

# USING DNA FINGERPRINTING TO DETERMINE IF THERE IS A LACK OF GENETIC VARIATION AMONG BLANDING'S TURTLES (*Emydoidea blandingii*)

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## ABSTRACT

The Blanding's turtle, *Emydoidea blandingii*, is an endangered species in Wisconsin. One reason is due to their populations being highly fragmented and their population sizes being relatively small. My goal was to use DNA fingerprinting to determine if there was a lack of genetic variability in this species' population in Southern Wisconsin (both between and within the populations). A pilot study was completed first using a turtle head and extracting the DNA from it. A polymerase chain reaction (PCR) was completed next using the DNA from the head, and the resulting DNA product was resolved on an agarose gel. This same experiment was then done using shell shavings from four different Blanding's turtles to determine if their DNA was genetically similar. However, when running the results of the PCR on an agarose gel for both the turtle head DNA and the Blanding's turtle DNA, there were no visible results. This meant that a DNA fingerprint of the Blanding's turtle was unable to be obtained.

## Introduction

Blanding's turtles are an endangered species in Wisconsin. Their population sizes are small and not very mobile. One of the reasons their population is declining is due to humans having an impact on their environment. Having a road developed or buildings constructed through their natural habitat disrupts the turtle's migration and emigration from one population to another. This in turn increases isolation among neighboring populations leading to population fragmentation. Because the turtles now have a barrier between them, this causes an increase in mating within the isolated populations (increased inbreeding) and a decrease in gene flow to and from other populations. This will then lead to a decrease in the turtle's genetic variation (Coffi 1999).

One study compared the DNA sequences of Blanding's turtles in separate populations of Wisconsin. Using such molecular techniques as DNA fingerprinting can greatly help to see a genetic distinctiveness of the endangered populations (Cummings et al. 1997). Since this species of turtle is endangered, my goal is to discover how much of a genetic relationship one population has to two others living in other parts of Southern Wisconsin. My objective is to determine how closely related the Blanding's turtles are within Genesee Depot, Delavan, and West Bend by studying their genetic material using DNA fingerprinting.



Blanding's Turtles

## Methods

- A pilot study was first completed before working on the DNA fingerprinting of the Blanding's turtles. A turtle head was donated from the physiology class at Carroll College to use for the pilot study.
- DNA was extracted from the turtle head using the Qiagen (Valencia, CA) DNA extraction kit.
- A polymerase chain reaction (PCR) was completed next to amplify the DNA from the turtle head. To analyze the PCR products, an agarose gel was run at 80 volts for one hour. Because the gel was stained with ethidium bromide, the results were examined under UV light.
- PCR cycling profile:  
Initial Denaturation: 95 degrees C for 3 min.  
Denature: 94.5 degrees C for 30 sec.  
Anneal: 56 degrees C for 30 sec.  
Extend: 72 degrees C for 30 sec.  
Final Extension: 72 degrees C for 5 min.  
Chilled: 4 degrees C
- For my experiment, shell shavings were obtained from four Blanding's turtles in Southeastern Wisconsin. Two of the Blanding's turtles' used for this experiment came from Genesee Creek, one from Maitland Ridge, and the fourth from Cedar Lake. The DNA from the shell shavings of the Blanding's turtles was extracted from the same kit (and procedure) that was used with the turtle head (DNeasy Tissue Handbook, 2004).

## Results

For my pilot study, DNA was successfully extracted from the turtle head. After running an agarose gel, the DNA was clearly observed as a solid pink band. If there had been pink streaks, this would have meant that the DNA had been degraded or had not been extracted properly. When using the primers (Crim et al., 2002) (Table 2) for the polymerase chain reaction (PCR) with the DNA from the turtle head, I was unable to isolate any DNA bands.

My actual experiment using the Blanding's turtles' shell shavings did not work successfully. When running an agarose gel of the DNA extraction of the shell shavings from the Maitland Ridge Blanding's turtle, nothing showed up in the gel lanes. A PCR was done to determine if a small amount had been extracted and just not visible on the agarose gel. However, when an agarose gel was run using the results of the PCR, but still nothing showed up in the lanes.

Table 1 PCR primer sequences used for both the turtle head and Blanding's turtle shell shavings DNA samples

Annealing Temperature (Degrees Celsius)	Primer Sequence	Allele Length (bp)
30	TCCTTAAAGTGATCCCTGTGAGTCC CAGTAGTTCGAGTTCATGTGTTCA	224-252 Expected
30	TCCTTTGACATGATGTCAGGAGTTG ATTGTTATAGCTTATTGTCAGGA	348-354

Table 2 Variations of the annealing step of the PCR for the turtle head DNA samples

Date	Step	Temperature (degrees Celsius)	Time (seconds)	Number of Cycles
2/11/05	Anneal	60	60	35
2/24/05	Anneal	56	30	30
2/26/05	Anneal	50	30	30
3/10/05	Anneal	45	30	35
3/18/05	Anneal	56	30	35
3/28/05	Anneal	45	180	35

\*Time Changed MgCl<sub>2</sub> amount to 1.5 microliters



## Discussion

I was able to successfully extract the DNA from the turtle head for my pilot study. However, I was unable to generate large quantities of the specific DNA sequences desired using the selected primers. One reason might have been due to the amount of MgCl<sub>2</sub> added into the PCR reaction. Another reason might have been due to the fact that I was working with Blanding's turtles, *Emydoidea blandingii*, and the primers I used came from an article where the researchers were working with a leatherback turtle, *Dermochelys coriacea* (Crim et al., 2002). These primers (Table 1) may have been species specific and may not have worked with the DNA found in the turtle head or Blanding's turtles that I was working with. Another factor may have been the annealing temperature. Variations of the annealing step of the PCR are described (Table 2).

Although there were some light pink areas found on the agarose gels after running a PCR, these areas being DNA bands were quickly ruled out when a control was run without the Taq polymerase and DNA added. The control was found to have the same pinkish area as that of a PCR with Taq polymerase and turtle head DNA. The nucleotides from the reaction were evident, but no DNA bands appeared when running either an agarose gel or acrylamide gel.

When isolating the DNA from the Blanding's turtle's shell shavings and loading the product into an agarose gel, there was no visible result. Either I was unsuccessful in extracting the DNA from the shell, or there was such a small amount that it couldn't be visibly detected. Because of this, I was unable to run an acrylamide gel to see the Blanding's turtle's DNA fingerprint, and was thus unable to determine if there is a lack of genetic variation within and between this species of turtle.

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## References

- Coffi C, Bruford MW (1999) Genetic Structure and Gene Flow among Konso and Diaga Populations Inferred by Microsatellite Loci Analysis. *Molecular Ecology*, 8, 517-530.
- Crim C, Milinkovich MC, Gibbs JP, Caccone A, Powell JR (2002) Microsatellite Analysis of Genetic Divergence among Populations of Giant Galapagos Tortoises. *Molecular Ecology*, 11, 2285-2288.
- Crim JL, Spotts D, Spotts JR, O'Connor M, Reza R, Williams CJ, Paladino PV (2002) The Leatherback Turtle, *Dermochelys coriacea*, Exhibits Both Polytypic and Polygeny. *Molecular Ecology*, 11, 2097-2106.
- Cummings SA, Branton JL, Adams KJ, Thorngard GH (1997) Genetic Analysis to Establish Captive-Breding Protocols for Endangered Snake River Sockeye Salmon. *Conservation Biology*, 11, 162-169.