Least Invasive Method for Collecting DNA in the Field for Eastern Box Turtles (*Tarrapene carolina carolina*)

RICHARD SINGISER[2], and DIANE E. DAY[1]
[1]Department of Biology, Clayton State University, Morrow, Ga, USA
[2]Department of Chemistry and Physics, Clayton State University, Morrow, Ga, USA
[DianeDay@clayton.edu]

Research of two box turtle populations separated by a ridge in the Fayetteville, Georgia area yielded two distinct observations. Some turtles in a specific community display unusual home range areas, with no apparent environmental pressures to cause the unusual behavior. Also discovered was that the turtles display various levels of shell pitting. Both observations could have significant implications on the turtle populations. First, the large home range could be caused by a number of different influences. Second, shell pitting is problematic because some turtles can no longer effectively defend themselves against predators. This results in the turtle suffering loss of eyes, toes, and limbs. Ultimately, it is hypothesized that something on the genetic level could be contributing to these problems. To test this hypothesis, DNA from the turtles of interest must be extracted, sequenced, and analyzed. This work explores the least invasive pathway for sample extraction methods using the turtle shell (scutes) and blood. Two methods of extraction were explored: Phenol/chloroform/isomamyl alcohol (PCI) extraction and a DNA extraction kit.

The quest of extracting DNA from the turtle shell was attempted because the scute is the hardest and outermost part of the turtle and seemed to be the least invasive pathway for extraction. Harvesting a small piece of scute was simple and did not harm the turtle. Due to a scale issue, it was necessary to switch methods to a DNeasy blood and tissue kit from Qiagen. The DNeasy kit was used because it seemed to be a more sensitive extraction technique. No results were detected in the scutes because it was found that the shell contains a large amount of mitochondrial DNA and our primers were suited for nuclear DNA. Also, no DNA was detected in the scutes because the size of our tissue samples were very small (~ .01g). Because of this, extraction from blood tissue was pursued via the DNeasy kit. Blood was obtained in the field using a pair of cuticle snips and sterile collections swabs.

Hatchling #1 Sept. 3, 2015

Hatchling #2 Sept. 8, 2015