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Do mitochondria recombine in humans?

Adam Eyre-Walker

Centre for the Study of Evolution and School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK
(*a.c.eyre-walker@sussex.ac.uk*)

Until very recently, mitochondria were thought to be clonally inherited through the maternal line in most higher animals. However, three papers published in 2000 claimed population-genetic evidence of recombination in human mitochondrial DNA. Here I review the current state of the debate. I review the evidence for the two main pathways by which recombination might occur: through paternal leakage and via a mitochondrial DNA sequence in the nuclear genome. There is no strong evidence for either pathway, although paternal leakage seems a definite possibility. However, the population-genetic evidence, although not conclusive, is strongly suggestive of recombination in mitochondrial DNA. The implications of non-clonality for our understanding of human and mitochondrial evolution are discussed.

Keywords: recombination; mitochondrial DNA; human

1. INTRODUCTION

It seems fitting in this special issue of the *Philosophical Transactions* that I should review an area of research in which John Maynard Smith is very much involved, namely recombination in mitochondria. Whereas the debate over the clonality of mitochondria has simmered in molecular biology (Gyllensten *et al.* 1991; Kaneda *et al.* 1995; Shitara *et al.* 1998) and human genetics (Howell 1997), it has been largely ignored by evolutionary biology; John Maynard Smith is one of those who have brought this debate to evolutionary biology and molecular anthropology. Maynard Smith first discovered potential evidence of recombination in mitochondria during his work on recombination in bacteria. In 1996 he had been working with Noel Smith on a method to detect recombination, 'the homoplasy test' (Maynard Smith & Smith 1998), and thought that he should check his method on something that did not recombine, as a negative control. He chose human mitochondrial DNA (mtDNA). He found an excess of homoplasies in the phylogenetic trees constructed with human mtDNA, just as many had done before him, but instead of interpreting this as evidence of hypermutation, he saw it as possible evidence of recombination.

It has been generally accepted for many years that mitochondria are inherited exclusively through the maternal line in most higher animals, and that their inheritance is therefore clonal (Birky 1995). The clonality of mitochondria has been used extensively in the study of human and mitochondrial evolution, yet the clonality of mitochondria has rarely been questioned within evolutionary genetics, despite clear and well-publicized experimental evidence that there might be paternal leakage, an obvious route by which mitochondria might become non-clonal (Gyllensten *et al.* 1991). In fact, what is perhaps most surprising about this subject is how strongly evolutionary biologists have believed in the strict clonality of mitochondria. It is not clear whether this faith arose through wishful thinking (after all, there is so much more that we can infer if mitochondria are clonal), the belief that a little recombination would have few consequences, or the perpetuation of a misconception, through the tangled web of article citation.

In this article I shall concentrate almost exclusively on the evidence that mitochondria recombine in mammals, and in particular in humans, because it is recombination in our species that is likely to have the greatest impact on our understanding of evolution.

2. PATHWAYS

There are at least three pathways by which the strict clonality of mtDNA can be circumvented: (i) recombination with paternal mtDNA, (ii) recombination with a nuclear encoded mtDNA 'pseudogene', and (iii) recombination between mtDNA molecules with new mutations in a heteroplasmic individual. Each of these pathways requires that mitochondria have the enzymes necessary for homologous recombination, or that DNA can be taken up from the cytoplasm by mitochondria. Thyagarajan *et al.* (1996) have shown that mitochondrial extracts from rat liver and from several human cell lines can promote recombination between plasmids *in vitro*, but there is no good evidence of recombination *in vivo* in mammals. Several heteroplasmic individuals have been tested for recombination (Ohno *et al.* 1996; Bidooki *et al.* 1997), but only one putative recombinant has been detected (Howell *et al.* 1996), and this might have been due to an experimental error (Howell 1997). The apparent lack of recombination in these individuals might be because recombination does not occur *in vivo*, recombination is occurring at a rate too low to be detected, or because mitochondrial fusion is rare in many tissues (see below). However, possible indirect evidence of recombination *in vivo* comes from variable-number tandem repeat sequences, found in the mtDNA of many vertebrates and invertebrates (Wolstenholme 1992; Hoelzel 1993). It is

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thought that the variation in copy number is generated by unequal exchange or gene conversion, in some species at least, and this has been recently confirmed *in vivo* in the phytonematode *Meloidogyne javanica* (Lunt & Hyman 1997).

