Hypotheses on the extended phenotype of the mitochondrion: sex, mortality, and aging

Gordon Irlam
gordoni@gordoni.com

Los Altos, California, United States

April 10, 2024

[***Pre-publication draft – feedback solicited***]

Abstract

How did sex evolve, how is sex evolutionary stable, and why do eukaryotes appear mortal. This paper presents a mitochondrial perspective on the evolution of the eukaryotic cell that appears capable of answering these questions. Rather than viewing a mitochondrion as a passive entity taken up by an archaeal host that remains in the driving seat, mitochondria are viewed as the key force driving eukaryogenesis. The proto-mitochondrion is presumed to have manipulated its archaeal host to engage in sex in order to replicate itself in a more and more beneficial environment. This process is hypothesized to still be operating today as a result of the mitochondrion's continued production of reactive oxygen species (ROS). The specific production of ROS by the mitochondrion appears to be an intentional mechanism to cause the organism ultimately to die. Faced with mortality, if the organism wishes to pass on its nuclear genes it will typically engage in sex as a means of resetting age. Eukaryotic species that instead reproduced parthenogenetically would find themselves out-competed by sexual species due to the reassortment of genes that comes with sex. The species benefits from a shortened time between successive sexual generations which creates an increased ability to adapt to a changing environment.

Keywords: evolution, eukaryogenesis, extended phenotype, mitochondria, sex, mortality.

Introduction

This paper offers a mitochondrial perspective on the evolution of the eukaryotic cell. It is not that the nuclear genome is unimportant, but that it might be over-emphasized, and that by giving due consideration to the role of the mitochondrion a clearer picture of eukaryogenesis, sex, and mortality emerges.

Many age-related diseases appear to be caused by cellular senescence, which appears to be activated by telomeric DNA damage, which in turn appears to be caused by reactive oxygen species (ROS). The core idea of this paper is that the production of ROS by the mitochondria can be viewed as an intentional mechanism by the mitochondria to cause the individual organism to die. A shortened lifespan will reduce the mean time between successive sexual generations, and thus increase the ability of the population to adapt to a changing environment. The species benefits from this increased ability to adapt.

Syngamy is the eukaryotic process that produces one diploid cell from two haploid cells. Meiosis is the process that produces four haploid cells from two diploid cells. As used in this paper, sex refers to the combination of syngamy and meiosis. Sex can be viewed as a mechanism by which haploid cells produce new haploid cells, or alternatively the process by which diploid cells produce new diploid cells.

The existence of sex is troubling to some biologists because of the two-fold cost it imposes; that is only half of a parent's alleles get passed on to each offspring[1]. Parthenogenetic reproduction has no such constraint. All of the parent's alleles get passed on to each offspring. This raises the question of how sex might have evolved, and how it might continue to exist, when the alleles for it would seem hellbent on their own demise.

Mortality is also troubling. Evolution appears able to produce a myriad of complex organismal forms, but unable to perform the seemingly much simpler task of keeping them working. The fact that two relatively recently diverged species, such as mice and men, have such widely different lifespans suggests mortality may be deliberate. But why and how?

The phrase "the extended phenotype" was developed by Richard Dawkins to refer to phenotypic effects beyond the boundary of the organism[2]. For example, the extended phenotype of the beaver includes the dams it builds. The extended phenotype of the mitochondrion is the phenotypic effects of the mitochondrion beyond the outer mitochondrial membrane. This paper considers extended phenotypic features of the mitochondrion, and in particular a role in the evolution of sex and mortality.

The eukaryotic cell is believed to have evolved from a symbiotic relationship between an archaeon and an alphaproteobacterion[3]. Most attempts to understand the eukaryotic cell focus on the nuclear chromosomes, treating the incorporation of the mitochondrion as an energy providing afterthought. In terms of size the mitochondrial genome is small, but in terms of what it brings to the equation, a 15-fold increase in ATP[4], it is large, and thus it should have been expected to play a major role in the evolution of the eukaryotic cell. In addition, the mitochondrial genome may be small today, but historically the proto-mitochondrial genome is likely to have been much larger. Alphaproteobacterial spotted fever group Rickettsia genomes are around 1.3M base pairs. And known Asgard archaeal genomes, putative eukaryotic ancestors[5], aren't a lot larger, ranging

Hypothesis 1: Sex evolved as a means for the proto-mitochondrion to increase the fitness of its host environment.
Hypothesis 2: Sex continues to be the means by which mitochondria increase the fitness of their host environment.
Hypothesis 3: By enforcing mortality, the mitochondria force the nuclear genome to engage in frequent sexual recombination.

