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Phylogeographic uniformity in mitochondrial DNA of the snapping turtle (*Chelydra serpentina*)

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Abstract

Previous studies have revealed considerable genetic variation, geographic localization, and genealogical depth for mitochondrial DNA (mtDNA) haplotypes within each of several species of freshwater turtles in the south-eastern United States of America. Here we report a notable exception to such phylogeographic patterns. In control-region sequences of 66 snapping turtles (*Chelydra serpentina*) collected from 10 south-eastern states, a single mtDNA haplotype predominated and the two rare variants detected were nearly identical to the common genotype. This pattern of low mtDNA variation *and* a lack of appreciable geographic population structure is extremely unusual for a widely distributed animal species. For purposes of taxonomy and conservation, these findings suggest the presence of only one 'evolutionarily significant unit' for *C. serpentina* in this otherwise phylogeographically rich region of the country. Possible explanations for this phylogeographic pattern in the snapping turtle are considered.

INTRODUCTION

Mitochondrial DNA approaches were introduced to natural population analysis in the late 1970s (Avise, Giblin-Davidson *et al.*, 1979; Avise, Lansman & Shade, 1979; Brown & Wright, 1979). Scores of mtDNA phylogeographic assessments are now available for a wide variety of vertebrate and invertebrate species (reviewed by Avise, 1994, in press). Nearly all assayed species display extensive intraspecific mtDNA variation, typically arrayed into one or another of the following categories of phylogeographic pattern as defined by Avise, Arnold *et al.* (1987): (I) discontinuous ('deep') phylogenetic separations between allopatric branches in a mtDNA gene tree; (II) discontinuous phylogenetic separations between sympatric gene-tree branches; (III) continuous ('shallow') phylogenetic separations between allopatric gene-tree branches; and (IV) continuous phylogenetic separations between sympatric gene-tree branches. A fifth conceivable pattern (category V) was outlined by Avise, Arnold *et al.* (1987) that has seldom been reported for any animal species: a near absence of detected mtDNA variation over a broad geographic range. Here we report this pattern for populations of the common snapping turtle in the south-eastern USA.

The range of *C. serpentina* within North America extends from the east coast to the Rocky Mountains and from southern Canada to the Gulf of Mexico (Ernst, Lovich & Barbour, 1994). Two morphological sub-species are recognized: *C. s. osceola* in peninsular Florida and parts of south Georgia, and *C. s. serpentina* across the remainder of the North America range (Ernst & Barbour, 1989). Richmond (1958) suggested that the Floridian form be elevated to species status. Feuer (1971) reported intergradation between the two subspecies near the Okefenokee Swamp.

Surveys of mtDNA restriction sites and/or nucleotide sequences in several other aquatic and terrestrial turtles in the south-eastern USA have revealed modest-to-high levels of intraspecific variation and strong geographic partitioning of gene-tree branches (Avise, Bowen *et al.*, 1992; Osentoski & Lamb, 1995; Walker, Burke *et al.*, 1995; Walker, Nelson *et al.*, 1997; Walker, Moler *et al.*, in press). Results reported here for the common snapping turtle depart rather strikingly from these conventional phylogeographic patterns.

METHODS

Samples and laboratory procedures

Tail snips or blood samples were taken from a total of 66 wild-caught individuals representing 38 locales and more than 20 drainages in 10 states (Fig. 1). Details of the collection sites are available from the senior author

