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Mitochondrial DNA and two perspectives on evolutionary genetics

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This essay reviews comparative studies of animal mitochondrial DNA (mtDNA), with emphasis on findings made and ideas developed at Berkeley. It argues that such studies are bringing together two previous paths of progress in evolutionary biology. One path is that of those who worked far above the species level and were concerned with genealogical trees, time scales and the accumulation of new mutations on surviving molecular lineages. The other path is that of those who worked at and below the species level and were concerned mainly with population structure, migration and the frequencies of alleles that existed in an ancestral population. This fusion of paths is made possible by the high rate at which mutations accumulate on mtDNA lineages and by this molecule's uniparental and apparently haploid mode of inheritance. These properties make mtDNA a superb tool for building trees and time scales relating molecular lineages at and below the species level. In addition, owing to its mode of inheritance, mtDNA is more sensitive to bottlenecks in population size and to population subdivision than are nuclear genes. Joint comparative studies of both mtDNA and nuclear DNA variability give us valuable insights into how effective population size has varied through time. Such studies also give insight into the conditions under which mtDNA from one species can colonize another species.

KEY WORDS—Restriction fragments – cleavage maps – DNA sequences – primates – rodents – *Equus* – *Gallus* – frogs – *Salmo.*

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CONTENTS

Introduction 376
Protein studies: temporal and population perspectives 377
mtDNA and both perspectives 379
Properties of animal mtDNA 379
Molecular structure and function 379
Mutation pressure and inefficient repair 380
Freedom from error catastrophe 380
Transmission genetics 381
Maternal inheritance 381
Homoplasmy and transient heteroplasmy 383
Recombination 384
Methods of comparison 384
Restriction enzymes 384
Restriction maps 385
Sequencing of cloned regions 385
Evolutionary implications 388
Rate of divergence 388
Accelerated coevolution 388
Temporal frameworks 388
Population size 391
Bottlenecks 391
Do founder effects accelerate mtDNA evolution? 392
Bottleneck during human evolution? 393
Differential introgression 394
Prospects 396
Acknowledgements 397
References. 397

INTRODUCTION

By 1979, it was clear that the mitochondrial (mt) DNA of animals was becoming the best known piece of the eukaryotic genome and would be of great interest to evolutionary geneticists because of its high rate of evolution (Table 1), unisexual mode of inheritance and high copy number. Since then, many laboratories that previously used proteins as a tool for studies of evolutionary genetics have become productively engaged in comparative studies of mtDNA. The present article reviews knowledge of the genetic properties of mtDNA, methods for comparing closely related mtDNAs, and evidence about the high rate of evolution of mtDNA. It also considers the potential value (and limitations) of mtDNA as a tool for building genealogical trees and time scales as well as for detecting bottlenecks in populations and tracing migrations.

Table 1. Relative rates of DNA evolution*

Type of mutation	Mitochondrial		Plastid	Nuclear
	Animal	Plant		
Point	High	Low	Low	Low
Length†	High	Low	Low	Low

*Sources: Brown *et al.* (1982), Cann & Wilson (1983), Clegg *et al.* (1984), Chao *et al.* (1984), A. C. Wilson, unpublished calculations.

†Deletions and additions in non-coding regions.

In addition, this article emphasizes the view that what proteins did for evolutionary studies above the species level, mtDNA may be able to do for the study of evolution at and below the species level. This point may be unfamiliar to many evolutionary geneticists who work mainly at the population level. To explain it, we review briefly how early comparative studies of proteins led to two contrasting perspectives on evolutionary biology.

Protein studies: temporal and population perspectives

Sequence evolution above the species level: Long before protein electrophoresis had become a tool for studies below the species level, comparative biochemists had searched for differences between species in the amino acid sequences of proteins like insulin, cytochrome *c*, ribonuclease and haemoglobin. Since the species compared (e.g. cow with human or horse) were distantly related, it was reasonable for these workers to ignore the small contribution of intraspecific polymorphism and gene flow to the sequence differences observed (Fig. 1), i.e. to ignore the realm of population genetics. The first generalization of evolutionary interest to emerge from these distant comparisons was that many of the amino acid substitutions accumulating as species diverge are functionally inconsequential or nearly so (Anfinsen, 1959). Quickly following this finding was the recognition that substitutions accumulate at fairly steady rates in proteins like cytochrome *c*, haemoglobin and albumin (Zuckerkandl & Pauling, 1962; Margoliash, 1963; Williams, 1964; Doolittle & Blombäck, 1964; Wilson *et al.*, 1964; Sarich & Wilson, 1967a).

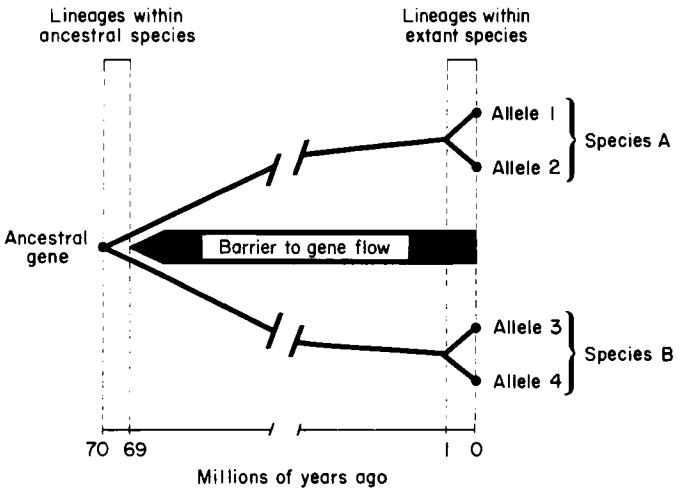

Figure 1. Molecular lineages leading from a common ancestral gene to alleles present in two extant species, A and B. The alleles within each species are assumed to have descended from a common ancestral allele 1 million years (Ma) ago. A similar extent of allelic divergence is assumed to have existed in the common ancestral species 69 Ma ago; thus the alleles within the ancestral species trace back to a common ancestral gene existing about 70 Ma ago. The point is that allelic variation can be virtually ignored, if the goal is to measure the mutational distance between the amino acid sequence of a protein picked at random from a species (A) and its homologue in another species (B) that diverged 69 Ma ago. The reason is that allelic variation is likely to contribute only 1 part in 70 to the observed distance.

The evidence that proteins behave as approximate evolutionary clocks came from direct sequencing methods as well as immunochemical methods and affected evolutionary biology in two ways. First, it led population geneticists to develop the neutrality hypothesis (Kimura, 1968; King & Jukes, 1969). Secondly, it led to the realization that chemically and immunologically estimated distances could give evolutionary biology above the species level a quantitative temporal framework. This new, explicitly temporal perspective had implications not only for reconstructing the evolutionary history of particular taxonomic groups but also for testing ideas about the genetic basis of evolutionary change. Accordingly, immunological distances and amino acid sequence differences were used to build trees and estimate divergence times for numerous taxonomic groups with poor or incorrectly interpreted fossil records (see, for example, Sarich & Wilson, 1967b; Sarich & Cronin, 1976; Beverley & Wilson, 1984). The next step was to use the temporal information provided by both protein clocks and fossils to fuel a new endeavour, that of estimating rates of evolution at supramolecular levels of biological organization. Comparison of rates of karyotypic evolution, anatomical evolution and speciation in various taxonomic groups allowed testing of old ideas and the generation of new ones about those factors, including population size and structure, which determine evolution's rate (Wilson *et al.*, 1977b; Wyles, Kunkel & Wilson, 1983; Larson, Prager & Wilson, 1984; Wilson, 1985).

