Reply to Macaulay *et al.* (1999): mitochondrial DNA recombination—reasons to panic

Adam Eyre-Walker*, Noel H. Smith and John Maynard Smith

Centre for the Study of Evolution and School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK

We recently presented evidence of recombination in human mitochondrial DNA (mtDNA) using a data set of largely complete human mtDNAs gleaned from GenBank and the literature. It was pointed out that some of the sequences are probably incorrect and that, when those sequences are removed, so too is the evidence of recombination. Nevertheless, we argue that there is still evidence of recombination. First, there are excessive numbers of homoplasies in other mtDNA data sets. Second, an expanded data set of our own, excluding the sequences which are the subject of suspicion, shows a significant excess of homoplasies and, hence, evidence of recombination.

Keywords: mtDNA recombination; homoplasies; phylogenetic tree

We recently presented evidence (Eyre-Walker *et al.* 1999) of recombination in human mitochondrial DNA (mtDNA) using a data set of 29 largely complete human mtDNAs gleaned from GenBank and the literature. Macaulay *et al.* (1999) pointed out that there are some anomalies in these data. First, we mistranscribed site 4985 from the data presented by Marzuki *et al.* (1991). Furthermore, there are several peculiarities in the sequences from the Marzuki *et al.* (1991) data set; at sites 3423, 11335 and 14365 the Marzuki *et al.* (1991) sequences have nucleotides segregating at high frequencies, which are otherwise absent or rare in human samples. It seems likely that these sites have been mis-sequenced. If these sequences are removed from the analysis, the level of homoplasy in the phylogenetic tree declines to levels which are consistent with clonal inheritance.

However, we believe there is still evidence of recombination for three reasons. First, mitochondrial data sets typically contain large numbers of homoplasies; for example, in a study of human mitochondrial control region variation, Vigilant *et al.* (1991) found 119 informative sites in 135 haplotypes. The shortest maximum parsimony tree they could find had 522 steps and, hence, 403 homoplasies. In the past these homoplasies have been attributed to hyper-variation (Hasegawa *et al.* 1993; Wakeley 1993), but there is no independent evidence that hypervariable sites exist. Jazin *et al.* (1998) recently showed that new control region mutations tend to occur at sites which are homoplastic, but they failed to take into account that some control region sites are under selective constraint and there is a strong base composition bias, both of which will lead to the clustering of mutations, but neither of which is sufficient to explain the level of homoplasy (Wakeley 1993).

Second, we have now collected an additional 25 complete mitochondrial DNA sequences (from Reid *et al.* 1994; Ozawa *et al.* 1995; Nishino *et al.* 1996; Polyak *et al.* 1998; Rieder *et al.* 1998). Omitting the Marzuki *et al.*

(1991) sequences from our original data set and incorporating these additional 25 sequences, there are 44 largely complete human mitochondrial sequences. There are 200 synonymous variants segregating across 3648 sites, of which 49 are informative. The shortest phylogenetic tree we have found using maximum parsimony has 76 steps and, hence, 27 homoplasies. The number of homoplasies expected if inheritance is clonal, taking into account base composition bias, is 9.28; the probability of obtaining a value of 27 or more on the basis of clonality is less than one in 10 000. So either there is recombination or there are hypervariable sites. In our previous paper we found no evidence of hypervariable sites.

Third, there are certain homoplasies which are difficult to explain in terms of hypervariability. Hagelberg *et al.* (1999) have recently shown that one particular single nucleotide variant segregates within three different haplotypic groups on the island of Nguna. This could be caused by recombination of the variant across haplotypes or by several independent mutations. The latter seems unlikely, since the variant nucleotide is very rare elsewhere in the world.

The high level of homoplasy in mitochondrial trees, the evidence that paternal mitochondria enter the egg (Kaneda *et al.* 1995; Ankel-Simmons & Cummins 1996) and the evidence that mitochondria contain the enzymes necessary for recombination (Thyagarajan *et al.* 1996) may not lead some to panic, but surely they should, because there is a very real possibility that an assumption we have held for so long is incorrect.

The authors are very grateful to Mark Rieder and Kornelia Polyak for providing their data.