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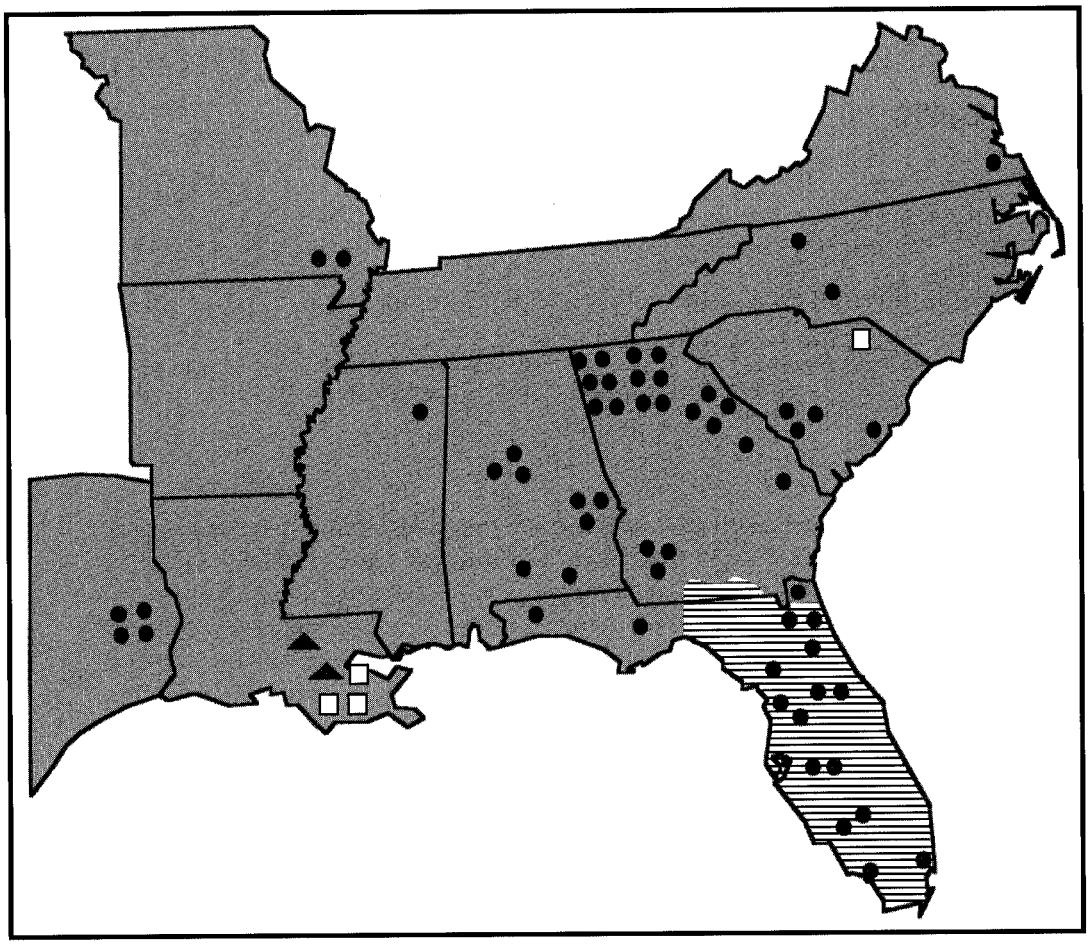

Fig. 1. Map of the south-eastern USA showing the collection sites for 66 snapping turtles. • represent individuals that displayed the common mtDNA haplotype; □ are specimens that differed from the common haplotype by a single assayed transition; and ▲ are specimens that displayed the indel (see text). Also indicated are the south-eastern ranges of the two traditionally recognized subspecies, *C. serpentina serpentina* (shaded) and *C. s. osceola* (striped). Note that the broader species' range also includes most of eastern and central North America, Central America, and north-western South America.

upon request. In addition, one sequence from a related species, the alligator snapping turtle (*Macroclemys temminckii*), was used for reference (Roman, 1997).

Total DNA was extracted following the methods of Taggart *et al.* (1992) and the samples were used for PCR-based sequencing of a portion of the $tRNA^{PRO}$ gene and the adjoining 5′ end of the mtDNA control region. The primers for initial PCR amplifications and sequencing were those used previously for map turtles (Lamb *et al.*, 1994). Under low stringency conditions, these primers amplified a product which then was sequenced to design a new set of primers specifically for snapping turtles: CS1 = 5′CTAGAA-TAATCAAAAGAGAAGG3′; CS2 = 5′GGACGCCA-TAACACAAT3′. The resulting PCR product in *C. serpentina* was approximately 480 bp in length.

Double-stranded PCR products were purified using Wizard PCR Preps (Promega) and then utilized for sequencing reactions with the *fmol* DNA Sequencing System (Promega). Both strands were sequenced. Owing to the unexpected uniformity of the mtDNA sequences, representative samples were also processed independently by two outside laboratories.

