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PRINCIPLES OF PHYLOGEOGRAPHY AS ILLUSTRATED BY FRESHWATER AND TERRESTRIAL TURTLES IN THE SOUTHEASTERN UNITED STATES

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KEY WORDS: historical biogeography, mitochondrial DNA, molecular evolution, conservation genetics

ABSTRACT

Geographic patterns in mtDNA variation are compiled for 22 species of freshwater and terrestrial turtles in the southeastern United States, and the results are employed to evaluate phylogeographic hypotheses and principles of genealogical concordance derived previously from similar analyses of other vertebrates in the region. The comparative molecular findings are interpreted in the context of intraspecific systematics for these taxa, the historical geology of the area, traditional nonmolecular zoogeographic information, and conservation significance. A considerable degree of phylogeographic concordance is registered with respect to (*a*) the configuration of intraspecific mtDNA subdivisions across turtle species, (*b*) the principal molecular partitions and traditional morphology-based taxonomic boundaries, (*c*) genetic patterns in turtles versus those described previously for freshwater fishes and terrestrial vertebrates in the region, and (*d*) intraspecific molecular subdivisions versus the boundaries between major zoogeographic provinces as identified by composite ranges of species in the Testudines. Findings demonstrate shared elements in the biogeographic histories of a diverse regional biota. Such phylogeographic concordances (and discordances) have ramifications for evolutionary theory as well as for the pragmatic efforts of taxonomy and conservation biology.

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> In the study of dispersal and distribution of animals, it is important to see that the physical conditions lead, and that in a more or less definite succession the flora and fauna follow; thus the fauna comes to fit the habitat as a flexible material does a mold. The time is passed when faunal lists should be the aim of faunal studies. The study must not only be comparative, but genetic, and much stress must be laid on the study of the habitat, not in a static, rigid sense, but as a fluctuating or periodical medium.
>
> Charles Adams, 1901

INTRODUCTION

The spatial genetic architecture of any species is likely to be a complex outcome of contemporary demographic and ecological forces acting upon a preexisting population structure that was molded by biogeographic factors operative throughout the evolutionary history of a species. Molecular methods are well suited for (*a*) describing current population genetic structures and (*b*) recovering historical components of those structures. A particularly useful molecule is animal mitochondrial (mt) DNA, which, by virtue of a rapid rate of change and a nonrecombining mode of asexual transmission through female lineages, permits powerful phylogeographic inferences at the levels of conspecific populations and closely related species (9, 39, 67). Given the great diversity of ecological and evolutionary factors that can influence genealogical structures, an idiosyncratic phylogeographic outcome might be expected for each species.

Nonetheless, comparative molecular assessments of many freshwater fishes, terrestrial vertebrates, and maritime species in the southeastern United States have revealed repeated patterns at several levels (reviews in 6, 8). These studies prompted the original formulation of phylogeographic hypotheses (9) and principles of genealogical concordance (8, 10), which are summarized in Table 1. Because these concepts were motivated (rather than independently tested) by the regional biogeographic data available at the time, they were considered provisional ideas pending further empirical evaluation. Thus, an important question is whether these phylogeographic hypotheses and concordance trends may prove generalizable to other taxonomic groups and to other regional biotas. Here we provide a summary of the results of one such extended set of independent tests: comparative evaluations of intraspecific phylogeographic patterns in the Testudines (turtles and tortoises) distributed across the southeastern United States.

BACKGROUND

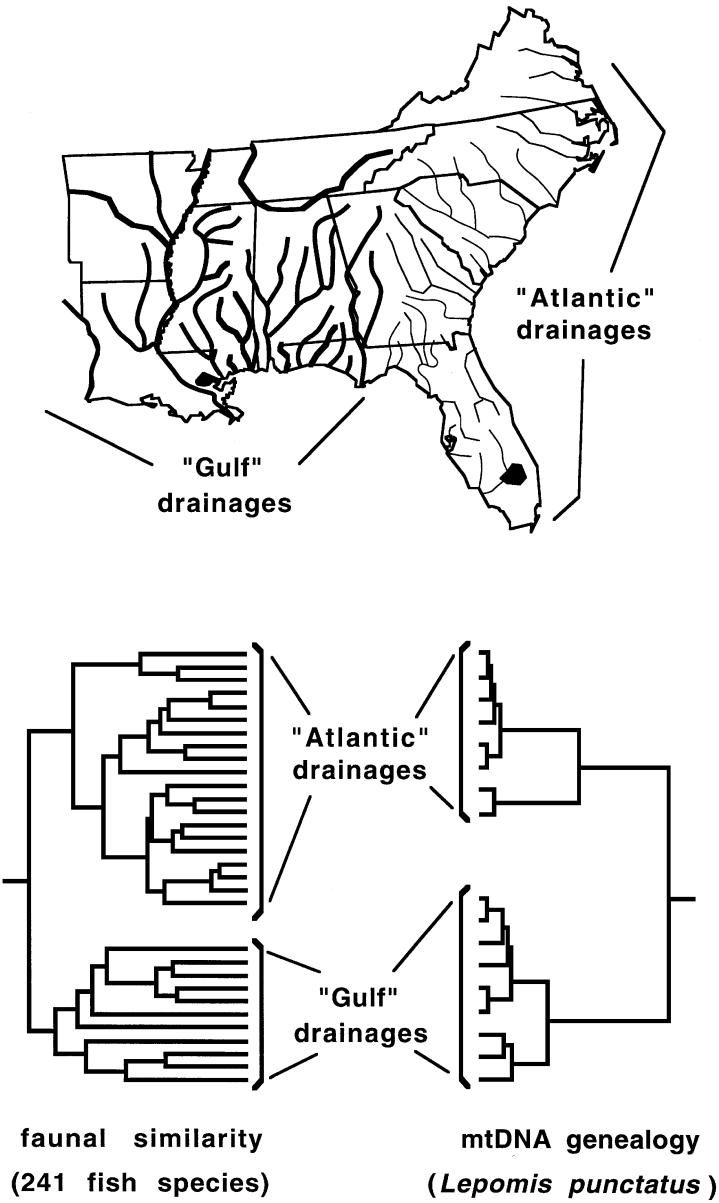
This research on the Testudines was motivated by comparative phylogeographic patterns reported for conspecific populations within each of several freshwater fish species in the southeastern United States (11, 14, 40, 54): bowfin (*Amia*

Table 1 Phylogeographic hypotheses and principles of genealogical concordance

Phylogeographic Hypotheses (from Reference 9)
I. Most species are composed of geographic populations whose members occupy recognizable genealogical branches of an extended intraspecific pedigree.
II. Species with limited or "shallow" phylogeographic population structure have life histories conducive to dispersal and have occupied ranges free of long-standing impediments to gene flow.
III. Intraspecific monophyletic groups distinguished by large genealogical gaps usually arise from long-term extrinsic (biogeographic) barriers to gene flow.
Aspects of Genealogical Concordance (theoretical corollaries of Phylogeographic Hypothesis III; after Reference 8)
A. Concordance across sequence characters within a gene (yields statistical significance for putative gene-tree clades).
B. Concordance in significant genealogical partitions across multiple genes within a species (establishes that gene-tree partitions register population-level phylogenetic partitions).
C. Concordance in the geography of gene-tree partitions across multiple codistributed species (implicates shared historical biogeographic factors in shaping intraspecific phylogenies).
D. Concordance of gene-tree partitions with spatial boundaries between traditionally recognized biogeographic provinces (implicates shared historical biogeographic factors in shaping intraspecific phylogenies and organismal distributions).

calva); mosquitofish (*Gambusia affinis/Gambusia holbrooki*); largemouth bass (*Micropterus salmoides*); and four species of sunfish (*Lepomis punctatus*, *Lepomis microlophus*, *Lepomis gulosus*, and *Lepomis macrochirus*). Within each of these species, deep and geographically oriented phylogenetic "breaks" typically have distinguished populations in the eastern portion of the species' range (river drainages primarily along the Atlantic coast and in peninsular Florida) from those to the west (most drainages entering the Gulf of Mexico from western Georgia or Alabama to Louisiana). An example involving the spotted sunfish (*Lepomis punctatus*) is presented in Figure 1.

Additional genetic substructure is evident within some of these east-west phylogeographic units, but these differences typically were "shallow" (low mtDNA sequence divergence) relative to the matrilineal separations between regions. In some of these fishes, notably *M. salmoides* (44), *L. macrochirus* (11), and the *Gambusia* complex (54), contact zones of introgressive hybridization also have been documented by allozymes (in conjunction with mtDNA) in geographically intermediate populations primarily in western Georgia and eastern Alabama.


"Atlantic"
drainages
"Gulf"
drainages
"Atlantic"
drainages
"Gulf"
drainages
faunal similarity
(241 fish species)
mtDNA genealogy
(*Lepomis punctatus*)

The major mtDNA phylogeographic subdivisions in freshwater fishes are consistent with historical biogeographic scenarios that invoke long-term (Pleistocene or earlier) separations of drainages and the fishes they contain into the Atlantic coast region and the Gulf coast region. The subvisions are also consistent with relatively recent physical connections permitting interdrainage exchange of fishes within each area. Such patterns are concordant with a traditional class of biogeographic information: the distributional boundaries of species. In a compilation of ranges for the 241 freshwater fish species native to the southeastern region, the most fundamental break was identified at the Apalachicola River it that forms part of the Alabama-Georgia boundary (58). Thus, a phenetic clustering of 31 drainages based on a presence-absence matrix of species revealed two basal assemblages (Figure 1): (*a*) an eastern (mostly Atlantic coast) group composed of all rivers from the Savannah to the Suwannee and (*b*) a western (Gulf coast) group composed of the Apalachicola and all drainages westward to Louisiana (Figure 1). Most of the intraspecific breaks in fish mtDNA reported previously (11, 14, 54) fall in this same general area as well, thus yielding concordance aspects C and D as defined in Table 1.

