Plant DNA detection from grasshoppers' gut contents: method and applications

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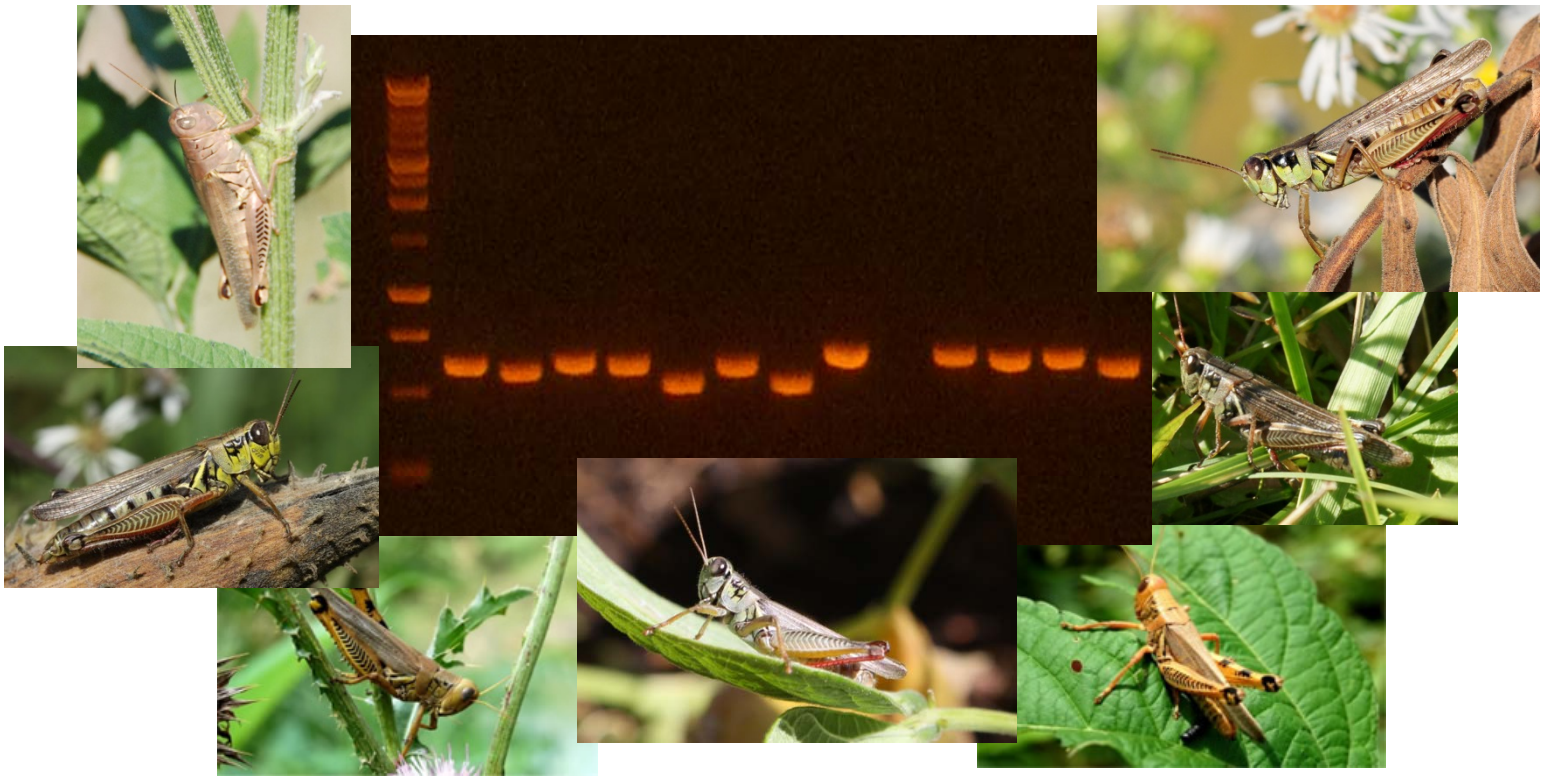
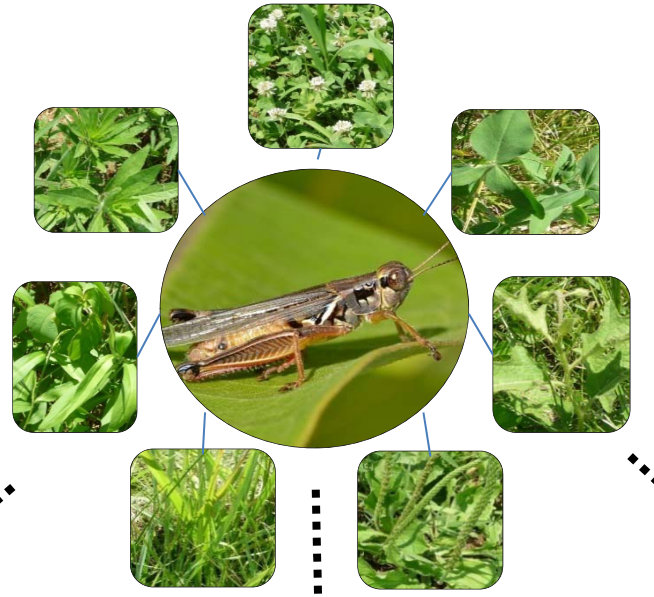


