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Mitochondrial DNA and the Evolutionary Genetics of Higher Animals [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1986 **312**, 325-342
doi: 10.1098/rstb.1986.0011

Phil. Trans. R. Soc. Lond. B **312**, 325–342 (1986)
Printed in Great Britain

Mitochondrial DNA and the evolutionary genetics of higher animals

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Mitochondrial DNA (mtDNA) in higher animals is rapidly becoming a well characterized genetic system at the molecular level. In this paper, I shift the focus to consider questions in organismal evolution that can be addressed by mtDNA assay. For the first time, it is possible to estimate empirically matriarchal phylogeny; to determine directionality in crosses producing hybrids; and to study the population genetic consequences of varying female demographies and life histories. The data obtainable from mtDNA may be especially well suited for studies of population genetic structure, dispersal, and historical zoogeography. The female-mediated, clonal transmission of mtDNA is also stimulating new ways of thinking about times to common ancestry of asexual lineages within otherwise sexually reproducing populations; about the possible relevance of mtDNA–nuclear DNA interactions to reproductive isolation; and about the very meaning of the phylogenetic status of related species with respect to particular kinds of genetic characters. These and other topics are reviewed.

Introduction

Interest in molecular evolution can centre on either of two fronts: (i) description of the molecular basis of variation in particular genetic systems; (ii) use of the information provided by these gene systems to study organismal evolution. Mitochondrial DNA (mtDNA) is a cytoplasmic gene system that in recent years has become very well characterized at the molecular level. Recent reviews by Brown (1983) and Avise & Lansman (1983) give details about the molecular features of mtDNA variation, major aspects of which are described below. In higher animals, mtDNA is a closed circular molecule encoding about 13 messenger RNAs, 22 transfer RNAs, and 2 ribosomal RNAs. There are no intervening sequences within transcribed genes, no spacer sequences between genes and no classes of repetitive DNA. MtDNA is very conservative in size (usually near 16 kilobase pairs), gene content, and gene arrangement, yet it is rapidly evolving at the nucleotide sequence level. Newly arising transitions greatly outnumber transversions, and base substitutions are far more common than additions or deletions. Progeny inherit most if not all mtDNA from their female parent. Individuals usually appear homoplasmic, that is they exhibit predominantly a single mtDNA sequence. None the less, genetic polymorphism among conspecifics is extensive.

The intent of this review is to focus on the second level of interest in higher animal mtDNA: the use of mtDNA genotypes as markers of evolutionary phenomena in the populations of organisms that carry these molecules. Although emphasizing this level here, I do not wish to imply any priority over the need for molecular characterization. Indeed, molecular- and population-level understanding often advance synergistically, and any final conclusions about mtDNA evolution must be compatible with phenomenological findings at both levels. Nor do I want to be interpreted as unquestioningly endorsing the conventional view about the

molecular basis of mtDNA variation. For example, much further study is needed of the possibilities that sperm-mediated transfer of mtDNA across female lineages may occasionally occur during zygote formation; that effective recombination among genetically distinct mtDNAs may sometimes take place; and that individual heteroplasmy in somatic or germ cells may be more prevalent than is currently thought (Hauswirth & Laipis 1982; Harrison *et al.* 1985; Solignac *et al.* 1983). New discoveries about these possible phenomena could have quantitative or even qualitative effects on some of the interpretations in this paper. None the less, to the best of current knowledge, mtDNA in higher animals appears to lack many of the genetic complexities that can make data on nuclear DNA difficult to interpret. In fact, the message from this review is that mtDNA is not 'just another' genetic system for evolutionary study. From a population biology perspective, it is a richly instructive molecule whose linear, matriarchal transmission across animal generations provides a class of information simply unobtainable from any nuclear gene system.

Background: evolutionary rates

Brown *et al.* (1979) were the first to document forcefully that the rate of mtDNA evolution appears to be several-fold (5–10 times) higher than that of typical single copy nuclear DNA. They examined the differences in thermal stability (Δt_m) between homoduplex and heteroduplex mtDNAs isolated from man and the guenon, and compared the results with similar thermostability analyses of single copy nuclear DNA from these and two other higher primates. Estimated sequence divergence between human and guenon mtDNA (assuming Δt_m equals percentage sequence divergence) was about 22%, a value consistent with independent estimates (ranging from 21 to 29%) derived from comparisons of restriction site maps of mtDNA from the four primate species. In sharp contrast, Δt_m values for the single copy nuclear DNAs for these same species were only 1.4–6.3%. Brown *et al.* (1979) went on to plot mtDNA sequence divergence (p) for 19 pairs of mammalian species against divergence times estimated from fossil or protein data, with results shown in figure 1. From the initial slope of the curve, the evolutionary rate of mtDNA was estimated to be about 2% (0.02 substitutions per base pair) per million years; from the plateau of the curve, it seemed likely that certain highly variable positions in mtDNA become saturated by substitutions within about 10–20 million years, after which the

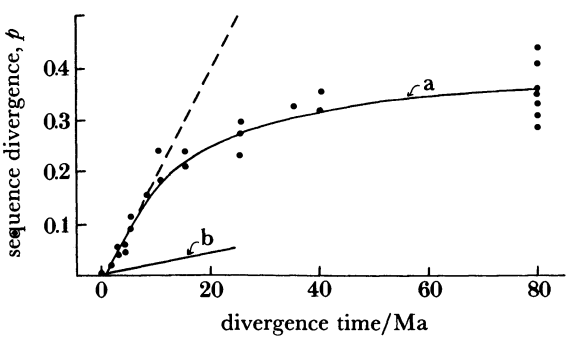

Figure 1. The dynamics of mtDNA sequence divergence (redrawn from Brown *et al.* 1979). Solid lines a and b plot the differentiation of mtDNA and single-copy nuclear DNA, respectively. The closed circles represent comparisons of mtDNA for various pairs of mammalian species whose divergence times were estimated from fossil or protein evidence. The broken line gives the initial slope of the mtDNA curve, from which sequence divergence was suggested to be about 2% per million years.

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accumulation of further sequence differences slows dramatically. This landmark paper still provides the paradigm of the dynamics of mtDNA differentiation.

