Mitochondrial DNA Phylogeography and Subspecies Issues in the Monotypic Freshwater Turtle *Sternotherus odoratus*

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Phylogeographic variation in mitochondrial (mt) DNA restriction sites is described for populations of the monotypic stinkpot turtle (*Sternotherus odoratus*) from the southeastern United States. Stinkpots display pronounced and hierarchical mtDNA phylogeographic differentiation, ranging from genetically shallow differences among local populations to genetically deep distinctions among regional assemblages of haplotypes. Both magnitude and general pattern of intraspecific mtDNA phylogeography in *S. odoratus* are remarkably similar to those in a related species (*Sternotherus minor*) that traditionally has been considered ditory based on morphological and genetic appraisals. The pronounced phylogeographic population structure in *S. odoratus* effectively falsifies prior hypotheses that extensive dispersal and gene flow account for the morphometric conservatism among geographic populations. These findings also raise broader issues concerning the significance of subspecies designations in testudine systematics.

Many species of turtles are partitioned into multiple geographic subspecies. For example, among 56 turtle species in North America, 24 (43%) are subdivided nomenclaturally, with an average of 3.9 described subspecies per polytypic species (Ernst et al., 1994). Further, many monotypic species (e.g., several map turtles of the genus *Graptemys*) have exceptionally narrow geographic ranges. One striking exception to the above involves the common musk turtle or “stinkpot” (*Sternotherus odoratus*). No subspecies of *S. odoratus* currently are recognized, yet the stinkpot’s range extends from Florida to southern Canada and west into Wisconsin and central Texas (Conant and Collins, 1991) and is broader than that of all except three other species of freshwater turtles in the United States. Stinkpots occur in many freshwater habitats but are most common in lentic water with a muddy or soft bottom (Ernst et al., 1994). Studies of geographic variation in life history (Tinkle, 1961), morphology (Reynolds and Seidel, 1983), and allozyme composition (Seidel et al., 1981) have not identified geographic differences deemed worthy of subspecific distinction (but see Neill, 1948). Cagle (1944) and Tinkle (1958) noted a proclivity in this species to migrate overland, a behavior that disputedly (Gibbons et al., 1983; Ernst et al., 1994) may be related to the observed paucity of geographic differentiation. Another suggested explanation for morphological similarity among stinkpot populations is “similarity of selective regimes throughout the range . . . Perhaps there has been widespread selection among *S. odoratus* populations for a general, conservative morphotype adapted for versatile habitat utilization” (Reynolds and Seidel, 1983).

In turtles as in other taxonomic groups (Ball and Avise, 1992), questions routinely arise concerning validity and meaning of morphological comparison as a sole guide to intraspecific taxonomy: (1) How adequately do monitored traits summarize overall patterns of morphological divergence? and (2) What evolutionary forces forge morphological differences, and how do the latter relate to detectable genetic differences? These and related questions are of academic interest and also have important ramifications in conservation biology and population management (Avise, 1994; Avise and Hamrick, 1996).

In this study, we employed restriction-site assays of mitochondrial (mt) DNA to assess matrilineal phylogeography of *S. odoratus* throughout the southeastern portion of its range. Results are compared to phylogeographic patterns reported previously (Walker et al., 1995) for a related species, *S. minor*, that is generally codistributed with *S. odoratus* in this region.

**MATERIALS AND METHODS**

Total DNA was extracted from heart, liver, and muscle; and mtDNA was isolated in closed-circular form following Lansman et al. (1981). Purified mtDNA from each individual was digested with each of 28 restriction endonucleases. Fragments were end-labeled using Klenow and $^{32}$P-labeled nucleotides and electrophoresed through 1.2% agarose gels together with a one-kilobase ladder that served as a molecular size marker. Fragments were visualized by auto-
Table 1. Mtdna Haplotypes Observed in Sternotherus odoratus. Haplotype codes represent digestion profiles for the following endonucleases: BamI, BclI, BseII, DraI, DraII, EcoRI, HindII, HindIII, NciI, PvuII, SpeI, StuI, and XbaI. Locales (lower case letters) are described in Material Examined.

