The Unmasking of Mitochondrial Eve

The use of mitochondrial DNA to trace the origin of modern humans has been a major advance for anthropology, but has left a trail of confusion in its wake

It was widely reported at the beginning of this year that the common mother of us all lived in Africa some 200,000 years ago. "All Family Trees Lead to 'Eve,' an African," ran one newspaper headline. " 'Super Eve' must have lived in East Africa," proclaimed another. The news was dramatic, not only because Africa was firmly stated as being the cradle of modern humans, but also because it indicated that our origins were much more ancient than had been supposed. But the news was also muddled.

"The publicity surrounding this work has been confusing," says Jon Marks, an anthropologist at Yale. "People have been going around talking as if there was a single African female living 200,000 years ago, from whom we are all descended—hence the catchy reference to Eve. Although it is true in a very restricted sense, it's also misleading." According to Allan Wilson of the University of California, Berkeley, not all of the confusion stems from the popular press. "That's regrettable," he comments.

The work that has caused all the fuss is the relatively recent technique of using mitochondrial DNA (mtDNA) to reconstruct "family trees"—phylogenies—of living organisms. In this case, the tree in question is that of the geographic populations of modern humans: how long have they been established, and where did they originally come from? Because mitochondria pass from generation to generation only through the female line (males' sperm simply do not have room to package these organelles), the phylogenies inferred from mtDNA data essentially trace maternal inheritance: ultimately, a single female is reached at the root of the tree, hence the reference to Eve.

"The problem," says Marks, "is that the mitochondrial Eve at 200,000 years ago is just not necessarily the same thing as the last common ancestor of all modern humans. Two separate issues have been confused." The two issues are as follows. First, it is true that mtDNA data can, under certain circumstances, give a good indication of when and where a now globally distributed species once originated. Second, mtDNA is nevertheless something of a passenger in the genetic processes that lead to the formation of a new species: it therefore neither contributes to the formation of a new species nor reveals anything about what actually happened.

"One consequence of the pattern of inheritance of mitochondrial DNA is that the mitochondrial Eve will almost always have existed a considerable time before a newly derived species becomes established," says Wilson. "For this reason, we see it as probable that the 200,000-year-old African female from whom we believe we all derive our mitochondrial DNA was a member of the archaic sapiens species, and was not yet an anatomically modern human." The headlines should therefore have indicated that the first members of anatomically modern humans, *Homo sapiens sapiens*, lived "somewhere between 100,000 and 200,000 years ago." Less catchy, but more accurate.

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Even so, the degree of confidence that one might wish to place in using the mtDNA technique for reconstructing phylogenies depends on the resolution of certain tricky problems. Some people even suggest that several of these problems are intractable, so that the technique is unlikely ever to reach any useful degree of accuracy.

For instance, the information one can deduce from the mtDNA data can in principle be produced by any one of several patterns of population dynamics. Determining which one has operated in any particular case is crucial to how the mtDNA data are to be interpretated. Wilson's suggestion that the mitochondrial Eve was an archaic sapiens rests on the assumption that one of the several possible models was operating. A second issue—and one over which there is significant dispute—is the extent to which mtDNA can be used as a molecular clock: does it tick regularly, and if so, at what rate?

In addition to its peculiar mode of inheritance, mtDNA also accumulates mutations at a much higher rate—some five to ten times faster—than does nuclear DNA. This combination makes mtDNA a good potential molecular clock for relatively short time periods, that is thousands rather than millions of years. Because the origin of modern humans was thought to have occurred at some time within the past half million years, mtDNA seemed to offer a genetic route to answering a question that had eluded anthropologists for decades.

The question was not just when the first modern humans evolved, but also how. Did they evolve simultaneously throughout the Old World, deriving from populations of archaic sapiens already established there from *Homo erectus* forerunners? Or did they arise in one location and then migrate throughout the rest of the world, replacing populations of archaic sapiens as they went? On the basis of the fossil evidence, the anthropologists could not agree (*Science*, 11 September, p. 1292).

The first foray into this new genetic territory was in 1980 by Wesley Brown, at the time a student of Wilson's, and now at the University of Michigan. Using a series of restriction enzymes, he chopped up the mtDNA from 21 individuals from diverse geographic and racial backgrounds. Because the enzymes cut DNA at specific sites determined by base sequence, differences in mtDNA structure between people from distant parts of the world will show up in the pattern of fragments produced for each individual. "One thing was clear," says Brown, "the degree of variation between individuals was much less than might have been expected, given the variation known for the great apes for instance."

