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Living fossils unearthed by blasting human chromosomes with Neanderthal mtDNA

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ABSTRACT

The successful retrieval of ancient mitochondrial DNA (mtDNA) from Neanderthals provides powerful experimental evidence that clarifies the arguments between the out-of-Africa and multiregional models of evolution. However, the lack of nuclear DNA from Neanderthal fossils and mtDNA of early modern human fossils dating back to approximately the same time in the Pleistocene constitutes a limitation that may compromise the significance of mtDNA phylogenetic analysis. In this report, we introduce a mitochromic analysis using Neanderthal mtDNA as a foreign transgene and humans as a naturally occurring transgenic species. Forty Neanderthal mtDNA retrievable nuclear fragments were identified by blasting human genome data with Neanderthal mtDNA. Five of the 40 fragments exhibited higher correlation with Neanderthal mtDNA than those with modern human mtDNA. Furthermore, these five nuclear fragments harbor Neanderthal mtDNA-unique haplotypes. Based on the 98%+ identity between Neanderthal and modern human mtDNA when compared by groups, we suggest that some of the modern human nuclear fragments retrieved using Neanderthal mtDNA may aid in decoding Neanderthal genetic information, and also may simultaneously demonstrate a close genetic evolutionary relationship between modern humans and Neanderthals.

1 Introduction

The successful retrieval of Neanderthal mitochondrial DNA (mtDNA) by PÄÄBO et al. in 1997 was a milestone in the genetic study of early human history [1-5]. The authenticity of these potential mtDNA sequences of Neanderthals has been independently confirmed by different groups using different fossils [6-8]. These Neanderthal mtDNA sequences enable the analysis of genetic diversity as early as 300 000 years ago [8]. The availability of ancient mtDNA sequences has promoted the anthropological study of modern human evolution. However, comparing the mtDNA of modern humans and Neanderthals does not solve the argument regarding the evolutionary relationship between the two. Whether Neanderthals contribute to the gene pools of modern humans is an important question, and is complicated by difficulties in the retrieval of chromosomal DNA from ancient fossils [9-13].

Considering endosymbiotic mitochondria as foreign invaders, human beings are a naturally occurring

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transgenic species. Based on this recognition, blasting human genome data against mtDNA provides a simplified transgenic human analysis, which we term mitochromics. Human mtDNA has migrated into chromosomes throughout evolution [14-18]. Once integrated, fragments of mitochondrial origin (mtDNA-like) may experience a slower mutation rate than their counterparts in the mitochondria. As a reverse assay, chromosomal sequences are a useful tool for tracing certain genetic records of mtDNA-origin fragments. If Neanderthals were the ancestors of modern humans, or if they interbred to produce fertile offspring, it is possible to decode some ancient information from the chromosomal sequences of modern humans. We hypothesize that the presence of Neanderthal mtDNA-like DNA fragments residing in chromosomes with high identity can provide evidence for solving the argument of whether Neanderthals passed any genes onto modern humans.

2 Nuclear (Nuc)-mtDNA fragmenting in modern human genome retrieved with Neanderthal mtDNA

A total of 40 *Homo sapiens* chromosomal fragments were retrieved. The shortest fragments consisted of 20 nucleotides, and the longest fragment comprised 250 nucleotides. Statistically, a DNA fragment of 20 nucleotides with specific sequences can be found randomly within a pool of 1.1×10^{12} nucleotides, which is 300 larger than the modern human genome. Therefore, it is reasonable to believe that a genetic relationship exists between the modern human nuclear mtDNA-like elements and the neanderthal cytoplasmic mtDNA. Recently, we identified more than 1 000 mtDNA-like fragments in the human genome by blasting human chromosomal sequences against modern human mtDNA. It is possible that the majority of these Nuc-mtDNA fragments may have resulted from recombination in the late stages of human evolution, which is supported by the observation that our older rodent counterparts, rats and mice, contain several-fold less Nuc-mtDNA elements in their genome. One crucial issue is whether the Neanderthal mtDNA-retrievable nuclear fragments are simply inserts that migrated from the mtDNA of modern humans or whether some of these inserts are potentially from Neanderthal mtDNA.

