

Prevalence of exotic and native plant food in the gut contents of *Melanoplus femurrubrum* grasshoppers: molecular confirmation of diet

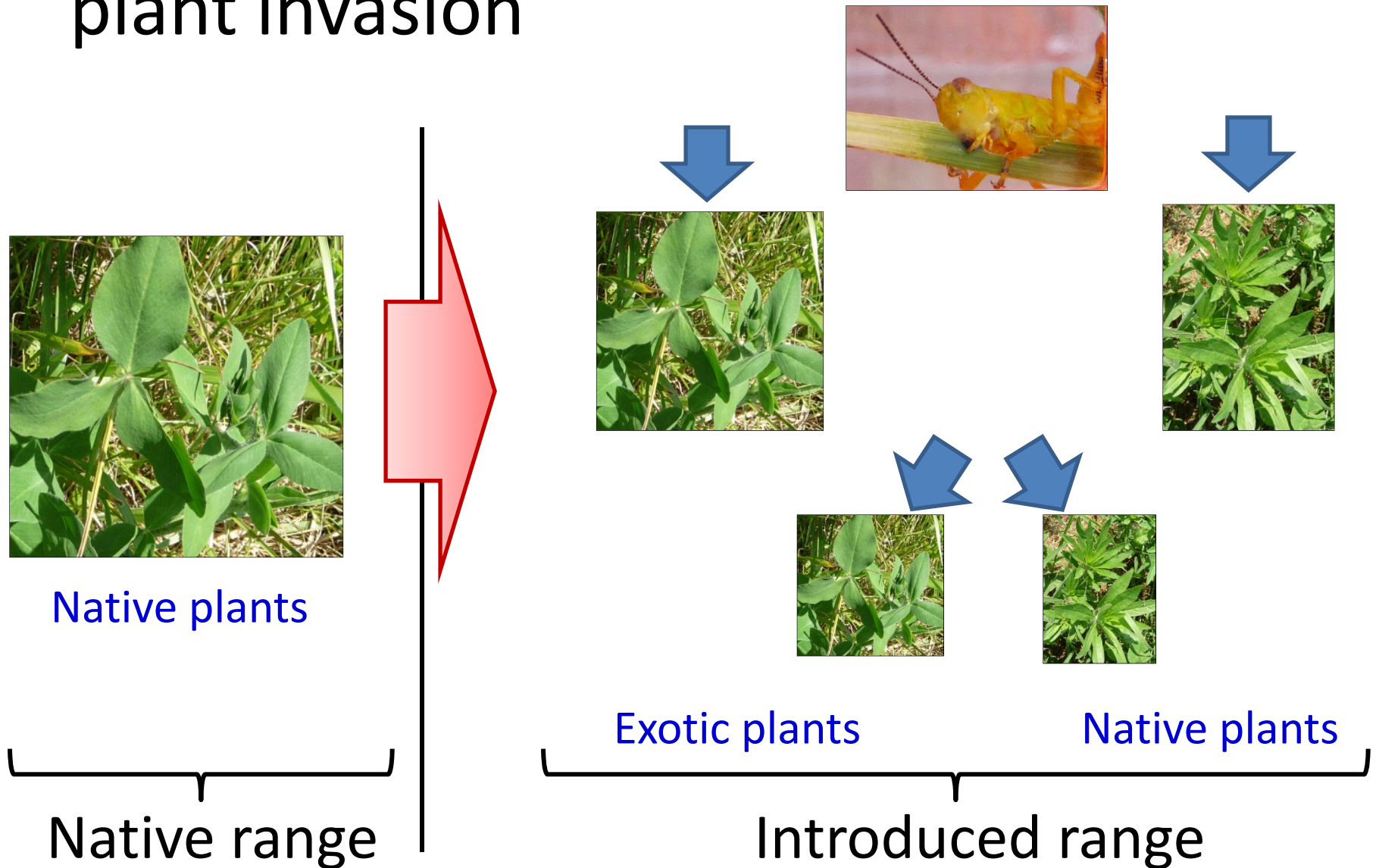
Alina Avanesyan and Theresa Culley