Entomology 2013

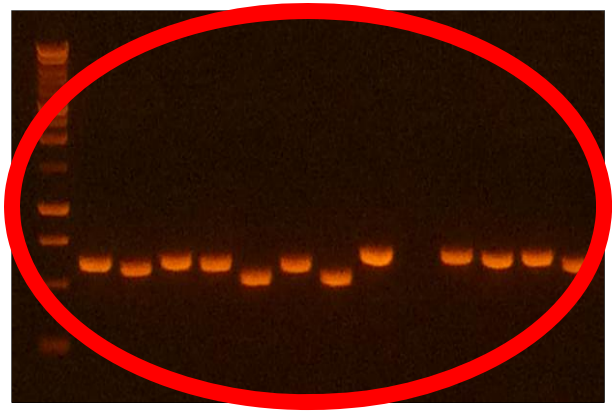
Detecting plant meals in insects guts

- understanding insect feeding preferences

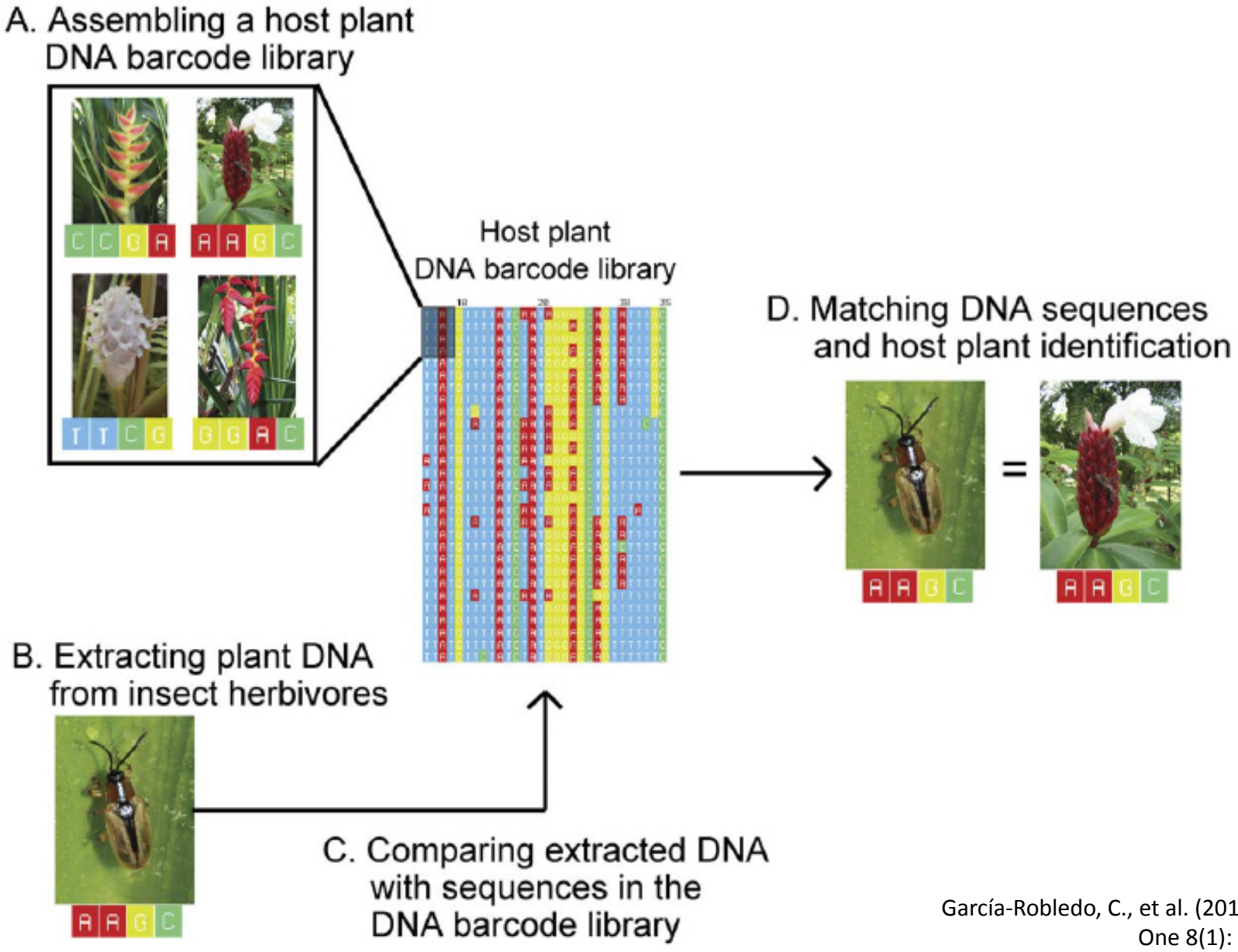


- detecting and predicting plant-insect associations

Accurate confirmation of plant food digestion is critical



Molecular confirmation of diet



García-Robledo, C., et al. (2013) PLoS One 8(1): e52967

Existing protocols for plant DNA detection within insect guts

Limitations:

- Protocols have been developed for a limited number of insect species – protocols for grasshoppers?

- Information about insect tissue preparation is limited – different size of insects?

- Plant DNA detectability in different parts of insect digestive system has not yet been described.

Research questions

1. Creating the protocol: Which region of plant DNA can be reliably detected in grasshoppers guts?

2. How long can plant DNA be detected in nymphs and adult grasshoppers of different sizes?

3. Is it possible to “follow” the plant DNA during the process of digestion?

Study species

Nymphs

Melanoplus sp.

