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## ► To cite this version:

Anne Atlan, Catherine Boyen, Laurence D Hurst. HOW TO LIVE WITHOUT SEX: THE ANSWER OF CYTOPLASMIC GENOMES. 2018. hal-01957601

**HAL Id: hal-01957601**

**<https://hal.science/hal-01957601>**

Preprint submitted on 17 Dec 2018

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# **HOW TO LIVE WITHOUT SEX: THE ANSWER OF CYTOPLASMIC GENOMES**

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**Unpublished manuscript, 2000**

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## **Abstract**

Uniparentally transmitted organelles are the best described anciently asexual genome and their analysis permits tests of models of the maintenance and long term consequences of sex. The genome size, copy number, substitution rate and topology of the organelle genomes of several different lineages are reviewed. These parameters appear not to be independent. Genome size is negatively correlated with substitution rate, copy number is positively correlated with substitution rate and genome size is negatively correlated with copy number. We note that this non-independence is consistent with the notion that for an asexual genome to persist, mutation accumulation, as envisaged for example in Muller's ratchet, must be slowed down. Cases where mutations do accumulate are also considered. Other mechanisms such as paternal contribution, the mode of transmission during cell division and RNA editing may also preserve the functionality of these genomes. We show that mutational meltdown is a problem for genomes with uniparental inheritance, and that they develop different strategies to stay fit.

Key words: organelle, sex evolution, mutation, Muller's ratchet

## Introduction

The prevalence of sex is an enigma for evolutionary biologists. Because sexual reproduction is costly compared to asexual reproduction (Maynard-Smith, 1978), it is a challenge to understand why most extant species are sexual. The debate around this problem has generated over 20 different hypothesis (Kondrashov, 1993). Indeed, there are so many good reasons why organisms ought to have sex (Hurst and Peck, 1996), it is considered that nothing could survive for a long time without sex. This led Maynard Smith (1986) to describe as an "evolutionary scandal" the persistence of asexuality in Bdelloid rotifers, a whole taxon in which no male has ever been observed.

In the long term, asexual species are expected to go extinct because of the accumulation of deleterious mutations. Muller's ratchet (Muller, 1964) is one formulation of this mutational meltdown. It is envisaged that in an asexual population the number of slightly deleterious mutations can only increase. Selection may remove the individuals with the lowest fitness but if the deleterious effect of a single mutation is relatively weak then selection will be unable to remove it. Over time the net accumulation of slightly deleterious mutations will have a large effect leading to the extinction of the species. By allowing recombination between two different genomes, sexuality permits the creation of individuals with fewer mutations than either of the parents and hence mutational meltdown is not inevitable. In a genome with clonal inheritance, however, weakly deleterious mutations progressively accumulate in an irreversible manner, just as the turn of a ratchet is irreversible.

For these reasons, non recombining genomes are often thought to be an evolutionary dead-end. In sexual species, nuclear genomes recombine regularly during meiosis. Similarly, prokaryotes, though not having sex in a conventional sense, do undergo related parasexual processes in which DNA from other cells is incorporated. Even viruses have the capacity to recombine with other viruses as well as with their host. In fact, very few species have been shown to be obligately clonal (Judson and Normark, 1996.). The rarity of such species ensures that they can be viewed as evolutionary curiosities. One type of asexual genome is however so widespread that its case cannot simply be so easily dismissed. These genomes are the uniparentally inherited cytoplasmic genomes of eukaryotes. As most cytoplasmic genomes have uniparental inheritance they do not have the opportunity to recombine with the genome of a different cell. As pointed out by Gabriel *et al.* (1993), if Muller's ratchet is really a problem for asexuals, the DNA from organelles should suffer from its effect. This raises two questions: is mutational meltdown a problem for these genomes and if so how do they manage to avoid the accumulation of deleterious mutations?

With the aim of answering these questions we review the structure and properties of the genomes of different types of mitochondria and chloroplasts. The parameters that we consider are the genome structure, copy number, and rate of substitution. We then argue that these parameters appear not be independent, and that the link between them may slow down the ratchet. Nevertheless, in some cases deleterious mutations do accumulate. Other mechanisms such as paternal contribution, the mode of transmission and RNA editing may also preserve the functionality of these genomes.

