Genetic and Phenotypic Consequences of Introgression Between Humans and Neanderthals

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ABSTRACT

Strong evidence for introgression of Neanderthal genes into parts of the modern human gene pool has recently emerged. The evidence indicates that some popula- tions of modern humans have received infusions of genes from two different groups of Neanderthals. One of these Neanderthal groups lived in the Middle East and Central Europe and the other group (the Denisovans) is known to have lived in Central Asia and was probably more widespread. This review examines two questions. First, how were these introgressions detected and what does the genetic evidence tell us about their nature and extent? We will see that an unknown but possibly large fraction of the entire Neanderthal gene complement may have survived in modern humans. Even though each modern European and Asian carries only a few percent of genes that can be traced back to Neanderthals, different individuals carry different subgroups of these introgressed genes. Second, what is the likelihood that this Neanderthal genetic legacy has had phenotypic effects on modern humans? We examine evidence for and against the possibility that some of the surviving fragments of Neanderthal genomes have been preserved by natural selection, and we explore the ways in which more evidence bearing on this question will become available in the future. " 2011, Elsevier Inc.

I. INTRODUCTION

A. The shape of the hominan family tree

The possibility that introgression has played a role in the history of our species has its beginnings in the long-continued controversy about single ("out of Africa") versus multiple origins of modern Homo sapiens (Relethford, 2008). It is clear from the fossil record that we are the surviving branch of a surprisingly luxuriant phylogenetic "bush" of close relatives.

Many of the branches of this bush overlapped spatially and temporally (Rightmire, 2009). For example, ancestors of modern humans that lived in Africa over the past million years are represented by finds at Klasies River and Elandsfontein in South Africa, Broken Hill in Zambia, and Herto and Omo in Ethiopia among others. These putative ancestors tended toward greater robust- ness than modern humans but are sometimes associated with stone tools that put them directly in the modern human lineage.

Members of other branches of the hominin bush that left Africa before the appearance of modern humans include several groups of Neanderthals and pre- Neanderthals (Fabre et al., 2009), Homo erectus (Anto ´ n, 2003), and possibly even-in the case of some of the Dmanisi remains from Georgia and Homo floresiensis from Indonesia—Homo habilis (Gordon et al., 2008). The details of these early migrations

continue to be revised. For example, revision of dates from Solo River terraces in Java now suggests that the most recent H. erectus finds may be as much as 150,000 years old, reducing the likelihood that this hominan persisted until the arrival of modern humans (Indriati et al., 2011). It is also clear, however, that H. floresiensis did persist until perhaps as recently as ten thousand years ago (Aiello, 2010).

Overwhelming evidence from mitochondrial and Y-chromosome DNA supports the out-of-Africa model (Penny et al., 1995), but none of this evidence has as yet provided evidence for introgression. Nonetheless, it is possible that some of the many other hominan branches, both within and outside of Africa, have contributed at least small amounts of nuclear genetic information to our gene pool.

B. Opportunities for introgression

Opportunities for introgression abound in our history. Modern humans and Neanderthals coexisted for between 4000 and 10,000 years in Western Europe. The exact nature of their interaction has been the subject of substantial controversy, summar- ized in Banks et al. (2008). In the Middle East, modern humans and Neanderthals may have overlapped for a longer period of time, between 135,000 and 100,000 BP (Gru ¨n et al., 2005). Modern humans and H. floresiensis also coexisted on the island of Flores east of Java for a substantial period of time, perhaps from 40,000 to 10,000 BP. Moving further back in time, robust and gracile Australopithecines coexisted for at least a million years in southern and eastern Africa, and H. habilis and Homo ergaster may have overlapped for a hundred thousand years or more in East Africa (Cameron, 2003). In Western Europe, a variety of hominins coexisted in what are now Spain, Germany, and Great Britain for indeterminate periods of time, starting more than a million years ago. Some of these peoples (undoubtedly with further input of waves of migrants from Africa via the

Middle East) contributed to the emergence of the Neanderthals. And the emergence of modern humans in southern and eastern Africa is similarly complex, involving a morphologically diverse collec- tion of hominins such as Homo rhodesiensis (Kenya; Hublin, 2009) and Homo sapiens idaltu (Ethiopia; White et al., 2003).

II. THE FIRST GENETIC EVIDENCE FOR INTROGRESSION IN OUR SPECIES

Direct genetic evidence bearing on most of these opportunities for introgression is unlikely to be forthcoming, because old bones in tropical climates quickly lose any traces of their original owners' DNA. But the availability of substantial amounts of DNA sequence information from bones that have survived under temperate and subarctic conditions has now provided direct evidence for introgression events.

The first compelling piece of evidence for a role of introgression in the history of our species comes not from an analysis of ancient hominin DNA but from a comparison of the divergences that have taken place between present-day human and chimpanzee genomes (Patterson et al., 2006). When these authors compared the two species' genomes, they found a substantial variation in diver- gence time among different genomic regions. This observation led them to propose that genetic exchanges between the two lineages took place over a span of time covering at least a million years, from about 7.4 to 6.3 million years ago. Additionally, they found an unusually small amount of divergence between the X chromosomes of the two lineages. This suggested that male hybrid sterility may have evolved toward the end of this period of repeated introgres- sions, as a result of the accumulation on the X of loci leading to male sterility in one of the hybrids. The result would have been that backcrosses of hybrids to one of the nascent species would have survived preferentially, so that both nascent species shared the same X chromosome sequences as they diverged.

This evidence from whole-genome comparisons suggests that humans and chimpanzees speciated parapatrically rather than allopatrically, with repeat- ed episodes of introgression. Such repeated patterns of introgression are being discovered in other cases of speciation, such as that taking place between Drosophila pseudobscura and D. persimilis (Kulathinal et al., 2009). This apparent widespread occurrence of introgression, and its important role in speciation, suggests the possibility that introgression may also have been common during the divergences of lineages in the hominan phylogenetic "bush."

In this review, I will examine the direct evidence for such introgression, and then turn to ways of detecting the possible genetic consequences (or possible lack of consequences)

of this introgression. If the introgression that can be demonstrated to have taken place can also be demonstrated to have had effects on the fitnesses of the recipients, then such a discovery would have large consequences for how we understand the evolution of our species. It is possible, though far from proven, that our genetic patrimony may turn out to be more extensive, with a greater effect on our own evolution, than the simple "out-of-Africa" model of our origins would imply.

