

**GENETIC DIVERSITY OF DIAMONDBACK TERRAPINS
(*MALACLEMYS TERRAPIN*) FROM PIERMONT MARSH,
HUDSON RIVER, NY**

A Final report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The *Malaclemys terrapin* population surrounding the Piermont Marsh complex is small and broadly dispersed, and it is unknown whether this population is reproductively isolated from other terrapin populations in the Hudson River estuary and elsewhere. To determine whether the Piermont Marsh terrapin population is distinct from other regional populations, the Inter-Simple Sequence Repeat (ISSR) technique for assessing genetic diversity of diamondback terrapins was developed and tested for terrapins from Piermont Marsh in New York and from terrapins collected in Connecticut, Rhode Island, and New Jersey. Isolation and PCR amplification protocols were developed successfully for the MAO primer sequence, but protocols for a second primer (844) could not be optimized. Results from the ISSR technique demonstrated a significant difference between terrapin and painted turtle DNA products, indicating that this technique can detect interspecific differences. Among terrapin populations the differences were less distinct, but a few polymorphic loci were evident both between regional populations and within the Piermont Marsh population. Using the MAO primer sequence the ISSR analysis could not identify the Piermont Marsh population as genetically distinct from other populations, suggesting low levels of population subdivision. Further ISSR analysis using additional primers or the application of other molecular techniques is required to determine the relationships between the Piermont population and terrapins in the Hudson River Bight and elsewhere.

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INTRODUCTION

The diamondback terrapin (*Malaclemys terrapin*) is an estuarine turtle that inhabits coastal marsh habitats ranging from as far south as Texas and the Gulf Coast, to as far north along the Atlantic Coast as Cape Cod. During the early 20th century, large numbers of terrapins were harvested for human consumption, thereby reducing terrapin population sizes and perhaps reducing terrapin distribution among estuarine wetland habitats. In recent years the perceived environmental threats to diamondback terrapin populations have prompted the New York State Department of Environmental Conservation (DEC) to list the terrapin as a species of special concern. Although some terrapin populations in the Hudson River Bight and around Jamaica Bay are considered large and healthy (Russell Burke, pers. comm.), other local populations face a number of threats to their survival. In Piermont Marsh, for example, reduced accessibility to the marsh surface and to high quality, upland nesting sites probably contributes to the small and broadly dispersed terrapin population (Simoes and Chambers 1999).

Given the possible geographic isolation of the Piermont terrapin population from other populations farther downstream and the environmental threats to population maintenance, an analysis of the genetic diversity of diamondback terrapins from Piermont marsh could determine whether these terrapins are reproductively isolated from other populations. For example, if the Piermont population is small, isolated, and exhibits extensive inbreeding, then the level of population subdivision would be expected to be high compared to other populations. This type of research has never been performed for

the northern terrapin sub-species (*Malaclemys terrapin terrapin*). In fact, terrapin sightings have been reported anecdotally along the lower Hudson River estuary (Boyle 1969; Klemens 1993; W.C. Nieder, pers. comm.), and are common farther south in the Hudson River Bight (Russell Burke, pers. comm.), but formal study of the Piedmont terrapin population has only occurred once (Simoes and Chambers 1999).

To date, only one study has been published on the genetic diversity of diamondback terrapins (Lamb and Avise 1992). In this study, mitochondrial DNA from heart and liver tissues were analyzed for the seven terrapin sub-species throughout their geographic range. This work identified broad genetic differences between northern and southern populations, but genetic differences among sub-species were not distinct (Lamb and Avise 1992). The variability in the mtDNA data was not great enough to allow population level comparisons. Similarly, Gray (1995) observed a lack of genetic variation within and among populations of the western pond turtle (*Clemmys marmorata*), but it is unclear whether these results are a consequence of low genetic diversity among turtles, or are caused by the limitations of the molecular methods employed.

The inter-simple sequence repeat (ISSR) technique developed by plant breeders (Zietkiewicz et al. 1994) has been applied successfully to answer ecological, evolutionary and conservation biology questions in a number of botanical studies (e.g., Hollingsworth et al. 1998; Wolfe and Liston 1998; Wolfe et al. 1998). Although developed in plant systems, use of the technique is now expanding to animal systems. To date, however, the ISSR technique has not been used in reptilian research.

