Evolution, 43(6), 1989, pp. 1192–1208

GENE TREES AND ORGANISMAL HISTORIES: A PHYLOGENETIC APPROACH TO POPULATION BIOLOGY[1]

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Abstract.—A "gene tree" is the phylogeny of alleles or haplotypes for any specified stretch of DNA. Gene trees are components of population trees or species trees; their analysis entails a shift in perspective from many of the familiar models and concepts of population genetics, which typically deal with frequencies of phylogenetically unordered alleles. Molecular surveys of haplotype diversity in mitochondrial DNA (mtDNA) have provided the first extensive empirical data suitable for estimation of gene trees on a microevolutionary (intraspecific) scale. The relationship between phylogeny and geographic distribution constitutes the *phylogeographic* pattern for any species. Observed phylogeographic trees can be interpreted in terms of historical demography by comparison to predictions derived from models of gene lineage sorting, such as inbreeding theory and branching-process theory. Results of such analyses for more than 20 vertebrate species strongly suggest that the demographies of populations have been remarkably dynamic and unsettled over space and recent evolutionary time. This conclusion is consistent with ecological observations documenting dramatic population-size fluctuations and range shifts in many contemporary species. By adding an historical perspective to population biology, the gene-lineage approach can help forge links between the disciplines of phylogenetic systematics (and macroevolutionary study) and population genetics (microevolution). Preliminary extensions of the "gene tree" methodology to haplotypes of nuclear genes (such as *Adh* in *Drosophila melanogaster*) demonstrate that the phylogenetic perspective can also help to illuminate molecular-genetic processes (such as recombination or gene conversion), as well as contribute to knowledge of the origin, age, and molecular basis of particular adaptations.

Received September 7, 1988. Accepted February 7, 1989

I will argue four main points in this essay: 1) population genetics and population biology are at a stage of development where these fields could profit from a greater infusion of phylogenetic principles and reasoning; 2) a powerful approach to this conversion will involve explicit focus on the phylogenetic histories of particular genes and gene products; 3) an analysis of one gene system already studied extensively under this philosophy—mitochondrial DNA (mtDNA)—suggests that the population sizes and the general demographic histories of most higher animals have been remarkably dynamic over recent evolution, both spatially and temporally; and 4) an extension of this phylogenetic approach to other genes, including those in the cell nucleus, may eventually yield a satisfying conceptual framework that links the mechanistic kinds of understanding possible in molecular biology with the higher-level phenomena that are traditionally the subjects of population genetics and evolution.

Population geneticists have conventionally been concerned with heredity and microevolutionary process and have maintained only tenuous communication with most systematists, whose primary focus is usually phylogeny reconstruction and macroevolutionary pattern. Elsewhere, my colleagues and I have discussed the history of and reasons for the gulf between population genetics and phylogenetic systematics (Avise et al., 1987); the gap has begun to narrow only in recent years (Felsenstein, 1982; Nei, 1987). There is irony in this state of affairs, because the branches in macroevolutionary trees have a substructure that consists of ever smaller branches and twigs which are ultimately resolved as generation-to-generation pedigrees through which genes have been transmitted. Thus, it would seem that considerations of gene history should provide a logical starting point for attempts to connect understanding of heredity with phylogeny and, thereby, to connect understanding of microevolution with macroevolution.

Some population biologists have gone so

[1] The substance of this paper was presented in the President's Symposium, "Phylogeny and Evolutionary Processes," organized by D. J. Futuyma for the annual meeting of the Society for the Study of Evolution, Asilomar, California, June 5–8, 1988.

far as to argue that, since evolutionary history can never be known with certainty, it should be largely discredited in the analysis of contemporary populations. For example, Birch and Ehrlich (1967 pp. 349, 351) state that "to investigate ecology and taxonomy through a series of inferences about the past is to base these sciences on non-falsifiable hypotheses," and "phylogenetic speculation(s) . . . do not help us to understand the distribution and abundance of plants and butterflies today, because they are not subject to testing." While I agree that great care should be exercised in phylogeny reconstruction (as in any scientific enterprise), evolution is ineluctably an historical process. Genes and populations, as well as species, do have histories, and to neglect historical considerations could also lead to erroneous conclusions.

For example, from a direct observation of limited contemporary dispersal in certain species of butterflies, Ehrlich and Raven (1969 p. 1230) conclude that "populations in Alaska are only slightly differentiated from those isolated in Colorado, indeed from those in Europe. Yet we would be greatly surprised if the Colorado populations (occurring as scattered isolates) receive a gene originating in Alaska once per hundred millennia." However, for extended periods in the last 100,000 years, massive glaciers covered much of the northern U.S. and Canada (Brown and Gibson, 1983). While it is possible that some butterfly populations survived in Alaskan nunataks (unglaciated refugia within an ice sheet), it is also possible that not only has gene flow occurred on a broad geographic scale, but that entire regional populations derive from extensive recolonizations after the retreat of the Wisconsin glaciers only some 18,000 years ago. Slatkin (1987 p. 791) makes clear the important distinction between contemporary and historical gene flow and concludes from a reanalysis of Ehrlich and coworkers' genetic data on butterflies (McKechnie et al., 1975) that "the current patterns are probably due to substantial gene flow in the recent past . . . due to the large-scale movement . . . permitted by unusual environmental conditions or major range expansions. . . ."

Whatever the outcome of this particular debate, it seems clear that the historical demographies of species must have left impressions on contemporary genome structure. The challenge is to use the clues of genome architecture to reconstruct historical events correctly. Traditionally, such attempts at the within-species level have been limited primarily to comparisons of allele frequencies, such as determined by protein electrophoresis. Here, I will suggest ways that a more explicit focus on intraspecific phylogeny through a complementary approach—*gene lineage analysis*—may further enrich understanding of population biology, as well as provide a continuous frame of reference for a hierarchy of evolutionary problems, ranging from concern with molecular mechanism to macroevolutionary pattern and including adaptation.

Gene Trees Versus Organismal Trees

There is an increasing awareness of the fundamental distinction between gene trees and population or species trees (Tateno et al., 1982; Tajima, 1983; Wilson et al., 1985; Neigel and Avise, 1986; Nei, 1987; Pamilo and Nei, 1988). A gene tree is the phylogeny of a particular gene or stretch of DNA. It can be estimated from nucleotide or amino-acid sequences, restriction-site maps, or other procedures which view the alleles themselves as the operational taxonomic units (OTU's). A population tree is the evolutionary pathway of a group of populations; populations or species are the OTU's. There are many gene trees within any population or species tree, and indeed, a population tree must in some sense represent a compilation of genealogies for many genes. Nonetheless, the topology of a given estimated gene tree can differ from that of the overall population tree due to: a) sampling error attributable to a small number of nucleotide or amino acids examined (Saitou and Nei, 1986); b) evolutionary rate heterogeneity across gene or organismal lineages; c) stochastic sorting of allelic lineages to daughter populations from a polymorphic ancestral population (Tajima, 1983; Neigel and Avise, 1986; Pamilo and Nei, 1988); and d) introgressive hybridization (e.g., Ferris et al., 1983*a*). The last three possibilities are not simply generators of "noise" in phylogeny estimation; rather,

TABLE 1. Selected characteristics of the conventional data provided by various molecular phylogenetic assays.