Department of Biological Sciences, University of Cincinnati



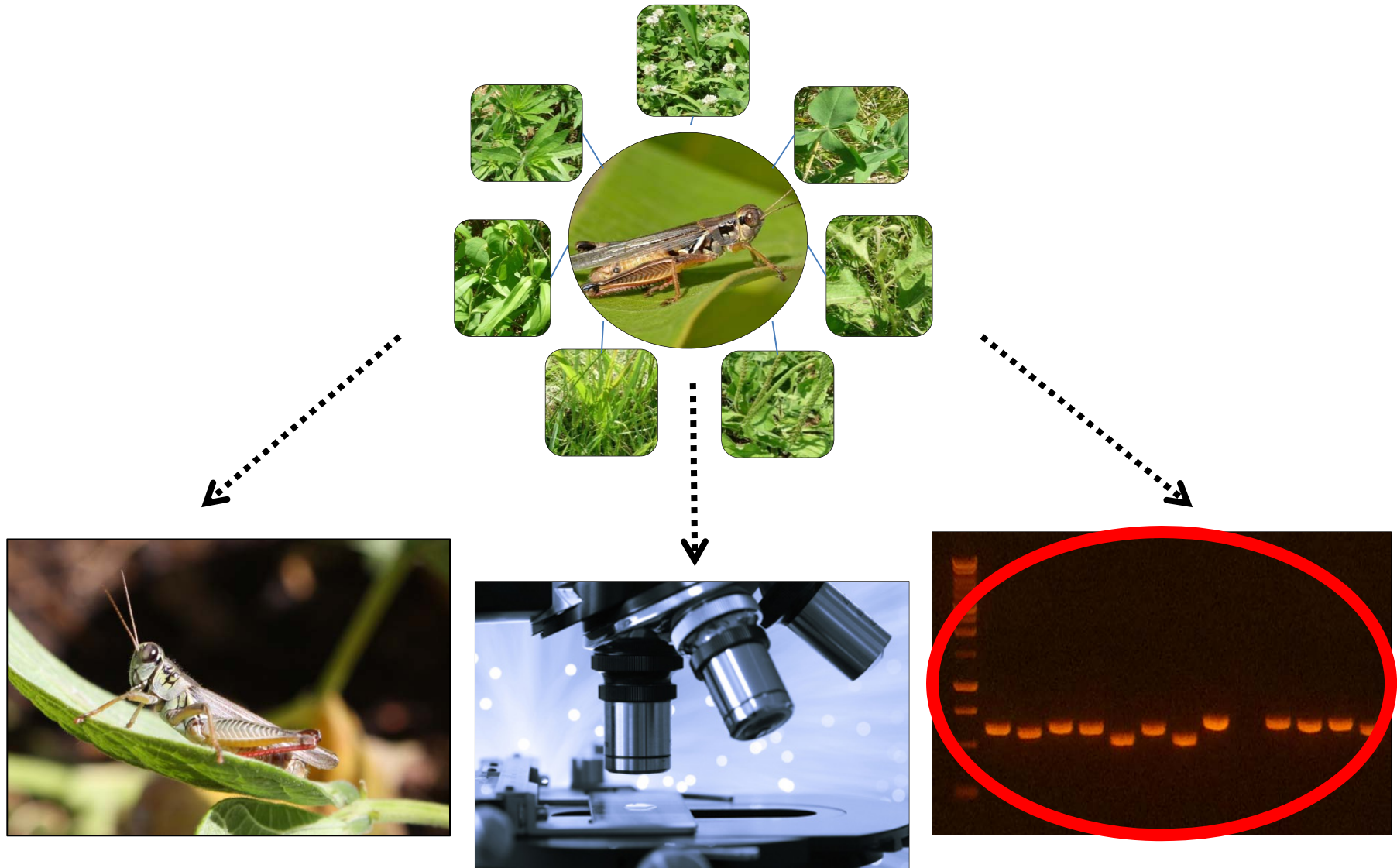
MGRS 2014

❖ Generalist herbivores might affect plant invasion



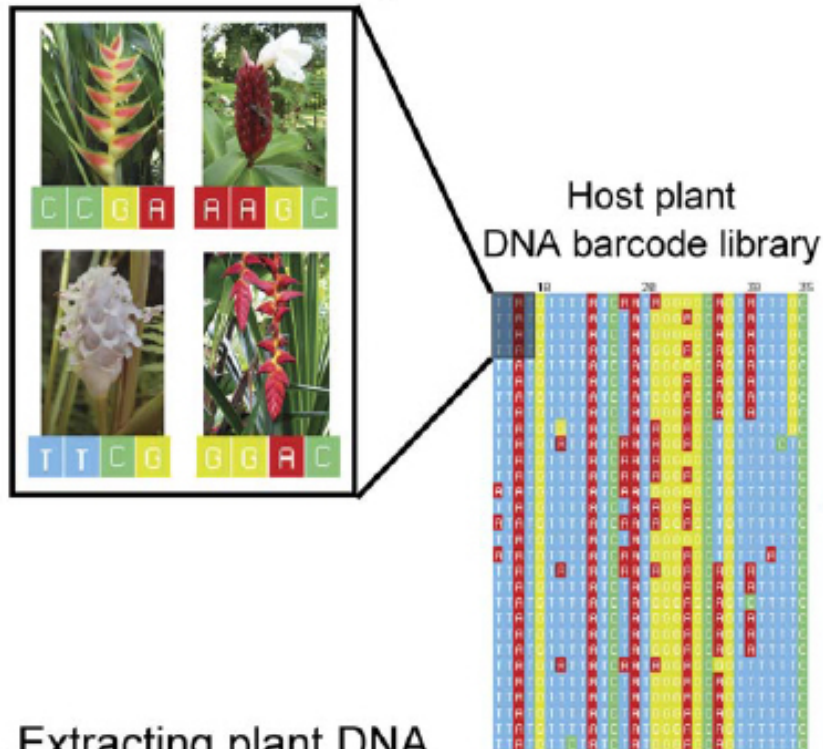
Modified from Keane & Crawley (2002)

