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A comparison of mtDNA restriction sites vs. control region sequences in phylogeographic assessment of the musk turtle (*Sternotherus minor*)

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Abstract

A total of nearly 800 base pairs of mitochondrial DNA sequence was assayed in each of 52 musk turtles (*Sternotherus minor*) collected across the species' range in the south-eastern USA. About one-half of the sequence information in effect was accessed by conventional recognition-site assays of the entire mtDNA molecule; the remainder came from direct sequence assays of a normally hypervariable 5′ section of the noncoding control region. The two assay methods produced essentially nonoverlapping sets of variable character states that were compared with respect to magnitudes and phylogeographic patterns of mtDNA variation. The two assay procedures yielded nearly identical outcomes with regard to: (a) total levels of species-wide mtDNA genetic variation; (b) mean levels of within-locale variation; (c) extremely high population genetic structure; (d) a phylogenetically significant separation of samples from the north-western half of the species' range vs. those in the south-eastern segment; and (e) considerably lower genetic variability within the north-western clade. The micro- and macro-phylogeographic mtDNA patterns in the musk turtle are consistent with a low-dispersal natural history, and with a suspected longer-term biogeographic history of the species, respectively.

Keywords: intraspecific phylogeny, mitochondrial DNA, phylogeography, population structure

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Introduction

For the past 16 years, mitochondrial (mt) DNA analyses have been widely employed to assess the intraspecific matriarchal phylogenies of numerous animal species (Avise 1994). The assays typically involved comparisons of restriction sites (or fragments) sampled across the whole mtDNA molecule, using techniques of gel electrophoresis. With the advent of PCR-based methodologies, nucleotide sequence analyses of particular segments of mtDNA have become more commonplace (e.g. Kocher *et al.* 1989; Meyer *et al.* 1990; Di Rienzo & Wilson 1991). In any such conversion between assay procedures, or between target sequences assayed, it is important to compare the new information against the old. However, surprisingly little attention has been devoted to direct appraisals of sequence vs. RFLP data in assessing levels of genetic variation and phylogeographic patterns within one-and-the-same array of individuals and populations.

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Here we apply both restriction site analyses of the whole mtDNA genome and direct sequencing of the mtDNA control region to assess genetic variation and intraspecific phylogeography of the musk turtle, *Sternotherus* (or *Kinosternon*) *minor*. This species inhabits lotic freshwater environments in the south-eastern USA, with two morphological subspecies recognized: *S. m. minor* ('loggerhead' musk turtle) in the south-eastern half of the range, and *S. m. peltifer* ('stripeneck' musk turtle) to the north and west (Fig. 1; Ernst & Barbour 1989). These two forms reportedly intergrade in the Florida panhandle and adjacent areas (Iverson 1977; Conant & Collins 1991). This species is strongly aquatic, with individuals rarely leaving water except during heavy rains, or briefly to lay eggs along stream banks.

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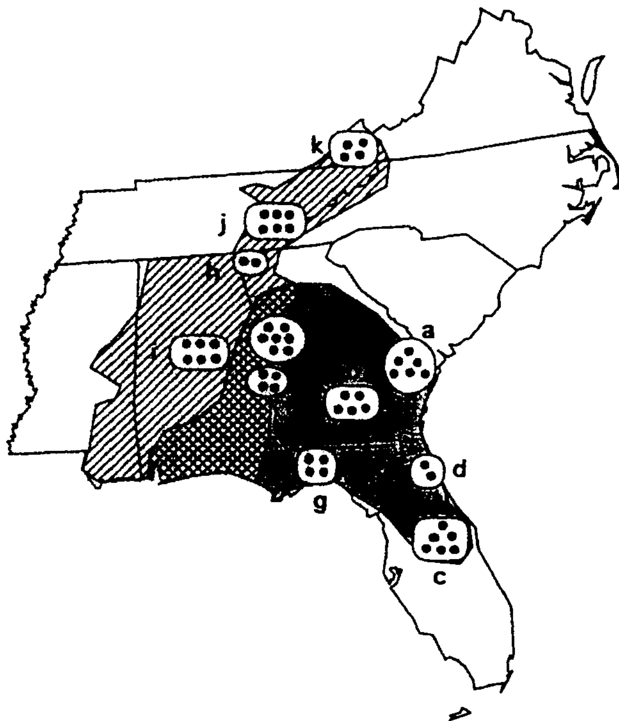

Fig. 1 Map of the south-eastern USA showing the 11 collection locales from which a total of 52 musk turtles was collected. Locales are lettered as in Materials and Methods. Also shown are the geographic ranges of the two conventionally recognized subspecies: *S. m. minor* (shaded), *S. m. peltifer* (hatched), and the presumed intergrade zone between them (cross-hatched) (after Conant & Collins 1991).