III. EVIDENCE FOR INTROGRESSION OBTAINED FROM PRESENT-DAY HUMAN GENOMES

Jeffrey Wall and his colleagues have pioneered statistical methods for estimating the amount of ancient admixture through examination of present-day genomes. They accomplish this by comparisons of single-nucleotide polymorphisms (SNPs) that are in linkage disequilibrium (LD) with nearby polymorphisms and that are found uniquely in particular human groups. These are compared with the distribution of

SNPs that are shared by all present-day African and non-African human groups (Wall, 2000; Wall et al., 2009). Their method depends on assumptions of random mating and selective neutrality for all the SNPs. They construct a maximum- likelihood model for the fate of the SNPs in the branching populations, assuming that these populations were small through most of their existence (and in the case of the non-Africans went through a severe bottleneck at the time their founders left Africa). They assume further that all populations then increased 100-fold, with the African populations beginning their increase before the non-Africans.

The authors examine the number of blocks of SNPs in strong LD that are possessed by one of the human groups and not possessed by others. If these numbers are higher than expected by chance, given the hypothesized population structures, then the group in possession of the excess may have received these blocks of linked genes through introgression from a divergent population. This hypothetical population is presumed to have diverged from both the human groups and subsequently come in contact with one of them, donating these strongly linked SNPs to it. The likelihood of finding blocks in LD depends on the rate of recombination, which is assumed to be constant within LD blocks but may vary among blocks.

The authors conclude, given their assumptions, that there is strong evidence for introgression in Yoruba genomes from sub-Saharan Africa, suggest- ing that introgression has played an important role during the recent divergence of human groups in Africa. This is a pattern that could have been predicted given the complex history attested to by human skulls with a variety of distinct morphologies that form part of the

fossil record of the emergence of modern humans in Africa. This complex history would have resulted in introgressive flow of genes that have survived in some members of the Yoruba population and not in others. And this in turn would suggest that the earliest stages of splits between gene pools, in particular the separation of ancient tribal lineages in sub-Saharan Africa, involved a good deal of parapatric gene flow.

They also conclude, using the same methodology, that there has been a substantial amount of introgression in Europeans, contributing about 14% of the current European gene pool. They estimate that there has been a much smaller amount of introgression in Asian populations, only about 1.5%. The European results strongly suggest that some introgression has taken place, but because the origin of the introgressed genomic segments cannot be inferred by these analyses, they do not directly implicate Neanderthals. These results leave open the possibility that introgressions between Neanderthals and modern humans are not the only gene flow among species and nascent species that might have taken place in Europe.

Until recently, there was apparent strong evidence against any Nean- derthal introgression. Estimates of the amount of introgression, based on large numbers of human mitochondrial DNA sequences that could be compared

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directly with Neanderthal mitochondrial sequences, showed no evidence for introgression. The first preliminary set of nuclear DNA data, from a Croatian Neanderthal bone, was obtained using PCR and first-generation massively par- allel sequencing. The million base pairs of sequence that were examined also provided no evidence for introgression (Teschler-Nicola et al., 2004). However, these first Neanderthal nuclear DNA samples were shown to be contaminated with modern DNA (Green et al., 2006; Noonan et al., 2006). Thus, conclusions drawn from this early sample of nuclear DNA were thrown into doubt.

Now, substantial amounts of higher quality Neanderthal nuclear DNA sequences have become available, along with well-annotated complete genomes from a number of African, European, and Asian modern humans. Molecular paleontologists are no longer required to use the distribution of present-day blocks of high-LD in order to detect possible past introgression, because direct estimates of introgression at individual sites can now be made. Nonetheless, the pioneering results obtained by Wall and his colleagues continue to be valuable and important, because they hint at the possibility of introgressions other than those between Neanderthals and modern humans. For example, waves of intro- gression between different groups of early human migrants into Europe may have taken place. Further, there may have been contacts between early modern humans in Europe and additional groups of hominans, such as late-persisting populations of pre-Neanderthals.

Arguments for introgression that are based entirely on information from present-day genomes are bedeviled by the fact that they are strongly dependent on assumptions about the demographic histories of the populations involved. In contrast, as we will see, the emerging genetic data from old DNA do not have these limits. These emerging data are so unexpected and have such revolutionary implications that the only one thing can be predicted with confidence about this field is that even more surprising revelations are in store for us as a result of continuing discoveries by molecular paleontologists.

IV. HISTORY OF HOMINANS IN EUROPE

A. Pre-Neanderthals

Hominans in Europe have a long history, and the first hominans to migrate into the peninsula appear to have settled in southern Europe. The earliest known indications of this occupation have been traced to as long ago as 1.3 to 1.7 mil- lion years BP, based on the dating of three stone tool cores and associated flakes that were found at the Pirro Nord site in Apulia in the heel of Italy (Arzarello et al., 2006).

The identities of these early stone tool makers remain unknown. The earliest hominan skeletal remains have been found in an infilled cave system near Atapuerca in northern Spain (Carbonell et al., 1995, 2008). A fragment of a child's upper jaw found there is older than 780,000 years, and a mandible from a nearby site has recently been dated to from 1.1 to 1.2 million years BP. These people were relatively light boned. In addition, a single cranium has been discovered from Ceprano in central Italy, dating from about 700,000 years ago (Manzi, 2001).

The penetration of hominans into northern Europe seems to have come much later than the earliest traces of human activity in southern Europe. The earliest finds so far from northern Europe are flint artifacts from Suffolk, UK, dating from about 700,000 years ago (Parfitt et al., 2005).

Who were these first Europeans, particularly the earliest settlers? Were they ancestors of the Neanderthals? This question has great importance, because molecular data clearly shows that these finds substantially predate even the oldest estimate of the time of divergence of modern humans and Neanderthals, which is currently 435,000 years BP (Green et al., 2010; supporting material, pp. 122–128). The question of how much, when, and how these mysterious early people contributed to the gene pools of the Neanderthals of Western Europe remains unresolved.

The fragmentary Atapuerca remains have been given the name of Homo antecessor. It

has been suggested by their discoverers that these people were ancestral to Homo heidelbergensis, which in turn could possibly be ancestral to the later Neanderthals. But the finds are few so far, making the affinities of this hominin uncertain.

The picture is further confused by a profusion of European fossil finds that have been made over the past century. Skull bones of a hominan with an apparently extremely robust skeleton have been found at Swanscombe in Kent, dated to 400,000 years BP (Stringer and Hublin, 1999). Similar robust bones have been found at other sites in Western Europe. Some of these peoples, like the Swanscombe hominan seem to have been even more robust than the later western Neanderthals. The first of these to be discovered, in 1908, was a massive mandible in a gravel pit near Heidelberg. This find has recently been dated to 600,000 years ago (Wagner et al., 2010). Hominans similar to this extremely robust H. heidelbergensis appear to have been widespread, because skulls with similar degrees of massiveness have been found not only in Europe (Arago and Petralona) but also in Africa (Bodo and Kabwe; Tattersall, 2007).