❖ Accurate confirmation of plant food digestion is critical

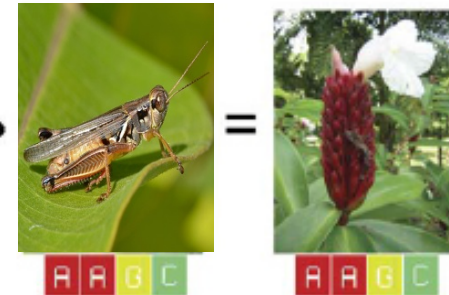


❖ Molecular confirmation of insect diet

A. Assembling a host plant
DNA barcode library



D. Matching DNA sequences
and host plant identification



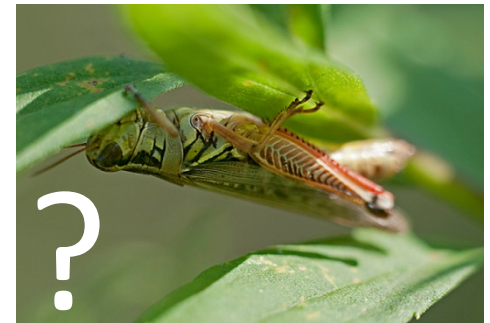
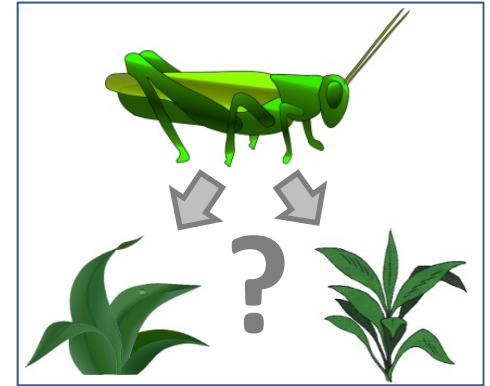
B. Extracting plant DNA
from insect herbivores



C. Comparing extracted DNA
with sequences in the
DNA barcode library

❖ Information about grasshopper gut contents is limited

- Grasshopper feeding preferences on native and exotic plants – **results from studies are mixed**
- Most of the studies have been done using field enclosures or greenhouse – **herbivory under natural conditions?**
- Studies on molecular confirmation of diet have been done for a limited number of insect species – **grasshoppers?**



Research questions/hypotheses

RQ 1. Do *M. femurrubrum* grasshoppers incorporate exotic plants in their diet?

Hypothesis 1. *M. femurrubrum* grasshoppers do not avoid exotic plants and their gut contents contain ingested exotic plants

RQ 2. If yes, do they prefer to feed more on exotic than on native grasses?

Hypothesis 2. Gut contents of *M. femurrubrum* grasshoppers contain a similar proportion of exotic plants and native plants.

Hypothesis 3. Gut contents of *M. femurrubrum* grasshoppers contain a greater proportion of exotic plants compared to native plants.

Study Sites



Cincinnati Center
for Field Studies (OH)

