

Transmission of the human mitochondrial genome

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The segregation and transmission of mitochondrial genomes in humans are complicated processes, but are particularly important for understanding the inheritance and clinical abnormalities of mitochondrial disorders. This review describes three aspects of mitochondrial genetics. First, that the segregation and transmission of mitochondrial (mt)DNA molecules are likely to be determined by their physical association within the organelles and by the dynamics of mitochondrial structure and subcellular organization. Second, that the transmission of heteroplasmic mtDNA sequence changes from one generation to the next often involves rapid shifts in allele frequency. For >20 years, the standard explanation has been that there is a developmental bottleneck in which, at some stage of oogenesis, there is a reduction in the effective number of mitochondrial units of inheritance. The third aspect is that ongoing analyses of the segregation and transmission of pathogenic mtDNA mutations indicate the operation of multiple genetic processes. Thus, the segregation and transmission of mtDNA mutations occurs predominantly, but not exclusively, under conditions of random

genetic drift. However, there is also evidence for bias due to incomplete ascertainment of pedigrees and for negative selection of pathogenic mutations in rapidly dividing somatic tissues such as the white blood cell population.

Key words: mitochondrial bottleneck/ mitochondrial disease/mitochondrial DNA/ mtDNA heteroplasmy/mtDNA mutations

Introduction

The human mitochondrial genome (mtDNA) is a small 16 569 bp DNA circle that encodes 2 rRNA, 22 tRNA, and 13 protein subunits of the respiratory chain complexes (reviewed in Howell, 1999). Mutations in the mtDNA cause a number of clinical disorders, and >200 different pathogenic mutations (single base pair substitutions and deletions as well as other rearrangements) have been identified (Chinnery *et al.*, 1999a; Howell 1999).

We are coming to realize that mitochondrial disorders are not rare, but that they are much more prevalent than we initially had thought and that they constitute a major public health risk (Majamaa *et al.*, 1998). However, mitochondrial disorders show marked clinical

heterogeneity and particularly complex genetics, and these two basic properties continue to hamper diagnosis and treatment. The purpose of this review is to summarize some of the current work on the transmission and segregation of mitochondrial genes, and to highlight those unsolved problems that will be the subject of future investigation.

Topological organization of mtDNA molecules

The genetics of mtDNA are markedly different from those of the nuclear chromosomes. In the first place, mtDNA is inherited exclusively, or almost so, from the mother; there is continuing debate over whether a small number of paternal mtDNA molecules can leak through into the zygote (for review, see Howell, 1999). This maternal mode of mtDNA inheritance has important ramifications for its genetics and evolution. As a general rule, mtDNA is considered to be an asexual, haploid genetic system in which mutations arise and are transmitted as clonal, maternal lineages; however, there have been other recent and controversial findings (Hagelberg *et al.*, 1999; Eyre-Walker *et al.*, 1999).

A second feature of mtDNA genetics is that there are multiple mtDNA molecules per cell. The numbers vary considerably, but mononuclear somatic cells typically contain on the order of 1000–5000 copies, whereas there are ~100 000 or more mtDNA molecules in the mature oocyte (R.P.S.Jansen, personal communication). If all of the mtDNA molecules in a cell are identical at the nucleotide sequence level, this condition is termed homoplasmy. In contrast, when there are two or more mtDNA sequence variants, the cell (or tissue, or organ, or organism) is said to show heteroplasmy. It is important to note that, although the same general terms (homoplasmy or heteroplasmy) can be applied to cells, tissues, organs or individuals, one must be

cautious about extrapolating tissue data, for instance, to the cellular level. For example, the white blood cell (WBC)/platelet fraction of whole blood, the most commonly used source of mtDNA for analysis of heteroplasmy, contains multiple cell populations. Furthermore, if one observes that WBC are heteroplasmic for a mtDNA sequence change, such a finding can mean either that the individual WBC are heteroplasmic or that the WBC are composed of both homoplasmic mutant and homoplasmic wild-type cells (a point discussed further below).