Adults

Melanoplus femurrubrum

Melanoplus differentialis

Miscanthus sinensis

Trifolium repens

Plantago lanceolata

Cichorium intybus

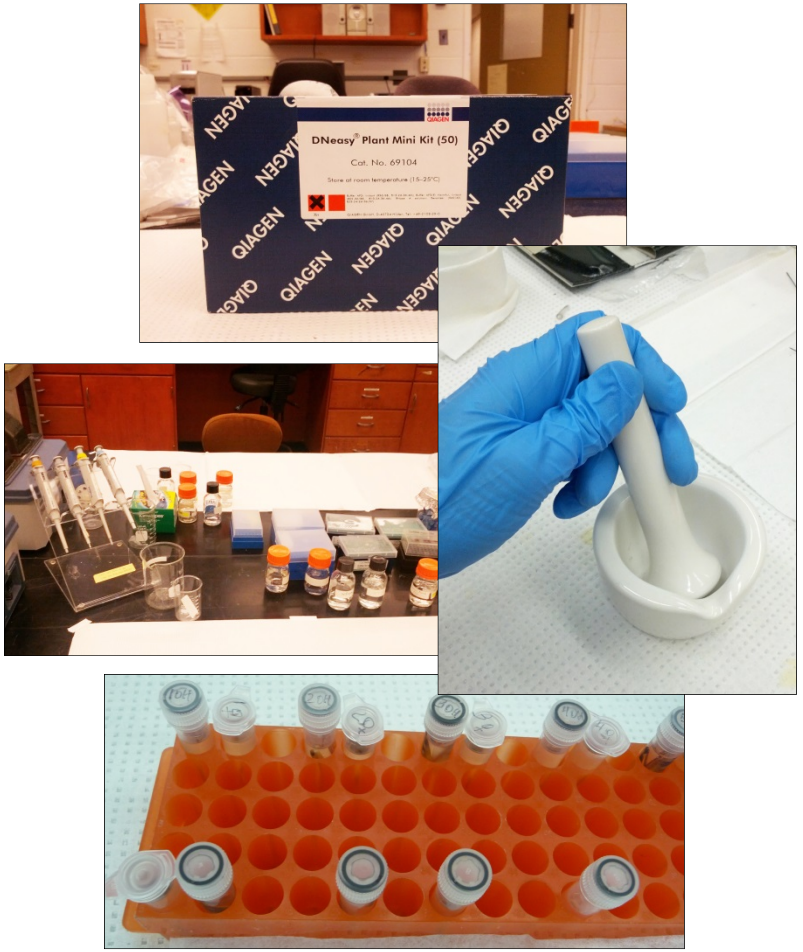
Bothriochloa bladhii

Bouteloua curtipendula

Creating a protocol for plant DNA detection within grasshopper guts

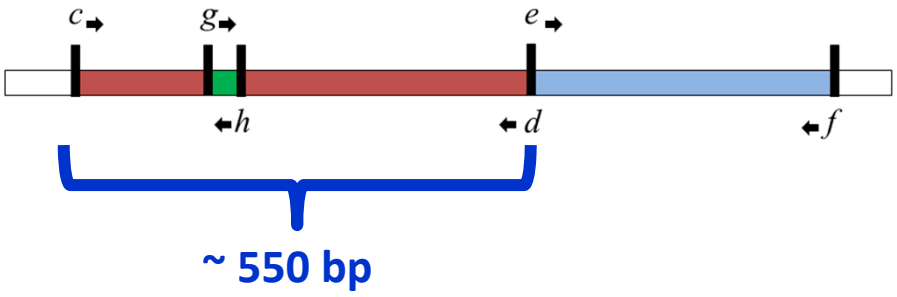
Step 1. Dissecting grasshoppers and isolating guts