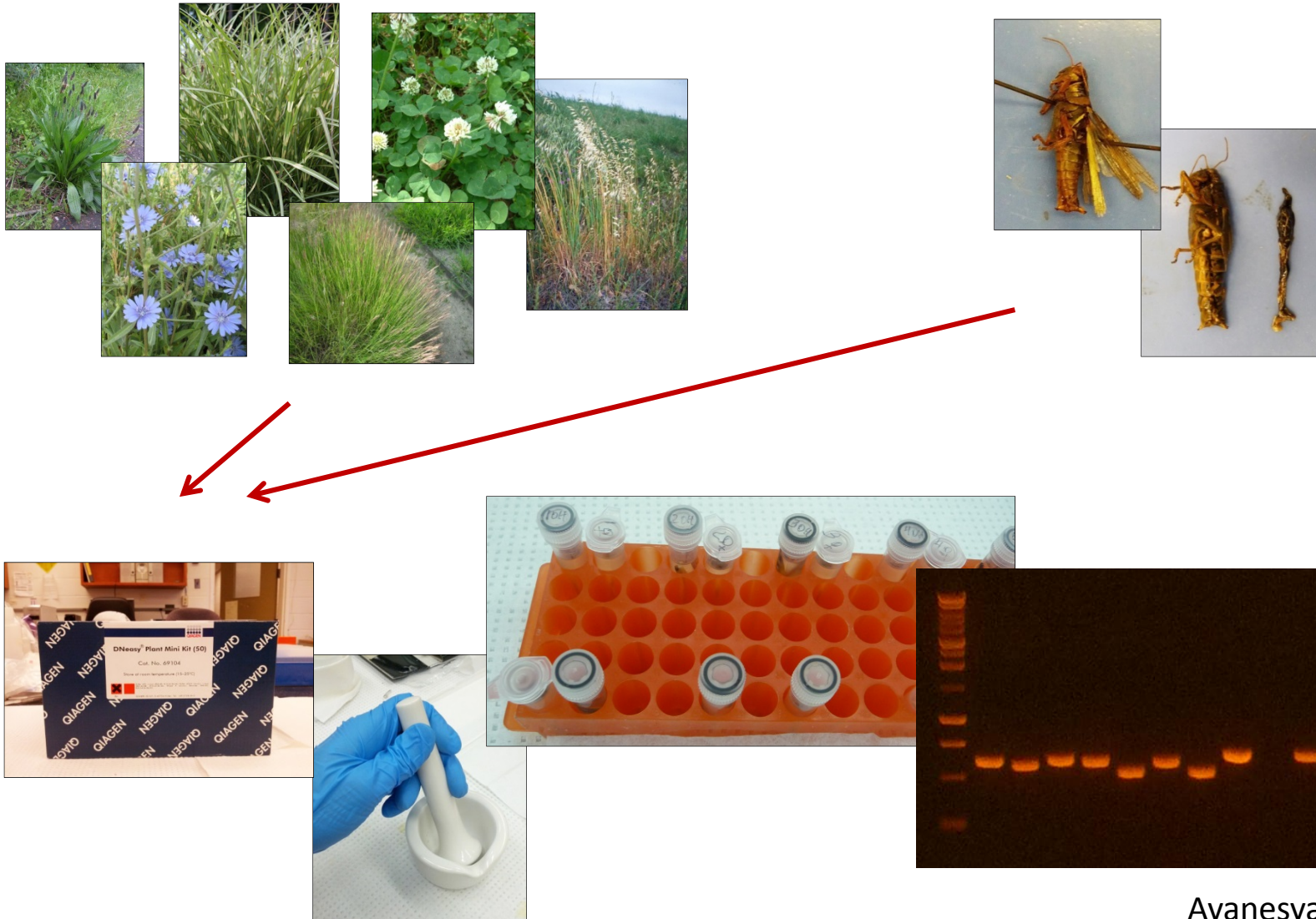


Western Maryland
Research and Education Center (MD)



Identification of ingested plants

Step 1. Amplification of *trnL* (UAA) intron



Avanesyan (2014)

Step 2. Creating a local plant reference database

The screenshot displays the BioEdit Sequence Alignment Editor interface. The main window shows a list of plant sequences on the left and their corresponding nucleotide sequences on the right. The NCBI Local BLAST window is open, showing the search parameters and the resulting BLAST output.

NCBI Local BLAST Parameters:

- Program: `blastn`
- Nucleotide Database: `Combined_MD.fas` (highlighted with a red circle)
- Protein Database: (empty)
- Query: `>I9`
- Expectation Value (E): `1.0E-10`
- Matrix: `BLOSUM62`
- Max number of hits to report: `500`
- Max number of alignments to show: `250`
- Threshold for extending hit: `0`

BLAST Output:

Sequences producing significant alignments:

Sequence	Score (bits)	E Value
Alopecurus pratensis (exotic)	1061	0.0
Lolium multiflorum (exotic)	710	0.0

Score = 1061 bits (53%), Expect = 0.0
Identities = 547/551 (99%)
Strand = Plus / Plus

Query: 3 ctaagtggtaacttccaaattcagagaaacccctggaattaaaaaggggcaatcctgagcc 62
Sbjct: 1 ctaagtggtaacttccaaattcagagaaacccctggaattaaaaaggggcaatcctgagcc 60

Query: 63 aaatccgtgttttgagaaggggttctcgaactacaatacaaggaaaggataggtgca 122
Sbjct: 61 aaatccgtgttttgagaaggggttctcgaactagaatacaaggaaaggataggtgca 120

The "Do Search" button is highlighted with a red arrow.