(a) *Paternal leakage*

Ankel-Simons & Cummins (1996) have laid the blame for evolutionary biology's belief in the clonality of mitochondria on the perpetuation of a myth, that paternal mitochondria do not enter the egg. Although it has been known for over 100 years (Lillie 1923) that paternal mitochondria enter the egg in both humans (Ankel-Simons & Cummins 1996) and mice (Gyllensten *et al.* 1991; Kaneda *et al.* 1995; Shitara *et al.* 1998), several textbooks and papers have stated that the mid-piece and tail of the sperm, containing all the paternal mitochondria, fall off outside the egg (see Ankel-Simons & Cummins 1996). There has been, and continues to be, overwhelming evidence that this is not so. However, questions remain as to what happens to the paternal mitochondria once they are in the egg.

Bi-parental inheritance of mitochondria is well established in fungi (Thomas & Wilkie 1968; Saville *et al.* 1998), and recombination is known to occur in natural populations (Saville *et al.* 1998). There are also examples of stable bi-parental inheritance in some animals, such as mussels. The pattern of inheritance in mussels is rather unusual: females receive their mitochondria from their mother, whereas males inherit mitochondria from both parents; however, the paternally derived mitochondria out-replicate the maternally derived mitochondria in the male germline, so males pass only their paternally derived mitochondria to their sons (Zouros *et al.* 1994; Skibinski *et al.* 1994). Paternal leakage also seems to be relatively common in interspecific crosses: in mussels, the elaborate inheritance system breaks down when *Mytilus trossulus* is crossed to either *Mytilus edulis* or *Mytilus galloprovincialis*, and paternal mitochondria are found in females; a similar effect is not observed when the much more closely related *M. edulis* and *M. galloprovincialis* are crossed (Rawson *et al.* 1996). Similarly, paternal leakage has been observed in crosses between *Drosophila mauritiana* and *Drosophila simulans* (Kondo *et al.* 1990).

The situation in mice, the only mammal that has been studied in depth, is unclear. Gyllensten *et al.* (1991) were able to detect the paternal transmission of mitochondria at a frequency of 0.01–0.1% during nine or 26 generations of backcrossing between two different species of mice, *Mus musculus domesticus* and *Mus spretus*. Furthermore, the paternally derived mtDNA was stably transmitted in two lines maintained by brother–sister mating. However, Shitara *et al.* (1998) failed to find similar results; they detected paternally derived mtDNA in the ovaries of *ca.* 25% of the females from a cross between male *M. spretus* and female *M. musculus*, but in none of the 78 unfertilized eggs from the females with paternal mtDNA in their ovaries. Because they showed their techniques to be capable of detecting paternal mtDNA at a concentration of less than 0.01%, the results suggest that the transmission of paternally derived mtDNA through the maternal line is at a lower level than suggested by Gyllensten *et al.* (1991).

The interspecific studies suggest that paternal leakage can occur. However, the one well-studied intraspecific cross suggests that paternal leakage within a species is much less common than between species. Kaneda *et al.* (1995) showed that paternally derived mtDNA was very rapidly eliminated from the egg in an intraspecific cross in *M. musculus*; paternal mtDNA was detectable after fertilization, but no paternal mtDNA was detectable after the first cell division (Kaneda *et al.* 1995). Similar results are obtained if paternal mitochondria are labelled with fluorescent dye and tracked; they tend to disappear early in embryogenesis, although some survive as far as the eight-cell stage (Cummins *et al.* 1997). Paternally derived mitochondria also seem to be rapidly eliminated in rhesus monkeys and cows (Sutovsky *et al.* 1999). In these species, and probably others, the destruction of the male mitochondria is due to the presence of a ubiquitin tag, which is placed on the sperm mitochondria during spermatogenesis (Sutovsky *et al.* 1999).