Table 1: Hypotheses on the extended phenotype of the mitochondrion

from 1.4-5.7M base pairs. In short, the proto-mitochondrial genome had a lot of bargaining power over the nature of the eukaryotic union.

After briefly reviewing some basic biological concepts and framing the problem, the bulk of this paper develops a series of four related hypotheses on the extended phenotype of the mitochondrion (HEPM). These hypotheses lay the foundation for, and formalize, the core idea that mitochondria causing the individual host organism to die is favored by evolution as it maximizes the ability of the species to adapt to a changing environment. These hypotheses are shown in Table 1.

Mitochondria

Mitochondria are reviewed in detail in Appendix A. Only the role of mitochondria in apoptosis and senescence is reviewed here.

Mitochondria play a key role in apoptosis[6]. The pathways leading to apoptosis all flow through the mitochondria, and the release of apoptotic inter-mitochondrial membrane proteins into the cytosol irreversibly starts the apoptotic process[7]. H_2O_2 oxidizes cardiolipin found in the inner membrane causing it to release bound cytochrome c[8]. Oxidized cardiolipin also helps open the mitochondrial permeability transition pore in the outer membrane[9, 10, 11]. Opening of the pore leads to a swelling of the matrix, rupturing the outer mitochondrial membrane, and the release of apoptotic intermembrane proteins into the cytosol including cytochrome c[12]. The reason why mitochondria play such a key role in apoptosis presently appears to be unknown.

Mitochondria also play a key role in cellular senescence, which is a state of seemingly permanent growth arrest of a cell. In cellular senescence mitochondria are enlarged, elongated, and hyper-fused[13]. Moreover they are less efficient at producing ATP, with a decrease in the mitochondrial membrane potential, and an increase in the production of reactive oxygen species (ROS)[13]. Mitochondrial ROS may cause DNA damage that leads to telomere shortening in replicative cellular senescence[13]. Senescent cells in which the mitochondria have been eliminated are capable of surviving via glycolysis[14]. Such cells remain at cell cycle arrest, but are unable to generate a senescence-associated secretory phenotype[14]. The reason why mitochondria play such an important role in senescence appears unknown.

Evolution of the eukaryotic cell

Alphaproteobacteria are a class of bacteria. The eukaryotic cell is widely believed to have evolved from a symbiotic relationship between an archaeon and an alphaproteobacterion, with the alphaproteobacterion becoming the mitochondrial organelle[3]. Much of the original DNA of the alphaproteobacteria is believed to have relocated to the nucleus, leaving behind only a small mtDNA remnant.

Exactly how an alphaproteobacteria ended up living inside an archaeon isn't certain. There seem to be few theories capable of explaining the mechanics of how eukaryotes originally evolved. Currently the only serious attempt to explain the mechanism of evolution of the eukaryotes appears to be the viral eukaryogenesis hypothesis.

The viral eukaryogenesis hypothesis posits that the eukaryotic cell evolved from a virus, archaeon, and alphaproteobacteria[15]. Difficulties with the viral eukaryogenesis hypothesis include viruses would need to evolve the means to replicate by themselves if they are to eventually evolve into gametophytes, and the lack of any known double stranded DNA viruses with segmented genomes; so the theory doesn't explain the origin of chromosomes.

An alternative line of reasoning starts with the archaeon's development of the cytoskeleton component actin[16, 17, 18]. Actin filaments would have allowed the archaeon to extend its plasma membrane to engulf large particles, which it would then attempt to digest, leading to the development of phagocytosis[19]. When that ingested particle happened to be a living cell, the ingested cell would evolve in such a way as to attempt to resist the full phagocytic effects of ingestion. An example of this is provided by *Rickettsia conorii*, an intracellular pathogen, which enters the host by inducing host phagocytosis, and then escapes from the phagosome into the host's cytosol[20]. It should be noted that Rickettsia is a genus of alphaproteobacteria.