3 Neanderthal mtDNA-origin fragments existing in modern human genome

The 40 Neanderthal mtDNA retrievable fragments were further analyzed to determine their correlation with Neanderthal mtDNA sequences and the relevant regions of mtDNA from modern humans and gorillas. The overall consensus rate of these fragments with Neanderthal mtDNA was higher than that of modern humans, with the lowest consensus rate being observed for gorilla mtDNA (Figure 1A and 1B). Among the 40 nuclear mtDNA-like fragments, 16 showed higher identities to Neanderthal mtDNA than the mtDNA reference sequence of modern living humans (NC_001807). With the exception of two fragments, the remainder had the same consensus rates as the mtDNA of Neanderthals and modern humans did. Figure 1C shows the consensus rates of these 16 fragments when compared with the mtDNA of Neanderthals, modern humans, and gorillas. The number of nuclear DNA fragments with a higher consensus rate to Neanderthal mtDNA varied depending on the haplotype of the modern human mtDNA used in the comparison. However, five chromosomal fragments showed considerably higher consensus rates with Neanderthal mtDNA than all mtDNA haplotypes of modern humans, as well as all mtDNA sequences searchable in GenBank. The five nuclear mtDNA-like inserts are highlighted in green in Table 1.

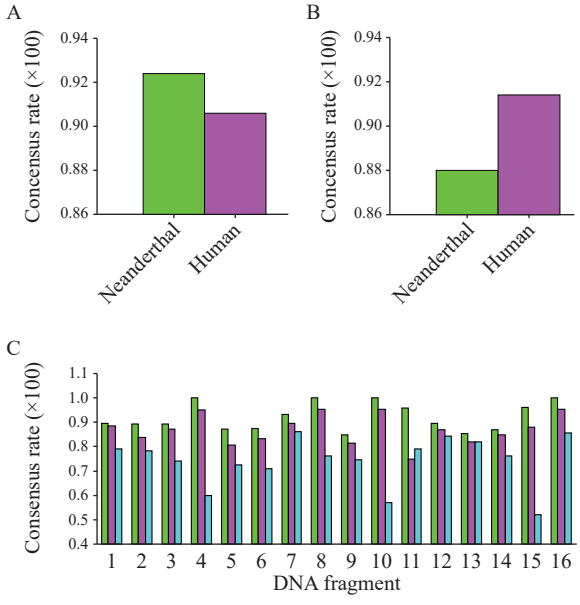

Figure 1 Consensus rate comparison of Neanderthal mtDNA retrievable genomic fragments with mtDNA of different species

A, Nuc-mtDNA retrieved by Neanderthal mtDNA vs two mtDNAs. The 40 fragments retrieved by Neanderthal mtDNA has a consensus rate with Neanderthal mtDNA that is higher than that with modern humans. B, Nuc-mtDNA retrieved by human mtDNA vs two mtDNAs. The 27 sequences retrieved using the relevant region of NC_001807 have a consensus rate with modern human mtDNA that is higher than that with Neanderthal. C, Nuc-mtDNA retrieved by Neanderthal mtDNA vs three mtDNAs. Chromosomal DNA fragments having higher consensus rates with Neanderthal mtDNA as compared with that of modern humans and gorillas. Green bar: with Neanderthal mtDNA; purple bar: with modern human mtDNA; blue bar: with gorilla mtDNA.

Table 1 Forty Neanderthal mtDNA retrievable homologues in the modern human genome (5'→3')

No.	Nucleotide sequence
1	tctgggggtgtgcacgcgatagcattgtgaaacgctggccccagagcacccctatgtcgcagtgtctgtctttgattcctgccccatcccattattgatcacacctacattcaatatcccaggcgagcatacctatcacaaggtgttaattaattaatgcttgtaggacataa
2	acatctttgccaaaccccaaaaacaaa
3	ctgctagccaccatgaatattatatagtactataatt
4	ccttgatagtacatagcacatatagtcattcatcgtacatagcacattatagtcaaatcatttctcgtccccacggatatccccctcagata
5	aaaccccaaaaacaaagaac
6	atctctgccaaaccccaaaaacaaagagccctaacatcagcctaaccagatttcaaat
7	ctttcatggggaagcaaatttgagtaccacccaagtattgacccaccccatcaatggccaccatgtatatcgtgcattactgccagccaccatgaatatt
8	gtgttaattaattcatgcttgtaggacataataa
9	tgccaaaccccaaaaacaaa
10	catagcacattatagtcaaa
11	ccatgtatatcgtgcattactgctagccaccatgaatattacatagtactataattgcttga
12	deleted
13	gccaccatgaatattgtatagtactataa
14	cccccatgcttacaagcaag
15	gtgttaattaattcatgcttgtaggacataacaataa
16	gaagtgtgttaattaattaat
17	tgccaaaccccaaaaacaaa
18	acatagcacatttcagtcaaatcc
19	gtgttaattaattcatgcttgaaggacataataat
20	tatgtacttcgtgcattactgctagtccccatgaataatatatagtactataattacttt
21	catagcacattatagtcaaat
22	atccacaccaaccccccccccca
23	cgtacatagcacattacagtcaaat
24	ttactgctagccaccatgaatattatatagtactataa
25	tatgtacttcgtgcattactgctagtccccatgaataatatacagtactataatt
26	gtgttaattaattcatgcttgaaggacataa
27	aattaattaatgcttgaaggacataa
28	tgccaaaccccaaaaacaaaga
29	actgctagccaccatgaatattatatagtactataaatgcttgact
30	acataaaaacctaatccaaatcaaa
31	tgttaattaattcatgcttgaaggacataa
32	tatgtacttcgtgcattactgctagcccccatgaata
33	gtgttaattaattaatgctt
34	catagcacattacagtcaaa
35	ttaattcatgcttgaaggacataataat
36	ctgccaaaccccaaaaacaa
37	ttaattaattcatgcttgaaggacataa
38	catagcacattatagtcaaat
39	catagcacattacagtcaaa
40	acatagcacatttcagtcaaatcc

