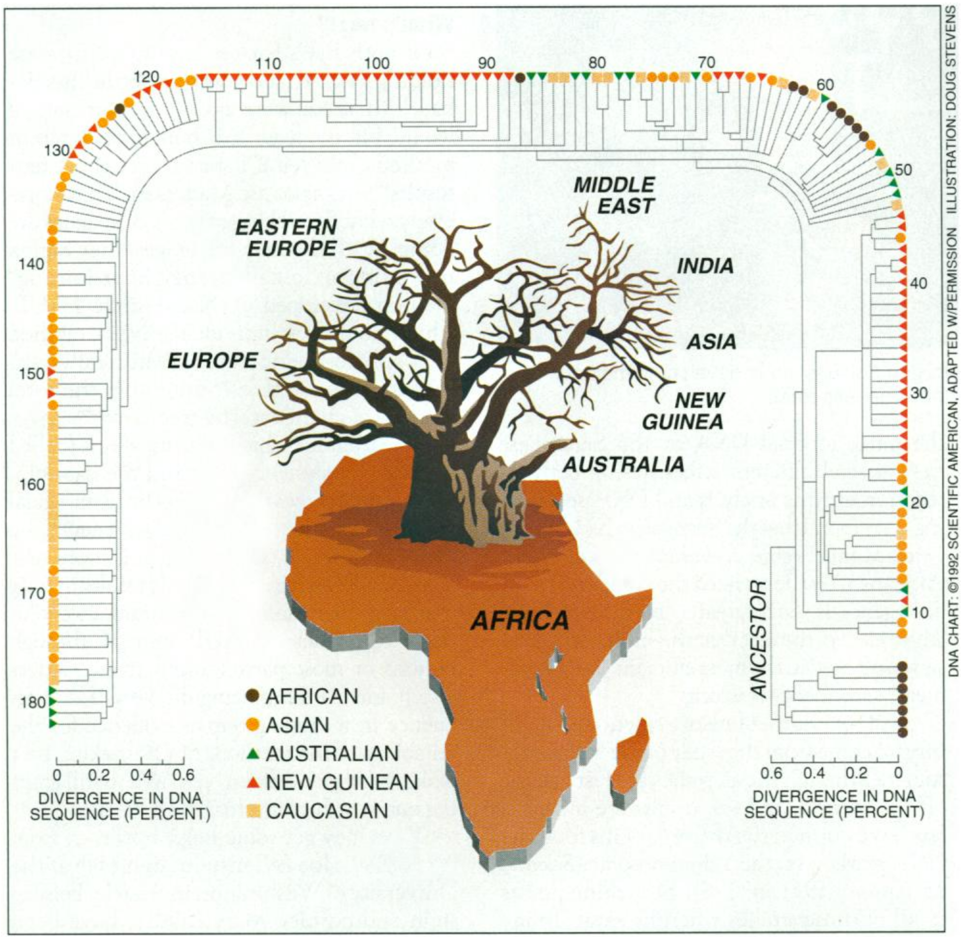
HUMAN EVOLUTION

Mitochondrial Eve: Wounded, But Not Dead Yet

It has been a tough year for mitochondrial Eve. After 5 years as the rising star of human evolutionary studies, Eve went into a free fall earlier this year when biologists found serious flaws in the evidence supporting the so-called Eve hypothesis, which holds that we all inherited our mitochondrial DNA from one woman who lived in Africa some 200,000 years ago. Eve is still reeling from the blow. Indeed, if the headlines in the popular press are any indication, she may not bounce back. This spring, *Newsweek* proclaimed "Eve Takes Another Fall," while *USA Today* announced that "anthropologists are saying it's time to write Eve's obituary."