The primary goals of this study were to: (a) assess patterns of mtDNA variation within and among populations of the musk turtle; (b) compare results from restriction site analyses to those from control region sequences; (c) consider the previous literature on this topic from other vertebrate species; and (d) contribute to a developing data base for comparative assessment of intraspecific phylogeography in other freshwater and terrestrial turtle species.

Materials and methods

Samples and laboratory procedures

A total of 52 musk turtles was collected from 11 locations (Fig. 1), as follows: *Georgia coastal basins* – (a) McKinney's Pond, Emanuel Co., GA ($n = 6$); (b) Oscheweeche Springs, Wilcox Co., GA. (5); *St. John's basin* – (c) canal south of Lake Jessup, Seminole Co., FL (6); (d) near Six Mile Creek, St. Johns Co., FL. (2); *Apalachicola basin* – (e) Flint River, Pike and Meriwether Cos., GA. (7); (f) Little Uchie Creek, Russell and Lee Cos., AL. (4); *Ochlockonee basin* – (g) Ochlockonee River, Leon and Liberty Cos., FL (4); *Mobile basin* – (h) Conasauga River, Murray Co., GA. (2); (i) Cahaba River, Jefferson Co., AL. (6); *Tennessee basin* – (j) Citico Creek, Monroe Co., TN. (6); and (k) Copper Creek, Scott Co., VA. (4). In some of the analyses, three individuals of a related species, the stinkpot turtle *S. odoratus* [from the Cahaba River, Jefferson Co., AL. (1); North Fork of the Holston River, Scott Co., VA. (2)] were employed as outgroups (Seidel *et al.* 1981, 1986; Iverson 1991). Sample sizes per locale were small (dictated by the biology and abundance of the species), so conclusions regarding local population structure are tentative.

Total DNA was extracted from heart, liver, and muscle, and mtDNA was isolated following procedures in Lansman *et al.* (1981). Purified mtDNA was used for RFLP analyses of the entire molecule, and as a source for PCR-based sequencing of an amplified 5′ portion of the control region [reported to be rapidly evolving in some mammals and fishes (e.g. Baker *et al.* 1993; Brown *et al.* 1993; Meyer *et al.* 1990)].

The 13 informative restriction endonucleases listed in Table 1 were employed to assay closed-circular mtDNA. Fragments were end-labelled using Klenow and either ^{35}S- or ^{32}P-labelled nucleotides before electrophoresis in 1.0–1.5% agarose gels (Lansman *et al.* 1981). Fragments were visualized by autoradiography, and sized by comparison to a 1-kb ladder. Although restriction sites were not mapped formally, the digestion profiles for all enzymes used could be interpreted in terms of restriction site changes. [Enzymes with complex digestion patterns (notably *Bst*EII and *Msp*I) were scored conservatively such that the minimum number of site changes consistent with the digestion profiles was assumed.] No mtDNA size differences among individuals were evident, although fragments smaller than 0.5 kb (or 1.0 kb in the case of *Bst*EII) were not scored.

Table 1 MtDNA RFLP haplotypes observed in musk turtles, as summarized in multienzyme letter codes. Letters, from left to right, represent digestion profiles for the following endonucleases: *Ava*II, *Bcl*I, *Bgl*II, *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Msp*I, *Pvu*II, *Stu*I and *Spe*I. Locales are labelled as in Materials and Methods

Haplotype code	Haplotype description	n	locale (n)
RS1	CCCCCCCCCCCCC	8	a (6); b (2)
RS2	ACDCCBCCCCCDC	2	b (2)
RS3	CCBCCCCCCCCCC	1	b (1)
RS4	CCDCCBCDCCCDC	8	c (6); d (2)
RS5	CCDCCCCCCCCDC	5	e (4); g (1)
RS6	CCDCCCCCCCCCC	9	e (3); f (4); g (2)
RS7	CDECCCCCCCCDC	1	g (1)
RS8	EGDADCCACFCBD	13	j (6); i (5); h (2)
RS9	EGCADCCACFCBD	1	i (1)
RS10	EGDADDCACFCBD	4	k (4)

The initial control region primers for the PCR reactions (LTCM1 and HDCM1) were designed for marine turtles by Allard *et al.* (1994). Under low-stringency conditions, these produced successful amplifications of control region sequences from *S. minor*, and permitted the subsequent design of refined internal primers near the 5′ end of the control region and extending into the adjacent tRNA^{PRO} gene. These were designated DW1 (5′-CCCTTTGATA-AAAGATACGGATCTTACGGC-3′; nucleotides underlined are in tRNA^{PRO}) and DW2 (5′-GATTAATAGTCTAG-AACTTACTGACCAAAGGC-3′). They span a stretch of approximately 450 bp of control region sequence, of which 429–430 bp were scored in all individuals. Sequence reactions were run on the double stranded PCR product using the fmol DNA Sequencing System from Promega (1991). All sequences were scored in both directions.

