Mitochondrial DNA Phylogeography and Subspecies Issues in the Monotypic Freshwater Turtle Sternotherus odoratus
Author(s): DeEtte Walker, William S. Nelson, Kurt A. Buhlmann, John C. Avise
Source: *Copeia*, Vol. 1997, No. 1 (Feb. 18, 1997), pp. 16-21
Published by: American Society of Ichthyologists and Herpetologists
Stable URL: http://www.jstor.org/stable/1447835
Accessed: 14/07/2011 15:29

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/action/showPublisher?publisherCode=asih.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

American Society of Ichthyologists and Herpetologists is collaborating with JSTOR to digitize, preserve and extend access to *Copeia.*

http://www.jstor.org

Copeia, 1997(1), pp. 16–21

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

DEETTE WALKER, WILLIAM S. NELSON, KURT A. BUHLMANN, AND JOHN C. AVISE

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 ditypic 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-

© 1997 by the American Society of Ichthyologists and Herpetologists

TABLE 1. MTDNA HAPLOTYPES OBSERVED IN *Sternotherus odoratus*. Haplotype codes represent digestion profiles for the following endonucleases: *Ban*I, *Bcl*I, *Bst*EII, *Dra*I, *Dra*II, *Eco*RI, *Hin*dII, *Hin*dIII, *Nci*I, *Pvu*II, *Spe*I, *Stu*I, and *Xba*I. Locales (lower case letters) are described in Material Examined.

Haplotype	Haplotype code	Individuals	Locale (no. individuals)
1	CCCCCCCCCCCCC	1	r (1)
2	CCCDECCCCCCBC	3	q (3)
3	CCCCDCCCCCCCC	9	b (8); p (1)
4	CCDCBDDCCCCDC	11	t (4); u (1); w (6)
5	CCDCBDCCCCCDC	6	v (6)
6	CBDCBDDCCCCDC	1	t (1)
7	CCECBDECCCCDC	1	t (1)
8	BCDCBDDCBCCDC	8	s (8)
9	BCDCBDCCBCCDC	1	s (1)
10	ECFECHCCCEBDC	15	k (1); l (1) m (5); n (8)
11	CCDDCFFDCDDDC	11	a (5); c (5); h (1)
12	CCDDCFBDCDDDC	18	d (6); e (1); f (1) g (5); h (4) i (1)
13	CDDDCFBDCDDDC	3	g (1); h (1); j (1)
14	CDDDCFBCCDDDC	1	j (1)
15	CCCDCFBDCDDDC	8	f (8)
16	ECFEDHCCCEBDC	1	o (1)

radiography; fragments smaller than 0.5 kilobases (kb) typically were not scored.

Data analysis.—Digestion profiles for most of the enzymes were straightforward and permitted provisional interpretation as particular restriction-site changes. Data were compiled into a presence/absence matrix of restriction sites. Genotypic- and nucleotide-diversity statistics were calculated following Nei (1987). Sequence divergence estimates (p) between haplotypes were calculated using the site approach of Nei and Li (1979). Similarities among haplotypes were summarized using neighbor-joining (Saitou and Nei, 1987). Phylogenetic relationships among haplotypes were inferred using the branch-and-bound search option in PAUP version 3.1 (D. L. Swofford, Illinois Natural History Survey, Champaign, 1990, unpubl.). Statistical support for putative clades was assessed by bootstrapping across 1000 replicates. Parsimony networks also were hand-generated by successively linking similar genotypes. Attempts to root *S. odoratus* networks with mtDNA haplotypes from *S. minor* failed because many mtDNA digestion profiles were too different to permit inference of homologous restriction sites. Networks are thus presented as unrooted.

RESULTS

Thirteen of 28 enzymes employed produced no or one cut in preliminary population screening and are not considered further. Two enzymes (*Ava*II and *Bst*NI) produced digestion profiles that were too complex to interpret as restriction-site changes but which were useful nonetheless in identifying additional haplotypes. The remaining 13 enzymes (Table 1) were informative in producing digestion profiles that were variable and interpretable as differing by specific restriction site changes.