Molecular assay	Single-gene versus multiple-gene information	Raw data	Allelic states	Usual level of application
Protein assays				
a) Multilocus protein electrophoresis	multiple	qualitative genotypes[1]	unordered	conspecifics and close species
b) Protein immunology (e.g., complement fixation)	single	phenetic distance	unknown	intermediate and higher taxa
c) Amino-acid sequencing	single	qualitative haplotypes[1]	ordered	intermediate and higher taxa
DNA assays				
a) DNA/DNA hybridization	multiple	phenetic distance	unknown	intermediate and higher taxa
b) Nuclear RFLP analysis (random probes)[2]	multiple	qualitative genotypes	unordered	conspecifics and close species
c) Restriction mapping, or nucleotide sequencing (after cloning or PCR amplification) of:				
i) Nuclear DNA	single	qualitative haplotypes	ordered (complications due to recombination)	potentially, any level; most often, higher taxa
ii) Mitochondrial DNA	single	qualitative haplotypes	ordered	conspecifics and close species

[1] Qualitative genotype or haplotype data can be converted to phenetic-distance estimates, if so desired, but the converse is not true (Avise, 1983).
[2] Use of several single-copy nuclear-DNA probes to reveal restriction-fragment length polymorphisms.

they are real phenomena, and a true and important part of phylogenetic history.

Various empirical issues are intertwined with these conceptual distinctions between gene trees and species trees (Table 1). Until recently, allozyme products of nuclear genes provided the major source of molecular genetic data at the intraspecific level. Allozymes of a given locus are distinguished by charge differences, but the evolutionary relationships (gene trees) of the alleles thus identified cannot be safely inferred from the observable property, electrophoretic mobility. Thus, it has been customary and, in most cases, necessary to restrict concern to allozyme frequencies, which represent composite attributes of assemblages of individuals. Composite attributes of assemblages of genes are reflected in estimates of genetic divergence derived from certain other molecular approaches, such as multilocus protein electrophoresis or DNA/DNA hybridization; but again, information about particular gene phylogenies is missing. On the other hand, molecular assays such as amino-acid or nucleotide sequencing, which do provide information relevant to construction of allelic genealogies, have previously been applied almost exclusively to the estimation of relationships among higher taxa (Dayhoff, 1972; Woese, 1981; Hori and Osawa, 1987), where the issue of intraspecific polymorphism can be safely neglected.

In recent years, with the advent of mtDNA assay techniques (which also have been applied to a limited extent to nuclear genes), it has become feasible to estimate gene trees even at the intraspecific level. The relevance of microevolutionary allelic trees (both mitochondrial and nuclear) to population biology is the subject of this report.

Mitochondrial DNA and Intraspecific Gene Phylogeny

Two major factors explain why mtDNA has been by far the most extensively exploited molecule for microevolutionary gene-lineage analysis in higher animals (Brown, 1985; Wilson et al., 1985; Avise, 1986): a) it evolves very rapidly, primarily through base substitutions (Brown et al., 1979); and b) it is cytoplasmically housed, maternally inherited, and effectively haploid in transmission across generations.

These latter attributes mean that individual organisms (as well as mtDNA alleles or haplotypes) can justifiably be considered as the OTU's in a phylogeny reconstruction, which is then interpreted as an estimate of a matriarchal tree (Avise et al., 1979; Lansman et al., 1981). From a functional perspective, mtDNA is composed of 37–38 genes or coding regions; but from a phylogenetic perspective, because mtDNA is nonrecombining, the entire 16–20-kilobase molecule represents one genealogical unit.

Within several assayed vertebrate groups, this mtDNA genealogical unit is reported to evolve at a mean rate of roughly one percent sequence divergence per lineage per million years (Brown et al., 1979; Ferris et al., 1983*b*; Higuchi et al., 1984; Wilson et al., 1985; Shields and Wilson, 1987). Beyond about 5–10 million years, however, the overall rate at which related mtDNA lines accumulate differences from one another gradually declines, as the faster-evolving positions in the genome become saturated with nucleotide substitutions (Brown et al., 1979). Base changes accumulate most rapidly in the D-loop (or control) region and most slowly in the tRNA and rRNA genes (Brown, 1985). Whether the mtDNA rate calibrations and patterns cited above apply to all vertebrate groups and to invertebrates are topics currently under debate (Brown and Simpson, 1982; Cann et al., 1984; Powell et al., 1986; Solignac et al., 1986; Vawter and Brown, 1986; DeSalle et al., 1987). In any event, it is abundantly clear that most species of higher animals harbor a wealth of intraspecific mtDNA polymorphism that can be tapped (most easily by restriction-endonuclease techniques) to reveal relationships among mtDNA alleles or haplotypes (Avise and Lansman, 1983; Wilson et al., 1985; Avise, 1986).

A mitochondrial-DNA phylogeny consists of the proposed historical relationships among haplotypes deduced from the numbers and sequences of mutational changes in the molecule. One common approach is to compile restriction maps or digestion profiles from many endonucleases, each of which can be considered as a character whose states often interrelate in a clear manner (Fig. 1). An example of a composite phylogenetic network for mtDNA of the spotted sunfish (*Lepomis punctatus*), based on information from 12 endonucleases, is shown in Figure 2. The restriction data can also be converted to estimates of nucleotide sequence divergence between mtDNA haplotypes (Upholt, 1977; Nei and Li, 1979) and interpreted against the mtDNA "clock" calibrations cited above. Of course, numerous algorithms exist for constructing trees or networks from qualitative and quantitative mtDNA data bases, but these various alternatives will not be addressed here.