Once inside the archaeon the alphaproteobacteria would have had three possible ways to propagate. Similar to the lytic or lysogenic cycles of bacteriophages and viruses, it could replicate until the host cell bursts and then find new host cells to infect, or it could attempt to ensure that it is faithfully propagated to each descendant of the host cell. Additionally the alphaproteobacteria could use actin based motility to spread from cell to cell. An example of this is again provided by the Rickettsia. The typhus group Rickettsia cause host cell lysis, while the spotted fever group spread from cell to cell by means of actin filaments[21]. It is worth noting that spotted fever group Rickettsia infection doesn't necessarily lead to host cell death, because avirulent strains of the spotted fever group exist that are capable of coexisting with their host in what might be described as a parasitic endosymbiosis[21].

The initially defenseless host might be expected to evolve defenses against the alphaproteobacterial parasites. These defenses are unlikely to be complete, we are still vulnerable to Rickettsia today, but are likely to be substantial. The exception being if the endosymbiont provides a benefit to the host. This proved to be the case with the mitochondria, which through oxidative phosphorylation increases the ATP available to the host by roughly a massive 15 fold over the glycolysis of anaerobic fermentation[22]. This then leads to a mostly cooperative relationship between the host and parasite. Over time the endosymbiont lost the ability to survive outside of the host.

Active germline replicators

An active germline replicator is an entity of which copies can be made and whose nature has some influence over the probability of it being copied[2].

Both the archaeal host and the alphaproteobacterial proto-mitochondrion are active germline replicators. The germ cells and zygotes of eukaryotes might be described as low fidelity active germline replicators. Germ cells and zygotes make copies of themselves in an environment made up of other germ cells and zygotes. The strategy they employ may involve creating zygotes, germ cells, somatic cells, and multicellular organisms, but in the end they produce more copies of themselves. They are low fidelity replicators in the sense that the DNA sequences of the copies only partially reflect the original due to homologous recombination of allelic sequences.

To be pedantic, the DNA should probably be viewed as forming the active germline replicator, and the archaeon or proto-mitochondria is just a vehicle for the replicator, but it is often easier to speak in terms of replicating proto-mitochondria than to spell out every time that it is the DNA of the replicating proto-mitochondria that is the replicator, with the rest of the mitochondrion existing because it assists in making copies of the replicator.

An important question in biology is: how can active germline replicators (the archaea and alphaproteobacteria) combine to make other active germline replicators (eukaryotes). This is the theme of the rest of this paper.

The problem of sex

For species in changing environments sex typically offers large fitness advantages, but at the nuclear genetic level it is difficult to understand how it evolved, and why it continues to exist.

Reported advantages of sex include the ability to combine the best mutations from several organisms, resistance to parasites, clearance of deleterious mutations, and an increase in the speed of evolution[23]. These are all advantages for the individual or the species. But microevolution is widely viewed as not working for the good of the individual or the species, but for the good of the gene[24].

The fundamental problem with sex is it results in only half of each parents' alleles getting passed on to each offspring. This includes the alleles promoting sex.

Consider a very simple scenario in which a single dominant nuclear allele for parthenogenesis arises in a large sexual population. At the population steady state each sexual female produces an average of two offspring; only one of which contains a given parent's allele. Meanwhile, assuming the male parental investment in the sexual case is zero, the asexual organisms will also produce two offspring, doubling the population of the allele. Consequently the allele for parthenogenesis should rapidly increase in the population and allele for sex should rapidly be lost from the population. This is J. Maynard Smith's classic argument for the two-fold cost of sex[1].

Care must be taken when using the phrase "two-fold cost". In the literature "two-fold cost" sometimes refers to gene dilution, as above, and sometimes it refers to the cost of producing

males[25]. For gene dilution, extra care must be taken with the phrase "two-fold cost" as it is not something that gets offset against the benefits of sex for a population. Rather it is a cost born by nuclear alleles in favor of sex. Even when sex is highly beneficial to the population, the two fold-cost can make nuclear alleles in favor of sex become extinct. It is true that parthenogenetic reproduction is associated with a small, gradual, loss in fitness, but this doesn't come close to the two-fold cost over the time frame in which nuclear alleles become extinct. The cost of producing males is something that does get offset against the benefits of sex for a population, making genome dilution the more fundamental problem.

