> The species phylogeny is more like a statistical distribution, being composed of various trees (the gene trees), each of which may indicate different relationships.
>
> —Wayne Maddison, 1995

5

GENEALOGICAL CONCORDANCE

GIVEN THE INHERENT stochastic elements of genetic transmission under Mendelian inheritance—random segregation of alleles and independent assortment of unlinked loci through extended organismal pedigrees—each piece of DNA within a species might be expected to trace an idiosyncratic genealogical history. Furthermore, given the great diversity of ecological and evolutionary factors likely to have impinged upon the historical demographies and genetic architectures of extant faunas, an idiosyncratic phylogeographic outcome also might be expected for each species. If the noise of genealogical idiosyncrasy is taken to be a general null expectation, then any pronounced departures from the null should assume special significance with regard to phylogeographic signal. Such departures are described generically as "genealogical concordance" (Avise and Ball, 1990).

Four distinct aspects of genealogical concordance of relevance to phylogeography can be distinguished (Table 5.1; Fig. 5.1). These will be described briefly as a prelude to empirical case studies from regional faunal assemblages.

TABLE 5.1 Four aspects of genealogical concordance in phylogeographic inference (after Avise, 1996b). See text for further explanation.

I	*Concordance across sequence characters within a gene.* Relevance: yields statistical significance for putative gene-tree clades.
II	*Concordance in significant genealogical partitions across multiple genes within a species.* Relevance: establishes that gene-tree partitions register phylogenetic partitions at the population or species level.
III	*Concordance in the geography of gene-tree partitions across multiple codistributed species.* Relevance: implicates shared historical biogeographic factors in shaping intraspecific phylogenies.
IV	*Concordance of gene-tree partitions with spatial boundaries between traditionally recognized biogeographic provinces.* Relevance: implicates shared historical biogeographic factors in shaping intraspecific phylogenies and organismal distributions.

ASPECT I: AGREEMENT ACROSS CHARACTERS WITHIN A GENE

Nearly by definition, a "deep" phylogenetic split deduced in the intraspecific mtDNA tree of any species will have been registered concordantly by multiple sequence characters. If this were not the case, such matrilineal separations would not be evident in the data analyses, nor would they have received significant phylogenetic support by criteria such as bootstrapping (Felsenstein, 1985a). Typically, at least three or four diagnostic characters (uncompromised by homoplasy in the broader data) are required for robust statistical recognition of a putative gene-tree clade in most phylogenetic appraisals.

All species described in Chapter 4 as belonging to phylogeographic Categories I or II display a mtDNA gene tree whose major branches were earmarked by multiple diagnostic characters (nucleotides or restriction sites) within the mitochondrial genome. These genetic markers are independent of one another in the sense of having had separate mutational origins, although once jointly present within a matriline they are cotransmitted as

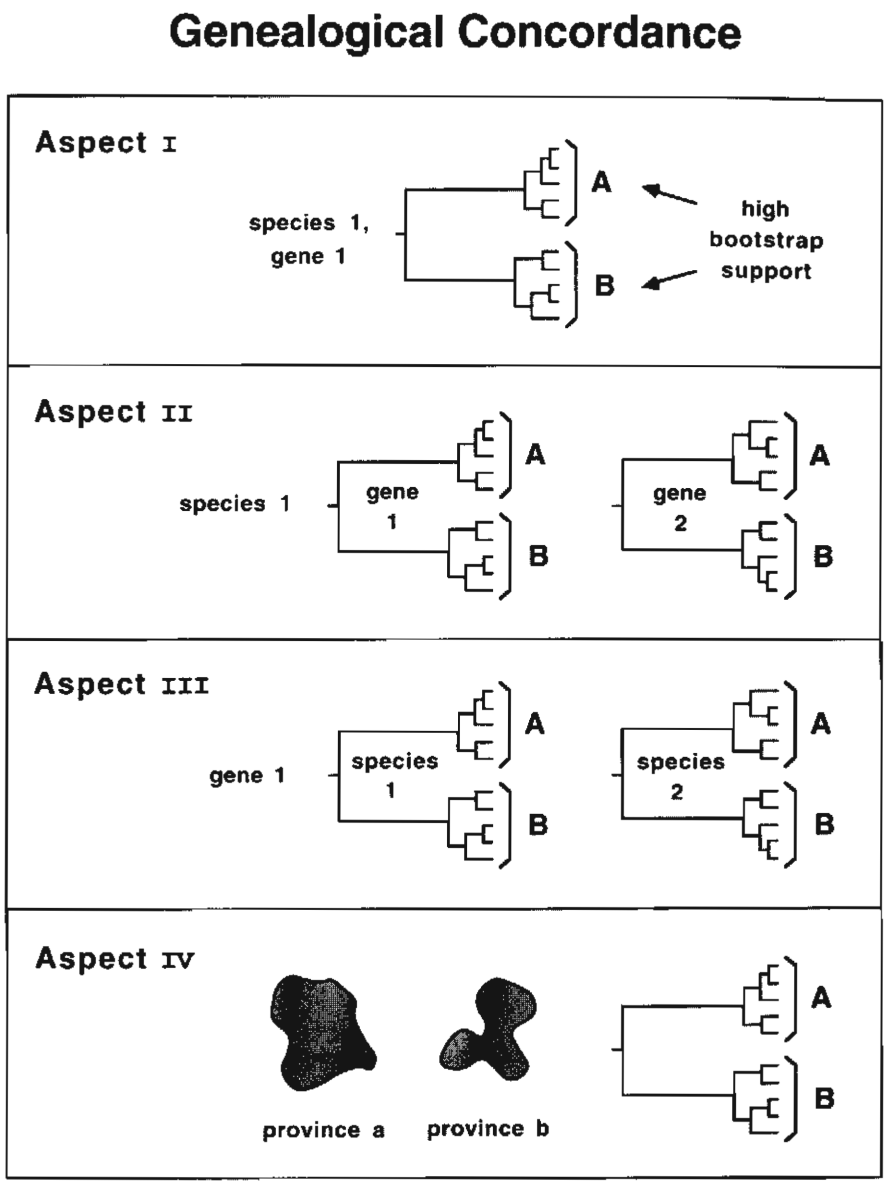


FIGURE 5.1 Schematic presentation of four distinct aspects of genealogical concordance (see text and Table 5.1 for further explanation). "A" and "B" are distinctive phylogroups in a gene tree.

a linked supergene. Similar statements would apply to tightly linked sequence characters within any nuclear gene.

Thus, this first aspect of genealogical concordance merely confirms the identity and distinctiveness of the principal branches or phylogroups within a gene tree. Furthermore, empirical experience shows that the major mtDNA phylogroups within a species nearly always show a strong geographic arrangement. Thus, such gene-tree branches are prime but provisional candidates for constituting major historical population units especially worthy of further consideration in a biogeographic context.

ASPECT II: AGREEMENT ACROSS GENES

Suppose that haplotypes were isolated, assayed, and gene trees estimated at each of several unlinked nuclear loci where intra-genic (inter-allelic) recombination had been absent within the species of interest. Suppose further that deep branch separations were apparent in each gene tree (concordance Aspect I), and that these major genealogical branches characterized the same sets of geographic populations. Aspect II of genealogical concordance ideally refers to such phylogenetic agreement across independent loci. The occurrence of concordance Aspect II demonstrates almost conclusively that particular partitions in the gene trees accurately register fundamental phylogenetic subdivisions at the population or species level. Several statistical approaches have been suggested to assess phylogenetic agreement among partitioned data sets from, for example, empirical gene trees from two or more loci (Day, 1983; Page, 1990, 1994; Bull et al., 1993; Farris et al., 1994; Lyons-Weiler and Milinkovitch, 1997).

An important theoretical qualification arises when Aspect II of genealogical concordance is addressed with respect to nuclear versus mitochondrial gene trees. Recall that under neutrality, the expected time for lineage sorting to reciprocal monophyly in two isolated populations is fourfold longer for nuclear lineages than for mitochondrial lineages, all else being equal (see Birky, 1991 and Hoelzer, 1997 for exceptions to this generality). In other words, due to a fourfold larger effective population size, the coalescent process in theory normally proceeds more slowly for alleles at nuclear genes than for those at mitochondrial loci.

This expectation has given rise to the so-called three-times ($3x$) rule, which predicts the level of phylogenetic concordance across nuclear gene loci as a function of relative branch lengths in a mitochondrial gene tree (Palumbi and Cipriano, 1998). The logic is that if a matrilineal tree for two isolated populations has just barely achieved a status of reciprocal monophyly at time x, then on average about $3x$ more time is required for a typical nuclear gene tree to achieve the same status through lineage sorting. The x in these comparisons can be estimated from the mean intrapopulation mtDNA diversity (e.g., as measured by pairwise sequence divergence, p), because p scales with N_e and provides a lineage-specific estimate of the time required for lineage coalescence. If the actual time of population separation is between x and $4x$, a mitochondrial gene tree likely will have achieved reciprocal monophyly whereas a nuclear gene will not. Thus, in theory, only when times of population separation fall outside the $3x$ window is it probable that major clades in many nuclear gene-tree lineages will display concordance with those in a mitochondrial gene tree (Fig. 5.2).

As discussed in Chapter 2, technical and biological complications have conspired to inhibit explicit genealogical appraisals of nuclear genes within and among populations of most species. These include the procedural difficulties of isolating nuclear haplotypes one at a time from diploid tissues, the challenge of identifying nuclear loci whose sequences have evolved rapidly enough to provide informative phylogenetic markers over recent evolution, and a requirement that the sequences have been mostly free of interallelic recombination (and/or gene conversion) over the historical timescale of interest. At the time of this writing, these hurdles have been overcome in relatively few instances. Recall, for example, the genealogical studies of human Y-linked loci and an autosomal globin gene (Chapter 3). These loci generally supported mtDNA data in suggesting a small evolutionary N_e and a relatively recent global population expansion for *Homo sapiens*.

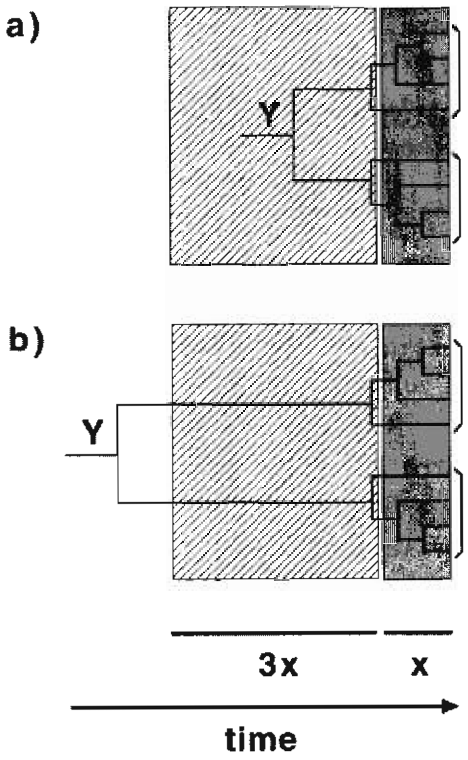


FIGURE 5.2 Diagrammatic representation of the 3*x* rule (after Hare, 1998). Shown are mtDNA gene trees whose major branches, stemming from node Y, characterize populations A and B. The mean lineage divergence time within populations (as might be estimated by intra-population mtDNA sequence diversity) is denoted by *x*. *(a)* A case in which node Y is within the 3*x* window. *(b)* The same pair of populations at a later point in time in which node Y now falls outside (is earlier than) the 3*x* time window. Only in the latter situation is it likely in theory that monophyletic clades at most nuclear loci will show concordance with those in a mtDNA gene tree.

Two studies that explicitly searched for (and found) phylogeographic concordance between mitochondrial and nuclear gene trees at the intraspecific level are summarized in Fig. 5.3. In the North American killifish, *Fundulus heteroclitus*, a pronounced phylogenetic distinction between northern and southern populations along the Atlantic Coast is registered

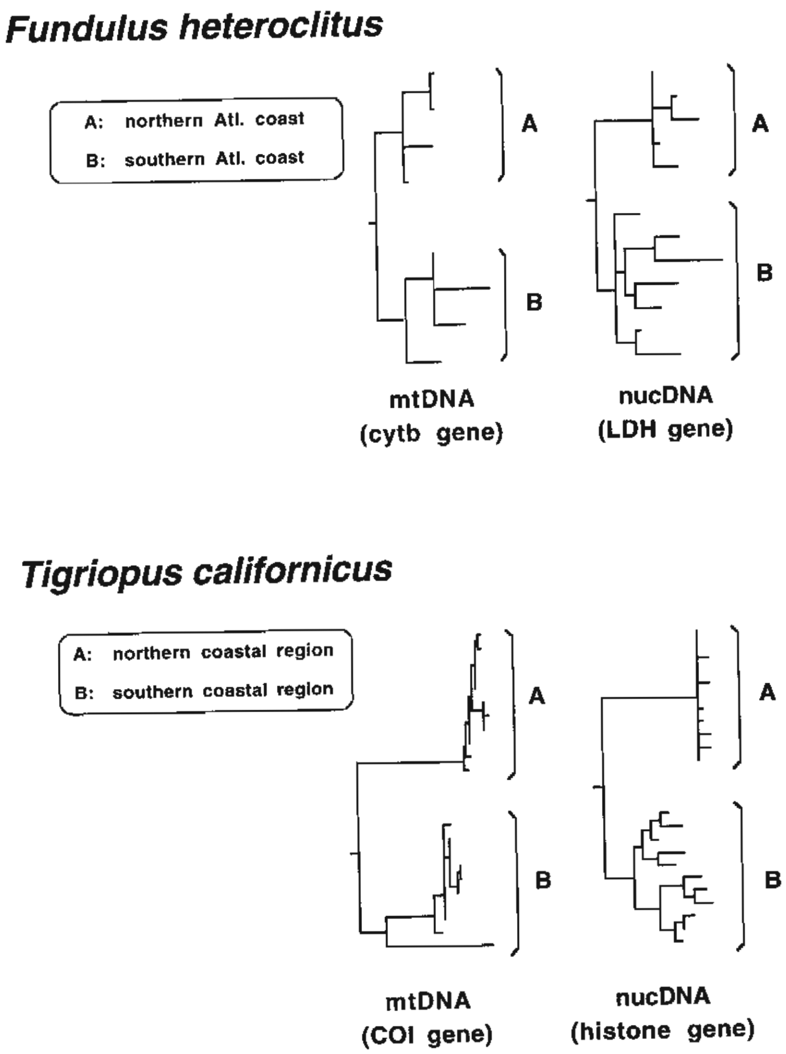


FIGURE 5.3 Empirical examples of Aspect II of intraspecific genealogical concordance. These cases involve general agreement between deep phylogeographic topologies in mitochondrial and nuclear gene trees. *Above:* Northern versus southern populations of a coastal killifish, *Fundulus heteroclitus* (after Bernardi et al., 1993). *Below:* Northern versus southern populations of a coastal copepod in California, *Tigriopus californicus* (after Burton and Lee, 1994; for a description of additional phylogeographic structure in this species, see Burton, 1998).

in a sequenced nuclear gene (encoding lactate dehydrogenase) as well as in mtDNA. Similarly, in a copepod, *Tigriopus californicus*, along the Pacific Coast, a phylogenetic split that distinguished northern from southern populations was detected both in mitochondrial and nuclear gene trees. Both of these cases exemplify what normally is implied by Aspect II of genealogical concordance: a general agreement in the principal phylogeographic units registered in gene trees from multiple unlinked loci.

Few such examples currently are available. In the absence of explicit genealogical evidence from nuclear genes to compare directly against phylogeographic data from mtDNA, surrogate information can be employed. This may come from phylogenetic appraisals of traditional population genetic data: e.g., allelic frequencies and genetic distances, preferably from multiple nuclear loci. Alternatively, the evidence may rest on geographic appraisals of organismal phenotypes—morphological or behavioral characters that might be presumed to register notable genetic differences among the populations monitored. As discussed in Chapter 4, the deepest phylogeographic partitions in intraspecific mtDNA gene trees often agree with traditional taxonomic partitions as reflected in subspecies' designations.

ASPECT III: AGREEMENT ACROSS CODISTRIBUTED SPECIES

Imagine that each of several codistributed species with comparable natural histories or habitat requirements proved to be phylogeographically structured in similar fashion. In particular, divergent branches in the intraspecific gene trees might map consistently to the same geographic regions. Aspect III of genealogical concordance presumably would reflect shared historical elements in the evolutionary or ecological factors that had shaped the intraspecific phylogeographic architectures within this regional fauna (Rosen, 1975; Platnick and Nelson, 1978; Cracraft, 1988). Concordance Aspect III has been documented for several regional biotas, as discussed later.

ASPECT IV: AGREEMENT WITH OTHER BIOGEOGRAPHIC DATA

The most important discoveries of molecular phylogeography often emerge when molecular genealogical data are integrated with independent biogeographic and systematic information. For example, historical vicariant events might have facilitated morphological or behavioral divergence between taxonomic subspecies with disjunct distributions. Or, the traditional data may pertain to collective information about historical biogeographic factors suspected to have shaped distributional boundaries in multiple species of a regional biota.

Major phylogeographic branches in a gene tree are most likely to register long-standing separations at the population level when they are concordant with morphological differences, or are consistent in position with obvious historical geographic or geologic barriers to dispersal. For more subtle cases, formal phylogenetic procedures have been suggested for evaluating correspondence between a molecular phylogeny's branching pattern and spatial relationships of physiographic regions (Wiley, 1988; Brooks, 1990; Brooks and McLennen, 1991).

With respect to multispecies patterns, biogeographers long have sought to identify unique or distinctive biotic assemblages at various spatial scales. Zoogeographic provinces, subprovinces, and areas of endemism traditionally are described from analyses of faunal lists, from which it is apparent that the distribution of the Earth's biodiversity is nonuniform and shaped in large measure by historical biogeographic factors. To pick an obvious example, the distinctive mammalian fauna of Australia (as well as its avifauna; Sibley and Ahlquist, 1986; Sibley, 1991) registers a long-standing isolation of the continent beginning in the early or middle Tertiary. An emerging generality from molecular phylogeographic studies is that deeply separated phylogroups at the intraspecific level often are confined to biogeographic provinces or subprovinces as identified from traditional faunal lists. This kind of agreement has proved to be another common element of Aspect IV of genealogical concordance.

REGIONAL PERSPECTIVES: THE SOUTHEASTERN UNITED STATES

Regional phylogeographic analyses consider the joint spatial patterns of intraspecific genealogies across multiple codistributed taxa. All four aspects of genealogical concordance can be illustrated by reference to the regional phylogeographic surveys currently available.

The first large collection of molecular phylogeographic appraisals involved faunas in the southeastern United States. Historical biogeographic factors played a major role in shaping the contemporary genetic architectures of numerous species in this region. A brief introduction to the physical environment of this area over the past few million years follows, indicating the types of physiographic factors that likely impinged on species' demographies and phylogeographic population structures.