Data analyses

Sequences were aligned easily by eye. For purposes of analysis, a gap was counted as a fifth base. Nucleotide and genotypic diversities were calculated from the observed sequence differences (p) following the methods of Nei (1987). A representative sequence from the snapping turtle is deposited in GenBank (accession number AF029986).

RESULTS

A total of 409 bp was sequenced from all individuals. Nucleotide sequences proved to be identical for 60 of the 66 snapping turtles assayed (see Fig. 2). Two additional haplotypes were observed in five specimens from Louisiana: three individuals differed from the most frequent *C. serpentina* haplotype by a single transition at position 206 (as numbered from the 5′ end in the reference sequence) and two individuals carried the same transition plus a 1 bp insertion/deletion (indel) at position 137. One individual from South Carolina shared the same transition with the three specimens

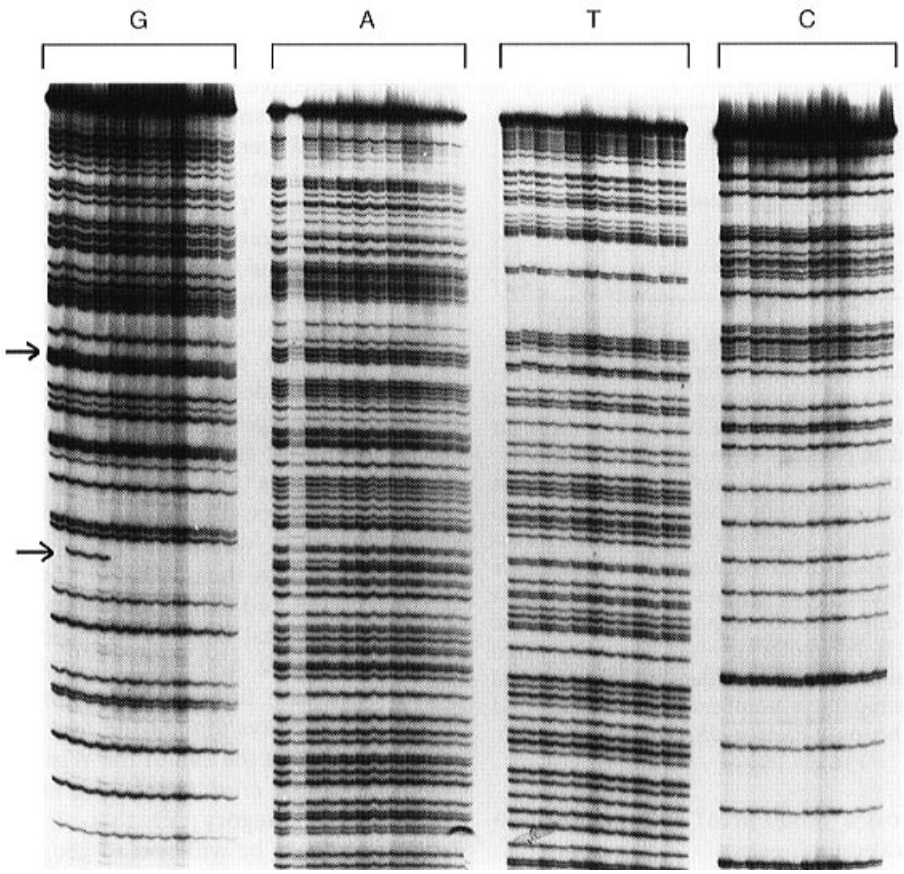

Fig. 2. Autoradiogram of mtDNA control region sequences from 12 snapping turtles. The lanes of the gel are grouped into four blocks of 12 each corresponding, from left to right, to the 'G', 'A', 'T', and 'C' bases. Also displayed are the common haplotype (lanes 1 and 5–12 in each block), as well as the two variant haplotypes (lanes 2–4). Arrows indicate the positions in the gel that distinguish these variant sequences from the common mtDNA haplotype.

from Louisiana and, thus, was identical to them in this control region sequence.

If the indel is counted as a single mutation event, mean and maximum sequence divergences between all pairs of individuals in *C. serpentina* are 0.0006 and 0.005, respectively. Genotypic diversity was 0.17. The haplotype from *M. temminckii* was identical in length to the common haplotype in *C. serpentina* but differed from it at an estimated interspecific sequence divergence level of 0.024 (Roman, 1997).