A more complex scenario than the one just considered, in which there are multiple dominant or recessive alleles for sex or for parthenogenesis, is unlikely to change the two-fold cost. The problem with sex lies with the fundamental nuclear genome sharing nature of sex, not with the genes promoting it. So how might sex have evolved, and how might it continue to exist?

Smith's argument depends critically on the amount of male parental investment. If male parental investment was 50% of the total parental investment in the offspring, the asexual organism would only produce one offspring. But even if male parental investment is 50% there are still problems with sex. A parthenogenetic reproducer could mimic a female and accept parental investment from a male, but then discard the male's genes. Or the allele for parthenogenesis could favor itself during or post meiosis by killing its siblings. Such an allele might be expected to spread within a population, even if it is harmful to the success of the species. Fundamentally, sex involves an allele sharing half of its accrued rewards with an unknown competitor. This does not seem a productive thing to do.

Despite the advantages of sex for a species, the two-fold cost to alleles in favor of sex, and hence Darwinian microevolution, seem to argue against it.

Sex

A proposed evolution of sex

It is proposed here that sex evolved as a means for mitochondrial active germline replicators to replicate themselves inside of more and more suitable hosts. The mitochondria were engaged in the ultimate selective breeding experiment, crossbreeding those host nuclear chromosomes that proved successful in previous generations.

> Hypothesis 1: Sex evolved as a means for the proto-mitochondrion to increase the fitness of its host environment.

The proposed route to sex is as follows. Imagine an actin propelled alphaproteobacteria attempting to spread from cell to cell by punching a hole in two apposed archaeal cells' plasma membranes. This would be similar to the way in which the spotted fever group Rickettsia are known to spread by punching a hole in their host by means of actin, and then entering a neighboring cell[21, 26, 27]. If the plasma membranes were close enough to each other when the holes were punched then there is a possibility that the holes might heal by joining together around the apposing points on their

rims. You would then end up with a single cell containing alphaproteobacteria and two copies of the archaeal genome. This would be much like the mitochondria and two copies of the nuclear genome found in eukaryotic cells today. When the cell next replicated the two archaeal genomes would become four genomes, which would be followed by cell division. All it would take is for this to be followed by a second cell division, and you would have something that is starting to resemble syngamy followed by meiosis – minus the important reassortment of nuclear genes. This is shown in Figure 1. Partial reassortment of genes could occur if the circular archaeal genomes were broken into distinct lengths: primitive nuclear chromosomes.

Reassortment of genes would bring huge benefits to the organism. Suddenly evolution can occur in parallel across all the genomes in the population, and the best features of each merged, instead of having to be evolved along a single lineage. Ortholog radA in archaea, recA in bacteria, and Dmc1 in eukaryotes, is a key gene in homologous recombination[28]. It is capable of scanning for double-stranded DNA homologous to a single-stranded DNA template. Since radA and recA are found in archaea and alphaproteobacteria, its recruitment to the process could thus quickly lead to full blown meiosis[28]. At first the process would be messy, with multiple archaeal cells sometimes merging, and chromosomes and proto-mitochondria not getting distributed evenly among descendants. Frequently failures would occur, but the sequence of steps leading to tightly regulated syngamy and meiosis is precisely the sort of thing evolution is good at climbing.

An evolution of sex like the one described would explain why anaerobic environments don't contain any anciently amitochondrial eukaryotic species[29]. The mitochondria was there at the start of eukaryogenesis.

The possibility that the alphaproteobacteria that evolved into the mitochondrion had a broad host cell range has the potential to explain difficulty in determining the archaeal ancestor of the eukaryotic cell. There need not be one single ancestor or ancestor species.

An actin-based alphaproteobacteria motility model of eukaryogenesis is a simple theory in which mitochondria replicate themselves inside more and more suitable hosts. Other theories of eukaryogenesis are possible. One such other theory is that sex somehow evolved as a means to provide compatibility between a rapidly mutating mitochondrial genome and a more slowly mutating archaeal genome[30]. Provided at least part of the mitochondrial genome survived the process of crossbreeding nuclear genomes intact, other such theories would lead to similar conclusions regarding the nature of mortality to those to be reached here.

