

Evidence that the adaptive allele of the brain size gene *microcephalin* introgressed into *Homo sapiens* from an archaic *Homo* lineage

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At the center of the debate on the emergence of modern humans and their spread throughout the globe is the question of whether archaic *Homo* lineages contributed to the modern human gene pool, and more importantly, whether such contributions impacted the evolutionary adaptation of our species. A major obstacle to answering this question is that low levels of admixture with archaic lineages are not expected to leave extensive traces in the modern human gene pool because of genetic drift. Loci that have undergone strong positive selection, however, offer a unique opportunity to identify low-level admixture with archaic lineages, provided that the introgressed archaic allele has risen to high frequency under positive selection. The gene *microcephalin* (*MCPH1*) regulates brain size during development and has experienced positive selection in the lineage leading to *Homo sapiens*. Within modern humans, a group of closely related haplotypes at this locus, known as haplogroup D, rose from a single copy $\approx 37,000$ years ago and swept to exceptionally high frequency ($\approx 70\%$ worldwide today) because of positive selection. Here, we examine the origin of haplogroup D. By using the interhaplogroup divergence test, we show that haplogroup D likely originated from a lineage separated from modern humans ≈ 1.1 million years ago and introgressed into humans by $\approx 37,000$ years ago. This finding supports the possibility of admixture between modern humans and archaic *Homo* populations (Neanderthals being one possibility). Furthermore, it buttresses the important notion that, through such admixture, our species has benefited evolutionarily by gaining new advantageous alleles. The interhaplogroup divergence test developed here may be broadly applicable to the detection of introgression at other loci in the human genome or in genomes of other species.

human evolution | introgression | admixture

Fossil records indicate that anatomically modern humans first emerged $\approx 200,000$ years ago in Africa and since then spread throughout the world (1). For most of the period since their emergence, anatomically modern humans are known to have coexisted with several now-extinct *Homo* lineages, such as Neanderthals (*Homo neanderthalis*). This long period of coexistence, including cohabitation in the Middle East and Europe, raises the intriguing possibility of genetic admixture between anatomically modern humans and archaic *Homo* populations, which could have resulted in contributions by these extinct lineages to the modern human gene pool.

The extent to which anatomically modern humans admixed with archaic *Homo* has been the subject of repeated speculation, particularly in regards to Neanderthals (2–22). Thus far, the mainstream view from fossil and genetic studies leans toward a model where anatomically modern humans fully replaced archaic *Homo* lineages rather than admixed with them (2–8). However, a number of investigators have voiced opposition to this total replacement model on a number of grounds, and the debate has yet to be resolved (9–22). Particularly needed to settle this debate is the identification of genetic loci that show telltale signs of admixture. There have been several reports of loci in the human genome that display unusually deep genealogy (15, 16, 23, 24), and in some cases,

admixture between humans and archaic *Homo* lineages has been invoked as a possible explanation. However, these studies cannot differentiate the admixture model from other possibilities, such as long-standing balancing selection, that also could contribute to deep genealogies (see *Discussion*). As such, proponents of the admixture scenario have yet to identify a concrete example of a genetic locus for which there is compelling evidence of admixture. Furthermore, most discussions of admixture tend to treat it as a selectively neutral event, one that happened simply as a byproduct of the geographical overlap between modern humans and archaic populations. Such discussions often overlook the possibility that admixture with archaic lineages, if it indeed occurred, might have brought adaptive alleles (along with the traits they determine) into the modern human gene pool, thus profoundly impacting the biological evolution of our species.

A major difficulty in looking for traces of ancient admixture in the modern human gene pool is that, under neutrality, very low levels of admixture are not expected to be readily detectable because of the effects of genetic drift. Indeed, simulations of ancient admixture showed that an archaic genetic contribution of $<0.1\%$ is unlikely to be detectable even in a very large set of polymorphism data (25). Thus, the absence at present of conclusive genetic data in support of the introgression scenario should not be taken as evidence against the possibility of any introgression.

If introgression of archaic lineages into the modern human gene pool indeed occurred, then genes that have been subject to recent positive selection in humans may be enriched for introgressed alleles. Although selectively neutral alleles introgressed from archaic lineages at low levels are likely lost by drift or swamped by the large influx of modern human DNA, an introgressed allele that is selectively advantageous could escape the effect of genetic drift and rise to high frequency. As such, these alleles might become detectable in the modern human gene pool.