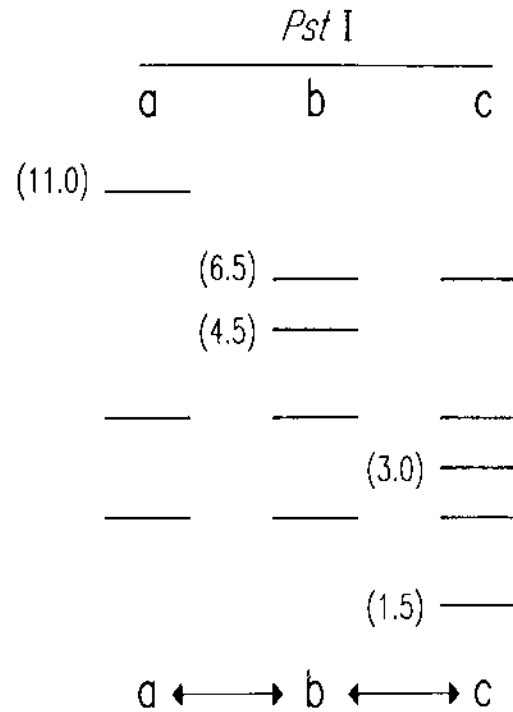

FIG. 1. *Pst* I digestion profiles observed in the mtDNA of spotted sunfish, *Lepomis punctatus* (from Bermingham [1986]). Consideration of mtDNA fragment sizes (relevant examples of which are indicated in parentheses; in kilobases) strongly indicates that the three haplotypes are genetically interrelated as shown at the bottom of the figure. For example, gain of a *Pst* I site in the 4.5-kilobase fragment in b could produce the 3.0-and 1.5-kilobase fragments in c.

Two major aspects of intraspecific mtDNA variability are of interest: a) the magnitude and pattern of phylogenetic differentiation among the mtDNA haplotypes themselves and b) the geographic distributions of the mtDNA phylogenetic groupings or clades. Together, these aspects constitute

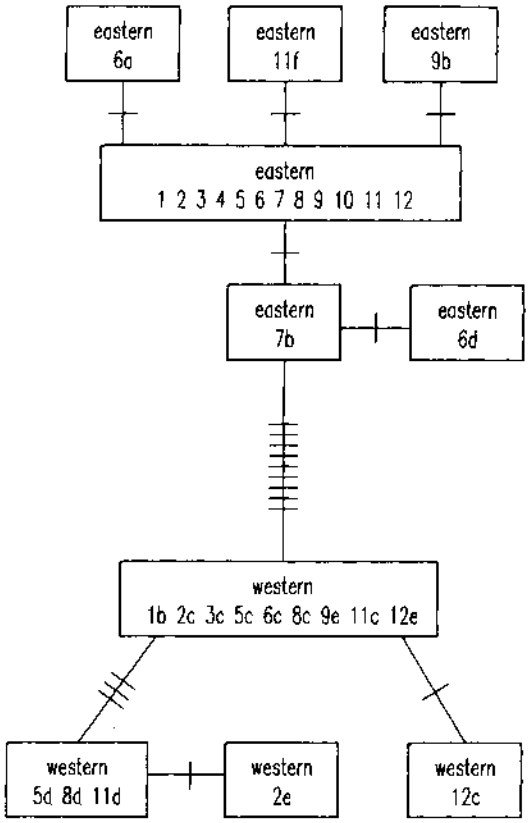

FIG. 2. Subset of the mtDNA phylogeny in the spotted sunfish, *Lepomis punctatus* (from data in Bermingham and Avise [1986]). One clonal genotype in the eastern range of the species serves as an arbitrary reference in which the numbers 1–12 refer to the standard mtDNA digestion profiles revealed by 12 respective endonucleases. Each digestion-profile change (usually resulting from a single restriction-site gain or loss) is represented by a lowercase letter. The sequence of mutational changes can be read cumulatively. For example, eastern genotype 6d differs from the standard by single restriction-site changes for *Hin*d III (enzyme number 6) and *Pst* I (enzyme number 7). Nine profile changes distinguish eastern genotype 7b from its closest relative in the western range of the species. Lines crossing branches of the network indicate numbers of digestion-profile changes along a path.

concerns of a broader discipline that my colleagues and I have termed *intraspecific phylogeography* (Avise et al., 1987). In principle, the magnitude of mtDNA phylogenetic divergence and the degree of spatial structure are independent variables among species, such that they can be plotted on orthogonal axes, as in Figure 3. Thus, for a given species, intraspecific nucleotide-sequence divergence between mtDNA haplotypes could be great, and the mtDNA clades could either be strongly partitioned geographically (phylogeographic category I of Avise et al. [1987]) or weakly partitioned geographically (i.e., mtDNA clades geographically widespread and in similar frequencies among populations; phylogeographic category II of Avise et al. [1987]). Conversely, sequence divergence between haplotypes could be small, and the mtDNA clades could either be strongly or weakly structured in geographic space (phylogeographic categories III and IV of Avise et al. [1987], respectively).

Empirical data on intraspecific mtDNA phylogeography, currently available for more than 20 species, have recently been summarized by Avise et al. (1987). Major features of these data are reviewed in Figure 3. Nine assayed species fall into phylogeographic category I, where mean mtDNA sequence divergence between mtDNA clades is great (more than about 2%), and the clades are geographically distinct, such that regional populations of a species belong to separate main trunks of the intraspecific mtDNA phylogeny. For example, there are two main branches in the mtDNA network for the spotted sunfish (*Lepomis punctatus*; Fig. 2) that differ by at least nine assayed mtDNA mutation steps and an estimated 4.4% sequence divergence (after correction for within-region clonal divergence; Bermingham and Avise, 1986). Representatives of one branch of the phylogeny appeared to be confined to drainages east of the Apalachicola River in the southeastern U.S., while members of the other phylogenetic branch were observed in all assayed fish from the Apalachicola west to Louisiana.

Several assayed species fall into phylogeographic category III (Fig. 3), where mean mtDNA sequence divergence is relatively low (less than about 1%), yet the mtDNA clades (which are now more weakly defined) remain geographically separate. A good example appears to involve the diamondback terrapin (*Malaclemys terrapin*), in which two mtDNA haplotypes, differing at a single assayed restriction site and with an approximate 0.2% sequence divergence, characterize populations in salt marshes north and

