Current versus Historical Population Sizes in Vertebrate Species with High Gene Flow: A Comparison Based on Mitochondrial DNA Lineages and Inbreeding Theory for Neutral Mutations[1]

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Using inbreeding theory as applied to neutral alleles inherited maternally, we generate expected probability distributions of times to identity by descent for random pairs of mitochondrial genotypes within a population or within an entire species characterized by high gene flow. For comparisons with these expectations, empirical distributions of times to most recent common ancestry were calculated (by conventional mtDNA clock calibrations) from mtDNA haplotype distances observed within each of three vertebrate species—American eels, hardhead catfish, and red-winged blackbirds. These species were chosen for analysis because census population size in each is currently large and because both genetic and life-history data are consistent with the postulate that historical gene flow within these species has been high. The observed molecular distances among mtDNA lineages were two to three orders of magnitude lower than predicted from census sizes of breeding females, suggesting that rate of mtDNA evolution is decelerated in these species and/or that long-term effective population size is vastly smaller than present-day population size. Several considerations point to the latter possibility as most likely. The genetic structure of any species is greatly influenced by historical demography; even for species that are currently abundant, mtDNA gene lineages appear to have been channeled through fairly small numbers of ancestors.

Introduction

In the study of molecular evolution, neutrality theory provides useful null hypotheses against which empirical experience may be evaluated. Important parameters in neutral models are effective population size (N_e) and mutation rate to neutral alleles (μ). For example, neutrality theory predicts that at equilibrium the effective number of neutral alleles per locus in a population is equal to $4N_e\mu + 1$ under an "infinite allele" model (Kimura and Crow 1964) and is given by $(8N_e\mu + 1)^{1/2}$ in a "stepwise-mutation" model (Ohta and Kimura 1973). Until recently, protein electrophoretic techniques provided the bulk of molecular data available *at the intraspecific level* to compare against these and other neutrality expectations (see, e.g., Ewens 1972; Ayala 1974; Soulé 1976; Nei 1983). Unfortunately, while protein electrophoresis yields information about numbers of alleles and their frequencies, the assay provides no direct measure of the mutational distance among different allelic types. An assay that also

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included information about the evolutionary relatedness among alleles would clearly be far richer in empirical content.

Newer techniques, including restriction-site mapping and nucleotide sequencing, have recently been employed to assess the magnitude and pattern of mutational differences among alleles at defined regions of DNA, such as the alcohol dehydrogenase locus in *Drosophila* (Kreitman 1983; Aquadro et al. 1986; Kreitman and Aquadé 1986; Schaeffer et al. 1987). A rich picture of molecular variability has emerged, including the documentation of common base substitutions, addition/deletion events, insertion of transposable elements, and nucleotide sequence alterations due to intragenic recombination or gene conversion (Stephens and Nei 1985; Aquadro et al. 1986). Some of these latter processes can, however, add complications to the interpretation of genetic distances and phylogenetic relationships among alleles of nuclear genes (Hudson and Kaplan 1985; Stephens 1985).

A simpler genetic system which appears to lack many of these complications is mitochondrial DNA (mtDNA). In higher animals, mtDNA is maternally transmitted, without recombination or conversion events between molecules from different female lines. Transposable or other moderately repetitive elements are unknown, and although addition/deletion events are not rare, they are usually greatly outnumbered by simple base substitutions which thus account for most of the mtDNA sequence differences among conspecific individuals. Several recent reviews summarize these and other molecular features of animal mtDNA (Brown 1981, 1983, 1985; Avise and Lansman 1983; Cann et al. 1984). There is a wealth of mtDNA genetic diversity within most species, and it has become common practice to estimate genetic distances and phylogenetic relationships among mtDNA "haplotypes" or "alleles" on the basis of restriction-fragment or restriction-site data (Wilson et al. 1985; Avise 1986; Avise et al. 1987*a*; Moritz et al. 1987).

Here we generate, under a neutral model, expected probability distributions of times to common ancestry of female lineages within a population or species. Several empirical mtDNA data sets will be compared with these expectations, and possible explanations for departures will be considered. We will be particularly concerned with the following question: do current census population sizes of extant species reliably predict observed frequency distributions of genetic distance among mtDNA alleles? If they do not, a likely explanation is that present-day population sizes are poor indicators of effective population sizes over recent evolutionary time.

Methods and Results

Theoretical Expectations

Since mitochondria are maternally inherited, we need only be concerned with demographic properties in a matriarchal phylogeny, which can be viewed as a history of reproductive success of various asexual, female lines within a species. Under simplifying assumptions, such as that the number of daughters produced by breeding females is Poisson distributed, several expectations about lineage ancestry—e.g., mean times to fixation or loss of newly arisen neutral mutations (Fisher 1930; Wright 1931; Crow and Kimura 1970), probabilities of survival of a given number of lineages through G generations (Avise et al. 1984), coalescence times at which all lineages converge to a single ancestor (Nei 1987), and times to reciprocal monophyly of two isolated populations (Neigel and Avise 1986)—have previously been addressed. Here we consider the probability distribution of times to common ancestry between a random pair

of individuals in a population by using a gene genealogy approach adapted from Tajima (1983).

