

Mitochondrial Steve: paternal inheritance of mitochondria in humans

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Mitochondrial DNA is the primary tool in the investigation of recent evolutionary history, particularly for uncovering human origins and expansion. Maternal inheritance of mitochondria enables models of population history to be much simpler than those needed for the analysis of nuclear DNA. The recent report of a man with his father's mitochondria suggests a rethink of mitochondrial evolutionary dynamics in humans might be necessary.

In August 2002, the *New England Journal of Medicine* published a brief report describing a 28-year-old man with a metabolic disorder that had resulted in a lifelong inability to exercise [1]. Although generally healthy, even minor physical exertion resulted in lactic acid build-up in his muscles, preventing him from running more than a few steps (the same process occurs in all people after intensive exercise). When his mitochondrial DNA (mtDNA) was sequenced, two distinct haplotypes (genetic variants) were found, a situation known as mitochondrial heteroplasmy (Box 1). The predominant haplotype in his muscles had a two-base pair deletion in the *ND2* gene, which encodes an essential metabolic enzyme, thus leading to his inability to exercise. Sequencing mtDNA from his mother, father, sister and uncle, all of whom are healthy, led to the stunning conclusion that most of the mitochondria in his muscles were inherited not from his mother, but from his father.

Paternal inheritance of mitochondria is so unexpected that the researchers were careful to verify this finding. They sequenced mtDNA from a range of the man's tissues (including blood, hair and skin) and found that in all tissues except muscle, the mitochondria were fully functional and exactly matched the maternal type. However, his muscle cells contained two distinct mitochondrial haplotypes, with the mutant mitochondria outnumbering the maternal type by ten to one. The mutant haplotype also differed from the maternal type by 18 separate genetic differences, which is the amount of difference expected between unrelated individuals, not between a mother and son. The mutant haplotype was, however, identical to the father's mtDNA (except for the *ND2* deletion, which must have arisen in the sperm that fertilized the ovum, or very early during embryogenesis). To rule out the possibility of contamination, the nuclear DNA of each sample was fingerprinted to confirm its identity.

This chance combination of events (mitochondrial

disease, heteroplasmy and paternal inheritance) makes this a unique case study in mitochondrial inheritance. How could this combination of chance events have occurred? Exercise intolerance diseases resulting from mtDNA mutations have been described previously [2], and there is a range of other diseases associated with mutations in mitochondrial genes including *ND2* (see <http://www.mitoresearch.org/MitochondrialDiseaseListing.html>). Heteroplasmy is also well documented, although the haplotypes are usually very similar, and therefore probably arose by mutation in the maternal line of mitochondria (Box 1). The observation of both disease and heteroplasmy revealed the paternal origin of some of the mitochondrial population in muscle cells. Routine inheritance of the father's mtDNA has been described in some species, most notably the mussel *Mytilus*, but evidence for paternal inheritance of mitochondria in vertebrates has been restricted to one or two examples of cross-species hybrids [3,4]. Indeed, there have been strong reasons to presume maternal-only inheritance.

Death to male mitochondria

On their journey to the ovum, sperm are powered by a tailful of mitochondria (Fig. 1). When the lucky sperm cell fuses with the egg, these 100 or so sperm mitochondria enter the egg cell, where they are vastly outnumbered by 100 000 or so maternally derived mitochondria. In

