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The Human Condition—A Molecular Approach

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Research into when and where modern humans originated and how they differ from, and interacted with, other now-extinct forms of human has so far been the realm of archaeologists and paleoanthropologists. However, over the past decade, molecular geneticists have begun to study genomes of extinct humans. Here, I discuss where we stand today with respect to understanding how modern humans came to differ from Neandertals and other human forms that existed until about 30,000 years ago.

Introduction

Humans are very special primates. Although apes such as chimpanzees are closely related to humans and use tools to solve simple tasks, like cracking nuts or catching ants, only humans develop highly complex tools, only humans actively teach each other the manufacturing and use of these tools, and only humans use language to transmit knowledge. In short, humans have developed a material and intellectual culture that is unique in its complexity. Most features of this culture are not only unique to humans but also rather recent developments in human history. We share a common ancestor with the chimpanzees on the order of 5 to 10 million years ago (Figure 1), but for the longest time after that, human ancestors continued doing pretty much what their ape-like ancestors did. Only some 2.6 million years ago did human ancestors start making stone tools that can be recognized as such when found by archeologists. But even then, the different tools produced did not change much for hundreds of thousands of years.

The situation changed shortly after 200,000 years ago when what archaeologists call "anatomically modern humans" appeared in Africa. They had skeletons very similar to those of present-day people, and they lived in Africa and the Middle East until sometime after 60,000 years ago. At that point, they started spreading across Eurasia, where they eventually replaced other forms of humans that already lived there. These modern humans showed clear signs of being just like present-day humans in their behavior. They produced figurative art that we find in the form of cave paintings and figurines made out of clay or bone. Their technology soon started changing rapidly, and they migrated across open water, eventually reaching not only all major continents but many tiny little islands in the Pacific Ocean. These behaviors were acquired by modern humans, presumably over a time of tens of thousands of years. But they were never acquired, even over hundreds of thousands of years, by the other, so-called archaic humans who were eventually replaced by the modern humans. The question of what made this explosive cultural development possible is one of the most important questions in human history. It is also a question with great ramifications because these technological leaps and the ensuing ability to multiply and colonize essentially all parts of the globe were the first steps in our species' tremendous influence on the whole biosphere. So far, research into how this revolutionary event came about has been the realm of archaeologists, paleoanthropologists, and evolutionary psychologists. However, over the past decade, molecular geneticists have started to contribute new data of relevance for understanding human uniqueness.

Comparison with Ape Genomes

Only 5 years after the completion of the first working draft of the human genome, the genome of the chimpanzee, one of the two closest living relatives of humans (Figure 1), was sequenced (Chimpanzee Sequencing and Analysis Consortium, 2005). The explicit goal was to illuminate human-specific biology (e.g., Olson and Varki, 2003). This was followed by the sequencing of the genomes of the macaque (Gibbs et al., 2007), the orangutan (Locke et al., 2011), the gorilla (Scally et al., 2012), and the bonobo (Prüfer et al., 2012). Comparisons among these genomes have revealed a great many things about the relationships and the evolution of the genomes of humans and other primates. For example, it has been shown that nucleotide substitutions accumulate at a rate that is approximately constant among the apes and humans but slower than in monkeys and rodents (Steiper et al., 2004; Wu and Li, 1985). Small deletions outnumber insertions by a factor of two or three and have occurred about twice as fast on the lineage to the chimpanzees and bonobos than on other ape lineages (Sudmant et al., 2013). So-called segmental duplications that are over a kilobase in length occurred four to ten times faster in the common ancestor of humans and the African great apes (gorillas, chimpanzees, bonobos) (Marques-Bonet et al., 2009b), and these duplicated sequences are more often interspersed across the genomes of primates, than in other mammals where they tend to be to be located in tandem arrays (Marques-Bonet et al., 2009a). The availability of ape reference genomes has also provided invaluable tools for studying genomic variation within these primate species (Prado-Martinez et al., 2013).

However, the insights into human-specific biology have so far been comparatively limited. One reason for this is that the large numbers of changes in the human genome since its divergence

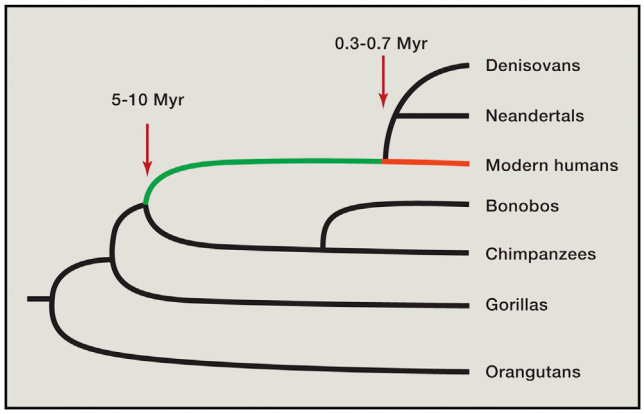

Figure 1. A Schematic Tree Illustrating the Average Relationships among the Present-Day Humans, Neandertals, Denisovans, and Great Apes