Electrophoretic variability below the species level: By contrast, a temporal perspective had been largely lacking in the evolutionary biology practised by population geneticists, ecological geneticists and 'new systematists' (e.g. Huxley, 1942; Dobzhansky, 1951; Simpson, 1953; Mayr, 1963). The fossil record is generally poor for the closely related populations or species with which most of these biologists worked. For a typical pair of species within a genus, for example, one could not estimate from fossil evidence whether their last common ancestor lived one million or ten million years ago. This level of uncertainty was so high that biologists of this type rarely based their evolutionary hypotheses on assumptions about times of divergence. Their perspective did not change radically when protein electrophoresis entered the field; rather, the focus stayed on gene frequencies and levels of variation within and between populations that exchanged genes (Lewontin, 1974).

It is true that the distance concept, generated by protein chemists and immunochemists who compared distantly related species, spread to those electrophoretic workers who compared the proteins of closely related species (Nei, 1975). These genetic distances were then calibrated against immunological and sequence estimates of protein divergence (King & Wilson, 1975; Sarich, 1977) and used to calculate divergence times. However, because the electrophoretic method is most suitable for comparing recently diverged species, the distances measured by this method are often dominated by differences in gene frequencies (Nei, 1975). Hence, sequence divergence (due to new mutations) can be confounded with changes in the frequency of alleles that pre-existed in the ancestral species. The relation of such frequency changes to time of divergence has not been investigated empirically. It is only sequence divergence which has been shown empirically to have a relatively simple dependence on time (Wilson, Carlson & White, 1977a).

For this reason, evolutionary biologists who work at or below the species level

continue to view evolution mainly as a process of changing gene frequencies. Because these frequencies are not expected to change at constant rates, much effort has been aimed in the traditional direction of finding out how they are affected by natural selection, genetic drift, gene flow and changes in population size. The power of electrophoresis to examine many proteins at once made this technique an extremely attractive one for measuring gene frequencies and analysing the genetic structure of populations. This gene-frequency perspective was dictated by the fact that the 'signal' due to the accumulation of new mutations was dominated by the 'noise' generated by the variation in frequencies of older alleles. It is understandable, then, that evolutionary geneticists who work mainly at or below the species level with the aid of protein electrophoresis have been unable to adopt the empirical goals of those who use immunological and sequence comparisons of proteins to study evolution above the species level.

mtDNA and both perspectives

Comparative studies of mtDNA allow simultaneous progress toward both of these goals. Populations, and even species, exchange intact mtDNA molecules in ways that can be modelled (Takahata & Palumbi, 1985). Yet each mtDNA molecule carries in its sequence the history of its lineage, not complicated by recombination. This paper explains what gives mtDNA this dual character and illustrates the applicability of mtDNA studies to evolutionary and genetic questions.

PROPERTIES OF ANIMAL mtDNA

Molecular structure and function

Animal mtDNA became the best known piece of eukaryotic DNA because it is easier to purify than any specific segment of nuclear DNA. This ease of purification is the result of an unusual buoyant density, a high copy number, and occurrence in an organelle other than the nucleus. mtDNA is also easy to characterize because it is small and lacks many of the complicating features of nuclear DNA (such as introns and repetitive sequences).

From the standpoint of suitability for comparative work, mtDNA has the virtue of being distributed universally in the animal kingdom and being remarkably uniform in gene content. The mtDNAs of all multicellular animals tested and some protozoans seem to have the same set of 37 genes specifying 22 tRNAs, 13 mRNAs, and 2 rRNAs (De La Cruz, Neckelmann & Simpson, 1984). These genes are tightly packed in about 15 kilobases of double-stranded DNA and their arrangement is very stable. Changes in gene order have been encountered only in comparisons of different phyla (Roberts *et al.*, 1983; De La Cruz *et al.*, 1984; Clary & Wolstenholme, 1984). The types of evolutionary change that animal mtDNA undergoes are relatively simple, being mainly base substitutions and length mutations (Table 1), the latter accumulating predominantly in the small non-coding regions.

Although mtDNA specifies the structures of only a small fraction of the macromolecules in a mitochondrion, some of the functions they serve are extremely important to the organism (cf. Fig. 5, below). We refer especially to

their role in energy-yielding metabolism and protein synthesis within the mitochondrion and allude as well to their possible contribution to the structure of the cell surface. Since the functions of all parts of the mitochondrial genome are under intense scrutiny by biochemists and cell biologists, animal mtDNA continues to provide perhaps the best hope of linking genetic change to organismal change, which is a prerequisite for understanding the genetic basis of evolutionary change at the organismal and population levels.

Several reviews are available on the molecular biology of the mitochondrion and its DNA (Clayton, 1982, 1984; Gray & Doolittle, 1982; Wallace, 1982; Brown, 1983; Yaffe & Schatz, 1984; Sederoff, 1984; Fischer Lindahl, 1985) as well as on the population genetics of mtDNA (Avise & Lansman, 1983; Birky, Maruyama & Fuerst, 1983; Takahata, 1984).

Mutation pressure and inefficient repair

The property that is probably most relevant for understanding why mtDNA evolves fast is the mitochondrion's apparent inefficiency (relative to the nucleus) as regards repairing replication errors and DNA damage (Brown, George & Wilson, 1979; Brown *et al.*, 1982; Clayton, 1982, 1984). The effective mutation rate is thus higher for mtDNA than for nuclear DNA. It follows that the evolutionary rate will be higher for mtDNA. The basic equation of evolution is $E = MF$, where E is the evolution rate, M is mutation rate per population, and F is the fraction of mutations fixed (Wilson *et al.*, 1977a). Hence the evolution rate is expected to be proportional to the mutation rate.

Consistent with the view that the high rate of mtDNA evolution is due to inefficient repair is the peculiarly high incidence of length mutations and transitions in relation to transversions (Cann & Wilson, 1983) that accumulate in mtDNA over evolutionary time. The mismatch repair system of bacteria (Glickman, 1979) and yeast nuclei (R. Kolodner, pers. comm.) is specifically designed to repair length mutations and transitions, which are the commonest types of replication error (Topal & Fresco, 1976). Microbial mutants lacking mismatch repair have a mutational spectrum like that observed during the evolution of mammalian mtDNA.

Freedom from error catastrophe

A second property that could be important for understanding the high rate of evolution of mtDNA is that mtDNA seems not to code for *proteins* involved directly in its own replication, transcription or translation (Clayton, 1982, 1984; Sederoff, 1984; Chomyn *et al.*, 1985). If so, its translation apparatus should be freer, as Fig. 2 implies, to be inaccurate than is the conventional translation apparatus in the cell sap. Less accuracy is expected to be tolerable in a small system that synthesizes, as the mitochondrion does, only 13 kinds of polypeptide chains (Cann, Brown & Wilson, 1982, 1984).

Consistent with this view are two additional observations about the evolution of mtDNA. First, its genetic code drifts, which implies a tolerance for inaccuracy during the switch from one code to another (Cann *et al.*, 1982). Secondly, the 22 tRNAs and two rRNAs specified by mtDNA evolve much more than 10 times faster than their nuclear counterparts. In contrast, the factor by which evolution

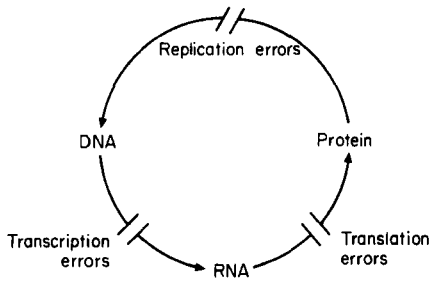

Figure 2. An error catastrophe loop for the three classes of proteins involved in the synthesis of DNA, RNA and proteins. This loop is intact for nuclear DNA, which encodes all three classes. By contrast, the mtDNA of animals appears not to encode any of these classes, so that the loop is apparently broken for mtDNA. In consequence, there is expected to be *stronger* selection pressure for accuracy in the conventional translation apparatus (in the cell sap), which makes proteins required for synthesis of DNA, RNA and proteins, than for accuracy in the mitochondrial translation apparatus, which appears not to make such proteins (Hasegawa *et al.*, 1984; Chomyn *et al.*, 1985).

at silent sites in the protein-coding regions is accelerated in mtDNA relative to nuclear DNA is barely 10-fold (Brown *et al.*, 1982; Cann *et al.*, 1984).