The gene *microcephalin* is a critical regulator of brain size. In humans, loss-of-function mutations in this gene cause a condition known as primary microcephaly, which is characterized by a severe reduction in brain volume (by 3- to 4-fold) but, remarkably, a retention of overall neuroarchitecture and a lack of overt defects outside of the brain (26). The exact biochemical function of *microcephalin* has yet to be elucidated, but this gene likely plays an essential role in promoting the proliferation of neural progenitor cells during neurogenesis (26). *microcephalin* has been shown to be the target of strong positive selection in the evolutionary lineage

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Abbreviation: MRCA, most recent common ancestor.

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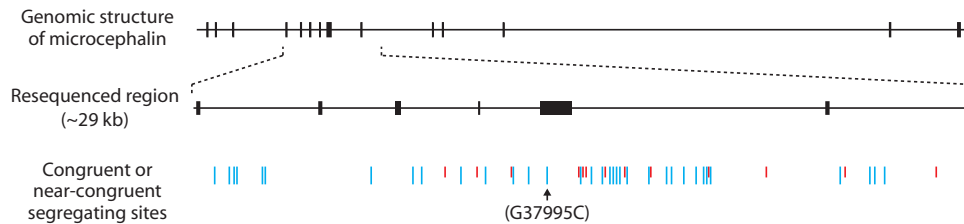


Fig. 3. Distribution of congruent or near-congruent segregating sites in the 29-kb resequenced region of *microcephalin*. Congruent sites are defined as showing consistently different alleles between D and non-D haplotypes; near-congruent sites are defined as having no more than four differences from congruent sites. Sites for which the D chromosomes are characterized by the derived allele are indicated by long blue lines, whereas sites for which the D chromosomes are characterized by the ancestral allele are indicated by short red lines (for exact positions of these sites, see Table 1). Also indicated is the G37995C nonsynonymous site used to define the D chromosomes (bearing the derived C allele) and the non-D chromosomes (bearing the ancestral G allele).

virtually no genetic exchange between the two clades except for a few rare recombinants (discussed below). These unusual features of the *microcephalin* genealogy suggest the possibility that the MRCA of the D clade introgressed into humans from a divergent *Homo* lineage at or some time before $\approx 37,000$ years ago. In the ensuing sections, we describe stringent statistical tests that support this introgression model.

Statistical Analysis of the Introgression Model. When two populations are isolated from each other for a prolonged period and are then followed by admixture, a key signature at the affected genetic locus is the presence of two haplotype clades bearing an excessive level of sequence divergence from each other (25, 30). In particular, the two haplotype clades should differ consistently from each other at a large number of sites, where the two historically separated populations have each fixed (or nearly fixed) a set of population-specific alleles during the time of separation before admixture. One way to test for admixture is to examine whether there is an excess of congruent segregating sites (i.e., sites having the same segregation pattern in the set of chromosomes sampled, as would be expected of sites that consistently differentiate two deeply divided clades), as compared with the null expectation of a panmictic model (25). A limitation of this approach is that it does not consider near-congruent sites whose perfect congruency has been slightly eroded when occasional recombination and gene conversion events occurred between the two haplotype clades since their admixture. We therefore used a modified approach called the interhaplogroup divergence test, which examines whether the divergence between two haplotype clades relative to the divergence within a clade exceeds the null expectation (see *Materials and Methods*). Our specific goal was to use the test to examine whether, at the time of coalescence of the D allele, the level of divergence separating the MRCA of D chromosomes from the non-D chromosomes, when scaled to the divergence within the non-D chromosomes, exceeds the null expectation.

To perform the test, we used the coalescent process as described (31, 32) to generate a large set of random genealogies containing the observed number of segregating sites over a 29-kb region under the observed recombination rate of the locus (see *Materials and Methods*). For each genealogy, we calculated the mean divergence between a given chromosome and the rest of the chromosomes (π_0), as well as the mean divergence among the rest of the chromosomes (π_1). A wide range of demographic scenarios was used in the coalescent simulations, including constant population size, exponential growth, and severe population bottlenecks (see *Materials and Methods*), all showing that the π_0/π_1 ratio of 3.3 observed in the real data is highly unlikely ($P = 0$ with 10,000 replicas). We also performed a much more conservative test by assuming that the mutational event that created the MRCA of the D allele landed on a chromosome, which, by chance, happened to be the most divergent chromosome in the genealogy (i.e., the chromosome with the

highest π_0/π_1 ratio in the genealogy). This test still produced highly significant results ($P < 0.0006$). Finally, we performed the test under the extreme condition of zero recombination, which makes the test highly conservative, because in the absence of recombination, the chance occurrence of an extreme genealogy will be shared

