References

Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol Biol Evol 13:494–504

Bellwood P (1978) The Polynesians: prehistory of an island people. Thames & Hudson, London

Bendall KE, Macaulay VA, Baker JR, Sykes BC (1996) Heteroplasmic point mutations in the human mtDNA control region. Am J Hum Genet 59:1276–1287

Bertranpetit J, Sala J, Calafell F, Underhill PA, Moral P, Comas D (1995) Human mitochondrial DNA variation and the origin of Basques. Ann Hum Genet 59:63–81

Forster P, Harding R, Torroni A, Bandelt H-J (1996) Origin and evolution of Native American mtDNA variation: a reappraisal. Am J Hum Genet 59:935–945

Francalacci P, Bertranpetit J, Calafell F, Underhill PA (1996) Sequence diversity of the control region of mitochondrial DNA in Tuscany and its implications for the peopling of Europe. Am J Phys Anthropol 100:443–460

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. Curr Anthropol 34:483–496

Hasegawa M, Di Rienzo A, Kocher TD, Wilson AC (1993) Toward a more accurate time scale for the human mitochondrial DNA tree. J Mol Evol 37:347–354

Horai S, Hayasaka K (1990) Intraspecific nucleotide sequence differences in the major noncoding region of human mitochondrial DNA. Am J Hum Genet 46:828–842

Howell N, Kubacka I, Mackey DA (1996) How rapidly does the human mitochondrial genome evolve? Am J Hum Genet 59:501–509

Imamura K (1996) Jomon and Yayoi: the transition to agriculture in Japanese prehistory. In: Harris DR (ed) The origins and spread of agriculture and pastoralism in Eurasia. UCL Press, London, pp 442–464

Maddison D (1991) African origin of human mitochondrial DNA reexamined. Syst Zool 40:355–363

Meadow RH (1996) The origins and spread of agriculture and pastoralism in northwestern South Asia. In: Harris DR (ed) The origins and spread of agriculture and pastoralism in Eurasia. UCL Press, London, pp 390–412

Melton T, Peterson R, Redd AJ, Saha N, Sofro ASM, Martinson J, Stoneking M (1995) Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis. Am J Hum Genet 57:403–414

Mountain JL, Hebert JM, Bhattacharyya S, Underhill PA, Ottolenghi C, Gadgil M, Cavalli-Sforza LL (1995) Demographic history of India and mtDNA-sequence diversity. Am J Hum Genet 56:979–992

Pääbo S (1996) Mutational hot spots in the mitochondrial microcosm. Am J Hum Genet 59:493–496

Phillipson DW (1993) African archaeology. Cambridge University Press, Cambridge

Redd AJ, Takezaki N, Sherry ST, McGarvey ST, Sofro ASM, Stoneking M (1995) Evolutionary history of the COII/tRNA(Lys) intergenic 9-base-pair deletion in human mitochondrial DNAs from the Pacific. Mol Biol Evol 12:604–615

Richards M, Côrte-Real H, Forster P, Macaulay V, Wilkinson-Herbots H, Demaine A, Papiha S, et al (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185–203

Rogers AR (1995) Genetic evidence for a pleistocene population explosion. Evolution 49:608–615

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Sajantila A, Lahermo P, Anttinen T, Lukka M, Sistonen P, Savontaus ML, Aula P, et al (1995) Genes and languages in Europe: an analysis of mitochondrial lineages. Genome Res 5:42–52

Sajantila A, Pääbo S (1995) Language replacement in Scandinavia. Nat Genet 11:359–360

Sherry ST, Rogers AR, Harpending H, Soodyall H, Jenkins T, Stoneking M (1994) Mismatch distributions of mtDNA reveal recent human population expansions. Hum Biol 66: 761–775

Shields GF, Schmiechen AM, Frazier BL, Redd A, Voevoda MI, Reed JK, Ward RH (1993) mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. Am J Hum Genet 53:549–562

Sykes B, Leiboff A, Low-Beer J, Tetzner S, Richards M (1995) The origins of the Polynesians: an interpretation from mitochondrial lineage analysis. Am J Hum Genet 57:1463–1475

Templeton AR (1993) The 'Eve' hypothesis: a genetic critique and reanalysis. Am Anthropol 95:51–72

