The Roles of Mutation Rate and Selective Pressure on Observed Levels of the Human Mitochondrial DNA Deletion mtDNA^{4977}

William C. Navidi, Simon Tavaré and Norman Arnheim

Abstract

The mitochondrial deletion mtDNA^{4977} has been found at high levels in individuals with certain neuromuscular and neurological diseases, and at lower levels in older normal individuals. We use experimental estimates of the mutation rate of mtDNA^{4977} and of the half-life of mitochondrial genomes to construct a model of mitochondrial replication and mutation that is consistent with observed levels of the deletion. We conclude that deleted genomes have a slight selective advantage, at least in some tissues. Our results suggest that for an individual to attain a clinically significant level of the deletion, between 0.2% to 0.5% of the mitochondrial genomes in the original oöcyte must have been deleted.

Keywords: branching process; Kearns-Sayre syndrome; mitochondria; selection

1 Introduction

The human mitochondrial mutation mtDNA^{4977} is a 4977 base pair deletion originating between two 13 bp direct repeats in normal mtDNA. This deletion is associated with the neuromuscular and neurological diseases progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (KSS) and Pearson's marrow/pancreas syndrome. Symptoms of these sporadic diseases range from mild to severe, depending on the level to which the deleted molecules have accumulated. For a review of diseases associated with mutations in mitochondrial DNA, see DiMauro and Wallace [10], Wallace [26], DiMauro [9] and Bianchi *et al.* [3]. MITOMAP (`http://www.mitomap.org`) is a very useful resource for human mitochondrial data and references.

The mtDNA^{4977} deletion has also been found at low levels in normal adults and appears to accumulate with age, primarily in non-mitotic tissues (Cortopassi *et al.* [8], Arnheim and Cortopassi [1], Corral-Debrinski *et al.* [7], Hattori *et al.* [14], Yen *et al.* [27], Zhang *et al.* [28]). The level of accumulation is found to vary among different tissues and even within tissues. For example, studies on the brains of old normal individuals has shown that the substantia nigra, caudate and putamen can have hundreds of fold higher levels of mtDNA^{4977} than the cerebellum (Corral-Debrinski *et al.* [7], Soong *et al.* [25]).

The degree of accumulation of mtDNA[4977], whether at the high levels found in patients with disease, or at the low levels found in normal adults, is determined by the mutation rate, by selective factors that may favor deleted molecules, and by the initial level of deletions present at conception. We describe models that enable us to discuss quantitatively the roles played by each of these factors in the accumulation of deletions in both growing and stable populations of cells.

2 A Stochastic Model for Deletions

The mitochondrial genomes in a human cell are distributed among many mitochondria, with an average of between four and ten genomes per mitochondrion. Mitochondria can turn over by being engulfed by lysosomes. In what follows we refer to this turnover as mitochondrial death.

Let ω_1 and ω_2 represent the probabilities that a nonmutant and a mutant, respectively, die before replication. Let λ represent the mutation rate, defined as the probability that replication of a nonmutant sequence produces a mutant. We assume that a mutant always gives rise to mutants upon replication.

Define one generation to be the length of time between replications of a nonmutant molecule. Let $2r$ be the mean number of descendants of a single mutant molecule after one generation, conditional on survival to replication of the original molecule. Then the unconditional expectation of the one generation clone size of a single mutant is $2r(1-\omega_2)$, and that of a single nonmutant is $2(1-\omega_1)$.

We define the selective advantage ν of mutants over nonmutants in terms of the ratio of the expected one-generation clone sizes: $\nu = r(1-\omega_2)/(1-\omega_1)$. Notice that any value of ν can be obtained by setting $r = 1$, and choosing ω_1 and ω_2 appropriately. For the sake of simplicity, we assume henceforth that $r = 1$, and that any selective advantage is due to differences in the death rates ω_1 and ω_2.

We model the evolution of the population of mitochondria as a two-type Galton-Watson process, the two types being nonmutant (type 1) and mutant (type 2). We calculate the probability $p_i(x,y)$ that a parent of type i ($i = 1,2$) produces x nonmutants and y mutants in a single mitochondrial genome replication. We have

$$p_1(0,0) = \omega_1, \qquad p_1(2,0) = (1-\omega_1)(1-\lambda), \qquad p_1(1,1) = (1-\omega_1)\lambda$$

$$p_2(0,0) = \omega_2, \qquad p_2(0,2) = 1-\omega_2, \tag{1}$$

and $p_i(x,y) = 0$ for other values of x and y.

