Agreements and Misunderstandings among Three Scientific Fields: Paleogenomics, Archaeology, and Human Paleontology
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Agreements and Misunderstandings among Three Scientific Fields

Paleogenomics, Archaeology, and Human Paleontology

by Carles Lalueza-Fox

The emergence of paleogenomics (the study and analysis of ancient genomes) has provided a new, powerful source of information that can be used to test previous hypotheses regarding human evolution. However, various misunderstandings concerning the interpretation of genetic data in an archaeological and paleontological context and the existence of different scientific goals tend to hinder the fluent and fruitful collaboration between these fields. Here we explore some of the subjects creating confusion, such as the problems associated with molecular clocks, the difference between sequence divergence and species divergence, and the limitations of the uniparental markers. Limited understanding of how the expression of a genome shapes the phenotype (including morphology and cognition) is the main obstacle to linking the genetic and the morphological evidence available. In the case of Neanderthals (and probably Denisovans, too), it is obvious that the conspicuous morphological differences cannot be explained by differences in a list of about 100 genes alone, thus suggesting that regulatory genomic elements must have been involved. A functional analysis of the genes involved as well as a study of the genomic architecture—a complexity level above the simple DNA message—could help us fill this gap. It is hoped that this future work will lead to the emergence of an interrelated and multidisciplinary view of the study of the past based on real collaborative efforts among disciplines.

Introduction

The interaction between archaeologists, paleontologists, and researchers from the emerging field of paleogenomics has traditionally been plagued by misunderstandings and a lack of collaborative efforts. Over the last three decades, molecular biologists working on population analysis of human samples have usually tried to fit their results to hypotheses proposed previously on the basis of morphological or archaeological studies. These hypotheses were often chosen at random from the available literature by the authors of these population genetics studies, who were clearly unfamiliar with the current state of the art in these other fields. Furthermore, the genetic results themselves—especially with data, such as mitochondrial DNA sequences, with limited phylogenetic resolving power—frequently did not allow the favoring of one hypothesis over another. For this reason, having possible support from another field (paleontology, archaeology, even linguistics or paleoclimatology) was seen as valuable and most-needed additional evidence. This kind of subjective multidisciplinary match was very convenient for increasing the chances of publication of those population genetic papers. However, many of these analyses were in fact purely descriptive and provided very limited insights into the genetic structure of past and extant human populations. As such, their real utility would be in providing a context that with increasingly large numbers of samples could be used for testing competing explanations in terms of past migrations and population affinities. When viewed from the other point of view, the behavior of archaeologists and paleontologists has often been equally biased, sometimes ignoring widely accepted genetic results in a rather condescending attitude of intellectual isolation. Many of these researchers seem to consider geneticists as newcomers to the study of the human past; this may be true, but they are here to stay.

These disagreements between different fields can be partially explained by some limitations associated with the genetic markers typically employed as well as problems in our current understanding of the relationship between genotype and phenotype. Here, I would like to highlight some of these difficulties and suggest how they can be overcome. In the future, we can expect that an interrelated and multidisciplinary view of our study of the past will be possible, and this can only be achieved with direct and real collaboration.

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Molecular Clocks, Sequence Divergence, and Species Divergence

We will start by discussing some of the problems associated with the evolutionary interpretation of the genetic data, as they are usually the subject of misinterpretations by archaeologists and paleontologists. The molecular clock hypothesis is based in the regularity of the mutation process in neutral genetic regions along time, thus involving the possibility of using it as a time estimator for molecular evolution. There are, however, some problems with the accuracy of a molecular clock. First, the current genetic diversity (either a population of study or a species) needs to be well characterized; second, the mutation rate needs to be known; third, we need to have precise dates to calibrate the clock (they are usually taken from the fossil record); and fourth, we need to work with selectively neutral genomic regions. All four factors can have their own limitations; for instance, there is conflicting evidence for estimating the mutation rates from family pedigrees and from evolutionary data (the former rate being usually much faster than the later). Also, because of the existence of ubiquitous regulatory elements and undetected selective sweeps, it is sometimes not so obvious that a particular genomic region is neutrally evolving. Thus, it is not surprising that time estimates can always be subjected to refinements and corrections. When we say, for instance, that the origin of the Neanderthal mitochondrial DNA variation can be dated to about 110,000 years ago (Briggs et al. 2009), we are assuming that the sampled Neanderthals are representative of the whole Neanderthal variation. If the next mitochondrial genomes to be sequenced turn out to be more variable than the currently available ones, the "Neanderthal Eve" will be moved dramatically back in time. Alternatively, if the future mitochondrial genomes are quite similar to the previous ones, the date will not be significantly altered. Therefore, another obvious trait of the molecular clock date is its capacity of being recalibrated depending on the sampling.