## Molecular and Structural Properties of Cytoplasmic Genomes

There exist a number of similarities between genomes of all organelles. Nearly all are circular DNA molecules (or at least have circular maps) and exist in multiple copies. Their endosymbiotic origin is now widely accepted (Gray, 1993); and phylogenetic analysis demonstrates that mitochondria are related to alpha-proteobacteria, while chloroplasts are

related to cyanobacteria (Loiseaux-de Goër, 1994). Contrary to what was previously thought, it is now thought that all mitochondria derive from the same endosymbiotic event (Leblanc *et al.*, 1997; Gray *et al.*, 1999). Genetic transfer to the nucleus has strongly reduced the size of the mitochondrial genome, with the smallest mitochondrial genome being observed within parasitic organisms. Because this implies that constraints on parasites' cytoplasmic genomes are relaxed, we will not incorporate these organelles in this review.

An increasing set of data on organelles from various independent eukaryotic lineages show that genome size, structure, organisation, and substitution rate may be very different, even among closely related taxa (Leblanc *et al.*, 1997). On the other hand, some similarities are striking e.g. the curious structural resemblance between the mitochondrial genome of oomycetes and the chloroplast genome of angiosperms. The amount of data available on the different lineages is, however, variable. Among animals, much more is known about the genomes of mammals than about those of ectotherms and invertebrates. Among plants, angiosperm cytoplasmic genomes have been extensively studied, but almost nothing is known about those of gymnosperms. Fungi and oomycetes are also well studied, but our knowledge of other lineages is very recent and limited to few species. Table 1 summarises the properties of the best known cytoplasmic genomes.

**Table 1:** Some properties of the best studied cytoplasmic genomes. Data for the number of copies come from concordant estimations given by specialists of each lineages. All other data come from references listed in the text. For the rate of point substitution, we arbitrary choose to give the value of 1 for the nucleus (which taking current averages roughly corresponds to 2 to 3 substitutions per site and per billion years, although no doubt there is variation between eukaryotes).

	Genome Size (in Kb)	presence of repeated sequences	Rate of point substitution compared to the nucleus	Frequency of rearrangements	Number of copies per cell (order of magnitude)
Mammals <sup>a</sup> mitochondria	16 - 23	none	5 - 10	-	10 <sup>3</sup> -10 <sup>4</sup>
Angiosperms <sup>b</sup> mitochondria	200 - 2500	many	0.1	+++	100-300
Plant <sup>c</sup> chloroplasts	120 - 217	0 or 1 inverted	1 - 2	+	10 - 10 <sup>4</sup>
Oomycetes <sup>d</sup> mitochondria	30 - 80	0 or 1 inverted	1 - 2	+	10 <sup>2</sup> - 10 <sup>3</sup>
Yeasts <sup>e</sup> mitochondria	18 - 85	many	1 - 2	++	10 - 40

<sup>a</sup>Up to 10<sup>5</sup> copies of the genome may be found in the oocytes. <sup>b</sup> The number of copies given here corresponds to copies of the "master circle", that is the molecule, often virtual, that contains at least one copy of each gene. <sup>c</sup>The estimation of the number of copies is highly variable depending on the tissue. It may vary from few dozen in the roots to hundred thousands in the green leafs. The number in the female gametes is unknown. <sup>d</sup>The number of copies is variable depending on the species. <sup>cd</sup>the substitution rate is smaller in the inverted repeat than in the single copy region. <sup>e</sup>yeasts are the only organisms present in this table that exhibit biparental inheritance of mitochondria.

## Genome structure

**Animal mitochondria:** Mammalian mitochondria have small genomes with minimal variability in genome size. They vary from 16 Kb for the smallest to 18 Kb for the largest. Furthermore, this genome is remarkably compact (Grivell, 1989; Boore, 1999). There are no duplicated sequences and non-translated 5' and 3' sequences are virtually absent. For instance, in humans, no more than 87 of the 16,569 base pairs can be regarded as lying in intercistronic regions. Five of the reading frames even lack a stop codon and terminate with either UA or U. However because mRNA must be polyadenylated prior to translation these sequences become UAA and hence a stop codon is produced without having to be coded for. This is clearly a base saving device. The same explanation can be mounted to explain the fact that some genes have an overlap between them. Often the start of one gene is the termination base of the previous gene. This compactness of the genome strongly suggests that selection on genome size has been an important force. Mammalian mitochondrial DNA does not normally exhibit recombination and gene arrangement is highly conserved (Boore, 1999).

