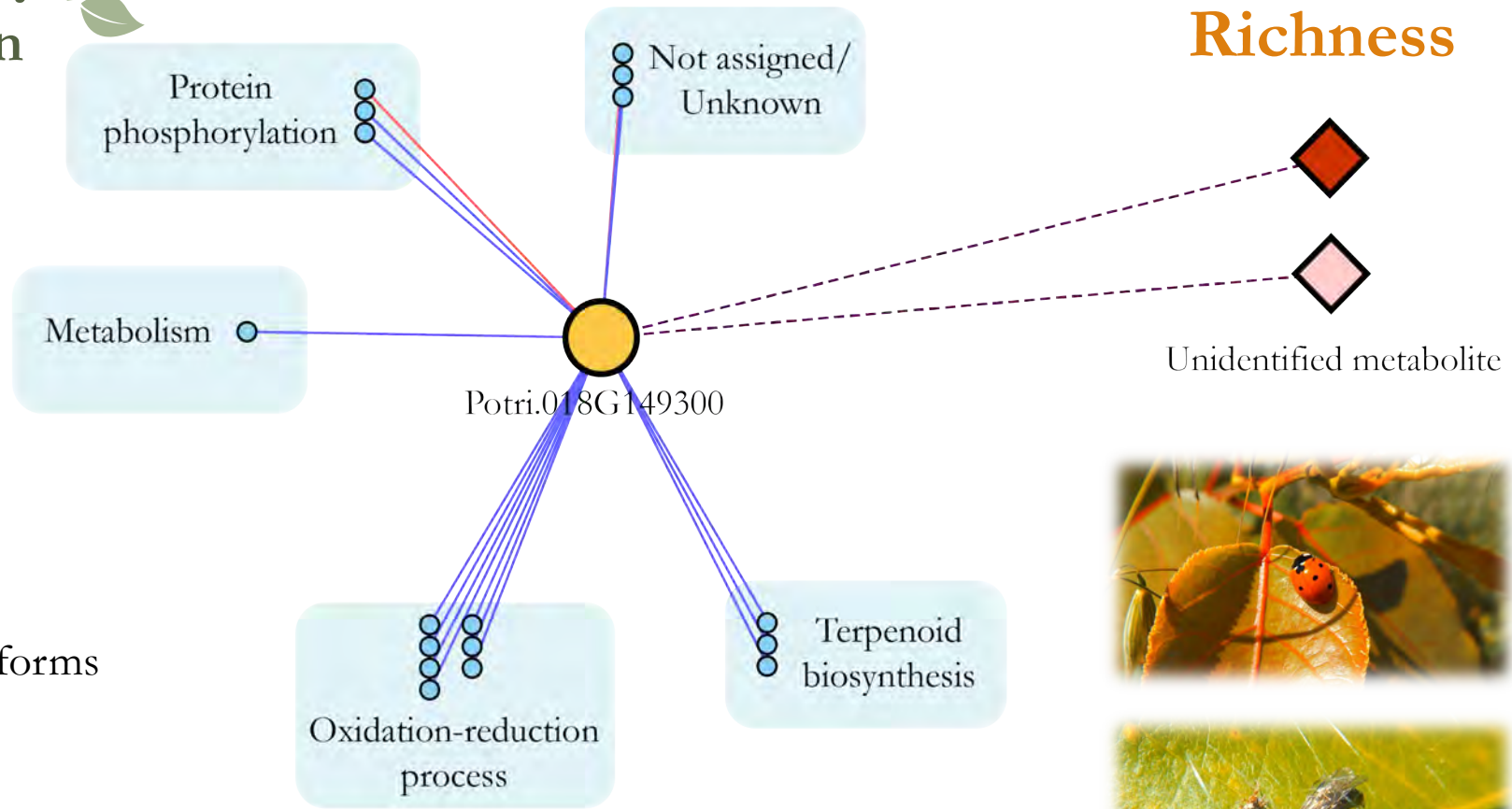
# Candidate gene appears to play a role in terpenoid production

## Potri.018G149300- cytochrome P450

- Wide range of biological activity
- Catalyst functions

## Terpenoids

- Consumption affects development
- Utilize as ovipositional signals
- Crushed leaf tissue releases volatile forms allowing prey location



Nodes

- Anchor gene
- Neighbor gene
- ◆ Single/Multitrait GWAS phenotype
- ◆ Metabolite phenotype

Edges

- Coexpression (+)
- Coexpression (-)
- - - GWAS





# Objective 2: Conclusions

1. How do different sites and genotypes compare in their arthropod communities?
  - Each site had unique assemblage of arthropods
  - Tree genotype influenced arthropod assemblage
2. Which candidate genes underlie arthropod associations?
  - 71 loci associated with arthropod traits
  - 3 strong candidate genes
3. What are the biological functions of candidate genes?
  - *Phyllonorycter* sp.- cell proliferation and growth
  - Richness- terpenoid biosynthesis important in both generalist defense, specialist host recognition, and predator location of prey



## What have we studied so far?

*P. trichocarpa* x *P. deltoides* hybrid backcross

- Fungal pathogens
- Insect herbivores

Association population *P. trichocarpa*

- Specialist insect herbivores
- Arthropod community composition

## Floral visitor communities are currently understudied in Salicaceae

Previous community genetics work primarily focused on species of *Populus*

- 30 species; Wind pollinated

*Salix* species

- 450 species; Wind and/or insect pollinated
- Beneficial insects for plant productivity



Sam Droege/Sandy Simon



**Objective 3:** Determine the impact of dioecy on assembly of floral insect communities in the widespread species *S. nigra*