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, et al (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835–1850

von Haeseler A, Sajantila A, Pääbo S (1996) The genetical archaeology of the human genome. Nat Genet 14:135–140

Wakeley J (1993) Substitution rate variation among sites in hypervariable region 1 of human mitochondrial DNA. J Mol Evol 37:613–623

Ward RH, Frazier BL, Dew-Jager K, Pääbo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724

Ward RH, Redd A, Valencia D, Frazier B, Pääbo S (1993) Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 90:10663–10667

Watson E, Bauer K, Aman R, Weiss G, von Haeseler A, Pääbo S (1996) MtDNA sequence diversity in Africa. Am J Hum Genet 59:437–444

Whittle A (1985) Neolithic Europe: a survey. Cambridge University Press, Cambridge

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0002-9297/97/6104-0031$02.00

Am. J. Hum. Genet. 61:986–990, 1997

Reply to Macauley et al.

To the Editor:

We wish to respond to the comments of Macauley et al. (1997) that address our recent report on the rate of

human mtDNA mutation and evolution. In that report (Howell et al. 1996), we observed a high rate of divergence within the D-loop among the members of a matrilineal pedigree, and we explored possible explanations for the disparity between phylogenetic and empirically determined pedigree divergence rates for the human mtDNA D-loop, the 1.1-kb noncoding control region. There was no intention to suggest that the pedigree rate of divergence should be used, instead of phylogenetic rate estimates, as a clock for timing events during human evolution. Macauley et al. (1997) note that our empirically determined D-loop divergence rate is almost ninefold higher than what they term the "conventional," or phylogenetic, divergence rate for the first hypervariable region (HVR1) of the D-loop. However, there is no gold-standard clock for the rate of mtDNA divergence, particularly within the D-loop. Standard phylogenetic estimates of the rate of human mtDNA divergence vary widely, and they are associated with high degrees of statistical uncertainty (e.g., see Adachi and Hasegawa 1996; Howell et al. 1996; Parsons et al. 1997), in part because of the different models of mtDNA evolution that have been used by different investigators. In addition, a different pattern of human evolution is often obtained after analysis of nuclear genes (in addition to the references in the article by Howell et al. [1996], see, for more recent studies, the reports by Cavalli-Sforza and Minch [1997], Harding et al. [1997], and Hey [1997], as well as the response by Richards et al. [1997] to Cavalli-Sforza and Minch). Furthermore, Tajima (1993, table 7) showed that the molecular-clock hypothesis was *not* supported among all subsets of hominoid mtDNA sequences that were analyzed with his statistical tests.

A high pedigree divergence rate in the D-loop has been supported by two other studies. Parsons et al. (1997), in a much larger study of normal matrilineal pedigrees, observed an empirical D-loop divergence rate of 1 substitution/33 generations, which is very similar to the rate that we obtained. In addition, Mumm et al. (1997) analyzed a matrilineal pedigree with an X-linked disorder, similar in size to the subset of TAS2 family members that we studied, and they observed one new HVR1 D-loop mutation, a result that also supports a rapid pedigree rate of divergence. Therefore, two possible limitations to the general significance of our results—that is, the relatively small number of family members that we analyzed and the presence of a pathogenic mtDNA mutation in the TAS2 pedigree—do not appear to have biased our estimate of the D-loop divergence rate (Howell et al. 1996).