Several studies of terrestrial vertebrates in the southeastern United States also have revealed strong intraspecific phylogeographic partitioning in mtDNA and a tendency (though not as consistent or well documented as in fishes) for concordant spatial patterns (8). Populations in peninsular Florida (and in some cases the adjoining Atlantic coast) tend to be strongly differentiated from those to the north and west. These results have been attributed to the insularization effects of a Floridian peninsula that was relatively isolated periodically during the Pleistocene and earlier (18, 23). Such isolating effects on faunal distributions were predicted many years ago: Remington (48) first emphasized the large number of species whose populations display a "suture zone" of secondary contact situated at the boundary between the Floridian peninsula and the continental mainland. Remington afforded this contact region a status equal to that of only five other major suture zones in North America.

←

Figure 1 (*Top*) Map of the 10 southeastern states that are of primary interest in the current analysis. *Heavy lines* depict most of the river drainages in the Gulf of Mexico freshwater biotic province, and *light lines* indicate river drainages in the Atlantic province. (*Bottom left*) Phenogram summarizing faunal similarities among 31 southeastern river drainages, based on a presence-absence matrix of 241 fish species in the area (after Reference 58). Note the basal split between the Atlantic and Gulf regions. (*Bottom right*) Phenogram summarizing relationships among mitochondrial (mt) DNA haplotypes observed in the spotted sunfish (*Lepomis punctatus*) sampled across more than a dozen major drainages in the region (after Reference 14). Note that the fundamental split in the mtDNA gene tree distinguishes conspecific specimens of the Atlantic coast drainages from those of the Gulf coast drainages.

Thus, in addition to tests of the general phylogeographic hypotheses and concordance aspects listed in Table 1, comparative studies of the Testudines in the southeastern United States permit independent evaluations of more specific biogeographic predictions. With respect to life history, aptitude for dispersal, and potential response to vicariant biogeographic effects, aquatic turtles as a group should be intermediate between freshwater fishes and low-vagility terrestrial mammals. Most turtles are associated with aquatic environments but are able to move on land; tortoises are slow moving and have no aquatic affiliations. Thus, if previous genetic findings for other freshwater and terrestrial vertebrate taxa in the southeastern United States are a guide, any deep phylogenetic partitions uncovered within species of Testudines might also tend to distinguish populations (*a*) in peninsular Florida from those on the main body of the continent and/or (*b*) in the Atlantic coastal region from those to the west and along the Gulf coast.

PHYLOGEOGRAPHIC OUTCOMES BY SPECIES

A total of 35 species of Testudines are native to the southeastern United States. Twenty-two of these (63%) have been the subject of molecular analyses based on mtDNA restriction sites or sequences (Table 2). Typically, a genetic study involved the assay of about 240–500 base pairs per individual either as an accumulation of data from multiple-restriction enzyme assays or as sequences obtained directly from particular mitochondrial genes (e.g. the control region). Many studies involved assays of scores of specimens, often sampled from populations scattered throughout the region. Table 2 is also a compilation of information on genetic variability either as reported directly in the original publications or as calculated by us from the data provided.

Following are brief species-by-species descriptions of major mtDNA phylogeographic studies. The original papers should be consulted for details. Typically, the authors used (*a*) parsimony and/or maximum likelihood (59) as applied to DNA sequence data or to presence-absence restriction-site matrices and (*b*) neighbor-joining (53) and/or phenetic clustering (56) as applied to genetic distance matrices. In no case did these alternative phylogenetic procedures yield inconsistent or conflicting outcomes with respect to the major mtDNA intraspecific phylogroups that are the focus of this review. Furthermore, these phylogroups invariably received strong statistical support by criteria such as bootstrapping (20) in phylogenetic appraisals presented in the original publications.

Thus, for simplicity and for ease of visual comparison across studies, results summarized below are presented in the form of cluster phenograms (and associated geographic maps) as plotted on common scales of estimated nucleotide sequence divergence. For the most part, we are not concerned with

genetic structure of local populations within the principal phylogroups, although (small sample sizes notwithstanding) such shallower genealogical structure was pronounced for most species in terms of spatial heterogeneity in haplotype frequencies (e.g. Figure 2).

Freshwater Turtles

STERNOTHERUS MINOR (MUSK TURTLE) In *Sternotherus minor*, a small-bodied turtle confined to the southeastern United States, two morphological subspecies have been recognized: *S.m. minor* to the east and *S.m. peltifer* to the west (Figure 3). In both restriction-site and direct-sequencing assays, Walker et al (61) observed numerous mtDNA haplotypes, all of which were spatially localized (Figure 2). These haplotypes cluster into two distinct phylogroups whose geographic orientations align well with these subspecies as traditionally recognized (Figure 3). Because the mean genetic distance between these intraspecific phylogroups is considerably greater than observed haplotype distances within either assemblage, net sequence divergence is large: $p \cong 0.032$ in the restriction-site assays (Table 2).

To explain the geographic distributions of the two subspecies, Iverson (25) suggested that an ancestral *S. minor* stock invaded southeastern North America during the Miocene and subsequently became vicariantly subdivided into two units—one in peninsular Florida and the other in what is now north-central Alabama. The current distributions were suggested to be a result of post Miocene-Pliocene dispersal from these refugial areas, probably facilitated for ancestral *S.m. peltifer* by a well-known historical connection of the current Tennessee River system to rivers draining southward through Alabama into Mobile Bay (37, 57).

STERNOTHERUS DEPRESSUS (FLATTENED MUSK TURTLE) The range of *Sternotherus depressus*, confined to the Black Warrior River in northern Alabama (Figure 3), is completely encircled by that of *S. minor peltifer*. The flattened musk turtle has been of questionable taxonomic status (see discussion in 65), but it is thought to be related closely to *S. minor* and is currently on the federal list of threatened and endangered species. Notwithstanding its odd distribution and an uncertain genetic etiology for a characteristic flattened carapace, this form is phylogenetically distinct in mtDNA composition from both *S. m. peltifer* and *S. m. minor* as well as from all other kinosternid turtle species in the southern United States (Figure 4).

STERNOTHERUS ODORATUS (STINKPOT) *Sternotherus odoratus* is traditionally considered monotypic: It is relatively uniform in morphology and life history features throughout its range, so no taxonomic subspecies have been recognized (49, 55, 60). However, striking geographic differentiation was uncovered

Table 2 Summary information for terrestrial and freshwater turtles genetically surveyed from the southeastern United States

Assay method	Number of base pairs[a]	Number of sub-species[b]	Phylogeographic unit (mtDNA)[c]	Number of individuals	Number of mtDNA haplotypes	Genotypic diversity[d]	Nucleotide diversity[e]	Net sequence divergence between units[f]	Reference
Whole-mtDNA restriction fragment length polymorphisms (RFLP)									
Sternotherus minor			A	18	3	0.451	0.001		
			B	34	7	0.817	0.004		
Total	367	2		52	10	0.859	0.017	A vs B = 0.032	61
Sternotherus odoratus			A	41	9	0.836	0.007	A vs B = 0.014	
			B	41	5	0.712	0.002	A vs C = 0.028	
			C	16	2	0.125	0.000	B vs C = 0.024	
Total	395	0		98	16	0.899	0.016		64
Kinosternon subrubrum			A	16	1	0.000	0.000	A vs B = 0.070	
			B	24	7	0.798	0.003	A vs C = 0.067	
			C	19	8	0.868	0.005	A vs D = 0.053	
			D	5	4	0.900	0.008	B vs C = 0.048	
Total	434	3		64	20	0.902	0.043	B vs D = 0.027	
								C vs D = 0.030	62
Kinosternon baurii			A	5	2	0.400	0.001		
			B	13	6	0.718	0.003		
Total	359	0		18	8	0.817	0.006	A vs B = 0.010	62
Trachemys scripta			A	40	1	0.000	0.000		
			B	25	1	0.000	0.000		
Total	240	2		65	2	0.482	0.003	A vs B = 0.006	12, C[g]
Graptemys pseudogeographica (A)		7		39	3	0.527	0.002	A vs B = 0.010	

								A vs C = 0.028	
Graptemys pulchra (B)		4		16	4	0.800	0.005	B vs C = 0.026	
Graptemys geographica (C)		1		12	1	0.000	0.000		
Total	400	12 species		67	8	0.803	0.013		31
Control-region sequence									
Gopherus polyphemus[h]			A	1	1	0.000	0.000		
			B	1	1	0.000	0.000		
Total	363–429	0		2	2	0.000	0.000	A vs B = 0.021	42
Sternotherus minor			A	18	4	0.661	0.003		
			B	34	13	0.915	0.014		
Total	430	2		52	17	0.925	0.017	A vs B = 0.013	61
Sternotherus depressus	402	0		6	4	0.866	0.007		65
Chelydra serpentina	409	2		66	3	0.172	0.001		63
Macroclemys temminckii			A	18	1	0.000	0.000	A vs B = 0.026	
			B	47	4	0.420	0.002	A vs C = 0.028	
			C	95	6	0.540	0.005	B vs C = 0.017	
Total	420	0		160	11	0.837	0.015		51, 52
Deirochelys reticularia			A	3	2	0.675	0.002		
			(B + C)	47	7	0.471	0.002		
Total	436	3		50	9	0.533	0.006	A vs (B + C) = 0.043	C[g]

[a]Mean number of base pairs assayed per individual (sequenced directly, or contained within the surveyed recognition sites).
[b]Number of subspecies conventionally recognized based primarily on morphology (from Reference 17).
[c]Number of moderately or highly distinct phylogeographic units as identified in the mtDNA assays (see text).
[d]$1 - \Sigma f_i^2$, where f_i is the frequency of the ith mtDNA haplotype.
[e]Mean pairwise sequence divergence between individuals within a mtDNA phylogeographic unit.
[f]Net sequence divergence $= p_{xy} - 0.5(p_x + p_y)$ where p_{xy} is the mean pairwise mtDNA sequence divergence between phylogeographic units x and y, and p_x and p_y are the mean pairwise sequence distances within x and y.
[g]C, current study.
[h]This species also was surveyed through polymerase chain reaction–based RFLP assays of mtDNA sequences (see Reference 42 for details).