These DNA fragments were arranged in the same sequences as those listed in the blasting results. All green highlighted fragments have consensus rates with Neanderthal mtDNA higher than the reference NC_001807. The five blue-highlighted fragments had consensus rates with Neanderthal mtDNA that were higher than all 34 types of modern human mtDNA haplotypes analyzed in this study.

4 Possible Neanderthal characteristic mtDNA haplotype identified

We aimed to further characterize the haplotype differences between the mtDNAs of Neanderthals and modern humans. The mtDNA haplotyping has been approved as a useful tool for molecular confirmation of the extinction of Lake Mungo 3. We compared Neanderthal mtDNA with all searchable mtDNA sequences within the Genbank, instead of directly sequencing mtDNA from a small group of modern humans. As shown in Table 2, the nucleotide substitution variances between Neanderthals and modern humans have overlapping ranges, with the exception of one Neanderthal mtDNA fragment sequenced to date. The minimal variation between Neanderthals and modern humans and the maximal variation between modern humans were approximately the same. Furthermore, we performed a search to detect subspecies-specific mtDNA variations between Neanderthals and modern humans. When taking Neanderthals as one group and modern humans as the other group, the nucleotide substitutions in the two comparable mtDNA regions dramatically decreased to 4 and 5 (with one gap). These small variations are frequently observed between two individual modern humans.

The maximum variance among human mtDNA was determined using short regions that were highly consistent with the Neanderthal sequences. The average value for variance was not calculated, as the sample number for Neanderthal mtDNA is very small, and the subpopulation of contemporary humans is not necessarily representative of the modern human mtDNA collection.

Comparisons between mtDNAs of Neanderthals and modern humans revealed some characteristic Neanderthal haplotypes that could be used as another standard to identify the origin of nuclear mtDNA-like elements. As shown in Figure 2, the five Nuc-mtDNA elements with greater identities to Neanderthal mtDNA than to modern living humans contain some of these unique Neanderthal haplotypes. None of the 15 000+ mtDNA sequences

Table 2 Variance range of nucleotide substitution between Neanderthal mtDNA and *Homo sapiens* mtDNA sequence collection of AF381992-AF382113 in GenBank

Sequence	Neanderthal vs Human		Human vs Human
	Minimum	Maximum	Maximum
AF282971	11	17	11
AF149291	18	29	18
AF254446	18	29	18
AF142095	11	17	11
AF011222	24	35	19

searchable in GenBank harbor these characteristic Neanderthal haplotypes. Blasting the human genome against Neanderthal mtDNA sequences revealed five out of 40 mtDNA-original fragments in our chromosomes that not only have relatively high consensus rates with Neanderthal mtDNA but also harbor characteristic Neanderthal mtDNA haplotypes. Neanderthal-unique mtDNA haplotypes offer strong evidence to support the authenticity of the Neanderthal mtDNA used in our mitochromics assay. These haplotype data also indicate that a small part of the 40 chromosomal fragments may have originated from Neanderthal mtDNA.

Figure 2 Selected base-pairing showing high species identities between Neanderthal mtDNA and modern human mtDNA

A, comparison of mtDNA hypervariable region II between Neanderthal and modern human. B, comparison of mtDNA hypervariable region II between two individuals of modern human with maximal variance. C, comparison of mtDNA hypervariable region I between Neanderthal and modern human. D, comparison of mtDNA hypervariable region I between two individuals of modern human with maximal variance. The blue highlighted mismatches are those that can be matched with another type of haplotype of modern human mtDNA. The red highlights are the nucleotides with no matches between the three Neanderthal mtDNA and the 34 types of haplotypes of modern human mtDNA analyzed in this study.