But the reports of Eve's death may have been greatly exaggerated. Indeed, no one argues with the idea that all modern humans inherited their mitochondrial DNA from one common female ancestor. But what is in dispute is the hypothesis first put forth in 1987 by molecular anthropologist Allan Wilson of University of California, Berkeley, who claimed to know Eve's age and whereabouts—that she lived about 200,000 years ago in Africa. And that's why so much is at stake here: If Wilson's thesis were to pass into oblivion, it could take with it a leading theory of modern human origins—the "out-of-Africa" model. At least that's what some of the theory's critics say, among them Washington University geneticist Alan Templeton. The "out-of-Africa" proponents, explains Templeton, relied heavily on the mitochondrial Eve data to claim that modern humans evolved first in Africa, and then spread out around the globe, replacing more primitive humans.



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Out of Africa. A disputed phylogenetic "tree" based on mitochondrial DNA (outer edge) points to a female ancestor whose female offspring left Africa to colonize other continents.

But scientists on the other side of the issue, like paleoanthropologist Christopher Stringer of the Natural History Museum, London, say it is far too soon to say the last rites for mitochondrial Eve, much less the out-of-Africa model: "Eve may have had a quick kick on the backside, but the out-of-Africa hypothesis certainly isn't dead," says Stringer. Other lines of fossil and genetic evidence, he adds, still point to Africa as the birthplace of modern humans. (The fossil evidence is discussed on p. 875.) Even University of Arizona systematic entomologist David Maddison, whose group found flaws in the mitochondrial Eve evidence, says: "The jury is still out."

Before the fall

Ironically, Eve's case never looked stronger than it did a year ago, just before she fell from grace. In the 27 September issue of *Science*, Wilson and his colleagues at the University of California, Berkeley, published a paper that purported to provide a stronger foundation for the Eve hypothesis first proposed by Wilson and his graduate students, Rebecca Cann and Mark Stoneking, in 1987 in *Nature*. In that paper, they claimed they could trace the origins of modern humans by examining the DNA of the mitochondria, the tiny structures within each cell that generate its energy and that are transmitted only by the mother. The underlying premise was that they could determine how closely related people of different ethnic origins are by comparing their mitochondrial DNAs (mtDNAs). By building ancestral trees based on those relationships, the researchers could then trace the branches backward until they reached the last common ancestor from whom we all inherited our mitochondrial DNA.

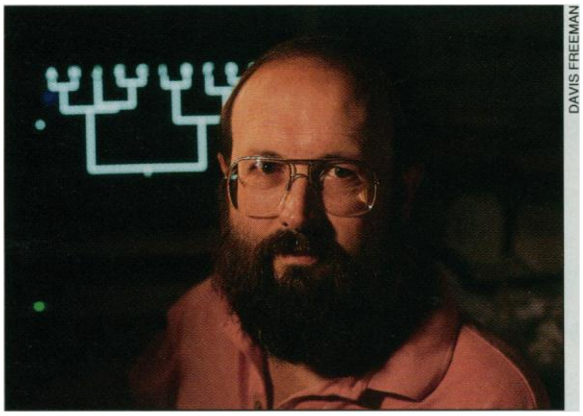
When the Wilson group first compared the mtDNAs of various peoples, they were struck by how vividly the Africans stood out: Their mtDNA was far more diverse than that of other regional groups, implying that people have lived longer in Africa, because it would take more time to accumulate the larger number of mutations in their DNA. And, when the Berkeley group calculated how long it would take to accumulate those mutations at a steady rate, they concluded that it would take between 140,000 and 280,000 years—thereby giving Eve's approximate age. But the critics launched several complaints: The researchers had detected the mtDNA variations by restriction analysis, an enzymatic method which is less thorough than actually determining the DNA sequences; they had used African Americans to represent native Africans; and they had used an inferior method to build a phylogenetic tree.

So, last September, the group came back with the *Science* paper, which appeared a few weeks after Wilson's death, hoping to address criticisms. They had sequenced more of the mitochondrial DNA from a larger number of people from diverse geographic origins, including several ethnic groups in Africa, Asia, and Europe. Then, they entered those data into a highly-regarded computational program called PAUP, or Phylogenetic Analysis Using Parsimony, which had been written to deduce evolutionary relationships between species, based on the assumption that the most "parsimonious" trees are the most likely to mimic best what happened during evolution. Their conclusion: "Our study provides the strongest support yet for the placement of our common mtDNA ancestor in Africa some 200,000 years ago."