# *Salix nigra* is a valuable resource for early emerging pollinators

**Widespread in eastern United States**

**Can help support polylectic insects**

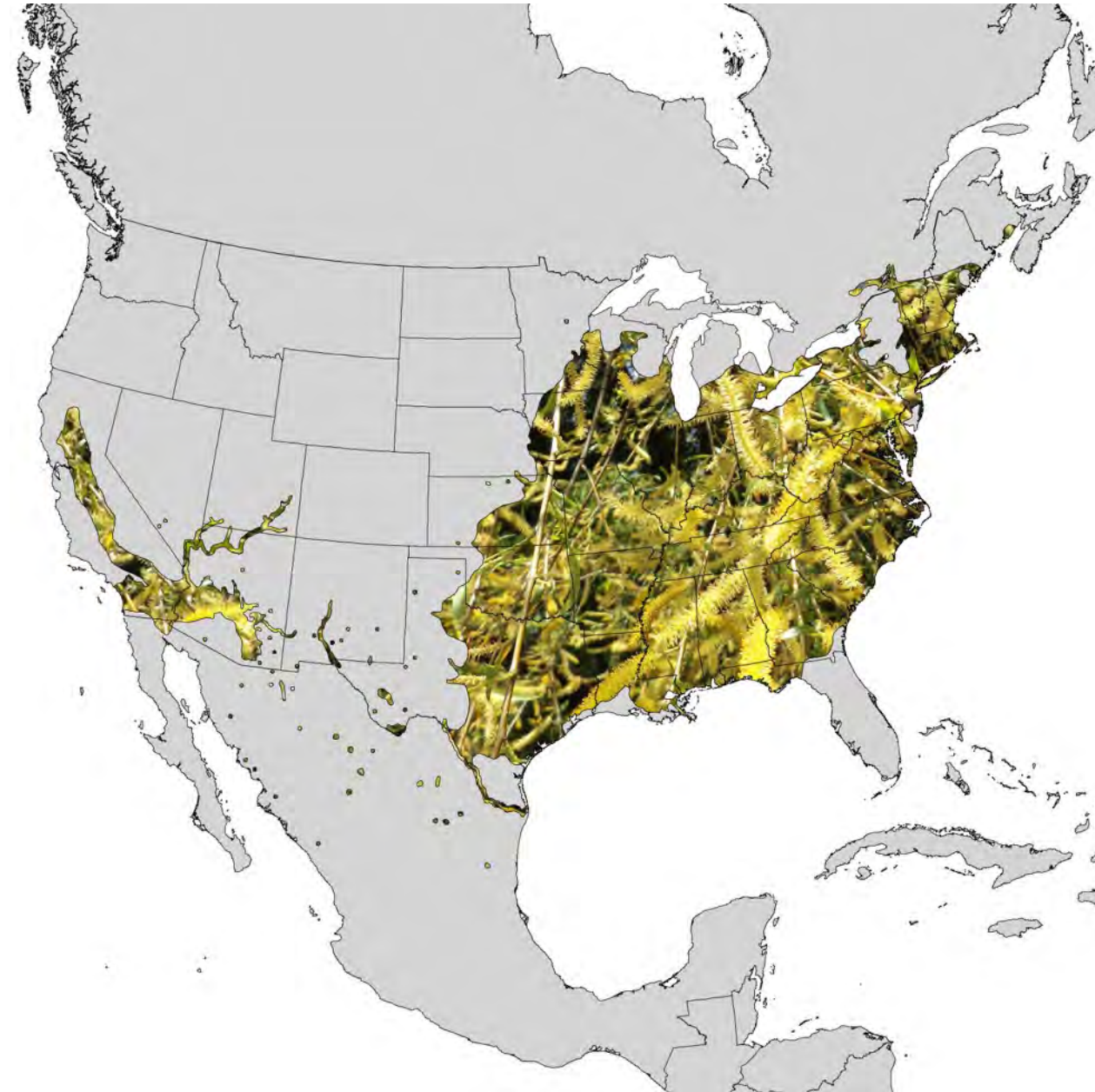
- Collect resources from unrelated flowering plants
- Honey bees (*Apis mellifera*)
- Agricultural pollinators

**Necessary for oligolectic bees**

- Only subsist on a single plant species or genus
- *Andrena* spp. willow specialists

**Dioecious species**

- Separate female and male individuals
- Not all insects will lead to successful cross-pollination





# Objective 3: Questions

1. How do tree characteristics such as dioecy, composition of volatile organic compounds (VOCs) and defensive chemistry influence floral visitor communities?
2. What insects are the main cross-pollinators in the *S. nigra* population and how do tree characteristics influence their behavior?
3. How does survey year impact distributions and activity of cross-pollinators?

# Floral surveys and methods

## Visual surveys

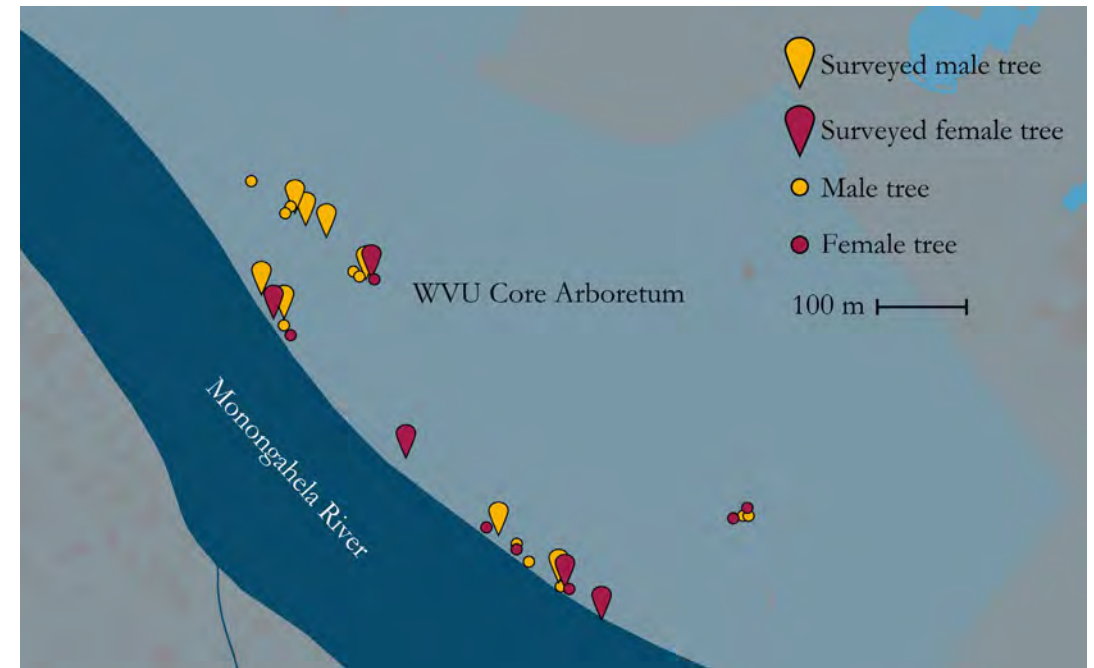
- Population of *S. nigra* WVU Core Arboretum
- 32 individuals in population; 12 females and 20 males
- 300 flowers observed for surveyed trees throughout bloom
- Specimens hand collected for identification
- Three years of surveys (2017-2019)