Further refinement in understanding mtDNA evolution has come from direct sequence comparisons. The complete nucleotide sequence of mtDNA has been determined for an individual human (Anderson *et al.* 1981), house mouse (Bibb *et al.* 1981) and cow (Anderson *et al.* 1982), and significant portions of the genome have been sequenced in additional humans (Greenberg *et al.* 1983), other primates (Brown *et al.* 1982) and rats (Brown & Simpson 1982; Wolstenholme *et al.* 1982). Brown *et al.* (1982) sequenced in primates homologous 896 base-pair regions coding for two proteins and three transfer RNAs. For human, chimpanzee, gorilla, orang-utan, and gibbon, estimates of p ranged from 0.09 to 0.19, a finding that, when compared with other data, was in agreement with their earlier conclusion that mtDNA evolves 5–10 times more rapidly than nuclear DNA. The rate of evolution was, however, variable across sites: in the protein-coding genes, the silent substitution rate was 4–6 times higher than the rate of base substitution leading to amino acid replacement; at the tRNA loci, the rate was similar to that for replacement substitutions in the protein-coding genes. Remarkably, the assayed tRNA genes in the mitochondria appeared to 'evolve at least 100 times faster than their nuclear counterparts' (Brown *et al.* 1982). A non-coding region approximately 900 base-pairs in length, which includes the origin of mtDNA heavy strand replication and the displacement (D) loop, is also known to evolve especially rapidly (Aquadro & Greenberg 1983).

On the other hand, Aquadro *et al.* (1984) point out that a substantial fraction of positions in the mtDNA must be under very strong selective constraints because it exhibits a negligible rate of substitution. For example, throughout the evolutionary lineages leading to six primates, mouse, and cow, 69% of the 195 nucleotides comprising the sequenced tRNA genes are completely conserved, as are 51 and 61%, respectively, of the first and second codon positions of sequenced protein-coding loci. Conservation of certain sites must certainly contribute to the plateau of sequence divergence exhibited in figure 1.

Two hypotheses, not mutually exclusive, are currently under consideration to explain the rapid average pace of evolution of mtDNA: (i) that there is an enhanced rate of mutation or (ii) that there are relaxed selective constraints on functional products of the molecule. Higher mutation pressures could in principle arise from such factors as deficient editing or repair during DNA replication (see Brown (1981) for mechanistic possibilities), or more rapid turnover of molecules with constant mutation rate per round of replication. Any proposed molecular mechanism for an enhanced mutation rate might also need to account for the recent observation that small length mutations appear to arise and survive at higher rates in non-coding mtDNA than in non-coding nuclear DNA (Cann & Wilson 1983). Alternatively, more rapid fixation of mtDNA mutants might occur because of relaxed selective constraints on components of the mitochondrial machinery, such as the translation apparatus, which has to process only a few kinds of messenger RNAs (see Cann *et al.* 1984). In any event, there is currently no compelling reason to suppose that most of the mtDNA variants routinely assayed cannot be interpreted as neutral markers of the female lineages in which they occur. This has been the working assumption in most population surveys of mtDNA.

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Phenomena in organismal evolution revealed by mtDNA

While nucleotide sequencing allows detailed characterization of the molecular basis of mtDNA variation, most studies at the population level have necessarily used the simpler and more rapid methods of restriction endonuclease fragment comparisons (fragment analysis) or restriction site mapping (site analysis). The 'raw' data in such studies consists of fragment digestion profiles on gels, or maps of restriction sites. Depending on the intent of a project, this qualitative raw data can be used directly to answer particular problems (such as the direction of crosses having produced interspecific hybrids), or can be converted to quantitative estimates of mtDNA nucleotide sequence divergence (p) between the mtDNAs under comparison (Upholt 1977; Brown *et al.* 1979; Gotoh *et al.* 1979; Nei & Li 1979; Kaplan & Risko 1981). For example, figure 2 shows the commonly used theoretical curves relating the fraction (F) of shared restriction fragments in two digestion profiles to p (Upholt 1977; Nei & Li 1979). These curves were generated under the assumptions that all fragment changes arise from base substitutions, that frequencies and distributions of cleavage sequences are similar to those expected in random sequences of the same base composition, and that non-homologous fragments of similar molecular mass are not scored as identical. Such mathematical derivations merit some scrutiny, because the underlying assumptions may be partly violated with real data and because the anticipated relationships in any event can have an important bearing on the acquisition and interpretation of data. In fragment analyses, for example, meaningful absolute estimates of p should preferably be attempted only when fragment identities are much greater than about 0.25; for more distantly related DNAs, even small errors in estimation of F will be translated into unrealistically large differences in p (figure 2).

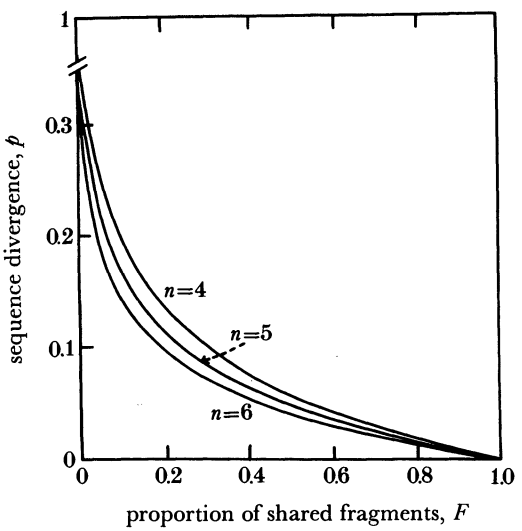

Figure 2. The theoretical relationship between the proportion of shared mtDNA restriction fragments (F) and the nucleotide sequence divergence (p) for restriction enzymes cleaving at 4-, 5-, and 6-base sites (see Upholt 1977; Nei & Li 1979).

The following sections do not review the literature exhaustively; they are intended to highlight the major classes of phenomena in organismal evolution that have been studied by fragment or site analyses of mtDNA.

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Phylogeny

Extensive assayable mtDNA polymorphism within and among very closely related species (Avise & Lansman 1983) has permitted estimation of matriarchal phylogeny on a micro-evolutionary scale. In one of the first such applications, Avise *et al.* (1979*a*) used six endonucleases to reveal 23 mtDNA genotypes among 87 pocket gophers (*Geomys pinetis*) collected from across the species' range in the southeastern United States. A parsimony analysis grouped these clonal genotypes into two distinct evolutionary assemblages which were also strongly patterned geographically. Fifteen closely related mtDNA clones in gophers collected from eastern Georgia and northern peninsular Florida differed by an estimated 3% sequence divergence from another phylogenetic assemblage of eight observed mtDNA clones in gophers collected from western Georgia, Alabama, and the Florida panhandle.