Paternal inheritance of mtDNA has not been observed in humans, either in studies of mitochondrial pedigrees (Soodyall *et al.* 1997; Parsons *et al.* 1997; Jazin *et al.* 1998; Sigurdardottir *et al.* 2000) or in recent analyses of artificial fertilizations perfomed with intracytoplasmic sperm injection (Houshmand *et al.* 1997; Torroni *et al.* 1998; Danan *et al.* 1999). However, the numbers of subjects are small: for intracytoplasmic sperm injection only 38 cases have been studied, and the number of generational events studied in pedigrees is probably less than 2500, even if we take into account studies that have established the maternal inheritance of various genetic diseases. Given that the sperm contains less than 100 mitochondria, in comparison with the 100 000 in the egg, and paternal mtDNA is preferentially degraded (Kaneda *et al.* 1995), it is perhaps not surprising that paternal inheritance has not been detected. Furthermore, paternal inheritance is rarely tested for. For example, Sigurdardottir *et al.* (2000) sequenced part of the control region from 705 generational events in 26 Icelandic pedigrees; they assumed that single base-pair changes were new mutations, whereas multiple changes were laboratory errors, or errors in the pedigree. Both could have been due to paternal inheritance, but this was not tested for.

In summary, it seems that paternal mitochondria do enter the egg, but in intraspecific crosses male-derived mitochondria and male mtDNA are usually eliminated rapidly. However, there is at least a window of opportunity for recombination between maternally and paternally derived mitochondria; in fact, the very act of destroying the mitochondria might release male mtDNA into the cytoplasm, where it can be taken up by other mitochondria. Furthermore, it is clear that the destruction mechanism can break down, as it seems to do in some interspecific crosses.

Once paternally derived mtDNA has entered the egg, it needs to enter a maternally derived mitochondrion; this can be achieved either by fusion between mitochondria or by the release of mtDNA into the cytoplasm and its subsequent uptake. Although it might not occur in all cell types or at all developmental stages (Yoneda *et al.* 1994), the fusion of mitochondria seems to be common at other stages, including both oogenesis and embryogenesis (Smith & Alcivar 1993).

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(b) *Nuclear pseudogenes*

There are many complete and partial copies of the mitochondrial genome in the nucleus of many organisms, including humans, other vertebrates, insects, other invertebrates, fungi and plants (Zhang & Hewitt 1996). These have the potential to provide a conduit for recombination between mitochondrial genomes from different mitochondrial lineages. Because copies of the mitochondrial genome exist in the nuclear genome, it is evident that copies of the mtDNA can pass to the nucleus, although the frequency of such events is not known. However, it is unclear whether the reverse process is possible; the most likely route would seem to be via transcription, and then reverse transcription of the nuclear encoded copy, either in the cytoplasm or in the mitochondria themselves. The crucial questions are whether mitochondrial DNA is transcribed in the nucleus and whether it can enter the mitochondria as RNA or DNA. Neither the transcription of mtDNA in the nucleus nor the ability for mitochondria to take up DNA from their environment has been demonstrated, to my knowledge. However, it does seem that RNA can be transferred (Takai *et al.* 1999).

(c) *New mutations*

The simplest route by which recombination can occur is between mtDNA molecules within the same mitochondrion or cell. This will naturally have no consequence if the mtDNAs are identical, and will only have limited impact if they differ by one mutation. However, if two independent mutations are generated within a mitochondrial lineage, recombination between them can lead to a loss of clonality. Recombination between mtDNAs in the same mitochondrion, or between mtDNAs in different mitochondria in the same cell, seems likely, given that mitochondria contain the enzymes necessary for homologous recombination (Thyagarajan *et al.* 1996) and mitochondrial fusion can occur, in some tissues at least (Smith & Alcivar 1993). However, it seems unlikely that such recombination events would be detectable. First, we must have two mutations segregating simultaneously in the germline cells of the same heteroplasmic individual; second, recombination has to produce all four haplotypes, and at least three haplotypes, containing the new mutations and recombinants, have to have descendants in our sample of sequences (the fourth, original haplotype, could come from another individual). This last requirement seems particularly unlikely; most individuals do not leave one allele in the population that survives for more than a few generations, so the probability of leaving three is likely to be very low.