Whilst the above appears to be also true for birds, i.e. the other homeothermic animals, it appeared that ectothermic vertebrates and invertebrates have a much larger **mt** size and size variation (Rand, 1993). Ectothermic vertebrates may have genomes over 10kB larger than that of the largest homeotherm, and some invertebrates have a genome over twice the size of homeotherms (e.g. 35 Kb for *Armadillidium vulgare* or 42 Kb *Placopecten magellanicus*). Large insertions or duplications, or large scale-variation of tandemly repeated sequences are not uncommon. Recombination through these sequences may generate heteroplasmy, as was first observed within crickets (Rand and Harisson, 1989) or scallops (Gjetvaj *et al.*, 1992), and are associated with the presence of short recombining repeats in direct orientation. However, changes in the order of the genes are rare among animal species as such are unlikely to arise independently in separate evolutionary lineages (Boore, 1999).

Fungal mitochondria have considerable variation in genome size, ranging from 18 to 174 kb (Clark-Walker, 1992). The increase in size partly results from the presence of introns and non-coding sequences. This suggest that selection for genome size is not as strong as in animal mitochondria. In fungal mitochondria, recombination is often facilitated by the presence of short directly repeated sequences, and it is noticeable that larger genomes are more prone to rearrangements than small ones (Clark-Walker, 1992). In *Saccharomyces cerevisiae* recombination has been extensively studied not least because it is involved in the generation of "petite" mutants. Recombination may be so frequent that up to 10% of petite mutants may be generated per generation (Dujon and Belcour, 1989). As a consequence, small differences in gene order may sometimes be observed within the same species.

Oomycete mitochondria and angiosperm chloroplasts have striking similarities. The size of the former varies from 30 to 80 Kb (McNabb and Klassen, 1988) while the size of the later vary from 120 to 217 Kb (Palmer and Stein, 1986). In both cases, most of the genome size variation comes from variation in the length of an inverted repeat, that may make up nearly three quarters of the genome. Apart from this variation, the order of the genes is generally conserved. When present, the inverted repeat leads to the coexistence of two isomers generated by recombination (Palmer, 1983; Boyd *et al.*, 1984). In both lineages, the inverted repeat always contain ribosomal genes, if nothing else.

Plant mitochondria have the largest genome and with it considerable genome size variation. It varies between 200 and 2500 Kb, of which a variable proportion is coding (Schuster and Brennicke, 1994). A comparison of different angiosperm species reveals that most, but not all

genome size variation is due to variation in the number of large duplications. Size variation probably often appears in evolution, since large variations were found among closely related species, and the whole range of variation was even found within the same genus, namely the Brassicaceae. This suggests an absence of selection on genome size, or at least that if such a selection exists, it is of little importance.

Angiosperm mitochondria have the highest rate of rearrangements, due to the presence of small repeated sequences with a very high rate of recombination. Recombination between these sequences is so frequent that a totally different order of the genes is often found between closely related species, within different individuals of the same species, or even within the same individual. This generation of diversity through recombination is well documented (Schuster and Brennicke, 1994.).

### **Rate of point substitution**

Animal mitochondria have an extremely high rate of substitution. In mammals, it is about ten times more than in the nucleus (Wolfe *et al.*, 1987). The substitution rate of vertebrate ectotherms, is highly variable and dependant on the species. The substitution rate is much slower, five to ten times less than in homeotherms, in some marine turtles (Avisé *et al.*, 1992), and all studied amphibians and fishes, from sharks to salmonidae (Martin and Palumbi, 1993). By contrast, the substitution rate is much higher (up to ten times higher than in homeotherms) in snakes, alligators (Kumazawa *et al.*, 1998) and the turtle *Pelomedusa subrufa* (D. Casane, pers. com.). The diversity of rates appears to covary with the thermal habits of the above species. While species of the first group do not control their temperature, species of the second group control their temperature by behaviour (warming themselves under the sun). The relationship between the rate of mitochondrial substitution and life history traits has already been studied (Martin and Palumbi, 1993; Rand, 1994), but discarded by the discovery of ectotherms with high mutation rate (*e.g.* Kumazawa *et al.*, 1998). Further analysis including these new data and taking into account the behaviour of the animals may be of great interest.

