

HUMAN EVOLUTION

The Mystery of Humanity's Missing Mutations

People like to think of themselves as unique individuals, but in one aspect our species is remarkably alike: our genes. When Harvard University anthropologist Maryellen Ruvolo looks at human DNA, she sees far more genetic similarity in our species than in our close relatives, the common chimpanzees. The story is the same for other apes. Ruvolo can look at genetic sequences from two humans from widely separated continents, and they appear more alike than do sequences from two lowland gorillas from the same forest in West Africa. Other scientists looking at different parts of ape genomes report similar results. "It's a mystery that none of us can explain," says Ruvolo.

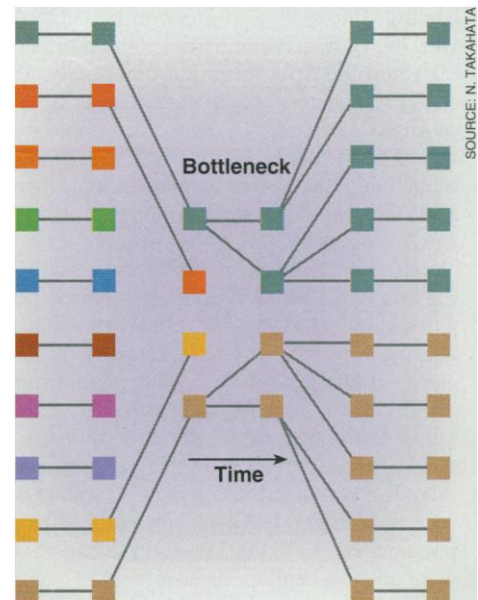
But even if there's no overall explanation for this mystery, a number of scientific sleuths say they have uncovered a clue that may help them solve it. If our ancestors went through a population "bottleneck," a sharp decrease in population over a short period, the squeezed-down group would emerge from the bottle having lost a lot of its genetic variability.

Using the mathematics of population genetics to calculate backward from our current level of genetic diversity, several teams of anthropologists, geneticists, and population biologists have recently concluded that

modern humans were indeed squeezed from a group of about 100,000 to a population of about 10,000 breeding men and women sometime during the last 400,000 years. That group of reproducing individuals is what biologists term an "effective" population (although the total population, including older people and children, would likely have been larger).

The genetic record "gives a remarkably consistent description for the effective population size," says Naoyuki Takahata, a population geneticist at The Graduate University for Advanced Studies in Japan, who has published a series of papers on the topic in the past 4 years. "Our ancestors survived an episode where they were as endangered as pygmy chimpanzees or mountain gorillas are today," agrees Pennsylvania State University anthropologist Henry Harpending. But while a consensus is emerging on the number, there is disagreement as to its meaning. Others, such as evolutionary biologist Jan Klein of the Max Planck Institute for Biology in Tübingen, Germany (who collaborates with Takahata), insist that 10,000 is too large a population to be considered a bottleneck.

Out of the bottle. Population biologists have been trying to figure out whether human ancestors underwent a bottleneck ever



Gene squeeze. This simplified population "bottleneck" shows how a reduction to a few individuals can eliminate genetic variability (different colors indicate different genetic alleles).

since 1942, when Harvard University biologist Ernst Mayr wrote a book in which he explained how new species arise when a few members of a population bud off from their ancestral lineage. "This was the paradigm—everyone assumed humans started with a bottleneck," says Klein.

To prove or disprove that idea, molecular evolutionists look at genetic patterns in modern humans to try to reconstruct their past. Many of these attempts have involved

Tracing Pedigrees of Genes

How does a scientist use contemporary genes to travel back through time to figure out that 5 billion people have all descended from 10,000 breeding men and women? First a population geneticist like Naoyuki Takahata of The Graduate University for Advanced Studies in Japan must find out how many different versions there are of a specific gene in modern humans. Takahata must also know how rapidly the nucleotides at that specific site in the DNA have been mutating over time. He gets this value by comparing how the genes have changed in several different species of apes since they split from a common ancestor—a date ascertained partly from the fossil record. Then he can take these numbers and plug them into an equation that's part of what's called "coalescence theory." It gives him the number of ancestors needed to produce the current genetic diversity.