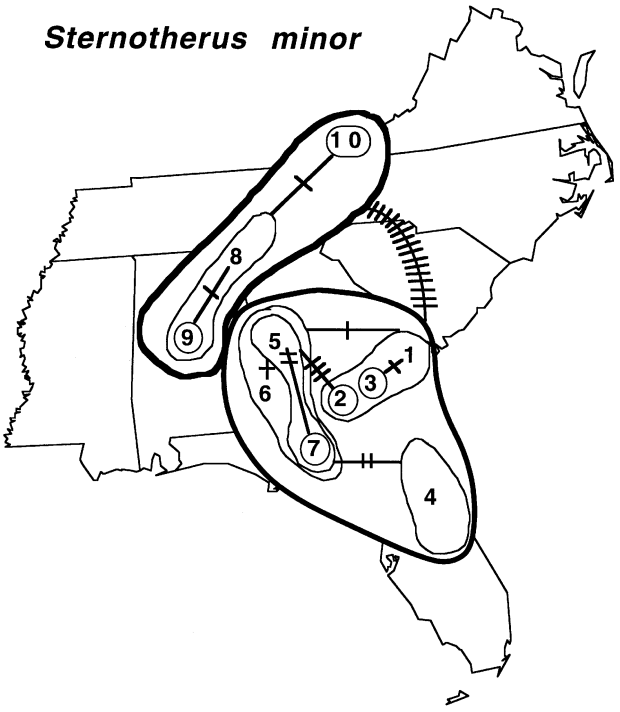


Figure 2 Parsimony network for the 10 different mitochondrial (mt) DNA haplotypes observed in restriction-site assays of the musk turtle *Sternotherus minor*, superimposed over the geographic source of the collections (after Reference 61). *Circles* and *other lines* encompass the ranges within which particular haplotypes were observed among the total of 52 specimens assayed, and *slashes* across branches of the network indicate numbers of restriction-site changes along each path.

in the mtDNA assays, with all haplotypes spatially localized. Three major phylogroups with regional distributions are evident (Figure 5): (*a*) group C in Florida and south Georgia, (*b*) group B along the Atlantic seaboard from Georgia to Virginia, and (*c*) group A in all locales to the west. Within the latter assemblage, two phylogeographic subgroups occur (64): one from northern Alabama to western Virginia and the other in western sites from southern Missouri through Mississippi and Louisiana.

KINOSTERNON SPECIES (MUD TURTLES) For reasons that will become apparent, two traditionally recognized congeners in the southeastern United States are considered together. The range of *Kinosternon subrubrum* encompasses most of the region, where three parapatric morphological subspecies typically

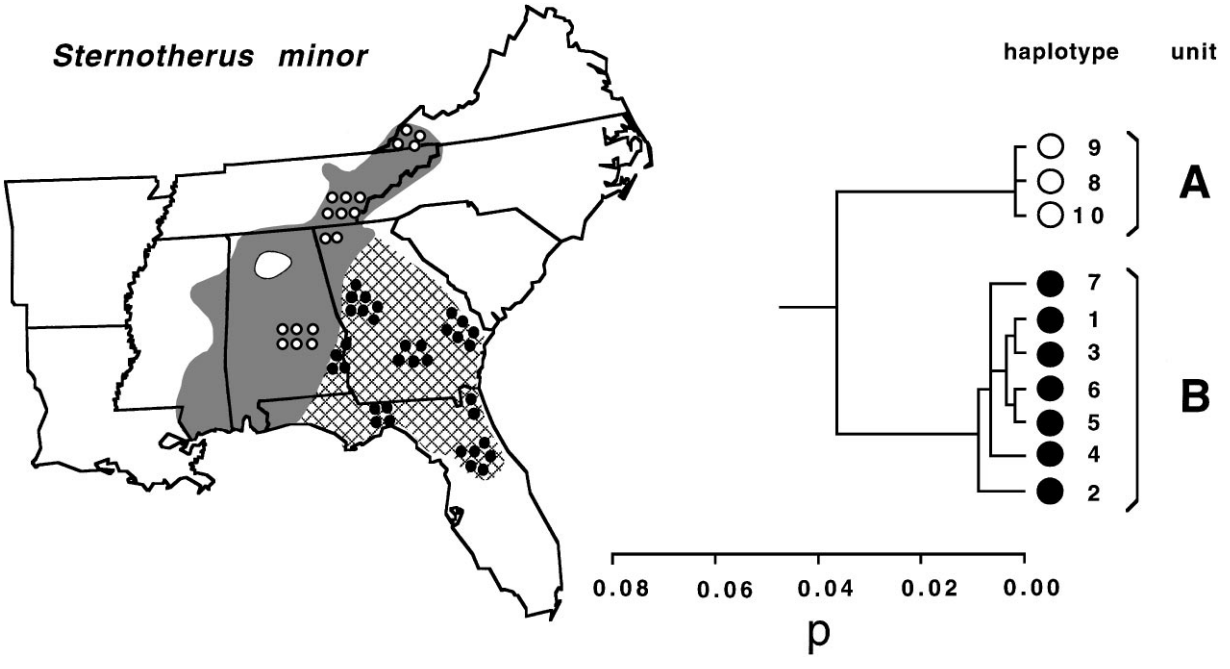


Figure 3 Phylogeographic pattern of mitochondrial (mt) DNA in the musk turtle, *Sternotherus minor*. (*Left*) Map showing the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of subspecies conventionally recognized by morphological criteria (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. The open area in northern Alabama describes the range of a related threatened species, the flattened musk turtle, *Sternotherus depressus* (see text).

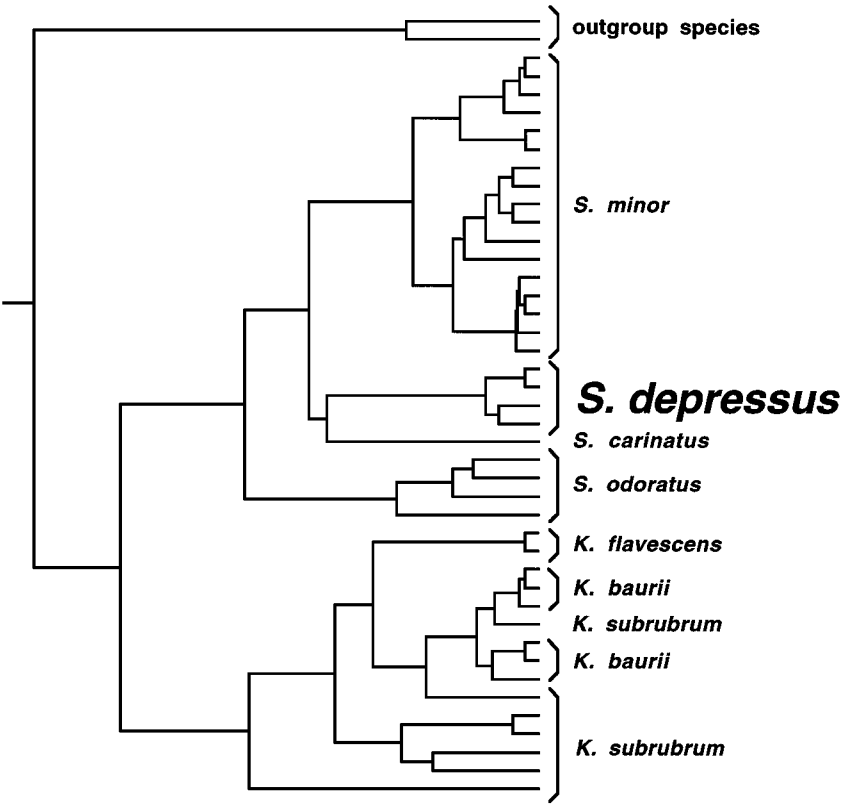


Figure 4 Distinctive phylogenetic position of the threatened flattened musk turtle, *Sternotherus depressus*, within a broader array of some presumed relatives in the genera *Sternotherus* and *Kinosternon* (Kinosternidae) (after Reference 65). These particular assays involved control region sequences of the mitochondrial mtDNA molecule. They were based on samples chosen to represent the major phylogroups identified by restriction fragment length polymorphisms (RFLPs) in more extensive geographic surveys.

have been recognized (Figure 6). In terms of mtDNA, four major phylogroups are evident (Figure 6): (*a*) group D, confined to the Florida peninsula (consistent with the traditionally described range of *K. subrubrum steindachneri*); (*b*) group C, along the Atlantic seaboard from south Georgia to Virginia (part of the traditional *K. subrubrum subrubrum*); (*c*) group B, in a central region from the Florida Panhandle and western Georgia to Mississippi (also *K. subrubrum subrubrum*); and (*d*) group A, from the west (in the traditional range of *K. subrubrum hippocrepis*).

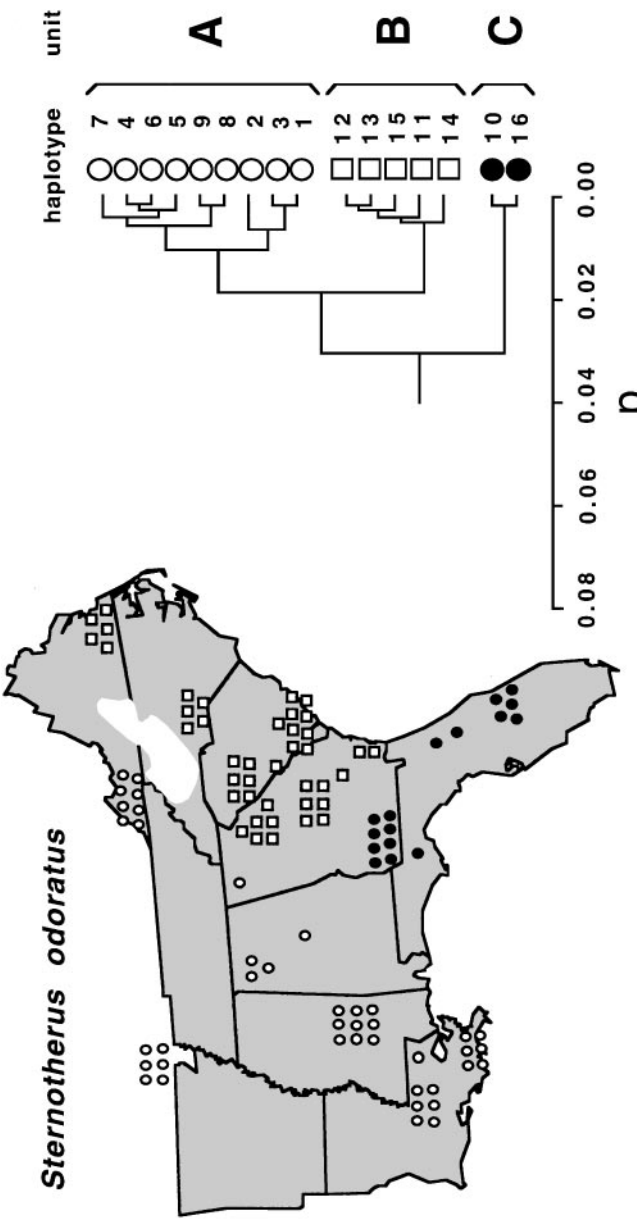


Figure 5 Phylogeographic pattern of mitochondrial (mt) DNA in the stinkpot, *Sternotherus odoratus*. (*Left*) Map showing the distribution of the species in the southeastern United States (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

