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Archaic admixture in the human genome

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One of the enduring questions in the evolution of our species surrounds the fate of 'archaic' forms of *Homo*. Did Neanderthals go extinct without interbreeding with modern humans 25–40 thousand years ago or are their genes present among modern-day Europeans? Recent work suggests that Neanderthals and an as yet unidentified archaic African population contributed to at least 5% of the modern European and West African gene pools, respectively. Extensive sequencing of Neanderthal and other archaic human nuclear DNA has the potential to answer this question definitively within the next few years.

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Introduction

Humans have had unparalleled success as a species — our ancestors developed the ability to live in diverse environments around the world and, today, our population size is nearing seven billion. Despite this success, humans are unusual among great ape species in not having much genetic variation. Chimpanzees, gorillas and orang-utans, three closely related species that have been confined to small geographic ranges, all harbor substantially more genetic diversity than our own species does [1–3]. How did this unusual situation arise, and has it always been the case?

Part of the answer lies in the fact that chimpanzees, gorillas and orang-utans all have multiple subspecies — diverged populations that historically have had little gene flow between them. Divergence between subspecies contributes a substantial fraction of the overall degree of species-level diversity [2,4]. Humans, too, used to be much more diverse. Recent fossil evidence suggests that multiple species were contemporaneous throughout a significant

portion of the time since humans and chimpanzees shared a common ancestor, with several distinct morphological forms of *Homo* emerging in the past 2.4 million years [5–10]. Some of these forms persisted until quite recently, well after the appearance of the first anatomically modern humans in East Africa ~195 thousand years ago (kya) [11**]. Moreover, there is evidence that anatomically modern humans and archaic forms were sympatric in some regions of the world. For example, fossil evidence suggests that both early modern humans and Neanderthals were present in the Levant by ~100 kya and that they probably coexisted in western Eurasia between 45 and 25 kya, just after the first arrival of anatomically modern humans in Europe, and around the time when Neanderthals went extinct [12–15]. Redating of *Homo erectus* fossils from Java to within the past 27–53 kya suggests that in Southeast Asia, too, anatomically modern and archaic humans might have overlapped in time and space [16]. Meanwhile, the striking discovery of *Homo floresiensis*, a species in the genus *Homo* remarkable for its small body, small brain, and survival until relatively recent times, provides even more convincing evidence that archaic forms were contemporaneous with modern humans on the Indonesian island of Flores [17**].

What happened to archaic human forms such as the Neanderthals or Asian *Homo erectus*? Did they go extinct without interbreeding with anatomically modern humans, or do their genes survive in the genomes of contemporary humans? Owing to advances in molecular sequencing technologies and in statistical methods for analyzing DNA sequence datasets, we now have a unique opportunity to re-examine this longstanding debate in human evolution. In this review, we examine recent inferences on the genetic relationships between modern humans and archaic forms, and outline what we might reasonably expect to learn given the flood of genetic data that will become available in the next few years.

Background

The question of the fate of Neanderthals and other archaic humans is just another way of phrasing an old debate on the origin of anatomically modern humans. One model, often called the recent African replacement model, posits that modern humans evolved in a single location in Africa and, from there, spread and replaced all other existing hominins [18]. The strict form of this model claims that anatomically modern humans formed a separate species, so were unable to interbreed with contemporaneous archaic human groups. A competing model, dubbed the multiregional model, claims that modern humans evolved in concert across the Old World from

various archaic human groups [19]. There are also a range of intermediate models that predict an African origin of modern humans but with some contribution from other hominin groups [20–23]. The fossil evidence in support of either model is mixed and subject to interpretation, but most studies seem to support the recent African replacement model [11^{••},24–26]).

From a genetic perspective, researchers have concentrated on what contribution, if any, archaic humans have made to the modern gene pool. Under the recent African replacement model, this contribution should be negligible, whereas the multiregional model predicts that this contribution would be substantial. One approach for answering this question is to make a direct comparison of DNA sequences from both archaic and modern populations. In the past decade, researchers have sequenced fragments of mitochondrial DNA (mtDNA) still present in fossilized bones from several different specimens, including Neanderthals [27–29,30^{••},31], ancient anatomically modern humans from a variety of sites in Europe [30^{••},32], and 10 ancient Australians [33]. These studies reveal that Neanderthal mtDNA is quite different from all present day human mtDNA sequences and from the mtDNA sequences isolated from fossils of anatomically modern specimens from comparable time periods. This pattern has been interpreted to mean that there was extremely little, if any, interbreeding between Neanderthals and their more anatomically modern contemporaries, and that Neanderthals represent a distinct species of *Homo* [28,30^{••},32,34]. Although these data are certainly consistent with the recent African replacement model, they do not prove that Neanderthals and modern humans did not interbreed; it is possible that the two groups did interbreed but that Neanderthal mtDNA was lost over time owing to the random effects of genetic drift. In fact, simulations suggest that the data are consistent with a Neanderthal contribution of up to 25% of the modern gene pool [30^{••},35]. Comparisons of Neanderthal and modern human nuclear DNA have the potential to clarify the precise genetic relationship between Neanderthals and modern humans (see below).

An alternative approach concentrates on examining the patterns of genetic variation in contemporary humans for signs of ancient admixture between genetically distinct hominin groups. Some recent studies of human nuclear genetic variation have estimated extremely old times to the most recent common ancestor (TMRCA), which are suggestive of ancestral structure in human populations [36–39]. However, owing to the inherent stochasticity of the evolutionary process, old TMRCAs are consistent with a wide range of demographic models.