REFERENCES

- Ankel-Simmons, F. & Cummins, J. M. 1996 Misconceptions about mitochondria and mammalian fertilization: implications for theories in human evolution. *Proc. Natl Acad. Sci. USA* **93**, 13 859–13 863.

*Author for correspondence (a.c.eyre-walker@sussex.ac.uk).

- Eyre-Walker, A., Smith, N. H. & Maynard Smith, J. 1999 How clonal are human mitochondria? *Proc. R. Soc. Lond. B* **266**, 477–483.
- Hagelberg, E., Goldman, N., Lio, P., Whelan, S., Schiefenhover, W., Clegg, J. B. & Bowden, D. K. 1999 Evidence for mitochondrial recombination in a human population of island Melanesia. *Proc. R. Soc. Lond. B* **266**, 485–492.
- Hasegawa, M., Di Rienzo, A., Kocher, T. D. & Wilson, A. C. 1993 Toward a more accurate time scale for the human mitochondrial DNA tree. *J. Mol. Evol.* **37**, 347–354.
- Jazin, E., Soodyall, H., Jalonen, P., Lindholm, E., Stoneking, M. & Gyllenstein, U. 1998 Mitochondrial mutation rate revisited: hot spots and polymorphism. *Nature Genet.* **18**, 109–110.
- Kaneda, H., Hayashi, J. I., Takahama, S., Taya, C., Fischer Lindahl, K. & Yonekawa, H. 1995 Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. *Proc. Natl Acad. Sci. USA* **92**, 4542–4546.
- Macaulay, V., Richards, M. & Sykes, B. 1999 mtDNA recombination—no need to panic. *Proc. R. Soc. Lond. B* **266**, 2037–2039.
- Marzuki, S., Noer, A. S., Lertrit, P., Thyagarajan, D., Kapsa, R., Utthanaphol, P. & Byrne, E. 1991 Normal variants of human mitochondrial DNA and translation products: the building of a reference data base. *Hum. Genet.* **88**, 139–145.
- Nishino, I., Seki, A., Maegaki, Y., Takeshita, K., Horai, S., Nonaka, I. & Goto, Y.-I. 1996 A novel mutation in the mitochondrial tRNA-Thr gene associated with a mitochondrial encephalomyopathy. *Biochem. Biophys. Res. Comm.* **225**, 180–185.
- Ozawa, T., Katsumata, K., Hayakawa, M., Tanaka, M., Sugiyama, S., Tanaka, T., Itoyama, S., Nunoda, S.-I. & Sekiguchi, M. 1995 Genotype and phenotype of severe mitochondrial cardiomyopathy: a recipient of heart transplantation and the genetic control. *Biochem. Biophys. Res. Comm.* **207**, 613–620.
- Polyak, K., Li, Y., Zhu, H., Lengauer, C., Willson, J. K. V., Markowitz, S. D., Trush, M. A., Kinzler, K. W. & Vogelstein, B. 1998 Somatic mutations of the human mitochondrial genome in human colorectal tumours. *Nature Genet.* **20**, 291–293.
- Reid, F. M., Vernham, G. A. & Jacobs, H. T. 1994 Complete mtDNA sequence of a patient in a maternal pedigree with sensorineural deafness. *Hum. Mol. Genet.* **3**, 1435–1436.
- Rieder, M. J., Taylor, S. L., Tobe, V. O. & Nickerson, D. A. 1998 Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human genome. *Nucl. Acids Res.* **26**, 967–973.
- Thyagarajan, B., Padua, R. A. & Campbell, C. 1996 Mammalian mitochondria possess homologous DNA recombination activity. *J. Biol. Chem.* **271**, 27 536–27 543.
- Vigilant, L., Stoneking, M., Harpending, H., Hawkes, K. & Wilson, A. C. 1991 African populations and the evolution of human mitochondrial DNA. *Science* **253**, 1503–1507.
- Wakeley, J. 1993 Substitution rate heterogeneity variation among sites in hypervariable region 1 of human mitochondrial DNA. *J. Mol. Evol.* **37**, 613–623.