In the last decades, a new population genetics approach with a strong mathematical base, know as coalescence, has also been developed. The coalescence theory allows us to go backward in time from the existing genetic variation until finding its common ancestors, providing inferences on population demography and genetic divergence. Another common misunderstanding with other scientists dealing with the study of the past is the confusion between sequence divergence and species (or population) divergence. The coalescence times obtained always predate the real species divergence simply because there is a certain genetic variation in any group of individuals at any given time. The Italian geneticist Guido Barbujani (Barbujani, Bertorelle, and Chikhi 1998:489) famously illustrated this point with the following remark: "Suppose that some Europeans colonize Mars next year: if they successfully establish a population, the common mitochondrial ancestor of their descendants will be Paleolithic. But it would not be wise for a population geneticist of the future to infer from that a Paleolithic colonization of Mars." Therefore, the smaller the ancestral population size, the closer sequence divergence times and species divergence time would be; but we have to keep in mind that both features do not need to be coincident.

Mitochondrial DNA: Limitations of Uniparental Markers

Before the mass availability of genome-wide data, people working on the genetics of human populations had to base their interpretations on single genetic loci, mainly uniparental markers such as maternally inherited mitochondrial DNA (mtDNA) and the paternally inherited Y chromosome. Although it is frequently stated that mtDNA is just a single, uniparental genetic marker, the limitations of the mtDNA for interpreting evolutionary processes are not fully recognized in the population genetics literature. Because of the stochastic factors associated with demography, some genetic markers may reflect population or species history and some may not (Balloux 2010). This is related to lineage sorting—in other words, the process of gene-lineage fixation along an evolutionary process. Incomplete lineage sorting occurs when a gene tree or genealogy differs from the species phylogeny, a phenomenon that produces conflicting phylogenies and nonmonophyletic groups for a particular genetic marker. The uniparental markers (mtDNA or Y chromosome) are greatly affected by these random processes. In this sense, a remarkable discrepancy between mtDNA and nuclear DNA phylogenetic trees has been recently described for polar bears (Miller et al. 2012). Of course, this variation in coalescence times along genetic markers would not be a problem if multiple nuclear genetic markers or even complete genomes could be generated, as is increasingly the case even for extinct hominin species.

In this regard, the estimate divergence times for the separation of Eurasian and African populations, generated from the observed mtDNA diversity, has yielded dates of <100,000 years ago (almost always around 60,000–80,000 years ago), while the time depth for the African populations has never been older than 200,000 years ago (the time of the so-called mitochondrial Eve). Nevertheless, nuclear DNA divergence times obtained from complete genomes are estimated to be around 600,000–800,000 years (Green et al. 2010; Reich et al. 2010). It is probably oversimplistic to directly interpret these molecular clock dates from the point of view of a simple speciation event because demographic events such as population fluctuations could have greatly affected the mitochondrial as opposed to the nuclear genome diversity. In fact, no bottleneck is even needed. If the effective population size was constant at the time of the emergence of modern humans, the mtDNA would still coalesce at some point in the past (Weaver 2012). If this was the case, anterior demographic events would have been "erased" and thus would be undetectable from the analysis of the mtDNA. The African thermal

conditions, unfavorable to DNA preservation, makes it likely that no ancient DNA would ever be retrievable from specimens before the "mitochondrial Eve," and thus we will need to rely more and more on the analysis of complete modern African genomes.