In summary, the high rate of mtDNA evolution seems to be a consequence of two forces, a general one (probably mutation pressure) affecting the whole base sequence and a specific one (relaxed constraints on components of the translation apparatus) affecting primarily the tRNA and rRNA genes (Cann *et al.*, 1984).

Transmission genetics

The two properties of most concern to population geneticists are the maternal mode of inheritance and apparent haploidy of mtDNA. In a typical mammalian individual, an average somatic cell contains thousands of mtDNA molecules. The corresponding number in a mature egg is about 10^5, while in the midpiece of a sperm there are about 50 mtDNAs (Hecht *et al.*, 1984). Yet, all of the mtDNA molecules in a typical individual appear to be identical (Potter *et al.*, 1975; Brown, 1980; Monnat & Loeb, 1985). The vast majority of individuals tested seem effectively haploid as regards the number of types of mtDNA transmitted to the next generation (although polyploid as regards the number of mtDNA copies per cell). If it were true that the sperm contributes no mtDNA to the next generation and the mtDNA population within the egg is homogeneous, mtDNA would in principle be a marvelous genealogical tool. As discussed below, we do not have proof that either of these propositions is strictly correct. Nevertheless, a theory is emerging that makes it understandable why both propositions should be correct (Palumbi & Wilson, unpubl.).

Maternal inheritance

The most thorough test for paternal inheritance of mtDNA in mammals (see Fig. 3) involved extensive backcrossing of female interspecific hybrids to males of the paternal species (Gyllensten, Wharton & Wilson, 1985). The species were *Mus domesticus* and *M. spretus* and reciprocal tests were done. In one case, an

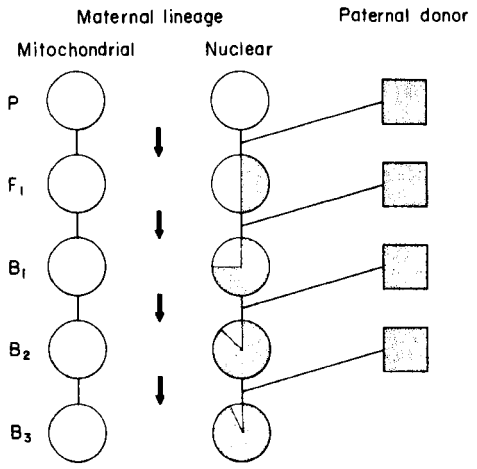

Figure 3. The concept of strictly maternal inheritance of mtDNA and biparental inheritance of nuclear DNA during backcrossing of a lineage derived from a female of one species (open symbols) to males from a second species (stippled symbols). P, parental generation; F_1, first filial generation; B_1–B_3, backcross generations. After three generations of backcrossing, the offspring carry predominantly the nuclear genes of the paternal species but exclusively the mitochondrial genes of the maternal species. Adapted from Gyllensten *et al.* (1985).

interspecific (F_1) female lineage initially bearing *domesticus* mtDNA was backcrossed for five generations to *spretus* males. In the other case, an interspecific (F_1) female lineage initially bearing *spretus* mtDNA was backcrossed for seven generations to *domesticus* males. The mtDNAs of these two species are readily distinguishable by restriction enzyme analysis (Ferris *et al.*, 1983c). No paternal contribution was detected in either case. Considering the sensitivity with which one mtDNA can be detected in the presence of the other, Gyllensten *et al.* (1985) concluded that the father contributes no more than one mtDNA per thousand maternal mtDNAs to the zygote.

A more extensive backcrossing test was done with a pair of moth species (Lansman, Avise & Huettel, 1983b). After 91 generations of backcrossing in one direction only, no paternal contribution was evident. There are two possible problems with this test. First, since reciprocal tests were not done, one cannot exclude the possibility of a replicative advantage for the maternal type. Secondly, in contrast to the mouse case, male progeny with the mtDNA of the maternal species and nuclear DNA from the paternal species are consistently sterile even after 91 generations of backcrossing (Makela & Huettel, 1979), which indicates a nucleo-cytoplasmic incompatibility.

A weakness in both the mouse and the moth tests is the implicit assumption that the effective size of the population of mtDNA molecules within a lineage of female germ cells is large, i.e. of the order of 10^5. If this assumption is correct, it is best to maximize the number of generations of backcrossing, so as to build up a detectable level of paternal mtDNA, as shown in Fig. 3. If, however, the effective number of replicating mtDNA molecules is low, and this is especially likely in mammals (Palumbi & Wilson, unpubl.), it is best to maximize the number of progeny tested. The tests conducted, so far, looked at too few progeny to rule out the possibility of a paternal contribution to mtDNA inheritance.

Homoplasmy and transient heteroplasmy

Individuals bearing two closely related types of mtDNA seem to occur rarely. Several cases exist in laboratory strains of *Drosophila mauritiana* (Solignac *et al.*, 1984), in dairy cattle (Hauswirth & Laipis, 1982; Olivo *et al.*, 1983; Hauswirth *et al.*, 1984), and in lizards (Densmore, Wright & Brown, 1985) and crickets (Harrison, Rand & Wheeler, 1985). Weak indications of heteroplasmy exist for some humans (Greenberg, Newbold & Sugino, 1983; Monnat & Loeb, 1985; Cann & Stoneking, unpubl.), rats (Brown & DesRosiers, 1983), and trout (Gyllensten, Wilson & Ryman, unpubl.). In theory, such states could arise by mutation or paternal contribution.

As shown in Fig. 4, segregation of the two types of mtDNA, A and B, present in one ancestral germ line could be expected to occur independently along different descendant lineages. In the example shown, B is lost along the lineages leading to individuals 1 and 3, while A is lost on the lineage leading to individual 2. This would have grave consequences for genealogical analysis. The tree based on mtDNA comparisons of individuals 1, 2 and 3 would link individuals 1 and 3 more closely despite the fact that individuals 1 and 2 are closer in terms of maternal genealogy.

The magnitude of this potential problem depends on the incidence of paternal contributions, on how long the heteroplasmic state persists, and on the resolving power of the method of mtDNA comparison. The persistence time in *Drosophila* seems to be about 400 generations (Solignac *et al.*, 1984). Given that heteroplasmic individuals seem rare in mammals and that the biggest divergence between the two coding sequences in heteroplasmic cattle is roughly 0.1% (Olivo *et al.*, 1983; W. Hauswirth, pers. comm.), we estimate that this

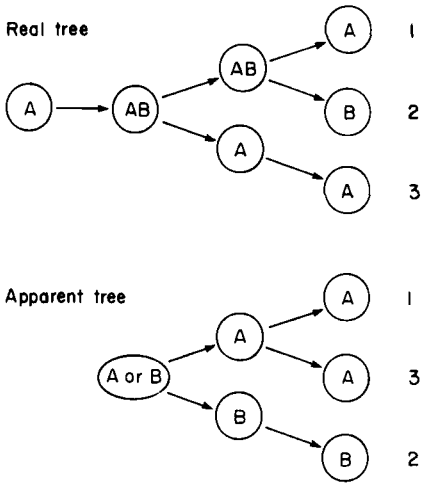

Figure 4. The possible effect of heteroplasmy on genealogical analysis. The true sequence of events relating individuals 1, 2 and 3 appears in the upper panel: first, in the ancestral lineage A mutates to B producing a mixed population of A and B; secondly, in one descendant the heteroplasmic state persists while in the other descendant lineage (leading to 3), B is lost; thirdly, the heteroplasmic descendant then produces two descendants (1 and 2), one of which (1) has lost B and the other of which (2) has lost A. The lower panel shows the result of a genealogical analysis, performed on the mtDNAs of the descendants; it groups individuals 1 and 3 because they both have mtDNA of the A type. In reality, however, 1 and 2 are related more closely by descent.

state usually persists for less than 50 000 years. This estimate is based on the assumption that sequence divergence is at the rate of 2% per Ma (see below) and that the second type was not introduced more recently by sperm.