To estimate how likely it is for an individual female to leave three different haplotypes, let us assume that heteroplasmy lasts for one generation, so that a heteroplasmic individual can have two or more offspring with different mtDNA types, but those offspring are not themselves heteroplasmic. If there are n sequences in our sample, there are $n-1$ internal nodes in the genealogy. If an individual is to leave two haplotypes she must be an individual at a node, and if she is to leave three, two of the nodes must be in the same generation. The probability that two nodes will be in the same generation is of the order of $1/N_f$, where N_f is the effective population size of females. Now let us consider the probability that the three mtDNA molecules that coalesce within a single generation are different; for this to be true, there need to be at least two new mutations and the mutations need to recombine. The rate of mutation in the hypervariable regions of the human control region has been estimated from human pedigrees as 6.4×10^{-6} point mutations per base pair per generation (Soodyall *et al.* 1997; Parsons *et al.* 1997; Jazin *et al.* 1998; Sigurdardottir *et al.* 2000). Hence, the probability of getting two new mutations in three mtDNAs is *ca.* $3\times16\,569\times6.4\times10^{-6}\times6.4\times10^{-6}=2.0\times10^{-6}$. If we assume that there is free recombination, the overall probability of a female producing three different haplotypes whose descendants appear in our sample is *ca.* $n-1/N_f\times2.0\times10^{-6}$. This will be increased by a small factor if heteroplasmy lasts more than a single generation. Given that the long-term female effective population size has been estimated to be *ca.* 10 000 (Jorde *et al.* 1998), the chance of a female's contributing three different haplotypes to a sample of sequences is vanishingly small.

3. HOMOPLASIES

Although mitochondria have been assumed to be clonally inherited by evolutionary biologists, they might have been looking at evidence of recombination for many years. Phylogenetic trees constructed with mtDNA sequences from a population usually contain many homoplasies; for example, in the human control region sequences presented by Vigilant *et al.* (1991) there were 119 informative sites. The shortest tree for the informative sites that they could construct had 552 steps, and therefore 433 homoplasies. Homoplasies can be caused by multiple mutation or by recombination; if the inheritance of DNA is clonal, they have to be produced by multiple mutation. Homoplasies in mtDNA trees had always been attributed to multiple mutation (Wakeley 1993; Hasegawa *et al.* 1993; Excoffier & Yang 1999; Meyer *et al.* 1999), until John Maynard Smith and colleagues suggested otherwise (Eyre-Walker *et al.* 1999*a*). This is surprising, given that it has been known for many years that homoplasies could be generated by recombination (see, for example, Hudson & Kaplan 1985), and there needs to be considerable variation in the rate of change between sites to explain the level of homoplasy that exists in the absence of recombination; i.e. some sites need to be hypervariable. Selective constraint and base composition bias can both generate variation in the rate of change across sites, but these factors are insuffient to explain the level of apparent hypervariability observed either in the control region (Wakeley 1993) or in the protein-coding regions of the mtDNA (Eyre-Walker *et al.* 1999*a*,*b*).

There are potentially two types of hypervariable site: those at which the rate of change is elevated in one direction only (e.g. C→T) and those at which the rate of change is elevated in multiple directions (e.g. C→T and T→C). There is no independent evidence of either unidirectional or multidirectional hypermutable sites in mtDNA. Furthermore, one line of evidence suggests that unidirectional hypermutable sites are either rare or absent in mtDNA, because if they were common they would be expected to depress the level of synonymous divergence at saturation below the level expected from

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Table 1. *The correlation between linkage disequilibrium and distance*

(The parameter ρ is the correlation between linkage disequilibrium, as measured by r^2, and the distance between sites. Probability is the probability of observing a correlation equal to or less than the observed value, calculated by using a one-tailed Mantel test (exactly equivalent to the randomization test used by Awadalla *et al.* 1999). All data sets are from humans except those of the chimpanzees. Probability values greater than 0.1 are given as not significant (n.s.).)