All these hominans had many points of difference from the Nean- derthals. And the earliest Neanderthals themselves may have undergone sub- stantial change. Perhaps, the most direct fossil connection with the later Neanderthals comes from a substantial find of parts of 28 skeletons at Sima de los Huesos near Atapuerca (Arsuaga et al., 1993). These people, who have been

dated to within a range of 200,000–350,000 years BP, had many similarities with the later Neanderthals, but they were on average substantially smaller and lighter-boned—quite the opposite of H. heidelbergensis.

It seems more likely that there were multiple migrations into Europe from Africa over the past one and a half million years, passing through the Middle East. Some of these migrants could have been H. heidelbergensis, while others appear to have been pre-Neanderthals who penetrated as far west as Spain before fanning north. Some of these immigrant groups in turn eventually evolved into the "classical" Neanderthals that had a robust phenotype and penetrated into northern and western Europe at a time when climatic conditions were challenging.

B. Neanderthals

The western Neanderthals persisted for at least 200,000 years. Possible fragmen- tary Neanderthal remains have been found in France dating from 230,000 years ago (Gru ¨n et al., 2008), and the most recent undoubted Neanderthal remains have been found at Vindija Cave in Croatia, dated to approximately 32,000 years BP (Wild et al., 2001). During this period, Europe passed through some of the most severe of the Quaternary ice

ages, and there is some evidence that the Neanderthals penetrated to the north and retreated to the south as the ice retreated and advanced (Skrzypek et al., 2011). But Neanderthals and their close relatives became much more widespread during this period. Neanderthals with less extreme morphologies than those in Western Europe have been found in the Middle East, most notably in cave sites in northern Israel, and in northern Iraq.

Early waves of Neanderthals or other hominans settled around the Israeli caves at least 400,000 years ago, a date based on a small number of artifacts that have been discovered deep in the cave deposits (Hershkovitz et al., 2011). There is clear fossil evidence for Neanderthal occupations of the caves starting from about 100,000 years ago. This later period overlaps in time and space with the occupation of nearby caves by modern humans, and both the Neanderthal and "modern human" remains show intriguing signs of morphological conver- gence. Each was morphologically distinct from western Neanderthals and from present-day humans (Rightmire, 2009).

This Middle Eastern overlap in time and space provides an opportunity for Neanderthal–human introgression. The fact that Europeans and Asians show the same amount of introgression suggests that this may have been the only region where introgression took place (Green et al., 2010). It may be that the apparent morphological convergence between humans and Neanderthals in the Middle East reflects a genetic exchange. There is no sign of modern human

introgression into the genomes of the Croatian Neanderthals, but this does not rule out the possibility of such introgression in the Middle East, where Neander- thal genomes are not yet available.

By routes and at times yet to be determined, Neanderthals and at least one other group distantly related to the Neanderthals spread into other parts of western Asia and at least as far as central Asia. Artifacts typical of Neanderthals have been found at Denisova Cave in the Altai Mountains of southern Siberia, which was first occupied 280,000 years ago (Derevianko, 1998). Genomic infor- mation extracted from a finger bone found at the site shows that the female to whom the finger bone belonged carried a mitochondrial DNA sequence that diverged from modern human sequences a million years ago (Krause et al., 2010). Coverage at 1.9 times of the nuclear genome from the same bone showed that this female shared some ancestry with the Neanderthals and that her nuclear genome was about as divergent from modern humans as are Neanderthals from central Europe (Reich et al., 2010).

The Neanderthals and the hominans who preceded them were geo- graphically dispersed, and they were also morphologically and culturally diverse. Although the first of these

peoples were able to settle in Southern and Western Europe for long periods, their descendants were only able to colonize the climatically more severe regions of Central Europe periodically, whenever the Arctic weather relented slightly (Skrzypek et al., 2011). Cooked plants were eaten by Neanderthals in Western Europe and in Iraq, as evidenced by trapped cooked plant particles that have been found in dental calculus (Henry et al., 2011). The extra energy released by cooking (Wrangham, 2009) must have aided their migrations, but may also have limited how far they could have spread into subarctic regions without sources of firewood once they became dependent on the technology of cooking.

We know little about the daily life of Neanderthals. The technology of the later Neanderthals was advanced, but it is unclear how many of these advances were the result of their own inventiveness and how many came from contacts with modern humans. Personal ornaments have been found in the Grotte du Renne along with Neanderthal remains. These ornaments are similar in complexity to those of the Aurignacian culture that is associated with the first modern humans in Europe. Such ornaments are absent from the more primitive Chatelperronian technology that is more often associated with western Nean- derthals. This apparent anomalous association at the Grotte du Renne may, however, be the result of mixing of the stratigraphic layers in the cave floor (Higham et al., 2010; Mellars, 2010).

Similar uncertainties surround indications of Neanderthal culture. One of the six skeletons, dating from 50,000 years ago, that were found at Shanidar Cave in northern Iraq (Stewart, 1977) may have been buried with some kind of ceremony involving bunches of flowers. The taphonomic evidence regarding

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this and other possible elaborate Neanderthal burials has also been called into question, but many disagreements remain (Gargett, 1999 and discussion following).

It is possible that many signs of Neanderthal culture and technology have been lost simply because they were unlikely to have been preserved. Remarkably sophisticated wooden spears from 400,000 BP have been found in a German peat deposit (Thieme, 1997). Such a find is rare, because wooden artifacts almost always perish quickly unless they are preserved under unusual circumstances. Nonethe- less, this discovery gives a tantalizing glimpse of what might have been lost.

There is a good evidence that pre-Neanderthal and Neanderthal tech- nology did change over time, as these peoples adapted to fluctuating climate and changes in animal and plant populations. Pebble tools with knapped flakes that first appeared in the fossil record about 1.3 million years BP were replaced by handaxes and cleavers about 600,000 years ago. Additional technological changes took place long before the invasion of Europe by

modern humans. For example, at Orgnac, a combined open air and cave site in southeastern France, a transition from periodic occupation to continuous occupation from 350,000 to 200,000 BP was accompanied by the replacement of primitive tools by more sophisticated tools of the Levallois type and by the emergence of systematic butchering methods that were used on carcasses of horses and bovids (Moncel et al., 2011).

In summary, the Neanderthals and the peoples who preceded them and followed them were a complex group of hominans with a history involving a series of migrations out of Africa. Some of these migrations may have resulted in replacements or near-replacements of earlier populations that were as drastic as the most recent replacement of Neanderthals by modern humans. This poten- tially complex history may help to explain why the Central European Nean- derthals and the Denisovans can be traced back to a last common ancestor with modern humans only half a million years ago, even though the first hominans appeared in Europe as much as 1.7 million years ago.