An issue of fundamental importance, our understanding of which is rudimentary at best, is how the number of mtDNA molecules is related to the number of units of mitochondrial segregation and transmission from one generation to the next. The available information indicates that the relationship is highly complex and dynamic, both spatially and temporally, because mtDNA molecules are topologically organized in several ways within the cell (reviewed in Howell, 1999). The available evidence strongly points against random and independent free mixing of mtDNA molecules within a cell (i.e. against there being a panmictic pool). From the first molecular studies of mammalian mtDNA, it was recognized that these genomes are packaged in somatic cells as clusters, rosettes or nucleoids in which 2–10 molecules are attached as a group to the inner mitochondrial membrane. Nucleoids cannot be static entities or there would be no way for mutations to spread through the mitochondrial gene pool. Whether this process involves exchange of mtDNA molecules between nucleoids and/or preferential replication or partitioning of mtDNA molecules is not known. In this regard, it has been concluded (Davis and Clayton 1996) that there is preferential replication of those mtDNA molecules that are in the perinuclear region of the cell, and that these molecules then migrate to the cell's periphery (see also

Meirelles and Smith, 1998). One important question that remains unanswered is whether the same mtDNA molecules are preferentially replicated throughout the entire cell cycle (or even in successive cell generations) or whether replication, though favoured by position, otherwise selects mtDNA molecules randomly.

Although mtDNA molecules usually exist as simple unicircular monomers, they can take a second and more complex level of organization through the formation of unicircular multimers and catenanes. Whether these complex forms are in some sort of dynamic equilibrium or whether they represent genetic 'dead-ends' is also not known, although evidence for the former possibility has recently been reported (Holt *et al.*, 1997). At a third level of organization, we must also factor in the dynamics of the organelles themselves. Fusion and fission of mitochondria is well documented (Bereiter-Hahn, 1990) and the mitochondria frequently take the form of a reticulum or network associated with microtubules or other components of the cytoskeleton (for review, see Yaffe, 1999).

The mitochondrial bottleneck: 'a mitochondrial bottleneck' or 'mitochondrial bottlenecks'?

In an article that was remarkably prescient on several points (Upholt and Dawid, 1977), a paradox was noted that has had far-reaching consequences for the analysis of mitochondrial genetics. On the basis of restriction site and heteroduplex mapping of the mtDNA D-loop in sheep and goats, it was concluded that the rate of sequence divergence within the D-loop was high but that individual animals were ordinarily homoplasmic for a single sequence variant. To explain the paucity of heteroplasmic individuals, they suggested that the effective number of mitochondrial units of inheritance was much smaller at an early stage

of oogenesis than the large number of mtDNA molecules in the mature oocyte. These observations were extended (Hauswirth and Laipis, 1982, 1985), by the observation of rapid shifts in mtDNA genotype within Holstein cow lineages. These investigators postulated that the rapid shifts were due to a developmental bottleneck in the number of mtDNA molecules transmitted to progeny, and that this effective bottleneck was the cumulative product of three different processes (Hauswirth and Laipis, 1985). Firstly, the number of mtDNA molecules per organelle drops to one or two during oogenesis (and persists until after the oocyte is fertilized), with later mtDNA repopulation within the organelle as somatic tissues form in the embryo (see especially Jansen and De Boer, 1998). Secondly, there is cytoplasmic partitioning of mitochondria among daughter cells during early embryogenesis. Thirdly, there is cellular segregation during later developmental stages of embryogenesis (embryonic partitioning). Thus, the term 'bottleneck', from the very earliest studies, has had specific mechanistic connotations that makes its current usage problematic, and still in need of experimental confirmation. It should be kept in mind that there may be multiple processes (occurring before, during and after fertilization) that determine the shift in mtDNA allele frequencies from that of a blood sample in one generation to a blood sample in the next. Furthermore, few investigators have been explicit about the mechanism(s) that they believe determine the shifts in allele frequency during transmission.