Step 3. Host plants identification (UCCFS, OH)

[illegible]

Host plants identification (WMREC, MD)

[illegible]

Step 4. Determining plant origin



Plant species	Origin	Grasshopper feeding choices	
		Number	%
<i>Alopecurus pratensis</i>	Exotic	4	16.67
<i>Cichorium intybus</i>	Exotic	9	37.50
<i>Lolium multiflorum</i>	Exotic	2	8.33
<i>Plantago lanceolata</i>	Exotic	5	20.83
<i>Pyrus pyrifolia</i>	Exotic	-	-
<i>Setaria sphacelata</i>	Exotic	-	-
<i>Setaria viridis</i>	Exotic	-	-
<i>Sorghum bicolor</i>	Exotic	-	-
<i>Stellaria media</i> (L.) Vill.	Exotic	2	8.33
<i>Symphotrichum novi-belgii</i>	Native	2	8.33
<i>Trifolium repens</i>	Exotic	-	-

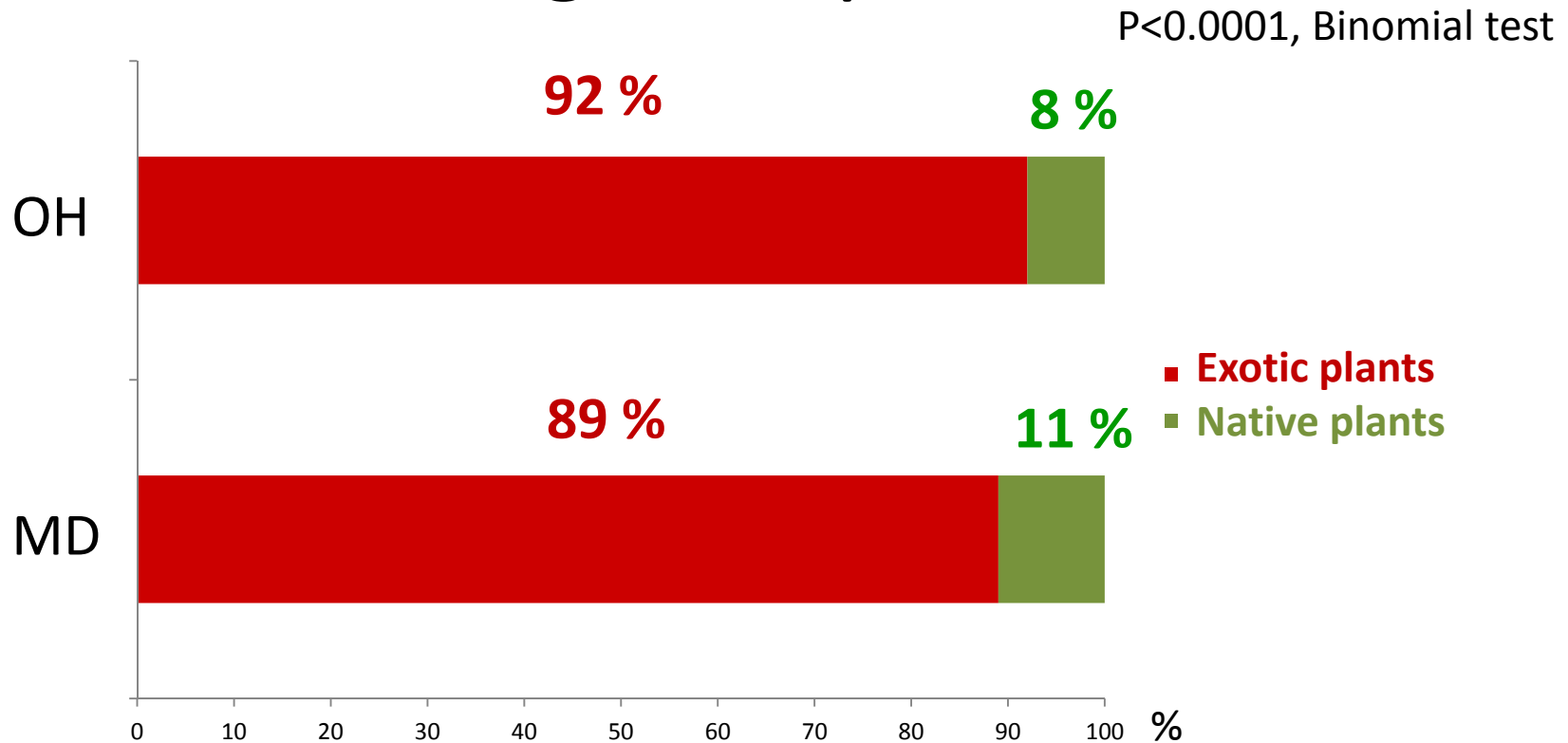
OH

Plant species	Origin	Grasshopper feeding choices	
		Number	%
<i>Allium ampeloprasum</i>	Exotic	-	-
<i>Amaranthus spinosus</i>	Native	-	-
<i>Arctium lappa</i>	Exotic	-	-
<i>Bromus arvensis</i>	Exotic	1	5.26
<i>Conyza sumatrensis</i>	Native	-	-
<i>Erigeron annuus</i>	Native	-	-
<i>Glycine max</i>	Exotic	1	5.26
<i>Hordeum vulgare</i>	Exotic	10	52.63
<i>Lamium amplexicaule</i>	Exotic	-	-
<i>Lobelia kalmii</i>	Exotic	-	-