Imagine an idealized population with nonoverlapping generations and a constant number N_f of breeding females. In each generation, these females contribute to a large mtDNA gamete pool from which N_f mtDNAs are drawn to form the female population in the next generation. The probability that two randomly chosen daughters share a mother is $1/N_f$. This is also the probability that the time to common ancestry of two randomly chosen mtDNAs is 1 generation ago ($G = 1$). The probability that the time to common ancestry is 2 generations ago ($G = 2$) can be derived in the following way: We first note that the probability that two randomly chosen mtDNAs are derived from different mothers is $1 - 1/N_f$. The probability that two randomly chosen mtDNAs come from two different females in the previous generation but from the same grandmother is therefore given by $(1 - 1/N_f)(1/N_f)$. It is now obvious that the probability $f(G)$ that two randomly chosen mtDNAs are derived from a common ancestor that existed G generations ago is

$$f(G) = (1/N_f)(1 - 1/N_f)^{G-1} \simeq (1/N_f)e^{-(G-1)/N_f}. \tag{1}$$

This is equivalent to Tajima's (1983) equation for the probability that two randomly chosen alleles at a nuclear gene are diverged from a common ancestor that existed t generations ago in a diploid population of size N (see also eq. 13.67 in Nei 1987). Indeed, the above equation can be derived by replacing $2N$ in his equation by N_f, and t by G.

Equation (1) gives the probability distribution of times to common ancestry in terms of the number of generations (G). The distribution is geometric with parameter $1/N_f$. Hence the mean time to common ancestry is approximately equal to N_f, with variance $N_f(N_f - 1)$.

The most important assumptions of the models, besides neutrality of mtDNA types, are the following: (*a*) in each generation there is a constant number of breeding females; (*b*) all females are replaced each generation; and (*c*) females attempt production of a large number of daughters but succeed with small probability. Assumption (*c*), in conjunction with (*a*) and (*b*) and a large N_f, implies a distribution of family sizes well approximated by the Poisson distribution, with mean (and variance) of 1.0 daughters/female.

Of course, no population in nature is likely to meet these assumptions. For example, there may often be large variances in progeny numbers, changes in population size through time, and overlapping generations. However, the impact of these factors on inbreeding is well-known via their influence on *effective population size* (N_e) (see, e.g., Gall 1987; Nei 1987). Thus, for any real population, if effective size of the female population is known or hypothesized, the expected times to common ancestry for mtDNA alleles can be obtained by substituting $N_{f(e)}$ for N_f in equation (1). Using this equation in programs written for the Zenith Z151 and Digital PDP-11/34A computers, we have computed expected probability distributions of times to common ancestry as a function of various female effective population sizes.

There are three potential sources of variation about an expected distribution of times to common ancestry. The first is due to stochastic variation in the underlying genealogy. The second is due to sampling error from a finite number of haplotypes assayed. The third is due to lack of fit to the model specified. The first source of variation would arise, for example, if a genealogical split at a particular locus happened

by chance to have occurred early in the pedigree. Thus, in principle, for comparisons of data to theory, it would be highly desirable to sample haplotype relationships at each of many independent loci (and through many independent pedigrees). Unfortunately, extensive haplotype data are currently available only for a single "gene"—mtDNA. Therefore we will proceed with these comparisons involving mtDNA, while recognizing and acknowledging that stochastic variation in the underlying genealogy is not fully captured.

Empirical Observations

Data sets appropriate to compare against these expectations involve mtDNA distances among individuals either (*a*) within an isolated local population of size $N_{f(e)}$ females or (*b*) within an entire species of total size $N_{f(e)}$ females, if historical levels of gene flow have been very high ($N'_{f(e)}m \gg 1$, where m is the migration rate between populations of size $N'_{f(e)}$) (Wright 1931; Maruyama 1970; Maruyama and Kimura 1974; Slatkin 1985). In practice, however, the former situation may seldom be especially informative, because effective population sizes in isolated local populations will usually be too low to have permitted the accumulation of extensive observable mtDNA differences among individuals, given known rates of mtDNA evolution (see below). In other words, the capacity of conventional restriction-site surveys to detect the small mtDNA allelic distances that are expected to have arisen de novo within a local isolated population is limited. We will therefore focus attention on mtDNA data sets for several large species (in terms of current census size) within which gene flow over historical times has apparently been very high. To a first approximation, each such species may be considered as a single population or demographic unit in evolutionary time.

1. *American Eels, Anguilla rostrata*

American eels exhibit a catadromous life-history pattern described in detail by Williams and Koehn (1984). In brief, juveniles inhabit freshwater and coastal habitats until sexual maturity, which for females in temperate waters is reached at ~10 years of age. Eels then migrate to the western tropical mid–Atlantic Ocean to spawn. Largely through passive transport by ocean currents, larvae disperse back to continental waters to complete the life cycle. Thus, "it is entirely possible that . . . collections of juveniles from any locality are all samples of the same breeding population" (Williams and Koehn 1984). Available genetic data are consistent with this view. In protein-electrophoretic surveys of eels from Newfoundland to Florida, the magnitude of allozyme differentiation was remarkably small (Williams et al. 1973; Koehn and Williams 1978; Williams and Koehn 1984); and in an mtDNA survey of eels from Maine to Louisiana, rarer as well as common mtDNA genotypes were geographically widespread (Avise et al. 1986). Thus, from life-history pattern and genetic evidence, the entire species *A. rostrata* may, for our purposes, be reasonably viewed as a single population.