## Pan-trapping

- Deployed below flowering branches
- Raised into canopy 9am and emptied at 7pm daily
- 2019 survey only
- Gather more complete view of insect community

## Volatile and defensive compounds

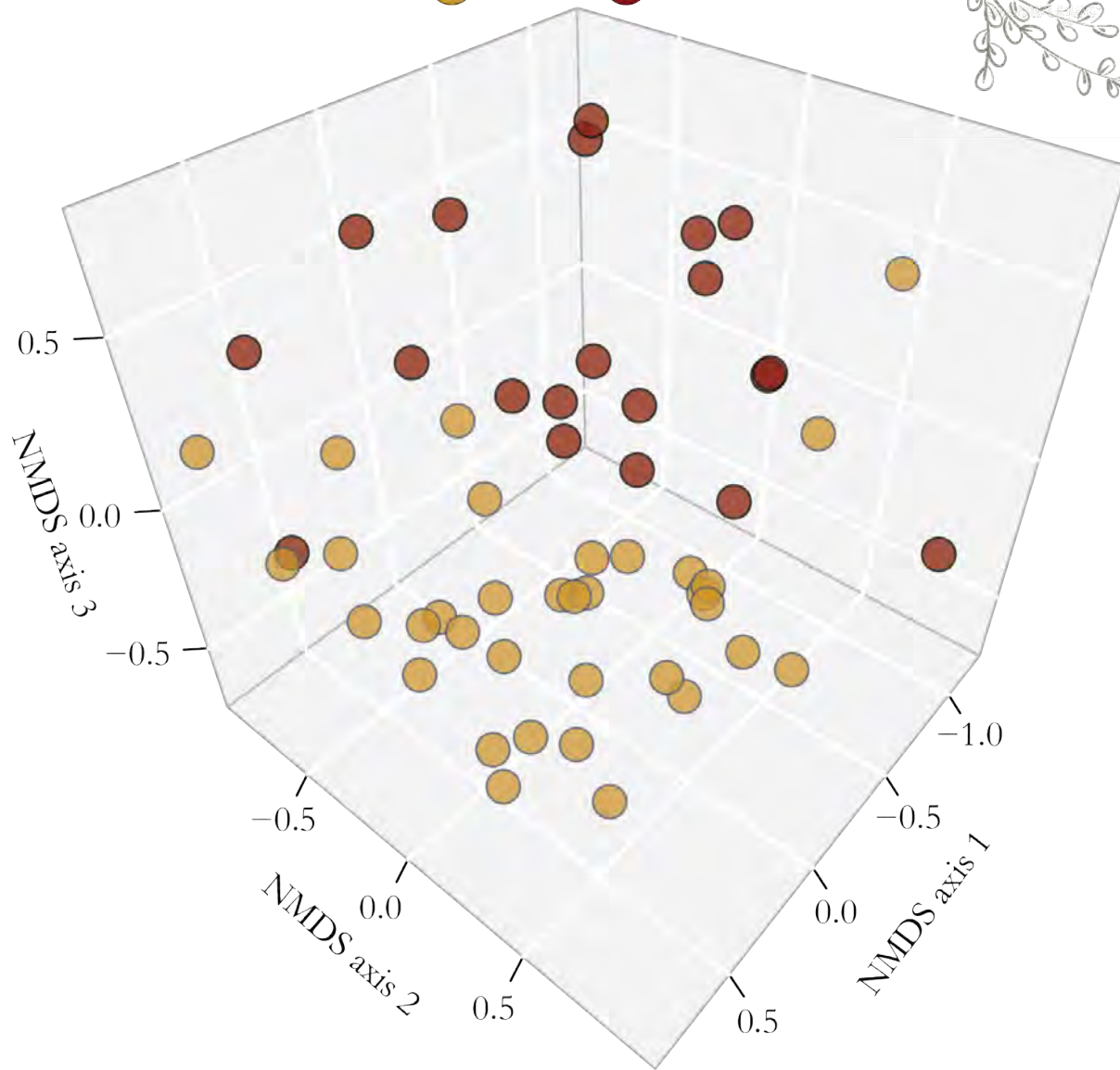
- Chemical analysis at University of Wisconsin-Madison
- Floral volatile organic compounds (VOCs)
- Flower secondary metabolites
- Leaf secondary metabolites





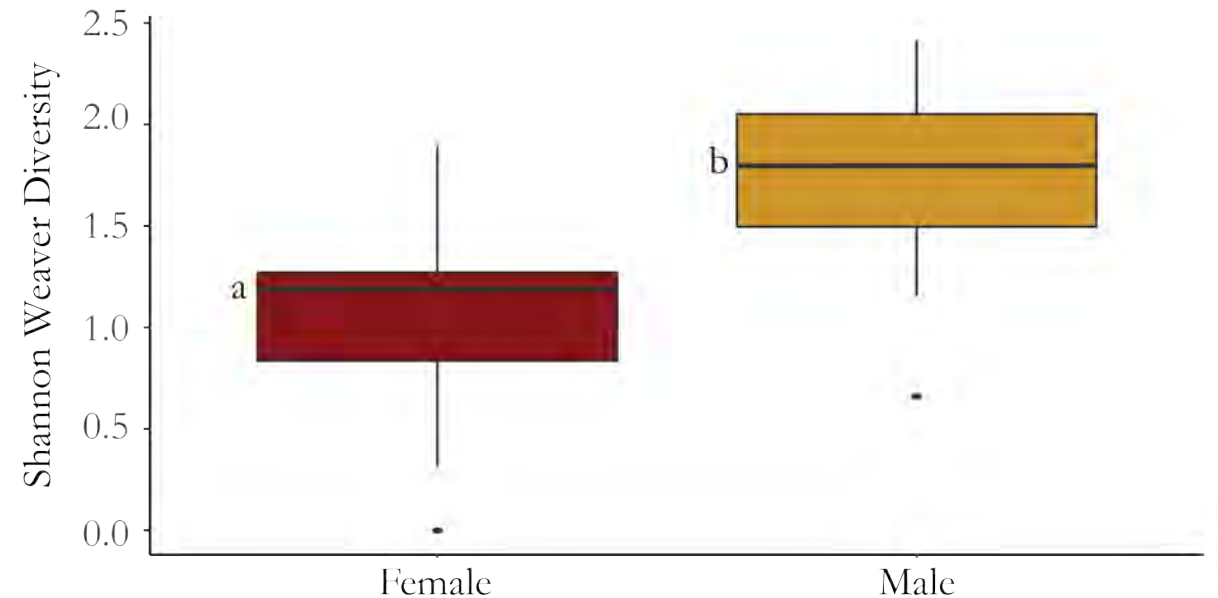
# Male and female trees assembled different floral visitor communities