The 15 variable enzymes uncovered 22 different mtDNA haplotypes (genotypic diversity = 0.931). The 13 enzymes for which site interpretations were possible revealed 16 haplotypes (Table 1), with genotypic- and nucleotide-diversity values of 0.899 and 0.016, respectively, in pooled samples. For the 13 endonucleases, 69 restriction site positions were scored; 31 were polymorphic. Genetic distances between haplotypes ranged from 0.002–0.037. The restriction-site and distance matrices are available from the senior author upon request. In addition to site differences, there was evident mtDNA size variation among individuals. Most notably, mtDNAs of samples from Florida were 0.5–1.5 kb larger than mtDNA of turtles sampled elsewhere. These size differences were apparent across digestion profiles of multiple enzymes and did not confound site interpretations employed in phylogenetic analysis.

Topologies of haplotypes produced by neighbor-joining and maximum-parsimony analyses (not shown) were similar to that produced by a hand-generated parsimony network (Fig. 1). All three topologies partitioned the 16 mtDNA haplotypes into the same three major groups: A

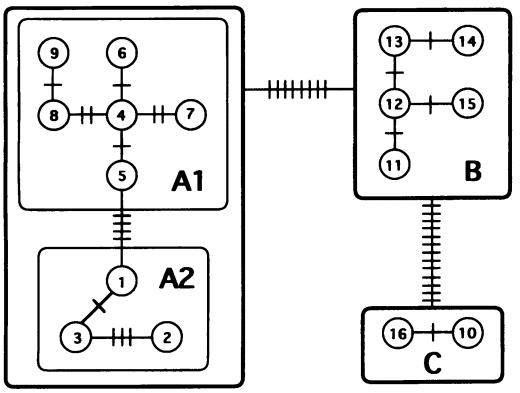

Fig. 1. Parsimony network summarizing interconnections among 16 mtDNA haplotypes in *Sternotherus odoratus*. Slashes crossing branches indicate numbers of restriction-site changes inferred along a pathway. Inferred changes within four portions of the network (A1, A2, B, and C) are additive. Slashes on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

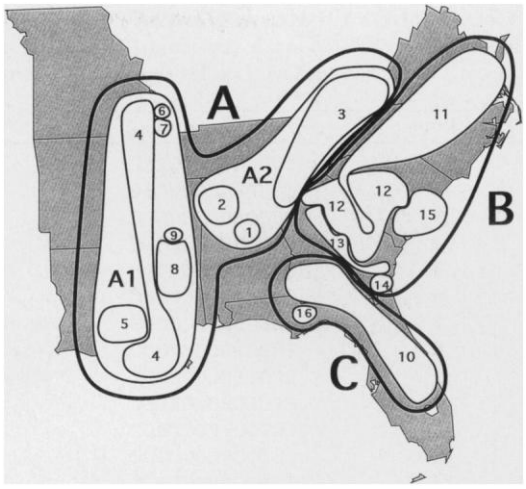

Fig. 2. Distributions of mtDNA haplotypes and their major phylogeographic assemblages in *Sternotherus odoratus*.

(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. peltifer* (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-

tween 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.

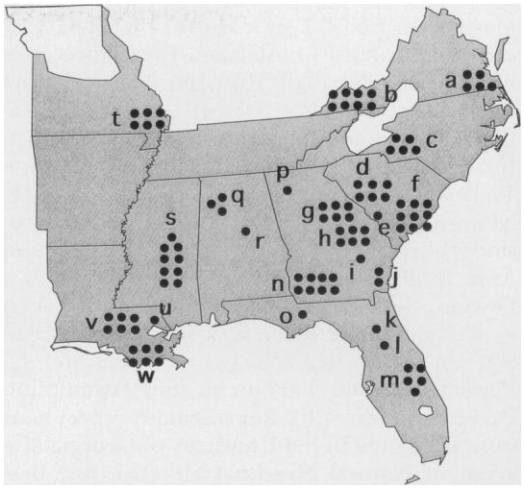

Fig. 3. Map of the southeastern United States showing localities (a–w; see Material Examined) of the 98 turtles collected.