The ISSR technique involves the amplification of regions of genomic DNA that lie between simple sequence repeats (SSRs) using the polymerase chain reaction (PCR). SSRs are composed of nucleotide repeats such as (AT)_n or (TGC)_n where the subscript represents how many times the sequence is tandemly repeated. These motifs are extremely common and uniformly distributed in most eukaryotic genomes. Instead of using two primers as in traditional PCR, ISSR uses a single primer that is anchored in a simple sequence repeat region of the genome (e.g., 5'CACACACACACAGT3'). The length of the primer allows for greater stringency of PCR conditions (i.e., higher annealing temperatures) compared to other PCR-based genotyping techniques (e.g., RAPDs). The higher annealing temperature results in high levels of band resolution.

The objective of the present study was to develop the ISSR technique for examining genetic diversity among diamondback terrapins from Piermont Marsh, Hudson River, New York. Terrapins from Piermont Marsh were captured and sampled for DNA, then the ISSR method was applied. Results of the ISSR analyses were compared with terrapins from other regional populations to determine whether the Piermont population represents an isolated group of terrapins. The goal was to determine the degree to which the small Piermont population might be threatened by ongoing disturbance of their feeding and breeding habitats.

METHODS

Study Site

Piermont Marsh is a 400-ha marsh complex located on the Hudson River in the Village of Piermont, approximately 4 km south of the Tappan Zee Bridge and 37 km up

from the mouth of the Hudson River, in Rockland County, NY (Figure 1). The marsh is transected in the northern portion by the Sparkill, which runs from the center of the Village out to the Hudson River, and in the central portion by the Crumkill. The marsh complex is protected to the north by a large earthen pier that extends out into the Hudson River. The dominant species of vegetation (currently covering approximately 80% of the marsh surface) is common reed (*Phragmites australis*). It appears that this plant is displacing stands of *Spartina patens* as well as other wetland species.

Trapping

Trapping was conducted using both baited (with menhaden) Maryland crab pots and 75-foot trammel nets, with 2-inch inner mesh, 4-inch outer mesh and a height of 6 feet. Simoes and Chambers (1998) were never able to capture terrapins in the Crumkill or the Sparkill, so trapping efforts were focused in areas outside the marsh. The crab pots were deployed in several areas, including the region just north of the pier ("pier" location, Figure 1). This small area is protected to the north by a rock jetty and to the south by the pier itself. It is a fairly shallow area of water approximately 7 feet deep at high tide containing a sandy substrate with much debris from what seem to be pilings used in a previous pier construction. Terrapins frequently are observed basking on the breakwaters north and south of the pier. The second crab trap location was south of the pier and adjacent to a rocky breakwater ("north sandbar" location, Figure 1). The third area where crab pots were deployed was off the old bridge over the Sparkill, adjacent to the Piermont Post Office. Reports by local fishermen suggested that terrapins were

occasionally observed swimming as far north on the creek as the sewage pumping station, and that some terrapins had been caught from the old bridge.