Such geographical patterning of the apparent branches in mtDNA phylogenies has so far proved to be the rule, with few exceptions. For example, figure 3 shows a parsimony-generated mtDNA phylogeny for the deer mouse *Peromyscus maniculatus* superimposed on the continent-wide sources of the collections (Lansman *et al.* 1983*a*). In this species, the 61 different restriction site maps revealed by eight endonucleases were grouped into five major phylogenetic assemblages. In particular, a large assemblage of related mtDNA genotypes in the interior and western portions of North America was clearly distinct ($p \approx 0.05$) from another major

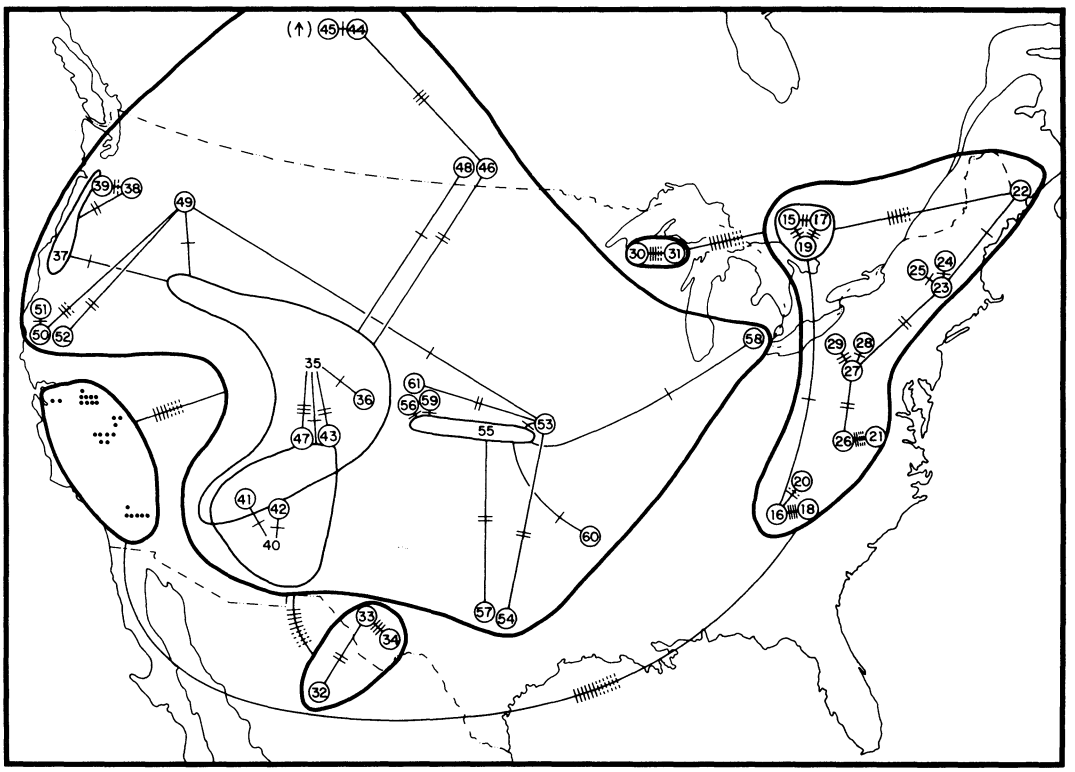

Figure 3. MtDNA-estimated phylogeny for the deer mouse *Peromyscus maniculatus* superimposed over the geographical sources of collections (from Lansman *et al.* 1983*a*). The numbers refer to distinct mtDNA genotypes, and branches interconnect related genotypes. The solid lines crossing branches represent the numbers of observed restriction enzymes producing gel profile changes; the solid and dashed lines represent the numbers of restriction site changes responsible for these profile differences.

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assemblage of genotypes in the eastern United States. Varying degrees of geographical structuring of mtDNA genotypes have also been reported for bluegill sunfish in the southeastern United States (Avise *et al.* 1984), water frogs in Europe (Spolsky & Uzzell 1984), whiptail lizards in Texas and Mexico (Wright *et al.* 1983), house mice worldwide (Yonekawa *et al.* 1981; Ferris *et al.* 1983*a*), rats worldwide (Brown & Simpson 1981), and apes in Africa and Asia (Ferris *et al.* 1981*a*).

These results suggest that for many species the geographical spread of mtDNA genotypes by dispersal is not sufficient to swamp the historical pattern of population subdivision supposedly revealed in mtDNA phylogeny reconstruction. While it may not be surprising that most species are geographically structured into phylogenetically distinct populations, this has not always been simple to demonstrate empirically. For example, the easily distinguishable assemblages of *Peromyscus maniculatus* revealed by mtDNA (figure 3) showed no major differences in the frequencies of allozymes encoded by nuclear genes (Avise *et al.* 1979*b*). The mtDNA assemblages of *P. maniculatus* also bear little or no correspondence to certain morphological characteristics (length of tail, size of ear, pelage colour) which probably represent phenotypic adaptations to specific habitats (Lansman *et al.* 1983*a*). Geographical surveys of mtDNA differentiation offer unprecedented opportunities for examining the phylogenetic component of differentiation among conspecifics.

One exciting arena for the interpretation of such results is zoogeographical reconstruction. If it could be demonstrated, for example, that populations with a series of independent species show concordant geographical 'tracks' of mtDNA divergence, some vicariant explanation would be strongly implied. In the southeastern United States, two subspecies of bluegill sunfish (*Lepomis macrochirus*) are distinct ($p \approx 0.08$) in mtDNA (and allozyme) genotype (Avise *et al.* 1984*a*). The Apalachicola drainage, which forms much of the boundary between Georgia and Alabama, is the eastern extreme of the western subspecies' range. Recent geographical surveys of four additional fish species show that at least two (the bowfin *Amia calva*, and the spotted sunfish *Lepomis punctatus*) also show major east–west disjunctions in mtDNA genotype in the Apalachicola region (Bermingham & Avise 1986). We suggest that these concordant patterns probably reflect historical patterns of connections between river drainages.

