INVITED EDITORIAL
The Mitochondrial Gene Tree Comes of Age

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Mitochondrial DNA (mtDNA) has been illuminating human evolution for almost 20 years, but it is only now that complete mtDNA sequences are finally being published in substantial numbers. One of the great virtues of mtDNA—arising from its cytoplasmic inheritance, lack of recombination, and high mutation rate—is the opportunity it provides for detailed estimation of the human maternal genealogy. With the arrival of complete sequences, it is timely to make explicit the issue of genealogical resolution, since these data will give us as much information as we can expect ever to obtain on the shape of the mitochondrial gene tree. The issue of genealogical resolution has been a major, often unacknowledged factor from the very beginning, and has underlain many of the debates about demographic history that mtDNA has provoked.

Different Levels of Resolution

Before the appearance of complete sequences, human mtDNA studies could be broadly categorized on the basis of how much of the molecule was assayed. "Low-resolution restriction analysis" constructed a restriction map of the molecule by cleaving with 5 or 6 restriction enzymes, whereas "high-resolution restriction analysis" used 12 or 14. Meanwhile, sequencing studies almost invariably focused on the control region (CR), or "D-loop," of the molecule, sometimes including both hypervariable segments (HVS-I and HVS-II) but usually including only the more informative HVS-I.

Research into human mtDNA as a molecular marker was pioneered by Wesley Brown and Douglas Wallace in the late 1970s. The earliest work began by digestion of the molecule, either with a single restriction enzyme in large numbers of samples (Denaro et al. 1981) or with many enzymes in a few samples (Brown 1980). Subsequent studies tended to use five or six enzymes on fairly large sample sets (Johnson et al. 1983; Santachiara-Benerecetti et al. 1988; Scozzari et al. 1988), and the results were presented as a phylogeny of global mtDNA variation. The structure of the tree was rather starlike; there was a single, central haplotype, shared among individuals from all over the world, that radiated other types, some of which were population specific. The central (so-called "universal") haplotype was assumed to be the type of the most recent common ancestor of all extant mtDNAs in the world. This suggested that all human populations might have shared a common evolutionary history for a very long time. It came to be interpreted by some as support for the "multiregional" model, the idea that modern humans had evolved from archaic ancestors in many different parts of the world (Excoffier and Langaney 1989; Templeton 1993).

mtDNA studies hit the popular consciousness in 1987, as a result of the publicity surrounding the debate on modern human origins and the African "mitochondrial Eve" (Cann et al. 1987). Rebecca Cann, Mark Stoneking, and Allan Wilson used high-resolution restriction analysis to obtain a much more detailed mtDNA phylogeny than had been seen previously. It was presented as an estimate of the maternal genealogy, the tree that connects us all through our mothers: a striking image that was to become—in the words of the leading British Palaeolithic archaeologist, Clive Gamble—"an icon in Palaeolithic archaeology."

The high-resolution analysis seemed to give a different picture from that inferred from low-resolution data. The tree was no longer so starlike, and it showed a deep split between sub-Saharan Africans and non-Africans. One of the two deepest branches in the tree led exclusively to African types, and the other to both Africans and non-Africans. Cann and her colleagues interpreted this as evidence for a recent origin, out of Africa (OOA), of modern humans, whose African mitochondrial ancestor was christened "mitochondrial Eve."

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Resolution and Genealogy

Why were the trees so different? Although little discussed at the time (but see Stoneking 1994), one answer lies in the issue of genealogical resolution.

The genealogy of a nonrecombining locus (the tree of all copies of the locus ancestral to those in a sample or a population) has become a cornerstone of modern population genetics. It provides the bridge between the evolutionary forces that we would like to know about, such as selection and migration, and the sequence variation that we observe. In a neutral setting, where selection is not playing a role, the mutation process can be seen as a convenient way that nature has devised for "lighting up" branches of the underlying genealogy for us. The structure of that genealogy tells us about the factors that influence genetic drift: principally, population size and migration. However, the mutation process at any locus only "lights up" certain branches of the genealogy, depending on the mutation rate at the locus. At one extreme, no mutations strike the genealogy, and we get a picture like that described by Dorit et al. (1995) on a segment of the Y chromosome. Slightly more informative is an autosomal single-nucleotide polymorphism (SNP), which has perhaps mutated only once in its genealogy. Fast-evolving micro- and minisatellites, on the other hand, will often have mutated many times in their genealogies. In nonrecombining genomes, it follows that we can illuminate more of the genealogy by sequencing more of the molecule.