On sex

Who benefits from sex? As hypothesized in this paper, sex evolved for the benefit of the proto-mitochondrial genome. Proto-mitochondrial genomes were manipulating their environment (the archaeal host) in order to make it more probable they will survive.

Do the nuclear genes of the originally archaeal host benefit from sex? A nuclear gene benefits relative to a nuclear gene in an asexual species by being placed in a fitter and fitter environment. This environment is created by the recombination of the other nuclear genes. But a particular nuclear allele does not benefit relative to some other allele of the same nuclear gene. Each nuclear allele would prefer to reproduce selfishly, asexually. This point is no different than a multi-player

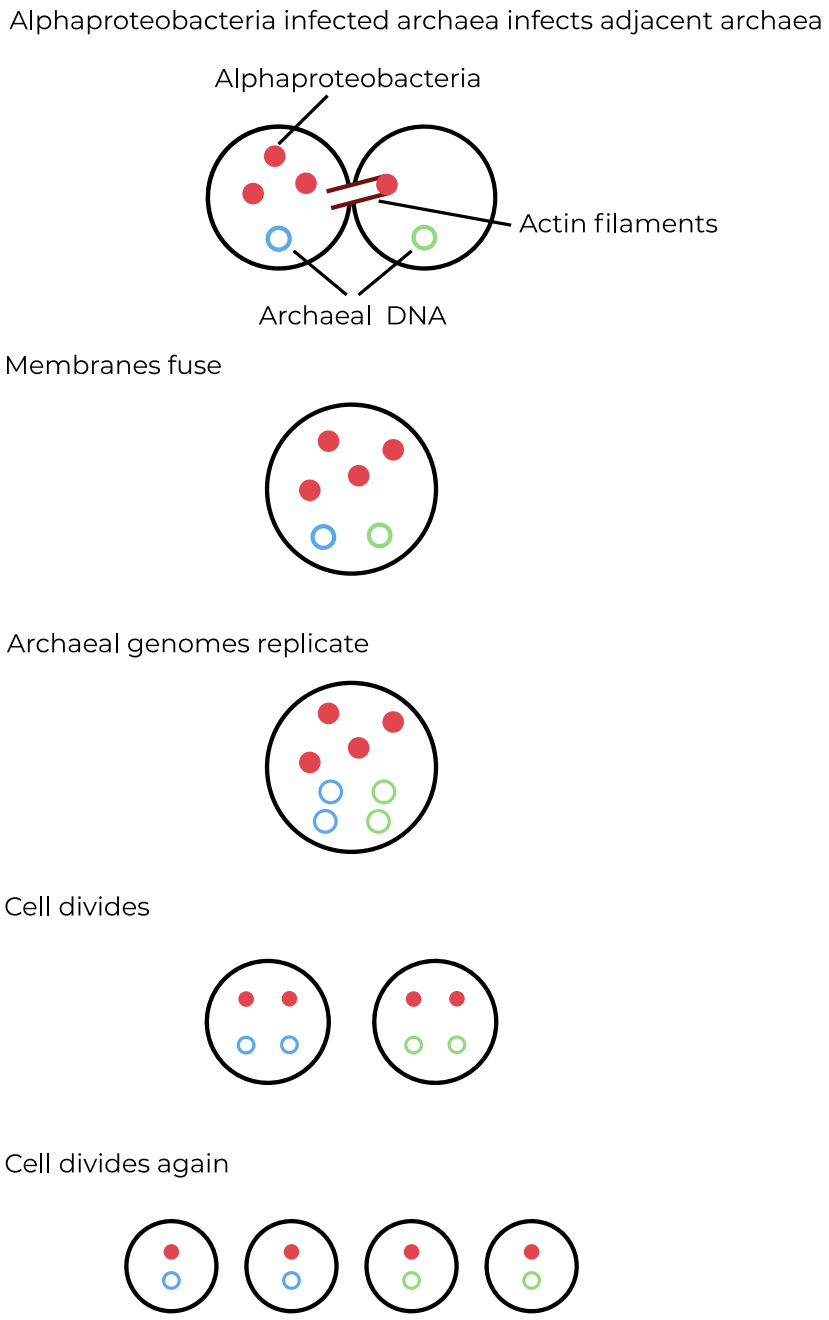

Figure 1: Proposed route to sex. An alphaproteobacteria from an infected archaea spreads to an adjacent archaea by means of actin filaments. The membranes of the two archaeal cells then fuse. The archaea genomes then replicate followed by cell division. If a second round of cell division then occurs the process starts to be reminiscent of syngamy followed by meiosis. The only major missing component is the reassortment of genes.

