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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 (mtDNA) variation within a population of smooth softshell turtles (*Apalone 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°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 $25\mu\text{l}$ volumes with $0.5\text{--}1.0\mu\text{g}$ DNA, 1X PCR buffer (Tris-HCl, 1.5mM MgCl₂ and 50mM KCl), 0.1mM dNTPs, $1.0\mu\text{M}$ 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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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 temperature-dependent 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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