Macauley et al. (1997) suggest that the high pedigree rate of D-loop divergence is due to the fact that the two new mutations in the TAS2 LHON pedigree arose within the second hypervariable region (HVR2) of the D-loop, which has been reported by Aris-Brosou and Excoffier (1996) to have a higher proportion of rapidly mutating sites than is seen in HVR1. However, their study examined a subpopulation of African mtDNAs, and their findings may not apply to the HVR2 region in other mtDNA haplogroups. Furthermore, the other pedigree analyses have observed newly arisen mutations at a number of different sites that are spread throughout HVR1 and HVR2. Parsons et al. (1997) thus detected 10 D-loop substitutions, 3 of which were in HVR1, as was the newly arisen mutation observed by Mumm et al. (1997). Phylogenetic analyses also point toward a relatively even distribution both of hypervariable sites and of overall mutation rates. Thus, Torroni et al. (1996) identified five hypervariable D-loop sites that had mutated in three or more European haplogroups, two occurring in the first hypervariable region and three in the second. Kocher and Wilson (1991) observed that 60% of all D-loop mutations in their sample of sequences arose in HVR1. Finally, one may use the analysis of Hasegawa et al. (1993), which explicitly incorporates site variability, to derive a phylogenetic HVR1 divergence rate of $\sim$1 substitution/1,300 generations (T. J. Parsons, personal communication). That rate is an order of magnitude slower than the pedigree HVR1 rate of 3 substitutions/327 generations (Parsons et al. 1997), results that yield the same disparity between the two types of divergence rates. Overall, these results do not support a simple explanation for the high pedigree divergence rate in which HVR2 hypermutational hot spots "swamp out" a slower overall rate of D-loop divergence. On the other hand, failure to correct for site heterogeneity of mtDNA mutation rates confounds *phylogenetic* analysis and produces serious biases in estimates of the overall mutation rate, the time of the last common ancestor, the transition-transversion ratio, population genetic parameters, and Tajima's *D* statistic for neutrality (e.g., see Hasegawa et al. 1993; Wakeley 1993; Bertorelle and Slatkin 1995; Aris-Brosou and Excoffier 1996; Yang 1996; Wakeley and Hey 1997). These problems are especially acute with highly diverged sequences.

Macauley et al. (1997) discuss their previous analysis of twin pairs (Bendall et al. 1996), which yielded an HVR1 divergence rate of 1 transition/2,000–20,000 years. Their original study concluded that the estimated divergence rate, when sampling error was included, was 1 transition/2,500–55,000 years (Bendall et al. 1996, pp. 1283 and 1285). It is not clear why the rate estimate is now expressed differently, but the approach of Bendall et al. (1996) entailed assumptions of the number of mitochondrial units of segregation, the detection threshold of their direct-sequencing approach, and the proportion of newly arisen mutations that will be fixed, presumably at the level of the pedigree. On the basis of these assumptions, whose validity and general applicability remain

to be established, Macauley et al. (1997) note that their divergence rates were skewed toward more-rapid values.

In these twin pairs, all seven of the newly arisen mutations occurred at sites with "higher than average" mutation rates, when the results of Hasegawa et al. (1993) were used, and Macauley et al. (1997) suggest that the higher pedigree rate of divergence thus reflects the greater influence of rapidly mutating sites. This conclusion merits closer scrutiny, however. Hasegawa et al. (1993) analyzed a set of highly diverged (mainly African) HVR1 sequences that spanned 385 bp. Their maximum-parsimony analysis yielded a total of 465 substitutions at 166 sites: 143 moderately variable sites (1–5 substitutions at each) and 23 highly variable sites (6–15 substitutions at each). Only two of the sites in the study by Bendall et al. (1996) fell into the latter category. The term "higher than average" mutation rate, used by Macauley et al. (1997), is misleading because it is based on all sites, the majority of which do not mutate. In fact, the spectrum of mutational changes observed by Bendall et al. (1996) is unremarkable, and there is no overwhelming contribution by the most highly variable sites. Second, Bendall et al. (1996) omitted the results for twin pair 1, in which two sites were heteroplasmic, from their divergence calculations. Inclusion of those data (e.g., as an eighth heteroplasmic individual) would further increase their divergence rate. Neither Bendall et al. (1996) nor Macauley et al. (1997) discuss the multiple observations of individuals who have undergone two D-loop mutations within a short period of time (also see Comas et al. 1995; Howell et al. 1996; Parsons et al. 1997). Those observations support a high pedigree divergence rate. Overall, one could conclude that the results of Bendall et al. (1996) support our suggestion (Howell et al. 1996) that it is the phylogenetic-divergence-rate estimates that are biased, possibly because they fail to adequately incorporate the effects of site variability in mutation rates.