The data obtained in mtDNA surveys are also suitable for estimating phylogenetic relationships among very closely related species. The most intensive utilization of mtDNA data for this purpose has involved comparisons among the living hominoid primates: man, chimpanzee, gorilla, orangutan and gibbon. The major intent has been to resolve the exact branching order of gorilla, chimp and man, which from analyses of a wealth of nuclear gene data had remained an unresolved trichotomy (Sibley & Ahlquist 1984).

Ferris *et al.* (1981*b*) studied the phylogenetic distribution of 132 restriction sites revealed among the hominoid primates by 19 endonucleases. Eleven sites that were invariant among species were used to align the restriction maps, and a parsimony procedure was used for tree construction. This approach involves comparing alternative trees with respect to the minimum number of mutation steps required to account for the evolutionary interconversion of the restriction maps. The most parsimonious tree, with 67 total mutations, grouped gorilla and chimp as the most closely related species. However, an alternative tree linking chimp and man required only 68 mutational steps, and Ferris *et al.* (1981*b*) speculated that 'at least 10 times more genetic information will be required to resolve the branching order for the gorilla, chimpanzee, and human lineages definitively'.

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Templeton (1983*a*) reanalysed the data of Ferris *et al.* (1981*b*) with a three-step algorithm involving (i) the construction of a series of parsimony networks, one for the site data produced by each restriction endonuclease; (ii) the use of a 'compatibility' method to determine the overall phylogeny consistent with the largest number of single-enzyme parsimony networks; and (iii) the comparison by non-parametric statistics of this overall phylogeny with alternative possible trees. Included in the statistical analysis is a ranking of the qualitative types of mutational events by their probabilities of occurrence: for example, convergent loss of sites is much more likely than convergent gain (Templeton 1983b). Templeton concluded that a phylogeny grouping chimp and gorilla was better (at the 5% level of significance) than alternative possibilities. For a critique of the Templeton approach, see Nei & Tajima (1985).

Brown *et al.* (1982) sequenced an 896-base-pair segment of mtDNA in the hominoid primates, and concluded from a parsimony analysis that while chimp and gorilla appeared most closely related, other phylogenies could not be ruled out. Goodman *et al.* (1983) used mtDNA sequence data from mouse and ox as outgroups to evaluate the available primate data, and also concluded that the order of branching of man, chimp, and gorilla could not be clearly resolved.

Taken together, these studies exemplify the kinds of controversies in phylogenetic methodology that inevitably arise when branch points close to one another in time are under debate. Ferris *et al.* (1981) and Templeton (1983a) justify the concern for delineation of the exact branching order of higher primates by pointing out that such information is critical for the correct reconstruction of behavioural and morphological traits in immediate human ancestors. None the less, it should be remembered that mtDNA data presumably reflects female ancestry only, and hence need not be perfectly concordant with nuclear DNA genealogy, even in principle. Furthermore, it is quite possible that mtDNA (or nuclear DNA) lineages in closely related species may be discordant with biological species boundaries owing simply to demographically influenced stochastic patterns of lineage survivorship (see Speciation). Thus the question of the exact branching order of species may be illusory in the sense that the answer may differ depending on the particular genetic bases, and hence genealogies, of the traits under surveillance.

Few other attempts have been made to generate between-species phylogenies from mtDNA data, perhaps because of a perception that arrays of species would commonly exhibit distances beyond the linear portion of the differentiation curve (figure 1). However, a recent restriction fragment analysis comparing mtDNAs of congeneric species of birds (in the genera *Anas*, *Aythya*, *Dendroica*, *Melospiza*, and *Zonotrichia*) revealed genetic distances ranging from $p = 0.007$ to 0.088, well within the expected linear region of divergence, and well below mean mtDNA distances among sunfish *Lepomis* or treefrogs *Hyla* (Kessler & Avise 1985*a*). By using maps of mtDNA restriction sites, Glaus *et al.* (1980) report mtDNA distances ranging from $p = 0.097$ to 0.175 in comparisons *between genera and subfamilies* of galliform birds. These results are significant because they parallel the conservative pattern of protein differentiation previously reported for many avian taxa (Avise & Aquadro 1982). Since both protein-coding nuclear loci and mtDNA seem to exhibit relatively small distances in several bird groups, it seems increasingly likely that many avian species have shared more recent common ancestors than have many of their non-avian taxonomic counterparts.

None the less, estimates of avian divergence times from mtDNA or protein-calibrated clocks could not readily be reconciled with some published dates based on limited fossil remains, so

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the possibility remains that both protein and mtDNA evolution are somewhat decelerated in birds (Kessler & Avise 1985*a*).

Whatever the reasons for the conservative magnitude of mtDNA divergence in birds, the data should prove useful for avian phylogeny reconstruction. Kessler & Avise (1984) applied parsimony algorithms, compatibility algorithms, and phenetic clustering procedures to qualitative and quantitative mtDNA restriction fragment data for 13 species of waterfowl in the genera *Anas* and *Aythya*. The resulting mtDNA phylogenies were highly concordant with one another, and with traditional phylogenies derived from independent sources of morphological and behavioural evidence.

Demography, life history and dispersal

Studies of two widely distributed species have suggested possible exceptions to the rule of extensive geographical structuring of mtDNA genotypes, and both cases appear to be illustrative of demographic and life-history characteristics which can importantly influence patterns of mtDNA differentiation. The first case involves humans. Brown (1980; Brown & Goodman 1979) digested mtDNAs from 21 humans of diverse racial (Caucasoid, Mongoloid, Negroid) and geographical origin with 18 restriction endonucleases. Seven of the enzymes produced identical digestion profiles in all individuals, while 11 enzymes revealed a total of about 50 scattered mtDNA variants, all of which could be attributed to single site gains or losses. In this small sample of humans, several of the mtDNA variants shared by two or more individuals appeared confined to a single race, but no race was consistently distinguishable from all others by any restriction enzyme. Six mtDNA variants were observed in two or more races. Overall, the estimated pairwise sequence divergence between samples was about 0.36%, roughly an order-of-magnitude lower than reports for several other vertebrates (Avise & Lansman 1983). Brown (1980) suggested that 'the amount sequence heterogeneity observed...could have been generated from a single mating pair that existed $180–360 \times 10^3$ years ago...'