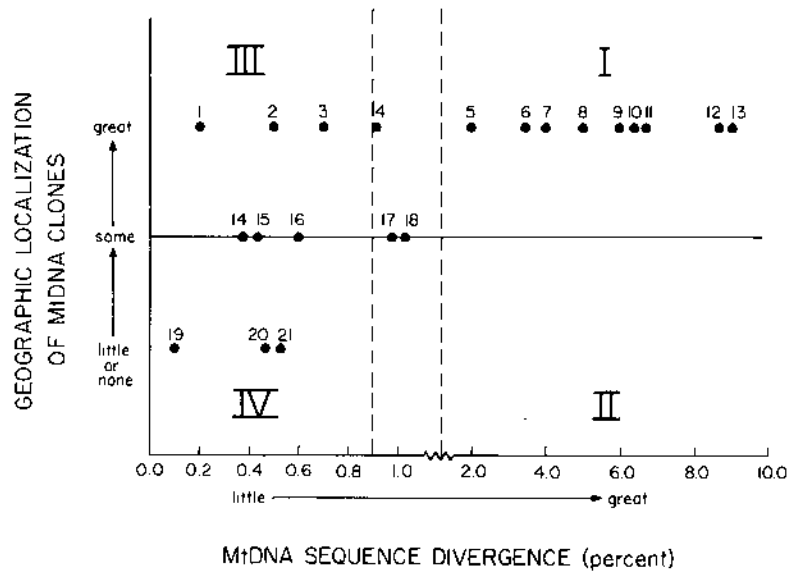

FIG. 3. Empirical relationship between the magnitude of mtDNA sequence divergence and the extent of geographic partitioning among mtDNA clades within each of 21 species of higher animals: 1) *Malaclemys terrapin* (diamondback terrapin); 2) *Opsanus tau* (toadfish); 3) *Trachemys scripta* (slider turtle); 4) *Amia calva* (bowfin fish); 5) *Limulus polyphemus* (horseshoe crab); 6) *Geomys pinetis* (pocket gopher); 7) *Peromyscus maniculatus* (deer mouse); 8) *Xerobates* (formerly *Scaptochelys*) *agassizi* (desert tortoise); 9) *Necturus* sp. (mudpuppy); 10) *Lepomis punctatus* (spotted sunfish); 11) *L. gulosus* (warmouth sunfish); 12) *L. macrochirus* (bluegill sunfish); 13) *L. microlophus* (redear sunfish); 14) *Opsanus beta* (toadfish); 15) *Agelaius phoeniceus* (Red-winged Blackbird); 16) *Homo sapiens*; 17) *Bufo terrestris* (southern toad); 18) *Peromyscus polionotus* (beach mouse); 19) *Anguilla rostrata* (American eel); 20) *Arius felis* (hardhead catfish); and 21) *Bagre marinus* (gafftopsail catfish). Data for *Homo sapiens* are from Cann et al. (1987). All other data are from my lab and are reviewed in Avise et al. (1987). Roman numerals I–IV refer to phylogeographic categories (see text). Notice a change in scale midway along the horizontal axis.

south of a region in northeastern Florida (Lamb and Avise, unpubl.). Some species exhibiting phylogeographic pattern IV have also been observed (Fig. 3). Here, sequence differences between mtDNA genotypes and clades are small, and geographic populations show little or no evidence of significant haplotype-frequency differences. The best available example involves the American eel (*Anguilla rostrata*), a catadromous species whose peculiar life history has the effect of providing extensive gene flow between geographic areas (Avise et al., 1986).

Since the positions of dividing lines between the phylogeographic categories in Figure 3 are to some extent arbitrary, some species may be expected to fall into gray areas in the classification scheme of population structure. For example, humans on a global scale (Cann et al., 1987), and Red-winged Blackbirds on a continental scale (Ball et al., 1988) both exhibit low mtDNA sequence divergence, and "some" geographic localization of haplotypes, that is, significant but not fixed haplotype-frequency differences distinguishing some regional populations. Of special interest in Figure 3 is the fact that no species as yet has been reported to show high intraspecific mtDNA sequence divergence in the absence of geographic localization of the mtDNA clones or clades (phylogeographic category II).

To interpret the phylogeographic patterns summarized in Figure 3 in terms of the demographic histories of species, various kinds of probability models addressing allelic relationships within and between populations are relevant (Cannings and Thompson, 1981; Kingman, 1982; Tavaré, 1984; Watterson, 1985). Consider, for example, the expectation for the mean probability distribution of times to identity-by-descent for pairs of selectively neutral mitochondrial DNA haplotypes within idealized random-

mating populations or within entire species characterized by very high gene flow. This can be obtained from a slight modification of conventional inbreeding theory. Suppose that in each generation G, females contribute to a large gamete pool from which a random draw of size N produces daughters. A randomly chosen pair of daughters then share a common mother with probability $1/N$, which is also the probability (F) that mtDNA haplotypes are identical by descent from the prior generation. Mitochondrial-DNA alleles could also be identical by descent through replication in prior generations. Thus F accumulates through time, eventually reaches 1.0, and in any generation is given by

$$F_G = \frac{1}{N} + \left(1 - \frac{1}{N}\right)F_{G-1}. \qquad (1)$$

The probability distribution of times to common ancestry, which can be generated from Equation (1), is geometric with mean N and variance $N(N - 1)$. In such idealized populations, it is assumed that constant numbers of breeding females produce random numbers of daughters and are replaced in each generation, such that the distribution of family sizes is well approximated by the Poisson distribution with mean 1.0. In practice, for comparisons with real populations in which these assumptions are unlikely to hold exactly, N in Equation (1) can be replaced by N_e, the evolutionary effective population size of females.

Times to common ancestors of different pairs of haplotypes from a single gene (such as mtDNA) are correlated due to coancestry in the population pedigree. Hence, the theory outlined above, which assumes genealogical independence among haplotypes, does not strictly apply for particular genealogies of genes within an organismal pedigree. Nonetheless, Ball et al. (1990) have recently shown that these theoretical expectations do hold reasonably well and that they can therefore be used to generate expectations for haplotype relationships to a first approximation.

Avise et al. (1988) have recently compared the current census breeding size (N) to the evolutionary effective population size (N_e) for each of three vertebrate species (American eels, hardhead catfish, Red-winged Blackbirds) in which both genetic and life-history data suggest historically high gene flow, such that the entire species is the relevant unit of analysis. Effective population size in each species was estimated from conventional mtDNA clock calibrations (see above) as applied under inbreeding theory to observed distributions of nucleotide-sequence differences among mtDNA haplotypes. In all cases, present-day census sizes proved to be much larger (by 100–1,000-fold) than inferred N_e's, indicating that mtDNA genotypes in these currently abundant species have been channeled through far fewer ancestors than might have been anticipated. An example involving the American eel is shown in Figure 4.

In fact, for any abundant species with high gene flow, the theoretical expectation is for many mtDNA haplotypes to be highly divergent genetically, provided evolutionary effective population sizes have also been large (Fig. 4). For example, in an annual species with $N_e = 5{,}000{,}000$ (not an unreasonable guess for the current breeding population sizes of eels, catfish, or Red-winged Blackbirds), mean time of separation of randomly drawn mtDNA alleles should be about 5,000,000 years, which translates under the conventional mtDNA clock calibration to $p \simeq 0.10$ base substitutions per nucleotide. However, as already mentioned, there are as yet no examples in the literature (apart from secondary hybrid zones [Avise et al., 1984*a*]) of panmictic populations or of quasipanmictic species exhibiting large (e.g., $p \gg 0.01$) mtDNA phylogenetic gaps (phylogeographic category II; Fig. 3). Thus, if one can generalize from available mtDNA data for species with large population sizes and high rates of gene exchange, N_e commonly appears to be vastly smaller than N. Possible explanations (not mutually exclusive) for this result include: 1) high variances in progeny survival among females; 2) intense selection resulting in the fixation of one or more advantageous mutants in the recent past; 3) dramatic fluctuations in population size, perhaps including very recent expansions to current levels; and 4) any other related demographic factors that have the