prisoner's dilemma. Everyone might benefit from cooperating, but an individual allele gains a strong initial advantage by defecting.

Note that although sex had benefits for the species, it need not. Sex could have been harmful to the success of the species, but so long as the proto-mitochondrial genome benefits, in the short run it would still occur. Depending on how harmful it was this could lead to the decline or extinction of the population. This is in contrast to most existing theories on sex, which attempt to divine how both nuclear alleles and the species benefit[23].

Why did the nuclear genes participate in sex then? This is a thorny question which will be addressed in the section dealing with mortality. For now, simply note that the species benefited from the recombination of advantageous alleles and from the energy provided by the mitochondrion: the primary beneficiary of sex.

How does the mitochondria cause sex? Today, this is difficult to see. Most mitochondrial genes that once existed to cause sex, say by riding an actin filament, punching a hole in two cell membranes, and causing recombination to occur, have probably nearly all long since migrated to nuclear genes, and been replaced by other nuclear genes. As will be explored in the section dealing with mortality, the mitochondria maintains a mechanism that causes sex to occur through the generation of reactive oxygen species.

Sex in present-day eukaryotes

One possibility is the alphaproteobacterion got the ball rolling with respect to the occurrence of sex, and then the alphaproteobacterion faded into the background becoming the mitochondrion, and the nuclear mechanisms of sex became self supporting. This however fails to explain how sex can continue to exist in unicellular organisms that are capable of parthenogenetic reproduction given the two-fold cost to alleles in favor of sex. Also, as explored in the section dealing with mortality, it fails to explain why eukaryotes appear mortal.

The near universality of mitochondria and sex in present day eukaryotes allows us to hypothesize that the crossbreeding of nuclear chromosomes by the mitochondrion isn't confined just to eukaryotic evolution, but that it continues to occur today.

> Hypothesis 2: Sex continues to be the means by which the mitochondria increase the fitness of their host environment.

How mitochondria achieve this will be explored in the section dealing with mortality.

Asexual eukaryotes

A nuclear gene that prevents sex and leads to parthenogenetic reproduction might be expected to propagate within a species, but represents an evolutionary dead end. The benefits of sex will be lost, and the species will be out-competed by other species. This is consistent with the observation

that it is very rare for a taxon higher than a species to consist entirely of asexual species[31]. The only reported exception appears to be the bdelloid rotifers:

- The bdelloid rotifers are famous as a class of ancient asexual eukaryotes[32]. Bdelloid rotifers appear to engage in interspecies and intraspecies horizontal gene transfer[33, 34]. Importantly bdelloid genomes have been found to contain pairs of homologous chromosomes and engage in occasional gene transfer between the homologous chromosomes[35]. This may go some way to explaining why the bdelloid rotifers don't need to engage in sex. They may get one of the benefits of sex through other means: the ability to try out multiple mutations without permanently losing the original genome.

Two taxa that were once thought to be asexual are aphids of the genus *Trama*, and bivalve crustaceans of the family Darwinulidae[32]:

- *Trama* was reportedly a genus of asexual aphids, but with the report of sex in *Trama troglodytes*, this is no longer the case[36].
- Like *Trama*, the darwinulid ostracods were long thought to be asexual, but males have now been found in one species, suggesting any loss of sex might have been more recent[37].

If the loss of sex is represents an evolutionary dead end, ancient asexual species should be rare. They are. Besides the bdelloid rotifers and darwinulid ostracods the only other well known ancient asexuals appear to be the arbuscular mycorrhizal fungi[32]:

- Like the bdelloid rotifers, arbuscular mycorrhizal fungi might have found an alternative to sex. In arbuscular mycorrhizal fungi, offspring receive hundreds of nuclei from their parent[38]. Thus there is a population of individually mutating nuclear genomes that might provide some of the benefits of sex seen in other organisms.