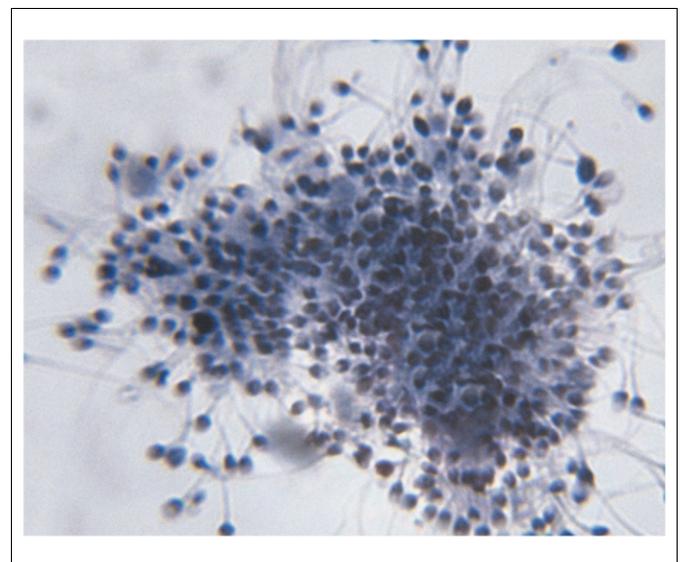


Fig. 1. Sperm are powered by a tailful of mitochondria, which enter the egg at fertilization, but are lost from the embryo soon afterwards.

Box 1. Mitochondrial heteroplasmy: a case study

Mystery surrounded the disappearance of the Russia's last Tsar, Nicholas II, and his family. The entire family was apparently executed by the Bolsheviks in July 1918, but, as their bodies had not been found, conspiracy theories abounded as to their ultimate fate. In July 1991, nine skeletons were exhumed in Ekaterinburg, Russia, not far from where the Romanovs had been held captive. To identify the remains, mitochondrial DNA (mtDNA) samples were taken [a]. mtDNA from four female skeletons exactly matched those of Prince Philip, a living maternal relative of Tsarina Alexandra, providing strong evidence that the remains were those of the Tsarina and three of her daughters. However, the analysis of the mtDNA from the presumed skeleton of the Tsar was more complicated, because the sample was heteroplasmic – two mtDNA haplotypes were present. The mtDNA matched a maternal relative of the Tsar, except for one site where two different nucleotides were segregating. The exact identity of the skeleton therefore remained uncertain until sequencing of mtDNA from the Tsar's brother, Georgij

Romanov, showed that he too was heteroplasmic for the same nucleotides at the same site [b].

mtDNA analysis has also been used to counter ongoing claims to the Romanov dynasty. In 1995, sequencing of mtDNA from Anna Anderson Manahan showed that she could not have been Anastasia, daughter of Tsar Nicholas II, as she claimed [c].

References

- a Gill, P. *et al.* (1994) Identification of the remains of the Romanov family by DNA analysis. *Nat. Genet.* 6, 130–135
- b Ivanov, P.L. *et al.* (1996) Mitochondrial sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. *Nat. Genet.* 12, 417–420
- c Stoneking, M. *et al.* (1995) Establishing the identity of Anna Anderson Manahan. *Nat. Genet.* 9, 9–10

addition, sperm mitochondria are actively targeted and destroyed by a mechanism that recognizes the ubiquitin that sperm are tagged with [5]. Paternal mitochondria do not therefore tend to persist beyond the first few cell generations of the developing embryo. Thus, all of an individual's mitochondria are inherited from their mother only, unlike nuclear DNA, which contains equal samples of both maternal and paternal chromosomes. Why should mitochondria have a dedicated system of maternal-only inheritance? Why not take mitochondria from both parents? The most common explanation is that it prevents selfish behaviour of mitochondria [6]. If mitochondria were inherited from both parents, each cell would contain a mixed population of at least two genetically different haplotypes, which would provide the raw materials for natural selection to occur within a cell, potentially at the expense of the overall functioning of the organism. For example, if one of these haplotypes was able to replicate faster because it had deleted a gene, it might increase in frequency in the cell even if it was less efficient at energy production.

An alternative hypothesis [7] is based on the idea that in the process of producing energy for the sperm cell, mitochondria inadvertently damage their own DNA through the production of free-oxygen radicals and other metabolic pollutants [8]. This could present a problem: if a gamete uses its own mitochondria in the pursuit of fertilization, it could damage the mitochondrial genome that it must pass on to the zygote. The problem might be solved by anisogamy (different roles for gametes of different sexes), if one gamete (the sperm) does all the swimming, then discards its used mitochondria, and the other gamete (the egg) sits still and keeps a healthy population of mitochondria in a quiescent and genetically pristine state [7].

