Applications in Plant Sciences

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Grasshoppers are what they eat

New method to extract plant DNA from grasshopper guts improves understanding of plant–insect interactions

Grasshoppers may be small, but the damages they are causing to the U.S. agriculture industry are anything but. Every year, they feed on crops and on rangelands needed for raising livestock, costing landowners millions of dollars. Although they pose a major threat, grasshopper populations play a positive role in cycling nutrients from decomposing plant matter back into the soil. A new method to investigate their feeding patterns could be the key to a better understanding of the impact of grasshoppers on plant communities.

"The main problem with current control methods is the damage done to non-target plant and insect species," says University of Cincinnati researcher Alina Avanesyan, who developed the new protocol while studying grasshopper leaf tissue consumption. "Accurately determining the feeding preferences of grasshoppers can help us to understand the magnitude of plant damage, and consequently, whether or not control of grasshoppers is needed in a given area."

The method recovers high-quality DNA of ingested plant tissue from grasshopper guts. This plant DNA offers valuable information about grasshopper diets because it holds more data than what can be observed by the naked eye. Scientists can use it to compare specific feeding patterns between different grasshopper species and uncover behaviors that might lead to intensive crop damage in certain areas. A detailed description of the dissection and DNA extraction, including a video illustrating the dissection technique, can be viewed in the February issue of *Applications in Plant Sciences* (http://www.bioone.org/doi/pdf/10.3732/apps.1300082).

According to Avanesyan, "With this protocol, a researcher can focus on a variety of research questions, such as detecting plant–insect interactions, determining how long the food has been digested, estimating the prevalence of different plants in insect guts, exploring the sequence of multiple plant species consumed, and inferring feeding preferences."

The protocol begins with a basic dissection kit used to isolate the grasshopper guts. A DNA extraction is then performed on the gut components, which results in a combination of grasshopper and plant DNA. Isolating the plant DNA involves a simple polymerase chain reaction, or PCR, which is used to amplify desired regions of genetic material for further research.

A major advantage of this method is that it can be completed in less than three hours and utilizes inexpensive laboratory equipment accessible to researchers with less funding. It also includes a new technique to divide the gut into sections, enabling researchers to track the step-by-step movement of plant matter through each gut compartment.

“We can follow plant food movement during its consumption, record the sequence of food digested (what plant was chosen to consume first) or the time needed for food digestion in each compartment, and ultimately better understand the insect food digestion process,“ Avanesyan explains. “It opens doors to a completely different research area—insect physiology.”

To demonstrate the utility of the protocol, Avanesyan successfully amplified the DNA of a noncoding region of a plant chloroplast gene and performed multiple feeding trials. Results indicated that plant tissue could be detected up to 12 hours after ingestion in nymph *Melanoplus differentialis* and *M. bivittatus* grasshoppers and adult *M. femurrubrum* grasshoppers. For adult *M. differentialis* grasshoppers, which were the largest in size, plant tissue was detected up to 22 hours post-ingestion. This information lets researchers know how to time the dissection with feeding experiments.

Findings from the gut separation technique uncovered interesting details about *M. differentialis* grasshoppers. They often did not switch between grasses during feeding, but instead consumed different plant species sequentially.

The proposed protocol is an effective, relatively quick, and low-cost method of detecting plant DNA from a grasshopper gut and its different sections. Benefits extend far beyond grasshoppers, as it can be adapted to any insect herbivores of interest. New information obtained from ingested plant DNA could ultimately lead to more targeted and sustainable methods of managing insect populations, making the new gut DNA extraction method a valuable tool for the scientific community.

“It would be great to know whether there is a difference in digestibility between native and exotic plants, which are morphologically and physiologically similar,” says Avanesyan, who plans to continue to use the protocol to investigate plant defenses against insect herbivores.

Alina Avanesyan. Plant DNA detection from grasshopper guts: A step-by-step protocol, from tissue preparation to obtaining plant DNA sequences. *Applications in Plant Sciences* 2(2): 1300082. doi:10.3732/apps.1300082.

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