origin	reference	ρ	probability
global	Awadalla *et al.* (1999)	−0.245	0.056
global	Cann *et al.* (1987)	−0.365	n.s.
Finns and Swedes	Torroni *et al.* (1996)	−0.464	0.066
native Siberians	Torroni *et al.* (1993)	−0.512	0.025
native Americans	Torroni *et al.* (1992)	−0.830	0.008
Adygei & Druze	Macaulay *et al.* (1999)	0.155	n.s.
Africans	Chen *et al.* (1995)	0.045	n.s.
Turks	Jorde & Bamshad (2000)	0.139	n.s.
Germans	Jorde & Bamshad (2000)	0.044	n.s.
Croatians	Jorde & Bamshad (2000)	−0.231	n.s.
Armenians	Jorde & Bamshad (2000)	−0.257	n.s.
Caucasians	Torroni *et al.* (1994)	−0.355	n.s.
South-East Asians	Ballinger *et al.* (1992)	−0.462	n.s.
Chukchi Siberians	Starikovskaya *et al.* (1998)	0.176	n.s.

the pattern of synonymous codon bias; this is not observed (Eyre-Walker *et al.* 1999*a*). We do not currently know the power of this test, but unidirectional hypermutable sites do have to be relatively common to explain the data. The reason is straightforward: if we have a hypermutable site at which the rate of, say, C→T is elevated, then the site is expected to be T most of the time; i.e. most hypermutable sites are expected to be in the non-hypermutable state. For example, in the revised data set used by Eyre-Walker *et al.* (1999*b*) there are on average 22.4 homoplasic sites in the 12 most parsimonious trees. To explain this level of homoplasy, there need to be at least 1300 hypermutable sites if we assume that there are just two categories of site, normal and hypermutable (calculated by using equations A2 and A3 in the appendix of Eyre-Walker *et al.* (1999*a*)); most of these are in the non-hypermutable state. It is expected that such a large number of hypermutable sites would significantly depress the level of divergence at saturation.

However, the homoplasies could be generated by multidirectional hypermutable sites; these are more difficult to test for. In principle, both types of hypermutable site should lead to an excess of sites that are homoplasic, or polymorphic, in both humans and other species, for instance chimpanzees. We performed this analysis with human and chimpanzee data (Eyre-Walker 1999*a*); however, some of the data used in that analysis were incorrect (Macaulay *et al.* 1999), and the power of the test was clearly weak. A test with data from multiple great ape species might have sufficient power to be useful.

Merriweather & Kaestle (1999) have argued that homoplasies in human mtDNA are caused by multiple mutation. They took a large data set of human control region sequences and noted that most of the sites were in complete linkage disequilibrium; most of those that were not were 'traditional' hypervariable sites. From this they argued that mutation is responsible for homoplasy and that there is no evidence of recombination. However, if there is recombination, then the sites that recombine will generate homoplasies, will seem to be hypervariable and will be in less than perfect linkage disequilibrium; so the analysis would seem to tell us nothing. It is also worth noting that sites are expected to be linkage disequilibrium in sequences that recombine (Riley *et al.* 1989; Schaeffer & Miller 1993), particularly if there is substantial population structure, as there is in Merriweather & Kaestle's (1999) data. The criticism by Arctander (1999) of the homoplasy analysis by Eyre-Walker *et al.* (1999*a*) is mysterious. He argues that the high level of homoplasy in mtDNA results sets is an artefact of maximum parsimony, because ancestral haplotypes cannot appear at the tips of the tree; this is clearly not correct. Hagelberg *et al.* (1999) presented an example of one particular homoplasy that seemed to be the consequence of recombination. Unfortunately, the proposed recombinant has turned out to be an alignment error (E. Hagelberg, personal communication).