More recent studies have investigated shifts in mtDNA allele frequencies in human pedigrees. We identified a small matrilineal pedigree in which the members were heteroplasmic for a silent polymorphism at nucleotide (nt) 14560 (Howell *et al.*, 1992). In contrast to the animal studies, in which rapid shifts in allele frequency were consistently observed, there was relatively little shift in

allele frequencies in this pedigree. Using the Wright–Solignac equation, we estimated the number of mitochondrial transmission units to be in the range of 36–180. This method uses the heteroplasmy ratio of the mother and the variance of these ratios for her first generation offspring to calculate the number of units at the bottleneck stage. Unfortunately, this approach has several serious limitations, including the fact that small pedigrees will have high sampling errors. In addition, one must make some assumption about g , the number of generations during which the population is going through the bottleneck. Thus, the range of units for the heteroplasmic nt14560 pedigree places the number of germ line generations between 10 and 50 (Howell *et al.*, 1992). Our subsequent studies of additional pedigrees that are heteroplasmic for a pathogenic mtDNA mutation have yielded examples within the same pedigree of both slow and rapid shifts in mutation load (e.g. Ghosh *et al.*, 1996; Howell *et al.*, 1996).

These cumulative pedigree data suggest to us that there is neither a precise bottleneck in humans that involves reduction to a very small number of units of mitochondrial transmission (say one or two), nor a precise maintenance of a very large number of units. No firm conclusion can yet be drawn as to the basis for the ‘mixed bag’ of both rapid and slow shifts of mtDNA allele frequencies, in large part because of the high (but not ascertainable) sampling error associated with small pedigrees. Thus, the pedigree results can indicate either that the number of mtDNA transmission units varies or that the number of units is precisely at some mid-range value (say, 10–20). It must be remembered, however, that mitochondrial genetic systems are ‘relaxed’ rather than ‘stringent’ (in the terminology of Birky, 1994), and some variability in the ‘size’ of the bottleneck seems likely. Such variability could arise

from fluctuations in the number and/or the topological organization of mtDNA molecules, or in the number of primordial germ cell divisions (see below).

More recently, a second method of estimation based on Bayes’ theorem has been developed (Bendall *et al.*, 1996). This approach has been used to estimate bottleneck size using the levels of rearranged mtDNA molecules in the mother’s ovary and in her individual oocytes (Marchington *et al.*, 1998). The most probable number of transmission units ranged between 1 and >100, but most of the values were between 6 and 21 (Marchington *et al.*, 1998). One limitation to the Bayesian approach is that it assumes the bottlenecking process is a single-step sampling, which does not seem biologically realistic (see below on the experiments of Jenuth *et al.*, 1996). Marchington *et al.* (1998) estimate that their bottleneck sizes are smaller than those obtained with the Wright–Solignac equation by a factor of 23.

Recent efforts to more directly investigate the transmission and segregation of mtDNA molecules, particularly during oogenesis, have used artificially constructed heteroplasmic mice (Lightowlers *et al.*, 1997; Howell, 1999). Thus, it was concluded that the predominant process that determines the variance in allele frequencies is random genetic drift during the divisions of the primordial germ cells as they give rise to early stage oocytes (Jenuth *et al.*, 1996). They further estimated, using the Wright–Solignac equation, that the number of transmission units was ~200 (using a value for g of 15 divisions), which was also their estimate of the number of mtDNA molecules in the primordial germ cells. It has been claimed (Jenuth *et al.*, 1996) that the shifts in mtDNA allele frequencies do not occur during the reduction in mitochondrial (and mtDNA) copy number that occurs during the divisions that separate the zygote from the generation of new primordial germ cells, at about the

time of gastrulation (Jansen and De Boer, 1998). Rather, it is during the subsequent divisions of the primordial germ cells that the smallest mtDNA copy numbers occur. A similar experimental approach has been used (Meirelles and Smith, 1997), showing rapid segregation to the homoplasmic state in some mouse lineages, but slow segregation in others. Furthermore, and in seeming contradiction to the results of Jenuth *et al.* (1996), Meirelles and Smith (1997) also observed extensive tissue variation in allele frequencies, which suggests a contribution from post-zygotic mtDNA segregation to the allele shifts that occur between generations.