<i>Morus rubra</i>	Native	-	-
<i>Oxalis corniculata</i>	Native	1	5.26
<i>Panicum dichotomiflorum</i>	Exotic	-	-
<i>Physalis heterophylla</i>	Native	-	-
<i>Poa pratensis</i>	Native	1	5.26
<i>Rhamnus davurica</i>	Exotic	3	15.79
<i>Setaria viridis</i>	Exotic	-	-
<i>Sorghum bicolor</i>	Exotic	-	-
<i>Veronica arvensis</i>	Exotic	1	5.26
<i>Veronica persica</i>	Exotic	1	5.26

MD

Results

Estimation of the prevalence of ingested plants



The number of grasshoppers = “number of trials”

Presence of exotic plants in grasshopper guts = “number of successes”

Conclusions

RQ 1. Do *M. femurrubrum* grasshoppers incorporate exotic plants in their diet?

Hypothesis 1. *M. femurrubrum* grasshoppers do not avoid exotic plants and their gut contents contain ingested exotic plants

Yes!

RQ 2. If yes, do they prefer to feed more on exotic than on native grasses?

Hypothesis 2. Gut contents of *M. femurrubrum* grasshoppers contain a similar proportion of exotic plants and native plants.

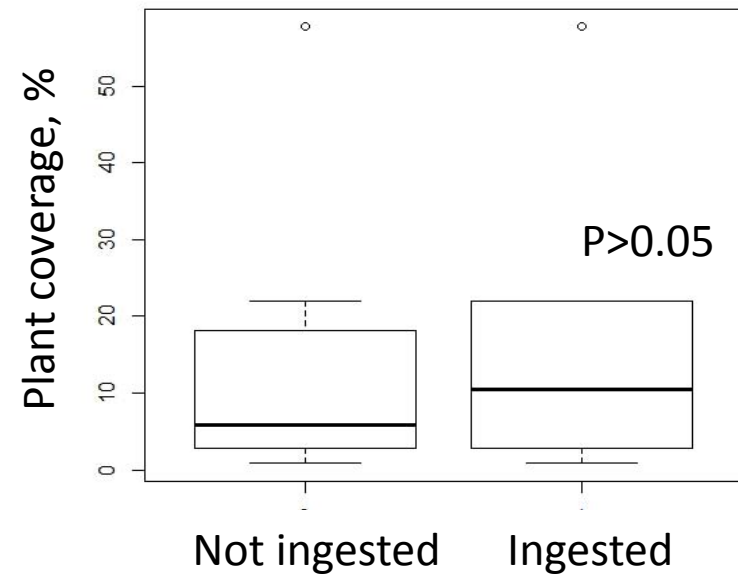
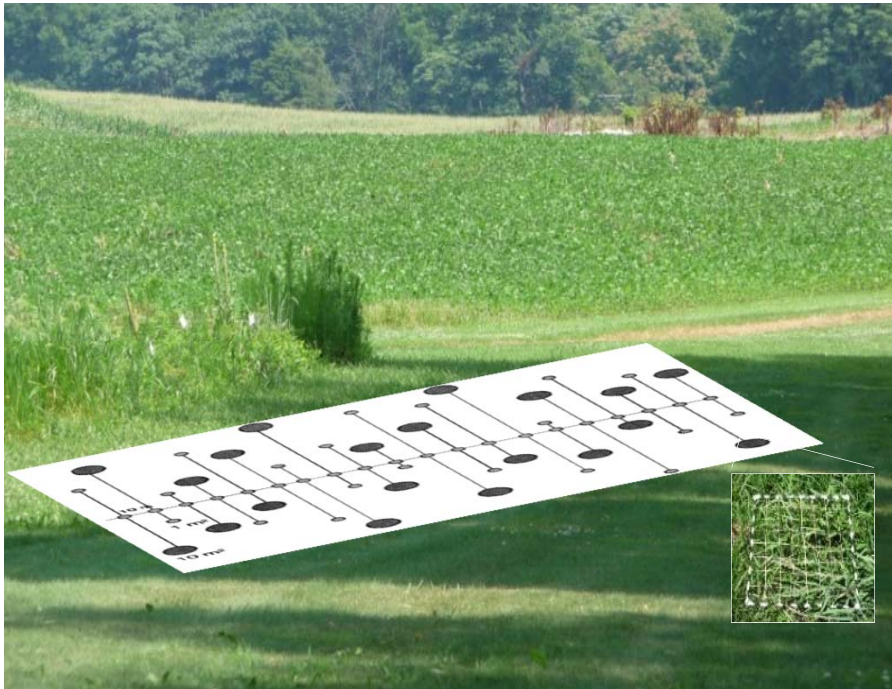
No, the plant proportion was different.