Step 2. Plant DNA extraction from guts



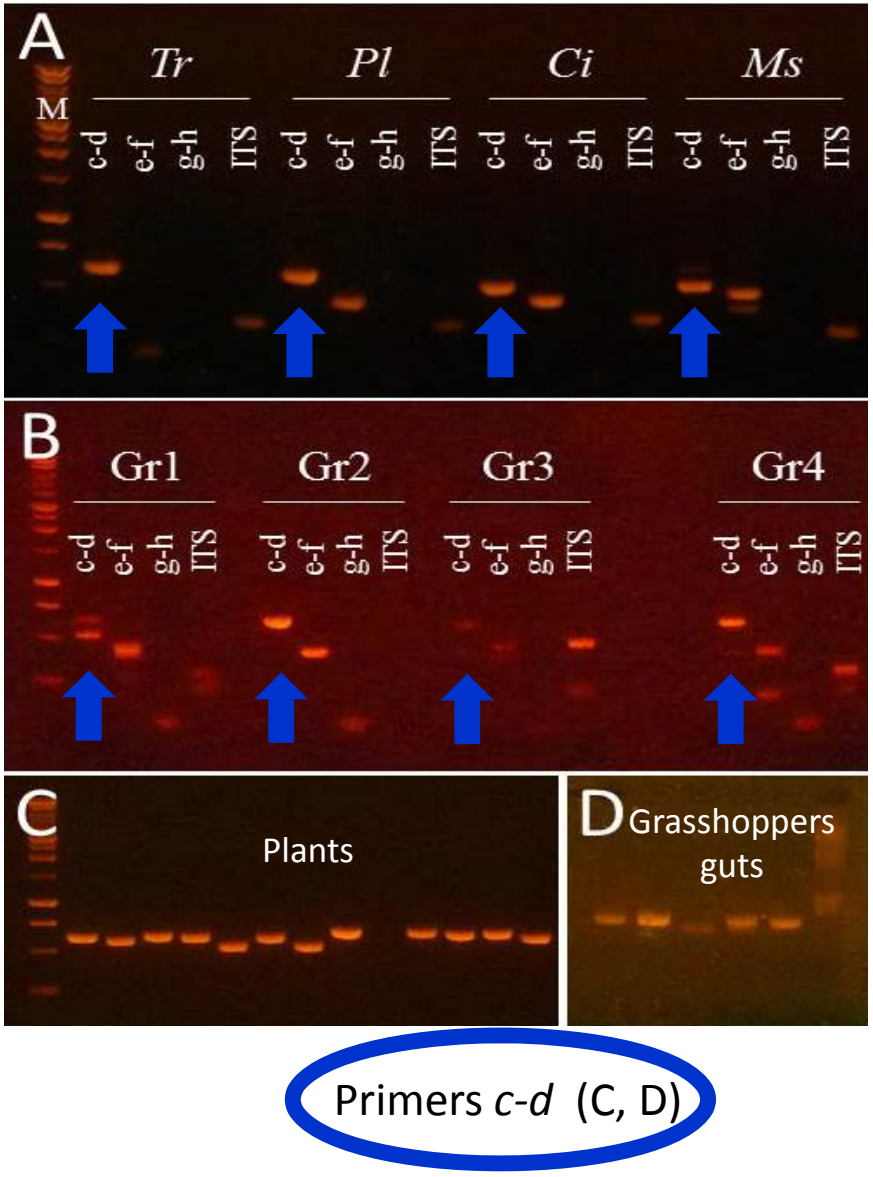
Step 3. PCR amplification: testing primers

Name	Target region	Sequence 5'-3'	bp
c	trnL (UAA) intron	CGAAATCGGTAGACGCTACG	500
d	trnL (UAA) intron	GGGGATAGAGGGACTTGAAC	500
e	trnL (UAA)3'exon/trnF(GAA)	GGTTCAAGTCCCTCTATCCC	400
f	trnL (UAA)3'exon/trnF(GAA)	ATTTGAACTGGTGACACGAG	400
g	P6 loop of trnL (UAA)	GGGCAATCCTGAGCCAA	40
h	P6 loop of trnL (UAA)	CCATTGAGTCTCTGCACCTAT	40
ITS1F	Nuclear, ITS	C TCCGTAGGTGAACCTGCGG	290
ITS2R	Nuclear, ITS	GCTGCGTTCTTCATCGATGC	290



Plants (A)