Although these various studies have contributed to our understanding of the inheritance of mtDNA mutations, we do not yet have an unassailable model. Considering all of the accompanying interpretive baggage, we should be cautious in our use of the term bottleneck until the mechanisms of mtDNA transmission and segregation have been further clarified. Ultimately, the most important reason for investigating these mechanisms is to improve the diagnosis and counselling of mitochondrial diseases and to develop therapeutic strategies. Therefore it is important to consider not only mtDNA segregation during the developmental bottleneck, but also the genetic processes that occur after development, because it is the combined effect of all processes that underlie expression of the clinical abnormalities. Furthermore, pathogenic mutations almost certainly behave differently than do neutral polymorphisms (see below for an example), and we do not currently have an animal model system that carries a pathogenic mtDNA mutation. For these reasons, it is important to obtain as much information as possible on the segregation and transmission of pathogenic mtDNA mutations in human pedigrees.

Segregation and transmission of pathogenic mtDNA point mutations

8344 MERRF and 3243 MELAS mutations

The hallmark of the mitochondrial encephalomyopathies is their marked clinical variability (Chinnery *et al.*, 1999a; Howell, 1999). Among this group of mitochondrial diseases, two of the most prevalent, as well as the most extensively analysed, are MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) and MERRF (myoclonus with epilepsy and ragged-red fibres). These two disorders are most often caused by mutations at nt3243 (tRNA^{LEU} gene) and at nt8344 (tRNA^{LYS} gene) respectively. The MELAS and MERRF mutations are invariably heteroplasmic (homoplasmy presumably being fatal), and it is likely that not just the degree, but also the cellular and tissue distribution, of heteroplasmy contributes to the clinical variability.

One of our analytical approaches to these disorders has been to carry out a large-scale retrospective analysis of the published MERRF and MELAS pedigrees. Although these studies are still in progress, some of our findings have been published (Chinnery *et al.*, 1997, 1998). One of the first results to emerge was that MELAS and MERRF are associated with an overlapping set of clinical abnormalities (Chinnery *et al.*, 1997). For most of these abnormalities, there was a clear relationship for both disorders between the frequency of expression and the mutation load in muscle (a post-mitotic tissue). However, there was no such relationship between clinical expression and MELAS mutation load in WBC and platelets. No clear trend was observed for the MERRF blood mutation load, but the number of data points is still too low to draw firm conclusions.

It has been observed that there is a lower mutation load in blood than in muscle for both MELAS and MERRF pedigree family members; this discrepancy occurs in 90 and 91% respectively of these individuals (Chinnery *et al.*, 1999b). The most likely explanation is that negative selection operates in the haematopoietic stem cell population (see below for a further discussion of this phenomenon), but not in the post-mitotic, syncytial muscle tissue. Furthermore, we have analysed five members of a MELAS family in whom the mutation load showed the following trend, from highest to lowest mutation load: muscle, hair follicles, buccal mucosa, and blood. These results indicate the presence of tissue-specific segregation patterns, and these are probably related to the rate at which these tissues turn over at the stem cell level (Chinnery *et al.*, 1999b). Tissue-specific segregation has also been observed in mice (Jenuth *et al.*, 1997), although in this case the mtDNA sequence basis for the selection pressures was not known.

If both MELAS and MERRF mutations occur in tRNA genes, and if both deleteriously affect mitochondrial biogenesis, then why are there significant differences in the pattern of clinical abnormalities? One possibility is that there are subtle differences in the regulation of organelle biogenesis among different tissues, such that these two mutations show tissue-specific differences in their phenotypes. The answer to this question is unlikely to be a simple one, however, and multiple processes could be involved.