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Fig. 1. Parsimony network summarizing interconnections among 16 mtDNA haplotypes in *Sternotherus odoratus*. Slashes crossing branches indicate numbers of restriction-site changes inferred along a pathway. Inferred changes within four portions of the network (A1, A2, B, and C) are additive. Slashes on branches connecting A (A1, A2) to B and B to C represent the minimum number of inferred restriction-site changes between any haplotypes within each assemblage.

(with subgroups A1 and A2), B, and C. Each of these groups and no others received > 90% bootstrap support. Within each of the major haplotype assemblages, branching topologies were strictly additive; whereas across the groups, some nonadditivity (homoplasy) occurred. The major mtDNA haplotype groups showed a strong geographic orientation (Fig. 2). In addition, local matrilineal population structure was evident (Fig. 2). Most haplotypes (10 of 16) were observed only at single collection sites, and four haplotypes were fixed in samples from those locales.

**DISCUSSION**

*Comparative mtDNA phylogeography in two *Sternotherus* species.*—Matrilineal population genetic structure of *S. odoratus* in the southeastern United States, as inferred from analysis of mtDNA restriction sites, shares general features with patterns reported previously for other terrestrial and freshwater vertebrates in the region (Avise, 1992, 1996). These include the following: (1) extensive intraspecific genetic variation, leading to high genotypic diversities in pooled collections; (2) strong local population structure, in which mtDNA haplotypes show narrow geographic distributions compared to the sampled range of the species; (3) high nucleotide sequence divergence between conspecific mtDNA haplotypes; and (4) significant geographic partitions in intraspecific mtDNA parsimony networks.

The genetic patterns for *S. odoratus* are similar to those reported previously for the musk turtle *S. minor* (Walker et al., 1995), a related species for which two well-demarcated subspecies in the southeastern United States are recognized from morphologic and biogeographic evidence (Iverson, 1977; Ernst and Barbour, 1989; Ernst et al., 1994). Genotypic diversities in the pooled intraspecific samples of *S. minor* and *S. odoratus* were 0.859 and 0.899, nucleotide diversities were 0.017 and 0.016, and the largest net mtDNA sequence differences between major intraspecific phylogeographic units in the two respective species were 0.032 and 0.031. The high levels of mtDNA variation within *S. odoratus* and *S. minor* provide a counterpoint to a previously reported tendency for many testudine species to display unusually low levels of intraspecific mtDNA polymorphism (Avise et al., 1992).

Similarities in intraspecific genetic profiles between *S. odoratus* and *S. minor* also extend to geographic distributions of identified mtDNA phylogeographic groups. The range of the morphological subspecies *S. m. pelifer* (with which mtDNA data are consistent—Walker et al., 1995) encompasses the western half of the species' distribution, notably the Alabama (Mobile) and Tennessee River drainages. This range corresponds approximately to the A phylogeographic assemblage in *S. odoratus*. In addition, the range of the eastern subspecies *S. m. minor* (as identified by morphology and mtDNA) corresponds approximately to that of the B + C phylogeographic assemblages in *S. odoratus*. Differences in patterns of mtDNA divergence be-
between the two turtle species include the following: (1) *Sternotherus odoratus* appears more strongly differentiated within the eastern region (between peninsular Florida and the Atlantic coastal drainages) than does *S. minor*, and (2) in contrast to *S. minor*, populations of *S. odoratus* in the Atlantic coast drainages (group B) are more similar in mtDNA composition to western populations (group A) than to those in southern Georgia and the Florida peninsula (group C). Nevertheless, like most other vertebrate species genetically surveyed to date in the southeastern United States (Avise, 1996), populations of *S. odoratus* in central Florida are strongly differentiated from others in the region, particularly those to the north and west.

Subspecies designations.—Evidently, the current status of taxonomic monotypy for *S. odoratus* in the southeastern United States belies considerable phylogeographic differentiation observed in mtDNA. The genetic results indicate that the paucity of morphological variation cannot be attributed to extensive contemporary dispersal and gene flow of matrilines among populations. Do the mtDNA findings therefore warrant a formal recognition of multiple subspecies for *S. odoratus* in the southeastern United States?