Recombination

The last genetic property of interest from the standpoint of using mtDNA as a tool for population genetic studies is recombination. The consensus at present is that recombination does not happen in animal mtDNA except perhaps in the displacement loop (Olivo *et al.*, 1983). This relative lack of recombination enhances the attractiveness of mtDNA as a genealogical tool and may contribute to its high rate of evolution through facilitating the fixation of mildly deleterious mutations by hitch-hiking (Cann *et al.*, 1984).

METHODS OF COMPARISON

Restriction enzymes

Restriction enzymes are particularly valuable for comparing closely related mtDNAs. Table 2 draws attention to four restriction methods. The method of highest resolving power (I) uses a set of about 10 four-base enzymes. Each such enzyme recognizes a specific sequence of four bases and cuts mtDNA wherever that recognition site occurs. The resulting fragments of mtDNA are made detectable by attaching radioactive phosphate to their ends. A typical four-base enzyme cuts vertebrate mtDNA into about 25 fragments, most of which are separable from one another by electrophoresis through a long (40 cm) polyacrylamide gel (Brown, 1980; Ferris, Sage & Wilson, 1982; Ferris *et al.*, 1983a, b, c; Cann & Wilson, 1983; Cann *et al.*, 1982, 1984). The fragment patterns, made visible with X-ray film, are then compared for different mtDNAs that have been treated with the same enzyme. Differences between the fragment patterns are usually due to base substitutions that cause restriction sites to be gained or lost but, sometimes, are the result of length mutations (Brown & DesRosiers, 1983; Cann & Wilson, 1983; Ferris *et al.*, 1983c; Greenberg *et al.*, 1983; Cann *et al.*, 1984). The four-base, end-labelling method (I) is capable of distinguishing between mtDNAs that differ by less than 0.05% in base sequence when a set of 10 such enzymes is used.

Six-base enzymes are also valuable for studies of mtDNA variation (see

Table 2. Four restriction methods of comparing mtDNAs

Method	Way of detecting DNA fragments	Type of restriction enzyme	Resolving power	Approximate extent of mtDNA divergence detectable (%)*	Corresponding time span† (years)
I	Radioactive labelling	4-base	High	0.05	25 000
II	Radioactive labelling	6-base	Intermediate	0.25	125 000
III	Southern blotting	6-base	Low	0.5	250 000
IV	Ethidium staining	6-base	Low	0.5	250 000

*Assuming the use of about 10 restriction enzymes.
†Assuming that the rate of divergence is 2% per Ma.

Table 3. Relative value of fragment patterns and cleavage maps for mtDNA comparisons

Feature	Fragment patterns	Cleavage maps
Effort and expertise needed	Less	More
Accuracy of distance estimates	Low	High*
Accuracy of genealogical trees	Low	High†

*Corrects for length mutations.

†Corrects for problem of fragments having the same size but coming from different parts of the mtDNA.

methods II, III, and IV, Table 2). Six-base methods require less technical expertise and have less resolving power because the number of six-base sites in mtDNA is usually only about three or four per enzyme. Moreover, some of the ways of detecting the fragments produced by six-base enzymes (e.g. ethidium staining and Southern blotting) ignore fragments less than 250 bases long. It is hard with the latter methods (III and IV) to distinguish between mtDNAs that differ at fewer than 0.5% of their base pairs with a set of 10 six-base enzymes.

Restriction maps

Table 3 helps to make the point that the relative locations of sites can be determined by comparing the fragments produced by digesting mtDNA with mixtures of two six-base enzymes to those produced by each enzyme alone. The resulting restriction maps improve the accuracy with which estimates of degree of sequence divergence can be made and genealogical trees built. End-labelling is advantageous for mapping work, because the fragments in double digests are smaller than those in single digests.

For the species studied in most laboratories, it has been impossible to do conventional mapping with four-base enzymes because double digests usually contain too many fragments to resolve electrophoretically. For humans, cows, house mice, *Xenopus laevis* and *Drosophila yakuba,* however, it is possible to map by the sequence comparison method (Cann *et al.*, 1982, 1984; Cann & Wilson, 1983; Ferris *et al.*, 1983a, b, c; Gyllensten *et al.*, 1985), because the complete base sequence of mtDNA is available for one individual of each of those species (Anderson *et al.*, 1981, 1982; Bibb *et al.*, 1981; Roe *et al.*, 1985; Clary & Wolstenholme, 1984). Figure 5 shows such a high-resolution map giving the locations of all 441 of the restriction sites found in a world-wide survey of 112 humans (Cann *et al.*, 1984).

Sequencing of cloned regions

Recombinant-DNA methods permit the cloning of specific regions of the mitochondrial genome from closely related individuals and species (Wolstenholme, Goddard & Fauron, 1979; Brown & Simpson, 1982; Brown *et al.*, 1982; Greenberg *et al.*, 1983; De Bruijn, 1983; Olivo *et al.*, 1983; Higuchi *et al.*, 1984). The sequencing of these cloned pieces of mtDNA gives insight into the nature of the evolutionary changes that mtDNA undergoes.

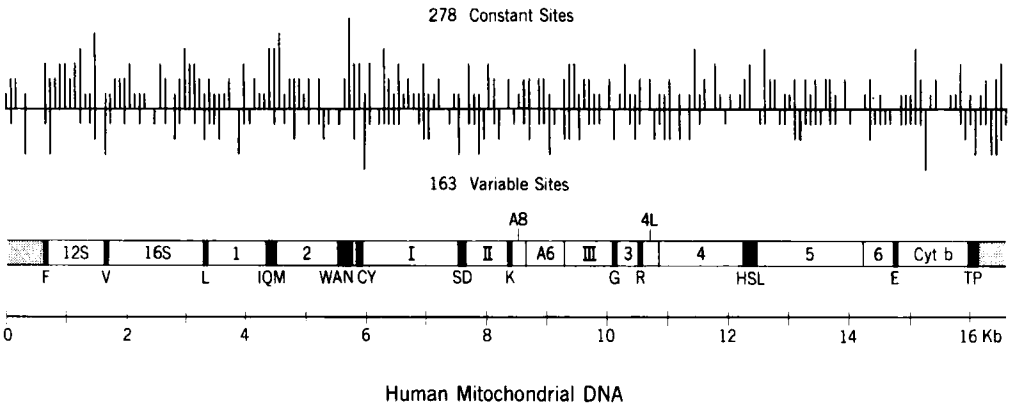

Figure 5. Locations of restriction sites in the 37 genes of human mtDNA. The 16 569 base pair circular genome sequenced by Anderson *et al.* (1981) is drawn in linear form. The major bar shows the regions of known function: 22 tRNA genes, each represented by a single letter and black shading, two rRNA genes (12S and 16S), and 13 genes coding for proteins, one of which is unidentified (6) and 12 of which are known, namely six NADH dehydrogenase subunits (1–5, 4L), three cytochrome oxidase subunits (I, II, III), two ATPase subunits (A6, A8), and cytochrome *b* (Cyt b). Dotted areas represent the large non-coding region, extending from 16 024 to 576 base pairs. The upper panel shows the locations of cleavage sites found in mtDNAs from 112 humans plus the Cambridge reference sequence with the aid of 12 restriction enzymes. Vertical lines below the horizontal line show the variable sites, i.e. those present in some but not all of these mtDNAs. The vertical lines above the horizontal line show those sites present in all of the human mtDNAs examined. Height of the vertical lines is proportional to the number of sites found within an 80 base pair segment. Adapted from Cann *et al.* (1984).