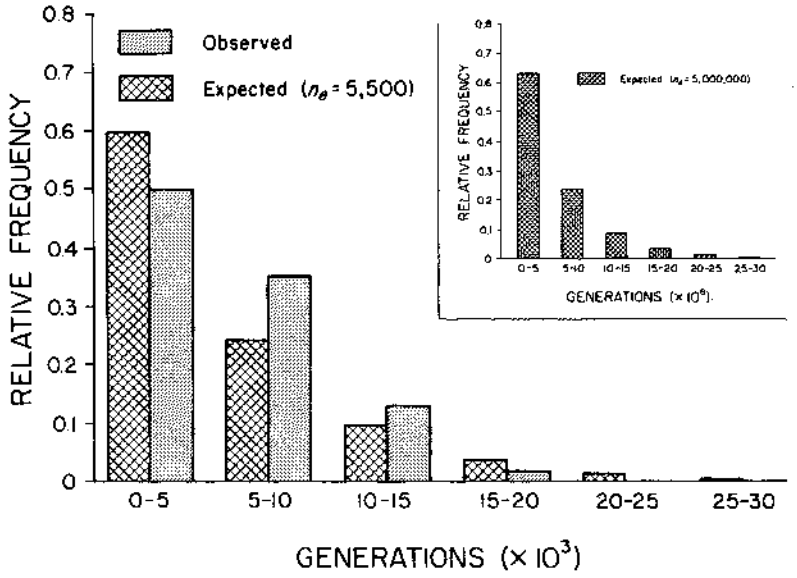

FIG. 4. Observed and expected frequency distributions of times to common ancestry for mtDNA haplotypes in the American eel, *Anguilla rostrata*. Expected times, generated from inbreeding theory (see text) are given for each of two conditions: 1) N_e = 5,000,000 (a rough estimate of the current breeding population of female eels; shown in the inset); and 2) N_e = 5,500 (a number which yields a good fit of expected mtDNA allelic distances to those actually observed; shown in the main graph). Observed times were derived from the data of Avise et al. (1986), using the mtDNA clock calibration of Brown et al. (1979). See Avise et al. (1988) for additional details.

effect of depressing the numbers of female ancestors that have contributed to the current gene pool through female lines.

Another class of useful probability models in the analysis of gene lineage relationships involves application of generating functions to distributions of family size in branching-process theory (Harris, 1963). If, for example, females again produce daughters according to a Poisson distribution with mean $\mu = 1.0$, the probability of random loss of any female line in one generation is $e^{-1} = 0.37$, or in general, $e^{-\mu}$. The probability of loss of a line after G generations is given by the generating function $P_G = e^{\mu(x-1)}$, where x is the probability of loss in the prior generation (Spiess, 1977). Avise et al. (1984*b*) used generating functions to examine the expected fates of neutral mtDNA alleles within populations as a function of demographic conditions such as population size and offspring distribution; results showed that random lineage extinction is a surprisingly important and usually rapid process. For example, for a stable-sized population initiated by N females or density-regulated at a carrying capacity $K = N$, after approximately N generations the probability is nearly 50% that all mtDNA alleles will trace back to one founder haplotype. Such alleles would then have a monophyletic origin within that time span. Generating functions are also available for family-size distributions other than the Poisson, such as the negative binomial. A general result is that, for any mean number of progeny, as the variance in progeny survival across families increases, the rate of mtDNA lineage extinction also rises, and the time to population monophyly decreases.

The extinction of mtDNA (or other allelic) lineages under branching-process theory is stochastic but, nonetheless, an inevitable consequence of population turnover through reproduction. Thus, gene trees within a population are continually "self-pruning," with the net effect of continually truncating the frequency distribution of times to common mtDNA ancestry. The limited mtDNA sequence divergence ob-

served within most local populations or within entire species characterized by high gene flow (Fig. 3), is likely to be attributable in large part to this process.

However, many species are probably comprised of geographic populations behaving as quasi-independent or completely independent demographic units because of severe historical restrictions on gene flow. For any such species considered as a whole, the above theory for individual populations does not apply directly and must be modified to take into account the fact that the times of separation of mtDNA lineages among completely isolated populations could be no less than the times since separation of the populations themselves. Furthermore, for any two populations that have separated recently, it is quite likely that some individuals in population A are more closely related in the mtDNA haplotype phylogeny to certain members of population B than they are to other members of A, due solely to stochastic sorting from the ancestral pool of lineages (Tajima, 1983; Neigel and Avise, 1986; Pamilo and Nei, 1988).

Using computer simulations in an extension of the branching-process theory outlined above, Neigel and Avise (1986) examined probabilities of various phylogenetic relationships (reciprocal monophyly, paraphyly, and polyphyly) of mtDNA lineages between two isolated populations descended from common ancestral stock. Outcomes were highly dependent upon the particular demographic conditions specified, but in general, populations tended to evolve to a phylogenetic status of reciprocal monophyly within at most about $4N$ generations of isolation. At times less than that, populations were commonly paraphyletic or polyphyletic with respect to the mtDNA haplotype lineages segregating within them.

Empirical data have demonstrated that local and regional populations within many assayed species occupy separate identifiable twigs and branches, respectively, in an intraspecific mtDNA phylogenetic tree (phylogeographic categories III and I in Fig. 3; see Avise et al., 1987). According to theory, such populations have likely been isolated from one another for more than at least N_e generations. For sedentary species with limited gene flow (such as the pocket gopher, *Geomys pinetis* [Avise et al., 1979]), genetic drift and population bottlenecking at a very local level probably yield quite small effective population sizes, such that mtDNA lineages become quickly but transiently fixed on a fine geographic scale. An extreme example is the geographic clustering and localization expected for individual family groups, some of which are detectable in conventional mtDNA assays. Over longer periods of time, however, many of these twigs of the tree are lost while others increase in frequency and geographic range, such that the kaleidoscope of local genetic differences is continually shifting.

On a broader geographic scale and time, local populations that were historically connected by gene flow group into more extended branches of the mtDNA phylogenetic tree. Not infrequently, populations over large geographic regions occupy phylogenetic branches highly distinct from other such regional assemblages (category I; Fig. 3). The separation of such major branches probably provides evidence of long-term zoogeographic barriers to gene flow, allowing accumulation of haplotype differences greatly exceeding those observed within local populations or regions. In Figure 3, species in phylogeographic category I all have between-region mtDNA differences greatly exceeding any known mean mtDNA distances within local populations or even within entire panmictic species. To return to the spotted sunfish example (Fig. 2; Bermingham and Avise, 1986), the mean mtDNA genetic divergence between phylogeographic assemblages ($\bar{p} = 0.062$) was considerably greater than the mean ($\bar{p} = 0.018$) or even maximum ($\bar{p}_m = 0.042$) genetic distance observed within a geographic clade. Using mean within-region genetic distance as a correction factor for the between-region differences, the mtDNA sequence divergence estimated to postdate regional population separation in *Lepomis punctatus* becomes $p = 0.044$, which according to the conventional mtDNA "clock" suggests that the populations diverged more than 2,000,000 years ago.