Age estimates for the population separation times between present-day humans and chimpanzees and bonobos and between present-day humans and Neandertals and Denisovans are given in millions of years (Myr). The ranges of these splits are large due to current uncertainties about the human mutation rate, which is used to estimate these dates (Langergraber et al., 2012; Scally and Durbin, 2012). The lineage leading to modern humans as well as the closest related extinct archaic hominins is indicated in green, and the lineage leaning exclusively to modern humans is in red.

from the ancestor shared with the chimpanzee genome—on the order of 20,000,000 single-nucleotide substitutions (Chimpanzee Sequencing and Analysis Consortium, 2005)—and changes in the numbers of repeated sequences and other insertions and deletions affecting at least as many nucleotides (Marques-Bonet et al., 2009b) make the functional study of all the changes impossible. Another reason is that the vast majority of the nucleotide substitutions observed have unknown consequences for the organism.

One way to identify changes of functional importance is to look for evidence of positive selection in the genome. Changes that are positively selected—that is, have effects that are beneficial to the individuals bearing that mutation—leave distinctive yet subtle signs in the genome, for example a reduced diversity and an increased frequency of rare variants around the change. However, tests to identify events of positive selection from such patterns work only for rather recent changes because over time new mutations arise and spread in the population, obscuring older patterns (Sabeti et al., 2006). To get at older instances of positive selection, one has to rely on comparing the numbers of substitutions likely to have functional consequences, for example by causing an amino acid substitution, to the number of changes that are unlikely to have such consequences in a gene or region of the genome. In this case, the tests can only detect cases where there have been multiple events of positive selection leading to functional changes. The unfortunate consequence is that in most cases, the human evolutionary lineage is too short to gain statistical power. For example, only 40% of protein-coding genes carry one or more substitution that will change an amino acid on the human lineage (Chimpanzee Sequencing and Analysis Consortium, 2005). In spite of these difficulties, some genetic changes that may have been of crucial importance have been identified on the human evolutionary lineage after it separated from the common ancestor with the chimpanzees.

One approach to find such changes has been to look for genes inactivated in the human genome given that the loss of a gene product is likely to have functional consequences. For example, a myosin heavy chain gene, *MYH16*, is inactivated by a frameshift mutation in humans. Because the myosin isoform encoded by *MYH16* is expressed in muscles involved in chewing, this may have contributed to the reduction in the masticatory apparatus in human ancestors (Stedman et al., 2004). A more systematic search for deletions in the human genome relative to other primate genomes revealed 510 deletions, which mostly fall in noncoding regions and often close to genes (McLean et al., 2011). Two of these deletions were investigated in some detail. One removes an enhancer from a gene involved in the development of penile spines, a part of the intimate anatomy of primate males that is lost in humans. Another deletion removes an enhancer near the gene *GADD45G* that may limit cell division in the subventricular zone during development of the cerebral cortex, a feature that may be associated with the expansion of the size of the human brain.

Another approach to identifying functionally relevant changes that occurred in humans since the separation from their common ancestor with chimpanzees has been to find "human accelerated regions" (HARs), i.e., genomic regions that are highly conserved among vertebrates yet have accumulated relatively many substitutions on the human lineage (Bird et al., 2007; Bush and Lahn, 2008; Pollard et al., 2006b; Prabhakar et al., 2006). The sequence conservation of HARs suggests that they are subject to functional constraints and are thus of importance, whereas the increased rate of substitutions on the human lineage may suggest that their function has changed or been lost in humans. Two HARs have been studied in some detail. One of these (Pollard et al., 2006b) encodes an RNA expressed in a subclass of neurons in the developing human cortex. The other one (Prabhakar et al., 2006, 2008) is an enhancer that in its human form is able to drive expression of a reporter gene in mouse limbs, whereas the ancestral version of the sequence carried by the apes is not. It is thus conceivable that this HAR has to do with the development of features unique to the human hand or foot. However, it is unclear to what extent the human-specific changes in HARs are generally functionally important. Nucleotide substitutions in HARs show an excess of A/T to G/C substitutions (Galtier and Duret, 2007; Pollard et al., 2006a) that appear to be due to GC-biased gene conversion (BGC), i.e., the nonreciprocal copying of a piece of DNA from one chromosome to the other that favors fixation of GC alleles over AT alleles (Duret and Arndt, 2008). Because recombination, and therefore BGC, tends to be localized to recombination hot spots (Paigen and Petkov, 2010), and because these hot spots often change their locations over short evolutionary times in primates (Ptak et al., 2005; Winckler et al., 2005), an increase in the rate of substitutions in a region of the human genome may be due to a recombination hot spot that has appeared in that region. It is thus likely that repeated events of BGC are the source of human-specific fixation of substitutions in many HARs (Duret and Galtier, 2009; Galtier and Duret, 2007).