The fall from grace

But when Maddison, then a postdoc at Harvard University, took a look at the phylogenetic tree, he realized right away that something was wrong—the 25 !Kung bushmen of Africa were split on the deepest branches of the tree, even though the !Kung are closely related. So he contacted Wilson's co-authors on the *Science* paper, Stoneking and Linda Vigilant, now at Pennsylvania State University, and got their data. After 4500 computer runs, Maddison ended up with thousands of trees that were even more parsimonious—and many showed non-African roots.

At about the same time, Templeton was doing his own PAUP run and coming to a similar conclusion. And an analysis by molecular systematist Blair Hedges and his colleagues in the laboratory of Masatoshi Nei at Penn State showed that the order in which the data were entered into the PAUP program influenced whether the best tree was rooted in Africa or somewhere else. Hedges showed the data to Stoneking, who then agreed to sign a letter admitting the error to *Science* (7 February, pages 636 and 737). Ever since, anthropologists and evolutionary biologists have been saying there's no proof that mitochondrial Eve lived in Africa about 200,000 years ago, and that the out-of-Africa theory is now seriously threatened.



DAVIS FREEMAN

Tree builder. Joe Felsenstein is developing methods to grow better phylogenetic trees.

The counterattack

But while Stoneking and Vigilant admit they made mistakes using PAUP, they maintain that other lines of genetic and fossil evidence still support putting Eve in Africa. The best evidence, says Cann, is the diversity in Africans' DNA, which has been found, not just by their group, but by others in both mitochondrial and nuclear DNA. "The tree is only one part of the argument," says Cann. "A tree is an abstraction from the sequences, and the sequences themselves are not disputed. The diversity of sub-Saharan African lineages is still there."

Among the other studies that Cann thinks buttress her case, the most extensive was performed by Stanford University geneticist Luigi Luca Cavalli-Sforza in collaboration with Yale University geneticists Kenneth and Judith Kidd. These researchers surveyed 100 polymorphisms—markers indicating mutations in the DNA—in the nuclear DNA of 5 different populations, including African pygmies, other Africans, Chinese, Melanesians, and Europeans. The result: the polymorphisms appeared far more frequently in the DNA of the Africans. Cavalli-Sforza says it is tough to quantify how much more, but estimates that the Africans have at least twice as much genetic variation as people from other continents—clearly implying, says Cavalli-Sforza, a longer human heritage in Africa.

And at Emory University, geneticists Doug Wallace and Antonio Torroni have similar results from their survey of genetic variation in the mitochondrial DNA of 186 Sénégalese from several different tribes. Using both restriction enzyme analysis and DNA sequencing, they found that the Senagalese had roughly twice as much genetic variation as did non-Africans. "You don't need the computer to see divergence is much greater in Africans," says Torroni. "So, the fact that the statistical analysis wasn't used in the most efficient way doesn't mean your theory is wrong."

And yet another line of evidence is beginning to come from the study of the Y chromosome: Although these studies are just getting off the ground, and the results are mixed, a few have pointed to African origins for some DNA markers on the Y chromosome (*Science*, 25 January 1991, p. 378). Stoneking points to all of those studies when he says: "In my opinion, out-of-Africa isn't in trouble at all. We have as much, if not more, evidence for African origins as we did 5 years ago."

Critics unmoved

But this staunch defense of Eve by her tribe of anthropologists and geneticists doesn't sway Templeton and the other critics; they still think the founding mother is dead. In a March 1993 special issue of the *American Anthropologist*, Templeton disputes a prediction made by the out-of-Africa hypothesis—that there should have been a rapid expansion in the range of modern humans living in the Old World about 100,000 years ago, and that they drove other populations to extinction. His analysis of the mitochondrial data from populations analyzed by Rutgers University geneticist Laurent Excoffier shows no sign of that. By tracing particular gene markers, about 70 of them, in different populations, Templeton sees no signs of a rapid range expansion, which would show up in the widespread dispersal of key variants. Instead, he sees indications that there were expansions of range within continents and low-level interbreeding among early human populations. That pattern, Templeton says, supports a competing theory, regional continuity, which claims that modern humans evolved in different geographic locations at the same time, and interbred to form a single species.