Let M denote the 2×2 matrix whose ij^{th} element m_{ij} is the mean number of offspring of type j produced by a parent of type i in a single replication. From (1) we have

$$M = \begin{pmatrix} (1-\omega_1)(2-\lambda) & (1-\omega_1)\lambda \\ 0 & 2(1-\omega_2) \end{pmatrix}. \tag{2}$$

Given values m_{g-1}, n_{g-1} for the expected number of mutant and nonmutant genomes, respectively, after $g-1$ generations, the values m_g, n_g can be computed as

$$(n_g, m_g) = (n_{g-1}, m_{g-1})M. \tag{3}$$

By repeatedly applying (3) starting from $g = 1$ and values for n_0, m_0, we can compute m_g and n_g for any value of g. We approximate the proportion of mutants in the population after g generations by $m_g/(m_g + n_g)$. For theoretical results in this spirit, see Olofsson and Shaw [19].

3 Constant-size Populations

We can use our model to evaluate the possible roles that both mutation and selection could play in the accumulation of deletions over time. We first investigate the relationships between mutation rate, selective advantage, and the proportion of mutants expected in regions of the brain. For example, the level of mtDNA^{4977} deletions in the substantia nigra has been estimated to be as high as 0.5% in an 80-year-old normal individual (Soong *et al.* [25]).

As before, let ω_1 and ω_2 represent the probabilities that a nonmutant and a mutant, respectively, die before replication, and let λ represent the probability that a replication of a nonmutant genome produces a mutant genome. Let m_0, n_0 be the initial number of mutants and nonmutants, respectively, present at birth, and let m_g, n_g be the expected numbers of mutants and nonmutants after g mitochondrial generations.

From (2) and (3), the quantities m_g and n_g satisfy the recursive equations

$$m_g = 2(1-\omega_2)m_{g-1} + \lambda(1-\omega_1)n_{g-1} \tag{4}$$

$$n_g = (2-\lambda)(1-\omega_1)n_{g-1}. \tag{5}$$

We use the ratio

$$\nu = (1-\omega_2)/(1-\omega_1) \tag{6}$$

to express the selective advantage of mutants over nonmutants. Values of ν greater than 1 correspond to an advantage for mutants, and values less than 1 correspond to an advantage for nonmutants.

Since the brain is primarily a non-dividing tissue, the number of genomes $G = m_g + n_g$ is assumed to be constant across generations. It follows that in each generation g, the death rates ω_1 and ω_2 satisfy the following equations:

$$1-\omega_1 = G/2(\nu m_{g-1} + n_{g-1}) \tag{7}$$

$$1-\omega_2 = \nu(1-\omega_1). \tag{8}$$

Given values of ν, m_0, and n_0, we can calculate values of m_g and n_g for any value of g by repeatedly calculating ω_1 and ω_2 from equations (7) and (8) and substituting into equations (4) and (5).

In our calculations, we took the mitochondrial generation time to be 45 days, based on experimental estimates of turnover in the rat brain (Gross *et al.* 1969). Table 3 gives values for the expected proportion m_{650}/G of mutants after 650 generations (about 80 years) as a function of the selective advantage ν for three values of λ, assuming no mutants were present at birth. The values of λ we chose bracket the estimate of Shenkar *et al.* [23]), who estimated the mutation rate of mtDNA^{4977} in cultured human cells to be $5.95 \times 10^{-8} \pm 2.28 \times 10^{-8}$.

It is clear that only a small selective advantage is needed to produce a large proportion of mutants, and that the value of the mutation rate has less impact. The second column in Table 3 shows that this is consistent with a selective advantage in the range of 1.012. Estimates of mtDNA^{4977} levels in putamen of old individuals may be as high as 12% (Corral-Debrinski *et al.* [7]) which would be consistent with a selective advantage in the range of 1.018.

The model yields a different conclusion regarding the cerebellar grey matter. Of thirteen regions of the brain studied by Soong *et al.* [25], this had the smallest fraction of deleted genomes, with the proportion being only 0.0013% in an 82 year old individual. Table 3 shows that this would be consistent with the absence of a selective advantage in the cerebellar grey matter, if we were to assume that the mutation rates in this region is the same as in the substantia nigra.