Some evolutionary changes of likely importance have also been identified through observation of human-specific changes in combination with prior knowledge of the function of the genes affected. One example is glycosylation, where humans lack N-glycolylneuraminic acid (Neu5Gc), a hydroxylated form of sialic acid, on the cell surface (Muchmore et al., 1998). The reason for this is a 92 bp deletion in the gene encoding the enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH) (Chou et al., 1998), which was caused by recombination between two *Alu* elements and occurred a little over 2 million years ago (Chou et al., 2002). This change is accompanied by multiple additional human-specific changes affecting at least 10 genes encoding lectins and other proteins involved in sialic acid function (Varki, 2009). Interestingly, mice that have been engineered to lack the gene encoding CMAH and that therefore, like humans, lack endogenous Neu5Gc have T cells that proliferate faster than wild-type T cells and mount greater T cell responses to a virus. It is thus possible that this mutation has led to a more active T cell response in humans than in apes (Buchlis et al., 2013).

Genes can gain functional differences either through substitutions of specific nucleotides or by incomplete duplications/deletions. An example of the latter is *SRGAP2*, a gene that is present once in apes and has duplicated three times in humans. One of the duplicated forms encodes a truncated form of the protein (Dennis et al., 2012) that binds to the nontruncated protein encoded by the parental gene. When expressed in mouse neuronal precursor cells, the truncated protein variant results in increased density of longer neuronal spines (Charrier et al., 2012), a feature which may be typical of human neurons, at least when compared to nonprimate mammals. *FOXP2*, another gene that appears to have undergone changes relevant for human evolution, represents an example of the former kind of mutation process. Inactivation of one copy of this gene in humans results in severe language and speech problems (Lai et al., 2001). *FOXP2* encodes a protein that is extremely conserved among mammals yet carries two amino substitutions in humans (Enard et al., 2002). When these two changes are introduced into mice, neurons in the striatum increase their synaptic plasticity and grow longer dendrites. The changes seem to involve cortico-basal ganglia circuits that are important for motor learning (Enard et al., 2009). It is tempting to speculate that *SRGAP2* and *FOXP2* are the first identified members of a postulated set of genes that changed function some 2–3 million years ago as human ancestors grew larger brains and started to use these brains in new ways to perform complex tasks. Traces of these complex abilities in the form of stone tools may be seen in the archaeological record, whereas other innovations, such as the beginning of complex oral communication, may have left no archaeological traces even though they are at least as important for the emergence of human culture.

Comparisons with Archaic Humans

Extinct hominins that lived during the past half-million years are collectively referred to as archaic humans. Most well-known among these are the Neandertals. The ancestors of Neandertals were the presumably last group of hominins to branch off the human evolutionary lineage before the appearance of anatomically modern humans (Figure 1). They appeared in the fossil record some 300,000 to 400,000 years ago, lived in western Asia and Europe, and became extinct around 30,000 years ago. They had bodies more robust and brains somewhat larger than those of present-day humans, and they produced technology not very different from the earliest modern humans. As far as can be judged from the archaeological record, Neandertal culture and technology were relatively uniform and showed only slow change over the hundreds of thousands of years of their existence (Mellars, 1996). Toward the end of their history, starting at about 45,000 years ago, several new behaviors emerged that parallel developments seen in modern humans. A lively discussion is still ongoing about how these behavioral changes relate to the appearance of modern humans in Europe at around the same time (D'Errico, 2003; Mellars, 2005). Nevertheless, the qualitative revolution in the rate and mode of cultural change that characterizes the last 50,000 years of human history never happened for the Neandertals.

Because Neandertals are our closest ''nonmodern'' relatives, their genome can be used to investigate what sets the modern human genome apart, not only from chimpanzees and other apes but from all other human forms that are now extinct. This requires recovering DNA from Neandertal remains. The retrieval of DNA from old tissues goes back over 30 years (Pääbo, 1984). However, for a long time, it was hampered by technical problems. The invention of PCR overcame many problems (Pääbo et al., 1989), but the study of human remains turned out to be complicated by the presence of present-day human DNA in most laboratory environments and reagents. Even when present in vanishingly small amounts, such modern DNA often swamps the even smaller amounts of endogenous DNA surviving in a fossil (Handt et al., 1996). Another limitation was that so little DNA survives in most ancient remains that only mitochondrial DNA could be retrieved by PCR. Among the rare exceptions were mammoths and other animals preserved in the permafrost for tens of thousands of years, allowing nuclear DNA sequences to be retrieved by PCR (Greenwood et al., 1999) and whole genomes to be sequenced (Poinar et al., 2006). However, thus far only human remains less than 6,000 years old have been found in the permafrost in a state that allows genome sequencing (Keller et al., 2012; Rasmussen et al., 2010). From the less well-preserved Neandertal fossils, only small parts of the mitochondrial DNA could be sequenced early on (Krings et al., 1997).

The advent of high-throughput sequencing technologies (Bentley et al., 2008; Margulies et al., 2005) changed this picture. Though attempts to sequence Neandertals by shot-gun approaches initially suffered from problems with human contamination (Wall and Kim, 2007), these were overcome by new techniques to make libraries (Green et al., 2009). A first draft of the Neandertal genome was produced in 2010 (Green et al., 2010). It showed that an average of ~2% of the genomes of people living in all parts of the world except sub-Saharan Africa are closely related to the Neandertal genome. This observation suggested that Neandertals had contributed to the genetic variation of present-day humans by mixing with modern humans when they came out of Africa. This hypothesis has since been borne out by population genetic simulations (Yang et al., 2012).