Gene Flow from Archaic Hominins

One interesting example of a coalescent discrepancy between an mtDNA and the nuclear genome has been described in the Denisova hominin (named after Denisova Cave in Siberia, Russia). The analysis of the complete mtDNA genome showed that Neanderthals were the sister group of modern humans and that the Denisova lineage diverged from those of Neanderthals and modern humans about one million years ago (Krause et al. 2010). This tree was misinterpreted to represent the evolutionary relationships between these three species. However, subsequent analysis of the complete Denisova nuclear genome produced a rather different picture in which Denisova was now the sister group of Neanderthals, and both lineages shared a common ancestor around 640,000 and with modern humans around 804,000 years ago (Reich et al. 2010). The authors suggested that the Denisovan mtDNA lineage could represent an archaic mtDNA that was introduced into Denisovan ancestors by hybridization with some archaic hominin and subsequently preserved in the population by incomplete lineage sorting. An additional individual from the same site showed an almost identical mtDNA (Reich et al. 2010), thus demonstrating that this discordance between mtDNA and nuclear DNA is not restricted to a single Denisovan individual. The authors suggested this kind of discordance is not outside the range of what could be expected within a population. However, the subsequent Denisovan high-coverage genome (Meyer et al. 2012) showed this individual had a remarkably low heterozygosity (only ~26%–33% of that seen in modern Eurasians), which seems to indicate a very small population size.

While Denisovans could be descendants of a morphologically unknown eastern form of hominin that inhabited large areas of Asia while *Homo neanderthalensis* was mainly evolving in Europe, the inferences drawn from the mtDNA alone, if nuclear data were unavailable, would have been rather different, pointing to a recent survival of more primitive hominin forms such as *Homo erectus*. A genomic comparison with modern humans also found that modern Melanesians, but not other non-African modern humans, share about 4.5% of their genomic regions with Denisova (Reich et al. 2010, 2011). If anything, the Denisova study shows that the emerging picture of human evolution is one in which gene flow between different hominin populations (or species) was common. A similar result was found previously upon analysis of the Neanderthal genome in which non-African modern humans share about 2.5% of their genomic regions with the former (Green et al. 2010). More recently, a genomic analysis in sub-Saharan populations has detected that about 2% of their genomes seems to be provided from yet another introgression event that took place around 35,000–50,000 years ago by contact with some archaic African lineage now extinct (Hammer et al. 2011). This archaic population, morphologically undetermined, would have split about 700,000 years ago from the lineage leading to the ancestors of modern humans (Hammer et al. 2011). Signals of admixture with Neanderthals have also been detected in North African populations, probably deriving from a back-to-Africa migration after the contact in the Near East (Sánchez-Quinto et al. 2012). Thus, the complex evolutionary events that took place in Africa during the Middle Stone Age are still being unraveled.

This is a crucial point, as some of the limitations of uniparental markers chiefly arise when they are used to detect gene flow and hybridization events. We therefore need to redefine the use of mtDNA and the Y chromosome in human evolution studies because they have clearly failed to detect the real evolutionary processes that took place in the out-of-Africa expansion of our species (see, e.g., Briggs et al. 2009; Krings et al. 1997; Serre et al. 2004). Moreover, thousands of modern human genomes from a large number of populations as well as new ancient hominin genomes will be available in the near future and will provide clearer answers to questions concerning the origin of our species than those obtained from uniparental markers. The scientific time of the mtDNA and Y chromosome as the main tool to correlate with the fossil record is coming to an end.

Limitations of the First Neanderthal Genome Draft

Some people may think that not much was discovered about Neanderthals themselves after release of the first genome draft. In fact, it is easier to understand ourselves by comparing us to Neanderthals than to understand what makes a Neanderthal a Neanderthal (or a Denisovan a Denisovan; Lalueza-Fox and Gilbert 2011). This problem is derived from the low genomic coverage of the first draft (Green et al. 2010). With a 1.3× coverage, if a particular read has an ancestral nucleotide in a position where modern humans have a fixed, derived nucleotide, it is likely that this read is neither the product of an unknown, chimpanzee-like contamination nor postmortem damage (fig. 1). However, those positions where modern humans have a fixed ancestral variant and Neanderthals a derived one are more difficult to validate (fig. 2). In this case, a Neanderthal read may harbor a novel variant, but it could also be simply due to damage or sequencing error. Of course, the damage tends to be template specific; therefore, increasing the coverage should make it possible to track those genes that have been modified in the Neanderthal evolutionary lineage only (Lalueza-Fox and Gilbert 2011). The analysis of segregating loci in Neanderthals suffers from a similar shortcoming. Thus, with the current low coverage, it is impossible to distinguish random damage (Briggs et al. 2007; Hofreiter et al. 2001) and/or background contamination (see,