Of course, differences in mutation rates in different tissues can also explain differences in accumulation. Table 3 gives values for the expected proportion of mutants after 650 generations as a function of mutation rate, assuming no selective advantage. Comparing Tables 3 and 3 shows that an increase of about two orders of magnitude in the mutation rate (from 6×10^{-8} to 5×10^{-6}) is needed to produce the same result as a selective advantage of 1%, i.e. $\nu = 1.01$.

4 Growing Populations

The mtDNA^{4977} deletion accumulates to at least a level of 40% of the mitochondrial genomes in muscle cells of the vast majority of children with KSS (Shanske *et al.* [22]). We develop a model to investigate the roles of selective pressure and mutation rate on the accumulation of mtDNA^{4977} in these children. In order to accomplish this we need to take into consideration three phases of the child's development.

The first phase begins with a single fertilized oöcyte (zygote) containing approximately 150,000 mitochondrial genomes (Chen *et al.* [6]). This cell divides until there are about 125 descendants, 40 of which comprise the inner cell mass and are destined to become the embryo (Hardy *et al.* [13]). We assume that a cell needs a minimum of about 7500 mitochondrial genomes to survive. It follows that the number of mitochondria in the 125 cells must be about 7500×125. Therefore the number of mitochondria in the original oöcyte (150,000) must have multiplied by a factor of 6.25 ($= 7500 \times 125 / 150{,}000$).

In the second phase the 40 embryo cells replicate to form a fetus which we estimate contains approximately 2.5×10^{12} cells at birth. During these two mitotic phases the

Table 1: Expected proportions of mutants after 650 mitochondrial generations for various values of the mutation rate and selective advantage.

Selective advantage (ν)	$\lambda = 1.0 \times 10^{-8}$	$\lambda = 5.95 \times 10^{-8}$	$\lambda = 1.0 \times 10^{-7}$
1.022	0.24010	0.65278	0.75960
1.020	0.08866	0.36663	0.49313
1.018	0.02930	0.15224	0.23185
1.016	0.00936	0.05327	0.08640
1.014	0.00299	0.01755	0.02914
1.012	0.00097	0.00574	0.00961
1.010	0.00032	0.00191	0.00320
1.008	0.00011	0.00066	0.00110
1.006	0.00004	0.00024	0.00040
1.004	0.00002	0.00009	0.00015
1.002	0.00001	0.00004	0.00007
1.000	0.00000	0.00002	0.00003

rate of mitochondrial turnover is likely to be insignificant. The third phase covers the period after birth where we only consider mitochondrial turnover and not cell replication. Our goal is to express the proportion of genomes that have the deletion in the muscle cells of a child 10 years of age as a function of the number of deleted genomes in the oöcyte and the selective advantage ν of deleted over non-deleted genomes, assuming a constant mutation rate.

Let N be the number of deleted genomes in the oöcyte. In the first phase, these N molecules multiply to a level of approximately $6.25N$ deleted molecules that are distributed among 125 cells. We assume that the distribution is random, so that the number of deleted molecules in a single one of the cells has a binomial distribution with parameters $6.25N$ and $1/125$. We approximate this with a Poisson distribution with mean $\mu = 6.25N/125 = N/20$.

To model the second and third phases, we focus on a single embryo cell and use the stochastic model described in equations (4) and (5). To apply the model, we specified values for the mutation rate λ, the death rates ω_1 and ω_2, the initial number m_0 of mutants and n_0 of non-mutants, and the number of generations g. We used the value 5.95×10^{-8} for λ, as estimated by Shenkar *et al.* [23]). For the second (mitotic) phase, we assumed there is no mitochondrial turnover, so that $\omega_1 = \omega_2 = 0$. Note that this implies an absence of a selective advantage in the mitotic phase. As explained below, the degree of selective advantage in the second mitotic phase has little impact on the final level of deletions, so this assumption is not crucial. We assumed that the cell contained a total of $m_0 + n_0 = 7500$ mitochondrial genomes. As described above, the quantity m_0 has a Poisson distribution with mean $\mu = N/20$. We assumed that

Table 2: Expected proportions of mutants after 650 generations for various values of the mutation rate assuming no selective advantage.