Data analyses

For the RFLP data, individual haplotypes were coded in a restriction site presence/absence matrix. Each restriction site map for a particular enzyme was also assigned an uppercase letter code, such that a string of 13 letters (one for each informative endonuclease) provided an abbreviated description of the RFLP clonal genotype for an individual. For the sequence data, haplotypes consisted of the 429–430 bp control region sequences, which could be aligned visually without ambiguity. Haplotypes identified in the restriction site assays were assigned 'RS' numbers, control region sequences 'CR' numbers, and composite data 'TD' numbers.

Estimates of nucleotide sequence divergence (p) between haplotypes were calculated using the site approach of Nei & Li (1979) for restriction site data, and by direct counts of nucleotide sequence differences for control region sequences. [Corrections for multiple substitutions at a site proved unnecessary because all p estimates were low.] Levels of genetic variation were summarized using genotypic- and nucleotide-diversity statistics (Nei 1987).

Phylogenetic estimates were obtained from distance-based and qualitative methods. Genetic distance matrices were clustered by UPGMA (Sneath & Sokal 1973) and neighbour-joining (Saitou & Nei 1987) using PHYLIP (Felsenstein 1991); restriction site and sequence matrices were analysed by parsimony using PAUP (Swofford 1990). Bootstrapping (100 replicates) assessed the statistical support for putative clades.

Results

For the restriction site data, a total of 367 bp was represented in the recognition sequences of the enzymes scored in each individual; for the control region sequence data, a total of 429–430 bp was scored per specimen. Thus, roughly similar amounts of genetic information were screened by the respective approaches. The raw data from the restriction site assays are summarized in Tables 1 and 2, and those from control region sequences in Table 3.

Levels of genetic variability

Overall levels of mtDNA variability as revealed by the RFLP vs. sequence assays were remarkably similar (Table 4). For example, for the pooled collection of individuals, nucleotide diversities as estimated by the two methods proved identical (0.017), and genotypic diversities were only slightly higher for control region assays ($h = 0.925$ vs. 0.859). Furthermore, although the sequence data revealed more haplotypes (17 vs. 10), the numbers of variable character states were similar (22 vs. 25, respectively).

The two data sets were also similar with respect to mean levels of variability *within* local population samples (Table 4). For example, mean genotypic diversities for the control region sequences as opposed to RFLP data were $h = 0.276$ vs. 0.231, and the respective numbers of different haplotypes were 1.73 vs. 1.55. Only in nucleotide diversity was there a hint of discrepancy in mean variability levels within populations (0.0017 vs. 0.0009), but even here the difference was not statistically significant ($t = 0.82$, d.f. = 20, $P \approx 0.4$).

Table 2 MtDNA RFLP haplotypes observed in musk turtles, as summarized in a matrix of presence (1) vs. absence (0) of restriction sites

mtDNA code	Restriction sites
RS1	111110111110010001110011111111011111011111111111111101111101111100
RS2	101010111110010101110011110111011111011111111111111101111101111110
RS3	111110111110011001110011111111011111011111111111111101111101111100
RS4	111110111110010101110011110111011111111111111111111101111101111110
RS5	111110111110010101110011111111011111011111111111111101111101111110
RS6	111110111110010101110011111111011111011111111111111101111101111100
RS7	111110111111010111110011111111011111011111111111111101111101111110
RS8	110111110110110101101110111111011111100111110011111111111111111101
RS9	110111110110110001101110111111011111100111110011111111111111111101
RS10	110111110110110101101110111111111111100111110011111111111111111101

Table 3 MtDNA control region haplotypes observed in musk turtles, as summarized in an abbreviated matrix showing only the variable nucleotide sites. Dashes indicate 1 bp deletions; dots indicate identity to CR1. The full sequence for CR1 is deposited in GenBank (accession number U19540)

mtDNA code	Sequence at variable positions	n	Locale (n)
CR1	CTTTTAATAAATCCCTAAGTGGACCAACTCTTACACCA	6	a (6)
CR2	................G...AA..........G.C...	1	b (1)
CR3	........-...T.......AA................	1	b (1)
CR4	............T.......AA..........G.C...	1	b (1)
CR5	................G...AA................	2	b (2)
CR6	........GG..T..C....AA..........G...T.	6	c (6)
CR7	........GG..T..C....AA..............T.	2	d (2)
CR8	T..C................AA..T.......G.....	4	e (4)
CR9	T..C.........T......AA..T.....C.G.....	3	e (3)
CR10	T..C.........T......AA..T.......G....G	4	f (4)
CR11	T..C.........T......AA..T.......G.....	2	g (2)
CR12	T..C...A.....T......AA..T.......G.....	1	g (1)
CR13	....C.......TT......AA..T.......G.C...	1	g (1)
CR14	..C..T..-G..T.AC....A...........G....G	6	j (6)
CR15	..C..T..-GG.T.AC....A...........G.....	1	i (1)
CR16	..C..T..-G..T.AC....A...........G.....	9	i (3); h (2); k (4)
CR17	.....T..-G..T.AC....AA..........G.....	2	i (2)
CR18*	.C...TG.T..-T.A..GCCAAGTTTTTCTCGGT.G..	3	

*Outgroup: *Sternotherus odoratus*.