Environmental Background

Freshwater Realm. About a dozen major rivers and numerous smaller streams currently traverse the southeastern United States, with eastern drainages entering the Atlantic Ocean and western drainages entering the Gulf of Mexico (Fig. 5.4). During the high sea-stands of the Pliocene and the moderate sea-stands of Pleistocene interglacials, smaller coastal streams likely were flooded, and freshwater faunas probably were isolated in the upper reaches of the larger rivers and perhaps in lakes and rivers of the Floridian peninsula itself (Wright, 1965). At these times, any interdrainage transfers of strictly aquatic species must have occurred via lateral stream captures. During the low sea-stands associated with the glacial episodes that dominated much of the Pleistocene, adjacent drainages also may have coalesced periodically as they meandered across the broader coastal plains. Such histories of drainage isolation and connection (the details of which remain poorly known) undoubtedly influenced the phylogenetic histories of aquatic populations. For example, if freshwater fishes periodically were isolated in separate refugial areas, the geographic strongholds involved and the subsequent range expansions should have been impacted by historical patterns of drainage connections.

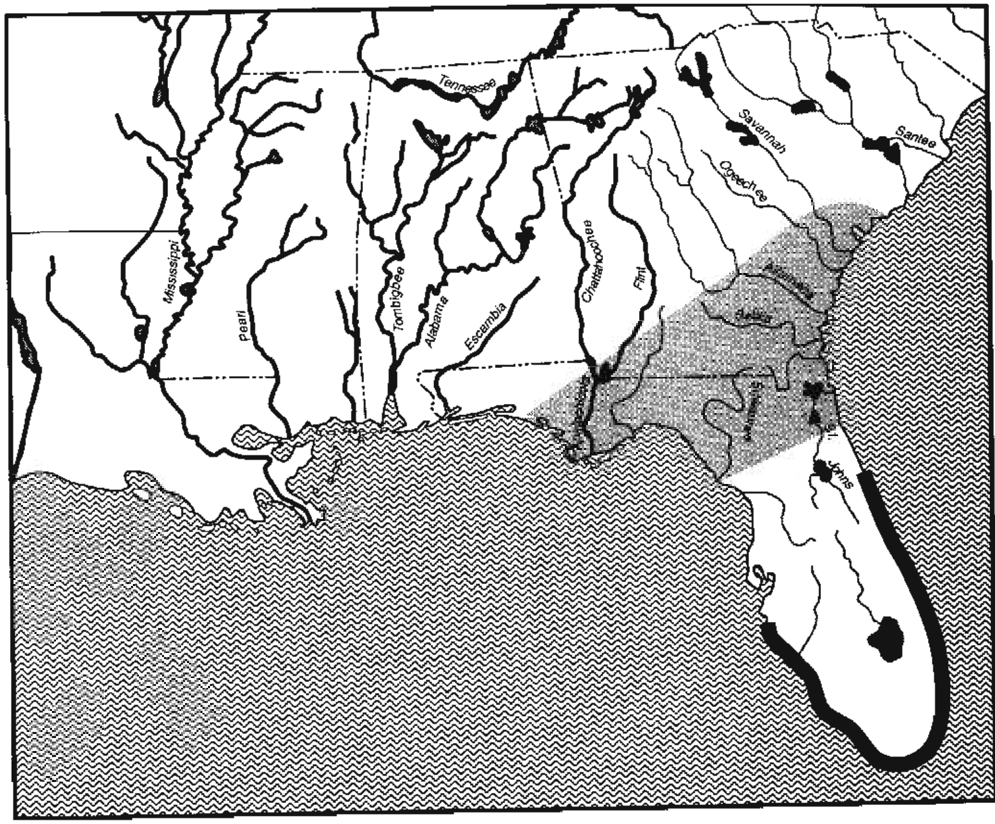


FIGURE 5.4 Map of the southeastern United States showing major environmental features rele vant to phylogeographic interpretations (from Avise, 1996b). For freshwater fish, the darker an lighter lines indicate, respectively, rivers in the distinctive western and eastern faunal zones as ev denced in a compilation of species' ranges (Swift et al., 1985). For terrestrial organisms, the shade area represents a region of presumed secondary contact and hybridization between many endemi Floridian forms and their continental counterparts as described by Remington (1968). In the mar time realm, the heavy line along the Florida coast indicates the distribution of a tropical fauna province as contrasted with the cooler zoogeographic zone further north (Briggs, 1974).

About 250 species of freshwater fishes inhabit coastal drainages of the southeastern United States, with the number of species per drainage ranging from 20 to 157. Swift et al. (1985) detailed these species' ranges and uncovered a great diversity of distributional patterns (as might be expected across a fauna this diverse). Nonetheless, the authors were able to define about nine faunal provinces that implicated significant historical and contemporary barriers to dispersal. One analysis grouped river drainages according to species' composition. Based on a presence-absence matrix across all fish species, the most basal split in a cluster phenogram distinguished all western drainages (western Georgia to Louisiana) from those to the east (Atlantic coast and throughout the Floridian peninsula) (Fig. 5.4). Thus, the geographic distributions of an unusually high proportion of fish species are confined to distinctive western or eastern freshwater provinces.

Terrestrial Realm. The Floridian peninsula (particularly at its southern extreme) is climatically, physiographically, and ecologically distinct from the more temperate continental realms to the north and west. Thus, many taxa that are adapted to tropical or subtropical conditions in the eastern United States are confined to southern and central Florida. During the Pleistocene glacial episodes, when sea levels were lower, the Floridian peninsula was much larger than it is today and may have served as an important refugium for temperate species whose ranges were compressed southward by climatic changes associated with glacial advances. Earlier in the Tertiary (as recently as the Oligocene), an uplift area in west-central Florida existed as one or more large islands separated from the mainland by a shallow marine incursion in the current area of northern Florida and southern Georgia (Webb, 1990; Randazzo and Jones, 1997).

Remington (1968) first emphasized that a diverse array of endemic plants and animals in the Floridian peninsula show morphological or other distinctions from their respective continental near-relatives. Remington observed geographically concordant contact regions of known or suspected hybridization between numerous Floridian forms (50 in his list) and their continental counterparts along a "suture zone" between the peninsula and mainland (Fig. 5.4). This secondary contact region was afforded a status equal to that of only five other major suture zones in North America. This suture zone demarcates a distinctive biotic province in peninsular Florida.

Maritime Realm. The Floridian peninsula now protrudes southward into subtropical waters, separating some but not all temperate faunas into allopatric units on the Atlantic coast and Gulf of Mexico. During the many glacial advances and retreats of the Pleistocene, climatic changes and sea-level fluctuations probably had great impact on coastal species in the area (Bert, 1986; Felder and Staton, 1994). Climatic cooling associated with glacial expansions pushed temperate populations southward and perhaps increased the opportunity for contact of Atlantic and Gulf populations around south Florida (Cronin, 1988). However, sea levels during the Ice Ages also were lower (by as much as 150 meters), exposing great expanses of the Floridian peninsula. At such times, the peninsula was more arid than now, and perhaps bordered by fewer intermediate-salinity estuaries and salt-marsh habitats favored by many coastal species. Thus, an enlarged Floridian peninsula during glacial advances may have promoted a physical separation between Atlantic and Gulf coastal populations of some maritime species.

Opposing influences on species' distributions also may have been at work during periods of climatic warming, when sea levels were higher and the Floridian peninsula perhaps was bordered by more extensive estuaries and salt marshes. During these interglacial periods (as now), some strictly temperate species probably were separated into disjunct Atlantic and Gulf populations by tropical conditions of southern Florida. Conversely, some eurythermal and estuarine-adapted species may have expanded out of the Gulf region to regain increased contact with Atlantic populations around the southern tip of a smaller Floridian peninsula. Which of these effects was dominant for the maritime fauna as a whole remains unclear. At present, marine currents moving out of the Gulf of Mexico contribute to the Gulf Stream that hugs the southeastern coast of Florida and may facilitate transport of Gulf-derived pelagic gametes or larvae into the southern Atlantic.

Faunal specialists have summarized distributional records for various marine taxa in the area (review in Briggs, 1974). The most striking pattern is a disjunction between the warm-temperate and tropical maritime faunas of southern Florida (and sometimes the Gulf of Mexico) versus the cooler temperate biotas farther north, particularly along the Atlantic coast (Fig. 5.4). For example, a temperate molluskan species assemblage extending down the northern third of Florida is replaced gradually by more tropical-adapted species to the south. A faunal transition for octocorals similarly occurs in eastcentral Florida. Many fish species also have northern or southern range limits in this area (Briggs, 1958). Thus, the eastcentral coastline of Florida describes a transitional boundary between distinctive Atlantic and Gulf faunal provinces in the maritime realm.

Genetic Findings

Freshwater Fishes. Bermingham and Avise (1986) used mtDNA restriction-site assays to examine four fish species collected from major river drainages across the southeastern United States: the bowfin (*Amia calva*) and three sunfish species (*Lepomis punctatus, L. microlophus,* and *L. gulosus*). Extensive variation and differentiation were detected, and the intraspecific matrilines clearly were nonrandom in geographic position. Notably, a major mtDNA phylogenetic break was evident within each species that distinguished nearly all individuals in eastern drainages (mostly along the Atlantic coast and peninsular Florida) from those to the west (entering the Gulf coast). The estimated magnitudes of inter-regional genetic separation in the intraspecific mtDNA gene trees differed considerably across the four species (Fig. 5.5), but invariably far surpassed genetic distances among haplotypes within either region. Geographic distributions of the two major mtDNA phylogroups within each species are summarized in Fig. 5.6.

Similar mtDNA phylogeographic analyses have been conducted on the bluegill sunfish, *Lepomis macrochirus* (Avise et al., 1984b), largemouth bass, *Micropterus salmoides* (Nedbal and Philipp, 1994), and a *Gambusia* mosquitofish complex (Scribner and Avise, 1993). A fundamental split in each mtDNA gene tree distinguished populations in the Floridian peninsula and along the Atlantic coast from those to the north and west (Fig. 5.5). Allozyme data also have been gathered for these three species (Avise and Smith, 1974; Philipp et al., 1983; Wooten and Lydeard, 1990; Scribner

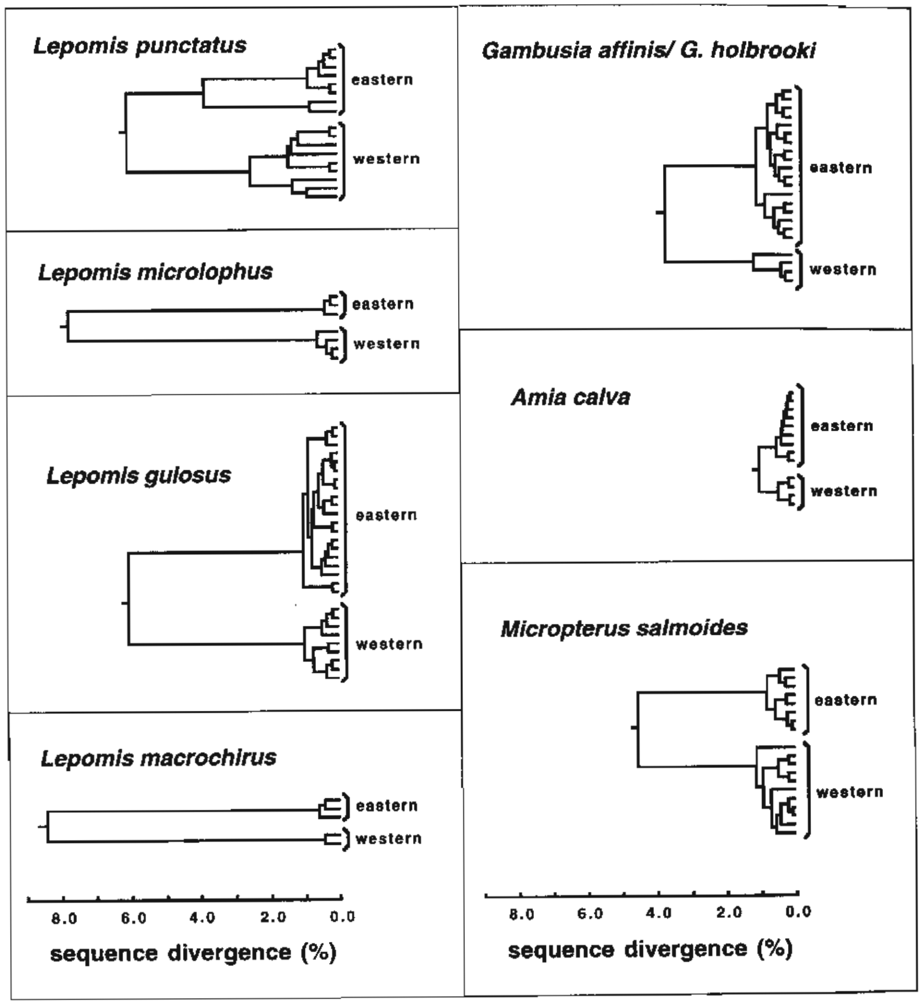


FIGURE 5.5 Phylogenetic relationships among mtDNA haplotypes within each of seven species (or species complexes) of freshwater fishes in the southeastern United States. For comparative purposes, all diagrams are plotted on the same scale of estimated mtDNA sequence divergence. "Eastern" and "western" refer to general drainage regions occupied by the two major phylogroups observed within each species (see Fig. 5.6).

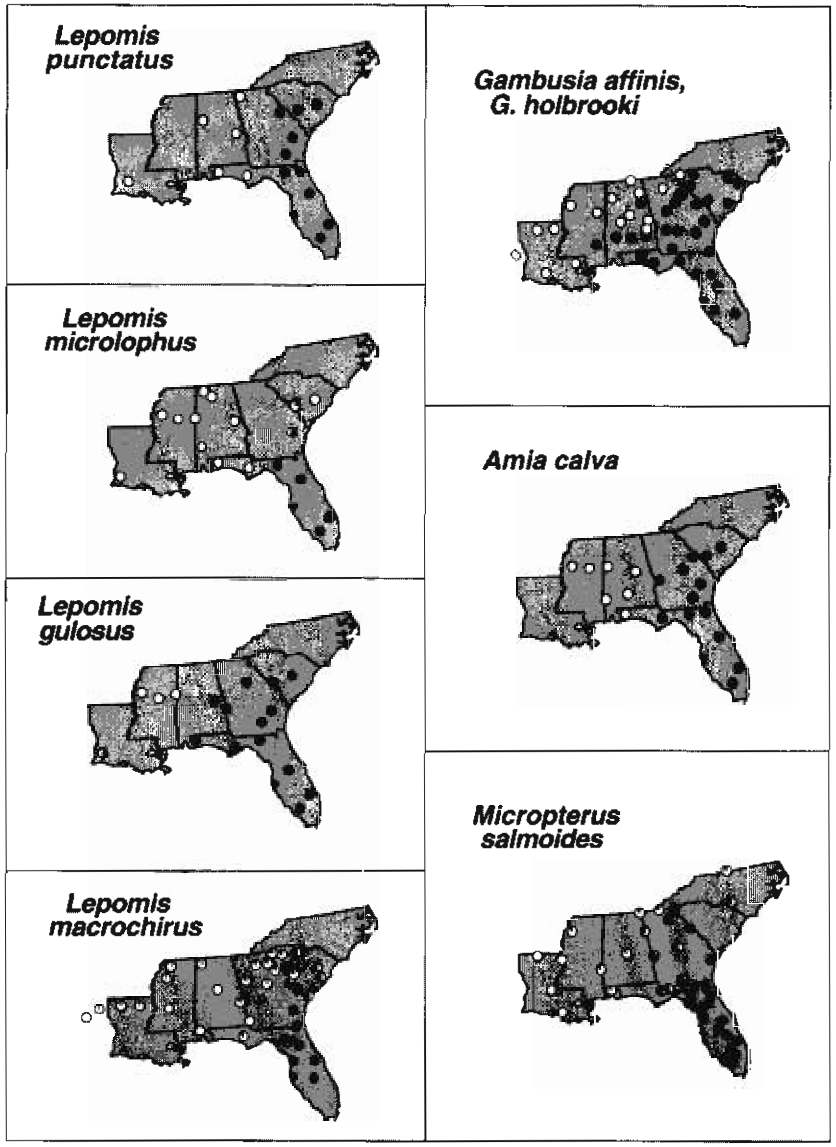


FIGURE 5.6 Pie diagrams summarizing the geographic distributions of the two primary phylogroups (Fig. 5.5) observed within each of seven freshwater fish species of the southeastern United States. Diagrams for five of the species refer to frequencies of diagnostic mtDNA haplotypes, whereas those for *Lepomis macrochirus* and *Micropterus salmoides* refer here to frequencies of diagnostic allozyme alleles.

and Avise, 1993), and in each case nearly fixed differences at several marker loci distinguished eastern from western populations. The molecular data further indicate that the phylogroups introgressively hybridize across parts of the Carolinas, Georgia, Alabama, and the Floridian panhandle. Some of these secondary contacts probably were facilitated by human-mediated transplantations (Nedbal and Philipp, 1994).

Overall, at least seven freshwater fish species or species-complexes have been assayed extensively for genetic composition across Atlantic and Gulf coastal drainages of the southeastern United States. Without exception, fundamental genealogical partitions distinguished eastern from western populations (Fig. 5.6), thus strongly suggesting shared historical biogeographic influences. The Floridian peninsula invariably is the geographic stronghold of eastern forms, whereas Gulf coastal drainages from Alabama westward house the western forms. The Mississippi or the Alabama-Tombigbee drainages may have been historical cradles for the western phylogroups (see Mayden, 1988).

In summary, all four aspects of genealogical concordance are documented abundantly in these freshwater fishes. Aspect I is evidenced by high sequence divergences between major branches in the intraspecific mtDNA gene trees. In three species surveyed also for allozyme variation, Aspect II is suggested by the general agreement between the geographic positions of the deep mtDNA phylogroups and those registered by dramatic shifts in frequencies of nuclear alleles. Aspect III of genealogical concordance appears in the similar geographic distributions of the two major phylogroups within each species. Concordance Aspect IV is evidenced by a close agreement between the geographic arrangement of intraspecific phylogroups vis-à-vis the major biogeographic provinces as identified from leading trends in the regional distributions of fish species (Fig. 5.7).

The magnitudes of the sequence divergence estimates between the major intraspecific phylogroups showed much poorer agreement across these fish species, ranging from $p \cong 0.006$ in *Amia calva* to $p > 0.050$ within each of the surveyed species of *Lepomis* (Fig. 5.5). Several hypotheses for this heterogeneity can be advanced. Perhaps the populations were sundered by

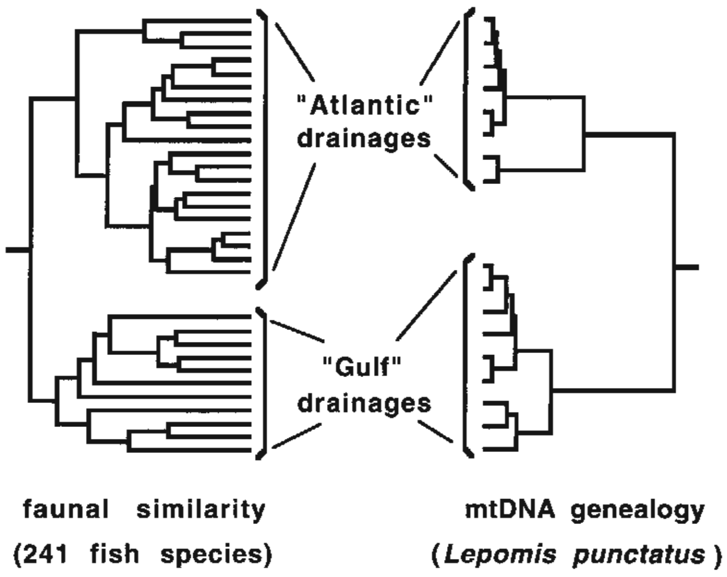


FIGURE 5.7 A clear example of Aspect IV of genealogical concordance (after Walker and Avise, 1998). Geographic distributions of the two major branches in the mtDNA gene tree for the spotted sunfish, *Lepomis punctatus* (Bermingham and Avise, 1986) agree perfectly with the two principal southeastern faunal regions for freshwater fishes (Fig. 5.4).

the same historical vicariant event but rates of mtDNA evolution varied among taxa. Or perhaps different levels of ancestral polymorphism were available for conversion to differences between phylogroups. All else being equal, larger ancestral populations or those structured more strongly in space will tend to contain gene-tree lineages with deeper historical separations, and some such lineages may be those that were destined to characterize sister populations arising from a vicariant event (Fig. 5.8).