Another factor that may explain the discrepancy between pedigree and phylogenetic divergence rates is the rate process for fixation of mtDNA mutations. The fixation of mtDNA mutations becomes entangled with several thorny issues, including a semantic one. The term "fixation," as defined for classical or diploid genetic systems with recombination, describes the process through which a new allele completely replaces the previous allele in a population (e.g., see Li and Grauer, p. 238). In contrast, human mtDNA is a high-copy-number genetic system that shows strict maternal inheritance and an apparent lack of recombination (Howell 1997). Some investigators use the term "fixation" when a newly arisen mtDNA sequence change becomes homoplasmic within an individual or within a pedigree (e.g., see Bendall et al. 1996; Howell et al. 1996), whereas others limit the use of the term to fixation of mtDNA sequence changes at the level of the species (e.g., see Templeton 1996).

The probability that a new allele will become fixed within the population, according to standard population-genetic theory, is a function of the initial frequency of the new variant, its selective value, and the effective population size (Li 1997, p. 47). In the case of mtDNA, however, effective population size will encompass both the number of mtDNA molecules in the germ line (which varies widely according to the developmental stage and which is influenced by the sequestration of mtDNA molecules into small membrane-bound clusters, or nucleoids; Howell 1996, 1997) and population demographics and history. Analyses of the mtDNA coding region indicate that a strictly neutral model of evolution is violated (e.g., see Ballard and Kreitman 1995, and references therein; Templeton 1996, and references therein), but relatively little is known about selection coefficients for D-loop mutations (e.g., see Aris-Brosou and Excoffier 1996). Furthermore, it is not yet clear at what level selection acts (replication, segregation, or phenotypic expression) or to what extent random drift predominates over selection, particularly during oogenesis (e.g., see Jenuth et al. 1996).

We suggested that the high pedigree D-loop divergence rate, relative to phylogenetic rates, may reflect the failure of a substantial proportion of new D-loop mutations to become fixed at the population level (Howell et al. 1996). Although definitive data are not available, it seems safe to posit that the fixation probability is <1 (unless one makes the unlikely assumption that all new mutations will become fixed). It then follows that the pedigree divergence rate must exceed the phylogenetic rate, because of the different time scales (see the further discussion below). An example of how the segregation of heteroplasmic alleles might affect fixation of new D-loop mutations is shown in figure 1, which combines data from figure 1 and table 1 of the article by Howell et al. (1996) and previously unpublished results. Female IX-240 was triplasmic and carried approximately equivalent proportions of three mtDNA genotypes: the preexisting genotype, one mtDNA subpopulation with an additional newly arising mutation at nucleotide 152, and a third subpopulation with a new mutation at nucleotide 195. Seven of her grandchildren showed varying proportions of the three genotypes, but XI-18 was close to homoplasmic for the mutation at nucleotide 195, whereas XI-21 was apparently homoplasmic for the original genotype (incorrectly designated "X-21" in the original publication), and XI-17 had a high proportion of the mutation at nucleotide 152. On the basis of these results, it appears that these three females will establish three different genotypes. However, the results obtained for XII-1, the son of family member XI-18, revealed that the segregation toward the homoplasmic state for

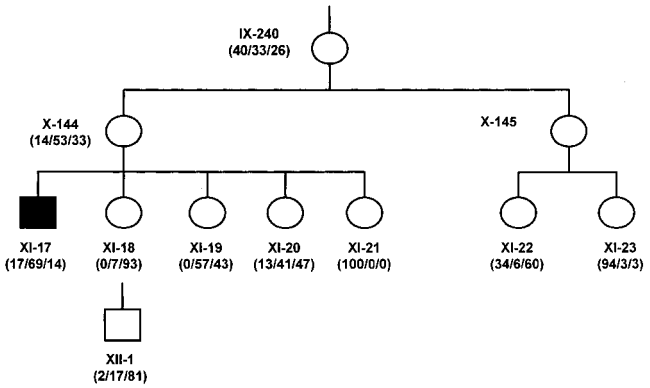

Figure 1 Transmission of three mtDNA genotypes in a subbranch of the TAS2 LHON matrilineal pedigree. Family members are designated with the numbering system used by Howell et al. (1996, fig. 1). The blackened symbol indicates a male who has been visually affected. The numbers in parentheses denote the percentages of the three mitochondrial genotypes in each family member, which are (left to right) the TAS2 genotype, the TAS2 genotype with the new mutation at nucleotide 152, and the TAS2 genotype with the new mutation at nucleotide 195. Because of rounding, some of the totals differ slightly from 100%.

the mutation at nucleotide 195 had stopped and that this individual has shifted back to a triplasmic state. The results for this family member thus exemplify the unpredictability of mtDNA segregation and underscore the danger of overreliance on simple models.