In a simple example, Takahata will take the sequences of two alleles for the same segment of DNA, known as a neutral gene (see main text). Then, he will count the number of differences in nucleotides between the two alleles and divide that number by the total number of nucleotides that make up that gene. That ratio gives him the genetic distance between the two alleles. And because he knows how long it took for those mutations to accumulate, he can deduce how long ago those two alleles coalesced in a common ancestor.

Those values are inserted into an equation that says that the species' effective population size for that gene is equal to the genetic distance divided by 4 times the mutation rate. (The time it takes to trace the two allelic lineages back to a coalescent gene is equal to twice the effective population size, according to coalescence theory. So, when the two are multiplied, the result is 4.) The premise of the equation is that the more diversity between two alleles, the more time has passed since they split from a common ancestor—and the larger the population needed to maintain the diversity over that time.

Looking at one pair of alleles is not enough, however. So Takahata and his colleagues did the same analysis for each of 50 pairs of alleles, to reach statistical significance. Each pair of alleles yields a slightly different result, because each of the genetic distances are different. But the mean genetic distance for all 50 pairs of neutral genes is 0.0008 substitutions per nucleotide site. And the estimated mutation rate for so many alleles is one substitution every 50 million generations (or 2×10^{-8} substitutions per site per generation). When those two values are plugged into the equation, the effective population size for all 50 neutral genes is 10,000 (divide the genetic distance of 0.0008 by 4 times the mutation rate of 2×10^{-8} to get 10,000).

—A.G.

tracing lineages of mitochondrial DNA (mtDNA), which come from cellular organelles and are only inherited maternally. In 1987, researchers constructed "family trees" of these lineages, showing they could be traced back to a single ancestral gene shared by a small number of women living about 150,000 years ago. This became known as the "mitochondrial Eve" hypothesis.

This hypothesis has been controversial—the size of the small founder population, in particular, has always been open to debate, because mtDNA only tells the story of one gene's heritage, not that of a population. Other mtDNA studies, such as those carried out by John Maynard Smith of the University of Sussex, as well as by Harpending and Alan Rodgers at the University of Utah (*Science*, 1 October 1993, p. 27), pointed to a population of 10,000 ancestors, but they were open to similar criticism.

The solution to this problem is to expand the analysis, taking in more of the 40,000 genes humans possess, most of them in the cell's nucleus. To do so, Klein and Takahata turned to the elegant mathematical equations of "coalescence theory." Much as a historian reconstructs the pedigree of royal hemophiliacs who all trace their disease to one influential ancestor, population geneticists use coalescence equations to trace the genealogy of two or more versions of a specific gene—known as alleles—back in time until they coalesce in a single ancestral gene.

The differences—among mutations—among alleles accumulate at a regular rate in neutral genes, which are defined by biologists as genes unaffected by natural selection because they do not give an individual a reproductive advantage or disadvantage. Researchers can therefore use the rate to determine approximately when that ancestral gene originated. That date, and the degree of diversity among alleles, helps to determine the minimum ancestral population size (see box on p. 35).

Sizing the ancestors. Takahata and Klein compared the variation within each of 50 pairs of neutral genes and then calculated the mean from all 50 pairs. They concluded that the effective size of the human population was about 10,000 individuals who lived sometime after our species appeared 400,000 years ago and before 10,000 years ago, when the human population began to explode.

But that still left open the bottleneck question, because it was unclear what came before this population. Was this group of

10,000 relatively stable, or did it crash from a larger, older population, losing genetic diversity in the process? So Takahata and Klein decided to look at the most diverse part of the human genome—the genes of the major histocompatibility complex (MHC).