e.g., Green et al. 2006; Wall and Kim 2007) from heterozygosity. Indeed, this can only be achieved with genome coverages of around 15–20 ×, something that is technically possible but exceedingly expensive in most cases (Denisovan and Neanderthal specimens from Denisova cave, by now at 30–50 × coverage, are a remarkable exception). Alternatively, targeted methods can be designed to retrieve a specific genetic marker several times, as was the case for the ABO blood group and bitter taste gene from two Neanderthal specimens (Lalueza-Fox et al. 2008, 2009, 2011). In any case, our understanding of the genomic diversity of these extinct hominins will, it is hoped, be improved with a high-coverage genome.

Beyond the Genome

Amino acid positions in about 80 genes have been found to differ between Neanderthals and modern humans by comparing both genomes with those of chimpanzees (Burbano et al. 2010; Green et al. 2010). With an increased coverage this figure will likely increase to around 100 genes, as in the case of Denisova (Meyer et al. 2012). These positions correspond to those where Neanderthals share the ancestral genetic variant with the chimpanzees but modern humans display a fixed, derived variant. This is, of course, suggestive of functional differences in these genes (although it is not always the case because the resulting proteins can have a similar efficiency albeit with some amino acid changes in the underlying genes). This list contains genes whose exact function remains unknown in most cases, and only when malfunction is somehow present (usually in the form of deleterious mutations) does a particular disease emerge as a consequence. However, these 80 preliminary genes include several associated with metabolism, physiology, and cognition and some with more precise roles, such as being involved in the movement of sperm, the expression and development of follicle hairs in the skin, or some olfactory receptors (Green et al. 2010). While this information offers an exceptional opportunity to create a list of genes shaped by recent selection in modern humans and thus genes modeled by the common meaning of humankind, it would be a mistake to believe that phenotypical differences between Neanderthals and modern humans can be explained by this short genetic list alone (Lalueza-Fox and Gilbert 2011).

Some years ago, the publications of the human (2001) and chimpanzee genomes (2006) failed to fulfill our expectations in terms of being able to understand the genetic basis of the conspicuous morphological (and cognitive) differences that exist between these two species. Indeed, those people who assumed that a quick look at the genetic differences would provide an easy answer to the evolutionary processes involved—for instance, in key hominin adaptations such as bipedalism or brain size and complexity—were certainly disappointed. The problem resides in both the difficulties in understanding gene function and also in the complexity of the genome operating above the simple DNA level. To start with, what was once called "junk DNA" was found to be functional even though these genetic regions do not code for any protein. This is partly due to the existence of many regulatory elements that interact with networks of genes, thus shaping the final organism resulting from expression of the genome (Carroll 2008). In other words, similar or even identical genomes could produce different phenotypes as a result of differences in the regulation of gene transcription (the process by which DNA makes RNA, the molecule from which proteins are subsequently generated).

An Example of a Regulatory Element: microRNA

There are many types of genomic regulatory elements, including the so-called microRNAs (miRNA). The miRNAs are small, noncoding RNAs with a length of 19–25 nucleotides in their mature form that act as posttranscriptional regulators of gene expression by acting on the DNA transcripts. It is estimated that miRNAs regulate more than 30% of all protein-coding genes, building complex regulatory networks that control almost every cellular process. One set of such miRNAs is present only in present-day humans and is thus a good candidate for having contributed to human-specific phenotypes. The discovery of one miRNA, namely miR-1304, that differs between two closely related species such as modern humans and Neanderthals is of special interest: modern humans carry what seems to be a fixed substitution, whereas Neanderthals present the ancestral allele in a nucleotide that is located just in the seed region of miRNA-1304 and is therefore likely to alter the spectrum of target genes for miR-1304 (Green et al. 2010).