We also observed that there was a relationship between blood mutation load in mothers and the frequency of affected offspring, although there was considerable scatter in the data (Chinnery *et al.*, 1998). On one hand, such a relationship might seem intuitively obvious. On the other hand, it suggests that rapid shifts in transmission due to the developmental bottleneck are not so prevalent as to

distort mtDNA transmission (see the further discussion of this point in the next section). A second result to emerge from these transmission analyses was that, for any one maternal mutation load, there was a higher frequency of affected offspring for the MELAS mutation than for the MERRF mutation. This trend suggests that the expression threshold for the former is lower, a suggestion that was also supported by the relationships in patients between muscle mutation load and expression of clinical abnormalities (see Figure 2 of Chinnery *et al.*, 1997).

LHON mutations

Leber hereditary optic neuropathy (LHON) is another mitochondrial disease (reviewed in Howell, 1999). LHON is a bilateral optic neuropathy of acute or subacute onset (typically in the mid-20s) which involves loss of function (and usually degeneration) of the retinal ganglion cells that subserve central vision. Penetrance in LHON pedigrees is incomplete. Males are affected more often than females. In Northern Europe, the respective penetrance in males and females is ~50 and 10%; among pedigrees in America and Australasia, the penetrance values for LHON are reduced to at least one-half of European values. The incomplete penetrance indicates that, although a mtDNA mutation is the primary etiologic component in LHON, secondary etiologic factors are required for manifestation of the optic neuropathy. The typical type of LHON, in which the optic neuropathy is usually the sole clinical abnormality, is caused in >95% of all pedigrees by one of three mtDNA mutations (these occurring at nucleotide positions 3460, 11 778 and 14 484). The 11 778 LHON mutation is the most prevalent, and it accounts for ~50–70% of all LHON cases.

The transmission of the 11 778 LHON mutation was analysed in pedigrees in which

one or more family members was heteroplasmic in WBC (Smith *et al.*, 1993). They observed that the mutation load increased in successive generations among this pooled dataset. Thus, the frequency of homoplasmic mutant family members increased from 0 to 35 to 74 to 94% through four generations. The number of family members was quite small (for example, only two individuals were included in the data for the first generation), but the trend was striking nonetheless. Smith *et al.* (1993) concluded that there was preferential replication or 'survivability' (their term) of the mtDNA molecules that carried the 11 778 mutation. In other words, these investigators reached the view that the 11 778 LHON mutation is transmitted from one generation to the next under conditions of positive selection.

We have recently extended the analysis of heteroplasmic 11 778 LHON pedigrees (P.F.Chinnery *et al.*, unpublished observations). One conclusion from this study was that the frequency of blindness in males was related to mutation load (see also above for our studies on MERRF and MELAS). Thus, if the mutation load was at least 80%, 44% of the male offspring lost vision: a penetrance essentially the same as that for the homoplasmic mutant male family members. In contrast, none of the males with mutation loads of $\leq 60\%$ or less lost vision. These results are in close agreement with those of Smith *et al.* (1993).

A second finding was that mothers with higher LHON mutation loads in blood tended to have a higher proportion of affected male offspring, but this trend was not statistically significant. This result is in contrast to the significant relationships obtained for MELAS and MERRF (see above). A likely contributing factor is that the penetrance of LHON in males is $<50\%$, so one is dealing with a truncated range of possible values. However, one is still left with a substantial number of affected males (and therefore of individuals with high

mutation loads) who are born to mothers with low mutation loads. A plausible explanation for this phenomenon is given below.

The third result was that the mean mutation loads of offspring were slightly greater than those in the mothers, but (and here the results contradict those of Smith *et al.*, 1993) the difference was not statistically significant. Thus, the frequency distribution of the offspring-mother differences was essentially symmetrical (but with a slight positive skew) and two-thirds of the offspring had a mutation load that was within 25% that of the mother. The slight positive skew arises from the fact that $\sim 60\%$ of the offspring had mutation loads greater than their mothers, whereas $\sim 40\%$ had lower loads.

