HETEROZYGOSITY OF A WOOD TURTLE POPULATION IN CENTRAL NY

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This presentation will report on the work and results of a genetic study on the wood turtle, *Glyptemys insculpta*, at two sites in Eastern Oswego County, NY for the Student/Faculty Challenge Grant. This study looked at tandemly repeating segments of nuclear DNA, referred to as microsatellites, to assess heterozygosity and relatedness of marked turtles at both sites. This data is part of a larger project assessing the status of wood turtles at both sites as a part of my honors thesis.

I. Introduction

Wood turtles, *Glyptemys insculpta*, are riparian species of turtle native to New York State and much of the Northeastern US, Great Lakes, and Southeastern Canada (Ernst et al., 1994). Declines have been documented in populations throughout their range in recent decades (Daigle & Jutras, 2005; Garber & Burger 1995; Harding, 1991; Saumure & Bider, 1998). As a result of the these declines, many states and Canadian provinces are providing protective status to wood turtles by listing them as endangered, threatened, or a species of special concern (Bowen & Gillingham, 2004). In New York State, the wood turtle is a species of special concern (Al Breisch, personal communication).

In 2005, Dr. Peter Rosenbaum and Kyle Pursel initiated surveys to find and study wood turtles in eastern Oswego County in New York State to determine the natural history, ecology, and status of this species in this part of their range. In 2007, a genetic proponent was added to the study to assess the genetic variation of turtles found at the study sites and to aid in the assessment. A genetic component was added due to the valuable data genetics can provide about a population. It was determined that microsatellites, which measure tandemly repeating sequences of nuclear DNA and are generally considered to be good measures of heterozygosity, were chosen to calculate the genetic diversity of the population. Heterozygosity is the measure of heterozygotes in a population, and a heterozygote is an individual which has two separate alleles for a given gene. With microsatellites, an allele can be considered to be any variation in the number of tandem repeats (Frankham et al., 2004). For example, an individual that is heterozygous can have one allele for 30 repeats and another allele for 33 repeats, which a homozygous individual would have either two alleles with 30 or two alleles with 33 repeats. Aiding in the decision was also a study released in 2005 which calculated the heterozygosities of six populations

in Quebec, Canada, which provided the data and names of primers proven to work with wood turtles for microsatellite studies (Tessier et al., 2005).

II. Methods

Blood samples were taken from each turtle using a vacuum syringe. Samples of 1/10 to ½ cc were taken depending upon the size of the turtle and ease of blood taking. Samples were preserved in a clotting buffer and stored in a -40^{o}F freezer until use. Thawed samples were extracted using a PureGene DNA extraction kit and the corresponding manufacturer's protocol for clotted blood. The general process consists of lysing the blood cells of the clotted blood were and protein denaturation for subsequent protein removal using proteinase K and centrifugation. For each extraction, roughly 50µL of clotted blood was added to 550 µL of cell lysis solution and 3 µL of proteinase K solution in a sterile 1.5mL labeled centrifuge tube. This was inverted 25 times and incubated at 55^{o}C overnight. The sample was then cooled to room temperature and 200 µL of protein precipitation solution was added. The solution was then vortexed at high speed for 20 seconds and then placed on ice for 5 minutes before being centrifuged on the highest setting for approximately 5 minutes. The resulting supernatant containing the DNA was then poured into a new sterile 1.5mL labeled centrifuge tube containing 600 µL of 100% isopropanol and was mixed by inversion 50 times. The sample was then centrifuged again on high for approximately 1.5 minutes. The new supernatant was poured out and the tube allowed to dry while inverted. Then, 600 µL of 70% ethanol was added and then centrifuged for another 1.5 minutes. The supernatant was again drained and inverted for about 10 minutes to dry. Once dry, 20 µL of DNA hydration solution was added to the DNA pellet and incubated at 65^{o}C for approximately one hour. Once hydrated, the solution was stored at 4^{o}C until needed. DNA was then quantified using a fluorometer.