Plant mitochondria have the lowest known substitution rate among living organisms: about ten times less than the nuclear rate, that is 100 times less than mammalian mitochondria! The fact that this low substitution rate is found even in the case of synonymous mutations (Wolfe *et al.*, 1987) indicates that the low substitution rate is not the result of selection on genomes with a low mutation number but rather that, above all, the mutation rate is low. This very low substitution rate, especially when interpreted as the consequences of a low point mutation rate, suggests that strong selection exists to lower the mutation load in this genome.

Yeast and oomycetes mitochondria and angiosperm chloroplasts do not differ very much in their rate of substitution, which is slightly higher than that of the nucleus. In oomycete mitochondria (Boyd *et al.*, 1984) and in angiosperm chloroplasts (Wolfe *et al.*, 1987) the substitution rate is significantly lower in the inverted repeat than in the single copy region. As a consequence, genes located in the inverted repeats, namely those coding for ribosomal RNA, are the least variable.

Variation in substitution rates may result either from variation in the rate at which deleterious mutations accumulate, or from variation in the rate at which they are generated. Comparison between synonymous and non-synonymous mutations allows one to differentiate these two mechanisms. The existence of variation in the rate at which mutations (synonymous or not) accumulate is suggested in mammals by Lynch (1996), and in aphid endosymbionts by

Lambert and Moran (1998). These variations may come either from difference in the fidelity of replication, or from differences in the efficiency of repair.

Cytoplasmic and nuclear genomes are replicated by different specific polymerases. In vertebrate, plant and fungi, a polymerase of the gamma type is devoted to mitochondrial DNA synthesis. This polymerase is capable of proof reading activity and exhibits a high in vitro fidelity (Lecrenier, 1997). Therefore the difference in in vivo mutation rate is thought to result from differences in the post-replication repair machinery, however, a DNA polymerase of the beta type that involves a DNA repair activity has been identified. The additional presence of a beta polymerase is suspected in trypanosomid *Crithidia fasciculata* and in yeast. The variation of the replication faithfulness might be more related to the absence or deficiency of a repair system, such as the *mutS*-like gene (Pont-Kingdon *et al.*, 1995) than to the efficiency of different DNA polymerases. The involvement of a post-replication repair mechanism is also consistent with the observed link between recombination and the mutation rate.

## The relation between genome parameters and Muller's ratchet

It is clear from table 1 and from the above descriptions, than the different types of genome structure and evolution don't follow the phylogenetic relationship between the lineages. However, the different parameters are not independent: the smaller the genome, the smaller the genome size variation and the frequency of rearrangements, the higher the rate of substitution and the number of copies per cell. Animal mitochondria are at one extreme with a small genome with high substitution rate, and plant mitochondria at the other extreme, with a large genome and a low substitution rate. Plant chloroplasts, along with oomycete and fungal mitochondria, lie in between.

Let us first consider the parameters involved in Muller's formulation of the problem of the accumulation of deleterious mutations (Muller, 1964). The rate at which the deleterious mutations accumulate is dependent upon the number of genomes in the population, or population size ( $N$ ), the number of mutable loci per genome ( $l$ ) and the rate of mutation at each locus ( $\mu$ ). Only slightly deleterious mutations are taken into account, because the ratchet will not advance if the deleterious effect of each mutation ( $s$ ) is sufficiently large for the corresponding genotype to be eliminated immediately by selection. The ensemble of these parameters determine  $N_0$ , the number of genomes which have the lowest number of mutations, which at equilibrium will be given by :

$$N_0 = N \exp -(\mu l/s)$$

The smaller  $N_0$  the greater the probability that all mutation free genomes are expected to be lost by drift. Hence, as  $N_0$  increases so Muller's ratchet is expected to slow. That is, the ratchet can be slowed by either increasing  $N$ , the population size, or decreasing  $l$ , the number of loci, or decreasing  $\mu$ , the mutation rate, or increasing  $s$ , the deleterious effect of a single mutation. This prediction holds even without considering the formal expression of Muller's equation. Furthermore, other formulations of mutational meltdown, such as those involving background selection, also link to the same prediction than for asexual organisms to persist, either the population size must be high, or the mutation rate low, or the genome must have few loci. For instance, asexual protists probably avoid mutational meltdown because their nuclear genome is relatively small and their population size is very high (Bell, 1988).