Figure 2. A Copy of the Fragment of the Last Phalanx of the Fifth Finger Used to Sequence the Denisova Genome to 30-Fold Coverage

In addition, the time at which Neandertal DNA entered the modern human gene pool has been estimated at between 40,000 and 90,000 years ago from the size distribution in present-day people of segments of Neandertal-like DNA, which is reduced in each generation by recombination (Sankararaman et al., 2012).

The new techniques to sequence old genomes were also applied to a tiny finger bone discovered in 2008 in the Denisova Cave in southern Siberia (Figure 2). Its genome sequence unexpectedly showed that it came from a population that shared a common origin with Neandertals but had separated from them early in its history. This previously unknown group was termed ''Denisovans'' after the cave where the finger bone and two teeth containing Denisovan DNA were found. It is the first extinct human group that is defined solely on the basis of DNA sequence data and in the absence of any skeletal morphology. Intriguingly, Denisovans contributed ~5% of the genomes of present-day people in Papua New Guinea, Australia, and some other parts of Oceania (Reich et al., 2010, 2011).

The first Neandertal and Denisovan genomes were only 1- to 2-fold genomic coverage. This meant that only a little over half of the genomes were covered by the DNA fragments sequenced. In addition, the Neandertal genome sequences were affected by nucleotide misincorporations caused by deamination of cytosine residues that affects DNA found in archaeological remains (Hofreiter et al., 2001). This results in uracil residues that appear as thymine residues in the sequenced DNA. The enzymatic removal of deaminated cytosine residues before sequencing (Briggs et al., 2010) and a novel technique that uses single-stranded DNA to produce sequencing libraries from very small amounts of damaged DNA allowed the determination of a 30-fold coverage genome from less than 10 mg of the Denisovan finger bone (Meyer et al., 2012). More recently, a Neandertal genome was sequenced to about 50-fold coverage from a toe bone found in a deeper archaeological layer in Denisova Cave (Prüfer et al., 2014). The analysis of these two genomes reveals that Neandertals contributed DNA to Denisovans to approximately the same extent that they contributed DNA to modern humans (Figure 3). In addition, Denisovans are likely to have carried DNA from yet another extinct hominin that diverged earlier from the human lineage. Thus, archaic and modern human groups have on several occasions mixed with each other. However, interbreeding appears to have been of comparatively limited extent. Thus, although the first individuals that were the result of interbreeding between Neandertals and modern humans had half of their DNA from each of the two groups, the number of such mixed individuals was small enough that after some generations, the Neandertal contribution in any one individual among modern humans ended up being only about 2%. So far, there is no indication that interbreeding with archaic humans has resulted in the introduction of more than about 8% of DNA in any present-day humans (Prüfer et al., 2014).

In addition to insights into the origins and mixing of various hominin forms over the course of evolutionary history, the archaic genomes can be used to look for genome changes that have been important for the evolution of present-day humans. For the approximately 1.9 billion bases in the genome to which Neandertal and Denisovan DNA fragments of an average length of 40 to 60 bp can be confidently mapped, the quality of the two archaic genome sequences is now as high as that of high-coverage genomes of present-day humans. For any nucleotide change of interest in these parts of the human genome, it can therefore now be determined whether it occurred or rose to high frequency before or after modern human and archaic ancestors separated some 300,000 to 700,000 years ago (Figure 1).

For example, it can now be determined that the two changes that resulted in an amino-acid substitution in the *FOXP2* gene in humans relative to chimpanzees and other apes are shared with Neandertals (Krause et al., 2007) and Denisovans, showing that these two changes predate the divergence of humans and Neandertals. However, another change in an intron of the *FOXP2* gene that affects a conserved binding site for the transcription factor POU3F2 changed in modern humans after their separation from the two archaic lineages. When the human-specific version at the POU3F2-binding site was compared to the ancestral version in functional assays, the latter was found to be more effective in activating transcription from a reporter construct (Maricic et al., 2013). It is therefore likely that subsequent to the substitutions that changed the encoded protein sequence, *FOXP2* was affected by a functional change that changed its expression level in some tissues (Figure 4). This illustrates how the archaic genomes can be used to decipher the temporal sequence of changes during human evolution.