The size of this population is currently very large. Eels are a common inhabitant of freshwater lakes and streams throughout eastern North America, and "each year's spawning migration . . . must include many millions of individuals" (Williams and Koehn 1984). Figure 1 plots the expected frequency distribution of generations to shared ancestry for mtDNA haplotypes under the assumption that the $N_{f(e)}$ for eels is 5,000,000 individuals.

To generate an observed distribution of times to shared ancestry for American eels, we used the mtDNA data set of Avise et al. (1986), which consists of information on approximately 78 scored restriction sites/individual in 109 specimens. Estimates

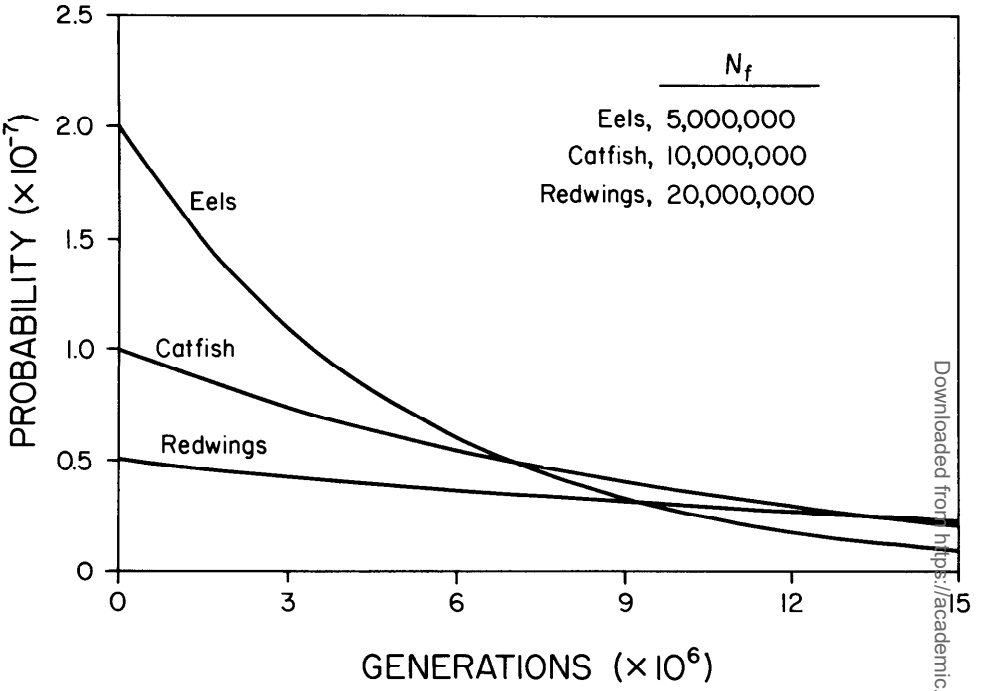

FIG. 1.—Probability distributions (plotted per generation) of times to shared ancestry of mtDNA haplotypes in American eels, hardhead catfish, and red-winged blackbirds, as calculated under the assumption that evolutionary $N_{f(e)}$'s are the same as present-day census N_f's, which are indicated. The lines cross one another because areas under each curve sum to 1.0.

of numbers of base substitutions per nucleotide site (p) between individuals were computed by the methods of Nei and Li (1979). To reduce the problem of dependence among haplotypes through their shared relationships in a common pedigree, no individuals were used more than once. These estimates were then converted to absolute time (t, in years) by

$$t = (0.5 \times 10^8)(p)\,, \qquad (2)$$

which assumes a "conventional" rate of evolution for vertebrate mtDNA, namely, 0.01 substitutions/bp/lineage/Myr (Brown et al. 1979; Wilson et al. 1985). These values were in turn converted to estimates of time measured in generations, assuming that the length of an eel generation is 10 years. Results are plotted in figure 2. The divergence times between mtDNA haplotypes, predicted from a face-value consideration of current breeding-population size, are much higher than the times to common ancestry inferred from the observed mtDNA differences (compare figs. 1, 2).

2. *Hardhead Catfish, Arius felis*

Avise et al. (1987*b*) surveyed an average of 57 mtDNA restriction sites/individual in 60 hardhead catfish collected from 10 coastal locales between North Carolina and Louisiana. Phylogenetic analyses of the 11 observed genotypes revealed two mtDNA groupings, both of which occurred throughout the assayed range of the species. Particular genotypes were also geographically widespread. These marine catfish are strong