The Neanderthals and their forebears had sophisticated and evolving technology that enabled them to penetrate into forbidding regions of northern Europe and survive in harsher climates than those of the present time. And, as has recently been discovered, their close relatives the Denisovans migrated into central Asia and possibly even further east, where they came in contact with modern humans (and perhaps earlier migrants such as H. erectus) in the process (Reich et al., 2010).

C. Modern humans in Europe

The earliest undoubted modern human remains in Europe, from Romania, date to about 42,000 years BP (Hoffecker, 2009). The high morphological diversity of early modern humans suggests that there may have been several migrations of

these groups from Africa into Europe (Gunz et al., 2009). The diversity of these lineages suggests that there may have been plentiful opportunity for introgression between different modern human and Neanderthal gene pools and indeed between disparate groups of modern humans as well.

The causes of the replacement of Neanderthals by modern humans remain essentially unknown, but are likely to be numerous, ranging from direct conflict over hunting sites to indirect competitions for food sources that would have been won by the superior technology of the new arrivals, to the introduc- tion of new diseases by the arrival of modern humans.

The extent and nature of the contacts between modern humans and Neanderthals remain unknown. As will be noted below, there is no current genetic evidence for Neanderthal–

modern human introgression in Europe or the Middle East, beyond the genes that were passed down to modern humans that resemble genes of Neanderthals from Slovenia. There is also no sign that any genes passed the other way, from modern humans to Neanderthals. Comparison of Neanderthal and Denisovan genomes shows some hints of introgression between them, but this may be artifactual because Neanderthal and Denisovan genome sequences were obtained using different massively parallel techniques (David Reich, personal communication). The possibility remains open that examination of further Neanderthal genomes will reveal evidence for both types of introgression. The recently discovered additional fragments of the Neanderthal type skeleton from the Neander Valley (Smith and Schmitz, 2002) might provide such an opportunity.

V. COMPARISON OF MIDDLE EUROPEAN NEANDERTHAL NUCLEAR DNA WITH MODERN HUMAN NUCLEAR DNA

Richard Green and his colleagues have obtained a set of Neanderthal nuclear DNA sequences, primarily from three bones of three different individuals who had died or been buried in Vindija Cave in Croatia (Green et al., 2010). Together, these sequences constitute about 1.3 coverage of the genome. Bones from other sites ranging from Spain to Russia were assayed, but the great majority of the sequence information came from these three bones. All three of the bones came from different females, and there was no detectable unique Y-chromosome DNA. This observation, along with an estimated upper bound of only 0.5% modern mitochondrial DNA in the samples, gave Green and his colleagues confidence that the bone DNA was virtually uncontaminated with modern human DNA (Green et al., 2006).

The fragments that were obtained were short, and they had undergone many chemical modifications. Most of these were deaminations of cytosine to thymine, especially near the 5^0 ends of the fragments. The fragments were also

badly contaminated by bacterial DNA, which was partially removed by digestion with restriction enzymes that rarely cut hominin DNA. Nonetheless, the restric- tion enzyme treatment must have resulted in the destruction of some Neander- thal sequences as well.

Although coverage was low and many of the Neanderthal reads were found only once, a sufficient number of matches to homologous human sequences were obtained to allow a good estimate of the time of divergence of these Neanderthals from the modern human lineage. That time of divergence is between 272,000 and 435,000 years BP. Even the older of these dates is far more recent than the arrival of the first pre-Neanderthals in

Europe, suggesting that the Neanderthals may displaced the European pre-Neanderthals rather than evolving from them (though see the discussion in the previous section about possible early introgressions).

VI. THE ABBA–BABA TEST FOR INTROGRESSION

Unlike the extensive amount of data from mitochondrial DNAs that had earlier shown no Neanderthal mitochondrial DNA in the modern human mitochondri- al DNA pool (Penny et al., 1995), the sequences from Vindija provided evidence that some Neanderthal nuclear sequences have been passed down to modern humans. The two ingenious analytical approaches that Green's group used were made feasible because of the large number of undoubtedly Neanderthal genomic sequences that could be joined into contigs and then aligned to the homologous sequences of modern humans.

The first of these approaches depends on two assumptions about muta- tion rates, assumptions that appear to be valid for this data set although they may not be for more divergent data sets. The test, ABBA–BABA, is based on a comparison of two types of single-SNP phylogenetic tree, which—given the validity of the assumptions—should be equal in numbers unless introgression from Neanderthals to humans has taken place.

The phylogenetic trees that were employed in each ABBA–BABA test consisted of homologous sites from two human sequences along with the homol- ogous Neanderthal sequence, using the homologous chimpanzee sequences as an outgroup (Fig. 2.1A). These nodes were always arranged in the following order: Human 1 (H1), Human 2 (H2), Neanderthal, and chimpanzee.

Sites were picked in which only two bases, such as C and T or A and G, were present in these four lineages. At these sites, the alternative bases possessed by the tree's four external nodes were designated A and B. The number of sites used was narrowed further by picking only sites in which the pattern of changes at the terminal nodes of the tree was ABBA or BABA. Thus, the majority of segregating sites in these trees could not be used in the test.

1)

Polymorphism arises at or before Neander thal–human split, new allele is passed to H2

2)

Polymorphism arises at or before Neander thal–human

X split, new allele is passed to H1

X

TCCTCTCT

Lineage H1 H2 Neanderthal Chimpanzee Lineage H1 H2 Pattern A B B A Pattern B A B

BTT

Chimpanzee A

Neander thal

1)

T T to C C T H1 H2 Neanderthal Chimpanzee

2)

Mutant allele

Mutant allele

arises in Neander- X thals subsequent

to split, then intro- gresses to H1

arises in Neander- X thals subsequent

to split, then intro- gresses to H2

Lineage Pattern A B B A

Lineage Pattern B A B

Figure 2.1.

A. If polymorphic mutations arise before or during the human-Neanderthal split and survive in only one of the human lineages, trees (1) and (2) should be found in equal numbers. Because such trees are rare, similar trees that result from two mutations are likely to be so rare that they can be ignored. B. If mutations arise in the Neanderthal lineage and introgress into one of the human lineages, there will be an excess of ABBA or BABA trees. The relative size of such excesses is a measure of the amount of introgression from Neanderthal genomes that has taken place. These figures (A) and (B), are adapted from Figure S38 of Green et al. (2010).

The assumptions of this ABBA–BABA test are, first, that at segregating sites that show these patterns only one mutation has taken place since the human–chimpanzee divergence, and second, that the two human sequences are known with an equal degree of precision.