Another possibility is that the times of population separation truly differed. Climatic changes of the Pliocene and Pleistocene were repeated many times, so successive cycles in some taxa might have overridden or erased (via population extinctions and recolonizations) the phylogeographic

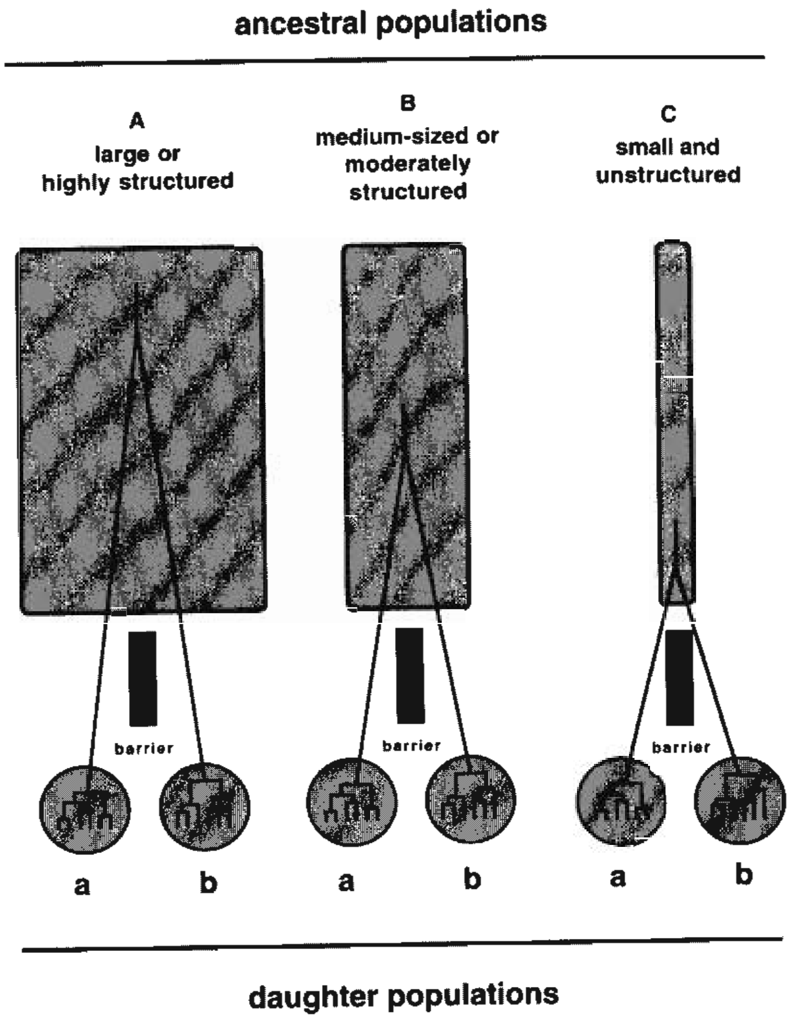


FIGURE 5.8 Illustration of how the intraspecific gene trees of codistributed taxa might show similar geographic patterns yet varying temporal depths as a result of differences in the sizes or degrees of spatial structuring of ancestral populations. All else being equal, lineage separations in a gene tree tend to be deeper when ancestral populations are larger or spatially structured.

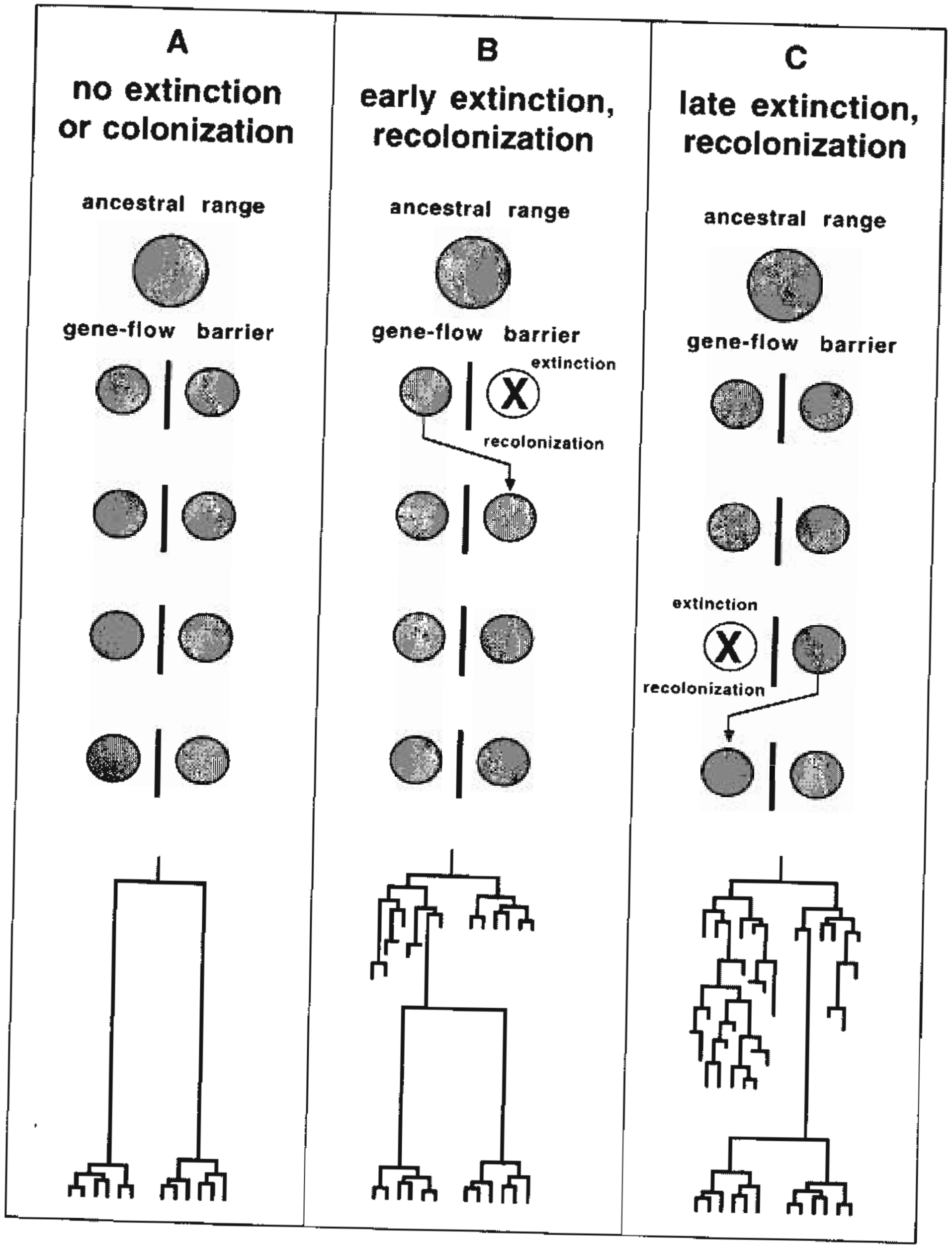


FIGURE 5.9 Illustration of how the intraspecific gene trees of codistributed taxa might show similar geographic patterns yet varying temporal depths as a result of cyclical vicariant events coupled with regional population extinctions and recolonizations (after Cunningham and Collins, 1998).

signals of earlier cycles (Fig. 5.9). Such recurring episodes of vicariance and interregional dispersal would produce a phylogeographic record that shows spatial concordance but considerable temporal heterogeneity across species.

The vicariant scenarios depicted in Figs. 5.8 and 5.9 also illustrate why the distributions of intraspecific phylogroups might tend to mirror biotic provinces as identified by species' distributional limits (e.g., Fig. 5.7). Suppose that in each of several species, population extinctions on one side or the other of the zoogeographic boundary were *not* followed by interregional recolonizations. Then, the range of each of these species today would be confined to one geographic area (either eastern or western in the case of freshwater fishes). An accumulation of such historical outcomes would produce a concentration of species' ranges that terminate at the boundaries between traditional zoogeographic provinces or subprovinces.

Maritime Species. All four aspects of genealogical concordance also have been uncovered in molecular phylogeographic surveys of maritime taxa in the southeastern United States. The major phylogeographic patterns described next are summarized in Fig. 5.10.

MtDNA genotypes were assayed from seaside sparrows (*Ammodramus maritimus*) representing seven named subspecies (Avise and Nelson, 1989). Most noteworthy was a substantial phylogeographic break (estimated 1.0 percent net sequence divergence) that distinguished Atlantic coast birds from all specimens collected along the Gulf of Mexico. This phylogeographic pattern had been anticipated by Funderburg and Quay (1983), who speculated from distributional and other zoogeographic information that these sparrow populations were split historically into Atlantic and Gulf units. Currently, there is a pronounced hiatus in the geographic range of the species with no extant populations in southeastern Florida.

Another coastal species with disjunct Atlantic and Gulf populations is the black sea bass (*Centropristis striata*). Here too, Atlantic and Gulf forms conventionally recognized as different subspecies proved to be quite distinct in mtDNA composition, with an estimated net nucleotide

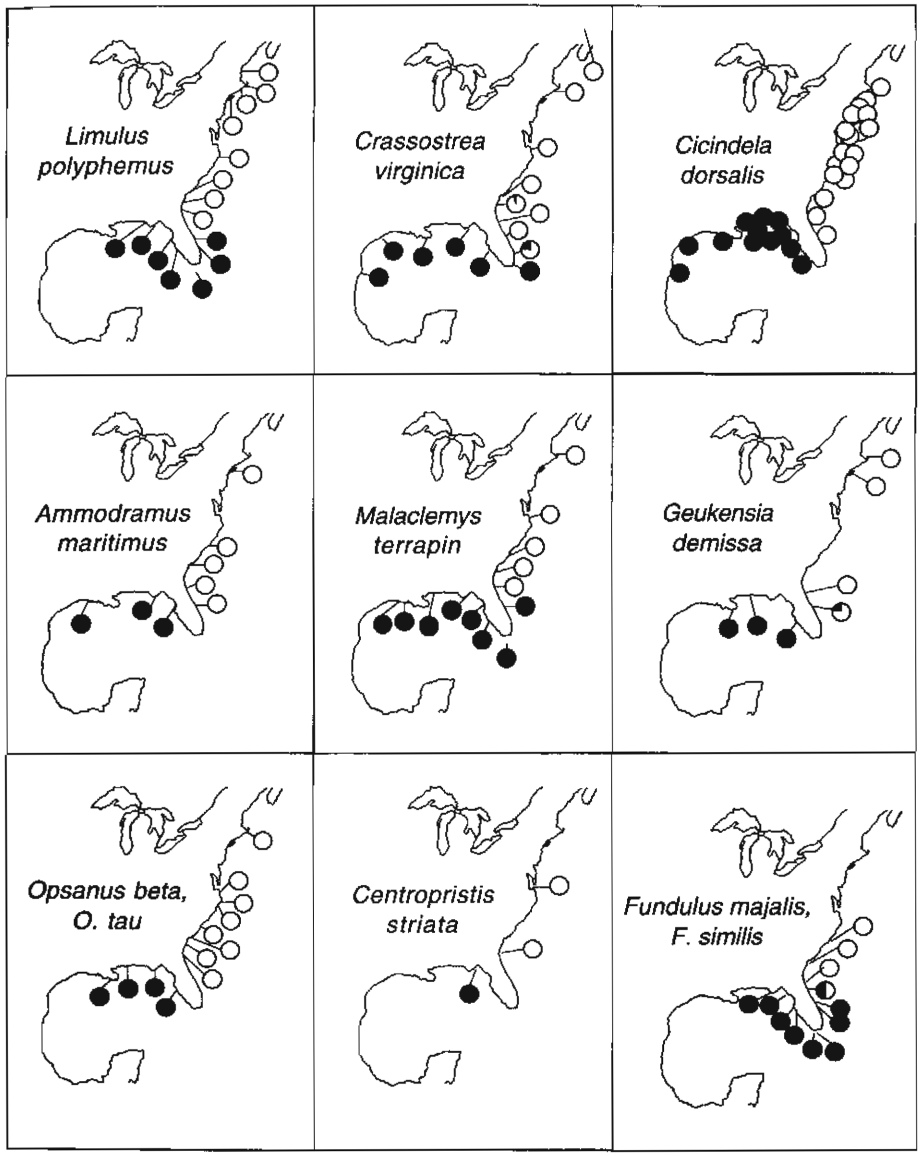


FIGURE 5.10 Pie diagrams summarizing geographic distributions of the two most fundamental phylogroups observed within each of several maritime species or species complexes along coastlines of the Atlantic and Gulf of Mexico in the southeastern United States. Diagrams for seven species refer to frequencies of diagnostic mtDNA clades (see text), whereas those for *Geukensia demissa* and the *Fundulus* species refer to frequencies of diagnostic allozyme alleles. The lower right panel exemplifies a common geographic pattern wherein one species is distributed primarily along the Gulf of Mexico and southeast Florida and a sister species occurs further north along the Atlantic coast.

quite distinct in mtDNA composition, with an estimated net nucleotide sequence divergence of 0.7 percent (Bowen and Avise, 1990).

Two other coastal vertebrates that show an Atlantic-Gulf disjunction in mtDNA phylogeny involve the toadfish complex *Opsanus* (Avise et al., 1987b) and the diamondback terrapin *Malaclemys terrapin* (Lamb and Avise, 1992). In the former assemblage, two related species (*O. tau* and *O. beta*) mostly are confined to the Atlantic and Gulf coasts, respectively, and differ by an estimated 9.6 percent mtDNA sequence divergence. In the latter species, only one fixed mtDNA restriction-site difference was detected, but it also distinguished Gulf samples from those along the Atlantic coast north of Florida.

Similar phylogeographic patterns in mtDNA have been documented for three invertebrates with quasi-continuous distributions along the coasts of the Atlantic and Gulf of Mexico. In the tiger beetle, *Cicindela dorsalis* (Vogler and DeSalle, 1993, 1994a), horseshoe crab, *Limulus polyphemus* (Saunders et al., 1986), and American oyster, *Crassostrea virginica* (Reeb and Avise, 1990), Atlantic versus Gulf populations proved highly distinct genetically. In the latter two species, the ranges of the Gulf forms extended into southeastern Florida (Fig. 5.10). In all three species, the level of genetic divergence between Atlantic and Gulf populations far surpassed observed differences within either region. In the American oyster, subsequent restriction-site assays of several anonymous nuclear loci added support for an Atlantic-Gulf population distinction (Karl and Avise, 1992; Hare and Avise, 1996). This distinction was not, however, apparent in molecular assays of other nuclear loci in this species (Buroker, 1983; McDonald et al., 1996; Hare and Avise, 1998). Hare (1998) concluded that the population separations in oysters may have occurred within the 3*x* temporal window where a split in a mtDNA gene tree is expected to be mirrored by only some modest fraction of nuclear genes.

Population studies based on allozymes or other genetic assays have uncovered evidence for similar Atlantic-Gulf distinctions in additional coastal invertebrates and vertebrates. In the ribbed mussel, *Geukensia demissa* (Sarver et al., 1992), and in a complex of killfishes (*Fundulus majalis* and *F. similis*; Duggins et al., 1995), a pronounced genetic discontinuity demarcated Atlantic from Gulf populations that the authors in both cases recommended be named separate species. The respective Gulf forms again extended along the southeastern coast of Florida in patterns reminiscent of those for the American oyster, horseshoe crab, and diamondback terrapin (Fig. 5.10). Similar genetic discontinuities also have been reported for some coastal invertebrates with disjunct Atlantic and Gulf ranges: *Hydractinia* hydroids, and crabs in the genera *Pagurus, Sesarma,* and *Uca* (Cunningham et al., 1991, 1992; Felder and Staton, 1994).

On the other hand, several genetically assayed coastal species have failed to show clear evidence for Atlantic-Gulf phylogenetic separations (Gold and Richardson, 1998). Examples among fishes include the red drum, *Sciaenops ocellatus* (Bohlmeyer and Gold, 1991), hardhead catfish, *Arius felis* (Avise et al., 1987b), a menhaden complex in the genus *Brevoortia* (Bowen and Avise, 1990), and the American eel, *Anguilla rostrata* (Avise et al., 1986). As described earlier, an absence of regional mtDNA divergence in the American eel probably is due to the species' catadromous life cycle. Lack of pronounced regional differentiation in the other groups probably is due to current gene flow around southern Florida or to retention of ancestral lineage polymorphisms by Atlantic and Gulf populations that separated recently.

Nonetheless, across a surprising diversity of species ranging from coastal invertebrates to maritime fishes and salt-marsh tetrapods, molecular genetic data reveal fundamental phylogenetic discontinuities and considerable concordance in the geographic distributions of the major lineages (Fig. 5.10). By these same yardsticks, little evidence exists for dramatic historical-genetic partitions within either the Atlantic or Gulf regions. Collectively, all four aspects of genealogical concordance have been evidenced: concordance across multiple mtDNA sequence characters in defining the principal mtDNA phylogroups; concordant support from nuclear genes in some of the species surveyed; general agreements across taxa in geographic boundaries between the intraspecific phylogroups; and a close agreement in the ranges of molecular phylogroups with traditional faunal provinces. Although the Atlantic-Gulf distinction is far from universal, the level of phylogeographic concordance across multiple taxa strongly implicates shared historical influences on the genetic architectures and gene-flow regimes of a substantial fraction of this maritime fauna.

Such historical influences probably have operated in collaboration with contemporary ecological conditions to influence the present-day distributions of genotypes. For example, the genetic discontinuities in several species localize to the eastcentral Florida coastline where a pronounced transition occurs between subtropical and cooler temperate waters. This ecological transition zone is mediated in part by warm waters of the Gulf Stream which flow out of the Gulf of Mexico into the coastal regions of southeastern Florida.