Table 4 Estimates of genetic variability in mtDNA restriction site data vs. control region mtDNA sequences in the musk turtle

	Assay method	
	restriction sites	sequences
All locales pooled		
no. individuals	52	52
no. bp surveyed	367*	430
no. variable characters†	25	22
no. different haplotypes	10	17
genotypic diversity‡	0.859	0.925
nucleotide diversity§	0.017	0.017
Mean values per locale ± SE		
no. individuals	4.7	4.7
no. bp surveyed	367*	430
no. variable characters†	1.00 ± 0.57	1.36 ± 0.66
no. different haplotypes	1.55 ± 0.25	1.73 ± 0.33
genotypic diversity†	0.231 ± 0.104	0.276 ± 0.118
nucleotide diversity§	0.0009 ± 0.0006	0.0017 ± 0.0008

* Total no. of bp in recognition sequences of the endonucleases employed.
† Restriction sites for RFLP data; nucleotide positions for sequence data.
‡ $h = [n/(n-1)](1 - \Sigma f_i^2)$, where f_i is the frequency of the ith haplotype.
§ mean $P = [n/(n-1)](\Sigma f_i f_j p_{ij})$, where f_i and f_j are the frequencies of the ith and jth haplotypes in a sample of size n, and p_{ij} is the estimated sequence divergence between the ith and jth sequences (Nei 1987).

Phylogeographic patterns

Restriction site data. The outgroup *S. odoratus* showed sufficiently large differences from *S. minor* that the fragment digestion profiles for most enzymes could not be interpreted in terms of particular restriction site changes. Thus, phylogenetic analyses were confined to the *S. minor* samples, and the resulting parsimony networks considered unrooted. A hand-generated parsimony network with restriction site changes is shown in Fig. 2(A), and this network is superimposed over the geographic sources of the collections in Fig. 3. An exhaustive search of the RFLP data using PAUP revealed 33 most-parsimonious networks, each of total length 27 steps. However, in bootstrap analyses, only one partition was supported at a level greater than 60% – that which distinguished haplotypes RS8, RS9, and RS10 from all others (100% support). A majority-rule consensus summary of the computer-generated networks is presented in Fig. 2(B).

Two major features of the RFLP data stand out. First is the striking geographic structure evident in the distribution of particular mtDNA genotypes (Fig. 3). Although local population samples were small, all observed mtDNA haplotypes appeared strongly localized geographically.

The second major feature of the data is the deep phylogeographic partition between haplotypes RS1–RS7 vs. RS8–RS10. This separation is evident in all analyses; maximum parsimony, where bootstrap support was at the 100% level; UPGMA analysis (not shown), where the level of clustering between the two groups was at $P = 0.036$ [more than four times higher than the maximum level of genetic clustering ($P = 0.008$) within either group]; and neighbour-joining analyses (Fig. 4). These two well-supported genetic subdivisions display a strong geographic orientation, with the latter confined to the north-western half of the species distribution (Mobile and Tennessee drainage basins) and the former confined to the south-eastern area (Fig. 3).