A corollary of this is that if the resolution is poor, the underlying genealogy may be so poorly estimated that demographic and historical interpretations can come unstuck. This problem underlies, at least in part, the controversy between proponents of the African-origin hypothesis and proponents of its multiregional alternative. In particular, it creates problems for phylogeographic approaches, in which it is essential that shallow and deep branches in the tree can be distinguished (Templeton 1993, 1997).

A related difficulty arises when assaying sites with a high mutation rate. This problem was highlighted when the Wilson team turned their attention to the control region, where the mutation rate is ~10 times greater than in the coding region (Vigilant et al. 1991). The high (average) mutation rate there, in conjunction with considerable variation in rate between sites, means that some sites mutate very fast indeed and that many have mutated many times in the genealogy. The result is that many tree topologies are equally plausible, and there may be no grounds—in parsimony analysis, at least—for deciding between them; hence, the genealogy can remain unresolvable. Vigilant and her colleagues had hoped to demonstrate the African root more persuasively, since this was one of the strong predictions of the recent OOA model. However, it was soon realized that there were millions of plausible alternative trees, many of which did not show the same pattern of an African root (Hedges et al. 1992; Maddison et al. 1992; Templeton 1992).

Part of the answer to this dilemma lay in developing new tools, for the analysis of such short, intraspecific sequence data, that sought to characterize the ambiguity in the tree. For example, Penny et al. (1995) explored the phylogenetic landscape of feasible trees in depth, whereas others developed ways to summarize many likely trees in a graph, called a phylogenetic network (Crandall et al. 1994; Excoffier and Smouse 1994; Bandelt et al. 1995). Both approaches aimed to identify which aspects of the phylogeny could be inferred robustly.

An analysis of a more comprehensive set of African mtDNAs using a network approach (Watson et al. 1997) supported the general structure of the mtDNA tree presented by Vigilant et al. (1991), with some interesting additional findings. Chief among these was that Eurasian mtDNAs all appeared to belong to a single clade of African origin. This raised the possibility that, within the OOA model, the migration out of Africa to found the Eurasian population may have involved a population small enough for all but a single mtDNA type to have been eliminated over a period of time. Further work confirmed this basic pattern while raising the possibility that the ancestors of two major mtDNA clades survived the OOA founding event (Quintana-Murci et al. 1999).

A more refined picture emerged during the early 1990s with the application of high-resolution restriction analysis to the analysis of mtDNA from one continent at a time (Schurr et al. 1990; Ballinger et al. 1992; Chen et al. 1995; Torroni et al. 1996). These analyses showed that mtDNAs could be classified into a small number of monophyletic clades, or haplogroups, defined by one or several restriction sites. These were usually restricted geographically: some to sub-Saharan Africans, others to Europeans, and yet others to East Asians. These clades were only rarely identified by low-resolution restriction analysis. Many of them could not be distinguished by control-region data either (despite the popularity of this approach in the 1990s), although they could often be picked out from control-region data after an exploratory combined control-region/restriction analysis (Torroni et al. 1996). The haplogroups could, moreover, be refined by inclusion of control-region information (Macaulay et al. 1999). The identification of robust genealogical groups has allowed the development of the phylogeographic approach to demographic history, in which questions of dispersals, migrations, and colonizations (distinguished in Gamble 1993) are addressed by study of the geographic distribution of lineages on

a gene tree, with a growing body of work exploring the colonization of the Americas, the Pacific, and Europe.

Genealogies and Phylogeography

In one form or another, the phylogeographic approach has been the major contribution of genetics to the study of prehistoric migrations. A bushy gene tree, such as that of mtDNA since ~20,000–30,000 years ago, provides the power to detect many individual migration events (when an ancestor of the modern sample is inferred to have moved from one place to another). Patterns in such data may reveal the existence of a genetic trail leading back to the source of the dispersals. In contrast, poorly designed summary statistics that are blind to the phylogeographic patterns within different populations will often fail to reveal these relationships, and the archaeological record can rarely provide unequivocal evidence for a movement of people, as opposed to cultural diffusion.