This raises the more general question of how far back in time pairs of extant organisms might have last shared a common female parent. One approach to an answer involves theoretical modelling of stochastic lineage extinction by using probability models of branching processes (Avise *et al.* 1984*b*). A parameter π, defined as the probability of survival of two or more independent female lineages over G generations, was monitored through time as a function of number of unrelated females initiating a population, the population size at carrying capacity, and the frequency distributions of surviving daughters. The general form of many π curves is illustrated in figure 4. For example, under the conditions specified in the figure, it is quite probable that a population founded by 1000 females will, within a few thousand generations, retain the descendants of only one foundress.

More generally, the rate of stochastic lineage extinction can be very rapid under certain biologically plausible demographic conditions (Avise *et al.* 1984*b*). Within roughly $4n$ generations (or less as the variance in progeny numbers increases), stable-sized populations started with n females or regulated about the carrying capacity ($k = n$) will be very likely to trace ancestries to a single female. In expanding populations, lineage extinction slows dramatically and π is more strongly influenced by k than by the founding n. Spatial isolation of populations will also have a dramatic dampening effect on the overall rate of lineage sorting

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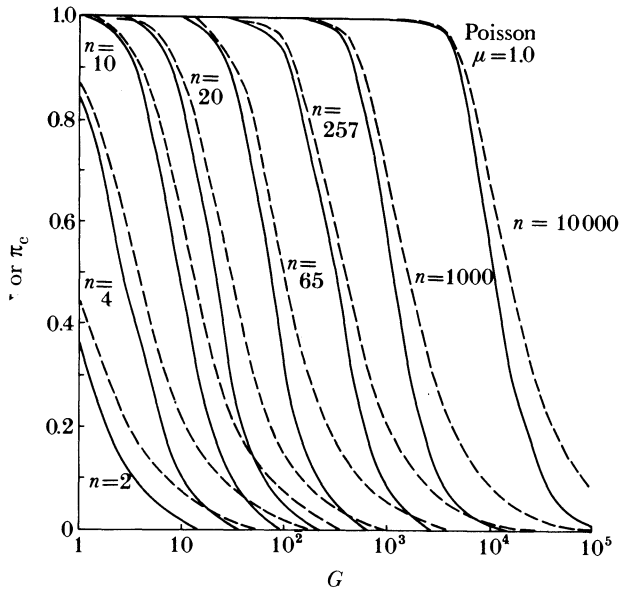

FIGURE 4. The theoretical probabilities (π) of survival of two or more female lineages through G generations in populations initiated with n females producing daughters according to a Poisson distribution with mean 1.0 (from Avise *et al.* 1984*b*). The dashed lines represent the conditional probabilities (π_c) that two or more lineages survive given that the population remains extant.

within a species, provided each population is buffered against extinction through density-dependent population growth. Although the diversity of original lineages within each population will rapidly decay, at least one lineage per extant population will be retained indefinitely. Such spatial population subdivision no doubt explains the usual empirical observation of major mtDNA differences across the range of most species.

As applied to Brown's (1980) scenario for human evolution, these theoretical models suggest that a dramatic reduction or bottleneck in absolute population size does not necessarily have to be invoked to account for the mtDNA data, even if it is true that all living humans trace to one recent female ancestor. For example, a stable-sized human population composed of 15000 unrelated females (or more as variance in progeny numbers increases beyond 1.0) will yield a value of $\pi \approx 0.5$ within 18000 generations (roughly 360000 years), owing simply to random lineage extinction associated with the vagaries of reproduction (Avise *et al.* 1984*b*). However, Brown's (1980) data do argue that human racial or population separation has been of fairly recent occurrence.

Brown (1980) also concluded that further human study would be likely to reveal some race-specific or group-specific mtDNA genotypes. While several recent studies have examined the issue, the data are still inconclusive. In a mtDNA survey of 200 humans with five restriction endonucleases, Johnson *et al.* (1983) identified 32 fragment patterns that, when analysed by parsimony methods, showed a mild but incomplete tendency to differentiate human groups. Cann *et al.* (1982, 1984; Cann & Wilson 1983) observed a total of 441 restriction sites (163 of which were variable) in a high-resolution screening of 112 humans with 12 restriction enzymes, and they also find only a weak tendency for geographical or racial structuring of mtDNA genotypes. Attempts to use certain algorithms to estimate phylogenies were hampered by the sheer mass of available data, and by the difficulty of unambiguous identification of

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parallel mutations that have probably arisen in separate lineages. None the less, Cann *et al.* (1982) suggest a mean human inter-racial divergence time of about 50000 years, based on the conventional mtDNA clock calibration of 2% per million years (Brown *et al.* 1979, 1982).

Another dramatic example of a lack of geographical differentiation in mtDNA genotype involves the American eel, *Anguilla rostrata*. A survey of samples collected from freshwater streams entering a 4000 km stretch of North American coastline from Louisiana to Maine has so far revealed no geographical differentiation at more than 80 surveyed mtDNA restriction sites (Avise *et al.* 1986). *Anguilla* has a life history that is remarkable if not unique among the vertebrates (reviewed by Williams & Koehn 1984). Juveniles inhabit coastal and inland waters until maturation, when they start an autumnal migration to the western tropical mid-Atlantic ocean, where spawning takes place. Each female may produce a million or more eggs, which hatch into leptocephalus larvae that disperse back to coastal streams, perhaps entirely through passive transport by ocean currents. As suggested by Williams & Koehn (1984), 'it is entirely possible that spawning is essentially panmictic...it means that collections of juveniles from any locality are all samples of the same breeding population'. Our mtDNA data are certainly consistent with this view; they dramatically exemplify a profound genetic consequence of this peculiar life-history. In the future, it will be most interesting to compare the effects of different life-histories on genetic structures and intraspecific phylogenies in a variety of species.

Lansman *et al.* (1981) considered another point about the use of mtDNA genotypes in studies of animal life-history and dispersal. Unlike diploid nuclear genotypes which can be altered by segregation or recombination during reproduction, mtDNA markers are transmitted intact (barring mutation); thus mtDNA analyses can more readily focus on the genealogies of particular individuals. To assess the applicability of this thesis, Kessler & Avise (1985*b*) studied fine-scale spatial and temporal mtDNA heterogeneity in a sample of 134 cotton rats (*Sigmodon hispidus*) collected from a single 3.2 Ha field. Inspection of the genotypes of individual animals allowed estimates of minimum numbers of female lines represented per nesting site, and allowed inferences about the microgeographical distributions of particular female lineages. However, an important caveat about the genealogical reconstruction was that, with respect to a given microgeographical area, shared mtDNA genotypes were not necessarily synapomorphs. In other words, individuals sharing a given genotype could represent descendants from separate immigrations from outside the area of study. In any event, it may no longer be entirely true, as lamented by Tamarin *et al.* (1983), that 'currently there are no effective field methods for determining mother–offspring relatedness in...small, secretive animals if one wishes to differentiate young from various females'.

