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# Lack of Genetic Variation in Cytochrome b in a Population of Smooth Softshell Turtles

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An important issue in intraspecific molecular phylogenetic studies concerns distribution of genetic variation within and among populations and, hence, within-population sample sizes used in analyses. To address this sampling issue, we sequenced a 795 base pair (bp) segment of the mitochondrial cytochrome b gene from 19 unrelated individuals from a Louisiana population of the smooth softshell turtle (*Apalone mutica* LeSueur). We found a complete lack of within-population variation in this large segment of mtDNA. This result supports the use of minimal within-population sample sizes in intraspecific molecular phylogenetic studies of *Apalone* using cytochrome b.

INDEX DESCRIPTORS: smooth softshell turtle, molecular phylogeny, mitochondrial DNA, genetic variation, Apalone mutica.

The advent and increased popularity of molecular techniques combined with cladistic methods has rejuvenated phylogenetics and systematics (e.g., Avise et al. 1994, Crother and Hillis 1995, de Queiroz and Lawson 1995). A particularly important assumption in intraspecific phylogenetic studies (i.e., constructing population "trees", see Avise 1989) is adequate sampling of individuals within populations. Because of factors such as gene flow, introgression, and lineage sorting, a given individual from a population could be more closely related to other populations than to its cohabitants (e.g., Avise 1989). This problem may be particularly acute in taxa with widespread distributions and great potential for migration. The use of minimal within-population sample sizes in intraspecific phylogenetic studies could therefore produce misleading results. Understanding how genetic variation is arranged both within and between populations can thus greatly increase the reliability of an intraspecific phylogenetic estimate.

Softshell turtles of the genus *Apalone* (formerly *Trionyx*) have a wide distribution in rivers across North America, from the Rocky Mountains in the west to the Appalachians in the east and as far north as southern Alberta and Quebec to as far south as Mexico and Florida (Ernst et al. 1994). The extensive geographic distribution and riverine habitats make *Apalone* a model organism to study the sampling assumptions of molecular phylogenetics in the context of intraspecific phylogeography (see Avise 1989).

This study estimated the amount of mitochondrial DNA (mt-DNA) variation within a population of smooth softshell turtles ( $Apa-lone\ mutica$ ) to address sampling issues involved in a larger phylogenetic study of the genus Apalone (sensu Meylan 1987). We specifically were interested in sequence from the cytochrome b gene, because it is the locus of interest in our larger phylogenetic analysis (Weisrock 1997). Although turtles are thought to evolve at relatively slow rates (Avise et al. 1992), we have nonetheless detected large amounts of genetic variation in cytochrome b at the intraspecific level between populations of Apalone (Weisrock 1997). Variation at this level accentuates the purpose of the present study. If large amounts of variation in cytochrome b exist between populations, does this variation reflect large amounts of within-population variation as well and, hence, interfere with our reconstruction of the historical relationships?

To address this specific question, we chose to extensively sample a population of A. *mutica* from Louisiana for which we had tissue from many unrelated individuals and sequence the homologous segment of mtDNA used in the larger study (Weisrock 1997). We assumed that this large sample size would maximize our probability of rejecting the null hypothesis that no genetic variation in cytochrome b existed within this population of A. *mutica*.

#### METHODS

We obtained 19 clutches of *A. mutica* eggs collected from 10 nesting beaches along a 3 km stretch of the Comite River in Baker, Louisiana. Eggs were incubated in the laboratory, and once all turtles had hatched, a 3 mm long V-shaped wedge was clipped from the edge of each turtle's carapace. Skin clips were stored at  $-80^{\circ}$ C until use.

Genomic DNA from one individual from each of the 19 clutches was isolated using a Proteinase K/NaOH and Phenol/Chloroform method modified from Hillis and Moritz (1990). Purified DNA was used in an initial PCR under the following thermal conditions: 95°C denature, 50°C anneal and 72°C extension for 35 cycles. PCR was conducted in 25µl volumes with 0.5–1.0 µg DNA, 1X PCR buffer (Tris-HCl, 1.5mM MgCl2 and 50 mM KCl), 0.1 mM dNTPs, 1.0 µM primers and 1 unit Taq polymerase (Boehringer Mannheim). The primers were developed to amplify an 800 base pair fragment of the mitochondrial cytochrome *b* gene. The forward primer (DW 2000; 5' ACA GGC GTA ATC CTA CTA A 3') was developed in our laboratory. The reverse primer sequence (DW 1594; 5' TCA TCT TCG GTT TAC AAG AC 3') was obtained from M.L. McKnight (pers. comm.). The 5' end of DW 2000 corresponds to position 16595 of the *Xenopus* mtDNA genome (Roe et al. 1985), while the 3' end corresponds to position 17415. The amplified fragment thus encompasses the entire 3' portion of cytochrome *b* and is separated