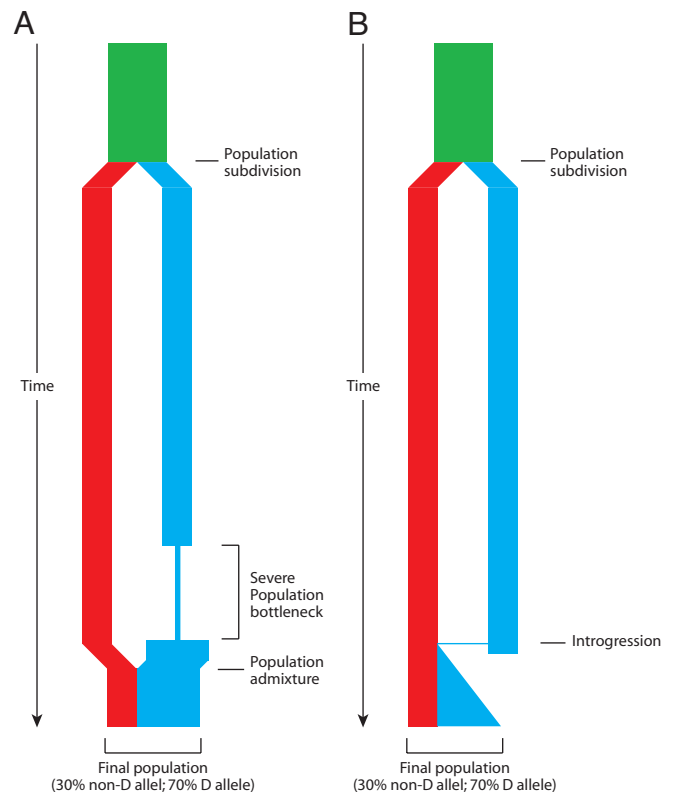


Fig. 4. Schematic depiction of two demographic scenarios compatible with the observed genealogy of the *microcephalin* locus. In both scenarios, an ancestral population, depicted in green, was subdivided into two reproductively isolated populations. One population, depicted in red, fixes the non-D allele, whereas the other population, depicted in blue, fixes the D allele. (A) In the first scenario, the blue population went through a severe bottleneck that dramatically reduced genetic diversity. It then expanded and merged with the other population. (B) In the second scenario, a rare interbreeding event occurred between the two populations, bringing a copy of the D allele from the blue into the red population. This copy subsequently amplified to high frequency under positive selective pressure. The first scenario depends on demography only and does not require selection. This scenario should therefore affect all sites in the genome. The second scenario requires the action of positive selection on the introgressed allele and is therefore not expected to have a genome-wide effect. The observation that the genealogy of *microcephalin* is not representative of the genome is consistent with the second scenario.

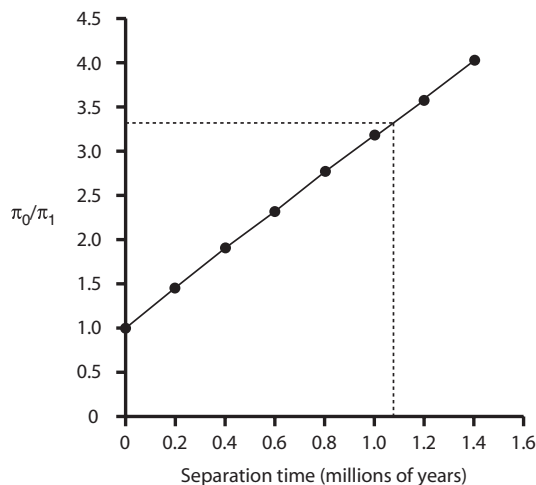


Fig. 5. Relationship between the time separating two populations and the π_0/π_1 ratio. Each circle represents the average π_0/π_1 ratio of 1,000 simulations at a given separation time (see *Materials and Methods*). Dashed lines show that a separation time of $\approx 1,100,000$ years produces the observed π_0/π_1 ratio. The average generation time is assumed to be 25 years.