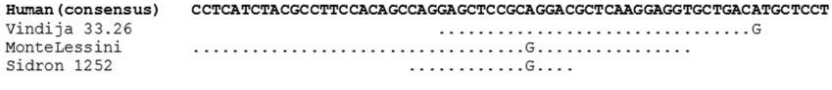

Figure 1. Only read of the Neanderthal genome draft (from Vi33.26) at the MC1R gene between positions 885 and 954. This illustrates the limitations of investigating Neanderthal-specific variants and also the heterozygosity using a low-coverage draft. The Vindija read does not have the Neanderthal-specific guanine substitution at nt 919 described in Monti Lessini and Sidrón 1252. However, it could have a nondescribed, synonymous substitution at nt 942 because A to G substitutions are not known to be associated with postmortem damage. However, only the genotyping of this position in additional Neanderthal samples or an increased coverage will allow us to confirm this new, Neanderthal-specific genetic variant.

The genomic search for target genes for this ancestral miR-1304 has shown an increase of more than 15 times the number of putative targets ($N = 515$) for the human miRNA, thus indicating an important functional evolution for miR-1304. The 36 predicted targets for Neanderthal miR-1304 include two important genes for teeth formation, namely enamelin and amelotin (Lopez-Valenzuela et al. 2012), and miRNA overexpression experiments using a luciferase-based assay confirmed that the ancestral version of miR-1304 greatly reduces enamelin- and amelotin-associated reporter gene expression by 50% (Lopez-Valenzuela et al. 2012). Interestingly, other genes in the Neanderthal miR-1304 list include cognitive genes such as TCF4 (associated with neuropsychiatric disorders such as schizophrenia and impaired verbal learning) or CD24 (associated with multiple sclerosis).

Although it is difficult to determine how this down regulation would affect the individual phenotype, it is known that the volume of coronal dentine in Neanderthal molars is larger than in modern humans. Because the absolute volume is similar for both hominin groups, this results in significantly thinner cuspal enamel in Neanderthals than in recent humans (Macchiarelli et al. 2006). Thus, although the ameloblast secretion rates are similar, the enamel cusp forms faster in Neanderthals than in modern humans (Smith et al. 2010). Another difference is found in the ameloblastic activity as reflected in the periodicity of long-period lines in the enamel (Retzius lines or perikymata; Aiello and Dean 1990). Several studies on dental growth have shown that the ontogeny in most Neanderthal dentitions examined was more rapid than that of *Homo sapiens* individuals, either recent or fossil (Smith et al. 2010). As a result, current dental eruption tables systematically overestimate the age of Neanderthal individuals at death while accurately predicting those of fossil *H. sapiens* (Smith et al. 2010).

However, the data generated from the 1,000 Genomes Project (released in 2011) have shown that the derived miR-1304, which was previously thought to be fixed in modern humans, is not; intriguingly, about 5%–7% of Asian individuals share the ancestral miR-1304 version with Neanderthals (Lopez-Valenzuela et al. 2012). This distribution schema fits the model of genetic introgression from archaic to modern humans as proposed in a recent study of certain alleles of HLA genes (Abi-Rached et al. 2011), although it could also be the result of selective sweeps within recent human populations, which would be compatible with a beneficial role for the new derived miR-1304 allele. Because of the relatively recent divergence dates between Neanderthal and modern human genomes (around 800,000 years), it is perhaps unrealistic to expect to find many fixed differences between both human groups, and even functionally important differences could be expected to segregate to some extent in both lineages. It could be that the conspicuous phenotypic differences among ancient human lineages are due to the summatory effect of a particular combination of genetic variants even if some of them segregate at low frequencies. If anything, the miRNA analysis again shows the complexity involved in unraveling the human evolutionary process.

Further functional studies could help our understanding of the link between regulation of the expression of genes associated with enamel formation and the final teeth morphology. In any case, this is a nice example of what can be expected in the future in terms of a view of the genomic architecture that goes beyond the simple reading of a DNA message.