This low level of mtDNA variation was a surprise, not least because it appeared to preclude deep genetic roots for *Homo sapiens*. "We had an estimate for the variation and an estimate for the rate of base substitution, so we decided to calculate how long it

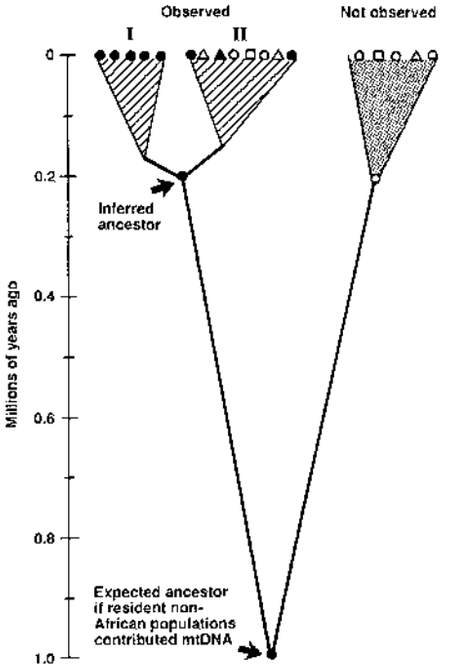
would have taken to produce that level of variation starting with a monomorphic population," recalls Brown. In essence he was asking, how long it would take descendants of a single mitochondrial Eve to accumulate the degree of mtDNA variation he had recorded in modern people? The answer was something between 180,000 and 360,000 years.

In reporting his results, Brown suggested that perhaps the human species had "passed through a severe population constriction ('bottleneck') relatively recently." In fact, that bottleneck would have had to have been a single mating couple in order that the maternal mtDNA would have been just one type. Brown also wrote that there were other population dynamic processes that might produce this pattern, but the caveat fell to the editor's pen and never saw the printed page. As a result the bottleneck idea became the focus of considerable discussion, reverberations of which linger on.

Brown's interests moved to other topics, and Rebecca Cann took over, later to be joined by Mark Stoneking. It was Cann, now at the University of Hawaii, Stoneking and Wilson who published the latest round of results at the beginning of this year. This dataset derived from 147 individuals from five geographic regions: Africans, Asians, Caucasians, Australians, and New Guineans. Again the mtDNA was cut with restrictions enzymes—12 in all—and the fragment patterns determined for each individual. On average each mtDNA was cut at 370 sites, which gives sequence information over about 9% of the 16,569-base-pair mtDNA genome. The result was that of the 147 mitochondrial genomes analyzed, 133 were different from each other.

Wilson and his colleagues were interested in two things here. The first, how the 133 different types might be assembled on a family tree, which should reveal the manner in which the separate geographic populations are related to each other. Many different trees are possible, of course, but the assumption is that the most likely one is that which minimizes the overall number of steps needed to link the complete set. The second is the absolute degree of site variation within and between the groups. Such information gives further insight into the evolutionary history of the different groups as well as a potential measure of the time since they all diverged from a common ancestor.

"We infer from the tree of minimum length that Africa is a likely source of the human mitochondrial gene pool," offer Wilson and his colleagues cautiously. The reason is that the 133 different types fall into two major groups, one of which is exclusively African in origin. Moreover, the degree of variation among the African individuals is greater than within the second group, which indicates that the African group has been established longer.



No Asian input. *If the founding modern human populations had interbred with resident Asian populations, mtDNA variation in today's population would be five times greater than is observed.*

The next question is, when did the common ancestor—the mitochondrial Eve—linking these two major groups live? Given a sequence divergence of 0.57%, and assuming a rate of sequence divergence of 2% to 4% per million years, "the common ancestor of all surviving mtDNA types existed 140,000 to 290,000 years ago," conclude Wilson and his colleagues. The lesser degree of variation within the non-African groups implies that the original founder populations might have left Africa between 90,000 and 180,000 years ago.