Such ecological conditions probably have two kinds of distributional impact on the genetic architectures of these maritime species. First, the ecological transition itself may generate distinctive selective pressures north versus south of eastcentral Florida that may further inhibit gene flow between historically differentiated Gulf and Atlantic phylogroups. Second, for some species such as the American oyster and horseshoe crab with mobile larvae, the Gulf Stream itself might have contributed to a "leakage" of Gulf genotypes into the Atlantic coast of southern Florida. These speculations emphasize a broader point. Present-day ecological and behavioral circumstances, as well as historical factors, impact the genetic architectures of all extant species. Thus, a full appreciation of why lineages occur where they do requires consideration of both contemporary and historical causation.

Freshwater and Terrestrial Turtles. The phylogeographic hypotheses in Table 4.1 and the four aspects of genealogical concordance in Table 5.1 were motivated largely by the findings just described on freshwater fishes and maritime faunas of the southeastern United States. Thus, these phylogeographic concepts were considered provisional pending further comparative evaluations. One faunal group subsequently assayed in explicit tests of phylogeographic concepts and concordance principles involved turtles (order Testudines) native to freshwater and terrestrial habitats of this continental region.

Thirty-five living species of Testudines inhabit the southeastern United States, of which about 20 have been subjects of molecular phylogeographic appraisal (Table 5.2). Nine assayed species have broad distributions across the area. Eight of these showed pronounced phylogeographic population structure in mtDNA, as predicted for relatively sedentary organisms under phylogeographic Hypothesis I (Table 4.1). Furthermore, highly divergent branches in the mtDNA gene trees were observed within these species, and the mtDNA clades invariably distinguished populations on a regional spatial scale (Fig. 5.11).

TABLE 5.2 Genetic summary statistics for several species of freshwater and terrestrial turtles examined for mtDNA phylogeographic patterns across the southeastern United States.

Species	Major Phylogeographic Units	Net Sequence Divergence between Units[a]	Reference
Sternotherus minor	A, B	0.032	Walker et al., 1995
Sternotherus odoratus	A, B, C	0.014–0.028	Walker et al., 1997
Kinosternon subrubrum	A, B, C, D	0.027–0.070	Walker et al., 1998a
Kinosternon baurii	A, B	0.010	ibid.
Trachemys scripta	A, B	0.006	Avise et al., 1992c; Walker & Avise, 1998
Graptemys (10 species)	A, B, C	0.010–0.028	Lamb et al., 1994
Gopherus polyphemus	A, B	0.021[b]	Osentowski & Lamb, 1995
Chelydra serpentina	A	—	Walker et al., 1998b
Macroclemys temminckii	A, B, C	0.017–0.028[b]	Roman et al., 1999
Deirochelys reticularia	A, (B + C)	0.043[b]	Walker & Avise, 1998

[a] All sequence divergence estimates within phylogeographic units were considerably smaller.

[b] From mtDNA control-region sequences. All other values in this column are based on RFLP assays.

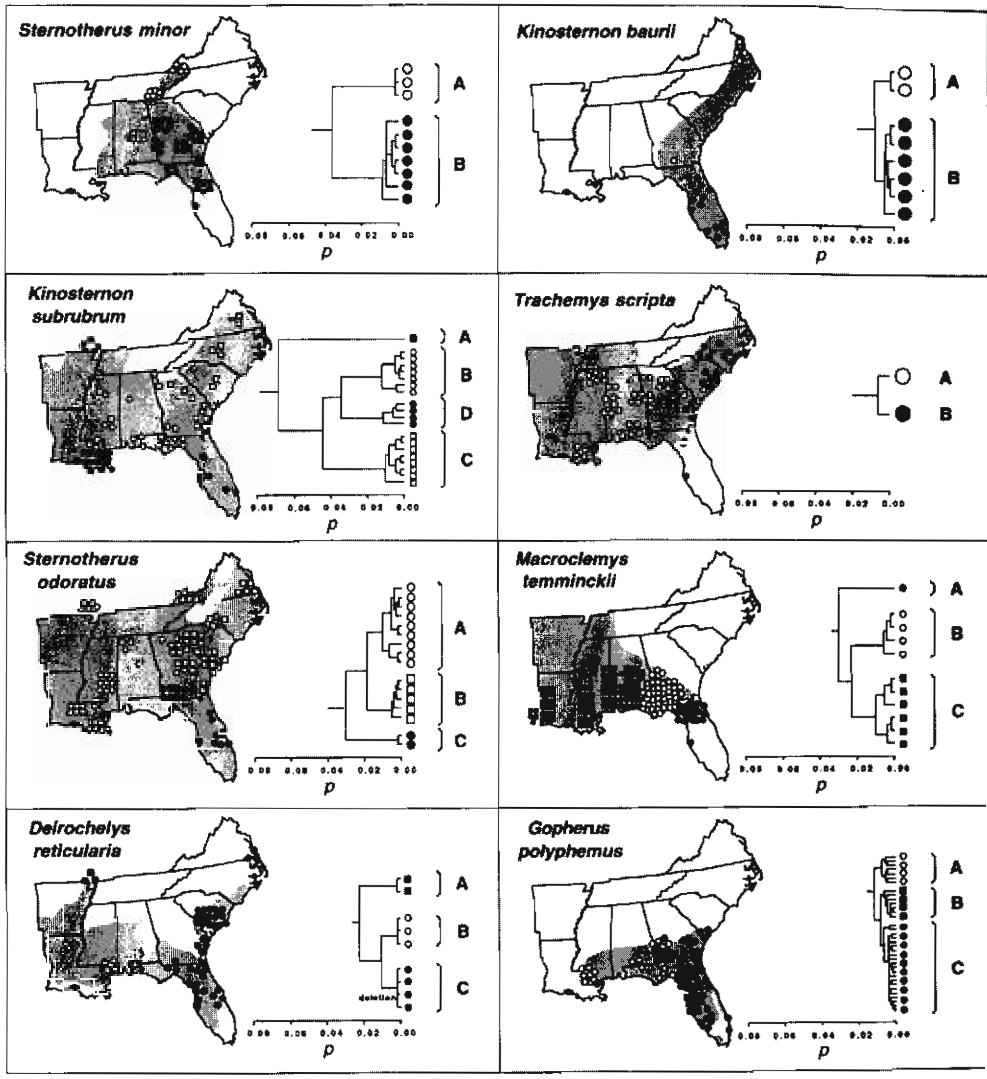


FIGURE 5.11 Intraspecific phylogeographic patterns in mtDNA for freshwater and terrestrial tu tles in the southeastern United States (after Walker and Avise, 1998). Note that all diagrams are plotte on the same scale of genetic distance between haplotypes. On the maps, each symbol denotes an ind vidual and the mtDNA phylogroup to which it belongs. Species' ranges are indicated by shading.

The evidence bearing on phylogeographic Hypothesis III for these turtles (that the major mtDNA phylogroups reflect historical population separations) consists of three aspects of genealogical concordance. First, the gene-tree separations themselves typically involved many mtDNA sequence or restriction-site characters (concordance Aspect I). Second, the mtDNA phylogroups often coincided reasonably well across multiple species (Aspect III). Thus, for seven species depicted in Fig. 5.11, populations in peninsular Florida and/or along the Atlantic coast belonged to mtDNA gene-tree branches that were highly distinct from those characterizing conspecific populations in coastal plains and Piedmont areas to the west. These phylogeographic patterns suggest genealogical separations between regions that are far older than those within regions.

Third, these intraspecific phylogeographic patterns agree quite well with traditional evidence on the major Testudine faunal provinces in the region (Aspect IV). Thus, a basal distinction in species' composition between Atlantic and Gulf zones (Fig. 5.12) parallels the intraspecific phylogeographic trends in mtDNA. Furthermore, these faunal provinces for turtles bear a remarkable resemblance to those described previously for freshwater fishes. All of these lines of evidence suggest that historical biogeographic factors had cardinal influences on the population genetic architectures and species' distributions of aquatic and semi-aquatic organisms in the southeastern United States.

OTHER MULTISPECIES REGIONAL APPRAISALS

Small Mammals of Lowland Amazonia

The lowland forests of the Amazonian Basin contain the world's richest biota. Several hypotheses have been advanced to explain recent speciations in this region. The Refuge Model (Haffer, 1969; Cracraft and Prum, 1988) states that populations were sundered by habitat vicariance associated with cyclical expansions and contractions of forests and savannahs during alternating wet and cool-dry Pleistocene episodes. Ecological models (Endler, 1982; Tuomisto et al., 1995) suggest that divergence has been

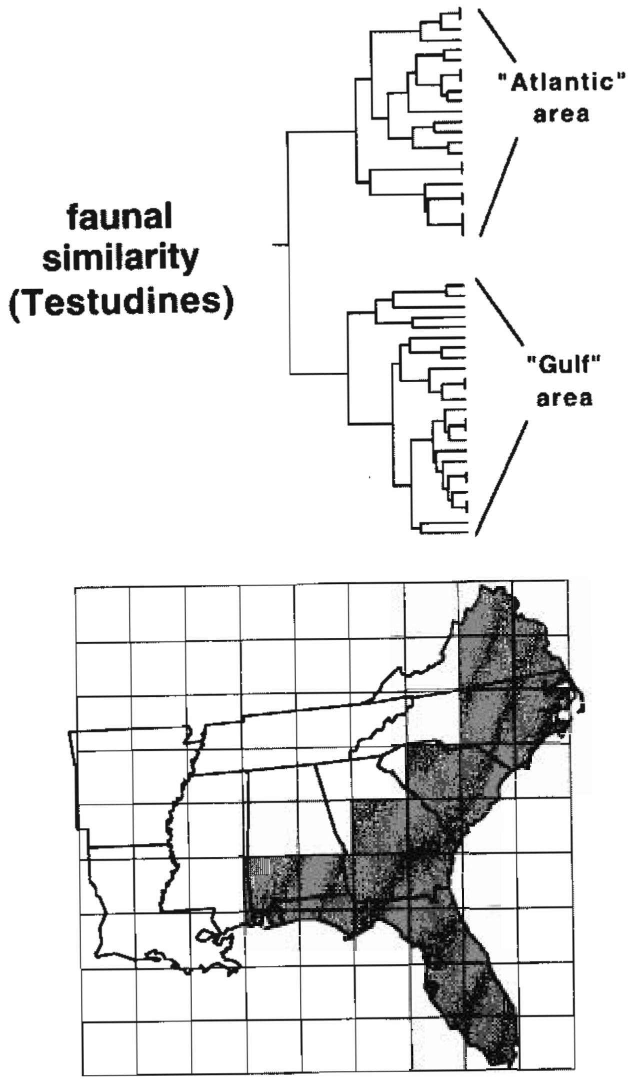


FIGURE 5.12 Faunal provinces for turtles and tortoises in the southeastern United States based on a composite assessment of species' distributions in the Testudines (after Walker and Avise, 1998). The map shows the two basal regions (shaded, "Atlantic"; unshaded, "Gulf") identified in a cluster analysis (above) of faunal similarity coefficients among areas demarcated by a grid superimposed on the map.

driven by selection pressures associated with high environmental and ecological heterogeneity in the region. The Riverine Barrier Model (Wallace, 1849; Ayres and Clutton-Brock, 1992) posits that large rivers promoted genetic divergence in terrestrial organisms by blocking inter-regional gene flow. These hypotheses are not mutually exclusive and multiple factors may apply (M. B. Bush, 1994). Nonetheless, these and related possibilities provide useful frameworks for biogeographic evaluations in the Neotropics (Simpson and Haffer, 1978; Prance, 1982; Whitmore and Prance, 1987).

For example, tests of divergence processes in Amazonia have been analyzed from mtDNA patterns within several nonvolant mammal species (Patton and Smith, 1992; da Silva and Patton, 1993; Patton and da Silva, 1997; Patton et al., 1994, 1996, 1997; Peres et al., 1996). An immediate goal was to test the Riverine hypothesis of population vicariance by examining conspecific populations along the 1,500-km length of the Rio Juruá, a major tributary of the Amazon River in western Brazil. More than a dozen species of marsupials and rodents have been assayed (da Silva and Patton, 1998). The Riverine Model predicts historical genetic separations of populations on opposite sides of the river, but this expectation was not met by any of the species. Instead, highly divergent mtDNA phylogroups were observed for upstream versus downstream regions in many of the assayed taxa (examples in Fig. 5.13). Sequence divergence estimates between these intraspecific phylogroups ranged from 4 percent to 14 percent across species, compared to genetic distances within clades that were typically less than 1 percent.

Although small sample sizes prohibited strong conclusions, the large genetic distances between phylogroups and the concordant geographic placements of the phylogenetic breaks supported the notion of common vicariant elements in the origin and history of this fauna. One intriguing possibility raised by da Silva and Patton (1998) is that Amazonia is composed of several historical drainage sub-basins lying in different tectonic settings, each separated by geological arches due to Andean uplifts of the mid- to late Tertiary. One of these major arches cuts perpendicularly across

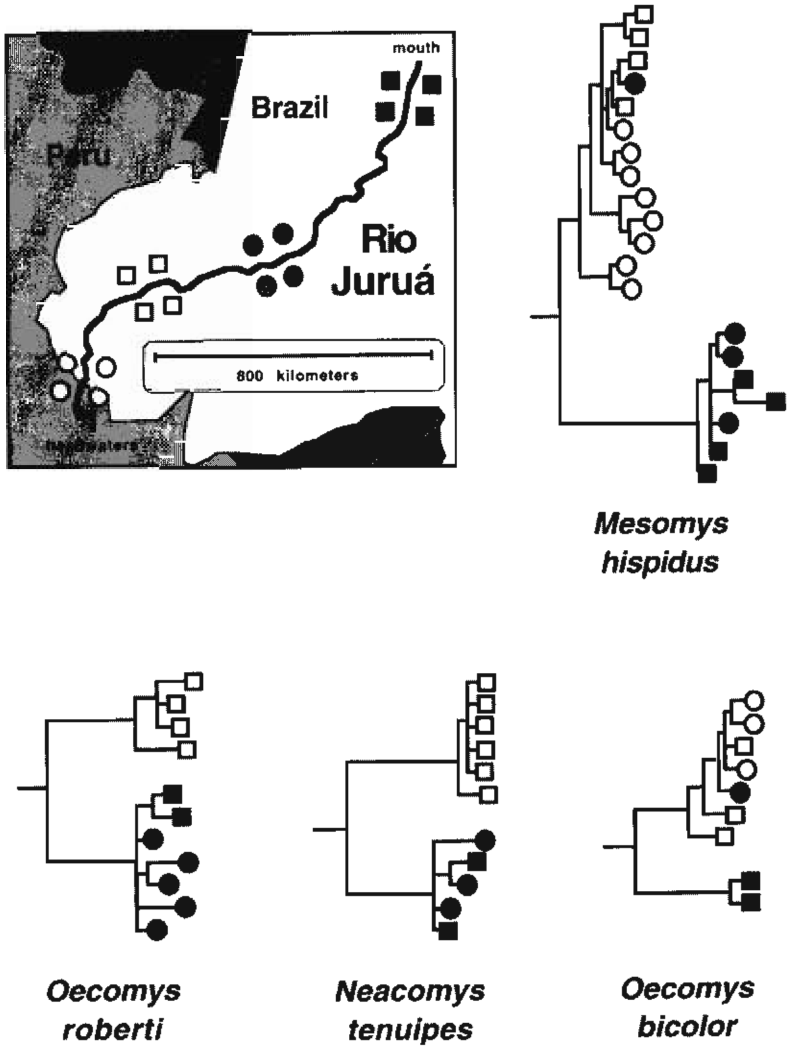


FIGURE 5.13 Phylogeographic concordance of major mtDNA phylogroups for four species of Amazonian rodents along the Rio Juruá in western Brazil (after Patton et al., 1994; da Silva and Patton, 1998; and Patton, pers. comm.). Members of *Oecomys* are semi-arboreal rice rats, *Mesomys* are spiny tree rats, and *Neacomys* are terrestrial spiny mice.

the central part of the Rio Juruá in a position coincident to and perhaps causally associated with the contemporary phylogeographic breaks observed in several of the species assayed (Fig. 5.13). If these paleobasins do constitute major historical centers for the diversification of recent Amazonian biotas, similar genetic patterns may emerge as additional species in the area are analyzed phylogeographically.

South American Cats

Another molecular genealogical study in the Neotropics revealed considerable phylogeographic concordance across species (Eizirik et al., 1998). In assays of the mtDNA control region, the margay (*Leopardus wiedii*) and ocelot (*L. pardalis*) each proved to be subdivided into 3–4 major phylogenetic units that agree closely in geographic placement (Fig. 5.14). For example, Central American populations in each species are highly distinct in mtDNA sequence from conspecific assemblages of haplotypes in northern and southern regions of South America. These genetic patterns are relevant to historical biogeographic reconstructions and also to conservation efforts for these cats.

Plants of the American Pacific Northwest

Given the relative paucity of molecular phylogeographic analyses at the intraspecific level in the botanical literature (Schaal et al., 1998), it is surprising that one of the earliest regional treatments of multiple codistributed taxa involved plants. Studies of restriction-site variation in chloroplast (cp) DNA have been conducted on several species native to the Pacific Coast of North America (Soltis et al., 1989, 1991, 1992b; Strenge, 1994). Aspects I, III, and IV of genealogical concordance emerged.

The evidence consists of more-or-less coincident distinctions in cpDNA clades between northern versus southern populations (Soltis et al., 1997). In six of the seven plant species assayed—three herbaceous perennials (*Tolmiea menziesii, Tellima grandiflora, Tiarella trifoliata*), a shrub (*Ribes bracteosum*), a tree (*Alnus rubra*), and a fern (*Polystichum munitum*)—deep separations in an intraspecific cpDNA gene tree distinguished molecular

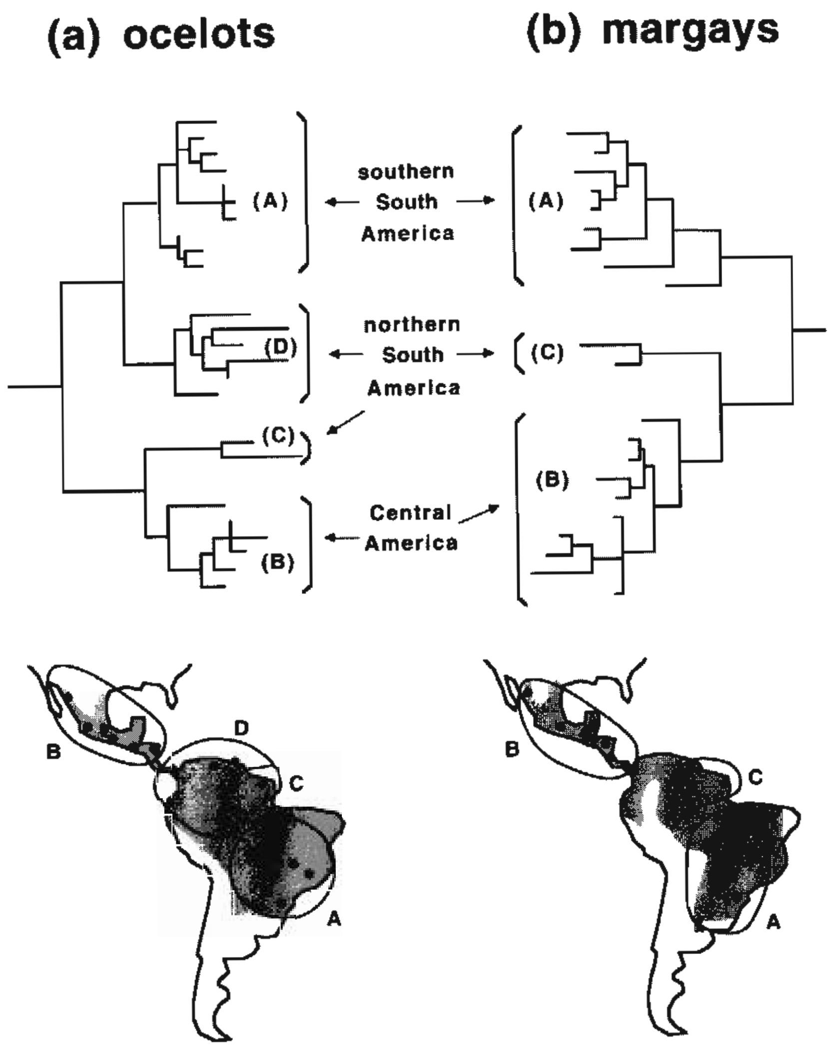


FIGURE 5.14 Concordant phylogeographic patterns within two species of Neotropical cats (after Eizirik et al., 1998). Shaded areas in the maps are species' ranges, and black dots are collecting sites for mtDNA haplotypes whose phylogenetic relationships are depicted in the maximum parsimony trees (*above*).

phylogroups centered in areas generally north versus south of the Oregon-Washington boundary (Fig. 5.15). In some of these species, "southern" genotypes also were observed in disjunct northern sites. The composite pattern suggests historical population separations tracing to previously known Pleistocene glacial refugia in the Pacific Northwest (Pielou, 1991; Soltis et al., 1997). Similar phylogeographic patterns for some animal species in the region (e.g., stickleback fishes [O'Reilly et al., 1993; Ortí et al., 1994] and brown bears [Talbot and Shields, 1996]) may register population differentiation in these same refugia (review in Byun et al., 1997).