Sex of tree ● Male ● Female



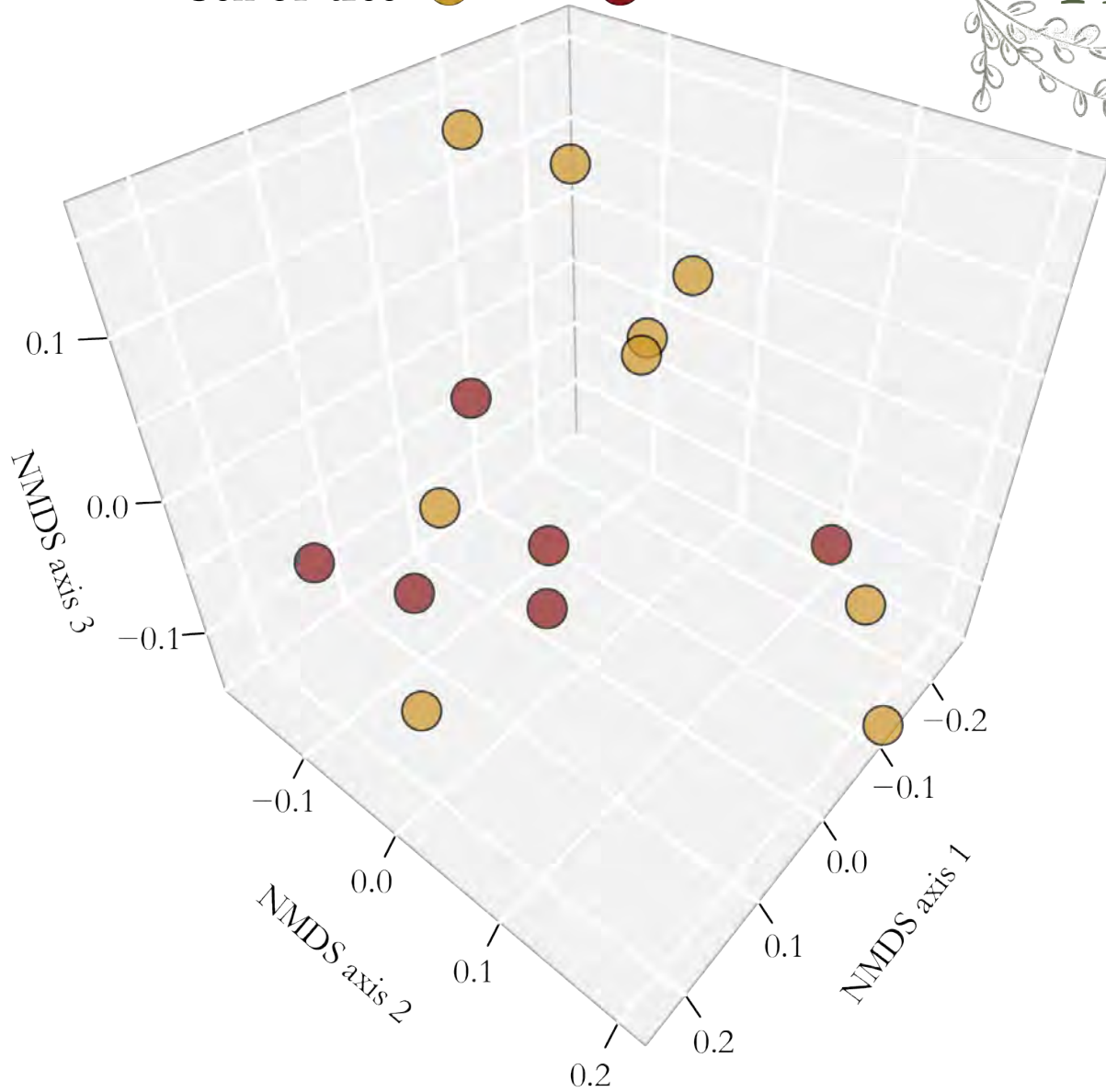
ANOSIM R = 0.301, p-value = 0.001

Male trees attracted a higher diversity of insects to their flowers when compared to female trees