These results and conclusions—if correct—strongly support the notion of a single geographic origin of modern humans: Africa. "The study . . . represents the strongest molecular evidence so far in favor of the African population being ancestral," notes Jim Wainscoat of the Radcliffe Hospital, Oxford. Wainscoat's own work on nuclear DNA also indicates an African origin of modern humans. But the Berkeley results go further, and also imply that when populations moved out of Africa they more or less completely replaced existing populations of archaic sapiens.

"The populations of archaic sapiens in Asia, for instance, would have been established from *Homo erectus*, which arrived there about a million years ago," explains Wilson. "If members of anatomically modern humans moving into Asia had interbred with these archaic sapiens, then the mitochiondrial DNA gene pool would show very deep roots. The failure to find any extremely divergent mtDNA lineage in surveys of 500 Asians makes it unlikely that Asian *Homo erectus* contributed much to the gene pool of anatomically modern *Homo sapiens*."

The question is, of course, are these results and conclusions correct?

The most contentious issue is the reliability of the mtDNA clock. Wilson points out that the great majority of mutations in the mitochondrial genome are base substitutions rather than chain-length changes. And most—about 70%—of these substitutions are in noncoding, and therefore neutral, regions. "The likelihood is that these would accumulate at a steady rate," says Wilson. Cann is more cautious. "I see big variation in rate in different parts of the molecule," she says. "We can make overall estimates, but they are still only probabilistic estimates."

Cann's caution over the regularity of the mtDNA clock is reflected in a paper she recently authored with Stoneking, which concludes: "Thus, we can probably only state with certainty that the common ancestor was present at least 50,000 but less than 500,000 years ago." Both Cann and Wilson agree that the only way to settle the issue of regularity of change is to sequence significant sections of the molecule so that the necessary detailed comparisons can be made.

In addition to doubts over the regularity of the mtDNA clock, some people challenge its calibration. "I question the value of 2% to 4% divergence rate that Wilson uses," says Masatoshi Nei of the University of Texas at Houston. "I believe the rate is about half that, 1% to 2% instead."

Wilson and his colleagues calibrated their figure for divergence rate using the variation they measured among individuals from New Guinea, and given a date for colonization of the region of 40,000 years ago: divide the degree of variation by 40,000, and you have a figure for the amount of mutation per year. Nei bases his criticism of Wilson's 2% to 4% figure on the fact that when the founding population arrived in New Guinea, their mtDNA genomes would have already begun to diverge. "The gene splitting is more ancient than the population splitting," he says. "Therefore to use 40,000 years to account for the variation you see there is incorrect. It should be more like 400,000 years."

If the 2% to 4% figure for the divergence rate is too high as Nei suggests, then the estimate for the time when the mitochondri-

al Eve lived would have to be pushed back, perhaps significantly. Many anthropologists would be more comfortable with the deeper roots for modern humans that this would imply.

Wilson is, however, puzzled by Nei's criticism. "We have analyzed 119 individuals from New Guinea," he explains, "and they group into 18 clans, presumably founded by 18 different females. What we measure is the variation within clans, not between them. And the variation within clans must have arisen since each founding female arrived in the region." This calibration is explained in a Cold Spring Harbor paper, which, says Wilson with evident frustration, "few people seem to have read."

Accepting for now Wilson and his colleagues' claim of a mitochondrial Eve around 200,000 years ago, one must ask, what does it really mean? What, if anything, did that female have to do with the origin of modern humans? The answer must be: "it depends." There are at least three populational and evolutionary processes that could have produced the pattern Wilson and his colleagues see, and each has a different implication for the origin of modern humans. Two of them involve population bottlenecks but the third does not (see diagram).

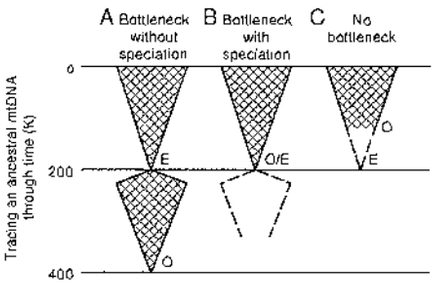
"It is tempting to relate the occurrence of an ancestral mitochondrial DNA type back to a severe constriction in population size," said Wainscoat in commenting on the Berkeley results. "If this assumption is correct, the timing of such bottlenecks may correlate with major evolutionary events." In other words, the origin of *Homo sapiens sapiens* from archaic sapiens might have been a highly restricted speciation event, occurring in a very small, isolated population—perhaps even a single pair. In this case, the mitochondrial Eve would also be the first modern human female, and the date of 200,000 years would mark the origin of *Homo sapiens sapiens*.