However, assays of nuclear genes (ribosomal DNA and/or allozymes) failed to identify clear phylogenetic separations in several of these plant species (Soltis et al., 1997). This might be attributable to either greater discriminatory power inherent in assays of cytoplasmic genomes (due to smaller effective population sizes for uniparentally-inherited genes, all else being equal), or higher levels of gene flow for nuclear genes (via pollen movement) than for cytoplasmic cpDNA (via seeds). In any event, cytoplasmic genomes appear to have recorded signal biogeographic events in the histories of many codistributed plant (and animal) species of the Pacific Northwest.

Marine Organisms and the Trans-Arctic Interchange

For most of the Cenozoic Era, the region that is now the Bering Strait was a land bridge between North America and Asia that effectively prevented any exchange of cold-water marine faunas between the North Pacific and the North Atlantic. This barrier to marine dispersal vanished abruptly about 3.5 Mya when the Earth's temperatures warmed and a sea channel opened (Fig. 5.16). Fossil evidence and current distributions of marine taxa point to an ensuing faunal interchange characterized mostly by unidirectional invasions of high latitude species from the North Pacific into the North Atlantic (Vermeij, 1991a,b). The initial opening of the Bering Strait was followed by Northern Hemisphere glaciations (beginning about 2–3 Mya) that probably influenced both regional extinction patterns and likelihoods of further biotic exchanges across the sub-Arctic region.

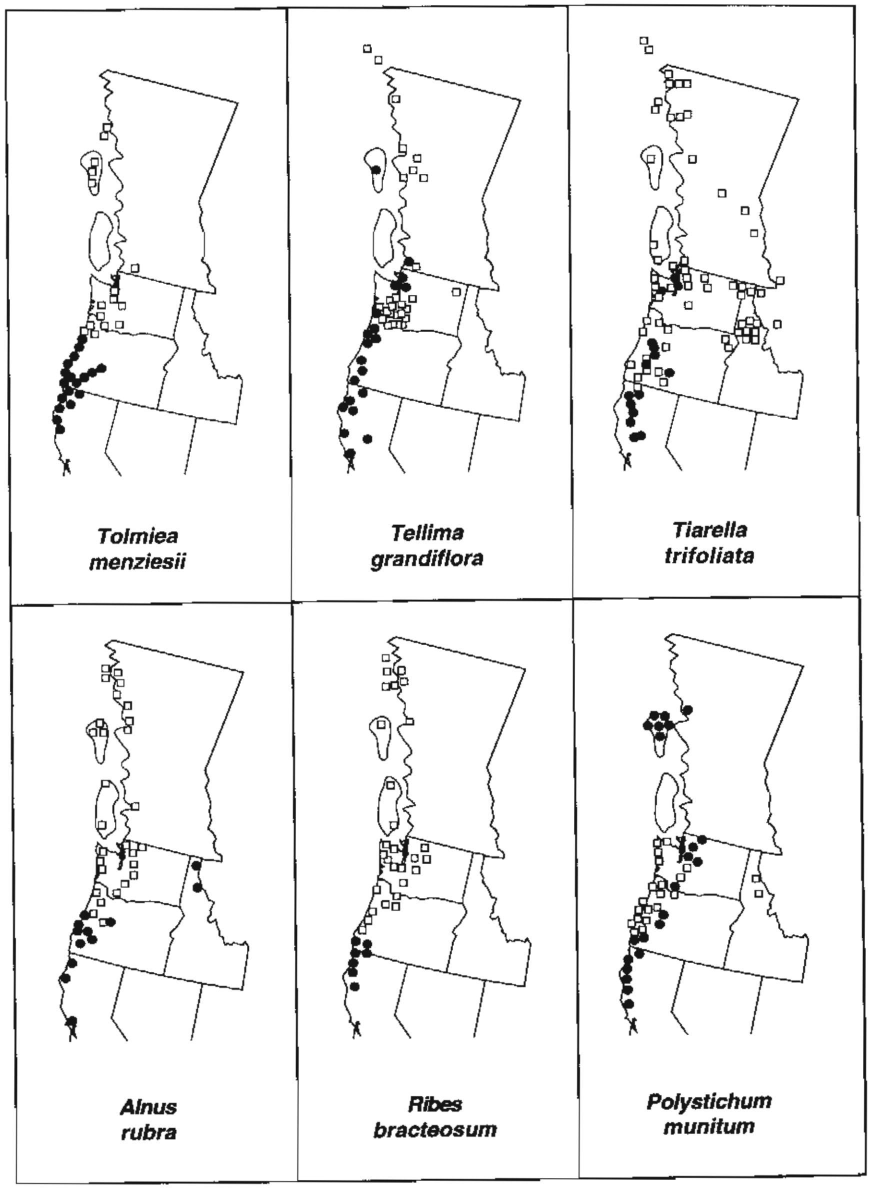


FIGURE 5.15 Phylogeographic concordance of major cpDNA phylogroups within each of six plant species along the Pacific coast of North America (after Soltis et al., 1997). In each case, open squares and closed circles denote the two principal cpDNA clades within a species.

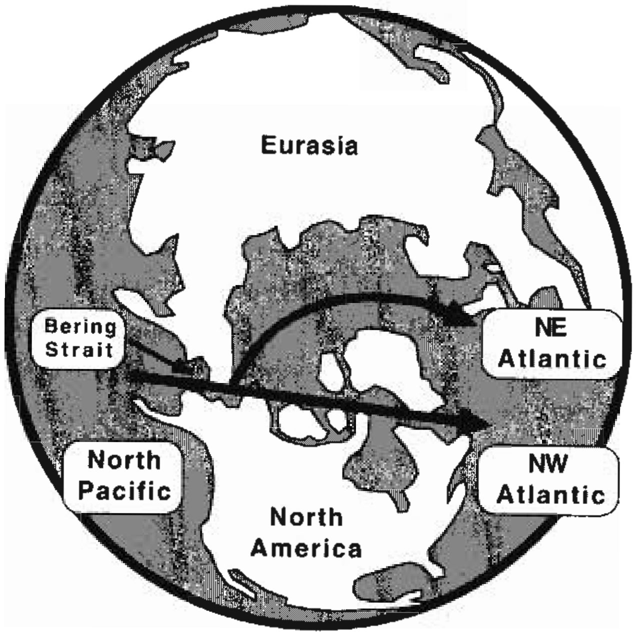


FIGURE 5.16 Circumpolar map showing avenues and suspected directions of gene flow (heavy arrows) from the North Pacific to the North Atlantic following the opening of the Bering Strait about 3.5 Mya (after Cunningham and Collins, 1998).

Molecular phylogeographic analyses have been conducted on several marine taxa in these high latitudes, and results often support the invasion scenarios described above. For example, a mtDNA-based phylogeny for rocky-shore snails in the genus *Nucella* displayed a paraphyletic pattern for North Pacific forms with respect to those in the Atlantic (Collins et al., 1996). Thus, Atlantic populations carry only a nested subset of the lineage diversity in the Pacific. The restricted position of the sole North Atlantic species within the broader phylogeny of *Nucella* snails in Pacific waters is consistent with a single successful trans-Arctic invasion of the Atlantic. Similarly, a molecular phylogeny for *Littorina* snails supports fossil-based hypotheses of two independent trans-Arctic invasions of the North Atlantic from the Pacific (Zaslavskaya et al., 1992; Reid et al., 1996).

A compilation of such case studies for high-latitude marine taxa has revealed a variety of phylogeographic outcomes that can be grouped into four major categories (Fig. 5.17). Classes *A* and *B* involve a secondary cessation of gene flow between the Pacific and Atlantic soon after the original opening of the Bering Strait, whereas Classes *C* and *D* involve recent genetic connections between these oceans. Classes *A* and *C* entail little or no recent genetic contact between populations in the Northwest Atlantic and Northeast Atlantic, whereas Classes *B* and *D* involve recent genetic connections between these two regions. Empirical examples of each phylogeographic outcome have been documented in genetic appraisals of Holarctic marine taxa ranging from fishes to mollusks, crustaceans, and algae (Table 5.3).

For example, a Class *B* pattern is suggested by a close phylogenetic relationship between populations of the alga *Acrosiphonia arcta* across the North Atlantic and a deep separation from populations in the Pacific. On the other hand, a Class *D* history is evidenced by the close phylogenetic connections between mussel populations in the *Mytilus edulis* complex across all three oceanic regions. Detailed inspection of phylogeographic patterns in multiple species (Table 5.3) led Cunningham and Collins (1998) to conclude that regional population extinctions, recolonization events, and vicariant processes have sculptured extant diversity in these trans-Arctic taxa. Furthermore, historical factors proved to be better predictors of phylogeographic outcomes than did intrinsic organismal vagility, including mode of larval dispersal.

Vertebrates in Fragmented Australian Rainforests

Wet forests were widespread across the Australian continent during the Miocene before they became much reduced during the mid- to late Tertiary and Quaternary. Today, rainforests in eastern Australia are confined to a fragmented arc of uplands and coastal lowlands mostly in Queensland (Fig. 5.18). These forest fragments house numerous endemic species as well as those with more catholic habitat tastes. Considerable interest has centered on the extent to which vicariant events dating to the Pleistocene or earlier contributed to contemporary biotic distributions via effects on

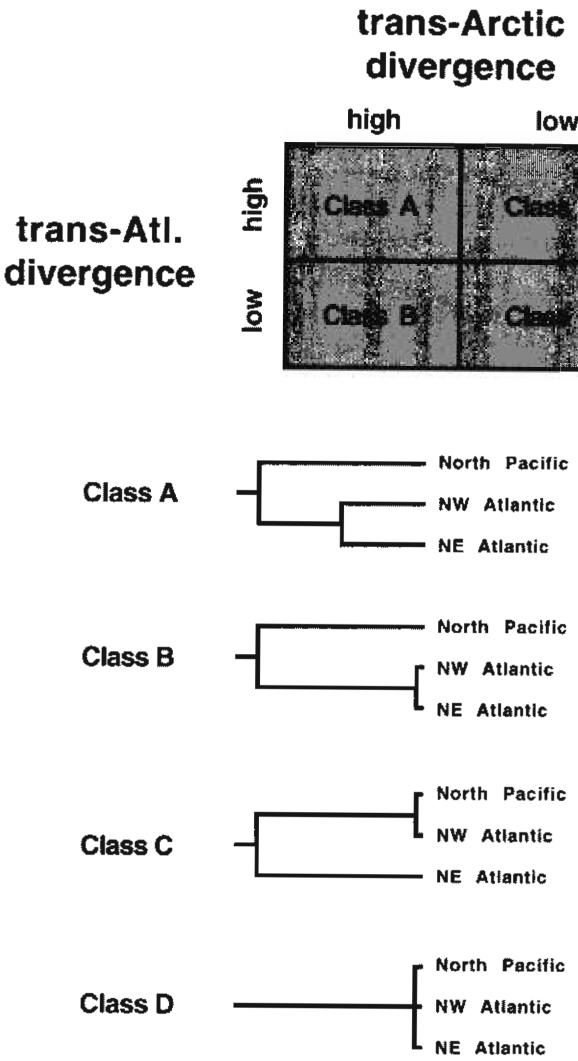


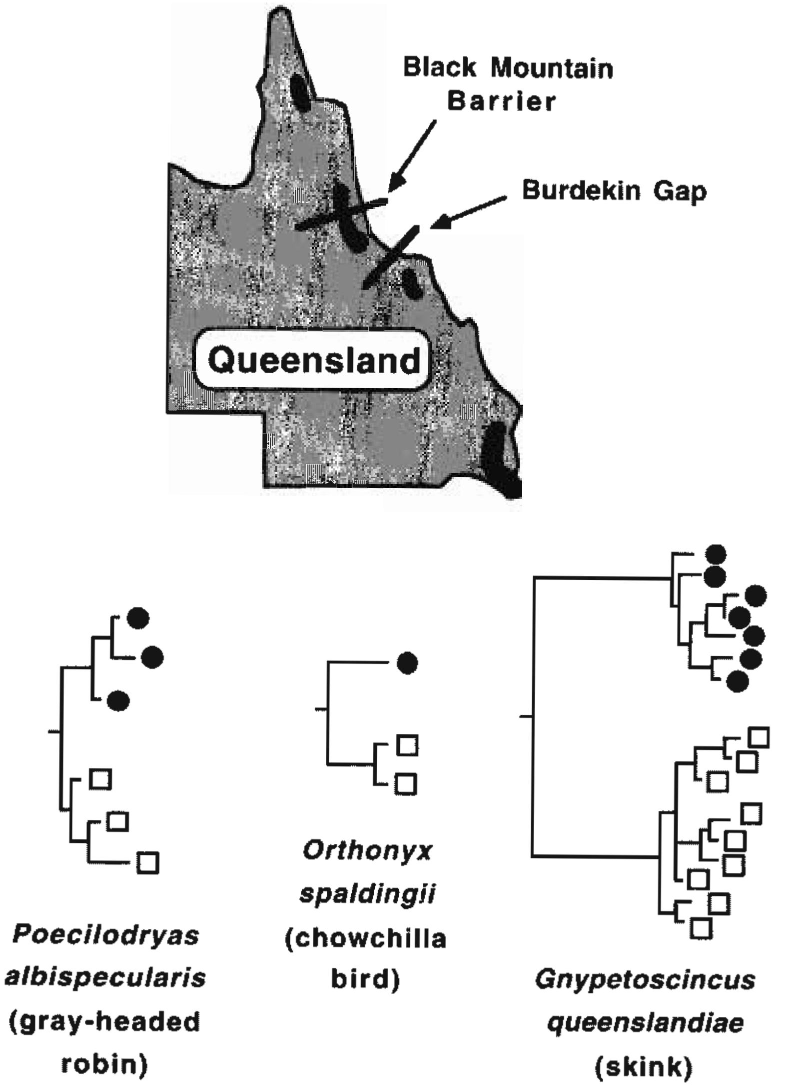
FIGURE 5.17 Four observed classes of phylogeographic history of Northern Hemisphere marine taxa as defined by degree of genetic divergence between regional populations (after Cunningham and Collins, 1998). See Table 5.3 and text.

TABLE 5.3 Marine taxa showing various classes of phylogeographic history as deduced from genetic divergence patterns across the Arctic and North Atlantic (see Fig. 5.17 and text).

Class A	Cessation of gene flow between Pacific and Atlantic soon after opening of the Bering Strait; continuous residence on both sides of Atlantic with little or no recent genetic connections across Atlantic. *Pagurus acadianus-bernhardus* complex of hermit crabs (Cunningham et al., 1992).
Class B	Cessation of gene flow between Pacific and Atlantic soon after opening of the Bering Strait; contemporary gene flow and/or recent colonization events across Atlantic. *Nucella lapillus* complex of snails (Collins et al., 1996); *Semibalanus balanoides* barnacles (cited in Cunningham and Collins, 1998); *Acrosiphonia arcta* algae (van Oppen et al., 1994).
Class C	Successive trans-Arctic invasions with recent genetic connections between Pacific and Northwest Atlantic and little or no contact across Atlantic. *Strongylocentrotus droebachiensis* sea urchins (Palumbi and Wilson, 1990); *Phycodrys rubens* red algae (van Oppen et al., 1995); *Macoma balthica* clams (Meehan, 1985; Meehan et al., 1989); *Osmerus* smelt fishes (Taylor and Dodson, 1994).
Class D	Recent genetic connections between the Pacific, Northwest and Northeast Atlantic. *Strongylocentrotus pallidus* sea urchins (Palumbi and Kessing, 1991); *Gasterosteus aculeatus* stickleback fishes (Haglund et al., 1992; Ortí et al., 1994); mussels in the *Mytilus edulis* complex (Varvio et al., 1988; McDonald et al., 1991; Rawson and Hilbish, 1995).

rainforest fragmentation (Joseph and Moritz, 1994). In particular, two areas unsuitable for wet-forest species (the Black Mountain Barrier and the Burdekin Gap) have been implicated as important historical-biogeographic features in patterning the current distributions of forest-restricted species.

Mitochondrial DNA patterns were summarized for seven avian (Joseph et al., 1995) and six herpetofaunal (Schneider et al., 1998) species in eastern Australia. The most dominant and consistent phylogeographic pattern was a deep split between northern and southern populations. For example, two rainforest-endemic birds (*Poecilodryas albispecularis* and *Orthonyx spaldingii*) showed deep and concordant phylogeographic breaks precisely at the Black Mountain Barrier (BMB), as did four herpetofaunal taxa



Poecilodryas albispecularis (gray-headed robin)

Orthonyx spaldingii (chowchilla bird)

Gnypetoscincus queenslandiae (skink)

FIGURE 5.18 *Above:* Map of Queensland (northeastern Australia) showing the current distribution of rainforest fragments (black areas) and two biogeographic barriers suspected to have played important roles in structuring current species' distributions. *Below:* Intraspecific mtDNA gene trees for two avian and one reptilian species that show concordant phylogeographic breaks north (black dots) versus south (open squares) of the Black Mountain Barrier. (After Joseph and Moritz, 1994 and Joseph et al., 1995.)

(examples in Fig. 5.18). North-south phylogenetic breaks in the other forest-endemic species were associated less obviously with the BMB. Two bird species (*Sericornis citreogularis* and *S. magnirostris*) not restricted to rainforests showed phylogeographic diversity apportioned mainly on either side of the Burdekin Gap. On the other hand, significant phylogeographic population structure was not detected in the forest-restricted *S. keri* or in the widespread *S. frontalis*.