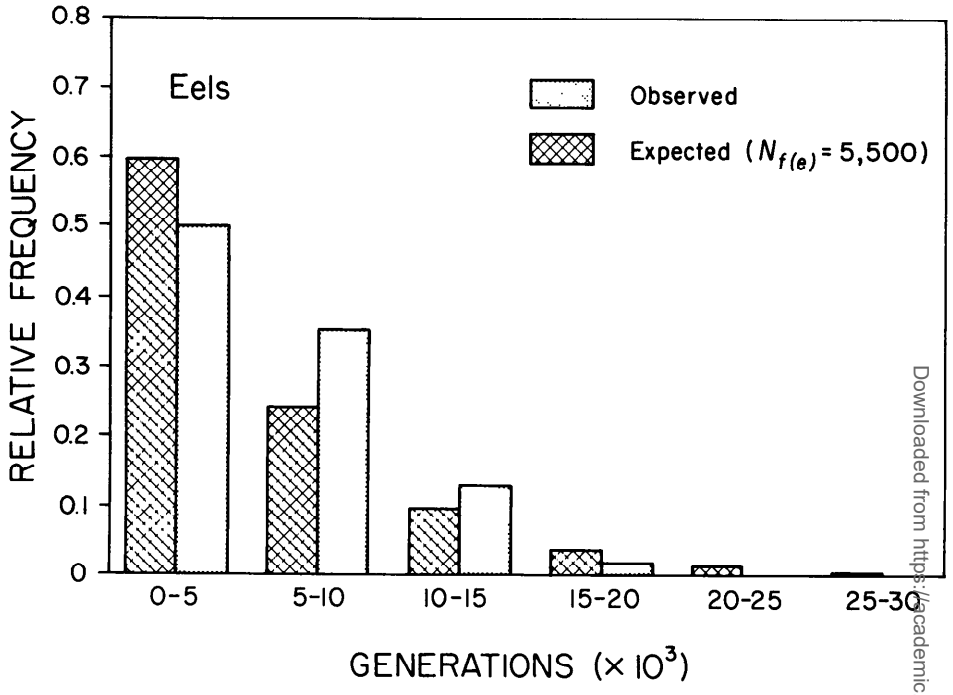

FIG. 2.—Frequency distributions of times to shared ancestry of mtDNA haplotypes in American eels. Solid bars represent observed times calculated, as described in the text, from the empirical restriction data of Avise et al. (1986); hatched bars represent expected times generated on the basis of inbreeding theory with $N_{f(e)}$ = 5,500.

and active swimmers as adults, although the actual extent of lateral movement along the coast has not been monitored directly. In any event, the life-history pattern in conjunction with the genetic data strongly suggest that historical interconnectedness among the populations has been extensive.

Arius felis is among the most abundant of nearshore marine fishes in the southeastern United States. Annual commercial landings in Florida alone total roughly 200,000 fish, and since catfish are only lightly fished, this number surely represents a miniscule fraction of the adult population (Muncy and Wingo 1983). For purposes of discussion, we will conservatively estimate that census size of breeding females in the species is 10,000,000 individuals annually. Under this estimate, the expected frequency distribution of generations to common mtDNA ancestry is plotted in figure 1. The observed frequency distribution (fig. 3), calculated from the mtDNA data of Avise et al. (1987*b*) by using equation (2) and assuming a generation length in catfish of 2 years, is far lower than these expectations (compare figs. 1, 3).

3. *Red-winged Blackbirds, Agelaius phoeniceus*

The redwing is another genetically assayed species in which historical interconnectedness among populations appears to have been quite high—and hence in which total species size may be the relevant parameter for current discussion. Ball et al. (1988) assayed an average of 63 mtDNA restriction sites in each of 127 specimens sampled from 19 sites across North America. Thirty-four distinct mtDNA genotypes

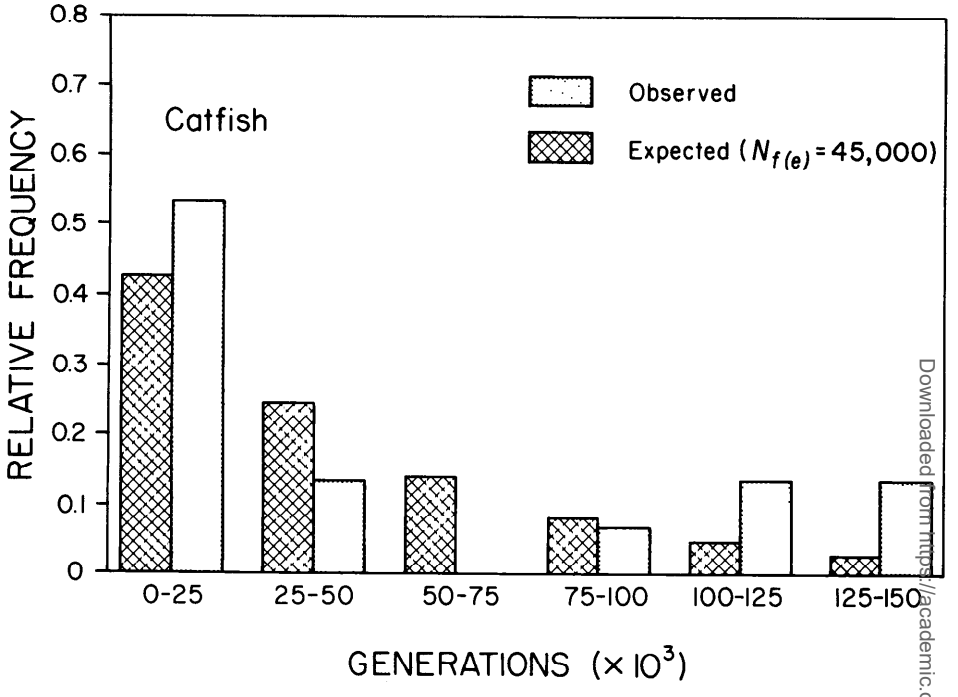

FIG. 3.—Frequency distributions of times to shared ancestry of mtDNA haplotypes in hardhead catfish. Solid bars represent observed times calculated, as described in the text, from the empirical restriction data of Avise et al. (1987*b*); hatched bars represent expected times generated on the basis of inbreeding theory with $N_{f(e)}$ = 45,000.

were observed. Even though sample sizes per locale were small, it was apparent that in most cases particular genotypes and phylogenetic arrays of genotypes were geographically widespread. The inferred distribution of times to mtDNA common ancestry, generated by application of equation (2) to the observed mtDNA genetic-distance estimates among random pairs of assayed individuals, is plotted in figure 4. This plot was derived from the reasonable assumption that generation length in redwings is 3 years.