Gene Phylogeny and Historical Population Demography

The overall picture to emerge from the numerous mtDNA studies of intraspecific phylogeography is one of dramatic dyna-

mism and flux of mtDNA lineages. Many species exhibit well-defined local structure with respect to mtDNA genotypes, suggesting that there are restrictions on gene flow and relatively small effective population sizes. On a regional geographic scale, the common presence of major mtDNA phylogenetic gaps indicates the profound effects of zoogeographic barriers in shaping extended pedigrees. Even for those species that are currently large and panmictic or quasi-panmictic, the time-depths of mtDNA lineage separations suggest evolutionary N_e's that are vastly smaller than current N's, perhaps because such species have fluctuated greatly in population size in the recent evolutionary past. Taken altogether, the results of the mtDNA lineage analyses imply that population demographies of many species have been unsettled and dynamic over time and space.

Is this picture of capriciousness in historical demography correct? Admittedly, for any of the species assayed, particular inferences about the demographic past were necessarily based on patterns of mtDNA lineage relationships in extant populations. However, it is a very simple matter to cite contemporary, directly observed examples of the qualitative kinds of irregular demographic events that we propose to have occurred in the evolutionary histories of many if not most species. The following few examples are from my own experience, or are well-documented cases in the literature; they are included here merely to convey an impression of some of the extreme sorts of demographic occurrences that may profoundly influence the structure of intraspecific gene phylogenies at both local and regional geographic scales.

Extinction or near-extinction of local populations is probably a common occurrence in many species, due to changes in climate and physical habitat or to biotic factors such as competition and disease. This point was forcefully driven home to me in August of 1980, when during a detailed study of microgeographic clonal diversity and recruitment in staghorn coral (*Acropora cervicornis*) at Discovery Bay, Jamaica (Neigel and Avise, 1983), the entire population (and associated forereef environment) was devastated by Hurricane Allen (Woodley et al., 1981). Other local *Acropora* populations are also known to have been decimated by hurricanes and cold fronts (Shinn, 1972). At any given reef locale, such major physical disturbances may well occur, on average, once every 100 or 1,000 years, well within time-scales relevant to influencing intraspecific genetic architectures.

Such extinctions need not be confined to small areas. For example, prior to 1983, the black sea urchin (*Diadema antillarum*) was one of the most abundant invertebrates on reefs throughout the Caribbean, sometimes reaching densities of 70 individuals per square meter. Within one year, mass mortality, apparently due to a virulent and species-specific waterborne pathogen, reduced population densities throughout the 3.5 million-square-kilometer area of the Caribbean to about one percent of former levels (Lessios et al., 1984). (Devastating disease outbreaks probably afflict a great many species over their evolutionary lifespans [O'Brien and Evermann, 1988].) Palumbi and Wilson (pers. comm.) have recently surveyed mtDNA variability in other sea urchin species (genus *Strongylocentrotus*) and, from an analysis similar to that discussed earlier in this paper for eels, catfish, and Red-winged Blackbirds, concluded that the observed sequence diversity was "... too low to be explained by a simple application of the neutral theory of genetic change, [suggesting] either that mtDNA variants have been under strong selection in the recent past, or that sea urchin population sizes have undergone dramatic fluctuations ...." Whether genotype-specific selection per se or population-size variation is the responsible factor in any of these instances will be difficult to determine, because both processes would have the net effect of channeling mtDNA lineages through fewer females and hence of reducing apparent N_e.

In any event, several other cases of mass mortality of marine species are known (e.g., Rasmussen, 1977; Lessios et al., 1983; Castilla, 1988). For example, the recent climatic changes associated with "El Niño" have had dramatic effects on many Pacific species (Robinson, 1987). Since physical environments in lakes and on land are usually even more variable than those in the ocean, freshwater and terrestrial species are probably at least as susceptible to dramatic population size reductions.

Conversely, dramatic population and range expansions are also certainly frequent enough, relative to the mean evolutionary lifespans of many species, to play a very significant role in shaping the intraspecific structures of gene lineages. The comparatively minor mtDNA haplotype divergences among humans on a global scale (Cann et al., 1987) and Red-winged Blackbirds across North America (Ball et al., 1988) may be examples of the expected consequences of extensive population expansions and movements in late Pleistocene and Recent times. The effects of such range expansions may of course also be exhibited at other geographic scales. For example, Bermingham and Avise (1986) propose that the regional uniformity of mtDNA haplotypes in *Lepomis punctatus* and other fish species in the southeastern U.S., relative to the magnitude of between-region haplotype differences, reflects zoogeographically constrained dispersal out of disjunct Pliocene or Pleistocene refugia.

Again, these particular inferences are based on present-day distributions of mtDNA lineages, but innumerable examples of directly observed range expansions in other species could be cited. For example, the cattle egret (*Bubulcus ibis*) is now the most plentiful egret in North America, but the continent was colonized only 30 years ago by as few as 14 immigrants to Florida, who themselves were descendents from 19th Century colonists of South or Central America from native African stock (Welty, 1982). Many such range expansions in birds are well-documented, such as the dramatic spread since 1942 of house finches (*Carpodacus mexicanus*) in eastern North America and the occupations of North America within the last century by the house sparrow (*Passer domesticus*) and European starling (*Sturnus vulgaris*) (Robbins et al., 1986). Birds typically have high dispersal capabilities, but even species with much more limited mobility unquestionably experience major shifts in geographic range through time. The past two million years, in particular, were a time of exceptional climatic instability, as the direct and indirect effects of glacial advances and retreats altered species' distributions in low as well as high latitudes (Blair, 1958; Selander, 1965; Wright and Frey, 1965; Martin and Wright, 1967; Haffer, 1969; Hocutt and Wiley, 1985).

The point of this argumentation is that much evidence points to the dominant role of historical demographic and zoogeographic factors in shaping intraspecific gene phylogenies. Direct observations of contemporary populations provide numerous examples of impressive fluctuations in population size and geographic distribution; climatic and geologic changes over recent evolutionary time have undeniably had major impact on species' abundances and distributions; and available data on the phylogeographic patterns of mtDNA genotypes in many species are consistent with the interpretation of historical demographic influence. Continued explorations of intraspecific gene phylogenies (in conjunction with the more traditional study of population trees through allele-frequency analyses) offer a realistic hope for reconstructing the historical events that have necessarily influenced genetic structure in contemporary populations of any species.