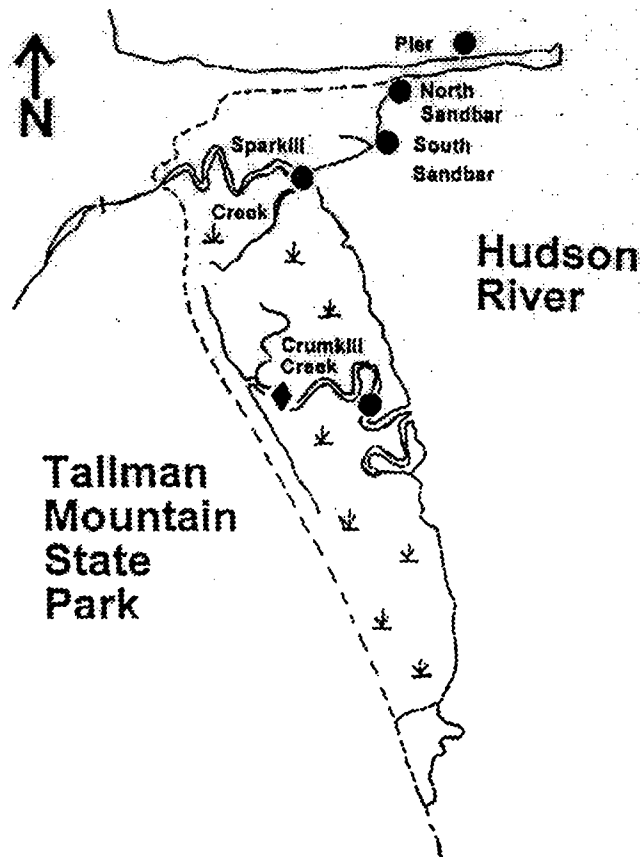


Figure 1. Piermont Marsh site-map Rockland County, NY (from Simoes and Chambers 1998).

Trammel nets were deployed on four dates at the “north sandbar” location and once at the mouth of the Sparkill (“south sandbar” location, Figure 1). During most dates for trammel netting and deployment of crab pots, the traps were set on an incoming morning tide and retrieved either two hours before or two hours after high tide. On one date, however, crab pots were set out overnight at the north sandbar and old bridge locations. On all trapping dates, trammel nets were cleared of by-catch on an hourly basis.

Tissue Sampling for DNA

Initially, both tissue and blood samples were used to extract terrapin DNA. Terrapin tissue was taken from the tip of the tail of captured terrapins. Approximately 2-4 mm of tissue was clipped off the end of the tails using a nail clipper. The tissue samples were stored in a DMSO field preservative (20% DMSO and 250 mM EDTA saturated with NaCl) for transport back to the laboratory. Blood draws were completed on some terrapins; venous access was gained by pulling the tail over the plastron of the terrapin. The tail was swabbed with alcohol in order to remove any potential DNA contamination by external organic matter. Using a heparinized, 22-gauge, 3cc syringe, venipuncture was achieved on the dorsal surface of the tail (Haskell and Pokras 1994). The heparinized syringes containing blood samples were labeled and stored on ice for transport back to the laboratory for DNA extraction. In order to minimize DNA degradation, if the blood samples were not able to be immediately processed, they were stored at -80°C once returned to the lab.

Blood samples yielded clean, high molecular weight DNA, but more equipment was needed in the field to conduct blood draws than to collect and process tail clips. Furthermore, ice was needed to transport raw blood samples back to the lab to keep DNA from degrading, a real possibility during summertime sampling. Although tail clippings may influence breeding activities by male terrapins (Russell Burke, pers. comm.) we obtained similar quantities of DNA from both techniques and therefore opted to use the simpler procedure of tail tissue sampling for all terrapins in this study.

DNA Isolation

DNA was isolated from both blood and tissue samples using a Qiagen™ Dneasy® kit. Different protocols were utilized for tail portion and blood sample DNA isolations. DNA isolates were stored in eppendorf tubes containing 200-300 µl of Qiagen elution buffer. Tubes containing DNA and buffer were stored at 4°C until which point they were utilized for PCR.

DNA Quantification

DNA was quantified using a standard UV Spectrophotometer. The Spectrophotometer was zeroed using a sterile H₂O blank and clean cuvette. Individual DNA isolates were placed in cuvettes in the same manner and DNA concentrations were gathered. Original DNA concentrations ran from 0.02-0.21 µg/µl. When these DNA samples were used in PCR and were later run on gels, a “smearing” was noted in the lanes of the gel, this smearing was traced back to the enormously high DNA concentrations being used. After observing this smearing, all DNA was quantified and diluted with sterile water down to a concentration of only 5ng/µl, which is orders of magnitude lower than original concentrations. Dilution of samples eliminated any smearing or streakiness on later gels.