The tree of Fig. 2.1A shows the interesting situation is one in which the site became polymorphic as a result of a single mutation in the ancestral lineage before the human–Neanderthal split took place, or during the split itself. If this polymorphism survived down the human lineage, one of the present-day human sequences might carry the new allele of the polymorphism and the other might carry the older allele. Further, the Neanderthal sequence that is being compared might carry the new allele (Fig. 2.1A). If no introgression from Neanderthals to humans has taken place, then it is these rather restrictive sets

T to C T C H1 H2 Neanderthal

T Chimpanzee

A

of conditions that yield the ABBA or BABA tree configuration. Whether the tree is ABBA or BABA depends on which of the human sequences carries the new allele.

These two cases, in which a polymorphism arose before or during the human–Neanderthal split and is preserved in the two human populations, are shown in trees 1 and 2 of Fig. 2.1A. Each case is equally probable, provided that there has been only one mutation since the human–chimpanzee common ancestor.

The essence of the ABBA–BABA test is that introgression from Nean- derthals to humans will cause deviations from equal numbers of the two trees, resulting in more of either ABBA or BABA configurations. Excesses of ABBA or BABA will in turn happen if one of the two human genomes, H1 or H2, is more likely than the other to carry one of the polymorphic Neanderthal alleles because it has gained that allele through introgression.

The test is able to detect such introgression events if a mutant allele arose in the Neanderthal lineage subsequent to the human–Neanderthal split and that allele was then introduced into one of the human lineages. The pattern expected from introgression of this recent allele into one of the two human lineages is shown in trees 1 and 2 of Fig. 2.1B. Without introgression, both of these trees would have been AABA and would not have been included in the test data. But introgression from the Neanderthal gene pool acts like a second mutation. In Tree 1, it has converted A to B in the H2 lineage, and in Tree 2 it has converted A to B in the H1 lineage.

Thus, if there has been introgression into H2 there will be an excess of ABBA trees, and if there has been introgression into H1 there will be an excess of BABA trees. Neither of these trees subtract from the original ABBA and BABA trees, because if there had been no introgression, both Tree 1 and Tree 2 of Fig. 2.1B would have been AABA and would not have been counted in the first place.

Note that many other trees are possible, but that the ABBA and BABA trees can only arise from events that lead to the patterns seen in Fig. 2.1A and B, provided that we assume only one mutation has taken place at this site since the time of the human–chimp common ancestor. Differences in mutation rate in the different human lineages do not affect the results. Because the mutation took place before or during the human–Neanderthal splits, or, in the case of introgression, in the Neanderthal lineage alone, any differences in mutation rate subsequent to those events would be immaterial. There might be such differences, but because no mutations are assumed to have taken place after the time of that single mutation, the question of any rate differences becomes moot.

The assumption on which the analysis is based is that for almost all ABBA and BABA trees, in which two of the sequences have one base and two have the other, only one mutation has taken place. This restriction does not pose a problem in this case, because the time back to the divergence of the two human lineages is relatively short compared with the rest of the tree. Two of a number of such

1)

C

2)

Two mutations take place

C

X

X_X

X

CTCTTCCT H1 H2 Neanderthal Chimpanzee H1 H2 Neanderthal Chimpanzee

Lineage Pattern B A B A A B B A

Figure 2.2. Two of the possible two-mutation trees that yield ABBA or BABA trees. Although the frequencies of such trees depend on the mutation rates of the human lineages, trees of this type are so rare that they can be ignored.

two-mutation trees, which yield either ABBA or BABA without introgression, are shown in Fig. 2.2. If there were different mutation rates in the two human lineages, then if such two-mutation trees occurred in substantial numbers this could lead to an excess of ABBA or BABA in the absence of introgression. Such configurations must arise occasionally, but they are likely to be rare. The ABBA and BABA configura- tions are only a small set of the total number of trees in which the four lineages carry two bases. Multiple-mutation trees that yield ABBA and BABA must therefore arise at a rate that is roughly the square of this small fraction, and even if there were a difference in mutation rates in the two human lineages this small effect can be neglected.

A second important assumption of the ABBA–BABA test is that the H1 and H2 sequences are known to comparable levels of accuracy (Liang and Nielsen, 2011). If they have not, then biases in base sequence ascertainment in one or both of the human sequences could lead to an excess of false positive ABBA or BABA trees. In the case of the human–Neanderthal comparisons, such biases have not been detected. For example, when the tests are confined to either transitions or to the less common transversions, the estimated amount of intro- gression does not change (Green et al., 2006).

VII. DIFFERENT REGIONS OF THE NEANDERTHAL GENOME ARE FOUND IN DIFFERENT MODERN HUMANS

The second test is a most ingenious one. It has a built-in control, which depends on the origins of one of the human genomes that have been used in the search for introgression.

RCPI11 is one of the "type" human genomes that were sequenced to a high level of redundancy by the Human Genetics Consortium of the National Institutes of Health. It contributed about two-thirds of the sequences that made up the final annotated genome. This individual had half-European and half- African–American recent ancestry (Reich et al., 2009a,b). Another thoroughly annotated sequence, that of Craig Venter, is entirely of European recent ancestry (Istrail et al., 2004). These different genomes provide an opportunity to compare the amounts of introgression from Neanderthals into Europeans and into sub- Saharan Africans, using the two halves of the single RCPI11 genome.

Green et al. divided the RCPI11 genome up into fragments that were either of European or of sub-Saharan African origin, then obtained the homolo- gous sequences of the Venter genome. They then estimated the divergence of the RCPI11 sequences and their homologous Neanderthal sequences. These diver- gence estimates were plotted on a graph in which the ordinate was the difference between the RCPI11 fragments and the Craig Venter fragments, and the abscissa was the difference between the Neanderthal fragments and the RCPI11 frag- ments (Fig. 2.3). Both of these sets of differences were normalized by the average

2.5

2

1.5

1

0.5

0

Comparisonsofthehuman–Neanderthaldivergencesofgenomicsegmentsofanentirely European genome (ordinate) with divergences involving homologous segments from a half-European and half-African–American genome (abscissa). Mismatches in the Euro- pean comparisons show that the two human genomes carry different segments of Neanderthal genomes. From Figure 5A of Green et al. (2010).