The redwing is perhaps the most abundant bird native to North America, nesting in all continental states and throughout Canada and Mexico. Audubon Christmas counts, which cover <1% of the U.S. land area, annually census nearly 9,000,000 individuals (Cruickshank and Cruickshank 1958). Under a conservative guess that 20,000,000 females breed annually, the expected probability distribution of times to common matriarchal ancestry is shown in figure 1. Again, the expected face-value allelic distances are much larger than those actually observed (compare figs. 1, 4).

The empirical distribution of mtDNA haplotype distances in redwings appears not to be strictly geometric (fig. 4). One possible explanation could be stochastic variation such that surviving substitutions arose early in the pedigree. A second possibility is sampling error in the number of haplotypes assayed, but this seems unlikely given the large number of individuals (127) surveyed. A third possibility relates to the fact that the mtDNAs of redwings do indeed evidence at least some degree of population subdivision across North America (see Ball et al. 1988).

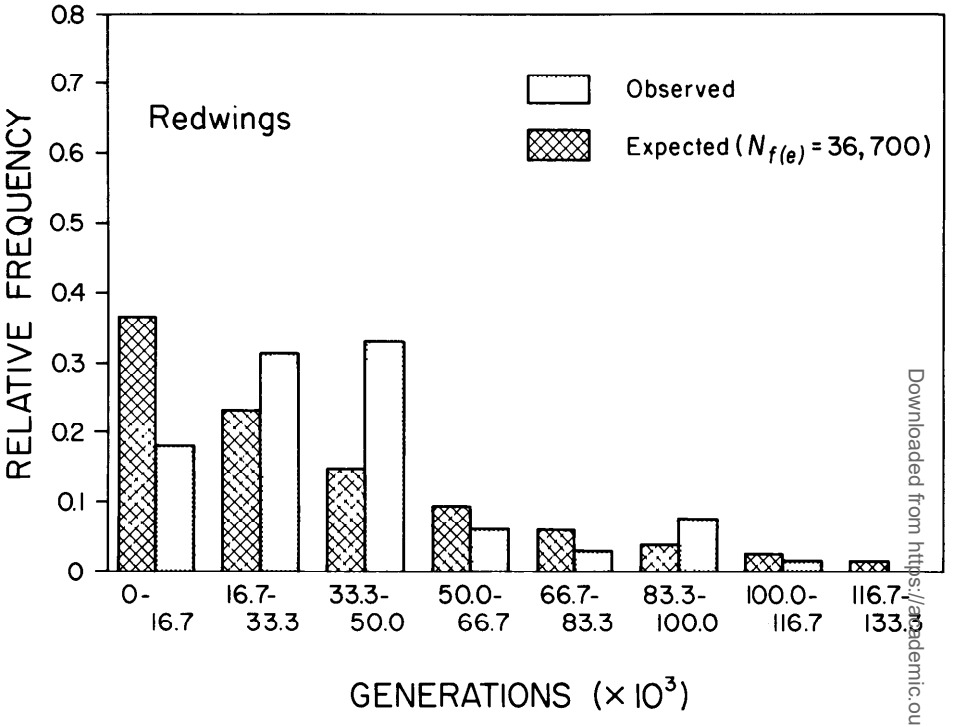

FIG. 4.—Frequency distributions of times to shared ancestry of mtDNA haplotypes in red-winged blackbirds. Solid bars represent observed times calculated, as described in the text, from the empirical restriction data of Ball et al. (1988); hatched bars represent expected times generated on the basis of inbreeding theory with $N_{f(e)}$ = 36,700.

4. *Other Species*

Genetic (and life-history) data for American eels, hardhead catfish, and red-winged blackbirds are consistent with the postulate that levels of gene flow in these species have been high over recent historical times. That is, many rarer as well as common mtDNA genotypes are found throughout the species' range. However, in most other species that have been similarly assayed for mtDNA variability, geographic population structure is far greater, suggesting that historical levels of gene flow have been much too low to allow consideration of each such species as a single demographic population (Avise et al. 1987*a*).

For example, in the redear sunfish *Lepomis microlophus* two phylogenetic arrays of mtDNA alleles differ by an estimated mean genetic distance of $\bar{p} \simeq 0.087$ base substitutions/nucleotide (roughly 10 times greater than the *largest* mtDNA distance observed in the American eels, hardhead catfish, or red-winged blackbirds), yet the two mtDNA allelic arrays were strongly patterned geographically (Bermingham and Avise 1986). One phylogenetic array appeared confined to the Florida peninsula and eastern Georgia and South Carolina, while the other was fixed in populations from western Georgia and Florida to Louisiana. It is likely that eastern versus western populations of *L. microlophus* have been strongly isolated since the late Pleiocene or Pleistocene and that the large mtDNA allelic distances have persisted and accumulated over several million years of separation (Bermingham and Avise 1986). Similar con-

clusions can be drawn for several other vertebrate species that have been surveyed for geographic variability in mtDNA (Avise et al. 1987*a*).

