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Protocol Note

Plant DNA detection from grasshopper guts: A step-by-step protocol, from tissue preparation to obtaining plant DNA sequences[1]

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- *Premise of the study:* A PCR-based method of identifying ingested plant DNA in gut contents of *Melanoplus* grasshoppers was developed. Although previous investigations have focused on a variety of insects, there are no protocols available for plant DNA detection developed for grasshoppers, agricultural pests that significantly influence plant community composition.
- *Methods and Results:* The developed protocol successfully used the noncoding region of the chloroplast *trn*L (UAA) gene and was tested in several feeding experiments. Plant DNA was obtained at seven time points post-ingestion from whole guts and separate gut sections, and was detectable up to 12 h post-ingestion in nymphs and 22 h post-ingestion in adult grasshoppers.
- *Conclusions:* The proposed protocol is an effective, relatively quick, and low-cost method of detecting plant DNA from the grasshopper gut and its different sections. This has important applications, from exploring plant "movement" during food consumption, to detecting plant–insect interactions.

Key words: grasshoppers; insect gut content; plant DNA barcoding; trophic interactions.

Knowledge of the diet of generalist insect herbivores is critical for understanding insect feeding preferences regarding different plants, as well as for detecting and predicting plant–insect interactions in natural communities. This becomes especially important when the insects of interest are agricultural pests, such as grasshoppers. Grasshoppers cause significant damage to crops and rangelands resulting in serious economic losses in the United States and worldwide. For example, in 17 western U.S. states, grasshoppers annually consume 25% of available rangeland forage, which averages about $1 billion per year (Hewitt and Onsager, 1983). Because of their important role in accelerating nutrient cycling, grasshoppers can influence plant community composition and, in particular, alter the abundance and species richness of plant species (Belovsky and Slade, 2000). Consequently, knowledge of the feeding preferences of grasshoppers can be important for control efforts and effective restoration of damaged areas (Branson and Sword, 2009).

The first step in any study on feeding preferences of insect herbivores is an accurate confirmation of food that is consumed. Among various techniques available for food identification (including direct observation, feeding trails, and microscopic gut content analysis), PCR assays have been shown to be an accurate and relatively quick method for detecting ingested plants, features that are especially important for large-scale studies (e.g., Jurado-Rivera et al., 2009; Garcia-Robledo et al., 2013). In particular, plant DNA sequences extracted from insect gut contents can provide information about insect feeding choices occurring under natural conditions, which can be hidden from direct observations of insects on plants, or may contradict feeding preferences of insects observed in laboratory feeding trials (e.g., Garcia-Robledo et al., 2013). Therefore, potentially erroneous plant–insect interactions can be corrected.

Previous studies on plant DNA detection from insect guts have been conducted on beetles (e.g., Jurado-Rivera et al., 2009; Wallinger et al., 2013), moths (Miller et al., 2006), flies (Junnila et al., 2011), and hemipterans (Matheson et al., 2008), but only Matheson et al. (2008) included one grasshopper in their study, dissecting it 4 h post-ingestion (PI). Studies that used small insects or insect larvae often obtain whole-body DNA extracts (e.g., Staudacher et al., 2011). The extraction of plant DNA from relatively large insects is complicated by the presence of excessive amounts of nontarget DNA of the herbivore; in this case, isolating the digestive system and preventing contamination of gut contents with possible plant material from the outside surface of the insect (e.g., Matheson et al., 2008) is critical for increasing the yield of target plant DNA. Grasshoppers that reach large sizes as adults are among the most important agricultural pests, with enormous economic costs (Hewitt and Onsager, 1983); therefore, information about their food consumption and, in particular, on tissue preparation and subsequent detection of plant DNA from their gut contents is much needed.

In addition, the availability of a protocol for plant DNA extraction from different parts of an insect gut has many advantages in terms of exploring new aspects of herbivore feeding, and is especially useful for insects of relatively large size. It can allow the researcher to "follow" the plant DNA during food consumption and, for example, (1) to determine the approximate time of food consumption from its location in each compartment of the insect digestive system, or in the case of mixed diet, (2) to infer the sequence of ingestion of different plant species.

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