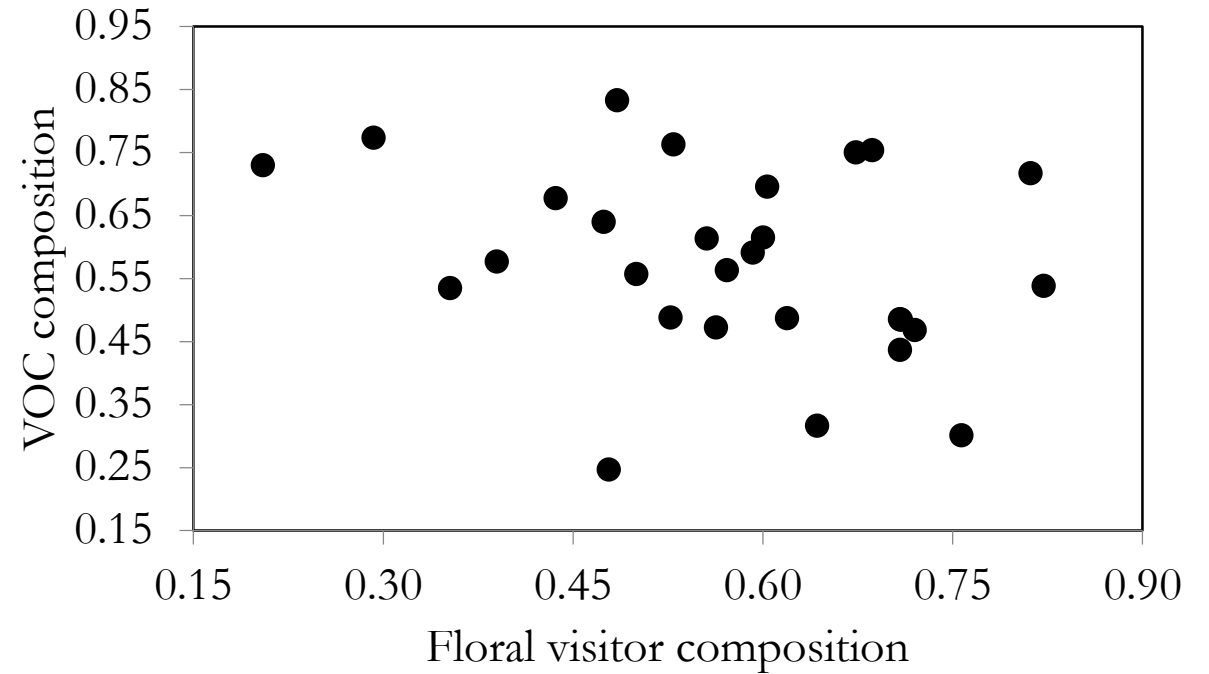
Sex of tree ● Male ● Female

# Floral scent composition (VOCs) was not different between males and females



ANOSIM R = -0.119, p-value = 0.914

There was no relationship detected between floral scent composition and floral visitor composition





**Visual cues are the most likely driver of differences  
between male and female floral communities**



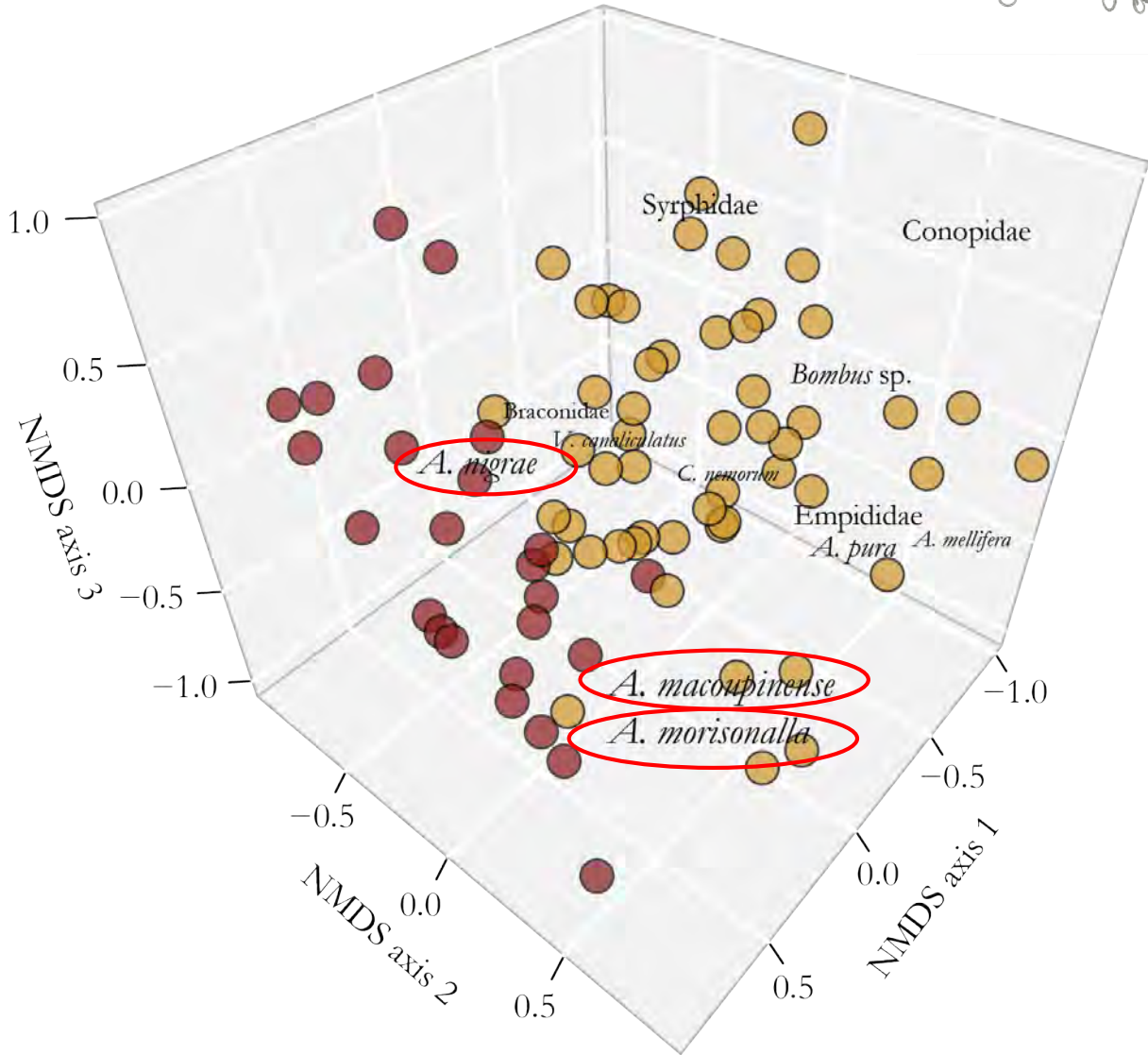
**Female flowers are green in color and only offer nectar rewards**



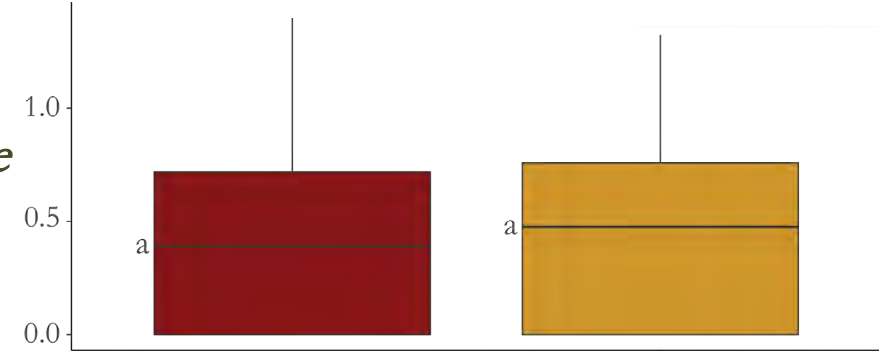
**Male flowers are yellow in color and offer both pollen and nectar rewards**