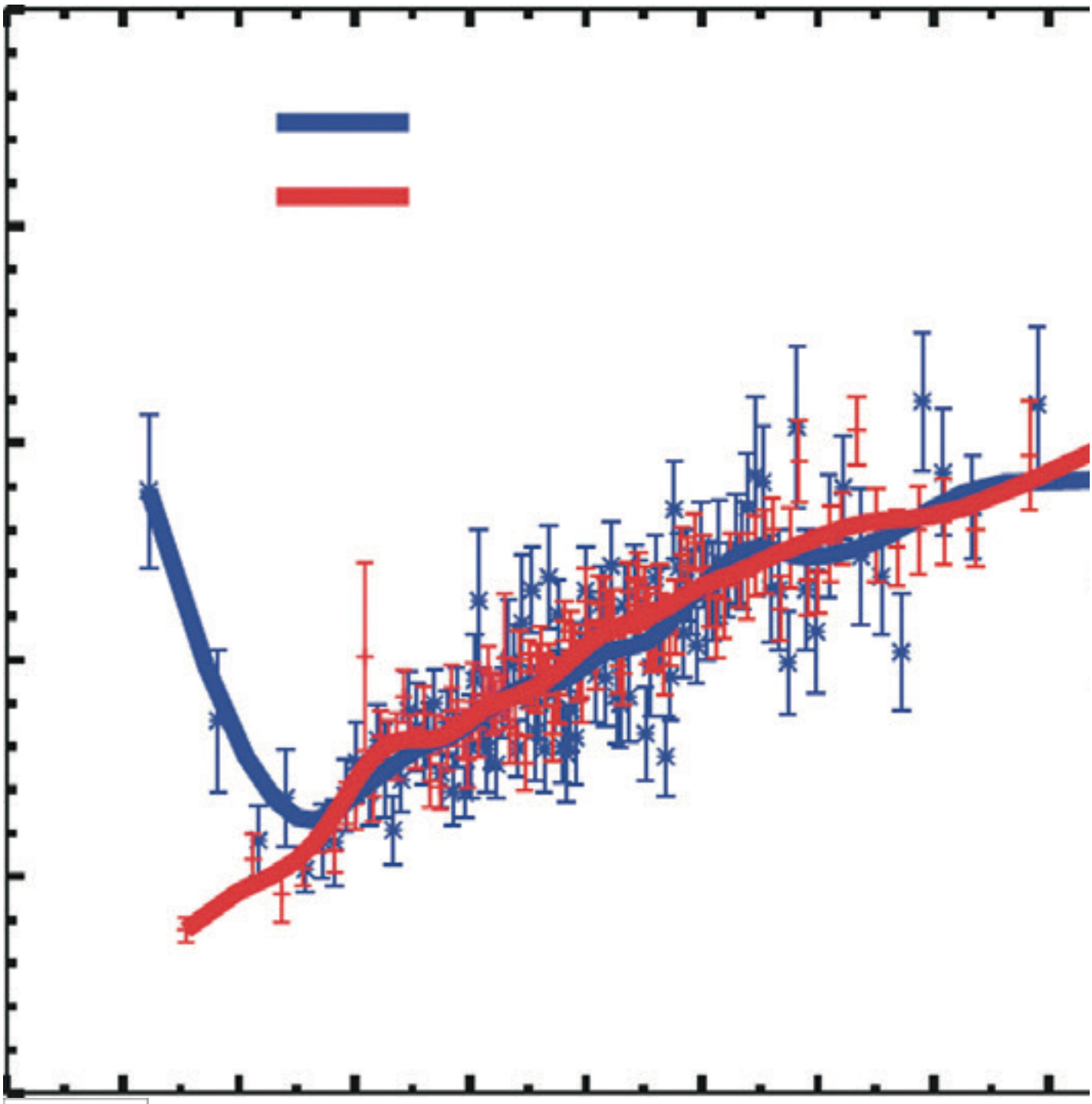

Figure2.3.

European African

1 1.2 1.4 1.6 1.8 hsRef-Neandertal divergence normalized by

2 2.2 2.4 2.6 human–chimp. divergence and scaled by the average

0 0.2 0.4 0.6 0.8

hsRef-Venter divergence normalized by human– chimp. divergence and scaled by the average

human-chimpanzee divergence. When this analysis was confined to sequences homologous to the African part of the RCPI11 genome, the divergence values formed a straight line. That is, RCPI11 sequences that closely resembled Nean- derthal sequences were homologous to Venter sequences that also closely resem- bled the same Neanderthal sequences. Sequences in one of the human genomes that were more distantly related to Neanderthals were also more distantly related in the other human genome.

A different pattern was seen when the European-origin segments of the RCPI11 genome were examined. Here there were a significant number of RCPI11 sequences that closely resembled the homologous Neanderthal sequences, while the homologous Craig Venter sequences did not resemble these RCPI11 sequences. This resulted in a distinct upward "hook" in the lower left corner of the graph for the European data, a hook that was not seen in the African-origin data.

The African-origin results clearly show that, as might have been pre- dicted from the fossil record of our species and that of the Neanderthals (Mellars, 2006), there has been no detectable introgression from Neanderthals into the gene pool of sub-Saharan modern humans. Such introgression might have happened if the ancestors of modern humans had encountered the ancestors of Neanderthals before they left Africa, but such encounters seem not to have taken place.

In contrast, the European-origin analysis leads to the conclusion that there are parts of the European regions of the RCPI11 genome that have introgressed from Neanderthals into the RCPI11 genome but that are not represented in the Craig Venter genome. Thus, introgression did take place between the ancestors of Europeans and the Neanderthals, and that introgression involved substantial parts of the Neanderthal genome that are scattered among modern humans.

In this analysis, the authors did not address the question of whether the reciprocal relationship is true: are there regions of the Craig Venter genome that carry introgressed Neanderthal regions that do not closely resemble Neanderthal sequences in the European part of the RCPI11 genome?

I have examined the published data (Figure 5B of Green et al., 2010) and find a small excess of Venter sequences that are different from RCPI11 sequences when the RCPI11 sequences are themselves different from Neanderthal sequences. These results are significant using a Chi-square test at the 0.01 level (unpublished results). The analysis in the paper was not designed to look at this possibility, however. We await an analysis in

which the Venter-Neanderthal divergence is compared with the European RCPI11-Neanderthal divergence. When this analysis is carried out, it may detect Venter sequences that resemble Neanderthal sequences closely, while the homologous European RCPI11 sequences do not. But, even before such tests are carried out, it is clear that these two human sequences have been shown to carry different regions of Neanderthal genomes.

These results raise two important questions. First, how much of the genomes of Neanderthals have introgressed into at least some members of the modern human gene pool? Second, what is the frequency distribution of those introgressions? Are some parts of the Neanderthal genomes more likely to have survived than others? I will address these questions and their consequences for the adaptation of our species in the last part of this review.