Hypothesis 3. Gut contents of *M. femurrubrum* grasshoppers contain a greater proportion of exotic plants compared to native plants.

Yes!

Future directions

- ❖ Explore whether the plant coverage (for both native and exotic plants) affects grasshopper feeding choice

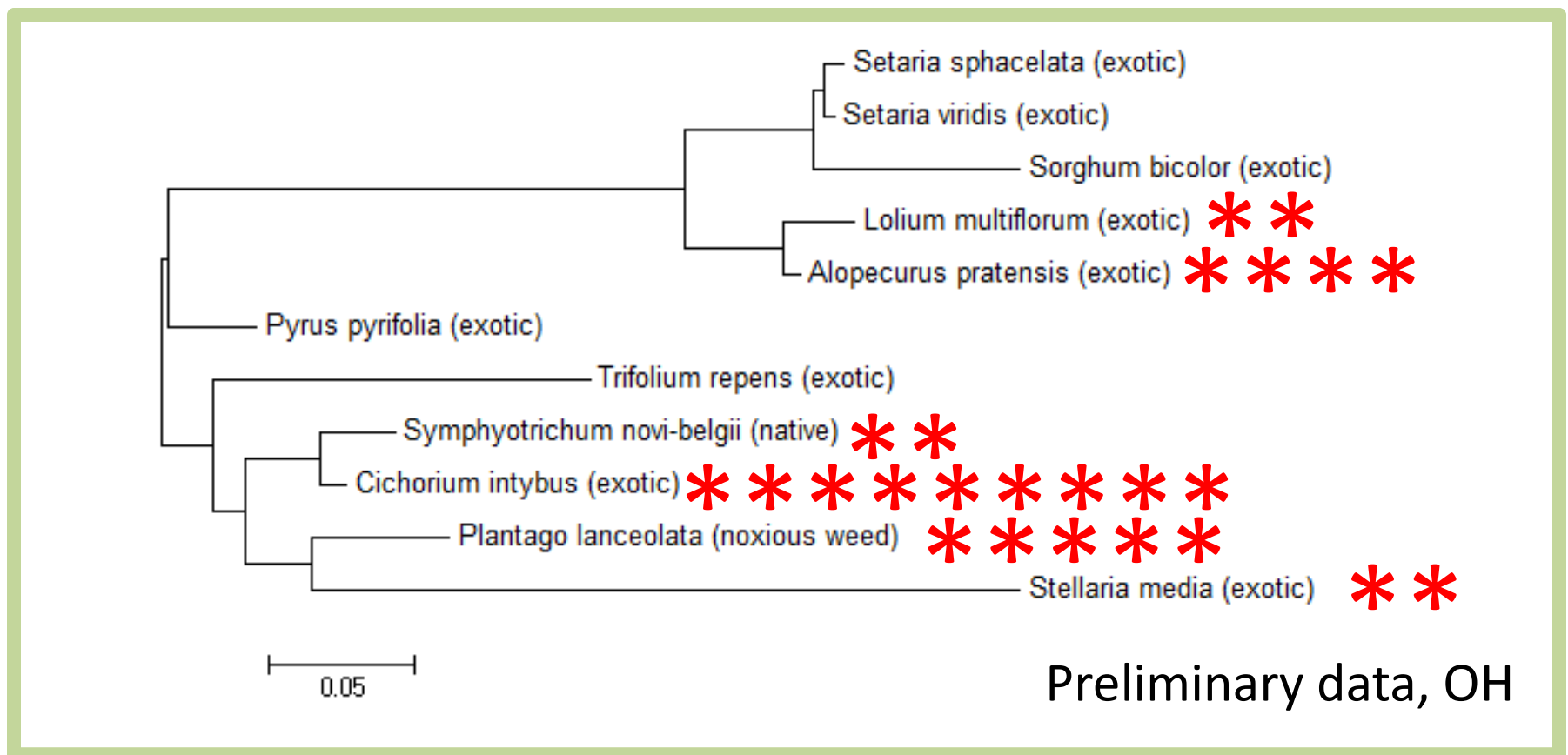


Preliminary data, OH

Native vs. exotic plants?