Genetic assessments have limitations as a guide to taxonomic inference when based on “single genes” (such as mtDNA). Avise and Ball (1990) suggest that the taxonomic category “subspecies” should be reserved for significant phylogeographic subunits within biological species, with “significance” assessed primarily by concordance in populational distinctions across multiple, independent, genetically coded characters. In the absence of intrinsic reproductive isolation, such multicharacter concordance in biotic delineations is anticipated only when populations have been isolated (typically by geography) for periods of time that are long relative to effective population size. Furthermore, populations separated for long times should display relatively deep divergences in particular gene genealogies such as that ensconced in mtDNA. In the (usual) absence of direct empirical evidence concerning multiple gene genealogies, useful surrogate sources of information must come from observed distributions of traditional systematic traits, under the working hypothesis that differences are genetically based.

As applied in the current context, the two recognized forms within *S. minor* warrant subspecies recognition because mtDNA lineage separations appear relatively deep, geographically coherent, and concordant with distributions previously described on the basis of morphological differences. However, “subspecies” distinctions within *S. odoratus* in our view are not yet warranted because concordant support from other lines of evidence (morphological or otherwise) is not yet available. The mtDNA data provide clear indications of where subspecies distinctions might be sought in phylogeographic appraisals of additional characters; but alone, they are insufficient to define subspecies status under a proper application of genealogical concordance principles.

**MATERIAL EXAMINED**

*Sternotherus odoratus* (sites labeled as in Fig. 3): York River basin: (a) Cohoke Mill Creek, King William Co., VA (n = 5); Tennessee River basin: (b) North Fork of the Holston River, Scott Co., VA (n = 8); Pee Dee River basin: (c) McKinney Lake, Richmond Co., NC (n = 5); Savannah River basin: (d) pond on Vaucelse Rd., Aiken Co., SC (n = 6); (e) Hwy 301 near Allendale, Allendale Co., SC (n = 1); Edisto River basin: (f) Orangeburg National Fish Hatchery ponds, Orangeburg Co., SC (n = 9); Altamaha River basin: (g) University of Georgia Golf Course, Clarke Co., GA (n = 6); (h) Long Creek, Oglethorpe Co., GA (n = 6); (i) Hwy 22 near Crawfordsville, Taliaferro Co., GA (n = 1); Ogeechee River basin: (j) Bo Ginn National Fish Hatchery ponds, Jenkins Co., GA (n = 2); St. John’s River basin: (k) River Styx, Alachua Co., FL (n = 1); (l) sinkhole pond near Hwy. 19, Putnam Co., FL (n = 1); (m) pond on 7th Ave., Seminole Co., FL (n = 5); Apalachicola River basin: (n) Warm Springs National Fish Hatchery ponds,
Meriwether Co., GA \((n = 8)\); (o) on SR 65, Liberty Co., FL \((n = 1)\); Alabama-Tombigbee (Mobile) River basin: (p) Hwy 136 near Villanow, Walker Co., GA \((n = 1)\); (q) Carbon Hill National Fish Hatchery ponds, Walker Co., AL \((n = 3)\); (r) Cahaba River, Jefferson Co., AL \((n = 1)\); Pascagoula River basin: (s) pond on Hwy 15, Newton Co., MS \((n = 9)\); Mississippi River basin: (t) cypress swamp, Big Cane Conservation Area, Butler Co., MO \((n = 6)\); (u) pond on East Pleasant Ridge Road, Tangipahoa Parish, LA \((n = 1)\); (v) Comite River, East Baton Rouge Parish, LA \((n = 6)\); and (w) L’Oursé and La Fourche Bayous, LaFourche and Assumption Parishes, LA \((n = 6)\). Representative specimens were deposited in the University of Georgia Museum of Natural History (reference numbers UGAMNH 24878–24898).

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LITERATURE CITED