PCR

PCR was run on DNA samples using two different primers named MAO and 844. Standard PCR reaction mixtures were set up using 3.5 mM MgCl₂, 1U Taq Polymerase, 0.2 mM dNTP's, 10X reaction buffer, 25 pmole primer, and 5 ng from each DNA

sample. The reaction mixtures were placed in the thermocycler and specific temperature protocols were utilized depending on which primer was being used. Successful amplifications with MAO involved the following cycling profile: 1.5 min at 94°C followed by 35 cycles of 40 sec at 94°C, 1.5 min at 45°C, and 1.5 min at 72°C. A final extension was performed for 5 min at 72°C. Products were stored at 4°C or -20°C until they could be separated by gel electrophoresis.

Gel Electrophoresis

Agarose gels were used for electrophoresis of PCR products. These gels were prepared using molecular grade agarose and 1x TBE Buffer and varied in concentration from 0.8%-1.2%. Gels were cast approximately 5-10 mm thick and were stained with Ethidium Bromide. A 100 bp size marker was loaded in all gels.

RESULTS

Trapping

On seven trapping dates in Piermont Marsh, a total of five diamondback terrapins were captured; all were captured on the first sampling date of the summer, in early June. Of the five, two were non-gravid females and three were males. None of the terrapins were marked, indicating they were not recaptures from an earlier terrapin study at Piermont marsh that marked eight terrapins (Simoes and Chambers 1998).

Maryland crab pots were fairly ineffective at trapping terrapins in this population. Of the five terrapins trapped, only one came from a crab pot that was placed at the "pier" location. The other four terrapins were trapped using a 75-foot trammel net deployed at

the "north sandbar" location (Figure 1). No terrapins were caught off the bridge over the Sparkill. All terrapin trapping dates after the first proved to be unsuccessful, although several species of by-catch were trapped in both crab pots and trammel nets. The complete list of these species included blue crab (*Callinectes sapidus*), Atlantic menhaden (*Brevoortia tyrannus*), common carp (*Cyprinus carpio*), white perch (*Morone americana*), white catfish (*Ameiurus catus*) and American eel (*Anguilla rostrata*).

Diversity Assessment

PCR reaction mixture concentrations and thermocycler protocols were optimized for primer MAO. A large portion of laboratory time was spent optimizing these procedures for MAO. For ISSR comparison, the DNA from terrapins from three other regional populations was extracted and analyzed using this primer. Terrapin tissue from the Barrington Marsh in Rhode Island was provided by Doug Rayner (The Barrington Land Conservation Trust, Inc.); tissue from the Milford Point Marsh in Connecticut and from the Stone Harbor marsh in southern New Jersey was provided by R.M. Chambers. Additionally, ISSR analysis of painted turtles collected by R.M. Chambers provided an "out group" comparison between turtle species.

Results of PCR with primer MAO showed several polymorphic loci (Figure 2). Interspecific differences are obvious, with at least 4 distinct loci unique to either painted turtles or terrapins, *i.e.*, bands appearing in one species and absent in the other. In addition to the expected interspecific polymorphism, small differences were observed among terrapin populations from different marshes (New York vs. Rhode Island, Connecticut and New Jersey) and even within the Piermont population (Figure 2). The

degree of variability seen using this primer, however, seems to be fairly low among terrapins used in this study. A second primer (844) was used for ISSR analysis, but PCR optimization is still in progress.