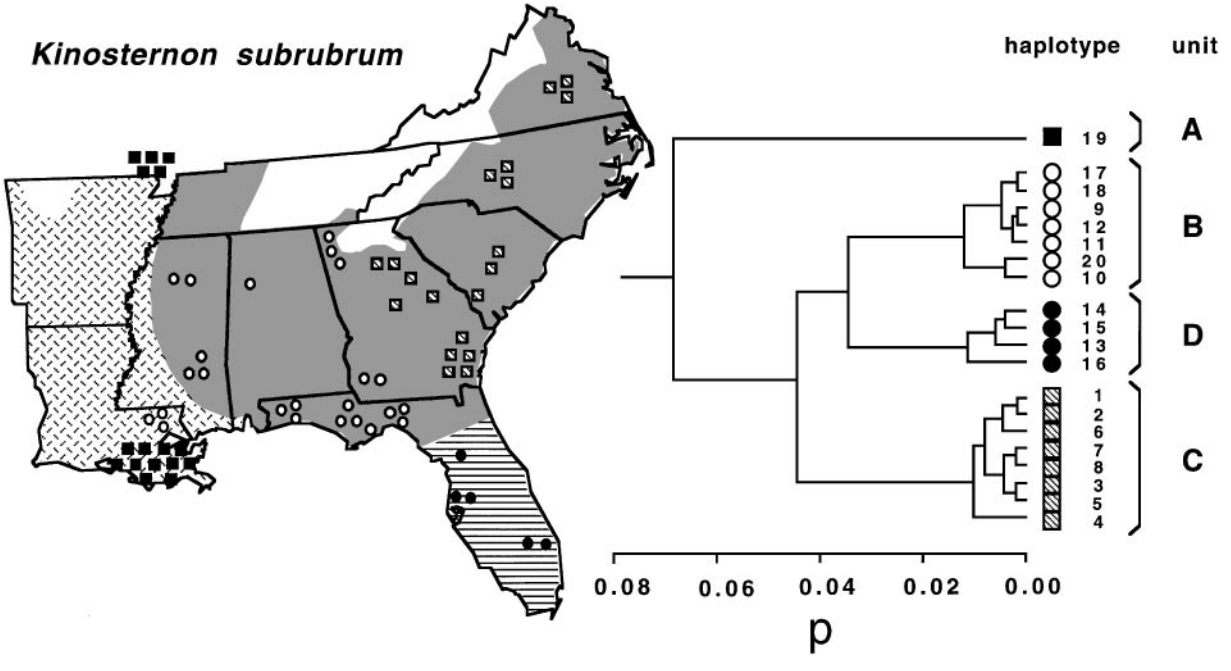


Figure 6 Phylogeographic pattern of mitochondrial (mt) DNA in the mud turtle, *Kinosternon subrubrum*. (*Left*) Map showing the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of subspecies conventionally recognized by morphological criteria (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

However, the genetic situation becomes more complicated when a close taxonomic relative, *Kinosternon baurii*, is included in the comparison (62). This taxon has two moderately different mtDNA phylogroups (Figure 7): One is confined to the Florida peninsula, and the other occurs along the Atlantic coast. Both of the *K. baurii* mtDNA units are related more closely to haplogroup C in *K. subrubrum* (the Atlantic coast assemblage) than is this C assemblage to other haplogroups within *K. subrubrum*. Indeed, all haplotypes in *K. baurii* are embedded phylogenetically within the broader diversity of the C clade of *K. subrubrum* (Figure 4).

Several explanations are possible (62). Perhaps hybridization has led to an introgressive transfer of mtDNA lineages between two otherwise long-separated biological species of mud turtles along the Atlantic seaboard. Alternatively, *K. baurii* may be a recent phylogenetic derivative of *K. subrubrum* in this area and has not yet evolved noticeable differences from its ancestor in mtDNA composition. Under this hypothesis, *K. subrubrum* and *K. baurii* might be good biological species with the former being paraphyletic to the latter in matrilineal genealogy. Under either of these scenarios, a secondary invasion of the Floridian peninsula by *K. baurii* could account for the sympatric occurrence there of highly divergent mtDNA lineages (the C and D phylogroups) within the *Kinosternon* complex. Another possibility is that *K. baurii* is confined to the Floridian peninsula and that turtles along the Atlantic seaboard represent *K. subrubrum* exclusively. Indeed, because of morphological similarity between the two species, particularly along the Atlantic seaboard, there has been much discussion in the literature as to whether the range of *K. baurii* truly extends into this area (21, 26, 27, 30). In the absence of direct evidence from nuclear genes, we cannot resolve these possibilities.

In any event, at least four major mtDNA phylogroups are present within this complex of *Kinosternon* mud turtles in the southeastern United States, and their distributions overall bear considerable likeness to those discussed above for *S. odoratus*.

GRAPTEMYS SPECIES (MAP TURTLES) Many turtle groups tend to be relatively conservative morphologically (relative to birds, for example). However, the carapaces and heads of *Graptemys* species display varied and strikingly beautiful designs, from which the moniker map turtles derives. About a dozen forms in the southeastern United States traditionally are recognized at the taxonomic level of species, and most are endemic to particular drainages along the Gulf coast (Figure 8). No species occur in Atlantic coastal drainages or in peninsular Florida.

Lamb et al (31) examined all of these forms for mtDNA restriction sites as well as nucleotide sequences from the *cyt b* gene and control region. About

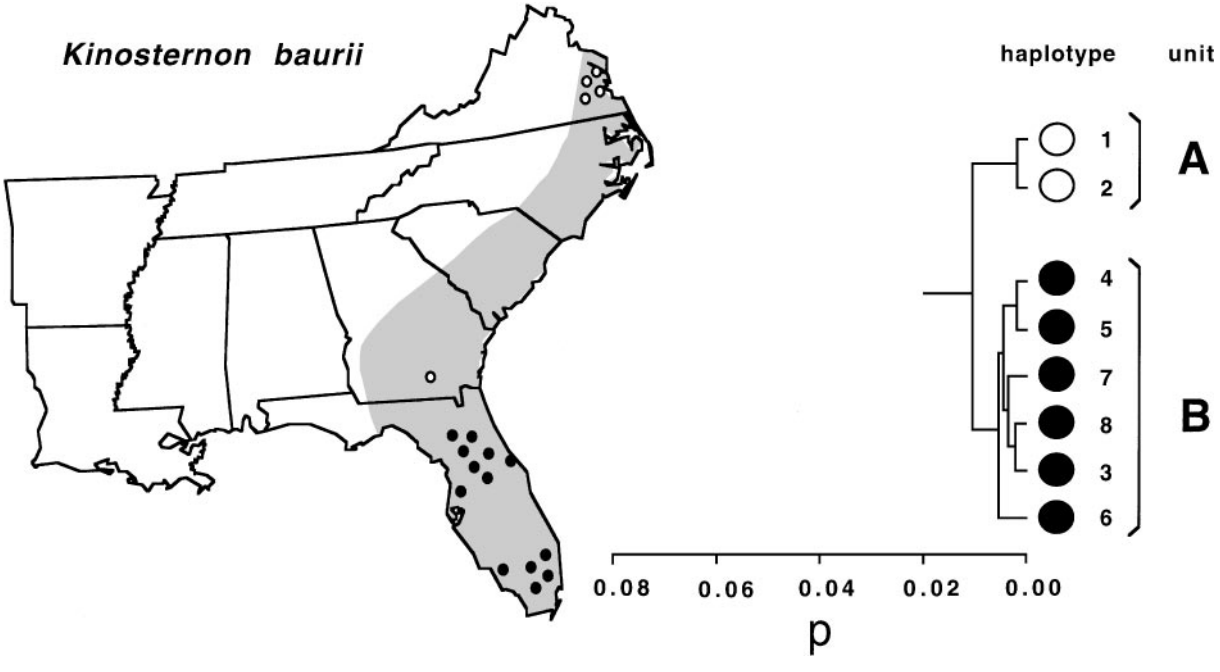


Figure 7 Phylogeographic pattern of mitochondrial (mt) DNA in the striped mud turtle, *Kinosternon baurii*. (*Left*) Map showing the distribution of the species in the southeastern United States (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