There are two problems for this approach that are not always appreciated, both relating to the issue of the genealogical resolution of nuclear loci. The first is the low mutation rate of nuclear loci. This is illustrated by the data set of Kaessmann et al. (1999), from a nonrecombining portion of the X chromosome (Xq13.3). Although it represents a worldwide data set, sequenced through a length of 10 kb and with a coalescence time of >500,000 years, the phylogeny shows only five major types, with a few offshoots from each. Four of the five are shared between Africans and Eurasians and, in an OOA scenario, would be good candidates for Eurasian founding types; only one major type is restricted to Eurasians. The reason is, simply, that so few mutations have affected this locus during its long history that only a tiny fraction of the branches of its genealogy have been resolved by the phylogenetic analysis. Beyond the conclusion that the data are consistent with a recent African exodus, there is little to be learned from these data with a phylogeographic approach. The second problem is recombination. Only nonrecombining loci have a single genealogical history; as soon as recombination is brought into play, the genealogy is broken up, and the length of sequence with a single history becomes shorter and shorter with each succeeding generation. Since there is no single genealogy—rather, an ancestral recombination graph—phylogenetic analysis becomes problematic and potentially misleading (Schierup and Hein 2000).

There is, of course, one nuclear locus that is exempted from these difficulties. Until recently, the nonrecombining portion of the Y chromosome, although it presents $\geqslant$30 Mbp of linked DNA sequence, appeared to be even less variable than autosomal loci. However, many polymorphisms have now been discovered, and the Y chromosome has rapidly emerged as a major tool for phylogeographic research. Researchers have moved from a genealogy with no branches highlighted by mutations (Dorit et al. 1995) to worldwide trees distinguished by 8 polymorphisms (Hammer et al. 1998) to trees with ~20 distinguishing mutations (Jobling et al. 1997). The current state of play is illustrated in the recent tree published by Underhill et al. (2000), which has been used to address a number of issues by means of the phylogeographic approach—for example, the question of modern human origins (strengthening the OOA perspective: Underhill et al. 2000), the settlement of Europe (Semino et al. 2000), and the settlement of the Pacific (Su et al. 2000; Capelli et al. 2001; Kayser et al. 2001).

Complete mtDNA Sequences and the Maternal Genealogy

The increase in genealogical resolution recently witnessed on the Y chromosome has been made possible by the use of denaturing high-performance liquid chromatography. This allows the recognition of polymorphisms on the basis of conformational changes in small DNA fragments, without the need for direct sequencing in every case. The application of this kind of technology has come late to the maternal genealogy but is now being exploited, so that we will soon be swamped by high-quality, complete mtDNA sequences.

Ingman et al. (2000) took the hard route of completely sequencing 53 mtDNAs, from a sample set comprising most of the individuals used for the Xq13.3 study referred to above (Kaessmann et al. 1999); the contrast between the two studies is striking. Although Xq13.3 has a poorly resolved genealogy, that of the 16.5-kb mtDNA (in fewer samples) is not far from bifurcating, and every individual presents a distinct sequence. Although the neighbor-joining tree shown by Ingman and colleagues does not clearly show the relations between the shallower branches, the deep branching structure is very clear. The first branches to diverge from the root (L1 and L2 in the nomenclature of Chen et al. [1995]; shown in purple in fig. 2 of Ingman et al. [2000]) emerge within sub-Saharan Africans. Non-Africans branch from a multifurcation with other Africans (the clade L3 in the nomenclature of Watson et al. [1997]; shown in green in fig. 2 of Ingman et al. [2000]) within the two clades M (shown in orange) and N (shown in blue). More-detailed analysis of the data confirms the branching order of more recent clades within L3, M, and N, previously inferred from combined control region/RFLP analyses (Macaulay et al. 1999; Quintana-Murci et al. 1999; Alves-Silva et al. 2000).

Besides confirming the African root of the mtDNA phylogeny, Ingman and colleagues also used their new data to challenge the recent suggestion of Eyre-Walker et al. (1999) and Awadalla et al. (1999) that mtDNA

may undergo recombination, a claim based partially on the meager set of complete sequences—including some of dubious accuracy—available at that time. In a recent study in this *Journal,* Elson et al. (2001) have used their own set of 66 new complete sequences to the same end. High-quality data of this kind will prove critical in settling this and other issues.