Geographic populations isolated by extrinsic (biogeographic) barriers to gene flow are expected to accumulate mtDNA allelic distances through time, regardless of their populations sizes (just as do "good" biological species isolated by intrinsic [genetically based] reproductive barriers). Such phylogeographically structured species are therefore not appropriately analyzed by the methods developed in the present paper (although particular populations within them could be). Here we are concerned with times to identity by descent of mtDNA haplotypes within a single demographic population or homogeneous species.

It might appear that there is an element of circularity in our reasoning: we consider times to shared ancestry of mtDNA alleles in species with historically high levels of gene flow, but such species have in part been identified by a geographic uniformity of mtDNA genotype frequencies. However, this problem is illusory only. We would view as appropriate for analysis any species within which most mtDNA haplotypes were geographically widespread, irrespective of the magnitude of genetic distance among the mtDNA genotypes involved. Thus, in principle, highly distinct mtDNA genotypes could certainly coexist within a large population or species. A widespread cooccurrence of highly distinct mtDNA alleles is exactly the expectation for a panmictic species with large effective population size over recent evolutionary history. In fact, however, a situation in which highly distinct mtDNA genotypes lack geographic localization (the phylogeographic category II of Avise et al. 1987*a*) has not yet been observed in any assayed species.

Discussion

The major result reported here is that for several large vertebrate species with high gene flow, observed intraspecific genetic distances among mitochondrial haplotypes are vastly lower than the expected distances derived from a consideration of the current breeding-population size of each species and from rate of evolution of the mtDNA molecule. Likely explanations for the discrepancy therefore involve (*a*) use of an incorrect calibration of the mtDNA evolutionary clock and/or (*b*) a gross disparity between present-day population size and long-term effective population size.

Rate of mtDNA Evolution

The original calibration of mtDNA evolutionary rate, ~0.01 substitutions/bp/lineage/Myr, was based largely on interspecies comparisons in primates (Brown et al. 1979). Similar rates were subsequently reported for rhinoceroses, horses, rats, mice, bovids, and geese (see references in Shields and Wilson 1987). However, many of the calibrations are very crude. Furthermore, the ratio of evolutionary rates for mtDNA versus scnDNA (single-copy nuclear DNA) can vary considerably among taxa (Powell et al. 1986; Vawter and Brown 1986), although it is debatable whether the heterogeneity is due to rate variation for mtDNA, scnDNA, or both. The slowest mtDNA clock calibration of which we are aware (one of several plausible rates suggested by Solignac et al. [1986], depending on the ages of species in the *Drosophila melanogaster* group) is ~0.001 substitutions/bp/lineage/Myr—or approximately one-tenth the conventional rate.

Even if we assume this latter, low rate of mtDNA evolution for the fishes and birds considered in the present report, the expected mtDNA allelic distances are still much larger than those actually observed. The mtDNA evolutionary rates required

Table 1
Approximate Long-Term $N_{f(e)}$ or Decelerated Evolutionary Rates for mtDNA (in Substitutions/bp/Lineage/Myr) Required to Make Expected Mean mtDNA Distances under the Neutral Model Compatible with Observed Mean mtDNA Distances in American Eels, Hardhead Catfish, and Red-winged Blackbirds

Species	Female Population Size		mtDNA Evolutionary Clock		Magnitude of Discrepancy (Population Size or Evolutionary Rate)
	Current (N_f)	Evolutionary ($N_{f(e)}$[a])	Conventional Rate	Decelerated Rate[b]	
American eel	5,000,000	5,500	0.01	0.000011	909-fold
Hardhead catfish	10,000,000	45,000	0.01	0.000045	222-fold
Red-winged blackbird	20,000,000	36,700	0.01	0.000018	545-fold

[a] Assumes the conventional mtDNA evolutionary rate.
[b] Assumes that $N_{f(e)} = N_f$.

to make expected mtDNA distances compatible with observations (under the assumption that rate heterogeneity alone is the responsible factor) are listed in table 1. They are 200- to 900-fold lower than the "typical" rate for vertebrates. Furthermore, from empirical evidence, it is unlikely that mtDNA evolution is severely constrained in the species considered here, because each does exhibit a large number of distinct mtDNA genotypes, and, in the cases of the hardhead catfish and American eel, each has closely related species that are known to differ by large mtDNA genetic distances (Avise et al. 1986, 1987*b*). Therefore, another explanation for the small intraspecific mtDNA distances is probably necessary.

Long-Term Effective Population Size

The long-term effective population size (N_e) of a species can be dramatically different from current breeding-population size (N). Usually, N_e is smaller than N because of (*a*) overlapping generations and non-Poisson distributions of family size, (*b*) fluctuations in population size through time, and (*c*) extinction and recolonization of subpopulations (for detailed treatments, see Gall 1987; Nei 1987). Table 1 lists the long-term female effective population sizes $N_{f(e)}$'s for the catfish, eels, and redwings required to bring expected mean mtDNA allelic distances into alignment with the observed mean values, assuming the conventional mtDNA clock calibration for vertebrates (Brown et al. 1979). These effective sizes are two to three orders of magnitude lower than current census sizes of the breeding populations. Using these lower values of $N_{f(e)}$ for eels, catfish, and redwings, we have calculated expected frequency distributions of times to mtDNA haplotype ancestry. Results are compared with the empirically based estimates of divergence times in figures 2–4, respectively. Although the expected distributions still differ significantly from the observed distributions as judged by χ^2 values (not shown), the agreements are far better than those based on the much larger census N_f's.