Genomic changes, like the one affecting the transcription binding site in the *FOXP2* gene described above, that are found in all, or almost all, humans today but appear in the ancestral or ape-like state in the Neandertals and Denisovans comprise a genetic definition of modern humans relative to our closest extinct relatives. Interestingly, such changes are not very numerous. There are 31,389 single-nucleotide positions in the genome where (as far as is currently known) all present-day humans carry only a novel or derived nucleotide, whereas both the Neandertal and Densiovan genomes carry only the ancestral nucleotide. Of these, 3,117 fall in regulatory regions (as defined by *Ensembl,* v. 67, http://may2012.archive.ensembl.org/index.html), 32 affect putative splice sites, and 96 affect amino acids

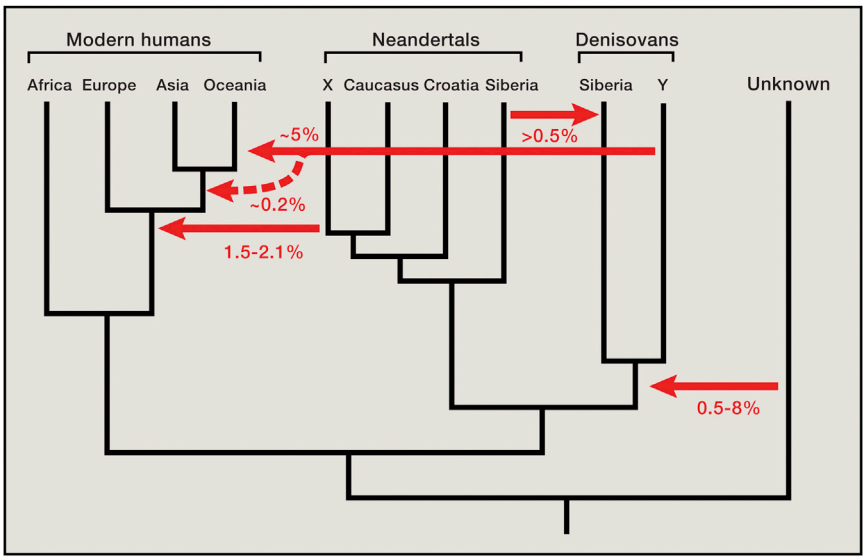

Figure 3. A Schematic Tree Illustrating Four, Possibly Five, Gene-Flow Events that Have Been Documented by Analyses of the Genomes of Archaic and Present-Day Humans

The approximate average fractions of the genomes contributed to the receiving group are given. X denotes the unknown Neandertal group that mixed with Eurasian modern humans, and Y the Denisovan group that contributed to ancestors of present-day people in Oceania. The dotted arrow indicates that people on mainland Asia also contain small amounts of DNA from Densiovans that may have been contributed by a separate mixing event(s) or by the same event that affected Oceanian ancestors (Prüfer et al., 2014).

in a total of 87 proteins (Table S1 available online) (Prüfer et al., 2014). However, our ability to identify functional variants in the genome is still very poor, so these may represent just a subset of the changes that could be important.

The challenge ahead is to find out which of these changes are functionally significant. Fascinatingly, the list of changes is so short that all changes can in principle be studied. Already a cursory inspection suggests that some may be interesting. For example, under the assumption that changes in brain development were important for the emergence of modern humans, it is interesting that of the 87 proteins carrying amino-acid changes, a larger number than expected are expressed in the ventricular zone when neurons of the cerebral cortex are formed during fetal development (Prüfer et al., 2014). Of the five proteins from Table S1 expressed in the ventricular zone (CASC5, KIF18A, TKTL1, SPAG5, VCAM1), three (CASC5, KIF18A, SPAG5) are associated with the mitotic spindle and the kinetochore. Because the orientation of the mitotic cleavage plane in dividing neural precursor cells may determine what type of neuronal precursor cell is formed (e.g., Fietz and Huttner, 2011), the functional consequences of these changes on neurogenesis will be interesting to explore. Another of the five genes, *VCAM1*, is involved in the maintenance of neural stem cells in the adult subventricular zone (Kokovay et al., 2012). These proteins may thus point to some aspect of cortex development that is unique to modern humans.

When considering how modern humans may differ from Neandertals and Denisovans, it should not be forgotten that these archaic humans are so closely related to present-day humans that for about 90% of the genome, they fall within the present-day human variation, i.e., for 90% of the genome, some humans today are more closely related to the Neandertals and Denisovans than to other present-day humans. In order to think about how modern humans may differ genetically from Neandertals and Denisovans, it may thus be useful to consider how present-day human groups differ genetically among themselves.

Comparisons among Modern Humans

Once humans became distributed on different continents, the possibility for any new mutation to spread to all humans was drastically reduced. Thus, for the past 50,000 years, species-wide fixations of alleles have likely ceased. However, humans have continued to accumulate mutations and to adapt to their local environments and cultures. This has produced phenotypic differences among people in skin color, body height, facial features, and other traits. Even closely related populations sometimes differ in such traits. Naively, one might therefore expect to find genetic differences that are fixed between different populations. However, the opposite turns out to be true.

An often overlooked insight from studies of DNA sequence variation on a global scale, for example, by the 1000 Genomes Project (Abecasis et al., 2010, 2012), is that there are no absolute genetic differences between continental populations. For example, when the genomes of 185 individuals from two African populations are compared to the genomes of 184 people representing European and Chinese populations, 38,877,749 positions in the genomes are found to vary. However, not a single nucleotide difference distinguishes all Africans from all Eurasians, and only 12 positions carry differences where one allele is present in 95% or more of Africans and in 5% or less of Eurasians or vice versa. How can this be when continental populations differ so obviously in features such as their physiognomy?