separate populations. Assuming negligible gene flow during separation, we simulated a range of separation times and calculated the π_0/π_1 ratio for each time point (note that a separation time of zero results in a demographic model that is the same as the null model of panmixia described above; see *Materials and Methods*). We found that a separation time of $\approx 1,100,000$ years best recapitulated the observed π_0/π_1 ratio; that is, simulations conditioned on this separation time produced a mean π_0/π_1 ratio that closely resembles the observed value (Fig. 5; see *Materials and Methods*). We also calculated the 5% confidence lower-bound of the separation time, which is defined as the separation time for which 5% of the simulations produced π_0/π_1 ratios at or greater than the observed value. We placed this lower bound at $\approx 530,000$ years. We note, however, that because these calculations are based on a number of assumptions, such as the size of historical populations, the results should be taken as rough estimates only that are broadly consistent with the data. Furthermore, the calculations assume complete reproductive isolation of the two populations. As an alternative, there could be a small amount of gene flow between the two populations, which would likely require an even longer separation time of the two populations to achieve the level of sequence divergence observed between D and non-D alleles. In this case, the amount of separation time estimated based on zero gene flow can be considered as conservative.

Discussion

In this study, we investigate the origin of the *microcephalin* D allele in modern humans. We show that the D allele is unlikely to have arisen within a panmictic population. Instead, our data are consistent with a model of population subdivision followed by introgression to account for the origin of the D allele. By this model, schematized in Fig. 4B, the lineage leading to modern humans was split from another *Homo* lineage, and the two lineages remained in reproductive isolation for $\approx 1,100,000$ years. During this period of reproductive isolation, the modern human lineage was fixed for the non-D allele at the *microcephalin* locus, whereas the other *Homo* lineage was fixed for the D allele. These two alleles are differentiated by a large number of sequence differences accumulated during the prolonged isolation of the two populations. At or sometime before $\approx 37,000$ years ago, a (possibly rare) interbreeding

event occurred between the two lineages, bringing a copy of the D allele into anatomically modern humans. Whereas the original D-bearing *Homo* population had since gone extinct, this introgressed copy of the D allele in humans had subsequently spread to exceptionally high frequency throughout much of world because of positive selection.

Several studies have reported loci in the human genome that are associated with unusually deep genealogies containing highly divergent clades (15, 16, 23, 24). Although this type of observation can result from the admixture of reproductively isolated populations, the observation is itself insufficient evidence for admixture. In particular, deep divergence can occur if two or more allele classes at a locus are maintained by balancing selection for a prolonged period. So, even for a locus whose genealogy is too deep to be statistically compatible with neutral evolution, it is essential to rule out balancing selection before the admixture model can be adopted. One notable example is the *MAPT* locus, which has two distinct haplogroups, H1 and H2, that diverged from each other ≈ 3 million years ago, whereas the coalescence age of H2 is much younger (24). One explanation for this unusual observation is that H2 introgressed into modern humans from an archaic *Homo* lineage (17). However, because H1 and H2 are inverted relative to each other and thus cannot recombine, deep divergence between them across an extend genomic region and the young age of H2 are also compatible with balancing selection that maintained H2 at low frequencies for extended periods (along with H1) followed by a recent rise in the frequency of H2 (24). For the *microcephalin* locus, in contrast, we were able to rule out the possibility of balancing selection with a high degree of confidence. As such, *microcephalin* shows by far the most compelling evidence of admixture among the human loci examined thus far.

Speculation about the identity of the archaic *Homo* population from which the *microcephalin* D allele introgressed into the modern human gene pool points to the Neanderthal lineage as a potential (although by no means only) candidate. Anatomically modern humans and Neanderthals shared a long period of coexistence, from as early as 130,000 years ago in the Middle East (39) to as late as 35,000 years ago in Europe (40), consistent with the estimated introgression time of the *microcephalin* D allele at or sometime before $\approx 37,000$ years ago. Furthermore, the worldwide frequency distribution of the D allele, exceptionally high outside of Africa but low in sub-Saharan Africa (29), suggests, but does not necessitate, admixture with an archaic Eurasian population. Finally, our estimate of the separation time between D and non-D alleles (i.e., $\approx 1,100,000$ years with a lower-bound confidence interval of $\approx 530,000$ years) is largely consistent with the divergence time between modern humans and Neanderthals based on mitochondrial DNA (mtDNA) sequence difference (320,000–740,000 years; refs. 41 and 42) and with the earliest appearance of Neanderthals in the fossil record $\approx 500,000$ years ago (43). It would be of great interest to sequence the *microcephalin* locus in Neanderthals or other archaic *Homo* lineages, should it become technically feasible to retrieve and analyze nuclear DNA from ancient hominid remains.