As a consequence of the necessity to avoid Muller's ratchet, a negative relation may be inferred between population size and genome size, and a positive relation may be inferred between genome size and mutation rate. We can relate these parameters to the properties of cytoplasmic genomes by making the assumption that the population size is related to the number of copies per cell, that the mutation rate is related to the synonymous substitution rate,

and that the number of loci per genome is related to the genome size. Then a negative relation is expected between the number of copies per cell and genome size, and a positive relation may be inferred between genome size and substitution rate. This prediction, deduced from the functioning of Muller's ratchet, appears to be consistent with the qualitative pattern between the above parameters as described in table 1.

For animal mitochondria, and especially for mammals, the large number of copies per cell and the small genome size is sufficient to slow the ratchet, as first suggested by Bell (1888). For plant mitochondria, by contrast, the number of copies of the genome is at least one order of magnitude smaller than in animals and there is no evidence of selection favouring the smallest genome size, which is one to two orders of magnitude greater than in animals. With such a genome and such a number of copies, it is to be expected that Muller's ratchet should click very rapidly. It would seem, in this case, that the low rate of mutation of this genome (100 times smaller than in homeotherms' mitochondria) is central to the prevention of the accumulation of deleterious mutations. The fact that chloroplasts and fungal mitochondria lie in between for both genome size and mutation rate may indicate that they follow the rule, although they have a different strategy. It is also possible that the existence of an inverted repeat, by allowing recombination and reducing the rate of point substitution of the genes within the repeat, is part of this strategy. Locating genes in this inverted repeat may have been selected because it protects these genes against mutational meltdown. The fact that the genes present in the inverted repeats (rRNA genes) are essential genes known to undergo strong selective pressure, are in favour of this hypothesis. It is also possible to interpret as the result of such a selection the fact that vertebrate ectotherms with a very high substitution rate (such as snakes) have a duplicated control region with concerted evolution (Rand, 1994).

We therefore propose that the rules underpinning the molecular evolution of cytoplasmic genomes (i.e. genome size, substitution rate and number of copies per cell), is in agreement with the necessity to prevent the accumulation of deleterious mutations within a genome with uniparental inheritance. To test this hypothesis consider one organism with biparental inheritance with clear evidence of frequent recombination between parental genomes. This is the case for yeast, where we may not consider the mitochondrial genome as asexual. Yeasts do not follow the above rule in sense that they have a genome size and substitution rate comparable to that of oomycetes with uniparental inheritance, but they have an especially low number of copies per cell: only a few dozen. This supports the hypothesis that selective pressures acting on the above parameters is dependent of the necessity to avoid mutational meltdown.

This does not mean that the relation between these parameters was directly selected for the avoidance of Muller's ratchet. Selection for metabolic efficiency may also produce comparable relationship. Consider an organism where, for one reason or another (maybe a high energetic demand), there is a selection for rapid replication and transcription of the cytoplasmic genome. This may be achieved by both decreasing genome size (for faster transcription), increasing copy number (for multiple simultaneous replication or transcription events) and increasing the rapidity of the DNA polymerase. As a consequence of rapid replication, the efficiency of repair is decreased and the mutation rate goes up. This alone may explain a non-independence between genome size, mutation rate and copy number. That animal mitochondria properties seem to be related with homeothermy or thermal habits of ectotherms would support such a metabolic explanation. Further, if allocation of DNA to a given organelle is a fixed parameter then the small size and copy number would be expected to covary.

Both mechanistic and mutational decay models predict that only a limited number of parameter combinations could be successful and that alteration of one parameter will select



for a co-alteration of others. If Muller's ratchet is a long term process only those genomes with an appropriate parameterization will survive in the long term. Alternatively, mutational decay, constraints on DNA replication and adaptation to metabolic demands may be short term forces. We remain agnostic as to whether Muller's ratchet is a strong selective force promoting adaptation or a long term filter.

## **Other ways to avoid mutational meltdown**

### **Increasing the efficiency of selection between gametes**

One way to increase the efficiency of selection against deleterious mutations is to allow their grouping in the same individual cell or organelle. In an asexual lineage, this can be achieved by the mode of transmission during cell division or by the germline bottleneck that occurs in mammals. This was first examined by Takahata and Slatkin (1983), and further modelled by Bergstrom and Pritchard (1998), who show that "while a bottleneck indeed increases the rate of genetic degradation within a particular (cell) lineage, it also serves to strengthen selection among lineages, and hence has a net effect of resisting genetic decay". A different model simulating the functioning of angiosperm mitochondrial genome (Albert *et al.*, 1996) showed that selection at the mitochondrial level may prevent the fixation of deleterious mutations (in this case, deletions). Most of work on this topic is however theoretical, since experimental data on the mode of transmission of the different cytoplasmic genome are rare (Boore, 1997; Yaffe 1999).