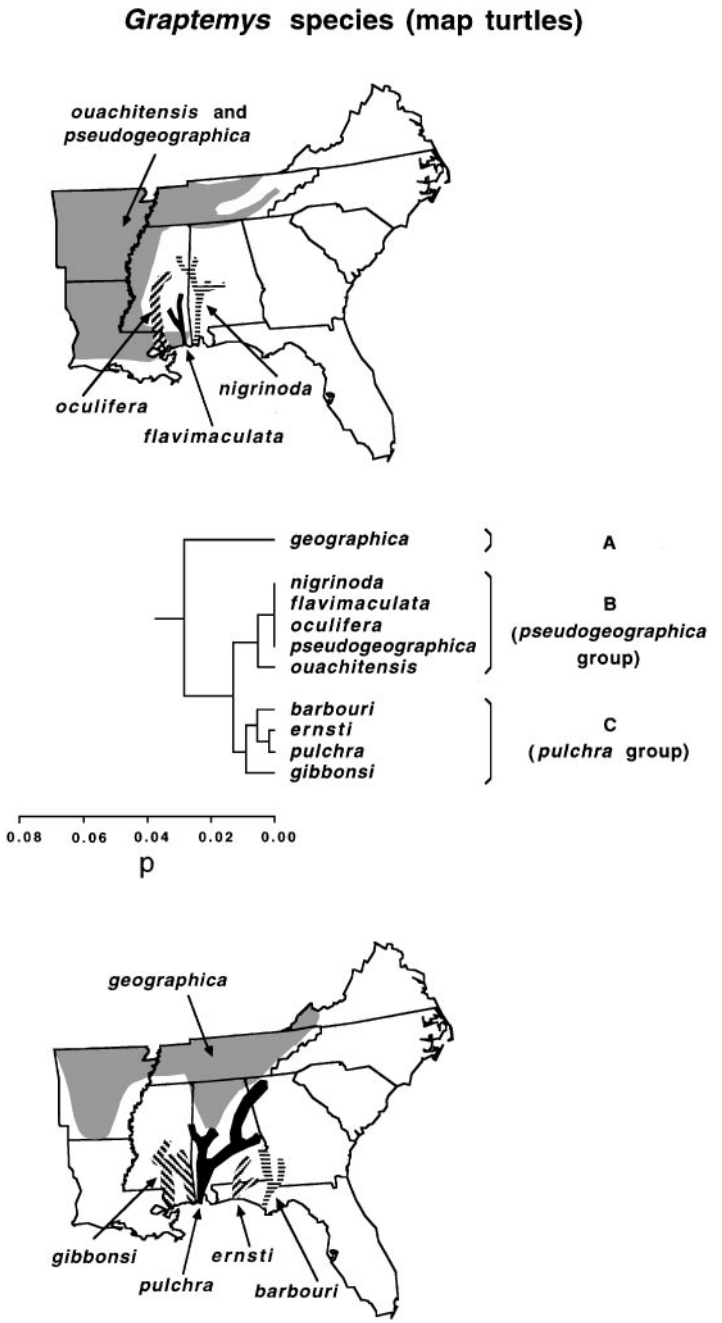
five of the taxa assayed could not be distinguished by restriction sites or *cyt b* sequences, and depths of genetic separations in the *Graptemys* complex overall were small by the standards summarized above for intraspecific phylogroups within other turtle species (Table 2). Nonetheless, three monophyletic lineages within *Graptemys* could be discerned in assays of mtDNA restriction fragment length polymorphisms (RFLPs) (Figure 8): (*a*) a *G. pulchra* group of four taxonomic species in central coastal rivers of the Gulf states; (*b*) a *G. pseudogeographica* group of five species mostly to the west but overlapping spatially with members of the *G. pulchra* group to some extent; and (*c*) *Graptemys geographica*, a widespread species in the central and northern United States.

The genealogical data taken at face value suggest that the *Graptemys* complex has been taxonomically oversplit at the species level. Perhaps this is because shell characters have been available to distinguish adjacent populations, many of which now appear from the molecular data not to have been long separated historically. Indeed, the *Graptemys* complex appears to display less mtDNA lineage diversification overall than do conspecific populations of several kinosternid species (compare Figure 8 with Figures 3, 5, and 6).

TRACHEMYS SCRIPTA (SLIDER) *Trachemys scripta* is widespread and abundant, with two named subspecies in the current coverage area: *T. s. scripta*, mostly along the Atlantic coast, and *T. s. elegans*, to the west. In field guides (e.g. 17), these forms usually are depicted as "intergrading" in western Georgia, Alabama, and the Florida Panhandle.

With respect to mtDNA, Avise et al (12) first surveyed a small number of specimens of this species, but additional specimens in the current study bring the total sample size to $N = 65$. Mitochondrial variation in this species is extremely limited: Two haplotypes were observed, and these differed by only three assayed restriction sites ($p \cong 0.006$). Nonetheless, the two lineages show a strong geographic orientation generally consistent with the described subspecies ranges (Figure 9). However, two individuals with the western haplotype A were observed in the Atlantic coastal plain.

DEIROCHELYS RETICULARIA (CHICKEN TURTLE) *Deirochelys reticularia* occurs primarily in coastal plain regions throughout the study area (Figure 10). In control region sequences, the mtDNA of some specimens display a variant feature of potential cladistic import that has not been reported in other turtle species: a relatively large (10 bp) deletion. This deletion, which appears to be a derived condition by reference to outgroup species, is present in all chicken turtles in the eastern portion of the species' range (Atlantic coast and peninsular Florida) but is absent to the west (Figure 10). However, within the western region, large sequence divergence estimates (mean $p \cong 0.045$) distinguish


Graptemys species (map turtles)
ouachitensis and
pseudogeographica
oculifera
flavimaculata
nigrinoda
geographica
nigrinoda
flavimaculata
oculifera
pseudogeographica
ouachitensis
barbouri
ernsti
pulchra
gibbonsi
A
B
(pseudogeographica
group)
C
(pulchra group)
0.08 0.06 0.04 0.02 0.00
p
geographica
gibbonsi
pulchra
ernsti
barbouri

samples in Missouri from those along the Gulf coastal plain. The pattern suggests an ancient separation in the mtDNA gene tree plus a more recent derivation for the distinctive matrilines along the Atlantic coast and peninsular Florida (Figure 10).

MACROCLEMYS TEMMINCKII (ALLIGATOR SNAPPING TURTLE) Striking phylogeographic structure in mtDNA is evident at two spatial scales within *Macroclemys temminckii*, a highly aquatic species (51, 52). First, samples from each of several river drainages entering the Gulf of Mexico display fixed differences in haplotype frequencies, suggesting severe restrictions on contemporary interdrainage gene flow. Second, much deeper phylogenetic separations in the mtDNA gene tree distinguish three regional population assemblages (Figure 11) that the authors (52) refer to as evolutionarily significant units. The most distinctive of these units is confined to the Suwannee River, the only major drainage in peninsular Florida currently inhabited by the species. The two other principal mtDNA units characterize populations in all drainages from the Pensacola River in western Florida to the Trinity River in Texas and all drainages in the Floridian Panhandle between the Pensacola and the Suwannee.

CHELYDRA SERPENTINA (COMMON SNAPPING TURTLE) *Chelydra serpentina* occurs throughout eastern and central North America. Two subspecies are recognized: *C. s. osceola*, in the Florida peninsula, and *C. s. serpentina*, elsewhere on the continent. A survey of mtDNA control-region sequences from samples across the southeastern United States revealed almost no variation within or between populations (63). A single haplotype characterized 60 of the 66 specimens surveyed, and two variant haplotypes differed from it by one and two mutational changes (Figure 12). This paucity of mtDNA variation could be attributed to some unknown molecular mechanism or selective peculiarity that has arrested mtDNA evolution in snapping turtles, but these reasons seem unlikely because a broader geographic survey of the species uncovered moderate mtDNA sequence differences between North American specimens and those in Central and South America (45). Also, in similar molecular assays, the snapping turtle proved to be highly distinct from its closest living relative, the alligator snapping turtle (51, 52).

←

Figure 8 Phylogeographic patterns of mtDNA in map turtles of the genus *Graptemys*. Maps show the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of species conventionally recognized by morphological criteria (after Reference 17). See text for further explanation. (Center) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes of these species. The scale is in units of sequence divergence (f) between species.

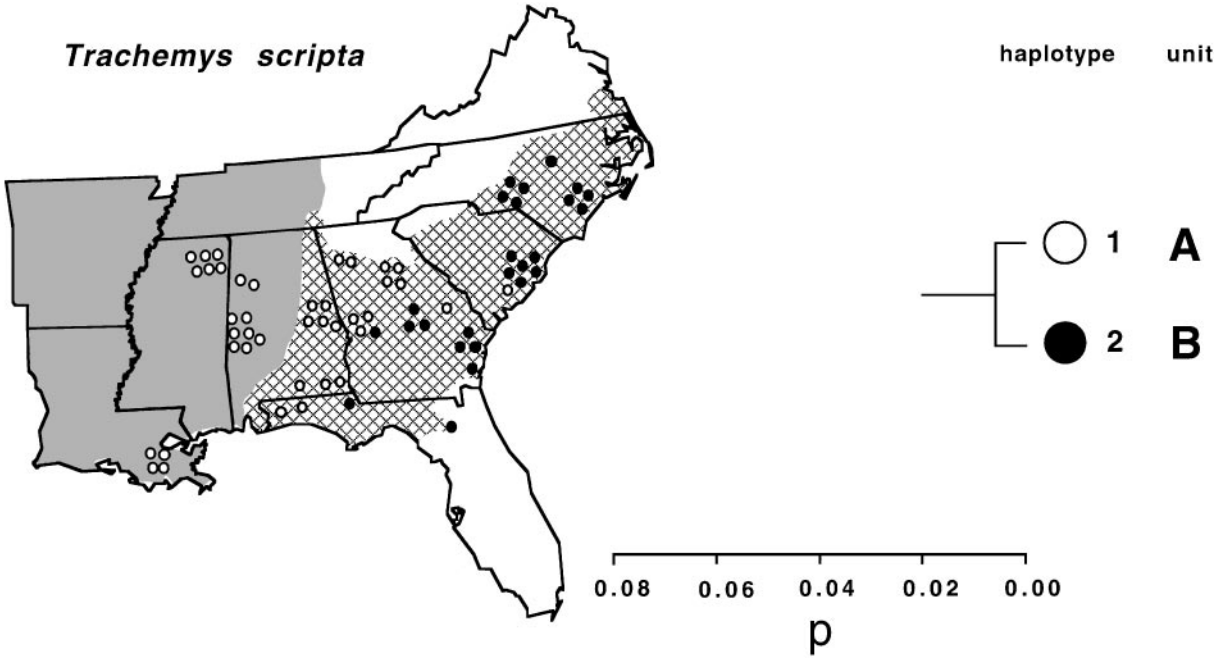


Figure 9 Phylogeographic pattern of mtDNA in the slider, *Trachemys scripta*. (*Left*) Map showing the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of subspecies conventionally recognized by morphological criteria (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