4. LINKAGE DISEQUILIBRIUM

Homoplasies are potentially a powerful method for detecting recombination (Maynard Smith 1999). However, they can be generated by systematic sequencing errors (Macaulay *et al.* 1999), and hypervariability will always provide an alternative explanation of homoplasy to recombination for any data set. An alternative, but less statistically powerful, approach is to examine the level of pairwise linkage disequilibrium (LD) as a function of the distance between sites; LD is expected to decline with increasing distance if recombination has occurred in a sequence, whether recombination is a reciprocal or a nonreciprocal process. Such a pattern has been observed in nuclear genes (Miyashita & Langley 1988; Schaeffer & Miller 1993; Awadalla & Charlesworth 1999), and recently Awadalla *et al.* (1999) have shown that LD declines as a function of the distance between sites in five human and one chimpanzee mtDNA data sets; the decline was statistically significant in the chimpanzee data set and two of the human data sets.

It is difficult to think of any other explanation for this pattern. It has been suggested that it might be a

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consequence of patterning in allele frequencies because the denominator of r^2, the LD statistic used by Awadalla *et al.* (1999), is a function of allele frequencies, and D', another measure of LD, shows no correlation with distance (Jorde & Bamshad 2000; Kumar *et al.* 2000). However, Awadalla *et al.* (2000) have shown that the reciprocal of the denominator of r^2 is positively correlated with the distance between sites in five of the six data sets analysed by Awadalla *et al.* (1999). Furthermore, it is not surprising that D' is not correlated with distance, because D' has a highly skewed distribution at low recombination rates and allele frequencies (Hedrick 1997), and D' is a poor measure of LD. Consider two populations in which the frequency of three haplotypes AB, aB and Ab are (0.49, 0.5, 0.01) in the first and (0.25, 0.5, 0.25) in the second. The absence of the fourth haplotype is only surprising in the second population, a fact reflected in the r^2-values, 0.01 and 0.33 respectively, but not in the D'-values, which are 1 in each case.

Although Awadalla *et al.* (1999) found a negative correlation between LD and distance in all the data sets that they considered, this consistency has not been found in other data sets (Jorde & Bamshad 2000; V. Macaulay, personal communication) (table 1). Out of the 14 human data sets that have now been looked at by us or other groups, only nine show a negative correlation between LD and distance, and if we use the sequential Bonferroni technique to correct for multiple comparisons, none are significant. However, the chimpanzee result is significant after Bonferroni correction, and there are some clear trends in the results, the significance of which is difficult to estimate because of the non-independence of the results. There is an excess of negative correlations, and the positive correlations are generally small: the highest positive correlation is smaller than the absolute value of the lowest negative correlation, with the exception of the chimpanzee result. It is possibly significant that the clearest indication of recombination comes from the chimpanzee data set, which has a very large number of variable sites, with sequences being collected from a single, although not necessarily small, locality, western Africa. The collection of samples from a restricted geographical area should help to minimize LD generated by population structure, which can obscure any patterns in LD due to recombination. For example, there is clear population structure in the African data set presented by Chen *et al.* (1995).

5. THE IMPLICATIONS OF RECOMBINATION

Although the evidence for recombination is not overwhelming, the potential consequences of recombination are such that it is imperative that we resolve whether mitochondria recombine, and, if they do, to determine at what rate. Mitochondrial DNA has been used extensively in the reconstruction of phylogeny between and within species, and in the study of demography, particularly in humans. It is also being used increasingly in forensic science, particularly for the identification of deceased individuals, the Russian royal family being a prime example (Gill *et al.* 1994).

The clonality of mitochondrial inheritance has been assumed in many of the analyses involving mtDNA. Fortunately, recombination is unlikely to have any great consequences for the field in which mtDNA has been used most, the reconstruction of species phylogeny. However, recombination could have profound consequences for our understanding of human evolution. Mitochondrial DNA has been used to trace the migration of *Homo sapiens* across the globe; so far, various events in human evolution, such as the age of our most recent female common ancestor, 'Eve' (Cann *et al.* 1987; Vigilant *et al.* 1991; Hasegawa *et al.* 1993), and the expansion of human populations in Africa, Asia and Europe (Rogers 1995; Jorde *et al.* 1998). All these analyses assume that mtDNA is inherited in a strictly clonal fashion.