Once quantified, DNA extractions were diluted for PCR. Frozen PCR materials were allowed to thaw and then mixed to make a master solution that could be added to the DNA samples. Added materials include 10X PCR buffer, dNTP, $MgCl_2$, and Taq polymerase. During this time, the forward and reverse primers for one of the five microsatellite primers was added to the master solution. The appropriate amounts of master mix and DNA solution were added to a sterile labeled 0.2mL centrifuge tube. This was centrifuged briefly before being placed in a thermocycler. The sequence of the program used for the thermocycler was adapted from that used by Tessier et al. (2005) and goes as follows: 2 minutes at 95^{o}C for one cycle, 35 cycles of 94^{o}C for 45 seconds, 54^{o}C for 45 seconds, 72^{o}C for one minute.

Samples were then prepared and ran on a Beckman-Coulter CEQ 8000 Genetic Analyzer. Samples were placed in trays and ran in acrylamide gels through capillaries. Fragments would travel at different speeds through the gel in the capillaries and, when they reached a certain point, a laser would detect the fluorescent tag in the fragments and determine the base pair composition. Once complete, the data came out in graphs as a series of one or two peaks, depending on if that individual was heterozygous or homozygous for that loci. From these peaks, the amount of base pairs or nucleotide

compliments, was taken and analyzed using the freeware program PopGene to determine the heterozygosity, allele numbers and frequencies, and Hardy-Weinberg Equilibrium for both sites. The freeware program Structure was used to determine if both sites compromised one large population or separate populations.

III. Results

Three of the five loci tested yielded recordable results. The loci which worked were GmuB21, GmuD16, and GmuD93. Not all turtle blood samples worked, with twenty-three different individuals working with one to all of the three loci. Base pair data for loci GmuB21 was obtained for 14 individuals from Sloperville and 3 from Little Grindstone, 13 in Sloperville and 4 for Little Grindstone for loci GmuD16, and 11 for Sloperville and all samples for Little Grindstone with loci GmuD93.

Analysis using the program Structure could not differentiate individuals form either site into separate populations. Fig. 1 clearly shows the close relatedness of turtles from each site to one another, with each turtle consistently having approximately 50% likelihood of being assigned to one site or the other during analysis. This data is consistent with both sites being part of a single larger population.

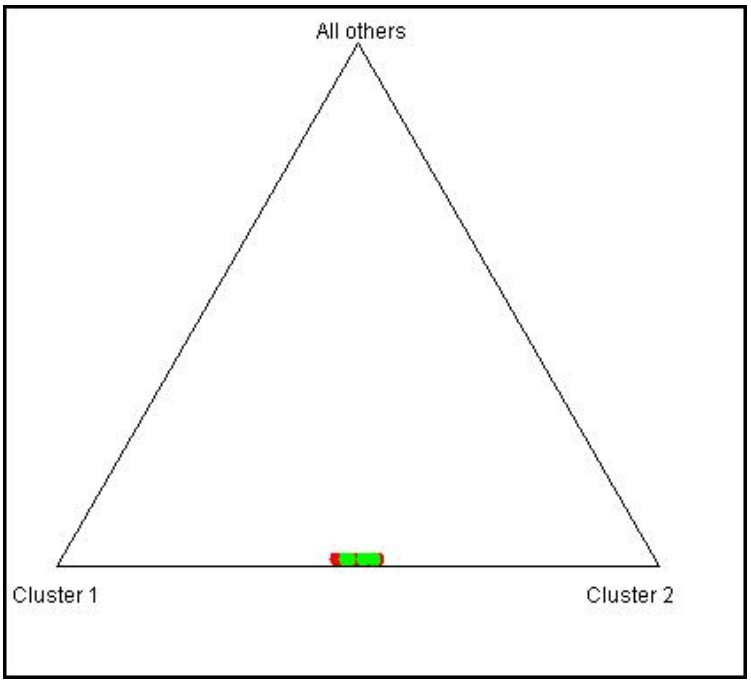

Fig. 1 Triangle Plot of the likelihood of turtle being in separate populations. Note that all points lie roughly in the center (50%) range of the plot. Red dots are individuals from Sloperville, green dots are individuals from Little Grindstone

Analysis using PopGene shows a total of 35 different alleles total from both sites together. Totals of 13, 9, and 13 different alleles were found for each loci (GmuB21, GmuD16, and GmuD93) respectively. Little Grindstone had fewer alleles per loci than Sloperville, but did contain 2 private alleles for GmuD93. Table 1 summarizes allele data for all loci and both site pooled together and separated. Sloperville consistently had larger numbers of alleles than Little Grindstone.