5 Modern human inherited Neanderthal genes is indicated

The data from crossover mitochromic analysis among Neanderthals, modern humans, and gorillas indicate that at least some of the DNA fragments in our chromosomes carry genetic information from Neanderthal mtDNA. Whether modern humans inherited genes from Neanderthals is a focus of debate in anthropological studies. Although we believe that the living fossils unearthed by mitochromic analysis support a close genetic linkage between Neanderthals and modern humans, the reason for its occurrence remains unknown. The simplest possibilities are either cross breeding of the two subspecies or Neanderthals being the ancestors of modern humans in Europe. This speculation of crossbreeding was supported by DUARTE et al., but it has not been supported by others in the field [11, 12]. Although less likely, we could not fully rule out the possibility that identical or very similar mtDNA sequences were shared by modern human ancestors and Neanderthals roughly 200 000 years ago. Additionally, fossil evidence supports the existence of a common ancestor of Neanderthals and modern humans [19].

The characteristic haplotypes of Neanderthal mtDNA described in this report provide strong evidence to support the authenticity of mtDNA sequences retrieved from Neanderthal remains. As living fossils, nuclear mtDNA-like elements residing in chromosomes unearthed by mitochromic analysis and the overlapping variances in nucleotide substitutions between mtDNA sequences of Neanderthals and modern humans support their close genetic linkage. In particular, the five highly convincing nuclear mtDNA-like fragments that contained characteristic Neanderthal haplotypes may putatively indicate their inheritance. These nuclear fragments harbor characteristic Neanderthal mtDNA haplotypes that always have higher consensus rates with Neanderthal mtDNA; their worst match is even higher than the best match between the nuclear fragments and mtDNA of modern living humans. The molecular evidence presented in this study is in accordance with data from an extensive phylogenetic analysis [20, 21]. In conclusion, mtDNA-original nucleic fragments may not only aid in decoding the genetic interactions between mtDNA and chromosomes, but also can potentially provide useful information in the study of human evolution.

Note: (i) There are several versions of this study. The first (WS-paper1), second, and fourth (WS-paper1b) versions were completed on 2003/11/8, 2004/2/10, and 2005/5/3, respectively. This published version used the title, text, tables, and figures from the first version, and the references and figure legends from the fourth version. (ii) When blasting the modern human genome with the full sequence of Neanderthal mtDNA that is currently available, many long fragments of genomic sequences showed 1% higher consensus rate with Neanderthal mtDNA (97.95%) compared to modern human mtDNA (96.92%).

Competing interests

The authors declare no conflict of interest.

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[18] When blasting genomes of modern human, rat, and mouse with their species-specific mtDNA sequences, different numbers of mt-original homologues were retrieved. With megsearch on, more than four hundred of mtDNA homologues were detected in human genome, which was several to more than ten times higher than the rodents of rat and mouse. The mtDNA retrievable homologues were significantly increased up to more than 1 000 hits in human genome when the meqsearch was off (word = 11 in default). In spite of the searching setting used in blast program, human genome always shows more mtDNA retrievable fragments, suggesting that mtDNA caused recombination rate is higher in human as compared to the rodents of rat and mouse. For the more than 1 000 fragments revealed in human genome, they are highly heterogeneous in their length and their consensus rates with human mtDNA, indicating their migration into chromosomes from mitochondria occurred at different stages of evolution. Those with higher consensus rates integrated into chromosome more recently, and those with low consensus rates recombinated at early stage of evolution. It is speculated that there are always some mtDNA-original recombinants not retrievable as they are fated by high mutation rates.

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现代智人染色体中存在源自古尼安德特人线粒体的 DNA 序列

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【摘要】古尼安德特人部分线粒体 DNA 测序成功，一定程度上终结了早期人类有关走出非洲与多中心起源的争论。但由于缺乏尼安德特人的染色体基因组序列，其线粒体 DNA 在古人类学领域的重要价值受到了限制。本研究引入核化线粒体组学分析法将线粒体 DNA 视为转基因和将人设定为转基因人。采用已有尼安德特人的线粒体 DNA 比对现代智人的基因组 DNA，得到 40 段同源性较高片段。其中 5 个片段与尼安德特人的同源性高于现代智人线粒体 DNA 且含有尼安德特人特有的单倍体序列。当将数据库中不同尼安德特人个体的线粒体 DNA 序列作为一个整体与现代智人线粒体 DNA 作为另一个整体比对时，高于 98% 的基因相似度不但说明已有尼安德特人线粒体序列是分析尼安德特人的有用数据，同时也说明尼安德特人与现代智人在进化上的近亲关系。

【关键词】尼安德特人；核化线粒体；人类进化；线粒体 DNA