Overall, the empirical results for these rainforest taxa show partial but incomplete congruence in phylogeographic patterns across species (concordance Aspect III), and between molecular phylogroups and biogeographic provinces identified by traditional nonmolecular evidence (Aspect IV). The authors conclude that much of the observed phylogeographic structure is consistent with Pleistocene-refuge models for rainforest fragments, but also that evolutionary histories in these taxa are complicated by idiosyncrasies of local population extinctions and recolonizations.

Geminate Marine Taxa across the Isthmus of Panama

Sometimes, vicariant barriers to dispersal are so firm and well dated that they provide critical geographic frameworks for evaluating molecular patterns (rather than the usual converse in which molecular data inform organismal biogeography). A case in point involves the rise of the Isthmus of Panama about 3 Mya, an event that sundered populations of many tropical marine organisms into geminates (Jordan, 1908) now found in the eastern Pacific and western Atlantic Oceans (Rubinoff and Leigh, 1990). Molecular data gathered for many of these pairs have been put to service in evaluating evolutionary clocks for allozymes and mtDNA in organisms ranging from shrimp (Knowlton et al., 1993) and sea urchins (Lessios, 1979, 1981; Bermingham and Lessios, 1993) to tropical fishes (Vawter et al., 1980; Grant, 1987; Bermingham et al., 1997).

Two general trends have emerged from the information published to date. First, as expected, appropriate genetic assays cleanly distinguish most (but not all) of the geminate taxa in the tropical Atlantic versus Pacific. Second, the genetic distances show great heterogeneity across the presumptive geminate taxa (Fig. 5.19). Thus, at face value, the phylogeographic footprints of the vicariant event on extant faunas appear similar in geographic pattern but perhaps different in temporal depth. This outcome is reminiscent of trends discussed previously for phylogroup separations in faunas of the southeastern United States.

Yet, even in the geologically ideal Panamanian setting, important qualifications apply to any conclusions about molecular evolutionary rates. First, some of the presumed geminates might not be sister taxa, and failure to appreciate this would bias genetic distances and inferred molecular rates upward, all else being equal (Bermingham et al., 1997). Second, gene lineages that were destined for alternative fixation in extant pairs of some of the true sister taxa might have separated long before the vicariant event itself (Fig. 5.8), again resulting in inappropriate upward biases on estimates of evolutionary rate (Knowlton et al., 1993). Third, post-vicariant genetic contact (Fig. 5.9) between some geminate taxa may have occurred via recent circumtropical gene flow perhaps through the Indian Ocean. If unrecognized as such, this secondary genetic contact would yield a false impression of decelerated molecular evolution.

Bermingham et al. (1997) suggested criteria for identifying pre- and post-vicariant contacts from genetic data on extant geminate taxa, and further suggested that such instances be eliminated (or corrected) before calibrating molecular clocks. Adoption of these protocols probably increases the realism of resulting calibrations, but it also entails a risk of circular reasoning. For example, if a lack of appreciable genetic divergence between a pair of geminate taxa is taken axiomatically to imply recent circumtropical gene flow, then no accumulation of such examples could reveal what alternatively could be decelerated molecular evolution following a true 3 Mya vicariant separation. Bermingham et al. (1997) conclude in reference to the Panamanian studies that "Insufficient data exist to test alternative hypotheses of rate variation."

The Hawaiian Volcanic Conveyor Belt

Another favorable geological setting for evaluating phylogeographic patterns is the Hawaiian archipelago, where evolutionary diversification

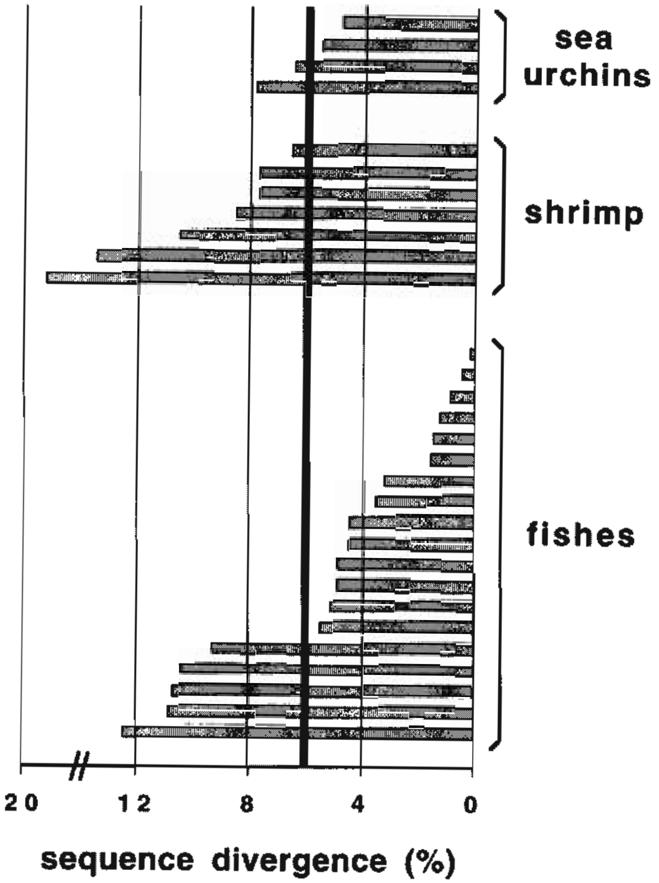


FIGURE 5.19 Differences in mtDNA sequence between presumptive geminate marine taxa separated by the Isthmus of Panama. Shown are mean genetic distances (Kimura, 1980) for four pairs of sea urchins (Bermingham and Lessios, 1993), seven pairs of shrimp (Knowlton et al., 1993), and nineteen pairs of marine fish (Bermingham et al., 1997). The heavy vertical bar indicates the expected genetic distance under a uniform molecular clock ticking at 2 percent sequence divergence per My, assuming that all geminates are true sister taxa that were sundered 3 Mya. See text for interpretations and qualifications regarding rate estimates.

within numerous taxonomic groups has occurred rapidly and recently (Wagner and Funk, 1995). The Hawaiian Islands form as the Pacific Plate moves over a volcanic hot spot that periodically extrudes island-building magma. The islands thus arose on a tectonic conveyor belt, and their times of origin are known from geological dating. These range from 0.4 My for the most recent island (Hawaii) in the southeast, to 5.1 My for the oldest major present-day island (Kauai) in the northwest of the chain (Fig. 5.20).

Molecular phylogenetic appraisals of several species-groups of birds and arthropods on the archipelago have yielded "serial area cladograms" in which the sequential order of clades in a gene tree generally parallels the linear physical (and, hence, temporal) arrangement of the islands (Rowan and Hunt, 1991; Tarr and Fleischer, 1993; Kambysellis et al., 1995; Wagner and Funk, 1995; Roderick and Gillespie, 1998). Such patterns have been interpreted as an expected result of successive lineage colonizations of younger islands. If these colonizations usually took place shortly after island formation, then ages of the islands provide a needed denominator of absolute time for assessments of molecular evolutionary rates.

Fleischer et al. (1998) used this approach to calibrate evolutionary clocks for several mitochondrial and nuclear genes in Hawaiian insects and honeycreeper birds (subfamily Drepanidinae). Results suggest that sequence divergence rates within some genes have been roughly constant over the past 4 My: e.g., 1.6 percent per My for the mitochondrial *cytb* gene in the honeycreepers, and 1.9 percent per My for the *Yp1* nuclear gene in *Drosophila* (Fig. 5.20). Nonetheless, as stressed by the authors, these calibrations remain provisional because several assumptions are entailed beyond the requirement that each island was colonized soon after its origin. Among these additional assumptions are that the geological dates of the islands are accurate, that the topology of a gene tree truly reflects serial island colonizations, and that levels of DNA sequence variation in extant populations (used as a correction factor for calculating between-island genetic distances) are similar to those in the ancestral populations.

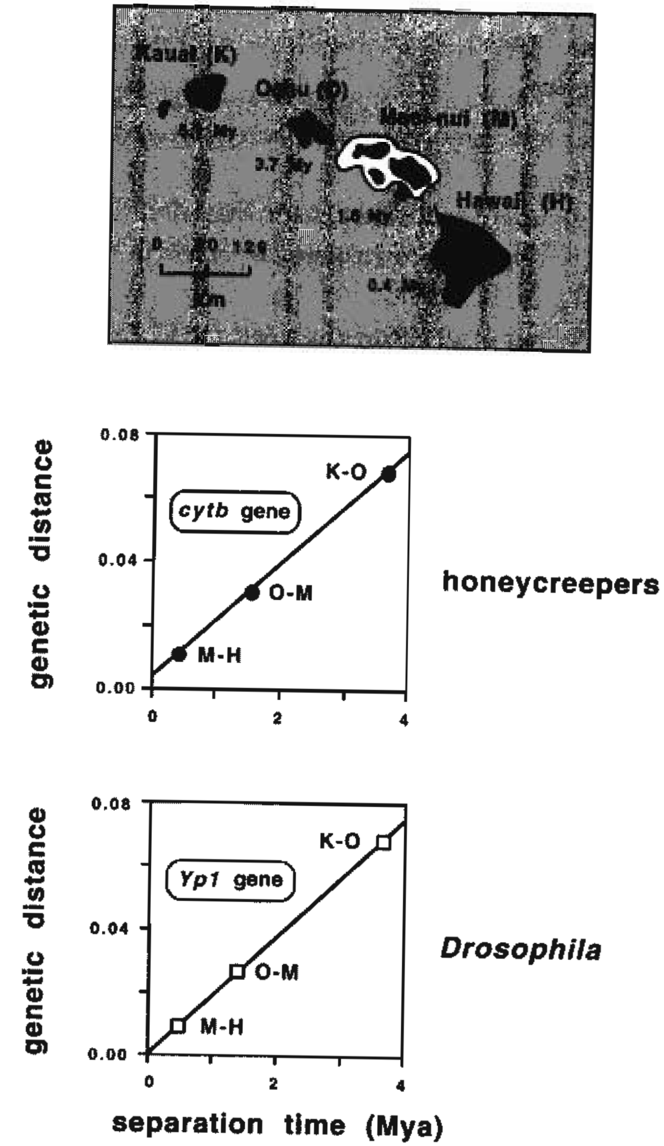


FIGURE 5.20 Molecular clock calibrations in Hawaiian birds and arthropods (after Fleischer et al., 1998). Shown in the map are major islands or island groups whose origination times are known from potassium-argon dating. Molecular data for *Drosophila* were from Kambysellis et al. (1995). See text for background and assumptions regarding rate estimates in the two graphs.

Additional Examples

At the time of this writing, few other molecular studies are available in *comparative* intraspecific phylogeography at regional levels. McMillan and Palumbi (1995) observed striking genealogical concordance in two monophyletic species groups of Indo–West Pacific butterflyfishes (*Chaetodon*): in each case, a genetic break (approximately 2.0 percent in mtDNA sequence) clearly separated individuals from the Indian versus Pacific Oceans. On the other hand, Turner et al. (1996) examined mtDNA and allozyme patterns in populations of five codistributed species of darter fishes (Etheostomatini) from the Ozark and Ouachita highlands of the southcentral United States and failed to discern phylogeographic concordance across species. Nonetheless, species-specific histories were recovered in the assays of these darters.

Similar conclusions regarding species-idiosyncratic population structures but absence of between-species phylogeographic concordance were drawn for the following: assayed herpetofaunas in deserts of the American Southwest (Lamb et al., 1992); some avian species in Central America (Brawn et al., 1996) and in the Caribbean (Bermingham et al., 1996); stream fishes in the central and eastern highlands of North America (Strange and Burr, 1997; but see Bergstrom 1997 for a different interpretation); a variety of marine invertebrates along the west coast of the United States (review in Burton, 1998); and freshwater fishes in Central America (Bermingham and Martin, 1998). In the latter study, close inspection of the molecular patterns led the authors to suggest that lower Central America was colonized by fishes from northwestern Columbia in perhaps three distinct waves of invasion dating respectively to the late Miocene, mid-Pliocene (coincident with the rise of the Panamanian Isthmus), and the Pleistocene.

Zink (1996, 1997) summarized mtDNA phylogeographic patterns in five avian species with broad ranges across North America (Fig. 5.21). Little or no phylogeographic structure was detected in the red-winged blackbird (*Agelaius phoeniceus*), chipping sparrow (*Spizella passerina*), or song sparrow (*Melospiza melodia*). On the other hand, the Canada goose (*Branta canadensis*) and fox sparrow (*Passerella iliaca*) displayed two and four deep

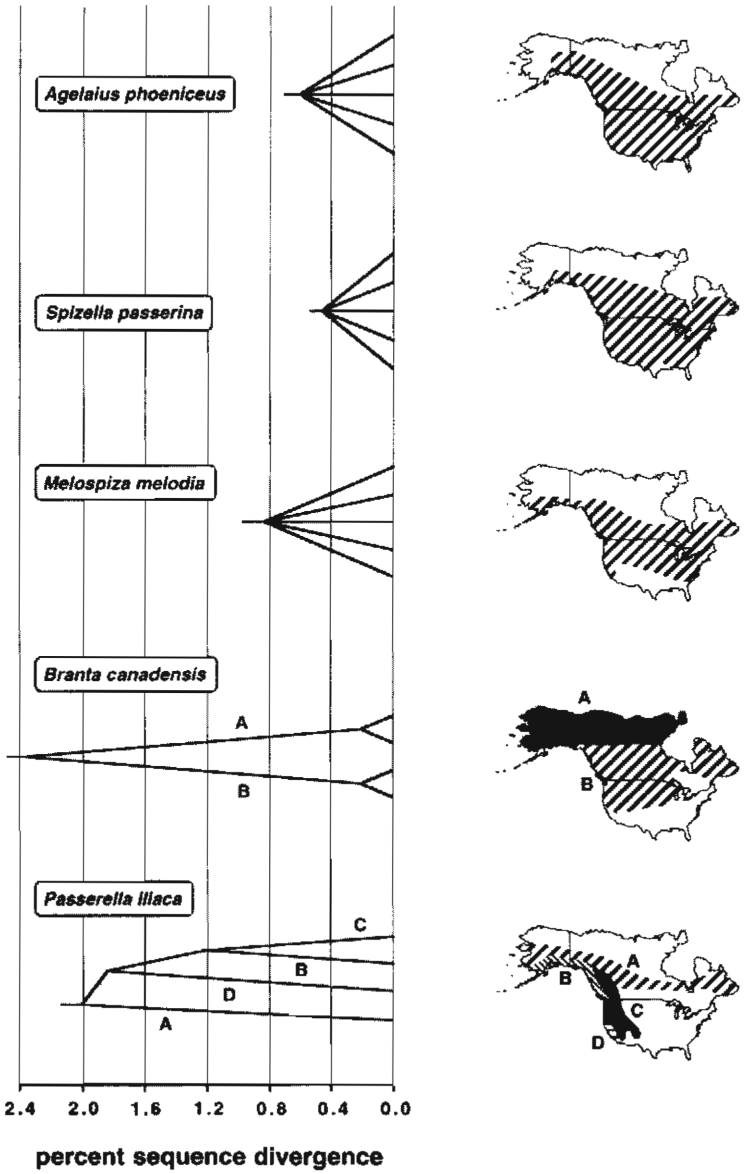


FIGURE 5.21 Approximate extent of breeding ranges and diagrammatic summaries of mtDNA gene trees for each of five avian species with wide distributions across North America (after Zink, 1996).

mtDNA clades, respectively, with strong but nonconcordant geographic orientations. In another continent-wide comparative summary, Taberlet et al. (1998) found little concordance in molecular phylogeographic patterns across 10 European taxa ranging from mammals and amphibians to arthropods and plants. Thus, in contrast to results for several regional biotas reported earlier in this chapter, these studies on broader continental scales have given little indication that populations of codistributed species have had similar phylogeographic histories.

GENEALOGICAL DISCORDANCE

Apparent and often real discrepancies among multiple gene trees, or between gene trees and traditional systematic characters, also are reported routinely in a phylogeographic context. Several aspects of genealogical discordance can be of interest in historical reconstructions.

Aspect I: Disagreement across Characters within a Gene

In principle, tightly linked sequence characters have experienced the same history of transmission through an extended organismal pedigree. Thus, any genealogical discrepancies among nonrecombined characters must in some sense represent phylogenetic "noise."

When (as is often the case) small numbers of different sequence characters earmark different but nonoverlapping clades in a gene tree, no explanations need be invoked other than the idiosyncrasies of mutational origin. The observed mutations considered individually merely may denote particular branches in a less-than-fully-resolved gene tree, without overt conflict in clade delineation. Thus, particular clades that happen to be revealed in an empirical molecular survey may vary according to vagaries of mutational origin and effects of finite genetic sampling. The net result is an estimated gene tree with unresolved branches but no topological discrepancies from the true gene tree.

On the other hand, different sequence characters within a gene may suggest conflicting or overlapping clades. If the locus was historically free of interallelic recombination (as normally is true for mtDNA), then the agents responsible must entail evolutionary homoplasy (convergence, parallelism, or reversal in particular character states). In phylogenetic reconstructions based on parsimony, the extent of homoplasy is estimated routinely as the number or fraction of extra steps in the gene tree beyond those that distinguish haplotypes in the matrix of raw data.

Sometimes, multiple sequence characters in different regions of a gene display overt disagreement with respect to inferred placement of haplotypes in a gene tree. For a nuclear locus, historical intragenic recombination (or, perhaps, gene conversion) could be responsible. Alleles that arose via intragenic recombination consist of amalgamated stretches of sequence that truly had distinct phylogenetic histories within a species. Cases are easiest to document empirically when recombination was between highly divergent alleles, and when recombination events were infrequent such that the odd recombinant haplotype can be seen to consist of two portions with distinct placements in a broader gene tree.

Aspect II: Disagreement across Genes

Some degree of phylogenetic discordance across gene trees is an inevitable consequence of Mendelian inheritance and the vagaries of lineage sorting at unlinked loci through a sexual pedigree. An example involving cytonuclear comparisons was discussed earlier under the 3*x* rule. This rule relates to the differing theoretical probabilities, at intermediate times of population separation, that nuclear versus mitochondrial lineages will have achieved reciprocal monophyly via random lineage sorting from a polymorphic ancestor. Indeed, genealogical discordances reported in the literature often involve a greater population structure inferred from cytoplasmic than from nuclear genes (although this also could result from a higher mutation rate in cytoplasmic genes).

However, other causes of cytonuclear discordance also are known (Palumbi and Baker, 1996; Rawson and Hilbish, 1998). In many species, males or their gametes tend to be more dispersive than females. Consistent gender biases in historical gene flow can result in distinctly different signatures of population structure in nuclear versus cytoplasmic loci. For example, macaque monkeys (Melnick and Hoelzer, 1992), green sea turtles (Karl et al., 1992; FitzSimmons et al., 1997b), and humpback whales (Palumbi and Baker, 1994; Baker et al., 1998) all display less geographic structure in nuclear genes than in mtDNA haplotypes, probably due at least in part to greater interpopulation movements or matings by males. In many plants, pollen are far more dispersive than seeds, one net consequence being a greater opportunity for the spread of nuclear alleles than of maternally transmitted cytoplasmic alleles (McCauley et al., 1996; Latta and Mitton, 1997).