Hybridization and introgression

Cnemidophorus neomexicanus and *C. tesselatus* are parthenogenetic species of whiptail lizards thought to be derived from past hybridization between the extant bisexual species *C. tigris* and *C. inornatus*, and *C. tigris* and *C. septemvittatus*, respectively. In one of the first applications of mtDNA analysis in evolutionary biology, Brown & Wright (1979) determined that *tigris* had probably been the maternal parent for both *neomexicanus* and *tesselatus*. *C. tigris* shares with the parthenogenetic species identical *Eco*RI and *Hin*dIII restriction site maps, which differ from those of *inornatus* and *septemvittatus*. A subsequent mtDNA and allozyme analysis showed that the direction of cross producing another parthenogenetic whiptail, *C. laredoensis*, was *C. sexlineatus* ♂ × *C. gularis* ♀ (Wright *et al.* 1983).

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The diploid, bisexual European water frog *Rana esculenta* is thought to have arisen through hybridization between female *R. ridibunda* and male *R. lessonae*, and is now maintained exclusively by hybridogenetic reproduction involving crosses of *esculenta* with *lessonae*. In this fascinating genetic system, the non-recombining *lessonae* chromosome set is excluded from germ-line cells during gametogenesis in *esculenta*, only to be restored again at the next fertilization. From behavioural observation, most matings take place between *esculenta* females and *lessonae* males, although the reciprocal matings (*lessonae* ♀s × *esculenta* ♂s) do occur at low frequency. It therefore might have been expected that *esculenta* would exhibit primarily *ridibunda*-type mtDNA, but Spolsky & Uzzell (1986) show that this is not the case. Instead, in study locales in central Europe, the great majority of *esculenta* has a *lessonae*-type mtDNA, which is easily distinguishable ($p \approx 0.08$) from the mtDNA of most *ridibunda*. The plausible interpretation of these findings was that at some point in evolution, for most of the *esculenta* lineages examined there had been at least one mating of male *esculenta* with female *lessonae*. Even if rare, such matings would act like a ratchet mechanism (somewhat analogous to unidirectional mutation pressure) in increasing the frequency of *lessonae* mtDNA in *esculenta*. In other words, following an aberrant mating, crosses in the normal direction would not re-establish the *ridibunda* mtDNA once lost from an *esculenta* lineage.

Spolsky & Uzzell (1984) also observed, in moderate frequency, a *lessonae*-type mtDNA in populations of *ridibunda*. They postulate an interspecific transfer of mtDNA from *lessonae* to *ridibunda*, probably mediated by the hybrid *esculenta*. Although *esculenta* normally cross with *lessonae*, a rare cross of an *esculenta* female with *ridibunda* male could have produced *ridibunda* progeny with *lessonae* mtDNA (Spolsky & Uzzell 1984). This is one of several reported instances (see beyond) in which the distribution of mtDNA genotypes lacks complete concordance with biological species boundaries.

The use of mtDNA markers to determine directions of crosses producing hybrids is of course not limited to parthenogenetic or hybridogenetic species. Avise & Saunders (1984) used a combination of allozyme and mtDNA markers to characterize genetically natural populations of five sympatric species of *Lepomis*, a group of fishes renowned for their propensity to hybridize. Among 277 fish assayed, 14 hybrids (all of which proved to be F_1s) were found. These hybrid fish came from crosses among various pairs of all five species, were produced preferentially between species differing greatly in abundance, and usually had a mother contributed by the rarer species in each cross. The results suggested that the absence of conspecific pairing partners and mating stimuli for females of the rarer species might be an important factor in increasing the likelihood of interspecific hybridization. The maternal markers provided by mtDNA should offer many opportunities for studies in behavioural genetics.

Avise & Saunders (1984) found no evidence for introgression among *Lepomis* at their study locales. Since the mtDNAs of the five surveyed species were highly distinct (estimated $p > 0.08$ in all cases), any introgressed mtDNA markers would have been readily identifiable. For species separated by large genetic distances, the linkage of markers imposed by the mode of mtDNA transmission should generally offer a better chance to distinguish effects of low-level introgression from symplesiomorphy or character convergence (Avise & Saunders 1984).

Other studies *have* found 'foreign' mtDNA genotypes within a species, attributed to invasion through introgressive hybridization. Ferris *et al.* (1983*b*) observed that Scandinavian house mice *Mus musculus* contain mtDNA genotypes very different ($p \approx 0.05$) from those of *musculus* elsewhere in mainland Europe, but very similar to the mtDNA of neighbouring *domesticus*. However, electrophoretic and immunological analyses of proteins confirmed that the Scan-

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dinavian *musculus* are allied in nuclear genotype to *musculus* elsewhere, and are quite distinct from adjacent *domesticus*. *M. musculus* and *domesticus* are known to hybridize in a region of central Denmark. Ferris *et al.* (1983*b*) postulate that colonization of Scandinavia by even a single successful *domesticus* female could have introduced the foreign mtDNA type via hybridization with *musculus* males. While the nuclear genome would have become enriched with *musculus* DNA with each successive backcross generation, the spreading *domesticus* mtDNA would have remained intact. Ferris *et al.* (1982) report another such 'takeover' event via introgression in which a laboratory strain of *M. molossinus* has acquired *domesticus*-type mtDNA within the recent past.

A less convincing case for interspecific mtDNA gene flow was presented for *Drosophila pseudoobscura* and *D. persimilis* by Powell (1983). Where these species occur sympatrically, most individuals had identical mtDNA genotypes as gauged by restriction fragment patterns for eight endonucleases. Allopatric samples of *pseudoobscura* usually differed by two or three restriction sites from the common genotype in sympatry. This finding was attributed to interspecific hybridization preventing divergence of mtDNA in sympatry. However, since mtDNA distances in the entire study were small, sampling errors in rank-ordering of p, or patterns of phylogenetic sorting of female lineages independent of introgression (see Speciation) could not, by hard criteria, be eliminated as alternative explanations.