# Three species of *Andrena* were identified as the main cross-pollinators

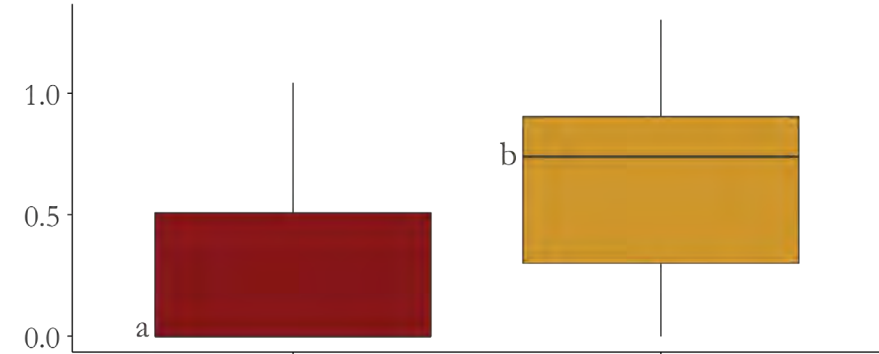
Sex of tree ● Male ● Female



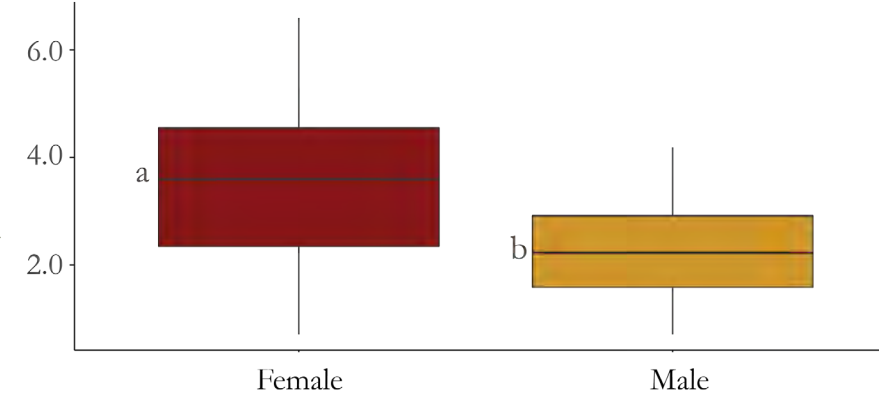
*A. macoupinense*  
WV state record



*A. morisonella*

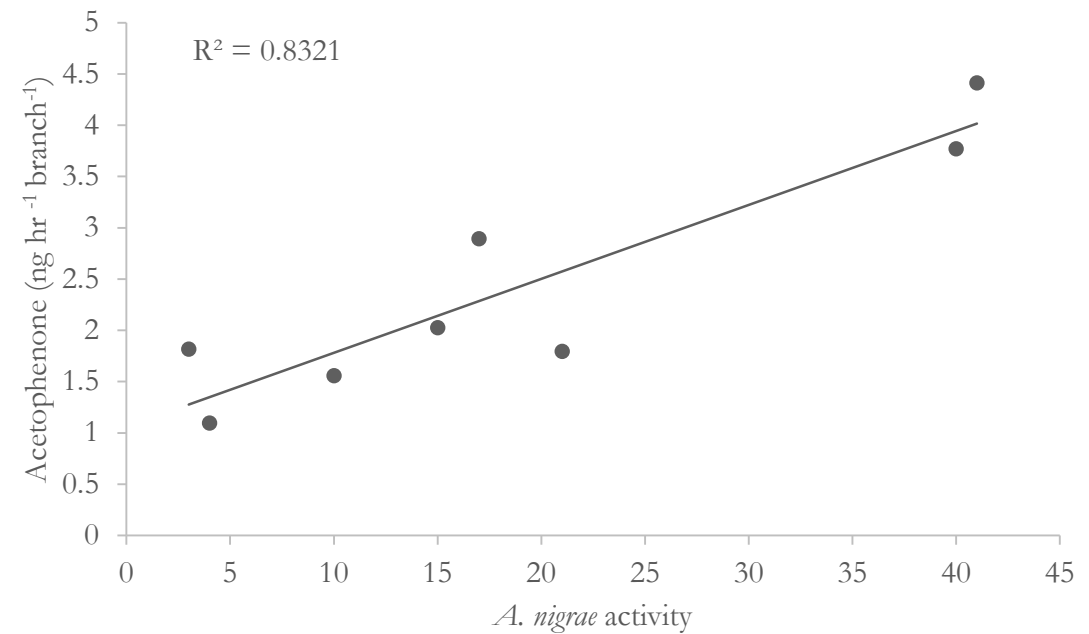
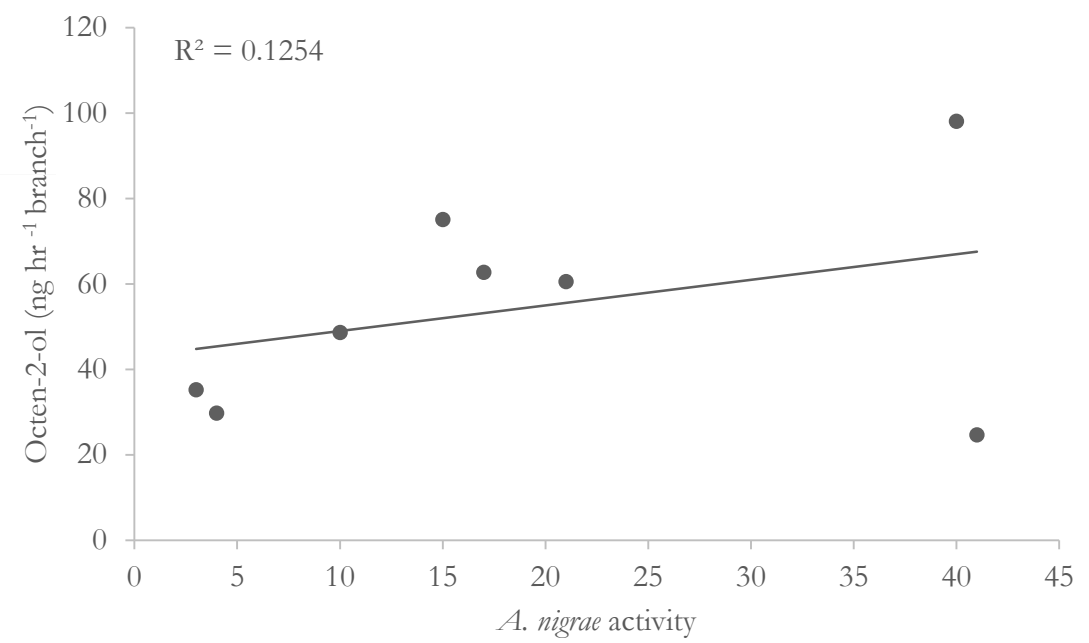
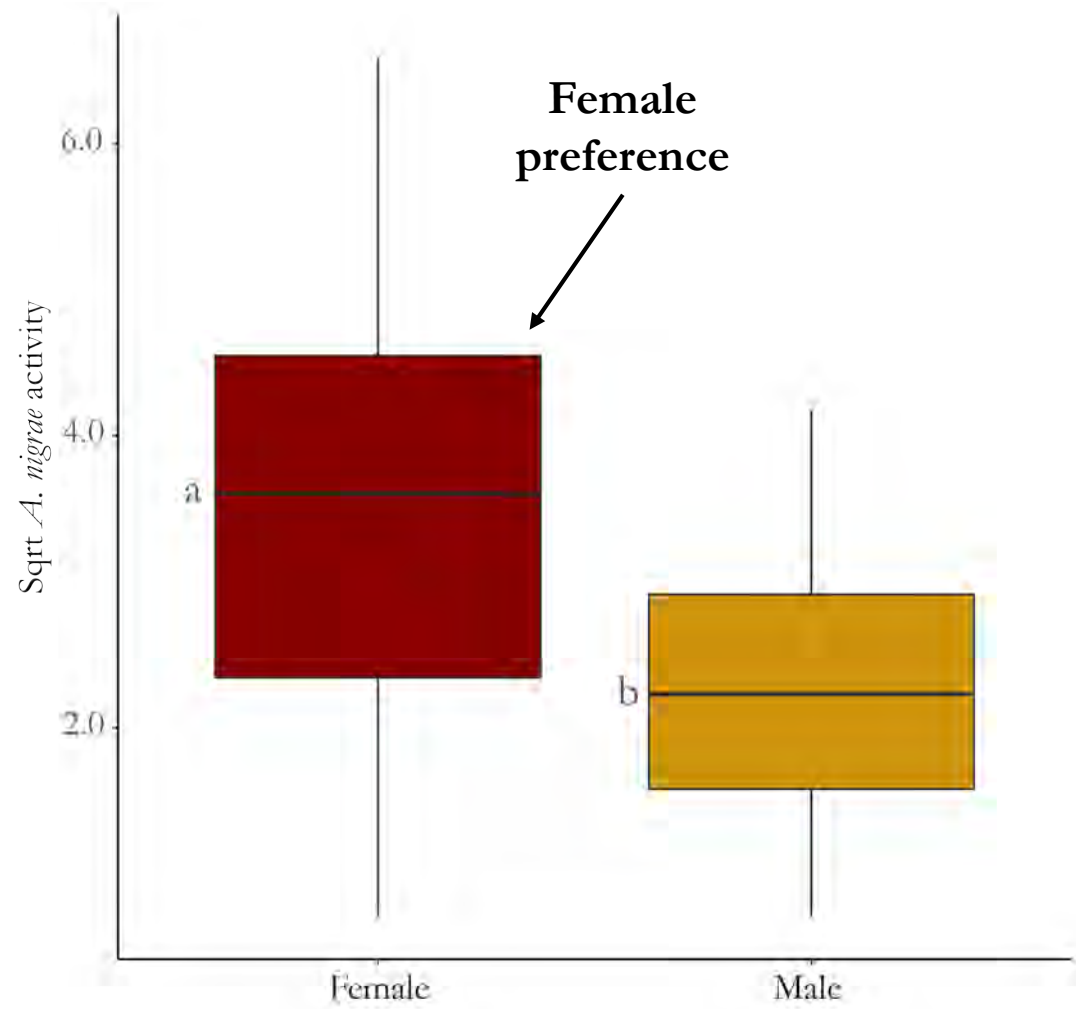