In some secondary hybrid zones also, gender-based asymmetries have been documented with respect to contemporary (Lamb and Avise, 1986) or historical (Dowling et al., 1997) mating patterns and gene flow regimes (Arnold, 1993; Mukai et al., 1997). Often in collaboration with selective influences (Boissinot and Boursot, 1997), sex-based asymmetries can have pronounced effects on cytonuclear associations and the spatial extents of introgression across hybrid zones (Arnold, 1993, 1997; Harrison, 1993). Ancient episodes of introgressive hybridization also can produce "ghosts of hybrids past" (Wilson and Bernatchez, 1998) sometimes recognizable as pronounced incongruences among gene trees (DeSalle and Giddings, 1986; Dowling and Demerais, 1993; Rieseberg et al., 1996; DeSalle et al., 1997; Dowling and Secor, 1997; Rieseberg, 1997; Bagley and Gall, 1998).

In nonhybrid settings also, various forms of natural selection may produce distinct phylogeographic patterns among unlinked loci. At some loci, balancing selection (review in Mitton, 1997) might inhibit the extinction of allelic lineages, thereby generating a misleading impression of moderate or high gene flow among historically isolated populations. One likely example involves an aspartate aminotransferase gene in the deer mouse (*Peromyscus maniculatus*). Notwithstanding strong evidence from mtDNA, karyotype, and morphology for pronounced historical restraints on gene flow in this species, populations across North America all maintain similar frequencies of the same two protein electromorphs at this locus (Avise et al., 1979c; Aquadro and Avise, 1982). Similar observations of geographic uniformity in allozyme allele frequencies, despite evidence from mtDNA and nuclear RFLPs for restricted historical gene flow, led to speculation that balancing selection may have operated on some protein-coding loci in the American oyster (Karl and Avise, 1992). In general, any strong and consistent heterogeneities in population allele-frequency variances across loci are suggestive of selective influence of some sort because alleles at all neutral loci should paint similar pictures of population structure (Lewontin and Krakauer, 1973). Perhaps balancing selection contributes to geographic uniformity of allele frequencies at some genes, or diversifying selection contributes to geographic heterogeneity at other loci.

At the nucleotide sequence level, single-copy nuclear DNA in animals often evolves slower than mtDNA, and this provides another reason why pronounced population structure detected in mtDNA assays may remain unregistered in some nuclear assessments. Conversely, nuclear genes underlying some phenotypic features traditionally used in subspecies' taxonomies, such as pelage color in mammals or plumage features in birds (Barraclough et al., 1998; Magurran, 1998; Price, 1998), may evolve rapidly under diversifying or sexual selection. This could produce the appearance of strong population structure notwithstanding shallow lineage separations that might be registered in neutral or selectively constrained molecular characters (Hillis, 1987; Avise, 1994).

In general, disparate classes of molecular and organismal features experience varied selective regimes that can generate heterogeneities in phylogeographic outcomes. Consider the spectacular radiation of cichlid fishes in some of the African Rift Valley lakes (notably Lake Victoria). There, as gauged by the extremely shallow mtDNA genealogies and other evidence (Meyer et al., 1990), many species with diverse morphological and natural-history adaptations appear to have arisen within the last few thousand years, probably under intense sexual selection by females for differently colored males (Seehausen et al., 1997; Galis and Metz, 1998). Near the other end of the spectrum of evolutionary patterns, horseshoe crabs that have been morphologically conservative for tens of millions of years display (in molecular assays) considerable phylogenetic depth both within and among species (Saunders et al., 1986; Avise et al., 1994).

At higher taxonomic levels also, gene trees can disagree with one another and with a species tree. Discussion of this expression of discordance will be deferred to Chapter 6.

Aspects III and IV

Evolutionary processes that can generate varied phylogeographic patterns across codistributed species (Aspect III of discordance) are as numerous and varied as the historical factors that may have impinged on the population demographies of different species over an extended time. Gene trees may conflict with other biogeographic information (Aspect IV of genealogical discordance) for several reasons, including chance historical events leading to phylogeographic patterns that at face value appear inconsistent with historical geography. For example, the cattle egret (*Bubulcus ibis*) is a common wading bird in the Americas, yet it recently colonized the New World from its ancestral homeland in Africa probably by way of a storm in the late 1800s. Such colonization events are on a long list of "stochastic" historical factors that can produce species-eccentric phylogeographic outcomes.

Nonetheless, even the most species-idiosyncratic of phylogeographic architectures can inform taxonomic decisions, historical reconstructions, and conservation efforts for the particular organisms investigated. This statement holds whether or not concordant phylogeographic patterns exist across taxa, and even when recognizable genetic lineages fail to map to traditional biogeographic provinces.

CONCORDANCE AND PHYLOGEOGRAPHIC DEPTH

The primary rationale for promoting genealogical concordance concepts is that various aspects of congruence are the best and perhaps only means to distinguish between temporally deep versus shallow population genetic architectures. In nearly all species, typical dispersal distances of individuals are far below the total geographic range occupied by that species. Thus, the existence of spatial genealogical structure is virtually axiomatic (whether or not it happens to have been detected in a particular genetic assay). Through genealogical concordance principles, attention is shifted from the mere genetic diagnosis of populations to an enlightened concern about the relative magnitudes of population genetic differentiation, and of the historical processes that have shaped the more salient of the biotic partitions.

Conservation Relevance of Phylogeographic Depth

Both shallow and deep genealogical separations within species can be informative in a conservation context. Furthermore, both idiosyncratic and concordant phylogeographic patterns across genes and across taxa can have relevance for conservation efforts, depending on the particular issue and biological setting. These sentiments follow from the theoretical ties between population demography and intraspecific genealogy discussed in Chapter 2, and now they can be revisited with the force of empirical examples.

SHALLOW SEPARATIONS: MANAGEMENT UNITS

Conspecific populations not clearly delimited by large phylogenetic gaps must be connected genealogically through ongoing or recent gene flow. Nonetheless, contemporary dispersal in many cases may be far too low to promote appreciable demographic connections between geographic demes. In genetic analysis, the logic underlying the concept of a "management unit" (MU) is as follows: Any population that exchanges so few migrants with others as to be genetically distinct from them normally will be demographically independent at the present time. In the literature of commercial fisheries, MUs traditionally are referred to as "stocks" toward which harvesting quotas and other management plans are directed (Avise, 1987; Ryman and Utter, 1987; Ovenden, 1990). For any species, populations that are demographically autonomous should qualify as distinct MUs.

Provisional MUs can be identified by significant divergence in allele frequencies at neutral loci, regardless of depth in the gene tree (Moritz, 1994b). Mitochondrial haplotypes are especially powerful for identifying MUs because of their typical fourfold smaller effective population size (compared to haplotypes at autosomal loci), and because of their special relevance to demographic and reproductive connections among populations. Even shallow matrilineal subdivisions can be relevant to conservation efforts. Demographically autonomous populations, if overexploited or extirpated by humans or other causes, are unlikely to recover via natural recruitment of foreign females over ecological timescales relevant to immediate management interests (Avise, 1995).

DEEP SEPARATIONS: EVOLUTIONARILY SIGNIFICANT UNITS

In concept, an "evolutionarily significant unit" (ESU) is one or a set of conspecific populations with a distinct, long-term evolutionary history mostly separate from other such units (Ryder, 1986). As such, ESUs are the primary sources of historical genetic diversity within a species (Moritz, 1995). The ultimate goal of conservation biology is to preserve biodiversity, an important currency of which is genetic diversity (Ehrlich and Wilson, 1991). A widely held sentiment in conservation biology is that added value or worth should be attached to organismal assemblages that are more rather than less distinctive phylogenetically (Vane-Wright et al., 1991; Barrowclough, 1992; Faith, 1992, 1994; see Erwin, 1991 for an opposing view). Various measures have been suggested to summarize this phylogenetic component of biodiversity (reviews in Crozier, 1992, 1997; Krajewski, 1994; Vogler and DeSalle, 1994b; Humphries et al., 1995). Conservation plans for conspecific populations would do well to recognize ESUs and interpret them as worthy of special consideration.

Issues of phylogenetic distinctiveness often arise in the context of triage deliberations about how best to apportion finite conservation resources on species-level or higher taxa. However, similar concerns also arise at the intraspecific level. Under the U.S. Endangered Species Act, for example, legal protection is afforded not only to listed species but also to "subspecies" and to "distinct population segments." The concept of ESUs provides a phylogenetic framework for deciding which population units are most distinctive.

Operational criteria ranging from broad to detailed have been suggested for recognizing intraspecific ESUs. A general suggestion is that ESUs must contribute substantially to the overall genetic diversity of a species (Waples, 1991). A more explicit recommendation is that ESUs be identified as groups of populations "reciprocally monophyletic for mtDNA alleles and also differ[ing] significantly for the frequency of alleles at nuclear loci" (Moritz, 1994a). Any such empirical suggestion is arbitrary to some extent because there can be no clean line of demarcation along the continuum of possible magnitudes of population genetic differentiation or inferred temporal depths of population separation.

Consider, for example, the criteria noted above that were intended to answer "how much difference is enough?" for ESU qualification. Under any such guidelines, uncertainties remain: How many or what fraction of nuclear loci must show significant allele frequency differences for populations to warrant ESU designation?; How many or what fraction of individuals who carry heterotypic mtDNA lineages would disqualify a population from ESU status?; What magnitude of nucleotide sequence divergence between mtDNA clades is necessary for ESU recognition? Any universal definition that demarcates ESU status from non-ESU status is arbitrary to some extent and fails to concede that some situations truly are intermediate.

Similar practical difficulties arise in the formal application of concordance principles in ESU recognition. For example, under Aspects I and II of genealogical congruence, only arbitrary minimum guidelines can be invoked in response to questions such as: How many concordant sequence characters are required to establish a meaningful separation in a gene tree? (at least a few, but the more the better); How many gene trees must show concordant phylogenetic partitions to register important historical separations at the population level? (ditto); How deep must be the branch separations in a gene tree for ESU recognition? (the deeper the better); and, How damning to ESU status are mild departures from perfect genealogical concordance across multiple characters? (less departure is better).

Such practical difficulties notwithstanding, the ESU concept (as a corollary of the recognition that population separations can be of varying evolutionary depth) remains one of the most important and revisionary of perspectives to have stemmed from intraspecific phylogeography. Its relevance to conservation efforts applies in the context of single-species issues, and to those of regional biotas (Avise, 1989a; Moritz, 1994b; Bernatchez, 1995; Avise and Hamrick, 1996; Smith and Wayne, 1996).

Conservation Issues in Individual Species. Consider an example involving the bananaquit (*Coereba flaveola*), a common land bird in Central and South America and on several Caribbean islands (Seutin et al., 1994). A gene tree was estimated for 58 different mtDNA haplotypes detected in this species (Fig. 5.22). Six major mtDNA phylogroups (putative ESUs) were observed, from which stemmed several suggestions relevant to conservation efforts (Bermingham et al., 1996). From the perspective of maintaining phylogenetic diversity within the species, it was deemed unwise to expend extraordinary resources in protecting small bananaquit populations from each of the northern Lesser Antilles. Birds on these small islands are close genealogically, as they are to populations on some of the larger islands such as Guadaloupe. If a bananaquit population became extinct, the phylogeny also could guide any proposed reintroduction program intended to restore the original genetic condition. For example, islands in the Lesser Antilles should not be repopulated with bananaquits from Jamaica because of a suspected long history of phylogenetic separation of birds from these regions.

This avian example involved genealogical inferences from a single locus, mtDNA, in conjunction with the geographic orientations of the inhabited islands. Ideally, proper identification of ESUs within any species should rest on at least two facets of genealogical concordance. Concordance Aspect I merely identifies likely candidates for ESU status. Confirmation then requires support from independent genetic characters (Aspect II), spatial agreement in phylogenetic partitions across taxa (Aspect III), or congruence of spatial pattern with that from other independent biogeographic evidence (Aspect IV).

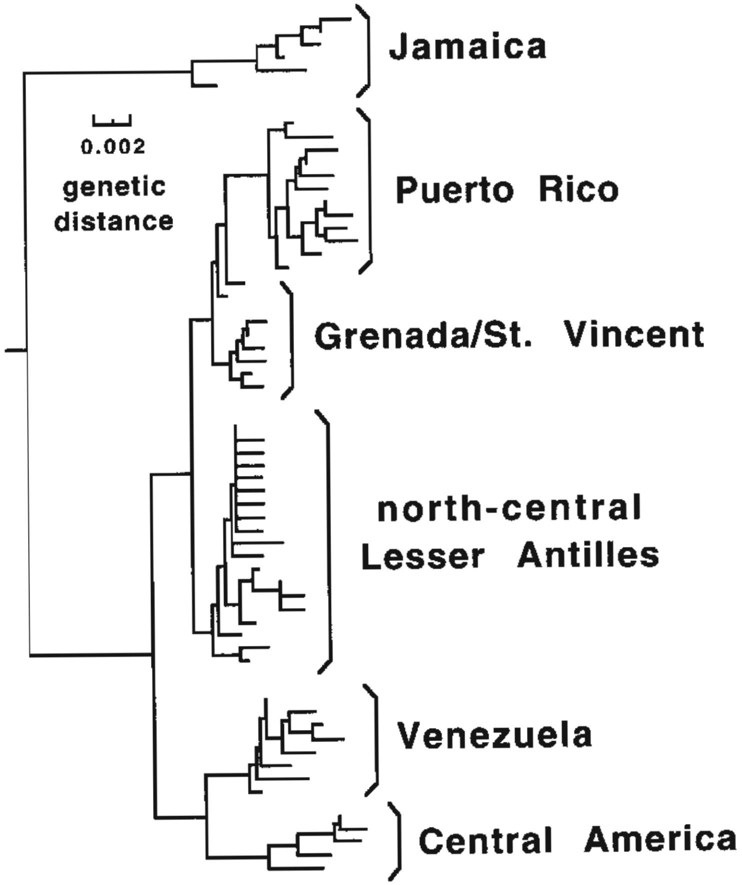


FIGURE 5.22 Mitochondrial gene tree for 58 haplotypes observed in a survey of 170 bananaquit birds, *Coereba flaveola*, in Central and South America and the Caribbean (after Seutin et al., 1994).

As detailed in Chapter 4, many species display deep separations in an intraspecific mtDNA gene tree. In such cases, the major genealogical branches by definition are registered concordantly by multiple mtDNA characters. These branches represent potential ESUs worthy of additional evaluation. As further detailed in Chapter 4, gene-tree phylogroups often display spatial distributions consistent with independent evidence on historical shaping processes. When such concordant support is available from multiple lines of biogeographic evidence, the putative phylogroups then warrant recognition as securely documented ESUs (or suggested terminological alternatives, such as phylogenetic subspecies [O'Brien and Mayr, 1991] or species [Frost and Hillis, 1990]).

Table 5.4 summarizes several empirical molecular studies that identified (or in some cases sought and failed to identify) ESUs within endangered or other species of special conservation concern. The phylogeographic outcomes in some instances challenged and in other cases bolstered the conventional taxonomies upon which conservation programs had been built. As noted by Daugherty et al. (1990), good taxonomies "are not irrelevant abstractions, but the essential foundations of conservation practice." Whether or not immediate conservation issues are involved, well-documented ESUs are relevant to lower-level taxonomic decisions.

Conservation Issues in Regional Biotas. Notwithstanding the phylogeographic idiosyncrasies expected across taxa, several of the comparative studies summarized above documented impressive levels of genealogical concordance in various regional biotas. An exciting application of molecular phylogeography in conservation biology is defining particular geographic regions within which multiple species display phylogenetically distinctive populations or ESUs. Such areas are special candidates for high conservation priority.

It remains to be seen how many regional biotas display the kinds of multi-taxa phylogeographic concordance currently best documented, for example, in the faunas of the southeastern United States, small mammals in Amazonia, vertebrates in rainforest fragments of northeastern Australia, or floras of the American Pacific Northwest. However, the prospect that disproportionate fractions of the Earth's biodiversity may be concentrated in recognizable historical biogeographic areas has obvious implications for conservation efforts at regional or ecosystem scales.

Analytical procedures have been introduced to formally assess genealogical data as a basis for prioritizing particular geographic areas for conservation focus. For example, Faith (1992, 1994; Faith and Walker, 1996) introduced a quantitative phylogenetic diversity measure that incorporates historical data from multiple taxa and can be adapted to summarize underlying "feature diversity" of geographic communities (Moritz and Faith, 1998). Such approaches can complement appraisals that seek to identify distinctive biogeographic provinces and subprovinces on the basis of species' richness or the distributions of taxa (Mittermeier et al., 1998; Olson and Dinerstein, 1998). They also can dovetail with a recent approach in conservation biology known as "gap analysis" wherein ranges of threatened species are compared in assessing priority locations for biological reserves (Scott and Csuti, 1997).

Regional hotspots of biodiversity or exceptional endemism often are promoted as special targets for focused conservation efforts (Scott et al., 1987; Margules et al., 1988; Myers, 1988, 1990; Dinerstein and Wikramanyake, 1993; Pressey et al., 1993; Bibby, 1994; Kerr, 1997). Governmentally sponsored "biogeographic reserves" or "biodiversity parks" could be designed to protect significant fractions of regional biotic diversity, important components of which clearly are historical. Private organizations such as the Nature Conservancy and a few countries such as Costa Rica already have championed similar ideas. A well-implemented system of biogeographic reserves also would promote public awareness of conservation issues, much as the current National Park System in the United States serves both to educate the public and to preserve exceptionally beautiful or unique features of the continent's geological history (Avise, 1996b).

Maiden analyses in comparative molecular phylogeography have yielded an encouraging observation of conservation relevance: a tendency toward spatial concordance (Aspect IV) between intraspecific phylogroups and biogeographic provinces or subprovinces as identified by species' distributions and historical physiography. Comprehensive molecular examinations of regional biotas are expensive and labor intensive, and thus can be contemplated only in model circumstances. Nonetheless, if trends from available phylogeographic studies can be generalized, an encouraging

TABLE 5.4 Examples of molecular phylogeographic appraisals of threatened or endangered species and their taxonomic relatives.