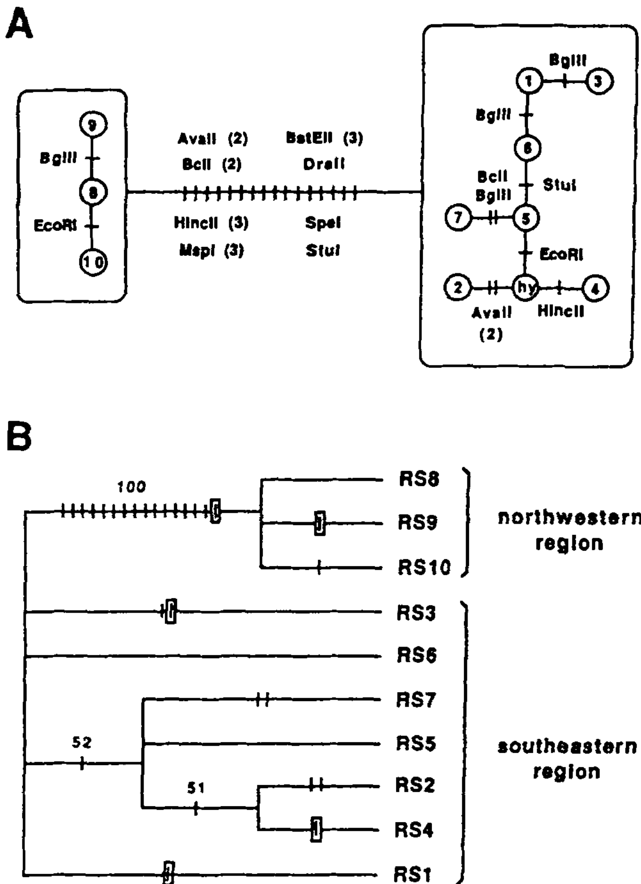

Fig. 2 Maximum parsimony networks summarizing relationships among mtDNA haplotypes in musk turtles as revealed by the restriction site assays. Hy is a hypothetical haplotype not actually observed, and slashes crossing network branches are inferred character state changes. (A) Hand-drawn network showing endonucleases responsible for the restriction site changes. (B) 50% majority-rule consensus network, with bootstrap support levels indicated. Rectangles enclose particular character state changes that occurred more than once on the network. The consistency index is 0.89, indicating relatively little homoplasy in the data.

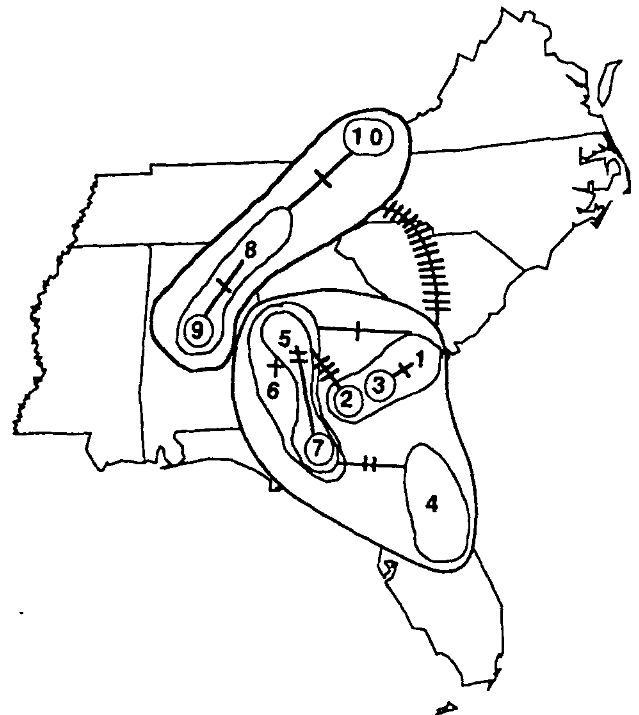

Fig. 3 Same parsimony network as in Fig. 2(A), overlaid on the geographic sources of the samples.

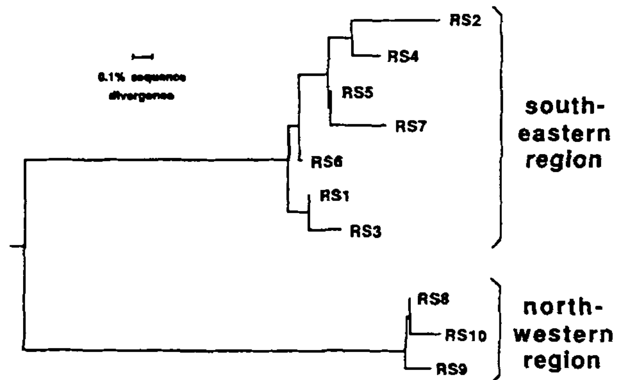

Fig. 4 Neighbour-joining network (midpoint rooted) for musk turtle mtDNA haplotypes as revealed in the restriction site assays.

Control region sequences. In these assays, all mtDNA haplotypes appeared geographically localized. Among the 17 haplotypes observed, only one (CR16) was observed at more than a single collection site (Fig. 5).

With respect to broader phylogeographic relationships, several similarities with the whole-genome restriction site data were evident (Figs 6 and 7). Most noticeable was the strong support (90% bootstrap) for the northwestern clade of musk turtles that also had been apparent in the restriction site information (Figs 2–4). On average, haplotypes within this array differed from those in the south-eastern region at more than nine nucleotide positions, whereas they differed from one another at only one or two nucleotide sites. This north-western assemblage was also characterized by a one bp deletion in the control region sequence (Table 3), a feature shared with only one haplotype (CR3) observed elsewhere.