Our results not only provide genetic evidence in support of the possibility of admixture between modern humans and an archaic *Homo* lineage but also support the notion that the biological evolution of modern humans might have benefited from the contribution of adaptive alleles from our archaic relatives. In the case of *microcephalin*, it is all the more intriguing given the fact that the adaptive allele is associated with an important brain development gene. As anatomically modern humans emerged from Africa and spread across the globe, the “indigenous” *Homo* populations they encountered had already inhabited their respective regions for long periods of time and were, in all likelihood, better adapted to the local environments than the colonizing humans, at least in some biological domains. It is perhaps not surprising then that modern humans, although likely superior in their own way, could in theory

benefit from adopting some adaptive alleles from the populations they replaced. That this might indeed be the case for the brain size-determining gene *microcephalin* should add an important new perspective to the discussion of human origins and the recent evolution of our species. Furthermore, any admixture between modern humans and archaic populations is likely to affect more than one locus in the genome. Our study thus provides a methodological template for identifying additional loci in the human genome that might harbor alleles from archaic populations through introgression and subsequent positive selection.

Materials and Methods

Resequencing of DNA Samples. The resequencing data were obtained as described (29). Briefly, a panel of 89 DNA samples was obtained from the Coriell Institute that broadly represents the global diversity of major human populations. It included nine sub-Saharan Africans, seven North Africans, nine Iberians, seven Basques, nine Russians, nine Middle Easterners, nine South Asians, eight Chinese, one Japanese, eight Southeast Asians, six Pacific Islander, and seven Andeans. The region of *microcephalin* studied encompasses exons 4–9 of the 14-exon gene and was 29,027 bp in length. Of this, 23,416 base pairs were fully sequenced with double-stranded sequencing in all individuals of the Coriell panel and a common chimpanzee. Inference of haplotypes was performed with the PHASE 2.1 software as described (44).

Calculation of Coalescence Time. The method for calculating coalescence time follows the described procedure (29), which is known to be unbiased by demographic history. First, the MRCA of all of the chromosomes in the sample was obtained by using chimpanzee sequence as the outgroup. The average sequence divergence separating the MRCA and each of the chromosomes was then calculated. Last, this average divergence was scaled to mutation rate as obtained from human–chimpanzee divergence in the region to produce coalescence time.

Coalescent Simulation. All software programs developed for the study are available upon request. The coalescent process as implemented in the ms software (31, 32) was used to simulate genealogies under the following parameters. The number of chromosomes was 50 (corresponding to the number of chromosomes present at the coalescence of the D chromosomes); the number of segregating sites was 178 (corresponding to the number of segregating sites present at the coalescence of the D chromosomes); and recombination (38) and gene conversion (45) rates were as described for the locus (29). Several demographic models were considered, including a constant population with an effective size of 10,000 individuals; population growth models with 10,000 individuals growing to 100,000 over 1,000, 2,000, or 3,000 generations; 10,000 individuals growing to 1,000,000 over 3,000 or 5,000 generations; and 10,000 individuals growing to 10,000,000 over 5,000 generations; and bottleneck models with 10,000 individuals dropping to 1,000 at 5,000 generations ago, then staying constant until 2,000 generations ago, at which time it exponentially grows to 10,000 or 10,000,000 at present. For each genealogy, a chromosome was selected, and the ratio of π_0 (average divergence between the chosen chromosome and the other chromosomes) to π_1 (average divergence among the other chromosomes) was calculated. This was repeated for all of the chromosomes and the highest π_0/π_1 ratio was recorded. SeattleSNP data were downloaded from <http://pga.gs.washington.edu/education.html>. Loci with <10 kb or 100 segregating sites were not considered. The *MAPT* locus was excluded, because it is known to contain a large inversion with suppressed recombination (24).

Estimation of Separation Time. A population with an effective size of 10,000 was split t generations ago into two subpopulations each with an effective size of 10,000. One subpopulation contributes one chromosome (i.e., the introgressing chromosome), and the other subpopulation contributes the remaining chromosomes. For any value of t , the average π_0/π_1 ratio for the introgressing chromosome was obtained from 1,000 simulations.

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