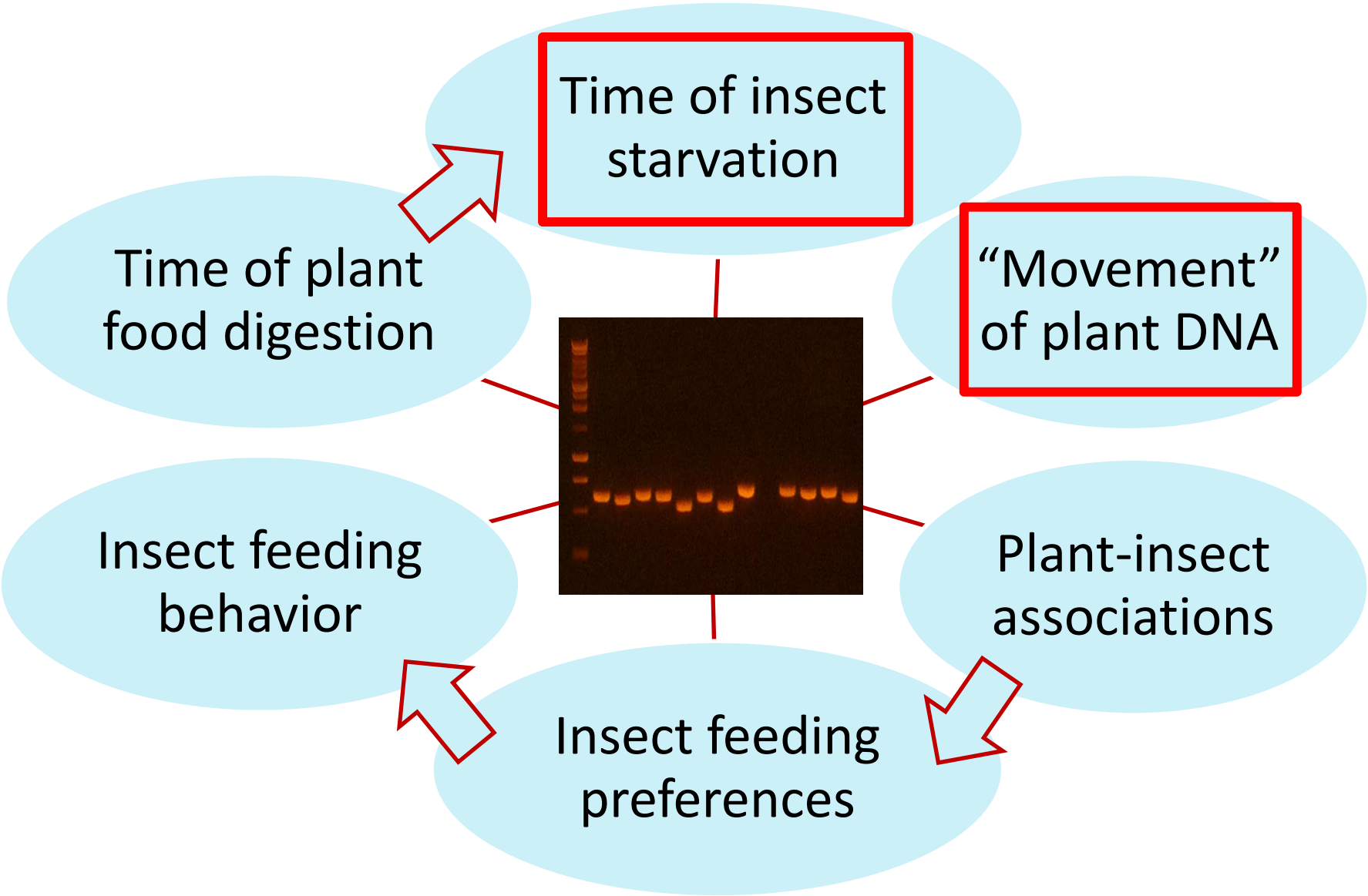
Grasshoppers guts (B)



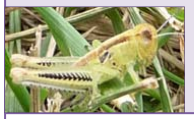
Primers *c-d* (C, D)

Avanesyan (2013), *Application in Plant Sciences – in review*

Applications of the protocol



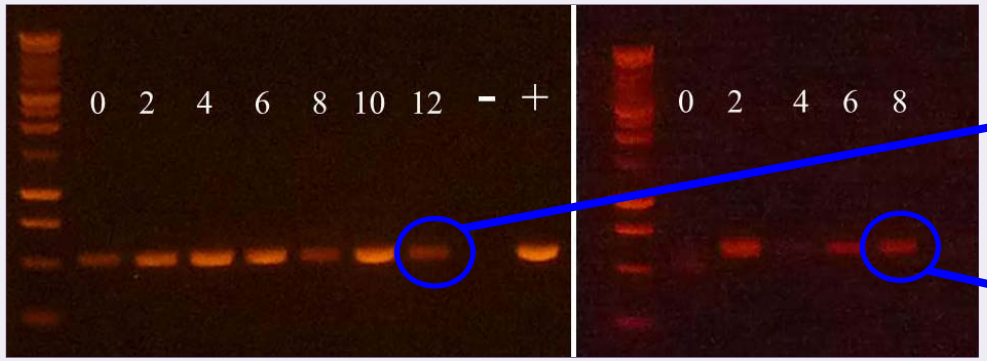
Time of starvation: How long can plant DNA be detected in nymphs and adult grasshoppers of different sizes?