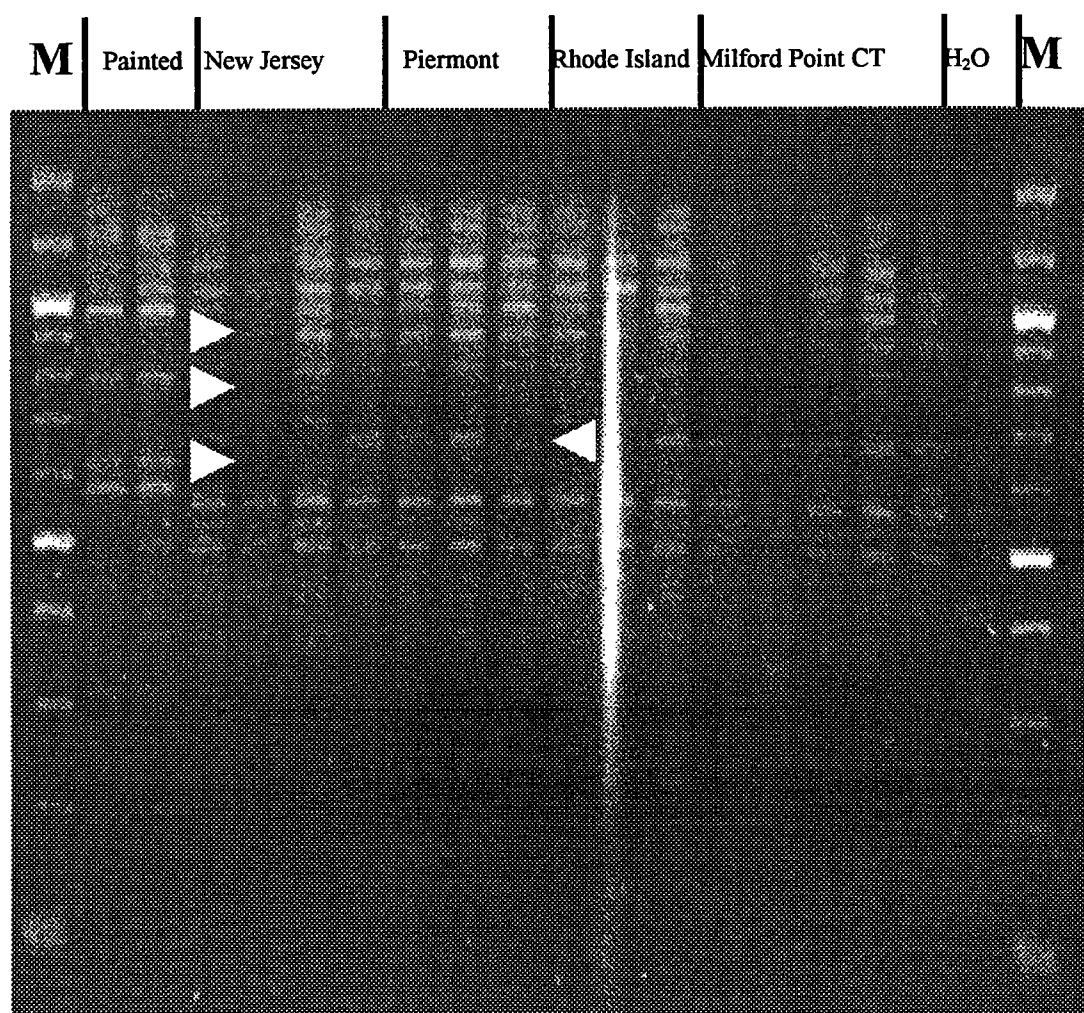


Figure 2. Picture of agarose gel containing separated MAO PCR products. Sources of terrapins are shown at the top of the gel. Gel lanes labeled M are 100-base-pair marker lanes. The H₂O lane is a negative control in which water was substituted for DNA in order to rule out any potential contamination. The Painted lanes are painted turtle (*Chrysemys picta*) added as an out group. White arrows pointing right identify three loci different between painted turtles and terrapins. The white arrow pointing left identifies variation within the Piermont population.

DISCUSSION

The ISSR technique offers the potential of identifying genetic differences among terrapin populations, but our study found the development of successful ISSR protocols was a lengthy process. Primer MAO has been optimized, the conditions used have been duplicated on numerous occasions, and results have proven to be reproducible. Optimization of Primer 844, however, was unsuccessful throughout the duration of this project. Many PCR trials were run with slight variations in reaction mixture concentrations and thermocycling conditions made each time. The optimum conditions for this primer and two other primers recently acquired have not been achieved at this juncture.

With only one primer optimized, the genetic distinction of Piermont terrapins could not be established. The similarity of results among terrapin populations from four states (New York, Rhode Island, Connecticut, New Jersey) relative to the out-group (painted turtles), however, indicates use of a single primer with the ISSR technique is sufficient to document genetic differences between species (Figure 2). Differences between terrapin populations were less obvious in our study; a few polymorphic loci could be observed among individual terrapins but they did not vary in any systematic, geographic fashion, *i.e.*, the variation between terrapin populations was similar to the variation within terrapin populations. Based on results obtained from ISSR using Primer MAO, the Piermont marsh terrapins do not appear to be genetically distinct from other regional populations.