Another way to increase the efficiency of selection is to decrease the number of surviving cells. A comparative analysis made among animals by Krakauer and Mira (1999) led them to conclude that the high level of germ-cell death in female germ lines may be considered as a "developmental solution" to avoid Muller's ratchet in mitochondria.

### **Paternal transmission**

The more obvious way to escape the ratchet is, of course, to have some level of paternal inheritance and recombination. Indeed, numerous models show that a low rate of recombination may be adequate to prevent mutational meltdown. Uniparental inheritance is not equally displayed through eukaryotic organisms. Some degree of biparental inheritance may be relatively frequent among sexual isogamous protists, where two haploid cells of equal size fuse to give a diploid. In angiosperms, maternal transmission is the rule, but there are some cases of rare paternal transmission, especially for chloroplasts (Reboud and Zeyl, 1994). In higher animals transmission is usually strictly maternal although rare paternal transmission may be found (Wallis, 1999). In gymnosperms cytoplasmic genes are usually uniparentally inherited, but paternal transmission of chloroplasts seems to be more frequent than in angiosperms (Chesnoy, 1987).

These paternal transmission events could play an important role in stopping the ratchet. It is however unclear just what level is necessary to have any effect. Furthermore, the parameter that has an importance here is the rate of paternal transmission that results in recombination between maternal and paternal mitochondria. In most lineages, this rate is almost impossible to estimate. However, we can predict that the higher the rate of paternal transmission/recombination, the lower the evolutionary constraints induced by Muller's ratchet.

## **Move of cytoplasmic genes to the nucleus**

The ancestral prokaryotic mitochondrial genome has undergone massive gene transfer from the primary symbiont to the nucleus. If the species is sexual, this results in reducing the number of genes that are not submitted to sexual recombination.

## **Post-replicative mechanisms**

In mammals, the ratchet is not totally stopped. As demonstrated by Lynch (1996), mitochondrial tRNA of mammals accumulated deleterious mutations that reduce their stability compared to their nuclear counterpart. This author, however, suggests that the functionality of the mitochondrial tRNA may not be necessarily affected, because compensatory mutations could prevent a net loss of fitness. Arguments in favour of this hypothesis came from the study of Lambert and Moran (1998). By comparing aphid endosymbionts and their closest free living bacteria, Moran found that the 16S RNA accumulate more mutations in five independently evolving symbiont lineages than in their free living relatives. Moran also found that some endosymbionts contain a very high concentration of a chaperone protein that may help stabilise mutationally decayed proteins.

RNA editing has been found in almost all lineages, although it is particularly common among angiosperms (Schuster and Brennicke, 1994) and trypanosomes (Arts and Benne, 1996). By modifying the messenger RNA to restore the ancestral sequence, it may also be viewed as a way to maintain the functionality of a genome, once a deleterious mutation has been fixed at the DNA level. The fact that editing results in reconstituting the original structure of a protein was first described in land plants (Gualberto *et al.*, 1989). In Kinetoplastida (Arts and Benne, 1996), it is even suggested that extensively edited RNA may be converted in DNA through retrotransposition. In that case, RNA editing not only allows the proteins to stay functional despite the ratchet, but would even allow the ratchet to go in reverse!

## **Conclusion**

Most current studies on cytoplasmic genome evolution are based on sequence data. This kind of data is powerful for estimating mutation and substitution rates, as well as estimating the number of loci under selection. However, cytological data on the number of copies per cell, their partitioning among organelles, and the way they segregate during cell division are necessary to understand the link between the above parameters. Furthermore, life history traits such as the rate of paternal transmission and recombination, metabolic demand, thermal habit, parasitic vs. free-living, and others may be of importance. It would also be useful to study cytoplasmic DNA of a phylogenetically broad representation.

Despite these limitations, and the imperfect correspondence between measurable data and the theoretical parametrization, it appears that molecular evolution of cytoplasmic genomes is, at the very least, enlightened by current theories of the evolution of sex. Conversely a comparative study of the structure and evolution of cytoplasmic genomes allows a better understanding of the evolutionary constraints leading to the prevalence of sex.

## **Acknowledgements**

We wish to thank Professors John Maynard Smith and Bengt Bengtsson for discussion. We also thank Professor Graham Bell for suggesting the problem.

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