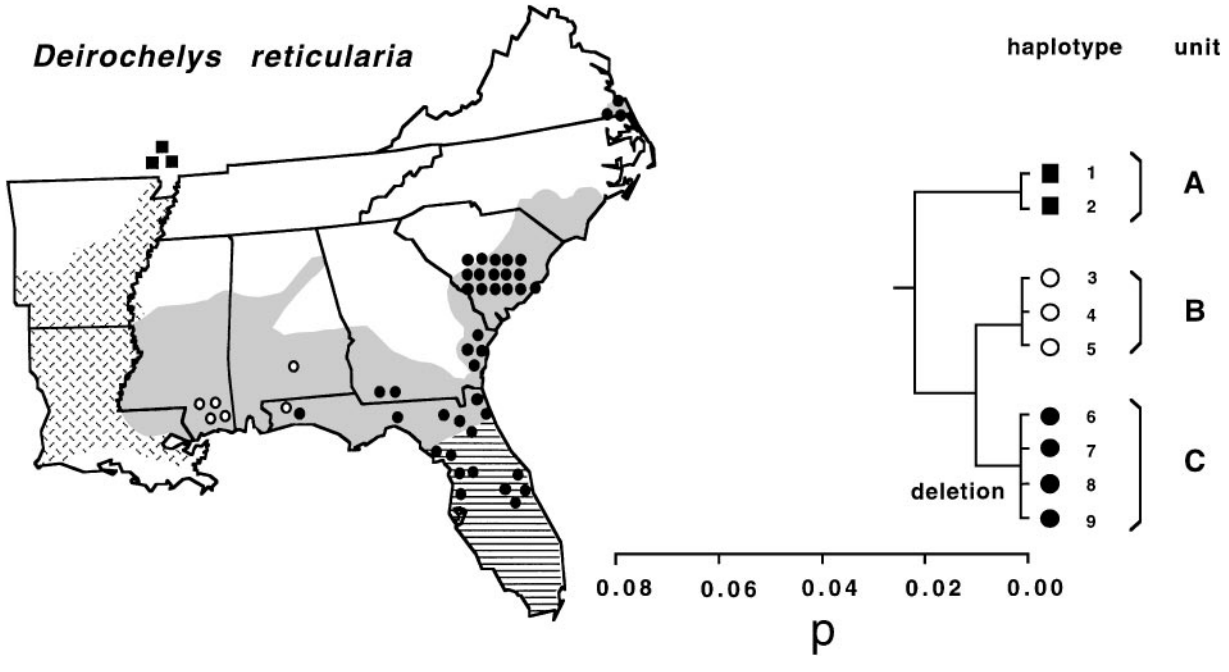


Figure 10 Phylogeographic pattern of mtDNA in the chicken turtle, *Deirochelys reticularia.* (*Left*) Map showing the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of subspecies conventionally recognized by morphological criteria (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

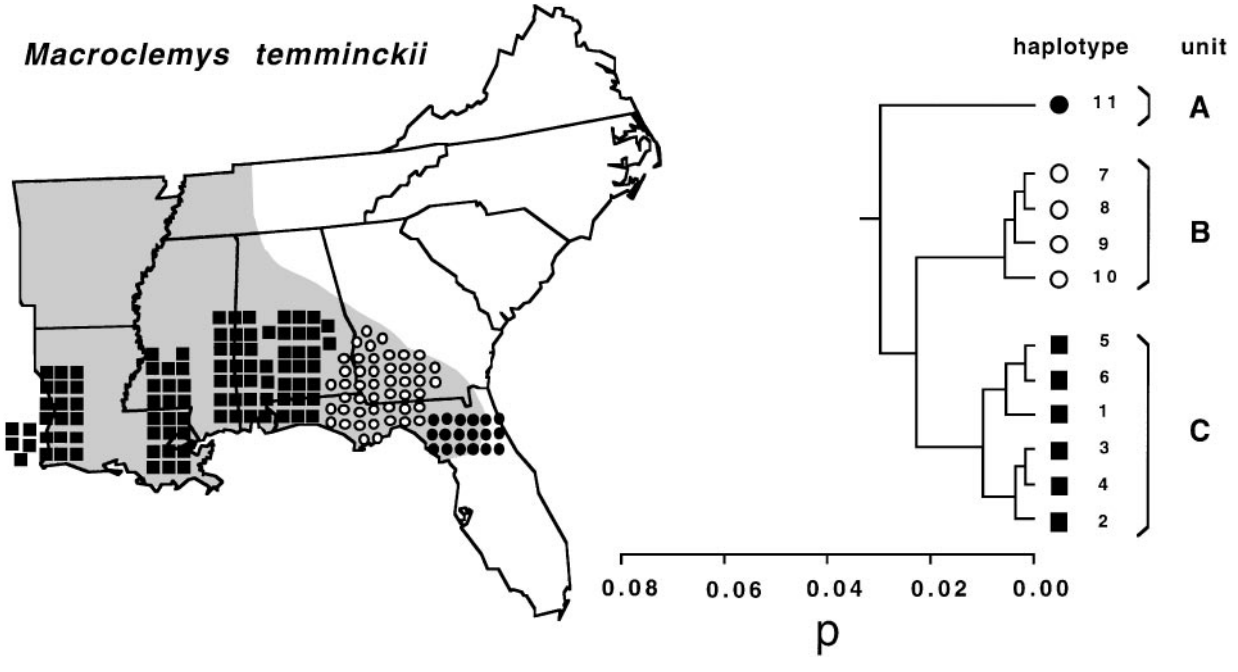


Figure 11 Phylogeographic pattern of mtDNA in the alligator snapping turtle, *Macroclemys temminckii*. (*Left*) Map showing the distribution of the species in the southeastern United States (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

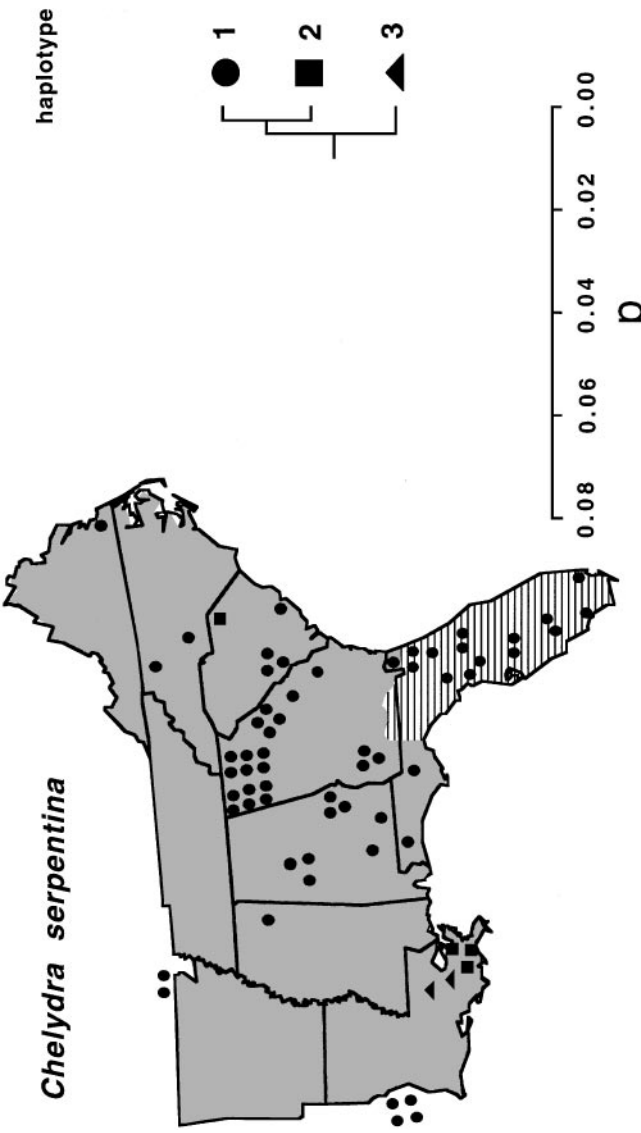


Figure 12 Phylogeographic pattern of mtDNA in the snapping turtle, *Chelydra serpentina*. (*Left*) Map showing the distribution of the species in the southeastern United States, with *shaded regions* indicating approximate geographic ranges of subspecies conventionally recognized by morphological criteria (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation.

Terrestrial Turtle

GOPHERUS POLYPHEMUS (GOPHER TORTOISE) The gopher tortoise is the sole land-confined turtle in the southeastern United States. This threatened species occurs in sand-scrub habitats in Florida and in the coastal plain from eastern South Carolina to Louisiana. A genetic survey (42) revealed numerous mtDNA haplotypes that are grouped into two major phylogenetic lineages with a strong geographic configuration (Figure 13). One phylogroup characterizes samples from western Georgia and the Floridian Panhandle to Louisiana, and the other is confined to peninsular Florida, southern Georgia, and South Carolina. The latter assemblage also consists of two recognizable subgroups, one of which is present only in mid-Florida.

PHYLOGEOGRAPHIC HYPOTHESES AND GENEALOGICAL CONCORDANCE

These comparative data on the intraspecific matrilineal histories of several species of Testudines in the southeastern United States permit independent tests of phylogeographic hypotheses and principles of genealogical concordance previously derived from similar genetic studies of other freshwater and terrestrial vertebrates in the region. These phylogeographic concepts and their corollaries are appraised in order of their appearance in Table 1.

Hypothesis I: Populations of Most Species Display Significant Phylogeographic Structure

Perhaps not surprisingly, the hypothesis that populations of most species display significant phylogeographic structure is supported abundantly by mtDNA data for the Testudines. With the exception of the snapping turtle, all broadly distributed species surveyed across the southeastern region show striking matrilineal population structure at various spatial scales and inferred temporal depths. Given the limited mobility of individuals in most turtle species, perhaps this local structure is to be expected, as is a window of opportunity for the evolution of deeper interregional separations in response to longer-term biogeographic barriers. This latter opportunity appears to have been realized, as evidenced by the major phylogeographic breaks identified within nearly all of the broadly distributed turtle species surveyed.