What's next?

So if both Eve's backers and her critics are holding fast, what's going to settle this debate? "If these were my molecular data, I would hit them with a bunch of different methods, and see if I start to get consistent results," says agnostic Maddison. That's precisely what Stoneking and his colleagues are doing: With the help of Hedges, they built a tree using a so-called "neighbor-joining" method developed by Nei. Unlike PAUP, which compares individuals, Nei's method builds phylogenetic trees by continually pairing sequences so that it minimizes the total amount of change in the tree.

The trouble is that a statistical test (called bootstrap analysis) of how well the data support the tree shows there is very little statistical confidence in the finding. Hedges defends the method, however, saying that it is more reliable than PAUP in this case. It results in only one or a small number of "minimum evolution" trees whereas PAUP must sift through billions of most parsimonious trees. Hedges also points to the placing of every !Kung sequence in a single group as evidence for the reliability of the method. But Stoneking isn't making any grand claims yet: "We're still groping our way through this," he admits.

They may get some help, however, from evolutionist Joe Felsenstein. In his lab at the University of Washington in Seattle, Felsenstein and postdoc Mary Kuhner have been working long hours at the computer to test out a method of drawing conclusions about population history known as maximum likelihood. Felsenstein explains that this method attempts to accumulate evidence about population sizes and migration rates by summing over all possible trees the probability of getting the observed molecular sequences. The advantage of this method is that it allows a researcher to test out a hypothesis and see how well it is supported by the data—something that, if it works, would impress the other phylogenetic modelers, including

Reading the Bones for Modern Human Origins

For decades, anthropologists have been arguing about whether Africa, Europe, or Asia was the birthplace of modern humans—and at least a few hoped that the genetic data pointing to a "mitochondrial Eve" in Africa would help them settle the debate. With that evidence now in dispute (see story on page 873), they are forced to go back and consider the fossil record again. Unfortunately, that record is sparse and incomplete—"nothing to write home about," in the words of paleoanthropologist Leslie Aiello of University College, London. Sparse or not, the fossil data do point to Africa as the best candidate for where we all came from, Aiello concluded after a review of the data for a special issue of *American Anthropologist* scheduled to be published in March, 1993. But like everything else in the field of human origins, Aiello's conclusion is wide open to debate.

In spite of the contention, all parties can agree on one thing. The proto-human fossil record begins in Africa, with a species now called *Homo erectus*. After evolving in an African homeland, all concur, *Homo erectus*, migrated to Europe and Asia about 1 million years ago. But after that, comes the Great Divide in paleoanthropology.

On one side of the divide is the Out of Africa group, counting among their number Christopher Stringer and Peter Andrews, both of the Natural History Museum, London, who think that only in Africa did the descendants of *H. erectus* give rise to modern humans. After evolving in Africa between 200,000 and 100,000 years ago, the out-of-Africa group says, modern humans spread throughout Europe, Asia and the rest of the Old World—replacing other less advanced humans, such as Neanderthals.

Part of the evidence they cite for their view are some key fossils found in Israel and Africa. These include skulls, a pelvis and leg bones from Qafzeh and Skhul in Israel, as well as pieces of skulls, mandibles and fragments of skeletons from Omo Kibish (1) in Ethiopia, and Klasies River Mouth and Border Cave in South Africa. These modern-looking fossils all date to about 100,000 years and appear at the end of a sequence of fossils that stretches back to 400,000 years ago, which seem to show a gradual transition from their *Homo erectus*-type forebears to early modern humans.