Taxon	mtDNA Gene-Tree Outcome	Concordant Support from . . .	Reference
Geomys colonus (pocket gopher)	no major phylogenetic distinction from problematic common congener	allozymes, karyotype, multivariate morphology	Laerm et al., 1982
Ammodramus maritimus (seaside sparrow)	two major phylogeographic units not coincident with subspecies' designations	phylogeographic partitions in codistributed coastal taxa, and historical biogeographic considerations	Avise & Nelson, 1989
Felis concolor (puma)	two phylogenetic units co-occur in an endangered population in South Florida	nuclear DNA markers, and macro-geographic ranges of ESUs	O'Brien et al., 1990
Lepidochelys kempi (Ridley sea turtle)	clear phylogenetic distinction from problematic congener	a biogeographic scenario from morphology and geology	Bowen et al., 1991, 1997
Canis rufus (red wolf)	close phylogenetic connections to gray wolf and coyote	microsatellite nuclear loci	Wayne & Jenks, 1991; Roy et al., 1994b
Chelonia mydas (green, black sea turtles)	two major phylogeographic units	geographic ranges (confined to Atlantic versus Indo-Pacific)	Bowen et al., 1992
Lycaon pictus (African wild dog)	three major phylogeographic units	geographic ranges (confined to distinct African regions)	Girman et al., 1993
Gopherus polyphemus (gopher tortoise)	at least two major phylogeographic units	phylogeographic partitions in codistributed taxa, and historical biogeographic considerations	Osentowski & Lamb, 1995
Macrotis lagotis (desert bandicoot)	no strong phylogeographic structure in Australian interior	microsatellite nuclear loci	Moritz et al., 1997
Sternotherus depressus (freshwater turtle)	clear phylogenetic distinction from problematic congeners	morphology	Walker et al., 1998c
Melanotaenia eachamensis (Australian rainbowfish)	clear phylogenetic distinction from a problematic congener	morphology, microsatellite nuclear loci	Zhu et al., 1998
Polioptila californica (Calif. gnatcatcher bird)	clear phylogenetic distinction from problematic congeners	morphology	Zink & Blackwell, 1998
Oreailurus jacobita (Andean mountain cat)	clear phylogenetic distinction from the ocelot and margay	morphology	Johnson et al., 1998

conservation message emerges: Much of the information needed to design historical biogeographic reserves already may be available in the form of traditionally recognized biogeographic provinces, subprovinces, or ecoregions (e.g., Scott et al., 1990; Abell et al., 1998). Thus, conservationists need not await complete genetic reexaminations of the biological world before embracing regional perspectives (in addition to traditional species-focused efforts) in preservation initiatives.

Absolute Times of Phylogroup Separation

Principles of genealogical concordance provide a conceptual framework for identifying the deeper phylogeographic units within species. What are the chronological times of the phylogroup separations? Temporal estimates are fraught with uncertainties, but provisional conclusions can be reached by appealing to molecular clock calibrations.

Avise and Walker (1998) summarized the mitochondrial literature on inferred separation times for intraspecific phylogroups in birds. Among 63 avian species surveyed for mitochondrial population structure across major portions of their respective ranges, 37 species (59 percent) displayed a Category I phylogeographic pattern: i.e., they were sundered into two or more significant (bootstrap supported) mtDNA phylogroups with a strong geographic orientation. In most cases, the assignment of a species to phylogeographic Category I was straightforward. Two examples involving *Ammodramus* sparrows are illustrated in Fig. 5.23.

For each such species that was phylogeographically subdivided for mtDNA, net sequence divergence between major phylogroups (corrected for within-phylogroup sequence heterogeneity) was converted to an estimate of population separation time using a conventional avian mtDNA clock: 2 percent sequence divergence between a pair of lineages per My (Klicka and Zink, 1997). The resulting histogram of estimated phylogroup separation times (Fig. 5.24) shows that 76 percent of the 37 inferred phylogroup separations date to the Pleistocene, and most of the remainder to the late Pliocene.

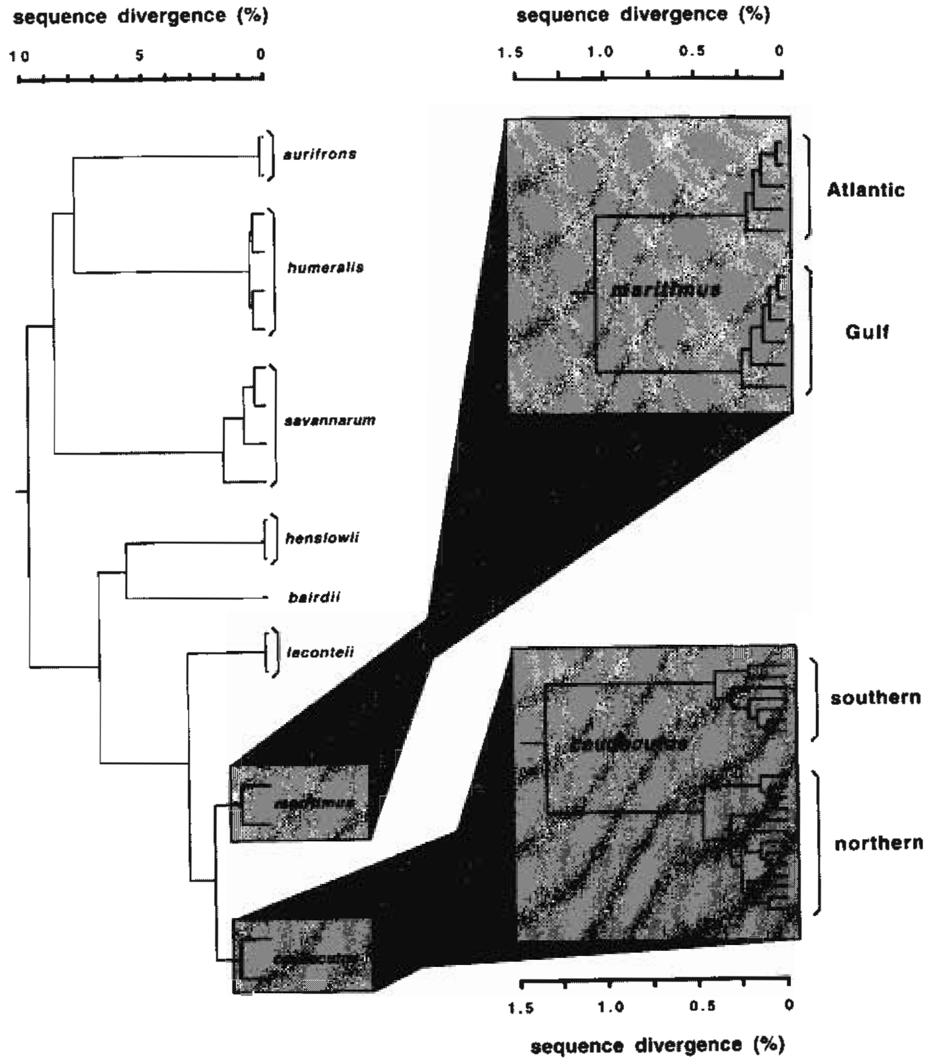


FIGURE 5.23 MtDNA phylogenies in *Ammodramus* sparrows (from Avise and Walker, 1998). *Left:* Matrilineal phylogeny for eight congeneric species as estimated by Zink and Avise (1990). *Right:* Magnified view of matrilineal relationships within each of two species surveyed across their ranges (*A. maritimus* from Avise and Nelson, 1989; *A. caudacutus* from Rising and Avise, 1993).

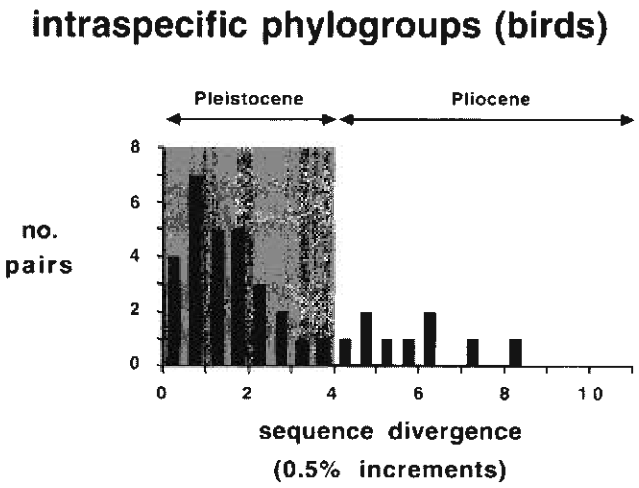


FIGURE 5.24 Histogram of estimated mtDNA sequence divergences (and inferred separation times) between pairs of major intraspecific phylogroups in avian species (after Avise and Walker, 1998).

Thus, the mitochondrial evidence is consistent with conventional wisdom that late Pliocene and Pleistocene events had great impact on the phylogeographic architectures of extant birds. Indeed, authors of the original studies often invoked explicit "Pleistoscenarios" to account for particular phylogeographic outcomes. For example, from an integration of mtDNA data with evidence from morphology and behavior, Rising and Avise (1993) hypothesized glacial refugia and subsequent range expansions of two major phylogroups within *Ammodramus caudacutus*. These forms subsequently were recognized as distinct taxonomic species (AOU, 1995).

Evolutionary separation times for primary intraspecific phylogroups similarly have been summarized in species representing other vertebrate classes (Avise et al., 1998). Among 189 nonavian vertebrates surveyed for mtDNA population structure across major portions of their respective ranges, 103 species (54 percent) displayed a Category I phylogeographic pattern (examples in Fig. 5.25).

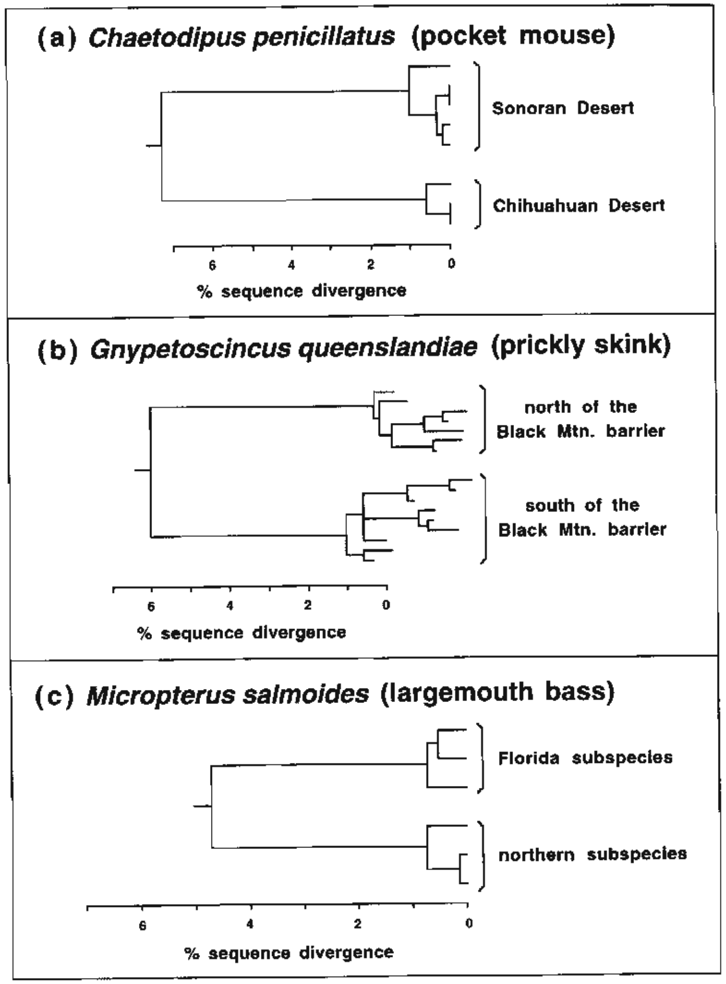


FIGURE 5.25 Typical examples of phylogeographic Category I as reported in mtDNA surveys of a mammal, reptile, and fish (after Avise et al., 1998). *(a)* cluster phenogram for the pocket mouse (Lee et al., 1996); *(b)* neighbor-joining tree for the prickly skink (Joseph et al., 1995); *(c)* cluster phenogram for the largemouth bass (Nedbal and Phillipp, 1994).

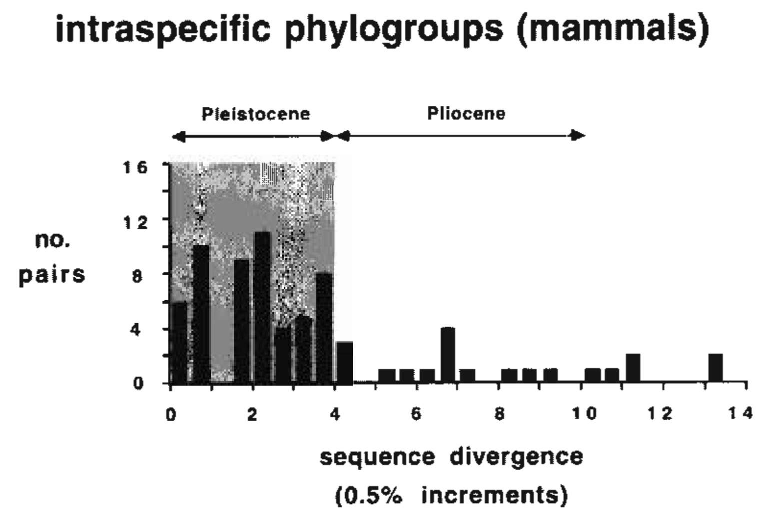


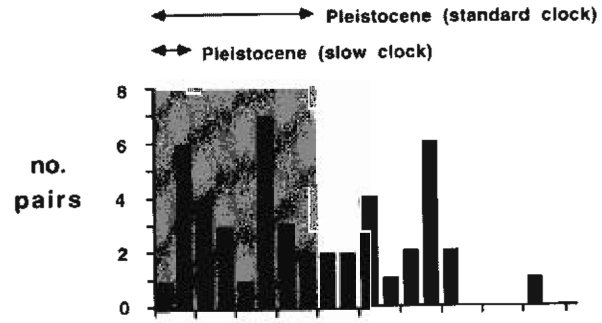
FIGURE 5.26 Histogram of estimated mtDNA sequence divergences (and inferred separation times) between pairs of major intraspecific phylogroups in mammalian species (after Avise et al., 1998).

Within the mammals (Fig. 5.26), 52 of the 72 inferred phylogroup separations (72 percent) date to the Pleistocene, and most of the remainder date to the Pliocene. These percentages are similar to those for avian taxa. For the other vertebrates (Fig. 5.27), interpretations are complicated by suspected slower mtDNA clock calibrations for some of these taxa (Avise et al., 1992c; Adachi et al., 1993; Martin and Palumbi, 1993; Rand, 1993, 1994; Martin, 1995). Under the standard mtDNA clock employed for birds and mammals, 27 of the 47 inferred phylogroup separations in amphibian and reptilian species (57 percent) date to the Pleistocene. In the fishes, 19 of 26 inferred phylogroup separations (73 percent) date to the Pleistocene. However, these fractions for herpetofauna and fishes drop to 15 percent and 31 percent, respectively, under a fourfold slower mtDNA clock calibration.

Three cautionary points should be made about these comparative analyses. First, estimates of absolute separation times are highly sensitive to the clock calibrations employed. By hard criteria, mtDNA evolutionary

intraspecific phylogroups

(a) amphibians and reptiles



(b) fishes

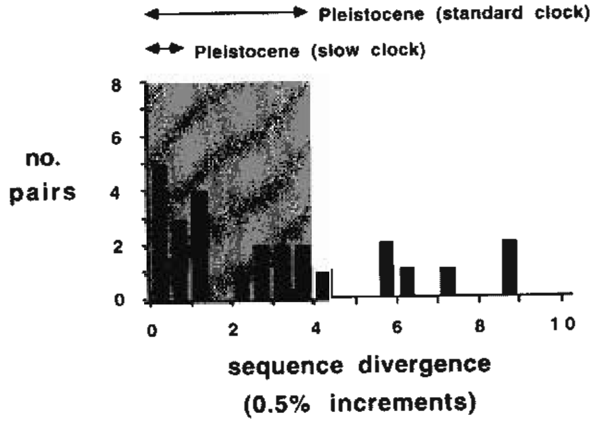


FIGURE 5.27 Histograms of estimated mtDNA sequence divergences (and inferred separation times based on two alternative mtDNA clock calibrations) between pairs of major intraspecific phylogroups in the indicated vertebrate assemblages (after Avise et al., 1998).

rates are poorly characterized for most animal groups, yet are suspected to differ by severalfold across some lineages. Second, a large variance clearly exists in the genetic distances (and inferred times of separation) across phylogroup pairs, and much of this probably reflects true heterogeneity in phylogroup separation times.

Third, a severe bias operates against the detection of any phylogroup separations that might date to less than about 200,000 years ago. About 500 bp of mtDNA sequence per individual was assayed in a typical study. Under a standard mtDNA clock, only about one nucleotide substitution in a sequence of this length is expected to distinguish two matrilines that separated 100,000 years ago. However, several concordant substitutions are required for statistical support of a putative clade in phylogenetic reconstructions. Thus, available data do not rule out the likelihood that late-Pleistocene and Recent events also initiated separations among many shallower phylogroups that remain poorly characterized with conventional laboratory efforts.

SUMMARY

1. Four distinct aspects of genealogical concordance are relevant to phylogeographic interpretations. These involve agreement in phylogenetic patterns: (I) across multiple sequence or other character states within a gene; (II) among multiple gene trees at unlinked loci; (III) across two or more codistributed species with similar ecologies or natural histories; and (IV) between molecular genetic data and more traditional classes of biogeographic evidence. Aspect I establishes that a gene tree displays significant phylogenetic subdivision. Aspect II confirms that gene-tree partitions register phylogenetic separations at the population or species level. Aspects III and IV implicate shared historical biogeographic impacts on regional biotas.
2. Regional phylogeographic surveys consider spatial patterns in the historical lineages of multiple codistributed taxa. All four aspects of genealogical concordance have been registered in one or another of the regional phylogeographic studies conducted to date. Notable examples include: freshwater and maritime faunas in the southeastern United States; mammals in the Neotropics; marine invertebrates of the trans-Arctic; vertebrates in fragmented Australian rainforests; geminate marine taxa that were sundered by the rise of the Isthmus of Panama into Atlantic and Pacific units; and diverse plants of the American Pacific Northwest.

3. Genealogical discordances also are relevant to phylogeographic interpretations. These involve disagreement in phylogenetic depths or patterns: (I) across multiple sequence or other characters within a gene; (II) among multiple unlinked gene trees; (III) across two or more codistributed species with similar ecologies or natural histories; and (IV) between molecular genetic data and traditional classes of biogeographic evidence. Aspect I of discordance often reflects homoplasy in gene-tree data. Aspect II can result from stochastic lineage sorting through sexual pedigrees, or from other biological factors including variable rates of sequence evolution, different effective population sizes for alleles at autosomal versus cytoplasmic (and sex-linked) loci, gender-based asymmetries in historical dispersal and gene flow, and several forms of natural selection. Aspects III and IV suggest that many species-idiosyncratic biogeographic factors have impinged upon regional biotas.
4. Concepts of genealogical concordance and phylogeographic depth are related intimately and carry special relevance in conservation biology. They have led to recognition of a distinction between management units (extant populations that are genealogically close but demographically independent now) and evolutionarily significant units (populations with longer-standing evolutionary separations). The nature of evidence for deep versus shallow phylogeographic separations, and the importance of such distinctions, are increasingly appreciated in ecology, systematics, and conservation biology.

5. Sidereal separation times for intraspecific phylogroups can be estimated from sequence differences and suspected evolutionary rates in mtDNA. Of more than 100 surveyed species of mammals and birds showing significant phylogeographic population structure, about 75 percent of the major phylogroups within species provisionally date to Pleistocene separations under a standard mtDNA clock for homeotherms. Similar conclusions apply to intraspecific phylogroups in reptiles, amphibians, and fishes under the same clock calibration. However, severalfold older separation times for poikilothermic vertebrates are implicated under the slower mtDNA clocks sometimes suspected for various of these taxa.