Another method exploits the fact that admixture causes extensive linkage disequilibrium (LD) between sites that differ in frequency between the two parental populations.

This LD is so pronounced that even tens of thousands of years of random mating are not enough to obscure it [40]. Consider, for example, the following crude approximation: at the time of (putative) admixture, strong LD would extend across the whole genome. After 1500 generations of random mating (i.e. approximately 37 500 years, assuming an average generation time of 25 years), linkage disequilibrium would still extend roughly 0.07 cM on average, equivalent to more than 50 kb, assuming an average recombination rate of 1.25 cM/Mb. Recent studies have looked for evidence of ancient admixture by examining patterns of LD at intermediate distances (e.g. 5–50 kb). For example, Garrigan and colleagues [41[•]] examined patterns of genetic variation over 17.5 kb of non-coding DNA on the X chromosome in a worldwide sample of individuals. They found that the patterns of LD were not consistent with the standard Wright-Fisher model and suggested instead that they were evidence of ancient admixture in human populations.

One drawback of this study is that it only looks at data from a single locus. Patterns of genetic variation are affected by many factors, including demography, natural selection and chance. Another concern is that, at most loci, the actual (unobserved) genealogy is consistent with a wide range of different demographic models. A rejection of the null model does not necessarily provide positive support for any particular alternative model. In any case, these difficulties can generally be dealt with by jointly analyzing sequence variation data from many evolutionarily independent regions. The benefits of analyzing multi-locus data are twofold. First, given that the effects of natural selection are localized — owing to recombination — and the effects of demography are global, multi-locus studies are more likely to capture the underlying demographic signal. Second, the evolutionary independence of the different regions gives one much greater power to distinguish between different demographic hypotheses. With these thoughts in mind, we are currently gathering resequencing data from 90 independent regions of the genomic in a diverse sample of individuals from a dozen different human populations (JD Wall and MF Hammer, unpublished). Our goal is to be able to answer a range of outstanding questions about human evolution, including the question of whether various archaic human groups contributed to the modern human gene pool.

Recent developments

In the past year, two promising developments have accelerated our understanding of modern human origins. First, new methods have been developed to accurately estimate demographic parameters from multi-locus sequence polymorphism data [42,43[•],44[•]]. In addition, new technical breakthroughs have led to the ability to sequence substantial amounts of nuclear DNA from fossil bones and teeth [45,46[•]]. We discuss both of these in greater detail below.

On the theoretical side, the development and application of more sophisticated statistical and computational methods has led to an increased ability to distinguish between different demographic alternatives. One recent study analyzed resequencing data — West African and European samples from the Environmental Genome Project — to more rigorously test the hypothesis of ancient admixture between archaic and modern humans [44*]. Plagnol and Wall [44*] took the following approach. First, to avoid possible confounding effects, they used the available data to estimate demographic parameters for a null model that incorporates several known features of modern human history: recent population growth, a bottleneck in European populations, and population differentiation between European and West African populations. They then tested and verified that the estimated null model provides an accurate fit to the data, including for summaries of the data that were not used in the fitting process. Next, they tested for archaic admixture using a statistic specifically designed to identify the polymorphisms most likely to have been inherited from an ancestral archaic population. Plagnol and Wall [44*] were able to strongly reject the null model ($p \sim 10^{-7}$), which they interpreted as evidence in favour of ancient admixture. They estimated that the archaic contribution to the modern gene pool was at least 5%. Though this study — like all studies — has caveats, it provides the strongest evidence to date against strict versions of the recent African replacement model.

Another recent development has been the application of new sequencing technologies that are more effective on the degraded DNA found in fossil teeth and bones. Researchers have performed a substantial amount of nuclear sequencing in cave bears, mammoths and Neanderthals. Although full details are not yet available, preliminary results suggest that modern humans and Neanderthals diverged from each other roughly 400 kya, in keeping with previous fossil-based estimates [47*].

Conclusions and prospects

Future studies will gather a wealth of genetic polymorphism data from a wide range of human populations. These studies will use improved computational and statistical methods, and will give us increasing power to infer specific facets of our species' demographic history. However, studies of extant populations will always have their limitations; there is no direct way to infer which of the polymorphisms we observe today in the anatomically modern human genome descend from populations that have gone extinct. This situation changes once the possibility of large-scale sequencing of ancient DNA becomes a reality. Indeed, a Neanderthal genome project is underway, with the aim of sequencing as much of the Neanderthal genome as possible from extant fossil remains. Although completion of this project will be a technological *tour de force*, the comparison of a single

Neanderthal genome with a single modern human genome will not immediately answer questions about the evolutionary fate of the Neanderthals. Regardless of what level of divergence between the two is found, it will not directly answer the question of whether the two hominin groups interbred and produced fertile offspring. However, a comparison between one Neanderthal sequence and multiple modern human sequences has the potential to be much more informative. For example, one could use the method of Plagnol and Wall [44*] to identify mutations that might have been inherited from Neanderthals, then look to see whether the Neanderthal genome contains these same mutations. Approaches such as these have the potential to conclusively determine what contribution, if any, Neanderthals (or *H. floresiensis*) have made to the modern gene pool.

The initial picture emerging from recent genetic analyses [38,41*,44*] is one that involves Africa as a possible source of admixture between anatomically modern humans and an as yet unidentified divergent taxon of *Homo* that might have survived until quite recently.

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