These several reports of introgressive exchange of mtDNA between species have understandably generated considerable excitement because of their potential general significance to evolutionary biology (Lewin 1983). Barton & Jones (1983) suggest that if mtDNA can commonly '...stray through taxonomic barriers (it)...may obscure the evolutionary history of diverging populations...' However, if the above scenarios are correct, introgressive hybridization *is* a very important component of the evolutionary history of many diverging species. Additional comparative studies of the phylogenetic association between mtDNA and nuclear DNA, and of possible patterns of reticulate evolution, should be most revealing.

Speciation

Another case of apparent discordance between biological species boundary and mtDNA genotype has been reported for sibling species of deer mice, *Peromyscus maniculatus* and *P. polionotus* (Avise *et al.* 1983). An analysis of 106 mapped mtDNA restriction sites from 203 animals suggested that some populations of *maniculatus* are genetically closer to *polionotus* than they are to other geographically separated populations of *maniculatus*. In this instance, the geographical ranges of the two species do not overlap, and there is no compelling reason to suppose that hybridization and introgression have ever taken place. As an alternative explanation, it was proposed that the phylogenetic sorting of mtDNA lineages during speciation might alone be responsible for the pattern. In principle, as shown in figure 5, two species can show any of three phylogenetic relationships with respect to one another: monophyly, polyphyly or paraphyly. By using this terminology, *maniculatus* appears to be paraphyletic with respect to *polionotus* in matriarchal ancestry; *polionotus* appears monophyletic with respect to *maniculatus*, but forms a subclade within the larger *maniculatus–polionotus* assemblage. This scenario, developed from the mtDNA data, is perhaps not inconsistent with conventional thought since *maniculatus* probably represents the ancient, geographically central evolutionary stock from which *polionotus* budded off via peripheral isolation.

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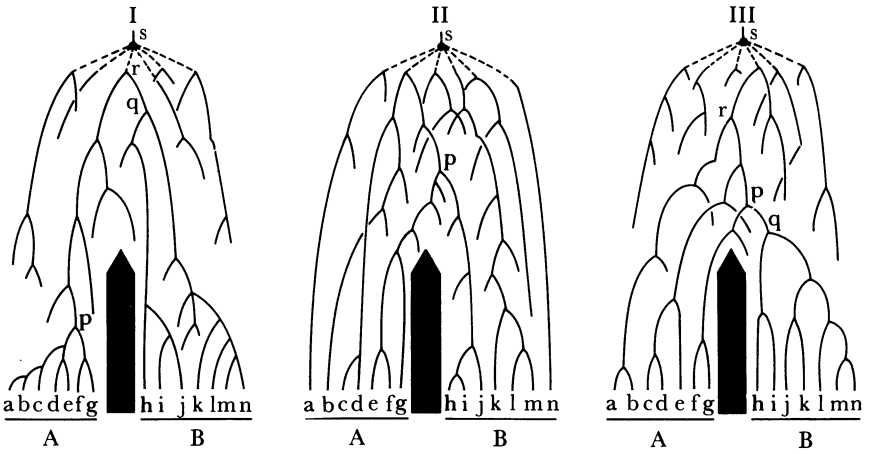

FIGURE 5. A diagrammatic representation of the possible mtDNA phylogenetic relationships of sibling species A and B (from Avise *et al.* 1983). The solid dark bars indicate barriers of reproductive isolation. In phylogenetic status I, A and B are both monophyletic in matriarchal genealogy; in status II, both species are polyphyletic; and in status III, A is paraphyletic with respect to B.

Neigel & Avise (1985) have recently extended the theoretical models of stochastic extinction of matriarchal lineages (Avise *et al.* 1984b; see Demography) to encompass speciation events. Computer simulations of the sorting of female lineages across speciations were used to generate expected probabilities of monophyly, polyphyly, and paraphyly of species-pairs under a variety of demographic conditions. An example of the kind of results obtained is shown in figure 6. Under the particular conditions specified in the figure, the probability is high that sibling species will be polyphyletic in matriarchal ancestry for roughly 2–4 k generations following speciation (k is the carrying capacity of each daughter species). Only later, as lineage sorting through random extinction continues, does the probability greatly increase that the sibling species will appear monophyletic with respect to one another. More generally, the qualitative conclusions from the simulations were as follows: (i) the phylogenetic distributions of

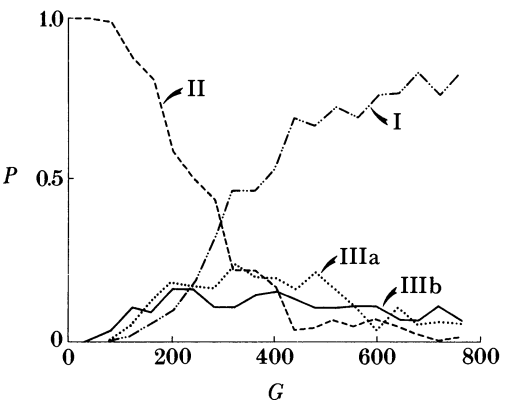

FIGURE 6. An example of computer output monitoring the probabilities (P) of a given phylogenetic status for a pair of species G generations following a simulated speciation (from Neigel & Avise 1985). Status I, both species monophyletic; status II, both species polyphyletic; status IIIa, species A paraphyletic with respect to species B; status IIIb, B paraphyletic with respect to A. In each of 100 replicate simulations, the daughter species A and B were founded by 30 and 20 individuals, respectively, drawn at random from a parental population. The daughter species were each allowed to grow to carrying capacity $k = 200$.

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matriarchal lineages can lack concordance with species boundaries, particularly when speciations have been very recent; (ii) the phylogenetic status of a pair of species is itself evolutionarily dynamic, with a common time-course of changes following speciation being polyphyly → paraphyly → monophyly; (iii) the demographic conditions associated with speciation exert an important influence on the expected phylogenetic status of related species.

By using a different, mathematical, model, Tajima (1983) also concluded that closely related species can exhibit a poly- or paraphyletic relationship in mtDNA sequence, even in the complete absence of secondary hybridization and introgression. These computer simulations and mathematical models were not intended to challenge or discredit all earlier conclusions that introgression has accounted for situations where mtDNA genotypes lack concordance with species' boundaries. Indeed, it is unlikely that such discordancies can be attributed solely to phylogenetic sorting when the speciation event was fairly old (as might be evidenced, for example, by large mtDNA distances between most of the taxa under study). None the less, it is clear that discrepancies between species boundary and mtDNA phylogeny do not by themselves necessarily signal effects of secondary hybridization and introgression, and the alternative possibility of phylogenetic sorting should be considered in evaluations of empirical data.