The answer is that most such features are formed by the interplay of many different genetic loci, and these loci carry alleles that vary in most populations. Therefore, a trait can change rapidly if selection slightly shifts the allele frequencies at many different loci that all contribute to a trait. For example, a modest shift in the allele frequencies toward alleles that favor lighter skin or less hair may thus "shift the balance" such that the appearance of many or most individuals in a population changes (Hernandez et al., 2011; Pritchard and Di Rienzo, 2010; Pritchard et al., 2010). That this can happen rapidly is obvious from the fact that even closely related populations sometimes differ in appearance or other features. An example is body height, a deceptively simple trait that has nevertheless been shown to be influenced by at least 180 different genetic loci (Lango Allen et al., 2010).

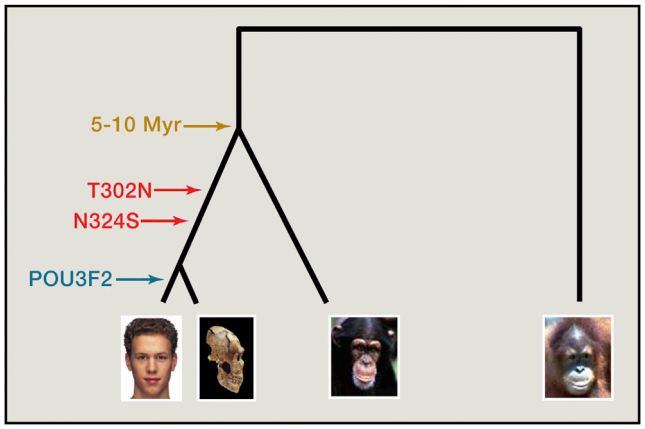

Figure 4. Schematic Illustration of the Two Amino-Acid Substitutions (Red) and the Change in a POU3F2-Binding Site (Green) that Have Affected the *FOXP2* Gene during Human Evolution
For changes of interest, humans are expected to be fixed or almost fixed for the derived state (>95%).

When people from northern Europe (where people tend to be taller) were compared to people from southern Europe (where people tend to be shorter), 85 out of 139 height-increasing alleles were found to occur at higher frequencies in northern Europeans than in southern Europeans (Turchin et al., 2012). Cultural or environmental factors apparently favored taller height in northern Europe or shorter height in southern Europe (or both), causing small shifts in allele frequencies at many of these loci, which together contributed to the differences in average stature observed today.

Because most methods used to detect positive selection are based on models where a novel mutation is swept to high frequency, positive selection that works by shifting frequencies of preexisting variants at many loci may often go undetected. Methodological advances (e.g., Peter et al., 2012) in combination with whole-genome sequence data from large numbers of individuals from many populations will hopefully eventually lead to a better understanding of the extent to which positive selection of preexisting variants affecting multigenic traits has shaped the current human gene pool.

However, classical selection acting on a single or a few genes has also played a role in recent human history. In fact, some studies have suggested that perhaps as many as 2,000 genes (Akey, 2009; Sabeti et al., 2007; Voight et al., 2006) and some 10% of the human genome (Williamson et al., 2007) are affected by positive selection. However, the limited overlap in genes identified among such studies is sobering (Akey, 2009) and suggests that their false-positive rate may be large (Teshima et al., 2006). Nevertheless, three organ systems seem to have been particularly susceptible to positive selection affecting few or single genes: the immune system, the digestive tract, and the skin with its appendages (hair, sweat glands, and sensory organs) (Grossman et al., 2013; Williamson et al., 2007). The reason may be that these parts of our bodies interact with the environment in very direct ways.

Pathogens cause disease and death when the immune system fails to deal adequately with them. It is therefore not surprising that variants of immune genes have been positively selected (Kosiol et al., 2008). This affects genes involved in innate immunity as well as both the cellular and humoral aspects of the adaptive immune response (Williamson et al., 2007). In addition, receptors or molecules that in various ways are necessary for serious infections to take hold in the human body have been targets of recent positive selection in humans (Kwiatkowski, 2005). An illustrative example is the C-C chemokine receptor 5 encoded by the gene *CCR5*, which is needed for entry into T lymphocytes by the human immunodeficiency virus (HIV)-1 (Carrington et al., 1999). A 32 bp deletion in *CCR5* confers protection from HIV infection and is thus currently positively selected in populations where HIV-1 infections occur. Another example is the gene *G6PD*, which encodes the enzyme glucose-6-phosphate dehydrogenase, which is involved in glycolysis. An allele carrying two substitutions that change amino acids in the protein and confer a 50% resistance to malaria infection reaches frequencies up to 20% in areas where malaria is prevalent (Cavalli-Sforza et al., 1996), although it reduces the activity of the enzyme by almost 90% (Hirono and Beutler, 1988). These and several other examples (e.g., Grossman et al., 2013) illustrate how pathogens by directly endangering our individual survival exert positive selection on genetic variants in the human population.