Grasshopper species	Life stage	Weight (g), Mean±1SE	Type of feeding experiment	Plant species used for feeding	Total time of feeding	Tissues for DNA extraction	Time intervals post ingestion (hours)	Plant DNA detectability (hours PI)
Melanoplus spp. 	Nymph	0.11 ± 0.02	Choice	*Bouteloua curtipendula* *Bothrichloa bladhii*	3.5 h	Whole body	0, 2, 4, 6, 8, 10, 12	12 h
Melanoplus femurrubrum 	Adult	0.35 ± 0.02	No-choice	*Bothrichloa bladhii*	3.5 h	Whole gut	0, 2, 4, 6, 8, 10, 12	12 h
		0.36 ± 0.01	Choice	Plant mixture	2 d		0, 2, 4, 6, 8, 10	8 h
Melanoplus differentialis 	Adult	1.66 ± 0.27	Choice	*Bouteloua curtipendula* *Bothrichloa bladhii*	3.5 h	Foregut and [midgut+ hindgut] separately	0, 1, 3, 22	22 h

Plant DNA detectability

Nymphs *Melanoplus spp.*

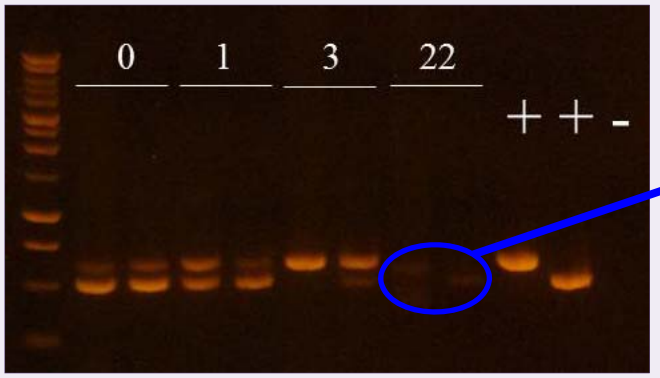
12 h PI: choice, two plants



Melanoplus femurrubrum

12 h PI: no choice, single plant

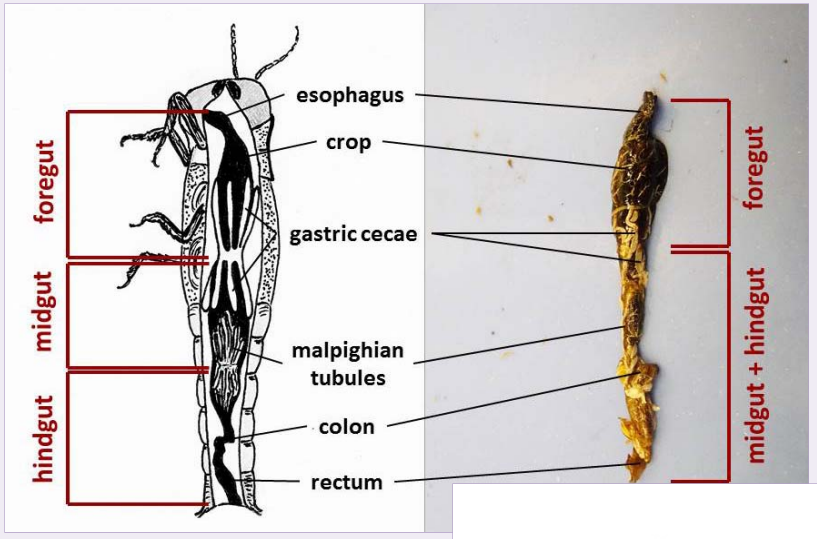
8 h PI: choice, plant mixture