What are the ramifications of genetic similarity among regional terrapin populations? From the perspective of the Piermont population, the implication is that

gene flow is occurring among these populations. Identifying the source of that gene flow is not possible with our data. However, we hypothesize that a small number of juvenile and/or adult terrapins are dispersing from the large "source" population in the Hudson River Bight and emigrating to Piermont marsh. Young terrapins may move upriver with the salt wedge during the summer and occupy Piermont marsh throughout their adult lives. We also suspect that the relative fitness (*i.e.*, survivorship and fecundity) of terrapins in the Piermont Marsh complex is low owing largely to the general absence of high quality nesting habitats, thus reducing breeding opportunities (Simoes and Chambers 1999). The Piermont Marsh complex may represent a "sink" for terrapins, and the population may be sustained primarily via dispersal from external populations and not via internal reproduction. ISSR analyses with additional primers will be required to determine whether the Piermont population of terrapins is genetically distinct. We suspect that without an external source of terrapins, the conservation status of the Piermont population would move to severely threatened since local reproduction is probably not sufficient for maintaining local population size in the absence of high quality nesting habitat.

CONCLUSION

The Inter-Simple Sequence Repeat (ISSR) technique for assessing genetic diversity of diamondback terrapins was developed and tested for terrapins from Piermont Marsh in New York and from terrapins collected in Connecticut, Rhode Island and New Jersey. Results from the ISSR technique demonstrated the significant difference between terrapin

and painted turtle DNA products and supported the validity of the method. Among terrapin populations the differences were less distinct, but a few polymorphic loci were evident both between regional populations and within the Piermont Marsh population. ISSR analysis could not identify the Piermont Marsh population as genetically distinct from other populations, suggesting the Piermont population is not reproductively isolated. Further ISSR analysis using additional primers is required to determine the relationships between the Piermont population and terrapins in the Hudson River Bight and elsewhere.

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REFERENCES

- Boyle, R.H. 1969. *The Hudson River: A Natural and Unnatural History*. W.W. Norton & Co., New York, NY. 304 pp.
- Gray, E. M. 1995. DNA Fingerprinting Reveals a Lack of Genetic Variation in Northern populations of the Western Pond Turtle (*Clemmys marmorata*). *Conservation Biology* 9:1244-1255.
- Haskell, A. and M. A. Pokras. 1994. Nonlethal blood and muscle tissue collection from redbelly turtles for genetic studies. *Herpetological Review* 25:11-12.
- Hollingsworth, P.M., M.Tebbitt, K.J. Watson, and R.J. Gornall. 1998. Conservation genetics of an arctic species, *Saxifraga rivularis* L., in Britain. *Botanical Journal of the Linnean Society* 128:1-14.
- Klemens, M.W. 1993. *Amphibians and Reptiles of Connecticut and Adjacent Regions*. State Geological and Natural History Survey of Connecticut, Bulletin No. 112, 318 pp.
- Lamb, T., and J.C. Avise. 1992. Molecular and population genetic aspects of mitochondrial DNA variability in the diamondback terrapin, *Malaclemys terrapin*. *Journal of Heredity* 83:262-269.
- Simoës, J.C., and R.M. Chambers. 1998. A Population Study of Diamondback Terrapins of Piermont Marsh, Hudson River, NY. Section I: 31pp. *In* J.R. Waldman and W.C. Nieder (eds.), *Final Reports of the Tibor T. Polgar Fellowship Program, 1997*. Hudson River Foundation.
- Simoës, J.C., and R.M. Chambers. 1999. The diamondback terrapins of Piermont Marsh, Hudson River, New York. *Northeastern Naturalist* 6:241-248.
- Wolfe, A.D., and A. Liston. 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. pp. 43-86, *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle (eds.), *Plant Molecular Systematics II*. Kluwer.
- Wolfe, A.D., Q-Y. Xiang, and S.R. Kephart. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable inter simple sequence repeat markers. *Molecular Ecology* 7:1107-1125.
- Zietkiewicz E., A. Rafalski, and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.