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Louisiana Arkansas	16613 TATAACATGGCAACAGCATTCATAGGATACGTCTTACCATGAGGCCAAATATCCTTCTGAGGGGCCACAGTCATCACAAACCTACTCTCAGCCATCCCAT TTAACCATGGCAACAGCATTCATAGGGTACGTCTTACCATGAGGCCCAAATATCCTTCTGAGGGGCTACAGTCATCACAAACCTACTCTCAGCTATCCCAT * * *********************************
Lou <b>isiana</b> Arkansas	16713 ACATCGGCACCACAATAGTACAATGAGTATGAGGTGGGTTTTCCGTAGACAATGCCACCCTAACACGATTCTTCACCCTACACTTCTTACTCCCATTCAT ACATCGGCACCACAATAGTACAATGGGTATGAGGCGGATTTTCTGTAGACAATGCCACCCTAACACGATTCTTCACCCCTACATTTTTTACTCCCATTCAT ******
Louisiana Arkansas	16813 ANTCCTAGGAATTGCAATAATCCACCTACTTTTTTCTCCACGAAACCGGATCAAACAACCCAACAGGACTTAACTCAAACACCGATAAAATCCCATTCCAC ANTCCTGGGAATTGCAATAATCCACCTACTTTTTTCTCCACGAAACCGGATCAAACAACCCAACAGGACTTAACTCCAAACACCGGATAAAATCCCATTCCAC
Lou <b>isian</b> a Arkansas	16913 CCCTACTTCTCATACAAAGACCTATTAGGATTTATAGCAATACTCACCGTGCTCCTATCAATTGCCATATTTCACCCCAAACCTATTAGGAGACCCAGACA CCCTACTTCTCATACAAAGACCTATTAGGGTTTATAGCAATACTCACCGTACTCCTATCAATTGCCATATTTCACCCCAAACCTATTAGGAGACCCAGACA
Louisiana Arkansas	17013 ACTTCACACCCGGCTAACCCGGCTATCTACACCCCCACACATCAAACCAGAATGATACTTCTTATTCGCCTACGCCATTCTACGATCTATTCCCAACAAACT ACTTCACACCCCGCTAACCCACTATCTACACCCCCCACCATCAAACCAGAATGATACTTCTTATTCGCCTACGCCATTCTACGATCTATTCCCCAATAAACT
Louisiana Arkansas	17113 AGGAGGTGTACTCGCCCTACTCATATCCATTCTAGTATTATTTAT
Louisiana Arkansas	17213 CAAACACTATTCTGATCATTCGTAGCTAACCTTGCCGTACTAACATGAATTGGAGGCCAACCAGTAGAAAACCCATTCATT
Louisiana Arkansas	17313 ССАССТТТТАСАТСТТАСААТСТТАСТССТАСТСАТАССААТСТСАААТАТААТА

Fig. 1. Light strand cytochrome b nucleotide sequences for a representative A. mutica from Louisiana and for a comparative A. mutica from Arkansas. There was no variation in this 795 bp sequence among 19 individuals within the Louisiana population despite the extensive cytochrome b divergence of these turtles from conspecifics in nearby Arkansas. Genetic differences between the homologous sequences for the two turtles are indicated by sites lacking a "\*". The number adjacent to the first nucleotide in each line indicates its reference position in the Xenopus mtDNA genome (Roe et al. 1985).

from the upstream d-loop region by approximately 140 bases of tRNA sequence (Roe et al. 1985).

PCR product was run on a 1.5% low melt agarose TBE gel and the 800 base pair fragment was excised from the gel. The low melt fragment was suspended in 1 ml dH<sub>2</sub>O and heated at 95°C for 5 minutes. This mixture was then used as template in a second PCR to generate double stranded DNA for sequencing. The second PCR product was run on a 1% TBE agarose gel and the band was excised. DNA was purified from the gel slice with 0.22 Micropure separators (Amicon) and then concentrated with M-100 microconcentrators (Amicon).

Template was sequenced at the Iowa State University DNA Sequencing Facility. DNA fragments were sequenced from both ends with the original PCR primers to verify the integrity of the sequence for each individual. Sequences were assembled with the use of Sequence Navigator version 1.0.1 (Applied Biosystems) and were subsequently aligned with the program Clustal W for the Power PC version 1.5 (Thompson et al. 1994).

#### **RESULTS AND DISCUSSION**

A contiguous stretch of 795 bases beginning from the 3' end of cytochrome b was analyzed for this population of A. *mutica*. Align-

ment of all 19 sequences revealed a complete lack of variation in cytochrome b within the population. This result is in marked contrast to the extensive differentiation of this segment of mtDNA between populations of *A. mutica* (Fig. 1; Weisrock 1997). The lack of multiple haplotypes of cytochrome b within the Louisiana population thus indicates that using a single individual from this population sufficiently represents the population in phylogenetic analyses.

The affinity of A. mutica for riverine conditions, which should promote gene flow, is common to this species. Yet despite these conditions, the presence of novel and potentially immigrant alleles was not detected in this Louisiana population. Upstream populations as close as the White River in Arkansas exhibit a large amount of sequence differentiation from Louisiana for this same segment of cytochrome b (Fig. 1). In principle, immigrant turtles from Arkansas and elsewhere could have been present in Louisiana, and, if these were sampled in a phylogenetic analysis, misleading results may have been obtained. This situation, however, can be ruled out in our study of cytochrome b because the 19 sequences were identical.