*A. nigrae*  
WV state record





# *A. nigrae* activity was positively correlated with two VOCs



# *A. nigrae* activity was lower in 2017

The bloom time of trees in 2017 was early

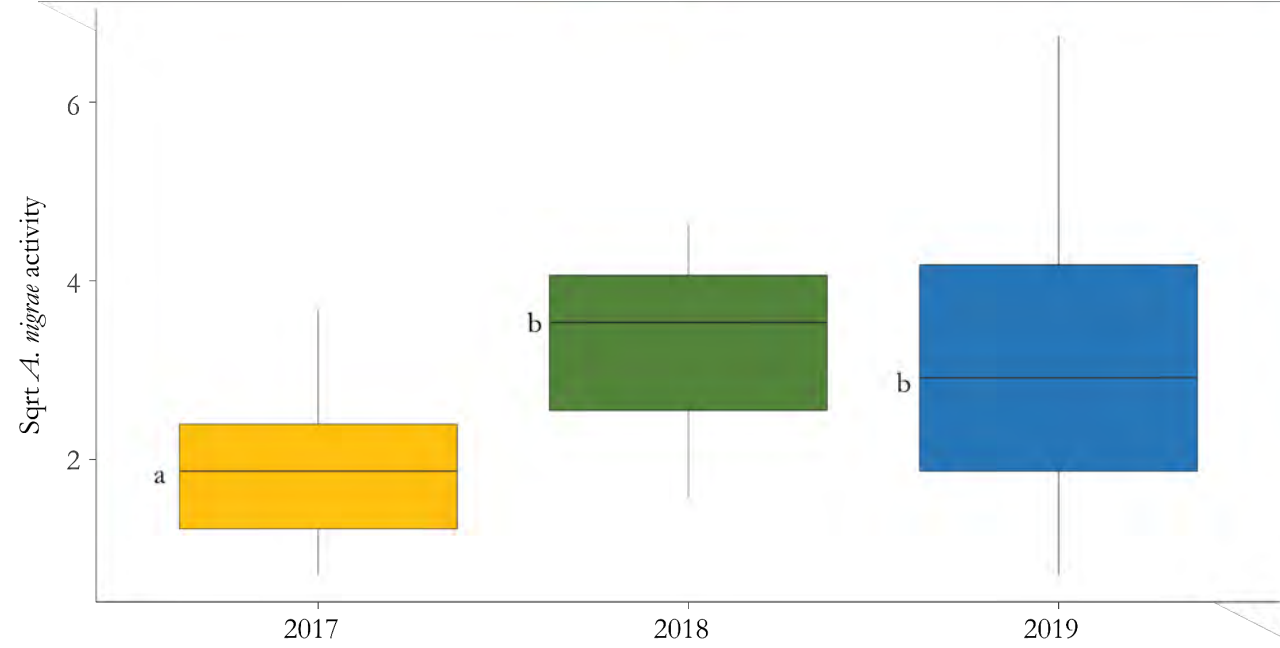
- Uncharacteristically warm weather in early March
- Cooler temperatures through April and early May

