G89 Detection of Wild Game DNA in Maggot Tissue

Jill C. Ross, BS, and John R. Wallace, PhD, Department of Biology, Millersville University, Millersville, PA 17551; and Jane E. Huffman, PhD, Northeast Wildlife DNA Laboratory, East Stoudsburg University, East Stroudsburg, PA 18301*

After attending this presentation, attendees will learn a new technique to assist conservation or game officers in the identification of illegally harvested wild game through the detection of DNA specific to several game species in maggot tissue. The attendee will learn how to rear, collect and preserve maggots, identify insect developmental stages as well as perform molecular analyses to identify non-human DNA.

This presentation will impact the forensic community as well as game law enforcement by demonstrating that entomological evidence can be useful in criminal investigations other than determining a minimum postmortem interval. To date, molecular analyses are useful to identify game species through their DNA; however, analyzing insect tissue for the presence of animal/bird DNA may provide another technique useful in wild game management and conservation.

Poaching wildlife is a problem faced by many conservation and game officers and many people are caught and convicted each year, but it is a crime that even more offenders get away with. Annually, there are approximately 1,000 big game poaching cases prosecuted. Unfortunately, many cases do not reach the court of law due to either the lack of personnel required to patrol over 26,000 square miles of forested game lands or lack of evidence required to identify the game species in question. The use of entomological evidence in human death-scene investigations in terms of estimating the minimum postmortem interval has been well documented as well as to some degree with wildlife. In addition, insect evidence can be used to differentiate between human and animal DNA through molecular analyses of the food stuffs in maggot crops. The purpose of this study was to examine maggot tissue (crop or entire body) and determine if wild game DNA could be detected using PCR analysis. The objectives for this study were to develop protocols using current PCR technology to identify and compare wild game DNA isolated from Dipteran larvae, and determine if larval developmental stage influenced the isolation and identification of wild game DNA.

Three species of forensically important flies were reared in the laboratory (Calliphoridae: *Calliphora vicina* and *Lucilia cuprina*; Sarcophagidae: *Sarcophaga haemorrhoidalis*) on approximately 350 g of deer, bear, coyote, bobcat livers. Bear, deer, coyote, fox, and bobcat livers were obtained from either euthanized animals or vehicle strikes. They were frozen immediately after removal. Fly larvae were collected at mid-molt from each larval instar, preserved in 95% ethanol and identified for species and age confirmation. After identification, maggots were individually preserved in 1.5mL of 95% ethanol and shipped in centrifuge tubes to the Wildlife Forensics Laboratory in East Stroudsburg University for PCR analysis.

Before DNA extraction, maggots were washed to remove potential external contaminants. Each maggot was individually soaked for 2 min a 1.5mL tube containing 1mL of 20% bleach. The bleach was removed and each maggot was rinsed twice with 1 mL of sterile distilled water. Each clean maggot was with iris scissors, then a ventral incision was made from the posterior to anterior end of the maggot. If possible, the crop was removed with forceps. In some circumstances, either the entire anterior inside of the maggot was removed or the entire maggot was extracted.

Amplifications were performed using Promega PCR Master Mix. Each reaction included 1 µL of each primer (5 pmol/µL) and 5 µL of DNA extract. The PCR program consisted of an initial denaturation cycle of 95ºC for 3 minutes, 45ºC for 1 min and 72ºC for 1 minute-30 seconds, then continued with 33 cycles of 94ºC for 1 minute, 45ºC for 1 minute and 72ºC for 1 minute-30 seconds, with a final extension at 72ºC for 3 min 30 s. The success of PCR reactions was determined using an agarose yield gel stained with ethidium bromide. Sequences were aligned and edited using Sequence Navigator software (Applied Biosystems). Quantitation of crop extractions showed the amount of DNA recovered varied with the species analyzed. The extractions produced at least 1.0 ng/µL. The samples analyzed produced the correct mtDNA haplotype for deer.

Maggot Tissue, Wild Game, DNA

 ** Presenting Author*