Hypothesis II: Nonsubdivided, High-Dispersal Species May Have Limited Phylogeographic Structure

The common snapping turtle, *C. serpentina*, is the only surveyed species without pronounced mtDNA phylogeographic structure. Although shallow or modest matrilineal structure might yet be detected in more sensitive molecular assays,

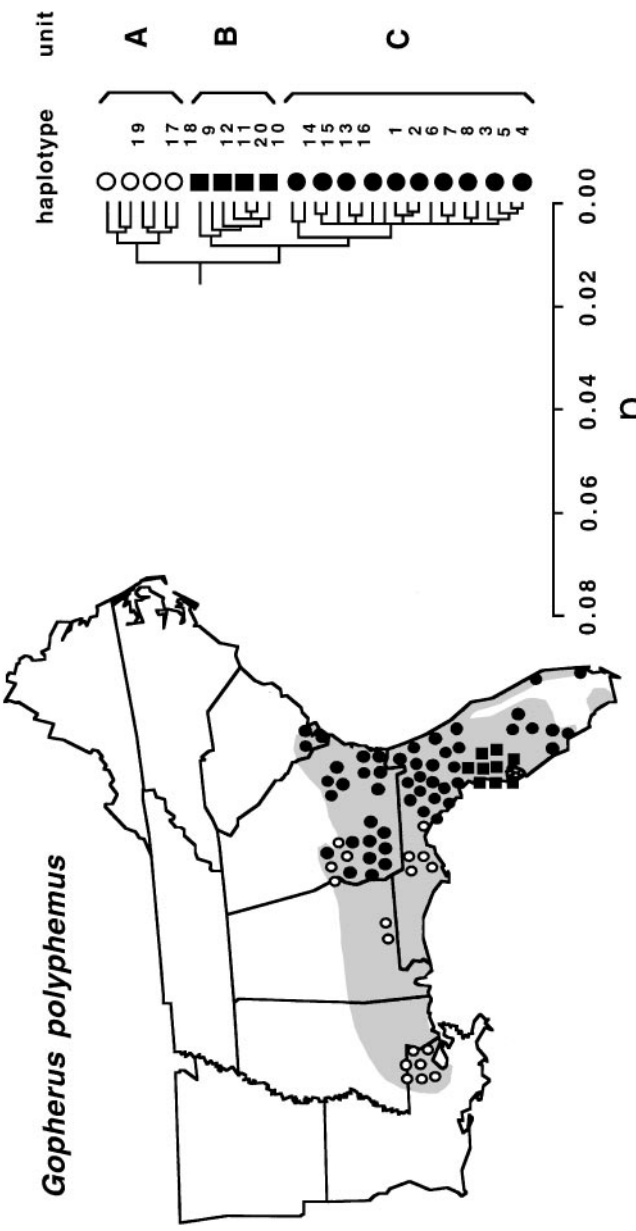


Figure 13 Phylogeographic pattern of mtDNA in the gopher tortoise, *Gopherus polyphemus*. (*Left*) Map showing the distribution of the species in the southeastern United States (after Reference 17). Also shown are locations and phylogroup membership of all individuals (*symbols*) assayed for mtDNA. (*Right*) Single-linkage cluster phenogram summarizing genetic relationships among the mtDNA haplotypes and highlighting (by different *symbols*) the principal divergent phylogroups. The scale is in units of sequence divergence (*p*) estimated between extant taxa. See text for further explanation. Because of large sample sizes, not all individuals are shown.

the available data strongly suggest that long-standing evolutionary separations have not been a part of the phylogeographic history of contemporary populations of the common snapping turtle in the southeastern United States. Perhaps individuals of this species are unusual among the surveyed turtles with respect to high dispersal capabilities (19) and attendant imperviousness to historical biogeographic barriers that appear to have affected other species.

A related possibility has to do with the observation that among all North American turtles, *C. serpentina* (and *Chrysemys picta*) are tolerant to cold and were "always among the first to invade formerly glaciated areas at the end of the Wisconsin" (24, p. 45). The southeastern United States was never covered by Pleistocene glaciers, but its climate was far cooler at times of glacial maxima, and some drainages traversing the South received frigid meltwaters from northern glaciers during warming episodes (37). The unusual cold tolerance and perhaps the high dispersal capability of *C. serpentina* may indicate that the species was not confined to isolated pockets of warm water habitat during the late Tertiary and Quaternary and thus was less subject to historical population subdividing by climatic events and/or shifting watersheds.

Hypothesis III: Major Phylogeographic Units Within a Species Reflect Long-Term Historical Barriers to Gene Flow

Most of the widely distributed turtle species assayed display deep matrilineal separations on a regional geographic scale. However, major splits in a gene tree (such as that for mtDNA) cannot automatically be equated with deep separations in a population tree (5, 22, 32, 41, 43). Thus, it has been argued (10), additional evidence in the form of genealogical concordance is required to establish by hard criteria that major phylogenetic branches in a gene tree register major branches in an organismal phylogeny. Four aspects of genealogical concordance (Table 1) can be distinguished, all of which represent logical corollaries of phylogeographic hypothesis III.

CONCORDANCE ACROSS CHARACTERS WITHIN A GENE Every deep phylogenetic split in the intraspecific gene tree of a turtle species is, by definition, supported concordantly by multiple restriction-site or sequence characters in mtDNA. Thus, this category of genealogical concordance merely identifies the major gene-tree phylogroups worthy of further biogeographic consideration.

CONCORDANCE OF GENEALOGICAL PARTITIONS ACROSS MULTIPLE GENES The concordance of genealogical partitions across multiple genes cannot be evaluated critically in the turtles studied because comparable genealogical evidence from multiple nuclear genes is unavailable for comparison. In the absence

of such direct information, a potential surrogate can be employed: traditional subspecies definitions. To the extent that morphology-based intraspecific taxonomy reflects substantial population-level differences in nuclear genomes, this category of genealogical concordance can be addressed, at least in part.

Agreement exists between mtDNA phylogeography and taxonomic definitions for several of the turtles assayed: within *Sternotherus minor* (Figure 3) and *Trachemys scripta* (Figure 9), to a partial extent within *Kinosternon subrubrum* (Figure 6), and with regard to the phylogenetic distinctiveness of *Sternotherus depressus* from the other species of Kinosternidae (Figure 4). However, in other cases such agreement is lacking. Thus, the mtDNA data provide no evidence for a special phylogenetic distinctiveness of the Floridian subspecies of *Deirochelys reticularia* (Figure 10) or *Chelydra serpentina* (Figure 12) or for long-standing evolutionary separations among several of the recognized species of *Graptemys* turtles (Figure 8). Conversely, relatively deep phylogenetic separations in mtDNA are apparent within the taxonomically monotypic *Sternotherus odoratus* (Figure 5), *Macroclemys temminckii* (Figure 11), and, to a lesser extent, *Gopherus polyphemus* (Figure 13).

For these cases of mtDNA discordance with traditional taxonomy, two primary possibilities remain: (*a*) The existing taxonomy does not reflect significant phylogeographic partitions or (*b*) the mtDNA phylogenies are misleading in this regard. Described next are two aspects of genealogical concordance that suggest that the mtDNA gene trees are meaningful registers of phylogeographic population histories and hence that current taxonomy in several cases may need revision.

CONCORDANCE OF GENEALOGICAL PARTITIONS ACROSS MULTIPLE CODISTRIBUTED SPECIES As was true in earlier studies of freshwater fishes, a remarkable result of the current review is the level of general agreement across species of Testudines in the spatial positions of major mtDNA phylogeographic units across the southeastern United States. In *Sternotherus minor* (Figure 3), *Sternotherus odoratus* (Figure 5), *Kinosternon subrubrum* (Figure 6), *Trachemys scripta* (Figure 9), *Deirochelys reticularia* (Figure 10), *Macroclemys temminckii* (Figure 11), and *Gopherus polyphemus* (Figure 13), recognizable phylogenetic separations in the mtDNA gene tree distinguish populations in peninsular Florida and/or those along the Atlantic coast from populations in western (Gulf coast) areas. These regions also bear striking resemblance to the areas inhabited by major mtDNA phylogroups within several southeastern US fish species (Figure 1).

Furthermore, as was the case for the freshwater fishes surveyed, in only a few cases of the turtles are additional deep mtDNA subdivisions evident within the surveyed region. Exceptions to this statement involve far western forms in

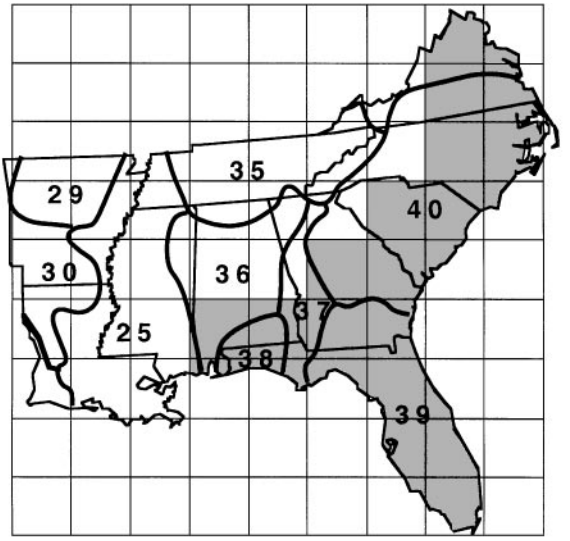
K. subrubrum (Figure 6) and *D. reticularia* (Figure 10) and a central Alabama form of *Sternotherus* (*S. depressus*; Figures 3, 4). These cases embellish but do not contradict the tendency for the above-mentioned phylogeographic distinctions between eastern and western regions.

CONCORDANCE OF GENEALOGICAL PARTITIONS WITH BIOGEOGRAPHIC PROVINCES IDENTIFIED BY INDEPENDENT EVIDENCE Faunal lists are the traditional data by which regional biotic provinces are identified. For example, as described earlier, an analysis of geographic ranges for the 241 species of freshwater fishes in the southeastern United States identified Atlantic (including peninsular Florida) and Gulf drainages as the two most distinctive regions faunistically (Figure 1). These two regions also show general agreement with the geographic distributions of major intraspecific mtDNA phylogroups in several fish species (e.g. Figure 1). Does a similar concordance between composite faunal distributions and intraspecific mtDNA phylogroups exist for the Testudines?

Following the general procedures employed by Swift et al (58) in analyses of fish faunal provinces, we compiled range information (17, 19) for all 35 native species of freshwater and terrestrial turtles that inhabit the southeastern United States. Presence or absence of each species was determined for each of 48 grids on a southeastern map (Figure 14), and similarity coefficients (Jaccard coefficients of association; see 56) between grids were calculated. The resulting similarity matrix was clustered phenetically (by UPGMA; see 50), with results shown in Figure 14.