Lower native bee activity

- Decoupled bloom from population emergence
- Negative effect on volatilization of floral scent
- Inability of bees to locate host



Sam Droege





# Objective 3: Conclusions

1. How do tree characteristics influence floral visitor communities?
  - Sex of tree influenced composition; male trees had higher diversity
  - No relationship with tree chemistry
  - Visual cues are the most likely driver
2. What insects are the main cross-pollinators?
  - Three species of *Andrena*
  - *A. nigrae* positively correlated with two VOCs
3. How does survey year impact cross-pollinators?
  - *A. nigrae* activity was lower in 2017
  - Decoupled bloom from local population emergence
  - Negative effect on volatilization of floral scent



# Final Conclusions

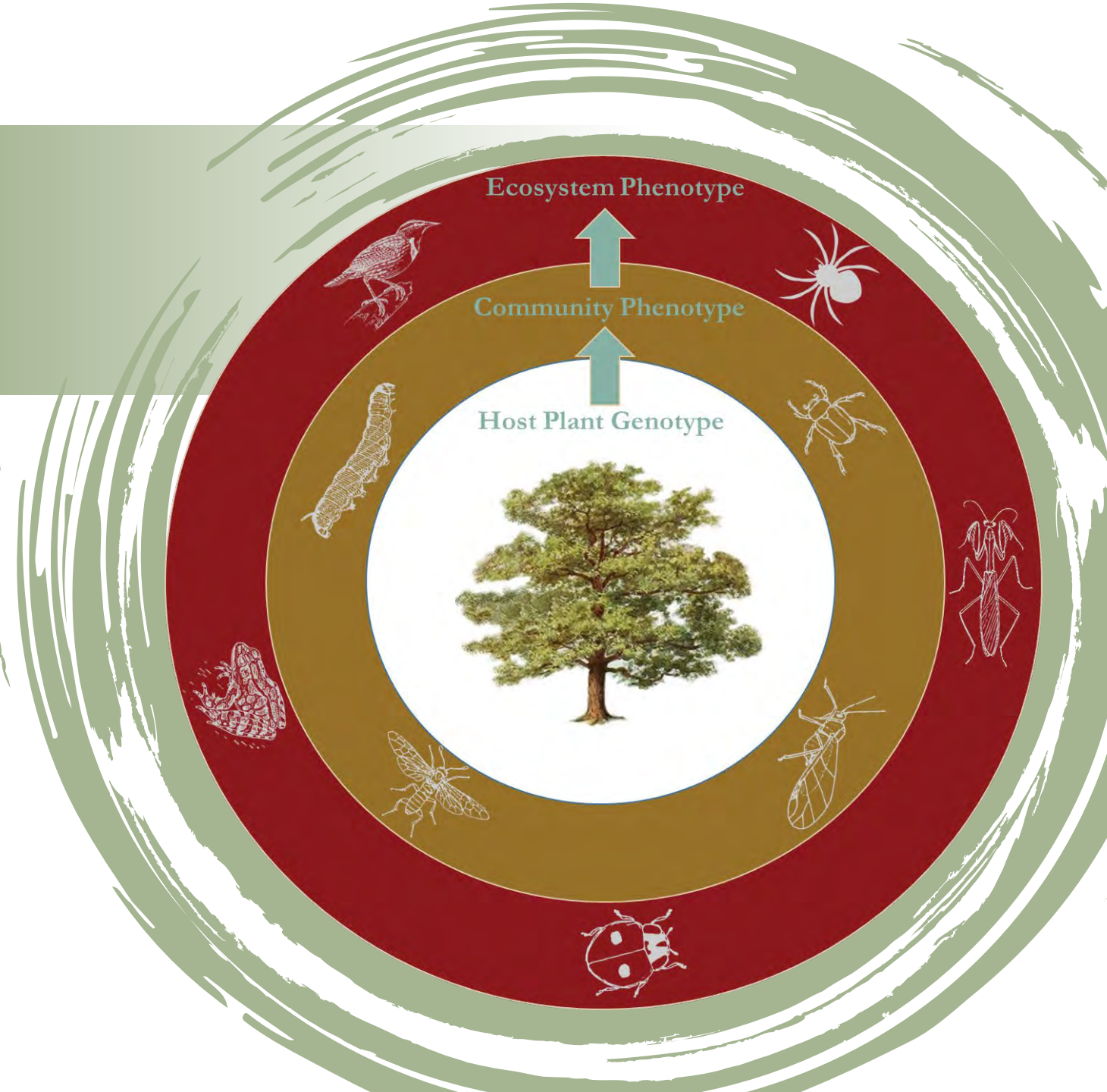
**Objective 1:** A hybrid genetic model demonstrated the importance of recent tandem duplication as a genetic signature of biotic interactions

**Objective 2:** New genetic tools are capable of detecting genes in single species populations underlying both individual insect distributions and entire community composition

**Objective 3:** Dioecy has a strong effect on floral community composition in natural populations and shifting seasonal transitions could negatively impact tree reproduction and activity of native pollinators

# Take home message

Continuing to study patterns using similar genetic tools will allow to not only be responsive to future changing conditions but also predictive, helping to better preserve our agricultural and forested ecosystems





# Acknowledgements

## Committee

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Questions?