Our interpretation of these data is that, rather than positive selection, the segregation and transmission of the pathogenic 11 778 LHON mutation are determined predominately by random genetic drift so that there is no change in mean mutation load in successive generations (P.F.Chinnery *et al.*, unpublished observations). The shifts in mutation load among individual family members, positive or negative, that occur during the transmission from one generation to the next result primarily from the drift that occurs during the developmental bottleneck (see previous section). As one consequence of the rapid drift between generations, a proportion of mothers with low mutation load will give rise to offspring with high mutation loads, thereby providing another part of the explanation for the absence of a clear relationship between maternal mutation load and the frequency of affected sons.

There is another point of interest in these results. As noted above, when a relatively large number of mother-offspring transmissions is analysed, there is $<25\%$ difference in mutation load in most instances. This result suggests that, on a population level, the effects of the bottleneck (that is, the effects of random genetic drift) are less marked than those

obtained with small pedigrees (see above), perhaps because the sampling error has been reduced.

In addition to random genetic drift, these results (and those of Smith *et al.*, 1993) suggest that there is a second process in operation. Although the difference was not statistically significant, we did note a slight increase in mutation load from one generation to the next. If only random genetic drift were operating then there should be no generational difference in mean mutation loads. As an explanation, we suggest that the analysis of these LHON pedigrees has been biased by incomplete ascertainment. The relatively high mutation load required for expression of the optic neuropathy (75–80%) means that we have only studied those pedigrees that contain at least one male in which the LHON mutation has segregated to high levels. We are unable to detect (because they are too rare to be recovered in population screenings) pedigrees that carry a LHON mutation but with no affected individuals. Turning this logic around, if LHON mutations are transmitted under conditions of random genetic drift, then there must be numerous pedigrees in the population in which a LHON mutation has arisen but in which it has been lost during transmission.

Longitudinal analysis: a difference between neutral polymorphisms and pathogenic mutations

In addition to the cross-sectional pedigree analysis of the LHON mutations, we have begun a longitudinal analysis (Ghosh *et al.*, 1996; Howell *et al.*, 2000). We have already described above the small matrilineal pedigree whose members were heteroplasmic for a silent polymorphism at nt14560 (Howell *et al.*, 1992). The original blood samples were obtained in June 1990 and additional samples were obtained from four family members in September 1997. Using primer extension

assays, we determined that there was no change in the allele ratios over a period of 7 years for any of these individuals. For example, the level of the polymorphic allele in family member II-3 was 62.5% in the 1990 sample and 63.5% in the 1997 sample. Thus, as expected, this silent polymorphism does not appear to be subject to selection in the WBC/platelet cell populations.

A different pattern of results, however, was obtained from our longitudinal analysis of a LHON family whose members are heteroplasmic for the 3460 mutation (Ghosh *et al.*, 1996). We have analysed eight family members, again using primer extension assays, at multiple time points for cumulative time spans of 5–6 years (Howell *et al.*, 2000). Two family members were homoplasmic for the 3460 mutant allele, whereas another family member was homoplasmic for the wild-type allele. In these three family members there was no change in mutation load. In addition, there were five family members heteroplasmic for the 3460 LHON mutation and who we have also analysed longitudinally. In all five there has been a slow, but statistically significant, decrease in mutation load over time, roughly 1%/year. For example, the mutation load in June 1991 for family member IV-2 was 32.1%, but the load had decreased to 26.1% by September 1997. Because of the slow rate of decline, a number of experiments have been carried out to eliminate experimental artefacts. The silent polymorphism at nt14560 showed no change over this time.