The two sequences compared in Fig. 6 illustrate this point by drawing attention to the high proportion of the base substitutions that are transitions at silent sites in codons. The cloned fragments bearing these two sequences come from part of the gene coding for subunit I of cytochrome oxidase. The species from which the clones came are two closely related members of the genus *Equus*, namely the extant mountain zebra and the extinct quagga. A similar bias toward transitions, as opposed to transversions, is evident in other regions of mtDNA and in mtDNA of other mammals (Brown & Simpson, 1982; Brown *et al.*, 1982; Greenberg *et al.*, 1983; Higuchi *et al.*, 1984).

The bias toward synonymous changes in codons is evident not only in Fig. 6 but also in all other comparisons of cloned fragments of coding regions from closely related mtDNAs. It is presumably the result of natural selection against substitutions that cause amino acid changes. In the example shown here, only one of the seven differences between the two *Equus* sequences shown (Fig. 6)

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Quagga   A GGA GGA TTC GTT CAC TGA TTC CCT CTA TTC TCA GGA TAC ACA CTC AAC CAA ACC
Zebra    G ... ... ..T ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ...

Quagga   TGA GCA AAA ATT CAC TTT ACA ATT ATA TTC GTA GGG GTC AAT ATA ATT TTC TTC CCA
Zebra    ... ... ... ..C ... ... ... ... ... ... ... ..A ... ..C ... .C. ... ... ...
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Figure 6. Sequences for the coding strand of a piece of the cytochrome oxidase I gene from a quagga and a mountain zebra. The 112 bases are arranged in triplets corresponding to the amino acids encoded. At seven positions the quagga sequence differs from that of the mountain zebra; only for these positions is the nature of the base specified for the zebra; a typographical error at position 97 in the original report by Higuchi *et al.* (1984) is here corrected.

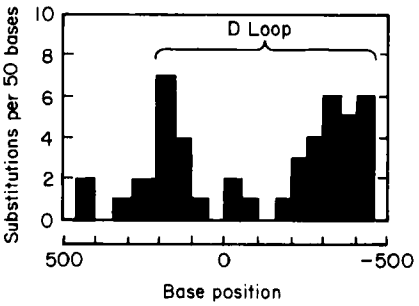

Figure 7. Sequence differences observed within the large non-coding region of human mtDNA. The ordinate gives the total number of variable positions observed in each block of 50 bases when seven individuals were compared. The base pair positions are numbered so that 0 corresponds to the origin of replication. Adapted from Greenberg *et al.* (1983).

would cause an amino acid substitution. This difference is probably an artefact caused by post-mortem change in the mtDNA of the quagga, whose remains sat in a museum for 140 years (Higuchi *et al.*, 1984).

The sequencing of cloned coding regions can be used to evaluate the precision of sequence-divergence estimates made from restriction map comparisons of whole mtDNA (Brown *et al.*, 1982; Higuchi *et al.*, 1984). Consider, for example, the quagga and mountain zebra sequences shown in Fig. 6. They differ in this region by evolutionary substitutions at 6% of the base positions, an estimate in agreement not only with that for another cloned coding region (Higuchi *et al.*, 1984) but also with the percentage divergence inferred from a restriction map comparison of mountain zebra mtDNA to that of Burchell's zebra (George & Ryder, unpubl.). The latter comparison is relevant in the light of our recent finding that quagga mtDNA is nearly identical to Burchell's zebra mtDNA (Higuchi, Wrischnik, Oakes & Wilson, unpubl.).

An added reason for sequencing cloned fragments is evident from the results of comparing the major non-coding region, which, in the case of vertebrates, contains the displacement loop. As Fig. 7 shows, Greenberg *et al.* (1983) found two hot spots for divergence within this loop. The extent of divergence in this region is at least 10 times higher than for the mtDNA molecule as a whole. Sequencing of these hot spots may raise the resolving power of mtDNA beyond that of the four-base restriction method (Table 2), not only for humans but also for other species (Olivo *et al.*, 1983; J. Hixson & W. Brown, pers. comm.).

Sequencing cloned fragments also facilitates testing and rooting intraspecific trees for mtDNA. Although restriction methods are valuable for building such trees, they have a potential weakness because the parallel loss of a site can be scored as a shared derived state; Templeton's (1983) tree-building method attempts to correct for this weakness. The sequences of fragments do not have this limitation and can reveal whether the mutations that caused a site loss in two different mtDNAs are due to the same base change.

If the sequences contain coding regions, another advantage accrues. By confining attention to silent substitutions, which accumulate rapidly, one can focus on recent branches of the tree. By working with the more slowly accumulating substitutions that cause amino acid replacements, one can focus on ancient times of divergence and the rooting of the tree.

The final reason for being enthusiastic about sequencing cloned fragments is that fragments are the only forms of mtDNA likely to be recovered from the organic remains of long-dead animals. These remains, which abound in museums, tombs, frozen soil, neutral peat bogs and amber, represent ancient and sometimes ancestral populations. Because of the high copy number of mtDNA per cell, the likelihood that these remains contain clonable fragments of mtDNA is thousands of times greater than for a fragment of any specific piece of single-copy nuclear DNA. Evolutionary geneticists of the future may be paying a good deal of attention to these materials because of the opportunity they may provide for calibrating mtDNA evolution and investigating the history of populations (Higuchi *et al.*, 1984; Pääbo, 1985).

EVOLUTIONARY IMPLICATIONS

Rate of divergence

The mean rate of divergence averaged over the whole mtDNA molecule is about 2% per Ma in primates (Brown *et al.*, 1979, 1982; George, 1982), rhinoceroses (George, Puentes & Ryder, 1983), rodents (Ferris *et al.*, 1983b,c), the genus *Equus* (Higuchi *et al.*, 1984; George & Ryder, unpubl.), artiodactyls (Upholt & Dawid, 1977; Tanhauser, 1985), gallinaceous birds (Helm-Bychowski, 1984), geese (Schields & Wilson, unpubl.), frogs (Carr, Brothers & Wilson, unpubl.), salmonid fishes (Gyllensten & Wilson, 1986), and Hawaiian *Drosophila* (De Salle, Giddings & Kaneshiro, 1986). These estimates come from groups of species for which there is evidence from fossils, biogeography or proteins concerning divergence times.

Accelerated coevolution

Although no major departures from this rate are known for the mtDNA molecule as a whole, one specific region exhibits perhaps the biggest departure from clock-like behaviour encountered in studies of vertebrate molecular evolution. Along the lineage leading to higher primates, mitochondrially encoded subunit II of cytochrome oxidase has undergone at least a 5-fold acceleration in rate of evolution at the amino acid sequence level (Brown & Simpson, 1982; Cann *et al.*, 1984). Paralleling this is an acceleration in the rate of evolution of somatic cytochrome *c*, a nuclearly encoded protein that interacts directly with this oxidase subunit in the electron-transport chain (Cann *et al.*, 1984). These two proteins have evolved at their standard rates in the lineages leading to rat, mouse, cow and *Equus* (Cann *et al.*, 1984; Higuchi & Wilson, unpubl.). Cytochrome *c* and cytochrome oxidase have also coevolved functionally along the primate lineage (Osheroff *et al.*, 1983). Although the driving force for this accelerated coevolution is a mystery, the fact that the mtDNA clock occasionally gets reset, so that it ticks at a new rate in certain parts of the molecule, must be borne in mind by those seeking to use mtDNA as a dating device.