Also in general agreement with the restriction site data was an assemblage of related haplotypes (CR8–CR12) characterizing samples from the Apalachicola and Ochlockonee drainages (84% bootstrap; compare to Fig. 3); and a group of two related haplotypes (CR6 and CR7) confined to the St. Johns drainage (76% bootstrap; compare to Fig. 3). No other putative clades were supported at bootstrap levels greater than 60% in the control region data (as also was true for restriction sites).

Regional levels of genetic variability also were qualitatively consistent between the restriction site and sequence data. Fewer genotypes were observed in the north-west

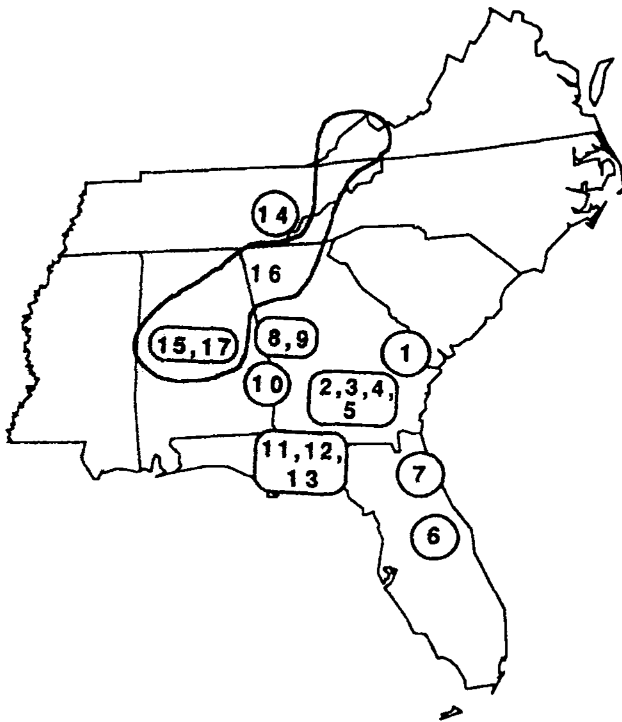

Fig. 5 Geographic distributions of mtDNA haplotypes in musk turtles as revealed in sequence assays of the control region.

sector than in the south-east (3 vs. 7 in restriction site assays; 4 vs. 13 in control region sequences), and five-fold lower nucleotide diversities characterized the north-western compared to the south-eastern regions (0.0007 vs. 0.0035, restriction sites; 0.0029 vs. 0.0139, control region

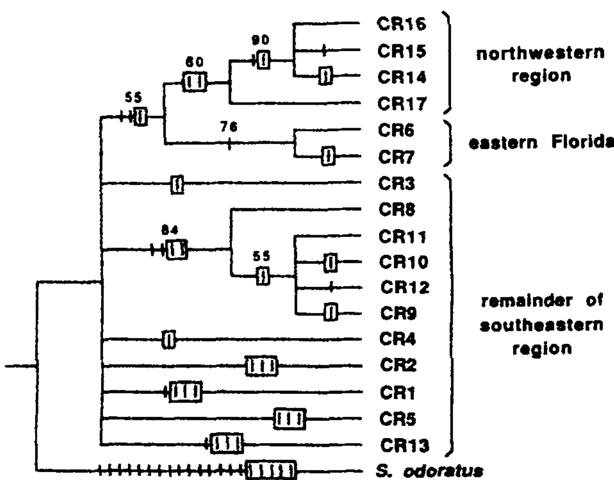

Fig. 6 Parsimony network (50% majority-rule consensus) for musk turtles based on mtDNA control region sequences, rooted using the outgroup *S. odoratus* (sequences identical in three individuals). Numbers indicate levels of bootstrap support greater than 50%. Also shown (slashes crossing tree branches) are inferred nucleotide substitutions, with those enclosed in rectangles indicating particular character state changes that occurred more than once on the tree. The consistency index is 0.69, indicating considerable homoplasy.

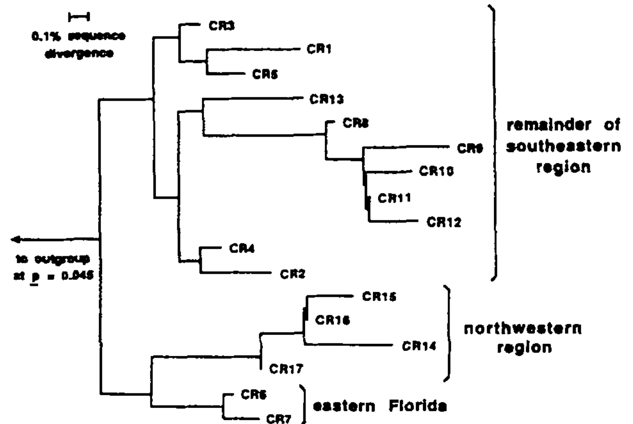

Fig. 7 Neighbour-joining tree for musk turtles based on mtDNA control region sequences, rooted using the outgroup *S. odoratus* (sequences identical in three individuals).

sequences). However, the depth of the genetic separation between the south-eastern and north-western genetic arrays was not as evident from the control region sequences, and indeed the eastern Florida samples tended to group with the north-western clade in some of the phylogenetic analyses (not at statistically supportable levels; Figs 6 and 7).