One must be cautious, even skeptical, pending further analysis, but selection may explain the observation, by Parsons et al. (1997), that some newly arisen D-loop mutations occur at sites with below-average levels of polymorphism within the population. One would predict, purely on statistical grounds, that pedigree analyses should provide a relatively unbiased indication of the most rapidly mutating sites (e.g., see Pääbo 1996) and that these sites should match the most rapidly mutating sites revealed by phylogenetic analyses, if selection is not a major determinant. The discrepancy observed by Parsons et al. (1997) may reflect the action of negative selection on some D-loop sequence changes, which mutate at high frequency and which are fixed, in the sense of becoming apparently homoplasmic at the level of the individual, but which subsequently fail to be fixed at the level of the population or species. Pedigree analysis may be more heavily dominated by random drift, whereas phylogenetic analyses may be more influenced by selection, because of the greater time spans inherent to phylogenetic analysis.

Macauley et al. (1997) use a set of Polynesian sequences to show that our D-loop pedigree divergence rate gives a coalescent time that provides a less satisfactory fit to the archaeological data than does their HVR1 "conventional mutation rate" (two rates are used in Sykes et al.'s [1995] table 3, depending on whether HVR1 or a subfragment thereof is analyzed). On the basis of this analysis, Macauley et al. (1997) conclude that the phylogenetic divergence rate is appropriate for times as recent as 1,000 years ago. It is difficult to assess this conclusion, because of the lack of experimental and analytical details. For example, it is unclear which set of 224 Cook Islands sequences (Sykes et al. 1995, table 1) were analyzed—and whether the authors used coalescent or relative-branch-length methods to derive the divergence rate (for a discussion of the difference between the two methods, which becomes substantial for short nucleotide fragments of low diversity, see the report by Ruvolo [1996]). Furthermore, Macauley et al. (1997) note that this lineage group is spread uniformly across Polynesia. As a result, it is not clear whether the Cook Islands sequences are an appropriate set for an analysis of this type. For example, one also needs to know the estimated origin times for this haplogroup, relative to the archaeological data, both among all Polynesians and among other Polynesian subgroups. Despite these concerns, we agree with Macauley et al. (1997) that the analysis of less highly diverged datasets is a fruitful avenue of investigation.

As Macaulay et al. (1997) mention, one expects that there should be a decline in divergence rates as the time depth increases, presumably as a failure of newly arisen mutations to become fixed, but there is not yet sufficicent information for us to expect the monotonic decline that they suggest. It is premature to make, as they do, these comparisons between phylogenetic analyses and pedigree studies. At this stage, further pedigree analyses should be undertaken to address the issues that have been raised by Macauley et al. (1997). However, there is sufficient data to suggest that there is a real discrepancy, in terms of evolutionary biology, between pedigree and phylogenetic rates of D-loop divergence (which perhaps extends also to the coding region, as discussed by Howell et al. [1996]). There is clearly a lot of work to be done in order to understand the basis of this discrepancy and to elucidate the details of mtDNA evolution. We look forward to further debate and discussion of these important issues.

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Acknowledgments

We gratefully acknowledge the assistance of Dr. Thomas J. Parsons (Armed Forces DNA Identification Laboratory) in the

preparation of these comments. The research of N.H. is supported by National Institutes of Health grant RO1 EY10758 and by a grant from The John Sealy Memorial Endowment Fund.