Our working hypothesis is that the 3460 LHON mutation segregates under conditions of negative selection in WBC/platelet cell populations. Clearly, this approach must be extended to other pathogenic mtDNA mutations, including other LHON mutations. It is relevant to note, however, that there is evidence (including data from longitudinal analysis) that the 3243 MELAS mutation also declines with time in WBC ('t Hart *et al.*,

1996; Sue *et al.*, 1998; and see also the previous section on MELAS and MERRF mutations). A general trend is emerging in which pathogenic mutations show segregational loss in WBC/platelets, but not in muscle, even under conditions where the mutation load is relatively low. It has been suggested (Sue *et al.*, 1998; Howell *et al.*, 2000) that, during development, heteroplasmic early stage zygotes give rise to homoplasmic haematopoietic stem cell populations. Thus, in the 3460 LHON family analysed, the heteroplasmic individuals carry two populations of stem cells, those that are homoplasmic for the 3460 mutant allele and those that are homoplasmic for the wild-type allele at this nucleotide. However, it is also possible that there is a population of heteroplasmic stem cells, and this is a question for further analysis. Because the 3460 mutation demonstrably compromises mitochondrial respiratory chain function (Howell, 1999), the homoplasmic mutant stem cells presumably divide more slowly and there is thus a slow decrease in the mutation load over time. Our hypothesis is supported by earlier work (Kobayashi *et al.*, 1994) showing that the leukocytes from a heteroplasmic 11 778 LHON patient were largely homoplasmic, and both homoplasmic wild-type and homoplasmic mutant cells were observed. The small proportion of heteroplasmic WBC suggests the presence of heteroplasmic haematopoietic stem cells. The failure to detect negative selection in the transmission analyses (see above) probably means that the effects of random genetic drift, and of the bias due to incomplete ascertainment, overwhelm the effects of weak negative selection, which can only be detected in the longitudinal analysis.

Why is there a mtDNA bottleneck?

A major role for random genetic drift in the segregation and transmission of the mitochon-

drial genome has profound implications for its evolution and population genetics. The human mitochondrial genome, because of its maternal inheritance and high rate of mutation, should be susceptible to Muller's ratchet (Howell, 1996; Loewe and Scherer, 1997; Lynch, 1997). Muller (Muller, 1964) postulated that asexual (no recombination) genetic systems with high mutation rates should become extinct because they accumulate deleterious mutations. However, as a consequence of drift, only a fraction of newly arisen mtDNA mutations will become fixed (that is, become homoplasmic) at the level of a female's germ line, although the rate at which those mutations is fixed is more rapid (Howell *et al.*, 1996; Howell, 1999). Thus the human mtDNA bottleneck is one way to slow Muller's ratchet (see Howell, 1996, for some others), by providing a mechanism to remove deleterious mutations that arise, or are present, in the zygote before they become fixed in the subsequent population of oocytes (Bergstrom and Pritchard, 1998). There might even be a benefit to the population as a whole if those deleterious mutations that slip through the bottleneck are rapidly fixed and, through markedly reduced fitness, a stop is thereby put to the mutant mtDNA lineage (Gabriel *et al.*, 1993).

The place of random genetic drift in the theory of evolution has been the subject of extensive debate (Rosenberg, 1994). From one perspective, drift appears to be a non-evolutionary process by which there are changes in allele frequencies, but not necessarily '... in the direction of greater adaptation to local conditions' (Rosenberg, 1994, p. 76). In view of the discussion above, the developmental bottleneck may perhaps be viewed instead as a counter-revolutionary process by which to avoid the drastic evolutionary consequences of Muller's ratchet.

There is one final question that can be posed, however. Is the developmental bottleneck a passive phenomenon, i.e. a secondary by-

product of oocyte differentiation? Alternatively, is the bottleneck an active process that has been the product of selection, because of its beneficial role in compensating for the high rate of deleterious mtDNA mutations (Jansen and De Boer, 1998)? Perhaps the human mitochondrial genetic system is an unusual situation where random drift turns out to be an evolutionary process!

From the microscopic (organelle dynamics) to the macroscopic (population genetics and dynamics), the segregation and transmission of human mtDNA molecules are complicated processes into which we are just beginning to gain some insights.

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