DISCUSSION

Shallow phylogeography

The absence of appreciable mtDNA variation and differentiation over a broad geographic range is an unusual finding in an animal species. To illustrate and emphasize this point, consider the contrast between the genetic results for *C. serpentina* and those for another co-distributed turtle species similarly surveyed, the musk turtle *Sternotherus minor* (Walker, Burke *et al.*, 1995). Both species were assayed for approximately the same section of the mtDNA control region, sample sizes were 66 and 52 individuals, respectively, and collections were taken from across the south-eastern USA. However, far greater mtDNA variation and phylogeographic differentiation were evident in *S. minor* (Fig. 3). We do not claim to have documented the 'null hypothesis' that mtDNA differentiation is absent in *C. serpentina* across the south-eastern USA. Nonetheless, the limited differentiation interpreted in a comparative context remains striking.

Our findings agree with those of Phillips, Dimmick & Carr (1996) who found minimal differentiation in a whole mtDNA restriction-fragment length polymorphism (RFLP) survey of 15 snapping turtles (total) from four widely separated states in eastern North America. In that study, ≤ 3 surveyed restriction-site differences ($P \cong 0.005$) distinguished mtDNA haplotypes from Florida, Missouri, Illinois, and Oklahoma. Phillips *et al.* (1996) concluded: 'the similarity of the mtDNA haplotypes among the North American samples ... is surprisingly high in view of the geographic distance between collection sites'; and 'The mitochondrial and allozyme data reported here do not distinguish the peninsular Florida populations from other North American populations'.

What could account for the currently-reported pattern of relative uniformity in mtDNA sequence in

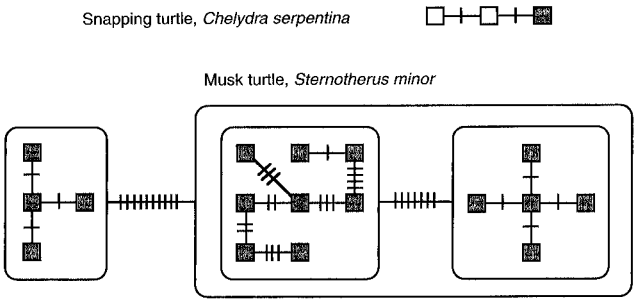

Fig. 3. Hand-drawn parsimony networks emphasizing the disparity in mtDNA phylogeographic patterns between conspecific snapping turtles (current study) and musk turtles (data from Walker, Burke *et al.*, 1995). Each square is a different haplotype, and shaded haplotypes were localized geographically. Larger boxes in the network for musk turtles encompass arrays of related haplotypes. Within a box, each haplotype is joined to its nearest genetic neighbors, with slashes across network branches indicating observed numbers of nucleotide differences. Between boxes, such slashes indicate the *mean* number of nucleotide differences distinguishing haplotypes in the respective arrays. Both studies involved sequences from approximately the same portion of the mtDNA control region, and entailed comparable numbers of individuals and sample locales across the south-eastern USA.

snapping turtles across a broad area? Several possibilities warrant consideration.

(a) *The portion of the control region sequenced is evolutionarily constrained.* This possibility is unlikely. The sequence assayed is located near the 5′ end of the control region and extends into the adjacent $tRNA^{PRO}$ gene. Typically, this section of the control region is considered the *most rapidly* evolving part of mtDNA in other animals (Greenberg, Newbold & Sugino, 1983; Kocher & Wilson, 1991; Brown, Beckenbach & Smith, 1993; Edwards, 1993). This region has displayed considerable variability in other turtle species as well (Lamb *et al.*, 1994; Walker, Burke *et al.*, 1995; Walker, Nelson *et al.*, 1997; Walker, Ortí & Avise, in press; see also Fig. 3). Furthermore, as mentioned above, the conservative pattern of mtDNA differentiation in *C. serpentina* also was registered in whole-mtDNA RFLP assays (Phillips *et al.*, 1996).