Mutation rate	Expected proportion of mutants
1.0×10^{-8}	0.00000
5.0×10^{-8}	0.00002
1.0×10^{-7}	0.00003
5.0×10^{-7}	0.00016
1.0×10^{-6}	0.00032
5.0×10^{-6}	0.00162
1.0×10^{-5}	0.00324
5.0×10^{-5}	0.01612
1.0×10^{-4}	0.03198
5.0×10^{-4}	0.15000
1.0×10^{-3}	0.27753
5.0×10^{-3}	0.80349
1.0×10^{-2}	0.96154

the second phase begins with 40 cells and ends with 2.5×10^{12} cells. It follows that the number of mitochondrial generations g satisfies the equation $40 \times 2^g = 2.5 \times 10^{12}$. This provides the estimate $g = 36$, to the nearest integer.

For the third phase, we used the method (described earlier for the brain) for a constant size population, in which we consider mtDNA turnover. We specify a value for the selective advantage ν, then compute ω_1 and ω_2 from equations (7) and (8). To estimate the number of generations g', we used an experimental estimate of a half-life of one week for mitochondria in muscle cells (Gross *et al.* [12]), which corresponds to a mean turnover time of about 10 days. Therefore 10 years corresponds to $g' \approx 350$ mitochondrial generations. The initial numbers of mutants and nonmutants for the third phase were of course the final numbers for the second phase.

Given values for the initial number N of deleted mitochondria in the oöcyte and the selective advantage ν, we computed the fraction of deleted mitochondria deriving from a single inner mass cell for all feasible values of m_0, as described above. We then averaged this fraction over the Poisson distribution of m_0 to obtain expected fraction $d(N,\nu)$ of deleted mitochondria. For many different values of N, we computed the value of ν for which $d(N,\nu) = 0.5$. This fraction is chosen to reflect the observed levels of deletions seen in skeletal muscle of a child with Kearns-Sayre syndrome.

Figure 1 presents the results for values of N ranging from 0 to 75,000. The latter number corresponds to the situation in which one half of the original genomes have the deletion. Figure 2 presents the results for values of N ranging from 0 to 1000. In order to check the impact of our assumptions concerning the lack of turnover in the mitotic

phase, we redid the calculations setting ω_1 to 0.1, and assuming the selective advantage was the same in both the mitotic and constant-size phases. The results (not shown) were nearly identical to those presented.

It is clear from Figure 1 that unless the initial level of deletions in the oöcyte is nearly one-half, the deleted molecules must have a selective advantage in order to reach the levels of 50% observed in a child. Figure 2 shows that a selective advantage of 4% would result in the proportion of deletion reaching one-half when there were no initial oöcyte deletions, and deletions arose post-zygotically by mutations alone. Our earlier analysis of deletion levels in the brain estimates selective values in the range 1.2% to 1.8%, suggesting that a selective advantage of 4% is implausible. If we assume that the selective advantage is indeed in the range 1.2% to 1.8%, then the number of initial deletions leading to a level of 50% after ten years is of the order of 300 to 750, or between 0.2% and 0.5% of the oöcyte's mitochondrial genomes.

There is some empirical evidence about the frequency of deleted molecules in oöcytes (cf. Chen *et al.* [6], Brenner *et al.* [4], Barritt *et al.* [2]). For example, Chen *et al.* [6] observed that in a sample of 15 oöcytes, approximately one half had no detectable deletions, while the rest had an average of roughly 50 deletions each. The maximum number of deletions observed was in the range 100–200. Figure 2 shows that if the selective advantage were greater than about 2%, this initial level of deletions in an oöcyte would lead to a level of 50% deletions by age ten, and thus a disease prevalence of about 7%. However, the observed prevalence of the disease is between 1/100,000 and 1/500,000 (Larsson *et al.* [16]). It follows that the selective advantage of the deleted molecules must be less that 2%, and we conclude that KSS is due to the very rare (frequency of around 1/100,000) oöcytes that have 350-700 deletions, with selective advantage in the range 1.2% - 1.8%.

5 Discussion

We have used a model of mitochondrial replication and mutation to evaluate the possible roles that both mutation and selection could play in the accumulation of deletions over time, in both expanding and stable mitochondrial populations. We note however that the mechanism by which mutant mtDNA accumulate in patients with mitochondrial diseases is a matter for debate (cf. Marchington *et al.* [17], Reynier *et al.* [21], Jansen [15], Shoubridge [24], Brown *et al.* [5], Elson *et al.* [11], Qintana-Murci *et al.* [20]), and that more details of this process will be needed for a definitive quantitative analysis.