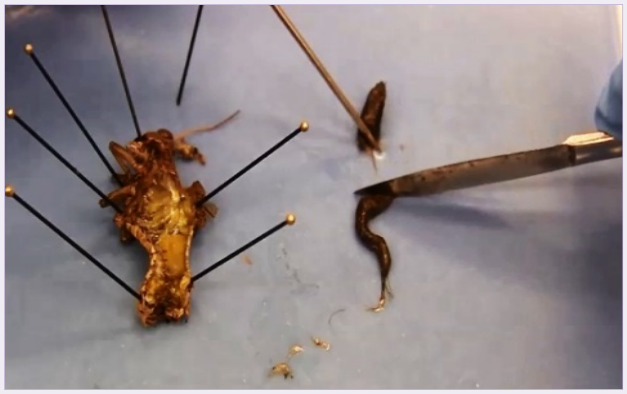


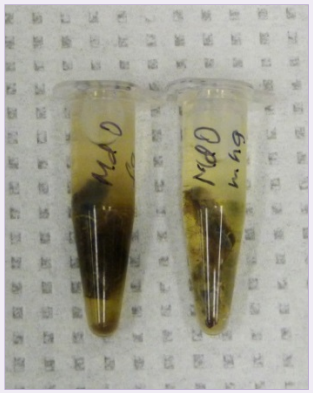
Melanoplus differentialis

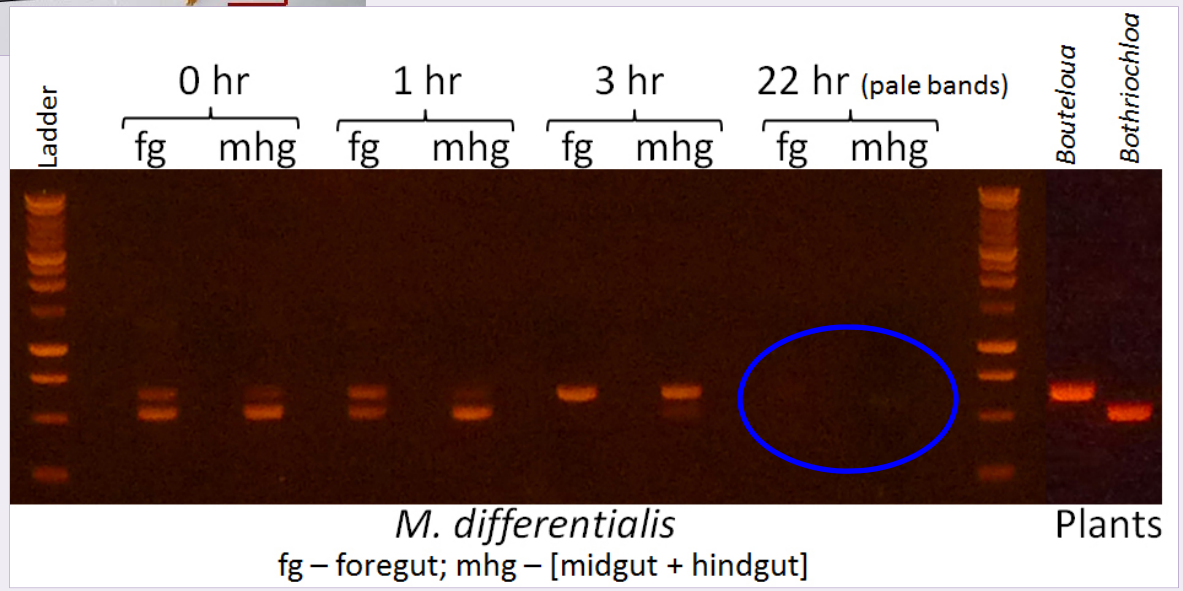
22 h PI: choice, two plants

Is it possible to “follow” the plant DNA during the process of digestion?









Conclusions

- We have developed an effective protocol for plant DNA detection from grasshopper guts: fragments (~550 bp) of the non-coding region of the chloroplast *trn*L (UAA) gene were successfully amplified.

- We have demonstrated the utility of this protocol for determining time of insect starvation before feeding experiments and for detecting plant food “movement” in an insect’s digestive system.

Future directions

Using the developed protocol:

- Determine plant-insect associations with regard to native and exotic plants

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

Thank you!

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University of Cincinnati