Table 1. Observed Allele Numbers per Loci

Locus	Diploid # of alleles	Observed allele total	Sloperville	LittleGrindstone
GmuB21	34	13	13	3
GmuD16	34	9	9	2
GmuD93	32	13	11	8
Mean	33	11.6667		
St. Dev		2.3094		

In all three loci, the observed heterozygosity was lower than the expected heterozygosity (Table 2). Tests of Hardy-Weinberg equilibrium within both sites pooled together as one population show that loci GmuB21 and GmuD93 were significantly out of equilibrium (B21 p=<0.001 Chi-Square=142.7; D93 p=0.004, Chi-Square=114.2), whereas loci GmuD16 was not significantly different from equilibrium (p=0.741, Chi-Square=30.2).

Table 2. Observed and Expected Heterozygosity of Both Sites

Locus	Diploid Sample Size	Observed Heterozygosity	Expected Heterozygosity
B21	34	0.5294	0.8663
D16	34	0.6471	0.7968
D93	32	0.6875	0.8851
Mean	33	0.6213	0.8494
St. Dev		0.0821	0.0465

Data for the individual loci and turtle were not obtained from the Quebec investigators (Tessier et al., 2005). A simple eye-ball comparison of data shows a similar trend amongst all populations (Table 3), in which all populations appear to have relatively high numbers of alleles and heterozygosity. However, thorough statistical analysis could not be conducted since only 3 of the 5 loci used in the Quebec studies yielded data for the NY population.

Table 3. Heterozygosity and Allele Comparisons Between Quebec & NY Populations

Site	Sample Size (turtles)	Observed Allele #	Observed Heterozygosity
Quebec FA*	46	50	0.561
Quebec SH*	39	46	0.673
Quebec SU*	8	40	0.886
Quebec MI*	19	45	0.804
Quebec TO*	6	36	0.767
Quebec DC*	18	54	0.837
Central NY	~23	35	0.621

*See Tessier et al., 2005. for in-depth site data and name meanings.

IV. Discussion

Turtles from both appear to be or recently were part of a larger population that has and continues to be fragmented by human development of the land. Although 2 out of the 5 loci failed to yield reliable data, a relatively large number of alleles were found for Sloperville and the overall population. This data indicates that turtles from Sloperville and the overall population are possibly genetically stable. However, Little Grindstone had lower allele numbers for 2 of the 3 loci. This may be an indication that Little Grindstone, through recent isolation and human impacts, may be facing an ecological and genetic bottleneck, and might be losing some of the genetic diversity that is present at other sites. This is consistent with the turtle collection data, which only yielded 5 turtles over 3 years. Since one loci, GmuD93, did yield a relatively decent number of alleles, it is possible that the addition of other loci may show that Little Grindstone turtles are more genetically variable than the current data suggests.

The heterozygosity and Hardy-Weinberg data suggests that the populations have fewer heterozygotes than expected for the number of loci present. However, Hardy-Weinberg is based off of many assumptions of nonrandom mating, no selection pressures (for that gene), no mutation, no migration, no genetic drift, and large populations. It is possible that sample sizes are not large enough to provide enough data and that the population is within equilibrium. However, it is also possible that there have been declines within the population, as appears to have happened at the Little Grindstone site, which could also lead to the elimination of some alleles and changes in allele frequency through genetic drift, since there should be little to no selection occurring for these genes, unless they are linked to selected genes.

V. Acknowledgements

I would like to thank the numerous volunteers who have helped me collect field data and turtles and the landowners who have graciously given me permission to conduct the field research and turtle surveys that led to this project. Funding for this work was provided by a Student/Faculty Challenge Grant. I would especially like to thank my two advisors, Dr. Peter Rosenbaum and Dr. Amy Welsh, both of whom have provided much help and advice.

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