We begin with the stable population case. Using experimental estimates of the half-life of a mtDNA genome (Gross *et al.* [12]) and of the mtDNA^{4977} mutation rate (Shenkar *et al.* [23]), we have shown that starting from a collection of brain cells that do not divide, each of which begins with no mutant mitochondria, after 650 generations (80 human years) we would expect no more than 0.002% mutant genomes if the mutants had no selective advantage.

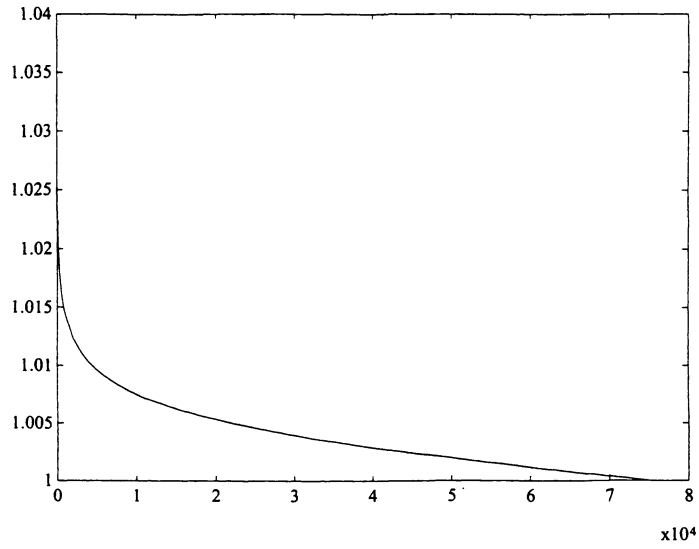

Figure 1: Value of selective advantage ν (y axis) required to give $d(N,\nu) = 0.5$ for different values of N (x axis).

That deleted genomes do have a replicative selective advantage is plausible *a priori*, since mtDNA^{4977} is two-thirds the size of normal mtDNA genomes. Using the estimates of the half life and the mutation rate as above, we calculate that even a selective advantage of as little as 1.2% could result in the levels observed in the substantia nigra of old individuals (Table 3). Experimental estimates of this selective advantage based on differences in the rates of completion of mtDNA circles during replication showed no evidence of any difference between deleted and undeleted genomes (Moraes and Schon [18]), but such studies could not have detected an advantage on the order of a few percent.

mtDNA^{4977} accumulation to the reported levels in some brain regions (0.5% to 12%) as a result of mutation alone would require a mutation rate about three orders of magnitude higher than the experimental estimate. It has been argued that the mutation rate may be high in the substantia nigra (as well as the caudate and putamen), due to the extra burden of oxidative damage that might result from the high levels of metabolism of the neurotransmitter dopamine by MAO-B (Soong *et al.* [25], Corral-Debrinski *et al.* [7]). This enzyme generates H_2O_2, which can react with iron deposits inside cells to produce hydroxyl radical leading to DNA damage. On the other hand, some brain regions show deletion levels hundreds of times smaller than in the substantia nigra and putamen. If mutation rates were the same, it is not clear how selective pressures could vary greatly among cells in different regions of the brain.

We applied our method to determining the extent to which mutations in oöcytes and selective pressure contribute to the deletion levels observed in the mitochondria of chil-

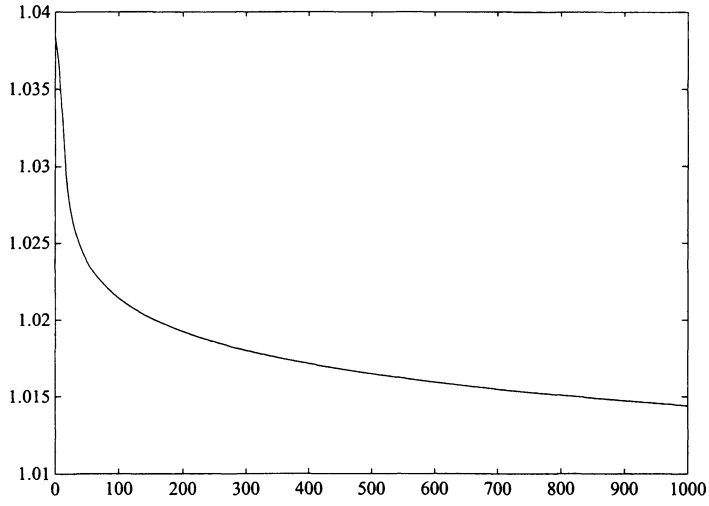

Figure 2: Value of selective advantage ν (y axis) required to give $d(N,\nu) = 0.5$ for different values of N (x axis).