Intraspecific Phylogenies of Nuclear Gene Systems

Surveys of mtDNA have provided most of the available data on intraspecific gene trees. What are the prospects for development of analogous approaches for the phylogenetic assessment of nuclear alleles and gene systems?

Chromosome Inversions.—Carson (1983, 1987) has examined the relationships of 103 species of Hawaiian "picture-winged" *Drosophila* from the perspective of chromosome-inversion phylogeny. To date, 214 gene orders have been observed. Because any change of gene order involves two simultaneous chromosome breaks, the probability seems high that each inversion records a unique historical event which is especially useful in clade delineation.

Figure 5 diagrams a subset of the chromosome-inversion phylogeny in a format that facilitates comparison with the properties of an mtDNA phylogeny (Fig. 2). In the inversion network, the chromosomes (labeled X and 2–6) can be considered as characters whose inversion states (lowercase letters) have evolved as indicated along

network branches. The mtDNA and inversion phylogenies appear to be remarkably alike in qualitative information content (compare Figs. 2 and 5), but the resemblance is partly superficial. As already indicated, an mtDNA phylogeny (Fig. 2) is an unequivocal example of a gene tree; however, the inversion phylogeny (Fig. 5) has an interesting mix of elements of a "gene tree" (or, in this case, a "chromosome tree") and a species tree. This is because inversions on different chromosomes (or on distant segments of one chromosome) segregate and assort independently of one another during meiosis and syngamy, so that the overall inversion phylogeny represents a summation of information across several nonlinked genealogical units. In this particular sense, such inversion data bear some similarity to multilocus protein-electrophoretic data, which also entail a compilation of information across nonlinked genetic units. But the inversion network is more like an mtDNA network in the sense that the qualitative character states observed within any genealogical unit can be ordered phylogenetically.

Nuclear Genes.—The same methods of restriction mapping employed in analyses of mtDNA can also be applied to particular segments of the nuclear genome. A technical complication in such studies relates to the fact that a preparation of nuclear DNA from any gene region of a diploid individual includes two haplotypes, whose nucleotide contents must be distinguished if the goal is to reconstruct allelic relationships. In some organisms such as *Drosophila,* this problem can be overcome by using controlled breeding schemes to construct strains in which chromosomes are identical by descent. Perhaps the most comprehensive study to date of haplotype evolution at a nuclear locus involves the alcohol dehydrogenase (*Adh*) gene region in *Drosophila melanogaster* (Langley et al., 1982; Kreitman, 1983; Aquadro et al., 1986; Kreitman and Aguadé, 1986). It is instructive to consider this model work, because it illustrates the kinds of results and considerations that will be likely to apply to most such nuclear gene surveys.

Aquadro et al. (1986) surveyed restriction-map variation in 49 *D. melanogaster* lines (from four populations in the eastern U.S.) over a 13-kilobase region surrounding the *Adh* structural gene. Variation due to base substitutions and insertions/deletions (including those of transposable elements) accounted for 20 distinct haplotypes in their sample. Most of the variants showed significant nonrandom associations (linkage disequilibria), so that an attempt could be made to construct a phylogenetic tree (gene tree) summarizing the haplotype interrela-

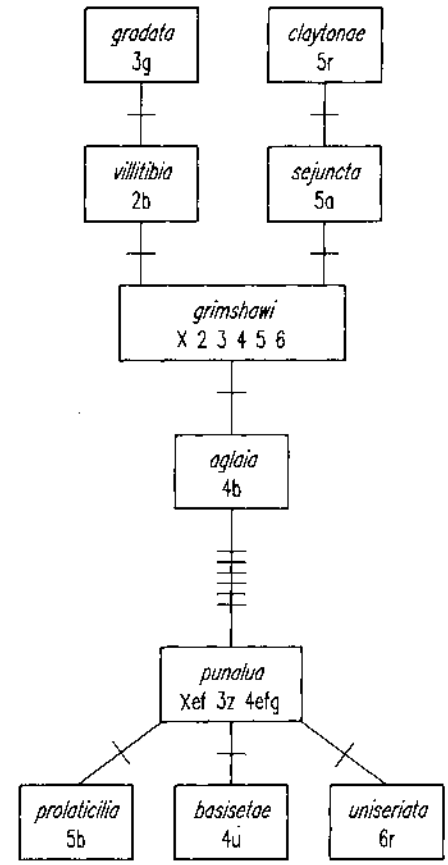

FIG. 5. Small subset of the chromosome-inversion phylogeny in Hawaiian picture-winged *Drosophila* (redrawn and recoded from Carson [1983, 1987]). The species *D. grimshawi* serves as an arbitrary reference in which the symbols X and 2–6 refer to the standard gene order on the X chromosome and the five autosomes. Each inversion is represented by a lowercase letter. The sequence of inversion changes in the network can be read cumulatively. For example, relative to the standard gene order, that in *D. gradata* (upper left) differs by inversion "g" in chromosome 3 and inversion "b" in chromosome 2. *D. punalua* differs from *D. aglaia* by six chromosomal inversions. Lines crossing branches of the network indicate numbers of inversion changes along a path.

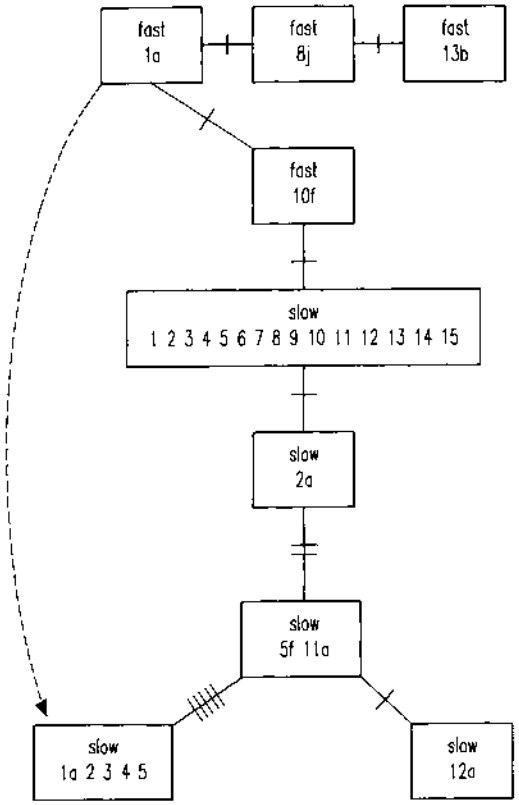

FIG. 6. Subset of the ADH haplotype phylogeny in *Drosophila melanogaster* (redrawn and recoded from Aquadro et al. [1986]). The haplotype labeled "slow" in the largest box serves as an arbitrary reference in which the numbers 1–15 refer to standard states of 15 characters (restriction sites, insertions, deletions, and the *Adh* allozyme), arrayed in the 5′ to 3′ direction. Each genetic variant from the reference is represented by a lowercase letter. "Slow" and "fast" refer to allozyme genotype (character 10). The sequence of genetic changes in the network can be read cumulatively. For example, haplotype "slow 5f 11a" differs from the reference in genetic states for characters 2, 5, and 11. Lines crossing branches of the network indicate numbers of character-state changes along a path. The haplotype at the lower left represents a probable recombinant between the two haplotypes to which it is connected by paths (see text).

tionships. A subset of this haplotype network is shown in Figure 6, in a format that facilitates comparisons with the mtDNA and chromosome-inversion networks (Figs. 2, 5).