VIII. THE GENETIC CONTRIBUTION OF THE DENISOVANS TO OUR SPECIES

A preliminary analysis of DNA from the child's finger bone that was discovered at Denisova Cave (Derevianko, 1998) showed that her mitochondrial genome had diverged from those of modern humans and the European Neanderthals about a million years ago (Krause et al., 2010). This finding indicated that she was on a clearly different hominan branch from the Neanderthals.

The bone has now been thoroughly scraped out to yield a 1.9 times coverage of her nuclear genome. These nuclear genes were found to have diverged from the human-Western Neanderthal lineage only about 200,000 years earlier than the human–Neanderthal split (Reich et al., 2010). There were two possibilities for this discordance between the mitochondrial and the nuclear results. Either old mitochondrial genomes that were descended from a million-year-old mitochondrial "Eve" had persisted in the Denisovan lineage, or there was introgression between the Denisovan child's lineage and another hominin lineage that had an older mitochondria "Eve." If the latter, then that lineage, perhaps H. erectus, H. habilis or a different lineage entirely, has also introgressed into our own ancestry.

The availability of the young Denisovan's nuclear genome provided an opportunity to examine, using the ABBA–BABA test, whether pieces of Deni- sovan genome have introgressed into modern humans (Reich et al., 2010). In the paper, the only human group that showed unequivocal introgression with the Denisovans was from Papua New Guinea. These results suggest that, at some point, the first small group of humans to migrate into Asia from Africa (Macaulay et al., 2005) encountered hominins who were closely allied to the Denisovans and hybridized with them.

The exact locations of the encounter or encounters may never be known, but genetic investigations of peoples who are descended from groups that were left behind during the first migration of modern humans into Asia— aboriginal South Indians (Consortium, 2009), Andaman Islanders (Reich et al., 2009a,b), Malaysian Orang Asli and Iban (Ang et al., 2011), Filipino and possibly Taiwanese aboriginal groups (Schanfield et al., 2002), and Australian

aborigines (Kayser, 2010)—will provide more evidence for when and where their interactions with the Denisovans took place. These and other studies that are currently ongoing (Reich, personal communication) will lay the groundwork for a detailed gene-based chronology of the events that took place during these early migrations. A recent report (www.sciencexpress.org / 22 September 2011 / Page 4 / 10.1126/science.1211177) indicates Denisovan intro- gression into the founding population of Australian aborigines, which would be expected.

IX. POSSIBLE RANGE OF PHENOTYPIC IMPACTS OF NEANDERTHAL AND DENISOVAN INTROGRESSIONS INTO MODERN HUMANS

A. Mechanisms for detecting natural selection after introgression

This final part of the review addresses the possible impacts of these remarkable introgressions. As was emphasized earlier, the fact that both Neanderthals and Denisovans contributed genes to at least some modern human groups greatly increases the possibility that our ancestors also received genes from earlier introgressions with other hominans. But, because only the central European and central Asian ancient hominin DNAs have been found to be sequenceable in investigations so far, direct investigations in the near future of the consequences of such introgressions are likely to be based on Neanderthal DNA.

Advances in sequencing of DNA from other hominins, in statistical methods, and in new fossil discoveries, are likely to open up further opportunities (Green et al., 2009). These possibilities are especially tantalizing in the case of the discovery of the recent remains of H. floresiensis, a hominin lineage that may have gone extinct as recently as 11,000 years ago. The phylogenetic relationship of H. floresiensis remains unclear, but bone measurements suggest possible affinities with H. erectus and H. habilis. The bones of this hominin that have been recovered so far have yielded no detectable DNA, because they have been badly damaged by long-term immersion in water under tropical conditions (Aiello, 2010; M. Mor- wood, personal communication), but when additional fossils of

this remarkable hominin are found they may yield better preserved bones.

In the meantime, an explosion of new information will soon be available about the fraction of the Neanderthal and Denisovan genomes that have survived in modern human gene pools. Assuming that the 1000-genome project (Stranger et al., 2010) eventually provides the 4 coverage that is currently envisioned for each individual in the project, the result will be high-quality sequence data that can be used to increase our knowledge about the number of segments of Neander- thal genome that have survived in the modern gene pool.

The distribution of ABBA and BABA sites along the chromosomes provides little information about the extent and location of pieces of introgressed Neanderthal or Denisovan DNA. This is because even in the most complete comparisons between genomes that show the strongest signal, there are only about 3000 “extra” ABBA or BABA sites in about 35,000 ABBA and BABA trees. It is therefore impossible to tell, using the ABBA–BABA test alone, which of these sites is truly extra and marks a chromosomal region at which introgres- sion has taken place.

The second approach that was taken by Green et al. (2010) to demonstrate introgression (Fig. 2.3) will yield more information about the frequency spectrum of surviving pieces of the Neanderthal genome. This ap- proach concentrates on searches for segments of the genome that have close resemblance to the homologous Neanderthal sequence in some individuals and not in others. The availability of more Neanderthal and Denisovan sequences (and perhaps of other as-yet-unknown groups allied to them who lived in Europe and Asia) will greatly increase this pool of information. Eventu- ally, we will have a clearer idea of which segments of the Neanderthal and Denisovan genomes have survived to the present time. We will also know the frequency distribution of each of these fragments in the non-African gene pool, and whether that distribution fits neutral expectation or shows signs that some segments of the introgressed Neanderthal genomes have been selected for or against.

Until this larger pool of information is available, population genetics approaches will have limited use in picking out the parts of the Neanderthal genome that are likely to have had an impact on the fitness of modern human groups. From the limited evidence so far, there is no obvious sign of selection acting on Neanderthal or Denisovan fragments, but this situation is likely to change in the future. (See added note at end of text.)

Sabeti and coworkers (Sabeti, 2006) have classified the effectiveness of different genomics methodologies in detecting the effects of positive natural selection in genomes, using the human genome as an example. The first test is whether an unusual proportion of

functional changes in genes or their closely linked regulatory regions has persisted in the human genome. This test for functional changes should pick up signs of positive selection that have persisted for millions of years, allowing differential selection in the human and chimpan- zee lineages to be detected. Although few amino acid changes have become fixed in humans from the time of Neanderthal divergence, there is strong evidence for acceleration of some parts of the human genome relative to chimpanzees, along with roughly equal amounts of accelerated evolution in chimpanzees relative to humans. Such accelerations have been detected in genes that are expressed in the brain (Lambert et al., 2011).

A second test looks for localized reductions in genetic diversity, indica- tive of selective sweeps. Signs of sweeps might be detectable for hundreds of thousands of years, until they fade away as a result of accumulating new neutral mutations. Surprisingly, signs of selective sweeps that involve alleles with non- synonymous substitutions are uncommon, no more common than sweeps of alleles that carry synonymous mutations (Hernandez et al., 2011).