dren with Kearns-Sayre syndrome. We described a hypothesis that quantifies the effects of the initial levels of deletions in oöcytes, the mutation rates, and the selective advantage of mutants. Although the number of mutant genomes in a person is random, we have based our analysis on expected values. We have estimated the selective advantage and the number of initial deletions necessary for the mean number of deletions after 10 years to amount to 50% of the total number of mitochondrial genomes. Of course, a few individuals will have deletion levels several standard errors above the mean. The standard error of the number of deletions produced in the second and third stages, however, is of the order of the square root of the mean, which is negligible in proportion to the mean. We conclude therefore that Kearns-Sayre syndrome cannot result solely from an unusually large number of mutations in the second and third stages.

This method can be generalized to situations involving several mutant types, with point mutations as well as deletions, and using time intervals other than the mitochondrial generation time. For example, the element in the second row and first column of the matrix (2) represents the rate of back point mutations multiplied by the mutant death rate.

The method outlined above is applicable in a wide variety of biological settings. Constant-size non-mitotic populations can be modelled by choosing values for ω_1 and ω_2 which indicate that on average half the genomes die each generation. Growing populations are modelled by choosing smaller values for these death rates. A selective advantage (for mutants, say) can be incorporated by choosing $\omega_2 < \omega_1$, indicating that mutants are more likely to replicate before dying than non-mutants are.

Dedication

It is a pleasure to dedicate this article to Terry Speed, friend, collaborator and teacher, on the occasion of his 60th birthday. The fields of statistics and genetics have been tightly bound together since their inception, and Terry's seminal contributions to the statistical analysis of molecular data stand in the forefront of this long tradition. Above all, Terry has shown us that one need not sacrifice mathematical rigor to obtain biological relevance. In this, he has set a standard toward which we continue to strive.

William C. Navidi, Department of Mathematics, Colorado School of Mines, wnavidi@mines.edu

Simon Tavaré, Program in Molecular and Computational Biology, University of Southern California, stavare@usc.edu

Norman Arnheim, Program in Molecular and Computational Biology, University of Southern California, arnheim@molbio.usc.edu

References

[1] N. Arnheim and G. Cortopassi. Deleterious mitochondrial DNA mutations accumulate in aging human tissues. *Mutation Research*, 275:157–167, 1992.

[2] J. A. Barritt, C. A. Brenner, S. Willadsen, and J. Cohen. Spontaneous and artificial changes in human ooplasmic DNA. *Human Reproduction*, 15(Suppl. 2):207–217, 2000.

[3] N. O. Bianchi, M. S. Bianchi, and S. M. Richard. Mitochondrial genome instability in human cancers. *Mutation Research*, 488:9–23, 2001.

[4] C. A. Brenner, Y. M. Wolny, J. A. Barritt, D. W. Matt, S. Munné, and J. Cohen. Mitochondrial DNA deletion in human oocytes and embryos. *Molecular Human Reproduction*, 4:887–892, 1998.

[5] D. T. Brown, D. C. Samuels, E. M. Michael, D. M. Turnbull, and P. F. Chinnery. Random genetic drift determines the level of mutant mtDNA in human primary oocytes. *American Journal of Human Genetics*, 68:533–536, 2001.

[6] X. Chen, R. Prosser, S. Simonetti, J. Sadlock, G. Jagiello, and E. A. Schon. Rearranged mitochondrial genomes are present in human oöcytes. *American Journal of Human Genetics*, 57:239–247, 1995.

[7] M. Corral-Debrinski, T. Horton, M. T. Lott, J. M. Shoffner, M. F. Beal, and D. C. Wallace. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nature Genetics*, 2:324–329, 1992.

[8] G. A. Cortopassi, D. Shibata, N-W. Soong, and N. Arnheim. A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. *Proceedings of the National Academy of Sciences USA*, 89:7370–7374, 1992.

[9] S. DiMauro. Lessons from mitochondrial DNA mutations. *Seminars in Cell and Developmental Biology*, 12:397–405, 2001.

[10] S. DiMauro and D. C. Wallace. *Mitochondrial DNA in human pathology*. Raven Press, New York, 1993.