These papers notwithstanding, it seems likely that the most economic approach to complete mtDNA sequencing will be to follow the Y-chromosome strategy and use mutation-detection technology. This approach was first adopted for mtDNA by Hofmann et al. (1997), who assayed a part of the coding region in a European population sample and helped to clarify aspects of the phylogeny (see also Macaulay et al. 1999). It has been developed and enthusiastically applied by Saara Finnilä, Kari Majamaa, and their colleagues at the University of Oulu in Finland. Their first paper in this vein was published in the *Journal* last year (Finnilä et al. 2000). It heralded a series of papers in which they have used the technology to carry out a comprehensive genealogical analysis of European mtDNAs—the latest of which is published this month in the *Journal* (Finnila et al. 2001 [in this issue]). They have used conformation-sensitive gel electrophoresis (CSGE), a mismatch technique originally developed with the detection of disease mutations in mind (Ganguly et al. 1993). Whereas early protocols rarely achieved 100% detection rates, it is claimed that refinements can detect virtually any mutation in any sequence context (Körkkö et al. 1998), and comparisons with complete direct mtDNA sequences support this. The method provides a means of sidestepping the expensive chore of sequencing fragment after fragment of invariant DNA sequences. Instead, one can use CSGE to screen fragments for variation and to sequence only those in which mutations are detected. This approach detects mutations in each DNA afresh, instead of detecting polymorphisms in a subset of samples and subsequently typing those mutations in the full sample of interest (as is often done in Y-chromosome studies), thereby avoiding a potential ascertainment bias that makes analysis by any number of population-genetic approaches problematic.

In this month's *Journal,* Finnilä and colleagues present complete sequences from 192 Finnish individuals encompassing virtually all of the major European clades, or haplogroups, bringing together their previous work on haplogroups JT (Finnilä and Majamaa 2001) and U (Finnilä et al., in press) alongside new data on haplogroups HV, I, W, X, and Z (a member of the Asian haplogroup M). They have presented their results in the form of two phylogenetic networks, one representing the coding-region variation and one the control region. This survey comes very close to representing the ultimate genealogical analysis of European mtDNAs, since it employs the highest level of phylogenetic resolution possible. Without the aid of a time machine (or ancient DNA), there is simply no more information to be had (although slight improvements in resolution may arise from the inclusion of minor haplogroups, such as European U3 and some of the rarer Near Eastern or Caucasian clades). Therefore, it is now possible to evaluate the effectiveness of previous analyses based on HVS-I and RFLP data, and it will also be possible in the future to devise more-effective screens of larger population samples on the basis of the detailed new information that the new genealogies based on complete mtDNA sequences provide.

The authors' initial evaluation of earlier phylogenetic work is, happily, rather positive. The previously inferred phylogenetic relationships between haplogroups, based on combined HVS-I and high-resolution restriction typing, stand up remarkably well. In the past, the use of either system alone clearly led to some errors of topology, usually attributable to hypervariable sites. But it is noteworthy that Finnilä and colleagues have been able to classify all of their sequences into haplogroups initially identified, by means of restriction analysis, by Antonio Torroni and his colleagues (Torroni et al. 1996). Within the haplogroups, the correspondence between coding-region and HVS-I phylogenies is less exact, but this is mainly a result of lack of genealogical resolution, rather than incorrect topology caused by recurrent mutation. Intriguingly, their results confirm earlier suspicions that certain sites can become unstable in particular sequence backgrounds.

The implications for future work are also positive. An approach adopted with some success in the last few years has been to use HVS-I sequences, supplemented by the typing of diagnostic mtDNA coding-region variants, to resolve the main branches in the gene tree that are not revealed by HVS-I variation alone (Rando et al. 1998; Kivisild et al. 1999; Richards et al. 2000; Wilson et al. 2001). The work of Finnilä and colleagues provides many such new markers for distinguishing haplogroups and also for important clades *within* the major haplogroups. A particular example is haplogroup H. Although it is the predominant European haplogroup, H has been difficult to subdivide, hindering detailed phylogeographic studies of Europe (Richards et al. 2000). The analysis of Finnilä and colleagues has revealed a number of major clades within haplogroup H that can now be studied for variation in their geographic distribution. Since it is thought that haplogroup H played a major role in the postglacial resettlement of Europe (Torroni et al. 1998), these new markers will have important implications for further study of this important demographic process.

For other parts of the world, this type of detailed

study is only just beginning. It seems there may be life in this old molecule yet.

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