Combined data sets. Direct inspections of control region sequences indicated that four (among 25) variable restriction sites scored occurred within the sequenced region. All remaining variable characters in the restriction site and sequence data bases were independent, and thus could be considered in composite. From this combined data set, a total of 21 different mtDNA haplotypes was observed among the 52 musk turtles, and genotypic diversity was $h = 0.944$.

A qualitative picture of the phylogeographic pattern remained much the same. Thus, samples from seven of the 11 locales were each fixed for a composite mtDNA haplotype observed nowhere else, and with one exception, all remaining haplotypes were confined to particular collection locales. The genetic distinctiveness of samples in western Georgia, eastern Alabama, and the Florida panhandle was again evident (75% bootstrap). Finally, the distinction between the north-western and south-eastern genetic arrays was supported at the 100% bootstrap level in an unrooted parsimony network (not shown).

Distance matrix correlations

One may also ask whether genetic distances between individuals quantitatively covary for the restriction-site vs. sequence data. Pairwise distances in a matrix are not independent values, so statistical tests of matrix correlations

(Sneath & Sokal 1973) were conducted using random permutations (1000 pseudo-replicates) of values in the relevant distance matrices. Because of small sample sizes and low genetic variability, only one such meaningful comparison was possible *within locales*. At locale b ($n = 5$), RFLP assays revealed three haplotypes that differed by as many as six restriction sites, and sequence analyses uncovered four control region haplotypes that differed at as many as five nucleotide positions. At this locality, the correlation ($r = 0.59$) between the genetic distances for pairs of individuals in these two data sets was not significant (marginally) as judged by the distribution of correlations in the null permutation tests. However, when all 52 individuals in the study were considered, the observed correlation between the restriction-site and sequence distance matrices was $r = 0.54$, a value well above the 95% confidence bounds registered in the permutation tests.

Discussion

Comparisons of methods

New or streamlined molecular assays frequently have been introduced into population genetics, often bidding to replace older (but sometimes 'tried-and-true') methods. Such is the case with the recent development of PCR technologies, which facilitate direct sequence analyses and threaten to make obsolete RFLP procedures which provided the great bulk of DNA-level population genetic data over the past 16 years. In evaluating the merits of any new approach, benefits (ease of use, reliability, etc.) must be weighed against costs (monetary and otherwise). One potential cost concerns whether the information generated by the new method can be effectively integrated with or compared to the old. This is of particular concern in evolutionary biology, where conclusions of broader biological significance frequently depend upon comparative analyses based on the accumulation of relatable data from multiple taxa or evolutionary settings.

In this study, we directly compared conventional mtDNA restriction site approaches against control region sequencing in assessing levels of genetic variation and phylogeographic patterns in the musk turtle. The two approaches yielded remarkably similar conclusions: (a) Nearly identical levels of variation were revealed, both with respect to the samples overall, and to mean values per locale; (b) Tremendous local population structure was evident; (c) In both assays, a phylogenetic array of samples in the north-western portion of the species' range was significantly distinguished from an array in the south-eastern portion; and (d) Levels of genetic variation were consistently much lower in the north-western assemblage.

One point of lesser agreement concerned the relative magnitudes of genetic separation between the north-western and south-eastern mtDNA arrays. In the RFLP data, the phylogenetic dichotomy was sharp, with more than twice as many restriction site changes inferred between the two regional populations, on average, than occurred maximally within either; but in the control region sequence data, some genetic differences within the south-eastern assemblage were equal to or exceeded the mean level of divergence between the two regions. This pattern contributes to the higher sequence diversity characterizing the south-eastern samples, and also suggests that the latter assemblage might be paraphyletic with respect to the north-western clade.

Because the restriction-site and sequence approaches yielded similar conclusions about the magnitudes and patterns of population genetic structure in *S. minor*, relative merits of the two assay procedures must be decided by other criteria. In general, control region sequencing would be favoured by the following: (a) non-destructive sampling of organisms may be facilitated; (b) inclusion of outgroups (particularly distant ones) is more feasible; and (c) refined knowledge of the molecular basis of polymorphisms is made available. Alternatively, restriction-site mapping also offers advantages: (a) the data come 'packaged' in a relatively simple manner (gel digestion profiles for particular endonucleases), which facilitates 'hands-on' appraisal of phylogeographic patterns for multiple specimens as the data are being gathered (e.g. compare Tables 1 and 3); (b) by sampling across many unadjacent positions in the mtDNA genome, the method avoids potential complications of compensatory changes among adjacent nucleotides; (c) there is indication of less homoplasy in the restriction site data for musk turtles (e.g. compare Figs 2B and 6); and importantly (d) the possibility of PCR contamination from nontarget source material does not arise.