This case study [1] stands alone as a demonstration of inheritance of paternal mitochondria in vertebrates. The only other documented evidence in mammals comes from a series of backcrosses of two mouse species, *Mus spretus* and *Mus musculus*: paternal inheritance of mitochondria was observed in these backcrossed interspecific hybrids at a frequency of one in 10 000 [3] (however, a subsequent study failed to repeat these results [9]). Naturally

occurring hybrids might also show occasional inheritance of paternal mtDNA: a great tit *Parus major* in the hybrid zone between two subspecies had mtDNA from both subspecies [4]. However, these rare cases of cross-species hybrids might tell us little about the occurrence of paternal inheritance in normal reproduction, as it has been suggested that the processes that destroy sperm mitochondria in the egg break down in hybrids.

Can mitochondria have sex?

One of the consequences of paternal inheritance of mitochondria is that it provides the opportunity for mitochondria, previously considered strictly clonal, to have sex. If mitochondria from two parents can coexist in one individual, as they do in this case study, then there is the possibility that mtDNA from two distinct lineages can recombine. In other words, any mitochondrial genome could ultimately be a blend of genes from more than one parent, just like nuclear DNA. How would this recombination take place? It might be possible for intact mitochondria to pick up pieces of DNA from lysed (destroyed) mitochondria, or recombination might occur through fusion of mitochondria in the early stages of embryogenesis, as observed in *Drosophila* [10].

The possibility of recombination in mitochondria has stirred heated controversy among population geneticists. Although several studies have been published claiming evidence for recombination of mtDNA [11–13], some have been discounted as based on faulty data [14], some criticized on methodological grounds [15], and the consensus in the field has been that recombination in mitochondria can be safely disregarded. Now this solid case study of paternal inheritance of mitochondria will reopen the debate. This is particularly important in light of the conclusions about human history derived from analysis of mitochondrial genes. Mitochondrial phylogenies, based explicitly on the assumption of strict maternal inheritance, have been used to tell the story of Mitochondrial Eve, the female who carried the last common ancestor of all human mitochondria [16]. Based on sequence analysis that assumes a molecular clock (constant rate of molecular evolution), Eve is thought to have lived in Africa 20 000 years ago, placing an approximate

date on the expansion of modern humans across the globe [16,17]. This story has been elaborated to trace the major lineages of European ancestry, and again female names have been given to these mitochondrial ancestors, such as Helena and Jasmine [18].

The picture changes if we allow for paternal mitochondrial inheritance, not least because our mitochondrial ancestor might have been Steve rather than Eve. The situation is complicated yet further if recombination has occurred between maternal and paternal mtDNA, in which case, our mtDNA would no longer have been inherited from a single ancestral individual. This could mean that the last common ancestor of human mitochondria could be up to four times older than current estimates based on the maternal inheritance model [13]. The older date would imply that the Out of Africa expansion of modern humans begun earlier than is currently thought, or that the population size of humans was never very small. This possibility might be welcomed by followers of an alternative hypothesis of human evolution, the multi-regional model [19,20].

Evidence for mitochondrial recombination could also shed light on patterns of molecular evolution in mtDNA. Human mtDNA trees contain many homoplasies, instances where the same mutation, or its reverse, occurs in several places on the tree. These homoplasies are commonly attributed to hypervariable sites, although the mechanism that would generate such hypervariability is currently unknown. Recombination, by shuffling mutations between mtDNA lineages, could provide an alternative explanation for the observed homoplasies [21].

This new case study [1] does not provide proof of recombination in human mitochondria, or even that paternal mitochondria can be stably inherited down multiple generations, but it does suggest that we should take the idea seriously when analyzing genetic data. This case demonstrates beyond reasonable doubt that it is possible for an adult human to carry his father's mitochondria. Although in this particular case, paternal inheritance was detected because of a disabling illness resulting from a mutated mitochondrial gene, it seems fair to assume that this illness was a co-incident that allowed detection, and that there will be other less obvious cases of healthy individuals with their father's mitochondria. Now that we know that paternal inheritance of mitochondria

can occur in humans, we should to look for it wherever comparison of parent and offspring mtDNA is possible.

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