Temporal frameworks

Trees relating mtDNA lineages: Assuming that most mtDNA divergence occurs approximately at the rate of 2% per Ma, one can build temporal frameworks

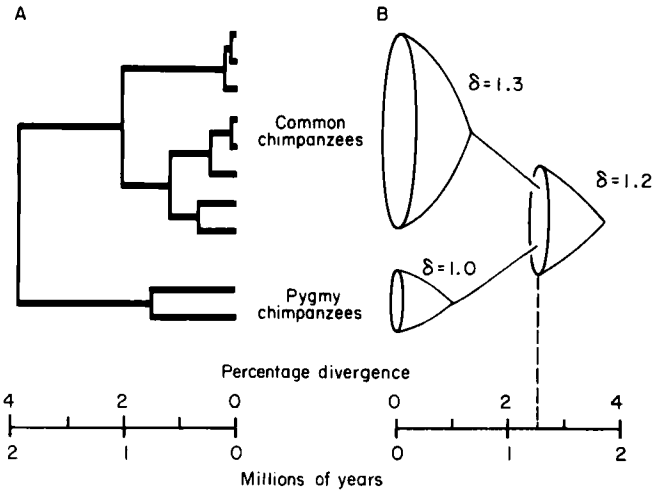

Figure 8. Tree for mtDNA molecules (A) contrasted with tree for species (B). These two ways of representing genealogical relationships are based on comparisons of chimpanzee mtDNAs reported by Ferris *et al.* (1981). The molecular tree in A depicts relationships among the eight types of mtDNA found in 10 common chimpanzees and two types found in three pygmy chimpanzees. This tree requires fewer mutations than do trees with other branching orders. The species tree in B shows how the two species are related and is based on the mean pairwise distances within and between the two species. The cones depict the variability observed within each species and the variability presumed to have existed in the ancestral species at the time of speciation, which is indicated by the dashed vertical line. The apex of each cone indicates the mean time at which two individuals randomly chosen from within each species had a common mother; this time is estimated from the mean pairwise distance, as described in the text and Table 4.

relating the mtDNAs found within species. Figure 8 shows, on the left, an example of a tree and time scale relating the eight types of mtDNA found in 10 common chimpanzees (*Pan troglodytes*) and the two types found in three pygmy chimpanzees (*P. paniscus*). This branching order accounts for the evolution of the 10 mtDNA types with fewer mutations than do other branching orders (Ferris *et al.*, 1981). It shows, first, that the mtDNAs within each species are related more closely to one another than to any of the mtDNAs in the other species, and, secondly, that all of these mtDNAs trace back to one female that lived roughly 1.9 Ma ago. Likewise, the common mother of all the mtDNAs found in common chimpanzees lived about 1.05 Ma ago and for pygmy chimpanzees the figure is 0.75 Ma ago.

Trees relating populations or species: What relation do these time estimates have to the time since these two species diverged? The right-hand side of Fig. 8 attempts to deal with this question, using Nei's concept of genetic distance, δ, between species, as expressed by equation (1),

$$\delta = \delta_{XY} - \delta_A, \tag{1}$$

where δ_{XY} is the mean pairwise divergence between individuals of species X and those of species Y and δ_A is the mean pairwise divergence between individuals within the common ancestral species, A. Since δ_A cannot be measured directly, we estimate it by assuming that

$$\delta_A = 0.5(\delta_X + \delta_Y), \tag{2}$$

where δ_X is the mean pairwise divergence between individuals in species X and δ_Y is the corresponding value for species Y.

The resulting estimate of genetic divergence between the two species (δ in per cent) is then converted to time (t in Ma) with equation (3),

$$t = 0.5\delta, \tag{3}$$

which assumes that the rate of evolution of mtDNA is 2% per Ma. The time of divergence between the common and pygmy chimpanzees estimated by this method is 1.3 Ma (Fig. 8B). (This estimate is uncertain because the assumptions underlying equations (1)–(3) may not hold. For further discussion of this point, see the section below on founder events.)

Time frames within species: One can also extract temporal information from the mean pairwise distance (δ_X) within a population or species. With equation (4),

$$t_X = 0.5\delta_X, \tag{4}$$

we can estimate the time (t_X) since two randomly picked individuals had a common mother (cf. Tajima, 1983). For common chimpanzees, this time is roughly 0.67 Ma (Fig. 8B and Table 4). Comparable estimates for 11 other species of mammal appear in Table 4. For higher primates, these times are generally at least 0.5 Ma, except for the cases of humans and gorillas, which are shorter (Table 4).

Table 4. Intraspecific divergence in mammalian mtDNA based on comparing restriction maps*

Species	Individuals surveyed	Base pairs surveyed per mtDNA	Mean pairwise divergence		Long-term effective population size	Source
			Sequence (%)	Time (Ma)		
Primates						
Human	111	1450	0.36	0.18	6 000	1,2
Common chimpanzee	10	282	1.33	0.67	45 000	3
Pygmy chimpanzee	3	285	1.00	0.50	33 000	3
Lowland gorilla	4	275	0.55	0.28	19 000	3
Orangutan	5	253	3.65	1.83	120 000	3
Crab-eating macaque	2	156	4.10	2.05	250 000	4
House mice						
Mus domesticus	32	707	0.67	0.34	670 000	5
Mus musculus	5	619	0.81	0.41	810 000	5
Black rats						
W Asian	16	132	0.18	0.09	180 000	6
E Asian	5	162	0.42	0.21	420 000	6
Brown rat						
Rattus norvegicus	21	144	0.37	0.19	370 000	6
Deer mouse						
Peromyscus maniculatus	135	240	2.90	1.45	2 900 000	7

*Calculations done by E. M. Prager & A. C. Wilson. In the case of house mice use was made also of supplementary unpublished information. Sources are: 1, Cann *et al.* (1982); 2, Stoneking & Cann (unpubl.); 3, Ferris *et al.* (1981); 4, George (1982); 5, Ferris *et al.* (1983c); 6, Brown & Simpson (1981); 7, Lansman *et al.* (1983a).

Population size

These times may also be related to the long-term effective population size of the species. The number of generations since two randomly picked genes had a common ancestor is expected to be about N (Tajima, 1983; Avise, Neigel & Arnold, 1984), where N is the long-term, effective population size. From the mean number of years per generation, g, and the percentage divergence values observed for mtDNA, we estimated effective population sizes for these species with equation (5),

$$N = 10^6 t_x/g, \tag{5}$$

and present them in Table 4. These estimates are generally in rough agreement with those of Nei & Graur (1984), which were based on electrophoretic comparisons of proteins encoded by nuclear genes, but agreement is not to be expected in all cases for the reasons given below.

Bottlenecks

Because a single breeding pair of diploid animals contains four nuclear genomes and one transmissible mtDNA, a population that goes through an extreme bottleneck could lose all of its mtDNA variability and retain a significant fraction of its nuclear variability (Nei, Maruyama & Chakraborty, 1975; Barton & Charlesworth, 1984). Figure 9B illustrates this concept and Fig. 9D draws attention to the expectation that a prolonged bottleneck will abolish nuclear variability as well; this has happened *within* inbred strains of mice (Ferris *et al.*, 1982).

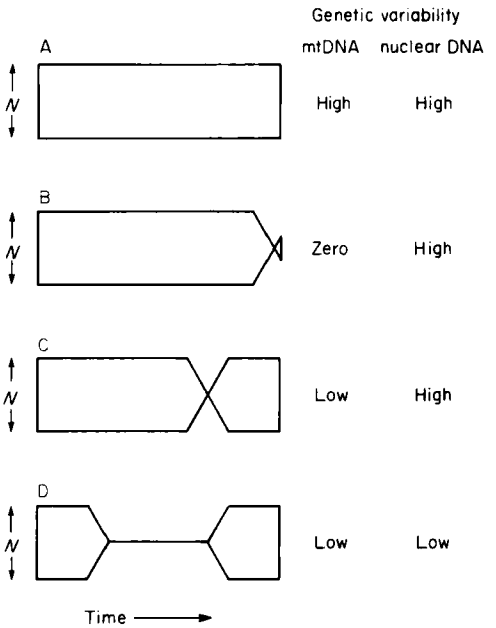

Figure 9. Expected effects of population size (N) on genetic variability in mtDNA and nuclear DNA. A, No bottleneck; B, transient bottleneck (recent); C, transient bottleneck (ancient); D, prolonged bottleneck (ancient).