If mitochondria are the cause of sex, it follows sex is more likely to be lost when mitochondria are delivering little or no value to the resulting organism, such as in anaerobic environments. Here the loss of mitochondria might be expected to lead to the loss of sex. It is thus worth considering a few other taxa within which sex appears to have been more recently lost:

- Further evidence that mitochondria have something to do with sex is provided by the microsporidia. Microsporidia are a group of fungi that lack mitochondria[39]. They do however have an organelle called a mitosome, that appears to have been derived from the mitochondrion[39]. Mitosomes appear to have lost their organellar DNA. This makes the fact that some species of microsporidia are entirely asexual interesting. Even more interesting is the fact that this loss of sex doesn't appear to have occurred in one ancient ancestral lineage, but to have occurred several times in different lineages[40]. This loss of sexuality has occurred in the absence of mitochondria.

- Diplomonads and trichomonads are two orders that have lost their mitochondria[41]. Despite some diplomonads having genes for meiosis, they are not known to be sexual[42]. Trichomonads are also believed to be asexual[43]. Once again the loss of mitochondria and the possibility for asexuality go hand in hand.

In conclusion there seems to be a relationship between missing or unusual mitochondrial systems and asexuality in eukaryotes. More specifically, asexuality rarely transcends taxa larger than a species, asexuality frequently appears to be an evolutionary dead end with very few ancient asexuals, and asexuality is particularly common in amitochondrial species.

Sex and mitochondrial genomes

Mitochondrial genomes function as selfish replicators, much like prokaryote genomes, but within a more complex environment. The nuclear genome pool forms the extended environment within which related mitochondrial genomes evolve, assisting some mitochondrial genomes, and impinging on others, and generally ensuring all the mitochondrial genomes associated with a nuclear genome pool remain sufficiently similar, that is, related.

If sex was lost in some species this would reduce the fitness for the embedded mitochondrial genomes relative to the mitochondrial genomes of other species as they would no longer benefit from the crossbreeding of the nuclear genomes. It would represent a rapid evolutionary dead end.

Mortality

So far we have seen how the mitochondrial genome might favor sex so that it gets to exist in a fitter and fitter environment. The theory we have developed so far however is incomplete. Nuclear alleles in favor of sex might be expected to be replaced by alleles favoring parthenogenetic reproduction. Or when parthenogenetic reproduction is not a possible option, nuclear alleles in favor of sex might be expected to be replaced by alleles favoring continued mitotic growth. In order to understand why this doesn't occur it will be necessary to examine eukaryotic mortality, and in particular to hypothesize a role for the mitochondria in bringing about eukaryotic mortality. How mortality promotes sex will be explained below.

Eukaryotic mortality refers to the existence of an apparent intrinsic time limit for which a eukaryotic organism can live, before death occurs. This time limit may be measured in terms of actual time, aggregate metabolic inputs, or some other organismal process. Here an organism that only dies as a result of extrinsic evolutionarily unavoidable misfortune is not viewed as being mortal.

From a microevolutionary perspective, the nuclear alleles desire immortality and asexual reproduction. Suppose after a certain time period without sex the mitochondria always sabotaged the existence of the organism. If this was the case, the nuclear alleles would have no alternative way to continue to exist other than to periodically engage in sex. This is precisely what appears to happen. Individual eukaryotes age and die, the mitochondrion appears to be implicated in organism mortality, and reproduction causes a resetting of the aging process.

Hypothesis 3: By enforcing mortality, the mitochondria force the nuclear genome to engage in frequent sexual recombination.

For single celled organisms, mortality might only come after several rounds of parthenogenetic reproduction. Sexual recombination, not parthenogenetic reproduction, is expected to reset the age of the organism.

For multicellular organisms, mortality means that the strategy of surviving by just continuously growing through mitosis is ultimately going to fail. If the nuclear genome is going to survive it ultimately has to engage in meiosis.

How mitochondria bring about mortality may not be obvious. For now, it is sufficient to recall that mitochondrial reactive oxygen species (ROS) are harmful to many cellular components, and that the mitochondria play key roles in apoptosis and cellular senescence.