Let us begin by considering the age of Eve, our most recent female common ancestor. If we assume that mtDNA does not recombine and we are wrong, it will make Eve seem younger for two different reasons. First, with recombination, the phylogenetic tree of the mtDNA will no longer represent the genealogy of the mitochondria, or the maternal family tree; the tree will represent an average of several different genealogies. As a consequence, dates will tend to be underestimated because recombination averages the distance between DNA sequences; all individuals end up being a similar distance from each other. To put this precisely, if population sizes have been constant, Eve lived on average $2N_f$ generations in the past; under clonal inheritance we would therefore expect the greatest distance between two sequences in our sample to be $4N_f u$, where u is the nucleotide mutation rate, the distance between sequences that are connected to each other through the deepest node, the node that represents 'Eve'. However, with free recombination the greatest distance between any two sequences is little more than $2N_f u$, the average distance between two sequences (Li 1997) (note that I am assuming that the paternal contribution of mtDNA to each generation is small, if it exists, so that the effective population size of mtDNA is still the effective population size of females). Hence, if we assume that a freely recombining mtDNA sequence is actually clonal, we underestimate the age of Eve by a little less than twofold. If the population size has expanded rapidly, the underestimation is less than this and might be negligible, and if the population size has been declining, the underestimation is greater than twofold.

The second reason that recombination will affect the dating of Eve is because homoplasies might not be due to hypervariable sites. When hypervariable sites are taken into account in estimating the rate of evolution, they lead to higher estimates of divergence, particularly between more distantly related sequences; this has the effect of making events in human prehistory seem more recent. The reason is as follows. The molecular clock within humans is usually calibrated by using the divergence between humans and chimpanzees; the divergence within humans, as a proportion of the divergence between humans and chimpanzees, decreases with the inclusion of hypervariable sites. For example, Hasegawa *et al.* (1993) estimated the age of Eve to be *ca.* 200 000 years ago when hypervariability was taken into account, but *ca.* 400 000 years ago when hypervariability was ignored. Eve might therefore be considerably older than currently estimated; this could have implications for debates on human

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origins, whether we evolved and spread out from Africa, displacing *Homo erectus* and other related hominids without interbreeding (the 'out-of-Africa' hypothesis), or whether *H. erectus* gradually evolved into *H. sapiens* by a process of adaptation and gene flow (the 'multi-regional' hypothesis) (see Jorde *et al.* (1998) for a review).

Rogers & Harpending (1992) and Rogers (1995) have shown that it is possible to infer the time of an expansion or contraction in population size from the distribution of pairwise divergences if the sequence is clonally inherited. Unfortunately, recombination generates exactly the same pattern as a population size expansion. The estimates of human population expansions in Africa, Asia and Europe, obtained from mtDNA, might therefore have to be revised (Jorde *et al.* 1998).

Mitochondrial DNA has also been used to trace the patterns of human migration across the world. The technique has been particularly successful in illuminating the way in which the Americas were colonized by successive waves of native Siberians (Torroni *et al.* 1992, 1993). Although it is expected that the interpretation of much of this work will remain unaffected if mtDNA is not clonal, recombination might have some subtle and important effects. The basic approach in human phylogeography is to divide mtDNA sequences into 'haplogroups', groups of sequences that share unique mutations not seen elsewhere in the human population, and then to trace haplogroups across the globe. In this process, mutations that are not unique are thought to be uninformative; they are assumed to be sites that mutate rapidly and therefore yield no phylogenetic information. However, it is possible that they are mutations that have recombined onto different genetic backgrounds; they might therefore be ancient and informative polymorphisms rather than rapidly mutating sites.

6. SUMMARY

Do mitochondria recombine? I think that the question is far from resolved: we have no definite pathway by which recombination can occur, although paternal leakage seems a definite possibility, and the evidence for recombination is strongly suggestive, but not conclusive. However, the question is clearly worth pursuing, given the potential consequences of recombination in mtDNA on our understanding of human and mitochondrial evolution.

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