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 Vaucluse 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, Tangipohoa Parish, LA (n = 1); (v) Comite River, East Baton Rouge Parish, LA (n = 6); and (w) L'Ourse 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).

Acknowledgments

We thank the following for assistance with the collecting effort: R. Babb, M. Case, J. Congdon, S. Doody, S. Emms, M. Goodisman, M. Hare, S. Hodges, C. Holod, T. Johnson, A. Jones, J. Mitchell, R. Nagle, P. Prodöhl, R. Vandevender, and D. Wilson; personnel from the Mississippi Museum of Natural Science and the University of Georgia Golf Course; and personnel from the US National Fish Hatcheries of Bo Ginn (Georgia), Carbon Hill (Alabama), McKinney Lake (North Carolina), Orangeburg (South Carolina), and Warm Springs (Georgia). Special thanks goes to V. Burke and P. Moler. All specimens were collected under relevant state permits. Work was supported by an NIH training grant to DW, by DOE contract # DE-AC09-76SR00819 between the US Department of Energy and the University of Georgia's Savannah River Ecology Laboratory and by an NSF grant to JCA.

Literature Cited

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

———. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

———. 1996. Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States, p. 431–470. *In:* Conservation genetics: case histories from nature. J. C. Avise and J. L. Hamrick (eds.). Chapman and Hall, New York.

———, and R. M. Ball Jr. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol. 7:45–67.

———, and J. L. Hamrick (eds.). 1996. Conservation genetics: case histories from nature. Chapman and Hall, New York.

———, B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. Mol. Biol. Evol. 9:457–473.

Ball, R. M., Jr., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk 109:626–636.

Cagle, F. R. 1944. Home range, homing behavior and migration in turtles. Misc. Publ. Mus. Zool., Univ. Mich. 61:1–34.

Conant, R., and J. T. Collins. 1991. A field guide to reptiles and amphibians. Houghton Mifflin, Boston, MA.

Ernst, C. H., and R. W. Barbour. 1989. Turtles of the world. Smithsonian Inst. Press, Washington, DC.

———, J. E. Lovich, and R. W. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Inst. Press, Washington DC.

Gibbons, J. W., J. L. Greene, and J. D. Congdon. 1983. Drought-resistant responses of aquatic turtle populations. J. Herpetol. 17:242–246.

Iverson, J. B. 1977. Geographic variation in the musk turtle, *Sternotherus minor*. Copeia 1977:502–517.

Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Ecol. 17:214–226.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

———, and W-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. (USA) 76:5269–5273.

Neill, W. T. 1948. The musk turtles of Georgia. Herpetologica 4:181–183.

Reynolds, S. L., and M. E. Seidel. 1983. Morphological homogeneity in the turtle *Sternotherus odoratus* (Kinosternidae) throughout its range. J. Herp. 17: 113–120.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Seidel, M. E., S. L. Reynolds, and R. V. Lucchino. 1981. Phylogenetic relationships among musk turtles (genus *Sternotherus*) and genic variation in *Sternotherus odoratus*. Herpetologica 37:161–165.

Tinkle, D. W. 1958. The systematics and ecology of the *Sternothaerus carinatus* complex (Testudinata, Chelydridae). Tulane Stud. Zool. 6:1–56.

———. 1961. Geographic variation in reproduction, size, sex ratio and maturity of *Sternothaerus odoratus* (Testudinata: Chelydridae). Ecology 42:68–76.

Walker, D., V. J. Burke, I. Barák, and J. C. Avise. 1995. A comparison of mtDNA restriction sites vs. control region sequences in phylogeographic assessment of the musk turtle (*Sternotherus minor*). Mol. Ecol. 4:365–373.

(DW, WSN, JCA) Department of Genetics, University of Georgia, Athens, Georgia 30602; and (KAB) Savannah River Ecology Laboratory, Drawer E, Aiken, South Carolina 29801. Submitted: 11 Dec. 1995. Accepted: 14 May 1996. Section editor: John R. Gold.