Just imagine, however, that modern humans evolved much earlier, say 400,000 years ago. Then, at 200,000 years ago the population crashed to a very small number. Once again the patterns of mtDNA in today's populations would point to a mitochondrial Eve at 200,000 years. In this case, mitochondrial Eve would substantially postdate the origin of *Homo sapiens sapiens*.

Although these two scenarios are described in extreme terms, they do at least illustrate possibilities. And for some—but by no means all—population geneticists, the coincidence of a bottleneck with a speciation event is very plausible, even likely. If a bottleneck had occurred in this manner, how would one know?

"You would have to compare the variability in nuclear and mitochondrial DNA," says Wilson. Bottlenecks of different degrees of severity will affect variability of nuclear DNA in different ways. By contrast, mtDNA variability will always look as if it passed through a single individual. "So it might be possible to get some information from the ratio of nuclear to mitochondrial DNA variability," he suggests.

How one might determine whether such a bottleneck had coincided with or significantly postdated the origin of the species is more problematical. "It would be hard to distinguish between the two possibilities," observes Rodney Honeycutt of Harvard University. "We are groping in the dark over this."



Three models of Eve. *In all cases Eve (E) appears at 200,000 years, with the appearance of modern humans (O) being earlier (model A), coincident with (B), or later (C), depending on the population dynamics and mode of species origin.*

"There is, however, no necessity to invoke a bottleneck in order to explain the existence of a mitochondrial Eve," says Wilson. "That single female may have been a member of a large population." The way in which one female among many could become the mitochondrial mother of us all was worked out by John Avise and his colleagues at the University of Georgia, principally in response to Brown's 1980 proposal of a bottleneck.

The process is best explained by analogy with the random loss of family names through time. Imagine a population of, say, 10,000 mating pairs, each with a different family name, maintaining itself by contributing on average two children to the next generation. A proportion of the family names would be lost each generation, and after 10,000 generations all but one family name would have gone extinct. "The same principle applies to mitochondrial DNA lines," explains Wilson.

Now, if the origin of *Homo sapiens sapiens* had occurred by phyletic transformation within a relatively large population—which, according to some population geneticists is highly likely—then both the nuclear and mitochondrial variability already existing within the founding population would derive from archaic sapiens ancestors. The mitochondrial Eve would have been one of those ancestors, having predated the new species by as much as 100,000 years. Eventually, by the stochastic loss of other mtDNA lines, Eve's would be the only one remaining. "I'm not arguing that there wasn't a bottleneck," says Wilson, "just that we don't know."

The application of mtDNA data to solving this anthropological problem brings molecular and fossil evidence in intimate contact. "Our tentative interpretation . . . fits with one view of the fossil record: that the transformation of archaic to anatomically modern forms of *Homo sapiens* occured first in Africa, about 100,000 to 140,000 years ago and that all present-day humans are descendants of that African population," note Wilson and his colleagues. In other words, the Berkeley group is adopting the nonbottleneck model, which gives the origin of modern humans significantly after the mitochondrial Eve lived, a date that is consistent with what some anthropologists are currently proposing. "Wilson and his colleagues are obviously trying to reconcile their genetic data with the fossil record," notes Marks, "and this shows a welcome degree of maturity in what was once a very contentious relationship."

In fact, it would surely be more satisfactory if the mtDNA data could produce clear inferences on its own merits, without reference to the human fossil record. Less than 10 years ago, interpretations of the fossil record would have conflicted with the mtDNA data. And the discovery of a single fossil of anatomically modern human, well dated at greater than 140,000 years, would dissolve the apparent agreement between the molecules and the fossils. Worked independently of each other, the fossil and genetic data would provide a test of each other. They are intertwined at the moment because the mtDNA technique is still in its infancy and the dates it produces are very imprecise.

"In order to improve on the dates we have," says Wilson, "we need to make comparisons of sequenced mitochondrial genomes, both of humans and apes." The ape data are necessary for making more reliable estimates of the shape of the genealogical tree. "This is the direction we are going in," says Wilson. "But it will take time." This first step by Wilson and his colleagues has taken anthropologists a long way in a short time. But, as two French researchers recently noted: "In this field, the best is yet to come." ■ **Roger Lewin**