Anthropologists do disagree about these "modern" fossils, Aiello says, because some retain primitive features such as pronounced brow ridges. The stronger evidence for the out-of-Africa model, she says, stems not from the fossils themselves but from recent advances in dating methods, including application of thermoluminescence and electron spin resonance analyses to fossils. These methods pushed the age of modern-looking remains from Qafzeh back from about 35,000 years ago to 92,000 years. Not far away are Tabun and Kebara, sites where Neanderthals have been found that have been dated to about the same period or earlier. Those new, simultaneous dates have dealt a blow to the notion that modern humans evolved from Neanderthals—and support the out-of-Africa model in which "moderns" replace Neanderthals rapidly in Europe

But those in the other camp, who favor the "Regional Continuity" model, can also find support for their position in the fossil record. Regional continuity supporters argue that once *Homo erectus* spread throughout the Old World, its descendants continued to evolve in different regions, but interbred sufficiently to give rise to "only one kind of modern human," according to Washington University geneticist Alan Templeton. The main fossil evidence, offered up by the University of Michigan's Milford Wolpoff, is a series of crania of ancient *H. erectus* specimens from various world regions that show similarities to modern human skulls from the same regions. The Lantian and Zhoukoudian *H. erectus* specimens from China, for instance, have features like those of modern Asians, Indonesian fossils share features with Australian aborigines, some Yugoslavian Neanderthal skulls show striking similarities with early modern Europeans.

The regional-continuity backers have more than one string to their bow, however. One thing that would obviously strengthen their case is a set of skulls that seem intermediate between *H. erectus* and *H. sapiens* from various world regions. And they think they've found just such skulls: Ngangdong skulls from Java and the Dali and Mapa specimens from China, found 20 to 50 years ago. Add to this new finds of *H. erectus* fossils from Yunxian, China, whose flattened faces look surprisingly modern—at a time when it would have been much too early in evolution for them to have interbred with modern humans coming from Africa.

But many researchers aren't ready to accept those claims, because few people have had access to the specimens, and the impression of remarkable continuity in the Far East is based only on a small number of sites, often poorly dated. For those reasons, concludes Aiello: "if you're going to put your money on [a place for modern human origins], Africa for now is the best place."

–A. G.

Maddison and Templeton. But at this stage, Felsenstein is still using simulated data. He expects to start using the method with real data next year.

Even if maximum likelihood does all that Felsenstein promises, it may take some doing to win back the trust of some of the anthropologists who felt burned by how long it took to expose the problems underlying the phylogenetic analysis. Says University of Chicago paleoanthropologist Richard Klein, who has cited the mitochondrial data to back up his arguments that the fossil data pointed toward an out-of-Africa model: "If the mitochondrial Eve theory can go for 5 years, and not only survive but grow in significance with no one pointing out that they were misusing the PAUP program, people like me are going to have to be very careful."

And, partly because their confidence in the tree-building methods also has been shaken, Cann and Stoneking, in fact, are among those looking at other ways to analyze DNA that do not rely exclusively on building phylogenetic trees. One possibility: comparing DNA sequences in modern humans whose migratory history is known, such as Polynesians who spread out over the Pacific, to see if the DNA variations reflect these population movements. If they do, that will help the researchers understand better the rates at which the mtDNA and nuclear DNA evolve, and improve the accuracy of their calculation of the age of a common ancestor, such as mitochondrial Eve. Cann and Stoneking also hope to sequence more segments of the mitochondrial DNA (not just the highly variable regions), and to complement that data with studies of the nuclear genome. At the same time, anthropologists also are working on ways to extract the DNA from fossils, with the idea that someday they may be able to analyze DNA directly from the remains of an early human.

With the emergence of new methods, and the persistence of those using old ones, the story of mitochondrial Eve is obviously still unfolding. Perhaps University of Texas molecular evolutionary biologist David Hillis, who is editor of articles on the Eve analysis in *Systematic Biology*, sums it up best: "The data are simply ambiguous. They don't argue that there wasn't an African origin, and they don't argue that there was one. It's like saying you can't solve a mystery after reading one page of the book."

–Ann Gibbons