Table 5. Nine examples of populations that appear to have passed through a bottleneck, reducing mtDNA variability drastically

Species	Populations	Number of mtDNAs examined	Approx. time of bottleneck	Source*
Mammals				
Homo sapiens	Aboriginal Venezuelans	30	Since 12 000 bp	1
Mus domesticus	Among old inbred strains	21	19th Century	2
Mus domesticus	Within chromosomal races†	14	Since 5000 bp	3
Mus musculus	Sweden	23	Since 5000 bp	4
Mus molossinus	S Japan	48	Since 5000 bp	2,5
Non-mammals				
Gallus gallus	Among domestic breeds	>30	Since 8000 bp	6,7
Xenopus laevis	Cape Town vicinity	10	20th Century	8
Rana lessonae	Central Poland	32	Since 8000 bp	9
Salmo salar	Within hatchery stocks	17	20th Century	10

*1, Johnson *et al.* (1983); 2, Ferris *et al.* (1982, 1983a,c, 1984); 3, Ferris *et al.* (1983c); 4, Gyllensten & Wilson (unpubl.); 5, Yonekawa *et al.* (1982); 6, Glaus *et al.* (1980); 7, Helm-Bychowski (1984); 8, Carr (1983); 9, Spolsky & Uzzell (1984); 10, Gyllensten, Wilson & Ryman (unpubl.).

† Having six or more fusions.

Table 5 lists nine examples of "populations" that appear to have experienced an extreme bottleneck in the recent past. The first example was found among the old inbred strains of laboratory mice. There are big differences *among* the old inbred strains as regards nuclear genes, and the study of mouse genetics has depended on this fact. Nevertheless, there is remarkable homogeneity among the 21 strains tested with respect to their mtDNAs, each of which has been examined at about 170 restriction sites (Ferris *et al.*, 1983c). In most of the examples in Table 5, nuclear variability has also survived while mtDNA variability has been drastically reduced. However, the level of nuclear variability is below normal in an isolated population of mice (in the Poschiavo Valley) with a highly derived karyotype (Sage, unpubl.).

The times at which these apparent bottlenecks took place are in most instances probably very recent and suspected to be due to the last ice age or to human influence (Table 5). We refer, for example, to the effect of the spread of the first farming families and their commensal mice into Japan and Europe within the past 5000 years and to direct human intervention in connection with the domestication of chickens and laboratory mice, the management of salmon populations, and the exploitation of female frogs in the vicinity of Cape Town during the 1930s and 1940s for pregnancy testing of women in many parts of the world.

Do founder events accelerate mtDNA evolution?

It is instructive to consider bottlenecks or founder events in light of the distinction made in the introduction between the two separate facets of evolution—the generally clock-like occurrence of mutations in the genes destined for survival, as opposed to the changes in gene frequency, whose rates can vary enormously. The extreme case of a single gravid female founding a new population demonstrates the separability of these two processes particularly clearly. The new population is likely to differ greatly from the parental

population in gene frequency by having only one, instead of many, types of mtDNA. Although an enormous change in frequency may have occurred within a single generation, there has been no change in the structure of any gene.

This sudden change in mtDNA frequency will not show up at all in a molecular tree like that shown in Fig. 8A. Such a molecular tree contains no information about frequency changes; it is simply a record of the mutational history of surviving molecular lineages. A founder event has drastic effects on the number of surviving mtDNA lineages but no direct effect on the number of mutations that accumulated on those lineages. Consistent with this expectation, we see no tendency for the lineages in Table 5, which have been identified as being the product of drastic reductions in population size, to have undergone more sequence change than lineages whose population sizes have remained high (Table 4).

By contrast, especially for mtDNA, a founder event can have an enormous (and instantaneous) effect on genetic distances, as usually calculated between populations, and on the apparent lengths of lineages in a tree relating populations. In the example of the gravid female founder, derived from a large parental population, there has been no molecular evolution. Yet, the routine application of equations (1) and (2) could yield a big genetic distance between her and a large sister population that descended within the past generation from the parental population. Since δ_X, the mean pairwise distance within the founder population, would be zero, the estimate of δ_A provided by equation (2) would be 0.5 δ_Y, where δ_Y is the mean pairwise distance within the sister population. This estimate would be too low by a factor of two. In consequence, the genetic distance (calculated with equations (1) and (2)) between the founder and the sister population would be greater than zero. Even if, instead, one used the true value of δ_A (which equals δ_Y), the genetic distance would seldom be zero. Indeed, the value would be enormous if the founder bore a rare type of mtDNA. So the calculation of interpopulation distances would generally obscure the fact that there was no genetic divergence due to new mutations as a gravid female founds a new population. This example draws attention to a problem of interpreting interpopulation distances and trees, a problem from which trees for molecular lineages, fortunately, are free.

Bottleneck during human evolution?

The human species has an anomalously low level of mtDNA variability, in spite of an apparently normal level of nuclear variability. This could be indicative of a transient bottleneck of type C in Fig. 9. Both the gorilla and human mtDNA values are low compared to those for other species of hominoids (Table 4) and monkeys (George, 1982), but it is the human value which we regard as anomalous. Since only four gorillas were sampled from only one small geographic area, their low mtDNA variability may not represent that of the gorilla as a species (Ferris *et al.*, 1981). Nevertheless, the mtDNA variability in these few gorillas exceeds that found in a worldwide survey of humans (Table 4). The idea that *Homo sapiens* is a younger species than is typical for other higher primates (Ferris *et al.*, 1981) and that a transient bottleneck was involved in its formation (Brown, 1980) merits continuing scrutiny.

Table 6. Differential introgression of mitochondrial and nuclear genomes

Species pair	Locality	Source*
Mus domesticus—M. musculus	Scandinavia	1
Mus molossinus—M. castaneus	Japan	2
Rattus rattus (I)—*R. rattus* (II)	S Asia	3,4
Gallus gallus—G. sonnerati	S India	5
Rana lessonae—R. ridibunda	Poland	6
Salmo gairdneri—S. clarki	Montana	7,8

*1, Ferris *et al.* (1983b,c); 2, Moriwaki *et al.* (1984); 3, Brown & Simpson (1981); 4, Baverstock *et al.* (1983); 5, Helm-Bychowski (1984); 6, Spolsky & Uzzell (1984); 7, Gyllensten & Wilson (1986); 8, Leary, Allendorf & Knudsen (1984).

Differential introgression

Perhaps the most surprising result of recent comparative studies of mtDNA was the recognition that there are individuals and populations bearing the nuclear genes of one species and the mtDNA of a closely related species. The first indications of this differential introgression came from studies of wild mice (Ferris *et al.*, 1982, 1983b) and *Drosophila* (Powell, 1983). Table 6 lists six possible cases for vertebrates. This phenomenon generated fascination on the part of evolutionary geneticists and consternation on the part of some molecular systematists, for reasons that are implied in Fig. 10. This figure shows the results of a phylogenetic congruency test applied to three species (or semispecies) of black rats. According to comparisons of proteins encoded by nuclear genes, the W Asian species is related most closely to the Sri Lankan one (Baverstock *et al.*, 1983). By contrast, mtDNA comparisons show that the W Asian species is closest to the E Asian one (Brown & Simpson, 1981). In principle, this contrast

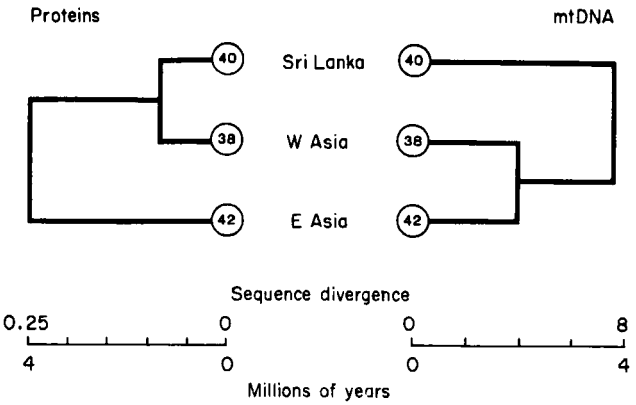

Figure 10. Example, in *Rattus rattus*, of incongruent trees for mtDNA and nuclear loci. The three groups of black rats are regarded here as separate semispecies or species because in nature they are isolated by partial sterility barriers. They also exhibit fixed differences in chromosome number ($2n$ = 38, 40 and 42) and mtDNA type as well as at several nuclear loci coding for proteins (Brown & Simpson, 1981; Baverstock *et al.*, 1983). *Rattus norvegicus* was used to root the two trees, one of which is based on an electrophoretic study of proteins encoded by 45 loci (Baverstock *et al.*, 1983) and the other of which is based on restriction maps bearing an average of 24 sites (Brown & Simpson, 1981). The time scales are based on an assumed rate of mtDNA divergence of 2% per Ma and the assumption that a Nei's D value of 1 corresponds to 16 Ma (Sarich, 1977).