Convergent Evolution

Another problem associated with our current lack of knowledge regarding the link between genotype and phenotype is the analysis of possible convergent evolutionary traits in hominin species. This is related to what is known as the evolution of "evolvability," that is, the limited physical and even chemical possibilities of a body design to create restrictions on the potential evolution of particular lineages. In the case of hominins, this could mean that only one set of adaptive traits can emerge with time, although it could also mean that similar traits are likely to appear independently in different hominin lineages.

One interesting example of this was described in the MC1R gene from Neanderthals (Lalueza-Fox et al. 2007). This gene codes for a protein in the membrane of melanocytes that regulates the synthesis of two different pigments in the hair and skin: the dark, brownish eumelanine and the fair, reddish pheomelanine. A Neanderthal-specific variant was found to produce a loss of function in the MC1R protein, thus resulting in fair skin and red hair in those Neanderthals carrying this variant. As in modern humans, it is likely that being heterozygous or homozygous for this particular mutation would

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Human          TCTCTGATGGTTCTGCTCATCTTTATGGGTTCGCCTGTCCTGTGTCCCACATGGA
Vindija 33.25  .....................C..............................
Chimpanzee     .....................C..............................
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Figure 2. Single read of Vi33.25 at position 735 of the RPTN gene (chr1 position 150393996) showing the ancestral (e.g., shared with the chimpanzee) variant C instead of the T fixed in modern humans. Even in light of the problems associated with low coverage, we can be reasonably certain about reads showing ancestral status because no chimpanzee contamination can be expected. Thus, and maybe a bit paradoxically, the low-coverage Neanderthal genome draft is more useful for determining modern human-specific changes than Neanderthal-specific changes.

produce phenotypes ranging from blond-reddish to "flame" red hair (Lalueza-Fox et al. 2007). However, it is worth emphasizing that the Neanderthal mutation is not found in present modern humans; therefore, living red-haired people would have a similar phenotype but for different genetic reasons (e.g., different mutations in the same gene). While this is somehow an anecdotic phenotypic trait that is marginally associated to adaptation to high latitudes, it is possible that other traits related to morphology and cognition could also be subjected to similar convergent processes. For instance, the increased cranial capacity and brain organization that lead to complex human cognitive functions could, to some extent, have evolved in parallel along different hominin lineages. This could explain the existence of aspects of modern symbolic behavior in Neanderthals well before the arrival of modern humans in Europe (Peresani et al. 2011; Zilhão et al. 2010).

It is likely that convergent evolution runs along particular gene networks that allow recurrent genetic modifications and that thus trigger the repeated opportunity for natural selection in the traits involved. This could explain underlying similarities in the phenotypical traits present in different hominin lineages. These traits, known as "orthologous phenotypes" or "phenologs," are defined as phenotypes related by the orthology of the associated genes in two different species (McGary et al. 2010). Although MC1R is an example of convergent evolution at a protein-function level, it is likely that this phenomenon could be more prevalent in regulatory circuits and could affect genes that tend to cluster together during the evolutionary process. The importance of convergent evolution in ancient hominins could be further explored when more is known about the precise genomic basis of specific human traits that can be observed in the fossil record.

Future Directions

The genetic basis of many of the traits observed in the fossil record is still unknown. This is partly due to the complexity of these traits but also to the problems associated with working with functional genomics, the branch that studies the biological function of the genes and their associated proteins. The study of most of the phenotypic traits will require the use of animal models (for instance the creation of transgenic mice) and molecular techniques not used by paleogeneticists. An even higher level of multidisciplinary effort will therefore be needed in the future.

Once this information becomes available, it will be possible to check the genomic regions involved directly in the phenotypic expression of extinct hominin genomes (specially those phenotypic traits that could be traced in the fossil record), and thus we will be able to finally understand some of the key issues of human evolution.

However, this complex enterprise can only be achieved with multidisciplinary teams and real collaborations among geneticists, archaeologists, and paleontologists. A profound understanding of the limitations and advantages of each discipline through interdisciplinary trainings and meetings will allow different hypotheses to be tested from the available evidence. More and more, all the disciplines studying the past will contribute from their own fields to the building of robust paradigms, and the current misunderstandings will, it is to be hoped, fade away.

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