Another way in which we directly interact with the environment is through eating and drinking. Diets have changed rapidly over time, for example, with the advent of agriculture and animal husbandry, and they differ drastically between cultures. As a consequence, several physiological aspects of food selection and processing have been the targets of positive selection. Examples are genes involved in smell and taste (Kosiol et al., 2008) as well as digestion. A well-described example is the gene *LCT*, which encodes lactase, an enzyme produced in the intestines that degrades the disaccharide lactose in milk to glucose and galactose. In most humans (as well as most mammals), *LCT* transcription ceases after weaning. However, in European populations that consume fresh milk, a mutation in an enhancer located 14 kb upstream of the lactase gene *LCT* causes the gene to continue to be expressed in adult individuals, allowing them to drink fresh milk without intestinal discomfort (Enattah et al., 2002). The *LCT* gene in European populations carries a strong signal of positive selection (Bersaglieri et al., 2004), and other *LCT* alleles conferring lactase persistence have been independently selected in African populations that use fresh milk (Tishkoff et al., 2007), suggesting that the consumption of milk has been a substantial advantage in the past. As expected, the Neandertal and Denisovan genomes do not carry substitutions associated with lactase expression after weaning (Green et al., 2010; Meyer et al., 2012).

Another dietary adaptation has affected the enzyme amylase in saliva, which helps digest starch. It is encoded by the gene *AMY1*, which occurs in up to nine copies per haploid human genome. Individuals in populations with starch-rich diets tend to have higher copy numbers than those in populations such as hunter-gatherers that eat little starch, whereas only between 1 and 2 percent of people carry a single *AMY1* gene (Perry et al., 2007). The apes, as well as the Neandertal and the Denisovan genomes, carry single AMY1 copies (Prüfer et al., 2014),

consistent with the idea that *AMY1* copy number increased with an increasing reliance on starch-rich food such as tubers in early modern humans; copy number then increased even further with the advent of agriculture, when starch-rich crops became important parts of the diets of many populations. Alcohol dehydrogenase that breaks down ethanol may similarly have been positively selected when agriculture made the production of fermented alcoholic beverages easy (Peng et al., 2010).

Skin and its appendages mediate many direct interactions of our bodies with the environment, for example, in the form of thermoregulation, detection of pain and pressure, and absorption of UV radiation. They also influence our appearance, which in turn may influence our reproductive success. Not surprisingly, several genes involved in pigmentation show evidence of positive selection (Williamson et al., 2007). For example, the gene *SLC24A5*, which encodes an ion transporter in melanosomes, has a variant that is associated with light skin pigmentation and shows signs of having risen to high frequency in Europe by positive selection (Lamason et al., 2005). Another example is the gene *EDAR*, which encodes the receptor for ectodysplasin, a protein involved in the development of ectodermal organs. An *EDAR* variant, the 370A allele, causes an amino-acid change in the receptor that is associated with increased hair thickness and shovel-shaped incisors. The 370A allele is present in frequencies close to 100% in many populations in Asia, where it shows signs of having been positively selected (Grossman et al., 2010; Sabeti et al., 2007). Recently, the human 370A allele has been engineered into mice. As expected, hair thickness was increased in the mice. However, the allele was also found to cause a higher density of ducts and smaller fat pads in the mammary glands and to increase the number of eccrine sweat glands. Prompted by this finding, an association study in humans found that individuals homozygous for the 370A allele had more eccrine glands than heterozygous individuals (Kamberov et al., 2013).

In addition to the immune system, the digestive tract, and the skin, some other aspects of human physiology have also been affected by positive selection. For example, 87% of people from the Tibetan Plateau carry a variant of the *EPAS1* gene, whereas only 9% of people in related populations at low altitudes carry this allele (Yi et al., 2010). The *EPAS1* gene encodes a transcription factor involved in response to hypoxia, and the variant common at high altitudes is likely to have been selected to cope with low oxygen pressure in Tibet.

Some alleles introduced into modern humans through interbreeding with Neandertals and Denisovans may also have been positively selected. For example, variants of immune response genes have entered the modern human population from archaic humans and risen to high frequencies in some regions of the world, suggesting that they confer some advantage there (Abi-Rached et al., 2011). Similarly, a Neandertal variant of the gene *SLC16A11,* which encodes a lipid transporter protein in the endoplasmic reticulum, has entered the human population and reached high frequencies in Native Americans, where it is associated with increased risk for type 2 diabetes (Williams et al., 2014). More examples of archaic alleles that influence the physiology of present-day people are likely to be found in the future because at the time when the archaic DNA sequences entered the modern human population, they had accumulated mutations independently of modern humans over the course of at least 300,000 years (Prüfer et al., 2014). Thus, although only about 2% of modern human genomes outside Africa derive from Neandertals, those 2% could in some cases have had a large impact on the physiology of the affected populations.

In summary, the overall picture emerging from genome-wide studies of variation in humans is that the parts of the human body or physiology where genetic differences between populations exist are those that are affected by the environment in very direct ways, such as the skin, the intestinal tract, or the immune system. In most cases, the aspects of our physiology affected are those that were known to differ between populations well before any genetic studies were performed. For example, it has been known for some time that skin color, hair structure, resistance to certain diseases, ability to drink fresh milk, or ability to physically perform at low oxygen levels differ among human populations. From a societal perspective, it is notable that other aspects of human physiology, in particular behavior or cognitive abilities, have not been revealed to be frequent targets of positive selection in human populations. The reason may be that the way in which environmental or cultural factors affect these traits are neither very direct nor consistently different between present-day human populations.