References

Adachi J, Hasegawa M (1996) Tempo and mode of synonymous substitutions in mitochondrial DNA of primates. Mol Biol Evol 13:200–208

Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol Biol Evol 13:494–504

Ballard JWO, Kreitman M (1995) Is mitochondrial DNA a strictly neutral marker? Trends Ecol Evol 10:485–488

Bendall KE, Macauley VA, Baker JR, Sykes BC (1996) Heteroplasmic point mutations in the human mtDNA control region. Am J Hum Genet 59:1276–1287

Bertorelle G, Slatkin M (1995) The number of segregating sites in expanding human populations, with implications for estimates of demographic parameters. Mol Biol Evol 12: 887–892

Cavalli-Sforza LL, Minch E (1997) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 61:247–251

Comas D, Pääbo S, Bertranpetit J (1995) Heteroplasmy in the control region of human mitochondrial DNA. Genome Res 5:89–90

Harding RM, Fullerton SM, Griffiths RC, Bond J, Cox MJ, Schneider JA, Moulin DS, et al (1997) Archaic African *and* Asian lineages in the genetic ancestry of modern humans. Am H Hum Genet 60:772–789

Hasegawa M, Di Renzo A, Kocher TD, Wilson AC (1993) Toward a more accurate time scale for the human mitochondrial DNA tree. J Mol Evol 37:347–354

Hey J (1997) Mitochondrial and nuclear genes present conflicting portraits of human origins. Mol Biol Evol 14: 166–172

Howell N (1996) Mutational analysis of the human mitochondrial genome branches into the realm of bacterial genetics. Am J Hum Genet 59:749–755

——— (1997) mtDNA recombination: what do in vitro data mean? Am J Hum Genet 61:19–22

Howell N, Kubacka I, Mackey DA (1996) How rapidly does the human mitochondrial genome evolve? Am J Hum Genet 59:501–509

Jenuth JP, Peterson AC, Fu K, Shoubridge EA (1996) Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. Nat Genet 14: 146–151

Kocher TD, Wilson AC (1991) Sequence evolution of mitochondrial DNA in human and chimpanzees: control region and a protein-coding region. In: Osawa S, Honjo T (eds) Evolution of life: fossils, molecules, and culture. Springer, Tokyo, pp 391–413

Li W-H (1997) Molecular evolution. Sinauer, Sunderland, MA

Li W-H, Grauer D (1991) Fundamentals of molecular evolution. Sinauer, Sunderland, MA

Macauley VA, Richards MB, Forster P, Bendall KE, Watson E, Sykes BC, Bandelt H-J (1997) mtDNA mutation rates—no need to panic. Am J Hum Genet 61:983–986 (in this issue)

Mumm S, Whyte MP, Thakker RV, Buetow KH, Schlessinger D (1997) mtDNA analysis shows common ancestry in two kindreds with X-linked recessive hypoparathyroidism and reveals a heteroplasmic silent mutation. Am J Hum Genet 60:153–159

Pääbo S (1996) Mutational hot spots in the mitochondrial microcosm. Am J Hum Genet 59:493–496

Parsons TJ, Muniec DS, Sullivan K, Woodyatt N, Alliston-Greiner R, Wilson MR, Berry DL, et al (1997) A high observed substitution rate in the human mitochondrial DNA control region. Nat Genet 15:363–368

Richards M, Macauley V, Sykes B, Pettitt P, Hedges R, Forster P, Bandelt H-J (1997) Reply to Cavalli-Sforza and Minch. Am J Hum Genet 61:251–254

Ruvolo M (1996) A new approach to studying modern human origins: hypothesis testing with coalescence time distributions. Mol Phylogenet Evol 5:202–219

Sykes B, Leiboff A, Low-Beer J, Tetzner S, Richards M (1995) The origins of the Polynesians: an interpretation from mitochondrial lineage analysis. Am J Hum Genet 57:1463–1475

Tajima F (1993) Simple methods for testing the molecular evolutionary clock hypothesis. Genetics 135:599–607

Templeton AR (1996) Contingency tests of neutrality using intra/interspecific gene trees: the rejection of neutrality for the evolution of the mitochondrial cytochrome oxidase II gene in the hominoid primates. Genetics 144:1263–1270

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, et al (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835–1850

Wakeley J (1993) Substitution rate variation among sites in hypervariable region 1 of human mitochondrial DNA. J Mol Evol 37:613–623

Wakeley J, Hey J (1997) Estimating ancestral population parameters. Genetics 145:847–855

Yang Z (1996) Among-site rate variation and its impact on phylogenetic analysis. Trends Ecol Evol 11:367–372

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0002-9297/97/6104-0032$02.00