might be explained in two ways. On the one hand, assuming the tree on the right in Fig. 10 represents the true pathway of divergence of the three gene pools, differential flow of nuclear genes (mediated by male introgression) from the W Asian species into the Sri Lankan form could have produced the protein tree shown on the left in Fig. 10. On the other hand, assuming the branching order shown on the left in Fig. 10 represents the true pathway of gene pool divergence, the W Asian species may have been colonized by mtDNA from one or more introgressing females of E Asian origin to yield the mtDNA tree shown.

The best-studied natural example of differential introgression involves wild populations of house mice in Scandinavia. Europe has two species of strongly commensal house mice that depend on human buildings and agricultural products, especially at high latitudes (Sage, 1981; Berry, 1981). *Mus domesticus* is the house mouse of the southern part of Denmark's Jutland Peninsula as well as of W Europe and the Mediterranean region. The other species is *Mus musculus*, the house mouse of the rest of Scandinavia and E Europe. The two species meet and form a narrow hybrid zone that runs southeast across the Jutland peninsula, south through Germany and Austria to S Yugoslavia and then northeast through Bulgaria to the Black Sea (Bonhomme *et al.*, 1984; Boursot *et al.*, 1984). These two species show fixed allelic differences at eight of 59 loci tested (Ferris *et al.*, 1983b) and their genetic distance based on electrophoretic comparisons of proteins encoded by 56 loci is about 0.2 (Sage, 1981), which corresponds probably to a divergence time of at least 2 Ma. In the laboratory, the two species interbreed and produce partially sterile males and fertile female offspring (Forejt, 1981; H. Winking, pers. comm.) but, in the wild, the productivity of both hybrid sexes is probably low (Sage, unpubl.). Hence, the hybrid zone acts as a genetic sink, across which there is severely retarded flow of nuclear genes.

Nevertheless, all of the *musculus* mice tested in Denmark and Sweden have *domesticus* mtDNA (Ferris *et al.*, 1983b; Gyllensten & Wilson, 1984). A tree relating all the known *domesticus* and *musculus* types of mtDNA (Fig. 11) draws attention to the existence of two *domesticus* types of mtDNA on the *musculus* side of the hybrid zone. Because these two types are related closely to one another and to a terminal branch on the *domesticus* mtDNA tree, they arose rather recently during the evolutionary history of *Mus domesticus*, probably about 100 000 years ago, i.e. long after the speciation event (Fig. 11).

It is also evident from Fig. 11 that these two Scandinavian types of mtDNA arose before 8000 bp, when the ice sheet retreated from N Europe and made Scandinavia available for colonization from the south by farmers speaking a Germanic language. The existence of only these two *domesticus* mtDNAs in the *musculus* mice of Scandinavia implies that the present-day population may stem from a very small number of colonists living slightly to the *musculus* side of the hybrid zone in N Germany (Gyllensten & Wilson, 1984). The founding populations presumably contained two backcross females bearing *domesticus* mtDNA and several *musculus* males. The resulting population in Scandinavia could then be exclusively *domesticus* as regards its mtDNA and predominantly *musculus* as regards nuclear DNA.

This and every other 'natural' example of differential introgression given in Table 6 are plausibly linkable to founder events, occurring in initially sparsely populated zones of contact between two species. By contrast, in the one case in which we are intensively examining a well-defined hybrid zone where mouse

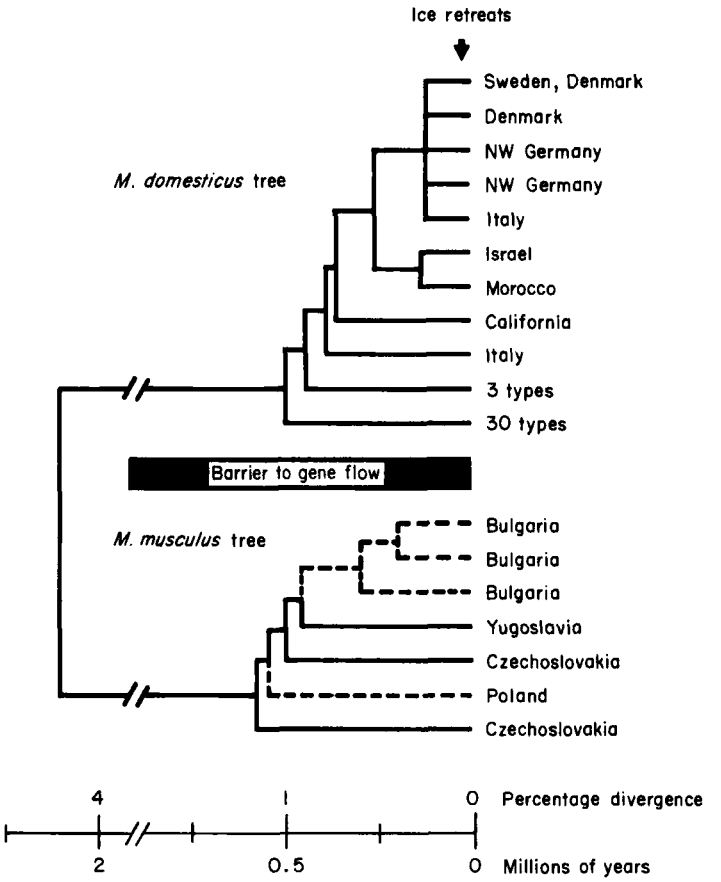

Figure 11. Tree showing how the 49 types of mtDNA found in two species of mice (*Mus domesticus* and *M. musculus*) are related genealogically. The dashed lines suggest how mtDNAs characterized with only a few restriction enzymes by Ferris *et al.* (1983b), Boursot *et al.* (1984) and Moriwaki *et al.* (1984) may be related to those described more fully by Ferris *et al.* (1983c). The top two lineages, although found only in the *musculus* mice of Sweden and N Denmark, are concluded to be of *domesticus* origin because they appear to have arisen recently (about 100 000 years ago), i.e. long after the speciation event (see thick horizontal bar) that separated *domesticus* and *musculus* gene pools (about 2 Ma ago). The time scale is based on the assumption that a 2% divergence inferred from mtDNA restriction maps (or fragment patterns) corresponds to a divergence time of 1 Ma. The time of divergence between the two species estimated from mtDNA comparisons (1.7 Ma) agrees approximately with that based on protein comparisons ($\geqslant$2 Ma; see text).

populations have probably been large throughout the period of contact, there is no evidence of differential introgression (Sage & Wilson, unpubl.). This result argues against the view that mtDNA is more capable of moving across a hybrid zone than is nuclear DNA.

PROSPECTS

To test the ideas in this review, further comparative studies of mtDNA are needed with both existing and new techniques. The full resolving power of this molecule will be available only when there is a quick way of sequencing it completely. Complete sequences for multiple individuals within populations would bring to evolutionary genetics an unprecedented resolving power,

allowing evolutionary questions to be asked on a historical time scale. N European populations will be particularly attractive; their history is relatively simple because many were founded within the last 8000 years. They will provide geneticists with a remarkable opportunity to calibrate and compare rates of mtDNA divergence in numerous species and to put population genetics on a quantitative temporal foundation.

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