Most of the interconversions among the *Adh* haplotypes could be accounted for by particular historical sequences of mutational events. However, in two or three cases, a given haplotype appeared to represent an amalgamation of pieces from unrelated haplotypes. Each such occurrence is best explained by a recombination (or gene conversion) event between divergent sequences. One example involves the haplotype in the lower-left corner of Figure 6, where the upstream (5′) segment of the molecule (characters 1a–5) is identical to the "fast 1a" genotype, and the downstream segment of the molecule (characters 6–15) is identical to the "slow 5f 11a" genotype. Such recombination or conversion phenomena are best distinguished from homoplasious events (arising from simultaneous parallel mutations) by the physical clustering of changes along the DNA molecule (Stephens, 1985; Aquadro et al., 1986; Templeton et al., 1987). Of course, if recombinational events had occurred more frequently in the recent evolutionary history of this gene region, reconstruction of the linear phylogenetic tracings of particular haplotypes (Fig. 6) would have been much more difficult and ambiguous.

In fact, preliminary indications are that the extensive disequilibria at *Adh* observed by Aquadro et al. (1986) may be the atypical situation in *Drosophila*. Similar restriction studies of the *Adh* region in *D. pseudoobscura* (Schaeffer et al., 1987) and of several other loci in *D. melanogaster* (e.g., Langley and Aquadro, 1987; Aguadé et al., 1989) have reported little or no disequilibrium, despite fairly extensive genetic variability. This has seriously compromised attempts to reconstruct evolutionary gene networks. The relatively high disequilibrium at *Adh* may reflect some unique evolutionary history for this gene region (see below), while the low disequilibrium yet high polymorphism at other loci perhaps indicates that population substructurings and size reductions have been minimal. However, I suspect that both *D. melanogaster* and *D. pseudoobscura* are unusual species in these latter regards, at least compared to many vertebrates (but see Hale and Singh [1987]). It would now be very interesting to gather similar nuclear DNA haplotype data for other organisms that are suspected to show greater geographic population structure (such as any of the species in phylogeographic category I in Fig. 3). Whether or not recom-

bination among nuclear haplotypes has shuffled allelic contents within a geographic region, disequilibria across regions (and the attendant possibilities for phylogeny reconstruction) might well be considerably greater for these species.

In any event, the *Adh* data for *D. melanogaster* also illustrate several ways in which the study of gene phylogeny can interface with the concerns of molecular biology and contribute to an understanding of molecular-level mechanisms and the basis of adaptation. For example, the "fast" and "slow" allozymes of *Adh*, which at the protein level differ only by a single amino-acid substitution, have long been the subject of intense scrutiny by molecular and evolutionary biologists because of their likely involvement in differential alcohol metabolism (review in Van Delden [1982]). Catalytic activity associated with the "slow" allozyme is usually much lower than that of the "fast" allozyme, and it was originally postulated that the amino-acid difference itself causes a major change in capacity to utilize and/or detoxify environmental alcohols. The phylogenetic analysis of *Adh* haplotypes (Fig. 6; see Aquadro et al. [1986] for the complete network) showed that the amino acids encoding the fast and slow allozymes occupy distinct phylogenetic branches in the *Adh* haplotype network and, hence, are in disequilibrium with other genetic markers in noncoding regions. On the basis of haplotype diversities and patterns of disequilibria within "slow" and "fast," several authors have concluded that "fast" is a derived condition having arisen from an ancestral "slow" allozyme within the last few thousand (Aquadro et al., 1986) or perhaps one million years (Ashburner et al., 1979; Stephens and Nei, 1985). Thus the fast-slow polymorphism is evolutionarily quite young, and selection acting on the "fast" haplotype probably has contributed to its rapid increase and spread. Furthermore, two recombinant haplotypes proved to exhibit the "wrong" activity for their allozyme (one instance is shown in Figure 6, where the "slow" allozyme haplotype in the lower left has ADH catalytic capacity that is normally characteristic of the "fast" allozyme). This strongly suggests that the genetic polymorphism(s) responsible for the high versus low activity profiles can be distinct from, but linked to, the nucleotide substitution underlying the amino-acid replacement (Aquadro et al., 1986).

Additional inferences about molecular process have been made through study of the phylogeny of *Adh* haplotypes. For example, from the observation that *Adh* haplotypes containing transposable elements are all located at the tips of tree branches (and from related considerations), Golding et al. (1986) concluded that such mobile elements are deleterious and selected against. The documentation of recombination (or conversion) in the history of the *Adh* region (Aquadro et al., 1986) is itself a conclusion about molecular process revealed by the phylogenetic analysis of haplotypes.

Study of the intraspecific phylogeny of nuclear haplotypes is an endeavor still in its infancy. It remains to be seen just how far such analyses can go in illuminating molecular-genetic processes, as well as in revealing the phylogeographic histories of populations.

Conclusions

Practitioners of any scientific discipline must decide what constitutes an adequate and satisfying level of understanding of natural phenomena. It is probably fair to say that systematists have set forth as a broad goal the elucidation of the phylogenetic and evolutionary histories of taxa; population geneticists are concerned with the processes responsible for gene-frequency changes within and among populations, and molecular geneticists are concerned with gene structure and function. A comprehensive understanding of evolution must include input from all of these (and other) perspectives. My aim in this paper has been to suggest ways that a relatively new kind of population data (on DNA haplotypes, both mitochondrial and nuclear) may provide empirical and conceptual connections between several disciplines. An expanded concern with this gene-phylogeny approach may accomplish several objectives. It may 1) enrich population biology and population genetics by adding the dimension of intraspecific evolutionary history (which has too often been neglected) to interpretations of contemporary population genetic structure;

2) provide feedback to molecular genetics by allowing deductions about molecular-level phenomena (such as recombination, or the molecular basis of particular adaptations); and 3) expand the sphere of systematics to include concern with microevolutionary phylogeny and with the intraspecific pedigrees which constitute the phylogenetic substructure of evolution.

ACKNOWLEDGMENTS

The author's research has been supported by grants from NSF.

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Corresponding Editor: D. J. Futuyma