[11] G. L. Elson, D. C. Samuels, D. M. Turnbull, and P. F. Chinnery. Random intracellular drift explains the clonal expansion of mitochondrial DNA mutations with age. *American Journal of Human Genetics*, 68:802–806, 2001.

[12] N. J. Gross, G. S. Getz, and M. Rabinowitz. Apparent turnover of mitochondrial deoxyribonucleic acid and mitochondrial phospholipids in the tissues of the rat. *Journal of Biological Chemistry*, 244:1552–1562, 1969.

[13] K. Hardy, A. H. Handyside, and R. M. Winston. The human blastocyte: cell number, death and allocation during late preimplantation development in vitro. *Development*, 107:597–604, 1989.

[14] K. Hattori, M. Tanaka, S. Sugiyama, T. Obayashi, T. Ito, T. Satake, Y. Hanaki, J. Asai, M. Nagano, and T. Ozawa. Age dependent increase in deleted mitochondrial DNA in the human heart: Possible contributory factor to presbycardia. *American Heart Journal*, 121:1735–1742, 1991.

[15] R. P. S. Jansen. Germline passage of mitochondria: quantitative considerations and possible embryological sequelae. *Human Reproduction*, 15(Suppl. 2):112–128, 2000.

[16] N-G. Larsson, E. Holme, B. Kristiansson, A. Oldfors, and M. Tulinius. Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayre Syndrome. *Pediatric Research*, 28:131–136, 1990.

[17] D. R. Marchington, V. Macaulay, G. M. Hartshorne, D. Barlow, and J. Poulton. Evidence from human oocytes for a genetic bottleneck in an mtDNA disease. *American Journal of Human Genetics*, 63:769–775, 1998.

[18] C. T. Moraes and E. A. Schon. Replication of a heteroplasmic population of normal and partially-deleted human mitochondrial genomes. In F. Palmieri, S. Papa, C. Saccone, and M. N. Gadaleta, editors, *Progress in Cell Research: Symposium on "Thirty Years of Progress in Mitochondrial Bioenergetics and Molecular Biology"*, volume 5, pages 209–215. Elsevier, 1995.

[19] P. Olofsson and C. A. Shaw. Exact sampling formulas for multi-type Galton-Watson processes. *Journal of Mathematical Biology*, 45:279–293, 2002.

[20] L. Quintana-Murci, A. Rötig, A. Munnich, P. Rustin, and T. Bourgeron. Mitochondrial DNA inheritance in patients with deleted mtDNA. *Journal of Medical Genetics*, 38:e28, 2001.

[21] P. Reynier, M-F. Chrétien, F. Savagner, G. Larcher, V. Rohmer, P. Barriére, and Y. Mathiéry. Long PCR analysis of human gamete mtDNA suggests defective mitochondrial maintenance in spermatozoa and supports the bottleneck theory for oocytes. *Biochemical and Biophysical Research Communications*, 252:373–377, 1998.

[22] S. Shanske, C. T. Moraes, A. Lombes, A. F. Miranda, E. Bonilla, P. Lewis, M. A. Whelan, C. A. Ellsworth, and S. DiMauro. Widespread tissue distribution of mitochondrial DNA deletions in Kearns-Sayre syndrome. *Neurology*, 40:24–28, 1990.

[23] R. Shenkar, W. C. Navidi, S. Tavaré, M. H. Dang, A. Chomyn, G. Attardi, G. Cortopassi, and N. Arnheim. The mutation rate of the human mtDNA deletion $mtDNA^{4977}$. *American Journal of Human Genetics*, 59:772–780, 1996.

[24] E. A. Shoubridge. Mitochondrial DNA segregation in the developing embryo. *Human Reproduction*, 15(Suppl. 2):229–234, 2000.

[25] N-W. Soong, D. R. Hinton, G. Cortopassi, and N. Arnheim. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. *Nature Genetics*, 2:318–323, 1992.

[26] D. C. Wallace. Mitochondrial DNA sequence variation in human evolution and disease. *Proceedings of the National Academy of Sciences USA*, 91:8739–8746, 1994.

[27] T-C. Yen, J-H. Sue, K-L. King, and Y-H. Wei. Aging associated 5 kb deletion in human liver mitochondrial DNA. *Biochemical and Biophysical Research Communications*, 178:124–131, 1991.

[28] C. Zhang, A. Baumer, R. J. Maxwelland A. W. Linnane, and P. Nagley. Multiple mtDNA deletions in an elderly individual. *FEBS Letters*, 297:34–38, 1992.