In a third test, linked groups of derived alleles may be towed by a selected allele to near fixation in one species or one population but not in others. A striking example of a cluster of derived alleles is found in the region that surrounds the Duffy-negative allele in sub-Saharan African populations (Escalante et al., 2005).

In a fourth approach, large differences in allele frequencies may be produced by different selective pressures that act on geographically separated human groups. Again, Duffy-negative provides a dramatic example. Admixture between these groups, however, will reduce such an association.

Fifth, haplotypes that have recently risen in frequency and therefore show LD, and that are found at the same genome location as other haplotypes that have reached linkage equilibrium (Sabeti, 2006), are a powerful indicator of either incomplete selective sweeps or movement of selected alleles to intermediate frequencies (Wills, 2011). These flags of partial selective sweeps, however, only extend back a few tens of thousands of years in humans before they fade away through the breakdown of LD.

A sixth indicator of selection, not considered by Sabeti et al., will become available when we are able to examine the frequency distribution of parts of the genome of one species that persist in another species after an introgression event. Consider the possibility that a number of segments of the Neanderthal or Denisovan genome are widespread in some current human populations, and that the rest of this introgressed genome is either at low frequencies or nonexistent. Such a bimodal distribution would be unlikely to arise through genetic drift in the relatively short time since the introgressions took place.

It will be of great interest to examine the fate of some of the Neander- thal versions of genes that are involved in disease resistance and neurological function and that show signs of strong selection in modern human ancestry. This will be particularly important because it is possible that Neanderthal introgres- sion may have had the effect of restoring levels of genetic variation that have been lost in isolated human groups.

As modern humans migrated further out of Africa, there has been a trend for genetic variation to be lost through the repeated bottlenecks that have occurred (Ramachandran et al., 2005). This increases the likelihood that when new genetic variants were introduced from Neanderthals and Denisovans, they might have added valuable variation on which natural selection could act. Such

introduced variation would have been less likely to survive and spread had they been introduced into more genetically variable human populations, such as those of sub-Saharan Africa.

B. The possible range of phenotypic impacts of introgressions

It is now becoming clear that the elaboration of human cultures over time is itself potentially a powerful source of new selective pressures. In an unusually perspi- cacious passage even for him, Darwin remarked in Chapter 5 of The Descent of Man (Darwin, 1871):

It deserves notice that as soon as the progenitors of man became social (and this probably occurred at a very early period), the advancement of the intellectual faculties will have been aided and modified in an important manner, of which we see only traces in the lower animals, namely, through the principle of imitation, together with reason and experience. Apes are much given to imitation, as are the lowest savages; and the simple fact previously referred to, that after a time no animal can be caught in the same place by the same sort of trap, shews that animals learn by experience, and imitate each others' caution. Now, if some one man in a tribe, more sagacious than the others, invented a new snare or weapon, or other means of attack or defence, the plainest self-interest, without the assistance of much reasoning power, would prompt the other members to imitate him; and all would thus profit. The habitual practice of each new art must likewise in some slight degree strengthen the intellect. If the new invention were an important one, the tribe would increase in number, spread, and supplant other tribes. In a tribe thus rendered more numerous there would always be a rather better chance of the birth of other superior and inventive mem- bers. If such men left children to inherit their mental superiority, the chance of the birth of still more ingenious members would be somewhat better, and in a very small tribe decidedly better. Even if they left no children, the tribe would still include their blood relations; and it has been ascertained by agriculturists that by

preserving and breeding from the family of an animal, which when slaughtered was found to be valuable, the desired character has been obtained.

In this passage, Darwin emphasized the differential effect that new technologies are likely to have on the chances of survival of members of human social groups. He also suggested that kin selection could pass on heritable inventive abilities, even if the inventor died without offspring.

The possibility that human bodies, brains, and genomes are in a feed- back loop with our rapidly evolving technologies (Wills, 1993, 1998) has recent- ly been raised as an explanation for apparent increases in the rate of evolution of modern humans over the past 80,000 years (Hawks et al., 2007). The latter analysis found several thousand cases of what appear to be partial selective sweeps in Africans, Europeans, and Asians, with little overlap among the sets of sweeps. The sweeps were of the form that was first explored by Sabeti (2006), in which one haplotype at a locus exhibits strong LD and the other haplotypes are in equlibrium. The strongest argument in the Hawks et al. paper for recent selection was that if such selective sweeps had been taking place for long periods of time in our own lineage, we would have lost most our genetic variability. Thus, they suggest, the sweeps must have begun fairly recently.

It will be most instructive to examine the population structure of other primates, when the data become available, to see whether they show a similar pattern of apparent partial selective sweeps, or whether this population architec- ture is unique to humans. The possibility will still remain, however, that these apparent partial sweeps are an artifact of the recent blending together in our species of inbred and outbred tribal groups. They would then be, not a mark of recent selection, but rather of recent increases in human physical mobility and consequent gene flow.

As information about the distribution of introgressed fragments of the Neanderthal genomes in present-day populations increases, these fragments too can be examined to see if they show signs that they have been swept up in frequency by natural selection. If some of them do, this will be prima facie evidence that these introgressed fragments have brought with them some adap- tive advantage. Unlike the events that were examined in the Hawks et al. paper, these would be unequivocal selective sweeps.

Will some of these Neanderthal fragments be found to be important in cognition, language ability, and other higher brain functions? To find out, it will be necessary to understand the human epigenome and transcriptome in detail, so that we can determine the true impact of both structural and regulatory genes on the development and function of the brain (Konopka and Geschwind, 2010). The results are likely to be complex. For example, a human-specific allele of ADRB2 appears to be associated with increased

intelligence in a young cohort and decreased intelligence in an older cohort (Bochdanovits et al., 2008). Such findings, if they turn out to be common, would suggest that at least some of the alleles that affect cognitive function may not be unreservedly advantageous. They may have moved to intermediate frequencies because of balancing selec- tion (Pritchard et al., 2010; Wills, 2011).

Subsets of randomly generated genetic variation have been shown to be adaptive in an artificial system with selection for altered ribozyme function (Hayden et al., 2011). Unlike these randomly generated variants, the

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Neanderthal genome belonged to highly adapted hominins. This fact increases the likelihood that parts of their genome will prove to have been advantageous for the evolution of recent human groups. If so, then the Neanderthals will truly have played an important part of our genetic patrimony.

Since this review was completed, evidence has emerged that "outlier" alleles of HLA may have been introduced into Eurasian modern humans from Neanderthals and been pushed to substantial frequencies by balancing selec- tion (Abi-Rached et al., 2011). However, these alleles are present in lower frequencies in African populations and may have been ancestral to both modern humans and Neanderthals.

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