While we strongly suspect that $N_{f(e)} \ll N_f$ accounts for most of the disparity between the observed and expected frequency distributions of genetic distances among mtDNA alleles, we can only offer conjecture about the specific reasons for particular values of $N_{f(e)}$. In the American eel, the spawn of each female may consist of a million eggs or more. Particularly in species with such high fecundities, the opportunity exists

for huge variances in progeny numbers among families, with consequent dramatic lowering of $N_{f(e)}$ relative to N_f. Marine catfish have moderately high fecundities, with females producing ~20–65 eggs/spawn. Adult male catfish mouthbrood the eggs and larvae. Since the success or failure of each entire brood may depend on the fate of the father, it is likely that the variance in progeny survival among families is great. On the other hand, redwing females have much lower fecundities (usually four eggs/brood). In this species, however, a significant fraction of the current geographic range has clearly been colonized since the retreat of the last Pleistocene glacier, so it is quite conceivable that population size has expanded greatly in the recent evolutionary past. For all three species, dramatic fluctuations in population size may well have played a role in reducing $N_{f(e)}$.

We are certainly not the first to utilize molecular data to test predictions based on intraspecific population size. Nei and Graur (1984) review the extensive allozyme literature on the subject—and they also conclude that observed variability is much lower than expected under neutral models. They state that "the level of protein polymorphism is actually much lower than the neutral expectation and that if the bottleneck effect is not sufficient for explaining the observed level, the type of selection to be considered is not diversity-enhancing selection but diversity-reducing selection." Results of the current report extend the original conclusions of Nei and Graur (1984) from the protein level to the mtDNA level.

At first thought, widespread diversity-reducing selection might appear to be even less likely for mtDNA than for proteins, since most mtDNA variants occur in nontranslated regions or in silent positions of protein-coding genes. However, mtDNA in higher animals is nonrecombining, so, even though many observed mutational variants are probably neutral in a mechanistic sense, their evolutionary dynamics might nonetheless be importantly influenced by linkage to occasional, beneficial mtDNA mutations. Such intermittent waves of selection would channel mtDNA lineages through smaller numbers of females and, in effect, also lower $N_{f(e)}$.

Results of the present study can be interpreted to fit well with a broader picture from mtDNA, one in which the intraspecific genetic architectures of species appear to be shaped to a large degree by historical demographic factors. In many species, geographic populations occupy distinct branches in an mtDNA phylogenetic tree (Avise et al. 1987*a*), suggesting extensive historical population subdivision, severe restrictions on gene flow, and relatively small local effective population sizes (Neigel and Avise 1986). Zoogeographic impediments to gene flow, which arise and disappear in idiosyncratic fashion with changing landscapes, climates, and biotic factors, should interact with life history–mediated dispersal capabilities to produce the phylogeographic structure of any species. Even for the large species with extensive gene flow that are considered in the present report, it now seems likely that population sizes have fluctuated dramatically in recent evolutionary times. With respect to genetic structure, natural populations of most species may seldom approach a stable, equilibrium condition.

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LITERATURE CITED

AQUADRO, C. F., S. F. DESSE, M. M. BLAND, C. H. LANGLEY, and C. C. LAURIE-AHLBERG. 1986. Molecular population genetics of the alcohol dehydrogenase gene region of *Drosophila melanogaster.* Genetics **114:**1165–1190.

AVISE, J. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Philos. Trans. R. Soc. Lond. [Biol.] **312:**325–342.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, and N. C. SAUNDERS. 1987*a*. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. **18:**489–522.

AVISE, J. C., G. S. HELFMAN, N. C. SAUNDERS, and L. S. HALES. 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. Proc. Natl. Acad. Sci. USA **83:**4350–4354.

AVISE, J. C., and R. A. LANSMAN. 1983. Polymorphism of mitochondrial DNA in populations of higher animals. Pp. 147–164 *in* M. NEI and R. K. KOEHN, eds. Evolution of genes and proteins. Sinauer, Sunderland, Mass.

AVISE, J. C., J. E. NEIGEL, and J. ARNOLD. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. **20:**99–105.

AVISE, J. C., C. A. REEB, and N. C. SAUNDERS. 1987*b*. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). Evolution **41:**991–1002.

AYALA, F. J. 1974. Biological evolution: natural selection or random walk? Am. Sci. **62:**692–701.

BALL, R. M., JR., F. C. JAMES, S. FREEMAN, E. BERMINGHAM, and J. C. AVISE. 1988. Phylogeographic population structure of red-winged blackbirds assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. USA **85:**1558–1562.

BERMINGHAM, E., and J. C. AVISE. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics **113:**939–965.

BROWN, W. M. 1981. Mechanisms of evolution in animal mitochondrial DNA. Ann. NY Acad. Sci. **361:**119–134.