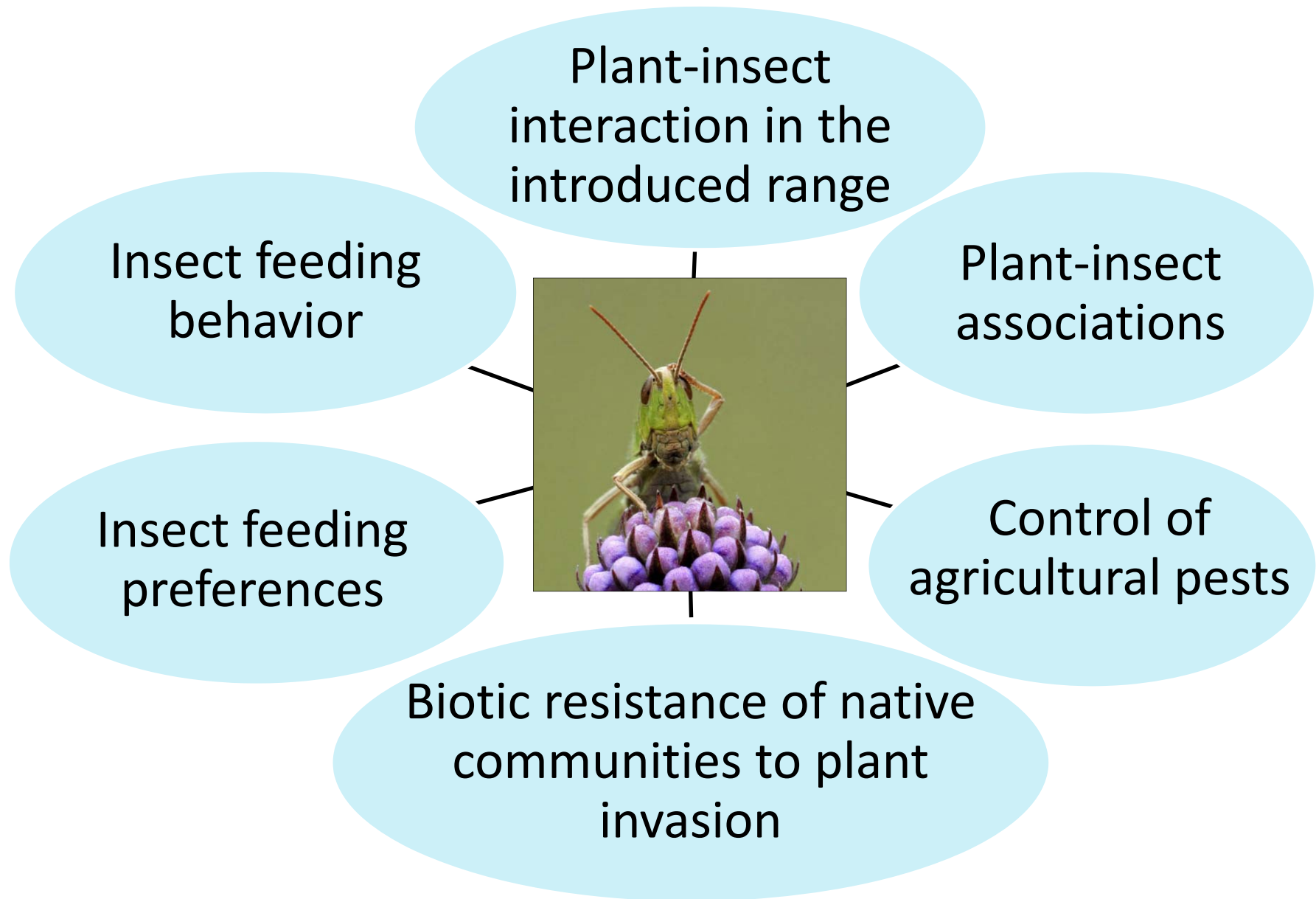
Future directions

- ❖ Explore whether the taxonomic relatedness of plants affects grasshopper feeding choice



Closely related vs. distantly related plants?

Applications of the study





Thank you!



University of Cincinnati:

Dr. Joshua Gross
Dr. Stephen Matter
Angelo Randaci
Roger Ruff
Dr. George Uetz



University of Maryland:

Tim Ellis
Dr. William Lamp

Wieman Wendel Benedict Award 2011, 2012, 2013, University of Cincinnati
Entomological Society of America 2013, ESA Eastern Branch 2013