The geographic picture of the Testudines bears strong resemblance to that of the freshwater fishes: A basal split distinguishes the Atlantic coast and Floridian region from locales to the north and west across most of the remainder of the survey area. As already described, this pattern agrees well with geographic trends in the distributions of major mtDNA phylogroups within several of the turtle species surveyed. Where deep phylogeographic splitting was observed, almost invariably the major phylogroups were oriented in an eastern (Atlantic)

Figure 14 Faunal assessments of Testudines in the southeastern United States based on composite species distributions. The map shows the two basal faunal assemblages (*shaded* and *unshaded*) for all native turtle species in the region as identified in a cluster analysis (phenogram shown in the *lower half of the figure*) of a matrix of faunal similarity coefficients for the grid squares. Note the basal distinction between "Atlantic" and "Gulf" areas. A somewhat different grouping method based on turtle "species richness" gives nearly identical results (1). *Numbered areas* on the map depict freshwater faunal ecoregions or aquatic ecological units as identified by differences in the assemblages and subassemblages of fish species (36).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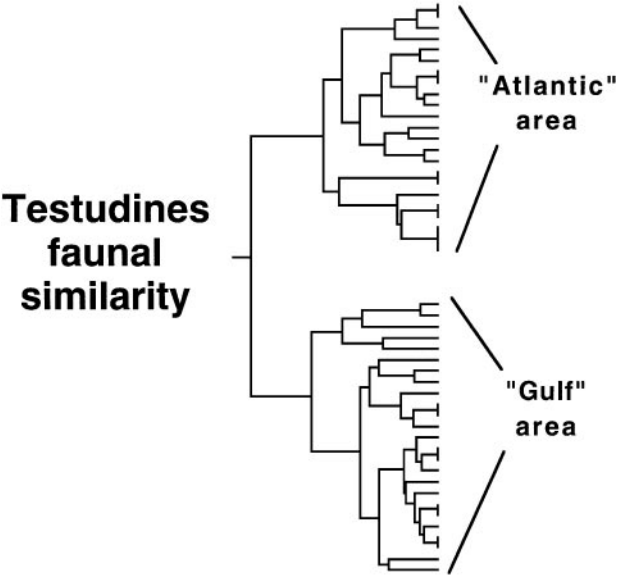
Testudines faunal similarity
"Atlantic" area
"Gulf" area

versus western (Gulf) pattern generally consistent with the major break in the overall faunal distributions.

We suspect that these two seemingly different sources of information on zoogeographic provinces—composite faunal lists and intraspecific phylogeographic partitions—may have similar evolutionary etiologies reflecting long-standing ecological or biogeographic impediments to interregional dispersal. The ranges of species clearly reflect dispersal barriers. Similarly, we suggest that phylogeographic breaks within species that are widely distributed across biogeographic provinces are due to evolutionary subdivision, with populations in both biotic provinces having survived for current observation. If populations in only one of the historical regions had survived, that species as a whole would be merely another contributor to the concordant distributional faunal lists upon which biotic provinces traditionally have been recognized.

On a finer geographic scale, Maxwell et al (36) identified several additional "ecoregions" or "aquatic ecological units" in the southeastern United States (Figure 14) that might be considered biogeographic "subprovinces" from traditional evidence. One of these (ecoregion 39) encompasses the Floridian peninsula and southern Georgia and is approximately coincident with Remington's (48) demarcation of the unique Floridian biome. Another of Maxwell et al's units (ecoregion 40) encompasses all of the Atlantic coastal plain from Georgia to Virginia. This distinction between peninsular Florida and the northern coastal plain was mirrored closely by phylogenetic partitions in mtDNA within *S. odoratus* (Figure 5), *K. subrubrum* (Figure 6), and *K. baurii* (Figure 7). To the west, a more complicated array of ecoregions appear in Maxwell et al's analysis, and in general these bear a less clear alignment to phylogeographic patterns observed in molecular studies of the Testudines.

PHYLOGEOGRAPHIC SEPARATIONS

The comparative phylogeographic patterns summarized here for the Testudines are similar in yet another regard to those reported previously for the freshwater fishes in the southeastern United States. In mtDNA studies of these fishes, the geographic distributions of the principal phylogroups were similar across the assayed species, but the absolute magnitudes of the estimated net sequence divergences between these mtDNA clades varied by more than an order of magnitude: e.g. from $p \cong 0.006$ in the bowfin (*Amia calva*) to $p \cong 0.082$ in the redear sunfish (*Lepomis microlophus*) (14). Under a conventional molecular clock calibration for vertebrate mtDNA ($p \cong 0.02$ between a pair of lineages per million years; Reference 16), these estimates at face value imply times of intraspecific phylogroup separation ranging from roughly 300,000 years ago (bowfin) to 4,100,000 years before the present (redear sunfish).

Similarly, net sequence divergence estimates between the major intraspecific phylogroups in the turtles differ by more than an order of magnitude (Table 2): from $p \cong 0.006$ in *Trachemys scripta* to $p \cong 0.070$ in *Kinosternon subrubrum* (phylogroups A and B). Under the conventional clock noted above, these translate into population divergence estimates ranging from 300,000 to 3,500,000 years ago. From mtDNA studies of other Testudine taxa, a fivefold to tenfold slower pace of mtDNA sequence evolution has been suggested (12, 15, 29; see also 33–35, 46, 47). As applied to the phylogroups under current review, these values imply intraspecific separation times that could range from 1.5 Mya to as much as 35 Mya.

Microevolutionary rate assessments are particularly challenging because de novo sequence change postdating population separations must be distinguished from sequence differences attributable to lineage sorting from a polymorphic common ancestor (7). In principle, the former is independent of effective population size whereas the latter is critically dependent on historical population demographic factors (that typically remain unknown from independent evidence). Our use of "net" sequence divergence (as defined in Table 2) is an attempt to correct for ancestral polymorphism, but it assumes that levels of variation in extant taxa are representative of that in an ancestral population.

Apart from these and additional concerns about estimation errors, at least two ad hoc biological explanations can be advanced for the large variance in the sequence divergence values. First, perhaps extant turtle populations were subdivided at widely different times. During the Pliocene and especially the Pleistocene, repeated episodes of climatic alteration promoted ecophysiographic changes in the southeastern landscape and forced either range shifts or extinctions on its biotic elements. Thus, lineage separations within various species might trace to different climatic cycles depending in part on the patterns of extinction of former regional isolates. Furthermore, some of the phylogeographic footprints might trace to earlier Tertiary times, when (for example) the central highlands of Florida probably existed periodically as one or more islands relatively isolated from the continent.

Second, perhaps a mtDNA "clock" ticks at widely varying paces in different turtle lineages. Considerable controversy (beyond the scope of this paper) exists about the calibration and reliability of molecular clocks (38), and considerable empirical evidence exists for severalfold mtDNA rate variation among taxonomic groups (e.g. 2, 4, 33–35, 46, 47, 66) and sometimes even among closely related lineages (68).

Our recent molecular findings on several kinosternid species (in *Sternotherus* and *Kinosternon*) run counter to our prior experience with several other marine, estuarine, freshwater, and terrestrial species of Testudines, in which unusually low (by vertebrate standards) levels of intraspecific and interspecific

mtDNA sequence divergence had been the norm (12, 15, 28, 29). The highly variable mtDNA genotypes in the Kinosternidae do not by themselves speak directly to molecular clock calibrations, but they do provide different impressions of the magnitudes of intraspecific population variation and differentiation as compared with several other turtle groups. One extinct kinosternid turtle (*Xenochelys formosa*) has been described from the Oligocene, and the extant species *Sternotherus odoratus* and *Kinosternon subrubrum* have been described from Pliocene deposits (19). This considerable antiquity for these kinosternid turtles provides an opportunity for ancient intraspecific lineage separations. On the other hand, *Chelydra serpentina* also has been reported from the Pliocene (19), yet no deep mtDNA lineage separations were evident.

RELEVANCE TO CONSERVATION BIOLOGY

Regardless of the particular historical scenarios invoked to account for the phylogeographic patterns in these terrestrial and freshwater turtles, the molecular data on matrilineal separations carry ramifications for management and conservation efforts in two principal regards (13). First, the data are germane to taxonomic and systematic issues for particular endangered (or other) species considered individually. For example, the federally threatened *Sternotherus depressus* was a problematic taxon because of its peculiar range and because of uncertainties about the genetic basis of its oddly flattened carapace. The mtDNA data indicate that the matrilines of this population had a relatively ancient evolutionary separation from those of other kinosternids in the region. To this extent, the basis for the existing taxonomic recognition is bolstered, as are special conservation efforts that have stemmed from it.

Second, the intraspecific genetic architectures of multiple species add to knowledge of the faunal provinces that, we argue, should be appreciated as major centers of biogeographic diversity in biome-based conservation efforts. With recognition of the relative integrity and tendency toward uniqueness of major historical biotic assemblages, conservation planning at the ecosystem level might be instituted in conjunction with traditional species-focused management efforts to enhance the effectiveness and impact of conservation programs on a regional scale (8).

SUMMARY

We have identified four aspects of genealogical concordance that apply empirically to several species of turtles (as well as to other freshwater and terrestrial vertebrates) in the southeastern United States. Many species-idiosyncratic phylogeographic outcomes also are apparent in the mtDNA analyses. Depending

on the context, both the idiosyncrasies and the generalized trends can be relevant to taxonomic and conservation efforts.

In the future, it will be of interest to evaluate phylogeographic hypotheses and principles of genealogical concordance by conducting similar comparative analyses on other regional biotas. Outcomes may differ. For example, perhaps high-latitude ecoregions will tend to lack genealogical concordance because no long-term in situ evolution can have taken place in recently glaciated areas. A more cosmopolitan view of phylogeography would recognize such trends as well. Regardless of the outcomes of such future studies, the comparative genetic analyses of faunas in the southeastern United States already have contributed insights into the historical nature of particular biotic provinces and of the evolutionary factors that can contribute to the composite architectures of species on a regional scale.

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