Modern Human Universals

Cognitive abilities are, however, likely to have been positively selected in early modern humans sometime after they separated from ancestors shared with Neandertals and Denisovans when perhaps subtle but novel cognitive abilities made the unique demographic and cultural expansion over the past 100,000 years possible. Can the genetic background for these traits be found, now that the genomes of our closest archaic relatives are known and the genomic variation among present-day humans is becoming well documented?

The answer to this question depends on whether one believes that a few crucial changes are responsible for these traits, as is the case for differences among present-day populations in pigmentation, digestion, and the immune system, or if one believes that each of these traits involves many genes where preexisting variants were selected, as is the case for body height. Unfortunately, the relatively short-lived nature of signals of positive selection (Przeworski, 2002) limit their helpfulness in discerning which genetic differences between present-day humans and Neandertals and Denisovans may have been positively selected sometime between 300,000 and 100,000 years ago. However, because the total number of such changes is just in the tens of thousands, a systematic functional analysis of all of them can be contemplated. An interesting question is then how traits that are specific to humans yet do not vary to any appreciable extent among humans should best be functionally studied.

Functionally Assessing Human Variants

At least three approaches for testing the functions of genetic variants specific to modern humans can be envisioned. One approach will be to introduce such genetic variants into mice.

Mouse models for *FOXP2, CMAH*, and *EDAR* have shown that rodent models can generate insights about the function of human variants. However, some human gene products may not function adequately in the genetic background of the mouse. This might be overcome by humanizing whole pathways or organelles in the mouse. For example, given that at least three proteins associated with the mitotic spindle and the kinetochore carry modern human-specific amino-acid substitutions (Prüfer et al., 2014), it might be interesting to humanize these organelles in the mouse in order to compare ancestral and derived variants.

A second approach will be to introduce the ancestral versions of genes of interest into human induced pluripotent stem cells (iPSCs). These cells can be coaxed to differentiate into neurons, hepatocytes, myocytes, and other cells in vitro, and methods to allow such cells to develop into more organ-like structures are being developed (Lancaster et al., 2013). Recent advances have made it much easier to both efficiently and specifically change nucleotides in eukaryotic genomes (Gaj et al., 2013), making this approach much more feasible.

A third approach will be to look for back-mutations in humans. The human genome is small enough that all mutations compatible with life are present in the current human population, albeit in most cases at low frequencies. In the future when millions of people will have their entire genomes sequenced as a routine medical procedure, it will therefore become possible to identify mutations back to the ancestral state and to study their effects in human individuals. This of course will require that their effects manifest themselves in heterozygous individuals and that the ethical issues about approaching affected individuals are solved. A complementary approach would be to incorporate the 31,389 alleles that are currently thought to be fixed in humans on arrays used to assess variable positions in the human genome.

Given the efforts necessary for these undertakings, it will be valuable to prioritize changes to be introduced into iPSCs or mice. A number of complementary ways to do this are possible. One way would be to sequence the genomes of very early modern humans. Because the changes that define modern humans must have arisen before some 50,000 years ago, modern human genome sequences from before this time are likely to refine the list of potential candidates. However, although under some circumstances DNA retrieval techniques now allow hominin DNA as old as 400,000 years to be retrieved (Meyer et al., 2014), such genomes will be very hard to sequence because of the hot environments wherein many hominin fossils are found.

A second way to prioritize changes will be to identify those that exist in the large regions where the Neandertal and Denisovan genomes fall outside the variation of present-day humans because in such regions, variants are likely to have swept rapidly to fixation in early modern humans, dragging along with them large parts of the chromosomes that did not have time to become reduced in size by recombination (Green et al., 2010; Prüfer et al., 2014).

A third prioritization strategy will be to use the fact that Neandertals and Denisovans mixed with modern humans. Intriguingly, a systematic analysis of where Neandertal DNA fragments occur in present-day humans suggests that certain regions of the genome are resistant to introgression from archaic humans (Sankararaman et al., 2014; Vernot and Akey, 2014). Perhaps gene variants in some of these areas led to sterility in the hybrids, but it is also possible that strong selection of a social or other nature made survival of archaic DNA in these regions much less likely or impossible. Such genomic regions might therefore encode features that defined modern humans as a group distinct from their archaic contemporaries.

No matter how this is approached, the search for the genetic changes that made modern human culture possible will be an expensive and labor-intensive undertaking but a worthwhile one given that understanding the biological basis for why modern humans came to explode in population size and eventually influence many parts of the biosphere is one of the most fundamental questions in human history. It may also provide new insights into our uniquely human biology and a new inroad into the etiology of diseases such as autism, which has been plausibly argued to affect recently evolved aspects of human cognition (Tomasello et al., 2005).

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.12.036.

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