(b) *Laboratory reagents were contaminated by sample DNA.* This is extremely unlikely for several reasons. First, negative controls were conducted and revealed no amplification products. Second, mtDNA variation was detected among some of the *C. serpentina* samples. Third, as a further precautionary check, small numbers of tissue samples from *C. serpentina* were assayed independently by two other laboratories (Riverbend Research Station at the University of Georgia, and the BEECS Genetic Core Facility at the University of Florida), and identical results were obtained.

(c) *A selective sweep in mtDNA has recently passed through the species.* If a mtDNA mutation under positive selection arose recently in *C. serpentina* and swept through the species, a near-absence of variation in mtDNA would result. Appropriate assays of the nuclear genome would be of interest to test this possibility because a selective sweep specific to mtDNA would not affect genes transmitted through both genders as profoundly. However, even if a selective sweep was involved, it alone is not sufficient to account for the mtDNA pattern. The sweep must have been accompanied by moderate to high gene flow in recent evolutionary time for its footprints to be registered across a broad geographic area.

(d) *The species is characterized by moderate to high gene flow and/or a recent range expansion.* Snapping turtles are considered habitat generalists because they occur in many types of freshwater and brackish environments from large rivers to seasonal wetlands. They also display a propensity to move across land, sometimes for several kilometers (Ernst *et al.*, 1994). As phrased by Cahn (1937), "a 'wanderlust' ... frequently attacks the turtles and drives them afield." The overland mobility of these turtles and their wide habitat usage suggest that few environmental barriers to dispersal exist. Furthermore, Holman & Andrews (1994) note that among all North American turtles, *C. serpentina* (and *Chrysemys picta*) 'were always among the first to invade formerly glaciated areas at the end of the Wisconsin'.

Avise, Arnold *et al.* (1987) also envisioned the possibility (their category V) that a common mtDNA haplotype could be widespread geographically within a species, with rare haplotypes (presumably recently arisen) localized within that range. This pattern suggests 'intermediate gene flow in a species not subdivided by long-term zoogeographic barriers', and is similar to the outcome for *C. serpentina* in the south-eastern USA. If the rare haplotypes were widespread geographically, high gene flow (or recent range expansion) would be implicated. Given our limited sample sizes, the data bearing on this point are ambiguous for *C. serpentina*. One rare mtDNA haplotype was observed both in Louisiana and South Carolina, but it may have arisen multiple times because it differs from the common haplotype by one assayed transition. A second rare haplotype was confined to Louisiana but more extensive sampling might reveal its presence elsewhere.

High gene flow will cause a species to approach panmixia such that mtDNA diversity under neutrality then will be governed by effective population size. If mtDNA in *C. serpentina* evolves at a standard pace and a selective sweep has not been involved, the paucity of mtDNA variation in this species would suggest a historical population bottleneck followed by a relatively recent range expansion.

In any event, the mtDNA data strongly suggest that long-standing evolutionary separations have not been a part of the phylogeographic history of contemporary populations of *C. serpentina* in the south-eastern USA. In this regard, this species departs dramatically from patterns reported for many other freshwater and terrestrial turtles in the area.

Conservation relevance

It has become customary to employ molecular markers to help identify 'evolutionarily significant units' (ESUs) for conservation (Avise, 1989; Daugherty *et al.*, 1990; Moritz, 1994). Molecular analyses sometimes reveal relatively deep phylogeographic partitions that were unrecognized by morphological evidence and unregistered in taxonomic assignments. Such was the case, for example, in a broader geographic survey of *Chelydra serpentina*, where Phillips *et al.* (1996) suggested from mtDNA evidence that populations in Central and South America should be recognized as two species distinct from the North American form. Such was also the case for several freshwater and terrestrial vertebrates in the south-eastern USA, where molecular evidence (reviewed by Avise, 1996) revealed relatively deep historical separations between eastern versus western arrays of populations in the region.

The converse outcome, in which molecular analyses fail to identify relatively deep phylogeographic partitions in widely distributed species, has seldom been reported. Although such outcomes will probably not serve to promote new conservation initiatives, they should not go unreported. Scientific re-appraisals of biodiversity patterns from molecular or other sources of information must, of course, remain fair and unbiased with respect to reported outcomes and conservation ramifications.

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