How do the current results compare to those of previous studies? Table 5 summarizes results of several published reports comparing whole-genome RFLP analyses against direct mtDNA sequencing. Outcomes have varied, as might be expected given the differing levels of relative effort expended among studies, and the different mtDNA regions sequenced. Direct sequencing of the control region has revealed considerably higher levels of mtDNA variation in several instances, but this trend fails to hold in the current case (and in some others listed in Table 5).

Population structure and phylogeography

One striking finding of this study is the degree of local population structure in *S. minor* as evidenced by the mtDNA assays. Nearly every local population sample was cleanly distinguishable from all others. Musk turtles are strongly aquatic, rarely leaving freshwater streams. 'The turtle is therefore confined to the same modes of distribution as fishes' (Iverson 1977), and might be expected to be

Table 5 Examples of additional population genetic studies that have compared results of restriction site vs. direct sequence analyses of vertebrate mtDNA. Most RFLP studies involved conventional multiple-enzyme assays of the whole mtDNA genome; the direct sequence analyses involved either coding or noncoding regions (as indicated)

Taxonomic group	Region sequenced	Does sequencing reveal greater, similar, or lower variation than RFLP's?*	Reference
Mammals			
humpback whales	control region	much greater	Baker *et al.* (1993)
humans	control region	similar†	Vigilant *et al.* (1989, 1991)
humans	control region	much greater	Aquadro & Greenberg (1983)
Birds			
babblers	cytochrome *b*	similar	Edwards & Wilson (1990)
Fish			
salmon	cytochrome *b*	similar	Birt *et al.* (1986); McVeigh *et al.* (1991)
cod	cytochrome *b*	greater	Carr & Marshall (1991)
rainbow trout	multiple coding sequences	lower	Beckenbach *et al.* (1990)
brook charr	control region	similar	Bernatchez & Danzmann (1993)
white sturgeon	control region	much greater	Brown *et al.* (1993)
Turtles			
marine turtles	control region	much greater	Norman *et al.* (1994)
green turtles	control region	much greater	Lahanas *et al.* (1994)
green turtles	control region	similar	Allard *et al.* (1994)
map turtles	control region	greater	Lamb *et al.* (1994)

*Because the comparative outcomes depend on several factors including the levels of effort expended and the amounts of sequence surveyed in the respective assays, the original papers should be consulted for details.
† RFLP assays in this case involved unusual effort in 'high resolution' mapping.

highly structured across drainages. Many of our collections came from separate drainages, or from separate major tributaries within the same drainage. The genetic results imply strong limitations to contemporary gene flow between locales, including those widely separated within a drainage.

On a broader phylogeographic scale, the north-west—south-east genetic separation in *S. minor* no doubt reflects longer-term historical disjunctions. The distributions of the two major genetic assemblages are consistent with the described ranges of the two previously recognized subspecies (Fig. 1), and have been attributed to historical biogeographic factors operating in this area over recent evolutionary time. According to one scenario (Iverson 1977), an ancestral *S. minor* stock that invaded the region during the Miocene subsequently became sundered into two separate units by the Suwannee Straits (a marine incursion across northern Florida). One population (ancestral to the present-day *S. m. minor*) presumably survived in peninsular Florida, whereas the other (ancestral to *S. m. peltifer*) occupied what is now north-central Alabama. Post-Miocene–Pliocene dispersal from these refugia would then account for the present-day distributions. One element of this range expansion must have been access by *S. m. peltifer* to the current Tennessee River system, which in preglacial times drained southward through Alabama into Mobile Bay (Stejneger 1923; review in Mayden 1988).

Finally, the north-west–south-east arrangement of genetic differentiation in the musk turtle strongly resembles intraspecific phylogeographic patterns previously reported in several other freshwater (and terrestrial) vertebrates in the south-eastern USA (Bermingham & Avise 1986; Avise 1992, 1995). We hope next to survey phylogeographic differentiation in other species of freshwater turtles in the region, and will therefore defer further discussion of comparative patterns.

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The work described in this report is the first instalment of a dissertation project by DeEtte Walker that involves molecular assessments of phylogeographic patterns in several species of freshwater turtles distributed across the south-eastern USA. This work is part of a broader research program in John Avise's laboratory on comparative phylogeographic patterns in freshwater, terrestrial, and marine organisms in this biogeographic region. Vincent Burke and Imrich Barák provided expertise in herpetology and molecular biology, respectively.