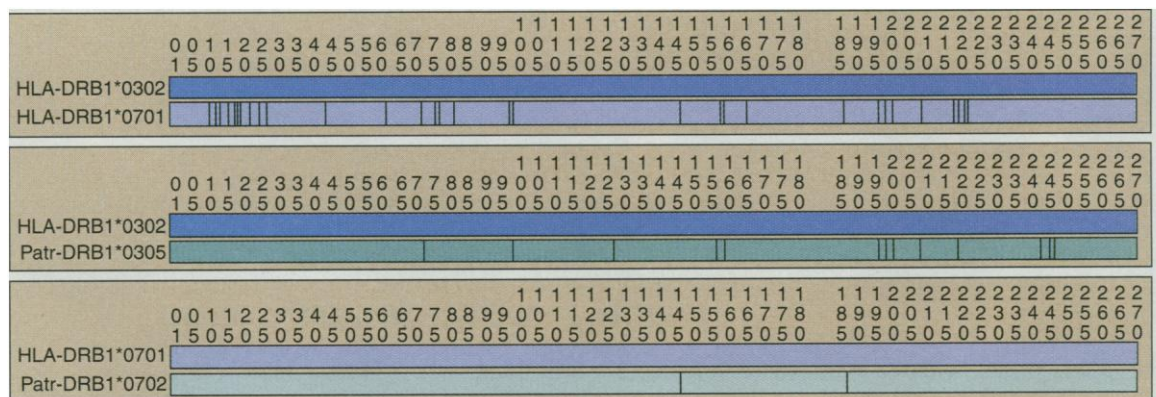
The diversity of some of the MHC alleles (sometimes known as HLA alleles) indicates a tremendous antiquity. Indeed, some of them—known as MHC-DRB1 alleles—are shared with apes and other primates, who split off from humans about 6 million years ago. This has prompted Klein to conclude that the alleles are older than our species. After adding estimates of the effects of natural selection on these MHC alleles to the coalescence equations, the researchers reported that to maintain that genetic diversity for so long, early human ancestors must have had a population size of 100,000 (plus or minus some fluctuations at various points) over the last few million years—before the emergence of modern humans.

But Takahata and Klein also wanted to know if the MHC genes showed a subsequent bottleneck. So they used computer simulations that took all the MHC-DRB1 alleles in the current population and calculated the minimum population size needed to get

another story. Takahata, along with Rogers and Harpending, claims that if the long-term population of the human lineage was closer to 100,000 over the past million years, then 10,000 was a significant reduction. Says Takahata: "Now that the HLA data suggest 100,000 breeding individuals over the longer period for the human lineage, I call the stage of 10,000 individuals a bottleneck."

But Klein and another collaborator, population geneticist Francisco Ayala of the University of California, Irvine, say 10,000 is no bottleneck. Why? Because—according to coalescence work by Pennsylvania State University molecular evolutionist Masatoshi Nei—10,000 is the standard breeding population of many species, from macaques to seals, yet these species have retained a high degree of genetic diversity.

If 10,000 doesn't equal a diversity-squeezing bottleneck, then what did wipe out human diversity? Klein thinks that the explosive population growth that occurred in the past 10,000 years may be the cause. According to computer simulations of population growth, the widespread reproduction that accompanies rapid growth mixes genes up throughout a population to a greater degree than in a small population that isn't repro-



Family resemblance. Comparisons of gene alleles in humans (HLA) and chimps (Patr.) reveals more sequence divergence—indicated by vertical lines—between the human alleles than between human and chimp counterparts. Such diverse human alleles probably originated before the species did.

these alleles back through the generations that lived since 400,000 years ago (when the first *Homo sapiens* arose). The answer was 10,000, prompting Takahata to conclude: "Before modern humans arose the size fluctuated around the mean of about 100,000 individuals. About 400,000 to 800,000 years ago the size dropped to approximately 10,000 individuals and remained around that mean until about 12,000 years ago."

Klein argues that these MHC findings are the third line of evidence—in addition to the mtDNA and neutral gene studies—that point to the same number: "By at least three different methods, we have come up with a similar estimate—10,000."

Making sense of the numbers. But what that number means for modern diversity is

ducing as frequently. The result is an overall genetic sameness.

Klein is skeptical that there ever will be complete agreement on what the population number means. Ruvolo thinks, however, that studies of more nuclear genes in humans and other species of primates will help refine estimates of current diversity and uniformity. But for now, while our genes appear similar, opinions on how we got that way remain as disparate as ever.

—Ann Gibbons

Additional Reading

N. Takahata, Y. Satta, J. Klein, "Divergence time and population size in the lineage leading to modern humans," *Theoretical and Population Biology*, in press, 1995.