Fort *et al.* (1984) describe another type of apparent anomaly in which two sibling species of *Mus* (*spicilegus* and *spretoides*) that are very close in nuclear allozyme frequencies are far more divergent in sequenced ribosomal mtDNAs than would have been predicted from supposed time of speciation. They propose that, in contrast to the divergence of allele *frequencies* at nuclear loci, which began, perhaps, at the onset of reproductive isolation, mtDNA *sequence divergence* was initiated with clonal separation vastly predating the speciation event. Since mtDNA sequences can be, in principle, much older than the species being compared, Fort *et al.* (1984) raise the possibility that nucleotide substitution rates for mtDNA may be considerably over-estimated.

Apart from their use as neutral markers of demographic and phylogenetic conditions associated with speciation, it is also conceivable that mtDNA genotypes may in some cases contribute directly to the origin or maintenance of reproductive isolating barriers. For example, a possible incompatibility between nuclear and mitochondrial genomes could be responsible for reproductive isolation between two species of tobacco budworms. Crosses between *Heliothus virescens* and *H. subflexa* produce sterile male hybrids, and the hybrid sterility is maintained in recurrent backcrosses of the fertile hybrid females to *virescens*. Lansman *et al.* (1983*b*) show that in male-sterile strains derived from 91 generations of unidirectional backcrossing, far more than 99% of the nuclear genome is derived from *virescens* while the mtDNA genotype remains exclusively that of *subflexa*. The reproductive isolation might also, however, result from other cytoplasmically inherited factors (micro-organisms), as has been shown for some dipteran and coleopteran insects (Wade & Stevens 1985).

Additional indirect evidence for incompatibilities between nuclear and mitochondrial genotypes comes from cultured somatic cell hybrids, in which chromosomes and mtDNA from the same parent species tend to be lost (de Francesco *et al.* 1980). On the other hand, the several case histories discussed above (see Hybridization and introgression) suggest that mtDNA genotypes may readily be able to traverse species boundaries through introgression. Barton & Jones (1983) conclude that 'mitochondrial DNA is able to penetrate the boundaries between

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species because it is not closely linked to genes responsible for maintaining reproductive isolation'. Clearly a great deal remains to be learned about the role that interactions between nuclear and mitochondrial genomes may play in speciation, and how these possible selective interactions could influence the phylogenetic distributions of mtDNA sequences.

Conclusion

Most of the conceptual models and data analyses reviewed in this paper have adopted the working hypothesis that the assayed mtDNA genotypes are effectively neutral markers of female lineages. To 'selectionists' and those interested in the study of adaptations, neutral variation is often viewed as irrelevant evolutionary 'noise'. But to 'neutralists' or those primarily interested in phylogeny reconstruction, adaptations can be a source of 'noise' drowning out the signal of evolutionary history and relationship. A broader perspective might welcome the integration of information from the distributions of both neutral and adaptive traits. As phrased by Selander & Whittam (1983), 'Natural selection for local adaptation can conceal the genetic history of subdivision', but '...in the absence of historical information, the interpretation of differentiation in terms of evolutionary factors is a difficult undertaking at best'. If most of the abundant assayable mtDNA polymorphism within and among closely related species does indeed prove to be selectively neutral, the study of the distribution of mtDNA genotypes may serve as a sort of historical 'null hypothesis' against which to evaluate the distributions of other characters. For example, since particular morphological forms within *Peromyscus maniculatus* bear little or no correspondence to the distribution of mtDNA genotypes (which presumably reflect matriarchal phylogeny), it seems likely that these morphologies represent adaptations that do not reliably indicate evolutionary relationship (Lansman *et al.* 1983*a*).

Admittedly, this review may be premature. I have attempted to paint, with a broad brush, an outline of evolutionary phenomena particularly amenable to analysis by mtDNA, yet only a small handful of examples is currently available to illustrate each topic. None the less, there is already a tangible excitement stemming from the findings of population surveys of mtDNA variation. For the first time, it is possible to estimate empirically matriarchal phylogeny; to determine directionality in natural crosses producing hybrids, and to relate it to the behaviours and morphologies of the species involved; and to study the population genetic consequences of varying female demographies and life histories. The data obtainable from mtDNA may be especially well suited for studies of population genetic structure, dispersal, and historical zoogeography. One unanticipated class of observations – the apparent frequency of interspecific transfer of mtDNA via hybridization and introgression – could, if verified and extended to other organisms, have profound implications for general evolutionary theory. It has usually been supposed that reticulate evolution is rare in higher animals, but this thesis may now require re-examination. Even a modest rate of interspecific exchange of genetic material could perhaps rival or surpass mutation as a source of novel genetic variation in a species.

The female-mediated, clonal transmission of mtDNA is also stimulating new ways of thinking about times to common ancestry of asexual lineages within otherwise sexually reproducing populations; about the possible relevance of mtDNA-nuclear DNA interactions to reproductive isolation; about the demographic influences on phylogenetic sorting of asexual lineages across speciations; and about the very meaning of the phylogenetic status of related species with

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respect to particular kinds of genetic characters. Overall, the extensive and assayable polymorphism of the mtDNA molecule is providing many exciting opportunities for the empirical and conceptual study of organismal evolution.

The mtDNA research of this laboratory has been supported by N.S.F. grants.

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Discussion

D. Ellis. (*The Galton Laboratory, Department of Genetics and Biometry, University College London.*) Is it necessary to assume, as was stated by Dr Avise, that mtDNA evolves neutrally for it to be of value in many areas of population genetics? For example, in historical population demography, it would be as interesting to demonstrate selection as to show species substructuring. Low sequence diversity can result both from founding events or bottlenecking, and from selection, In principle, these alternatives may sometimes be distinguished by comparing the relative frequency of the particular mtDNA form within populations in nearby, ecologically similar areas. Is neutrality crucial?

J. C. Avise. If mtDNA genetic markers are under selection, the modes of selection and their intensities of course become of immediate interest. An understanding of such selection pressures would be of direct value to population genetics, and as well might provide predictions about expected geographical and phylogenetic distributions of mtDNA genotypes. The point is that the interpretations about distributions of genetic markers may well differ depending upon whether such markers are neutral or selected.

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