Our results may also be informative about the mitochondrial demographics of other species of *Apalone*. *Apalone spinifera* (LeSueur) and *A. ferox* (Schneider) are found not only in rivers and streams, but also in slow-moving aquatic environments. Thus dispersal among populations is possible in these species as well. However, the leathery skin of softshell turtles has a high rate of water exchange with the air, which limits the amount of time turtles can spend out of water and thus their overland dispersal ability (Ernst et al. 1994). Consequently, gene flow might be minimal in these species. Besides, overland dispersal in other species of turtles is most commonly undertaken by males rather than females (Tuberville et al. 1996), and these migrant males will have a negligible impact on the mitochondrial demographics of a population because mtDNA is maternally inherited. We therefore infer that the lack of genetic variation at cytochrome b within the Louisiana population of A. mutica is likely paralleled by little or no variation within populations of A. spinifera and A. ferox as well.

The assumptions made about the environmental and demographic conditions that turtles experience are necessarily general. The actual behavior of a particular population may not be as clear. Turtle populations may be affected by numerous other factors such as dams, habitat destruction, and introduction by humans, which could influence dispersal and the genetic variation present in a population. Small population size or strongly male-biased sex ratio could also eradicate the presence of a stray mtDNA allele due to genetic drift. The likelihood that these phenomena have affected this population of A. mutica is not estimable. Studied populations of A. mutica are large (e.g., Plummer 1977) and were chosen primarily because of these excellent sample sizes. Consequently, published evidence that populations of softshell turtles are typically large could represent investigator bias, including the population studied here (Doody 1996). Furthermore, although A. mutica does not have temperaturedependent sex determination (Janzen 1993), strongly male-biased sex ratios could nonetheless be present (e.g., Plummer 1977). Genetic similarity due to sibship is an unlikely complication in this study because we analyzed individuals from separate clutches.

In conclusion, our results indicate that minimal within-population sampling of *Apalone* in molecular studies of intraspecific phylogeny may be warranted. Although the complete lack of variation at cytochrome b implies that the Louisiana population is homogeneous at this locus, it certainly does not preclude all genetic variation within this population. Faster evolving loci such as the d-loop region in mtDNA or neutral nuclear loci may exhibit within-population variation, as they are not under the selective constraints that limit molecular evolution of cytochrome b.

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#### LITERATURE CITED

- AVISE, J. C. 1989. Gene trees and organismal histories: A phylogenetic approach to population biology. Evolution 43:1192–1208.
- AVISE, J. C., B. W. BOWEN, T. LAMB, A. B. MEYLAN, AND E. BER-MINGHAM. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Molecular Biology and Evolution 9:457–473.
- AVISE, J. C., W. S. NELSON, AND C. G. SIBLEY. 1994. DNA sequence support for a close phylogenetic relationship between some storks and New World vultures. Proceedings of the National Academy of Science USA 91:5173–5177.
- CROTHER, B. I. AND D. M. HILLIS. 1995. Nuclear ribosomal DNA restriction sites, phylogenetic information, and the phylogeny of some xenodontine (Colubridae) snakes. Journal of Herpetology 29:316–320.
- DE QUEIROZ, A. AND R. LAWSON. 1994. Phylogenetic relationships of the garter snakes based on DNA sequence and allozyme variation. Biological Journal of the Linnean Society 53:209-229.
- DOODY, J. S. 1996. Summers with softshells. Bulletin of the Chicago Herpetological Society 31:132–133.
- ERNST, C. H., J. E. LOVICH, AND R. W. BARBOUR. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, District of Columbia.
- HILLIS, D. M. AND C. MORITZ. 1990. Molecular Systematics. Sinauer Associates, Inc., Sunderland, Massachusetts.
- JANZEN, F. J. 1993. The influence of incubation temperature and family on eggs, embryos, and hatchlings of the smooth softshell turtle (*Apalone mutica*). Physiological Zoology 66:349–373.
- MEYLAN, P. A. 1987. The phylogenetic relationships of softshell turtles (Family Trionychidae). Bulletin of the American Museum of Natural History 186:1-101.
- PLUMMER, M. V. 1977. Activity, habitat, and population structure in the turtle, *Trionyx muticus*. Copeia 1977:431-440.
- ROE B. A., D. P. MA, R. K. WILSON, AND J. F. WONG. 1983. DNA sequence of the Xenopus laevis mitochondrial heavy and light strand replication origins and flanking tRNA genes. Nucleic Acids Research 11: 4977–4995.
- THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.
- TUBERVILLE, T. D., J. W. GIBBONS, AND J. L. GREENE. 1996. Invasion of new aquatic habitats by male freshwater turtles. Copeia 1996: 713-715.
- WEISROCK, D. W. 1997. Molecular phylogenetics and phylogeography of North American softshell turtles (*Apalone*). Unpublished Masters Thesis, Iowa State University, Ames, Iowa.