———. 1983. Evolution of animal mitochondrial DNA. Pp. 62–88 *in* M. NEI and R. K. KOEHN, eds. Evolution of genes and proteins. Sinauer, Sunderland, Mass.

———. 1985. The mitochondrial genome of animals. Pp. 95–130 *in* R. J. MACINTYRE, ed. Molecular evolutionary genetics. Plenum, New York.

BROWN, W. M., M. GEORGE, JR., and A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA **76:**1967–1971.

CANN, R. L., W. M. BROWN, and A. C. WILSON. 1984. Polymorphic sites and the mechanisms of evolution in human mitochondrial DNA. Genetics **106:**479–499.

CROW, J. F., and M. KIMURA. 1970. An introduction to population genetics theory. Harper & Row, New York.

CRUICKSHANK, A. D., and H. G. CRUICKSHANK. 1958. 1001 Questions answered about birds. Dover, New York.

EWENS, W. J. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. **3:**87–112.

FISHER, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

GALL, G. A. E. 1987. Inbreeding. Pp. 47–87 *in* N. RYMAN and F. UTTER, eds. Population genetics and fishery management. University of Washington Press, Seattle.

HUDSON, R. R., and N. L. KAPLAN. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics **111:**147–164.

KIMURA, M., and J. F. CROW. 1964. The number of alleles that can be maintained in a finite population. Genetics **49:**725–738.

KOEHN, R. K., and G. C. WILLIAMS. 1978. Genetic differentiation without isolation in the

American eel, *Anguilla rostrata*. II. Temporal stability of geographic patterns. Evolution **32:** 624–637.

KREITMAN, M. 1983. Nucleotide polymorphism at the *alcohol dehydrogenase* locus of *Drosophila melanogaster*. Nature **304:**412–417.

KREITMAN, M., and M. AQUADÉ. 1986. Excess polymorphism at the ADH locus in *Drosophila melanogaster*. Genetics **114:**93–110.

MARUYAMA, T. 1970. Effective number of alleles in a subdivided population. Theor. Popul. Biol. **1:**273–306.

MARUYAMA, T., and M. KIMURA. 1974. Geographical uniformity of selectively neutral polymorphisms. Nature **249:**30–32.

MORITZ, C., T. E. DOWLING, and W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18:**269–292.

MUNCY, R. J., and W. M. WINGO. 1983. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico)—sea catfish and gafftopsail catfish. U.S. Fish and Wildlife Service, Division of Biological Services, FWS/OBS-82/11.5. U.S. Army Corps of Engineers.

NEI, M. 1983. Genetic polymorphism and the role of mutation in evolution. Pp. 165–190 *in* M. NEI and R. K. KOEHN, eds. Evolution of genes and proteins. Sinauer, Sunderland, Mass.

———. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NEI, M., and D. GRAUR. 1984. Extent of protein polymorphism and the neutral mutation theory. Evol. Biol. **17:**73–118.

NEI, M., and W.-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76:**5269–5273.

NEIGEL, J. E., and J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 513–534 *in* E. NEVO and S. KARLIN, eds. Evolutionary processes and theory. Academic Press, New York.

OHTA, T., and M. KIMURA. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. **22:**201–204.

POWELL, J. R., A. CACCONE, G. D. AMATO, and C. YOON. 1986. Rates of nucleotide substitution in *Drosophila* mitochondrial DNA and nuclear DNA are similar. Proc. Natl. Acad. Sci. USA **83:**9090–9093.

SCHAEFFER, S. W., C. F. AQUADRO, and W. W. ANDERSON. 1987. Restriction-map variation in the alcohol dehydrogenase region of *Drosophila pseudoobscura*. Mol. Biol. Evol. **4:**254–265.

SHIELDS, G. F., and A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. J. Mol. Evol. **24:**212–217.

SLATKIN, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. **16:**393–430.

SOLIGNAC, M., M. MONNEROT, and J.-C. MOUNOLOU. 1986. Mitochondrial DNA evolution in the *melanogaster* species subgroup of *Drosophila*. J. Mol. Evol. **23:**31–40.

SOULÉ, M. J. 1976. Allozyme variation: its determinants in space and time. Pp. 60–77 *in* F. J. AYALA, ed. Molecular evolution. Sinauer, Sunderland, Mass.

STEPHENS, J. C. 1985. Statistical methods of DNA sequence analysis: detection of intragenic recombination or gene conversion. Mol. Biol. Evol. **2:**539–556.

STEPHENS, J. C., and M. NEI. 1985. Phylogenetic analysis of polymorphic DNA sequences at the Adh locus in *Drosophila melanogaster* and its sibling species. J. Mol. Evol. **22:**289–300.

TAJIMA, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics **105:**437–460.

VAWTER, L., and W. M. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science **234:**194–196.

WILLIAMS, G. C., and R. K. KOEHN. 1984. Population genetics of North Atlantic catadromous eels (*Anguilla*). Pp. 529–560 *in* B. J. TURNER, ed. Evolutionary genetics of fishes. Plenum, New York.

WILLIAMS, G. C., R. K. KOEHN, and J. B. MITTON. 1973. Genetic differentiation without isolation in the American eel, *Anguilla rostrata.* Evolution **27**:192–204.

WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, JR., U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, and M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linnaen Soc. **26**:375–400.

WRIGHT, S. 1931. Evolution in Mendelian populations. Genetics **16**:97–159.

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