Death as a means of promoting a fitness increase that comes from sex is capable of explaining why unicellular eukaryotes are capable of committing apoptosis[44].

That the population will benefit from a shorter generation time than desired by microevolution may appear fairly obvious. Sex provides an increase in fitness as a result of the combination of advantageous alleles and the overcoming of Muller's ratchet. A full analysis would however need to take into account changing population sizes, organism sizes, niche sizes, and mutation rates that may be associated with a change in the generation time.

In summary, microevolution strives for near immortality at a cost to the species, while the mitochondria support frequent mortality. Given mortality the genes will bend to the interests of the mitochondria, and support sex as a means of resetting mortality.

Mortality and metabolism

So far mitochondria have been viewed as imposing a time limit on the life of the organism. But mitochondria aren't armed with a stopwatch, and even if they were this might not be the most appropriate way of doing things. Instead it seems more likely that lifespan might be tied to something easier to measure such as the aggregate inputs or outputs of the mitochondria. This might explain the observation that lifetime energy consumption per unit of body weight is roughly constant for related species[45]. This might also explain the correlation between obesity (the result of greater metabolic inputs), and shortened lifespan. The world beyond the organism might be viewed as containing energy, and the evolutionary mandate is to gather up enough energy to create another organism as quickly as possible.

Mitochondrial genome kin selection

Group selection is the hypothesis that natural selection acts for the good of a group. Group selection is widely dismissed by evolutionary biologists[46]. Kin selection is the hypothesis that evolution is

capable of acting not just for the benefit of an organism and its offspring, but also for the benefit of the organism's relatives. Kin selection is widely accepted by evolutionary biologists[47].

So far the argument in favor of sex and mortality amounts to mitochondrial genome kin selection. By killing the host after some time, kin mitochondrial genomes benefit from reproducing within a more fit environment. The kin mitochondrial genomes are the other mitochondrial genomes making up the species.

Mitochondrial genome kin selection could be viewed as an example of group selection in so far as it can be viewed as occurring for the benefit of the species, which is a group. However, mitochondrial genome kin selection does not require the invocation of group selection to explain it, as it is fully explained by kin selection. Evolutionary biologists presumably don't object to group selection occurring when the group is comprised of fully related kin.

Discussion

Hypotheses on the extended phenotype of the mitochondrion (HEPM) offers answers to the important questions of how did sex evolve, how is it sex appears evolutionarily stable, and why eukaryotes are mortal. These have been thorny questions that for a long time have gone unanswered.

The proto-mitochondrion was engaged in the ultimate crossbreeding experiment for its own benefit, and ultimately for the benefit of the species. This involved the crossbreeding of nuclear genomes to produce a more beneficial environment within which for it to reside. The proto-mitochondrion achieved this crossbreeding through the production of ROS, which rendered the organism mortal, and left sex as the only long term option for the nuclear genome to pass on, at least some of, its genes. The results of this crossbreeding experiment are all the varied eukaryotic forms that now exist, and within which trillions of trillions of mitochondrial genomes now reside.

Treating the mitochondrial genome as a selfish replicator offers a different way of looking at the eukaryotic cell, and with it new understanding. Sex might have evolved as a means for the proto-mitochondria to propagate itself into a more and more competent host. Thus creating the defining advantage for eukaryotes relative to prokaryotes: meiosis. Mortality exists as a means of ensuring meiosis occurs at a near to optimal frequency for the species. And the whole eukaryotic cell can be viewed as the extended phenotype of the mitochondrial genome. This isn't the only way the eukaryotic cell should be viewed, but it adds an interesting new perspective.

Materials and methods

For the bioinformatic analysis of mitochondrial gene frequencies in Appendix A, annotated mitochondrial genomes from the NCBI Reference Sequence Database (RefSeq) release 214 were used. See Supplement 1 for a copy of this data[48]. After filtering for data quality the 14,062 genomes were reduced to 13,959 genomes. Different names used in the annotations for the same orthologous genes were mapped to the most common name. This process may have missed some annotated names that only occurred once, and orthologs that have split into multiple separate genes. The frequency of different genes was then computed.

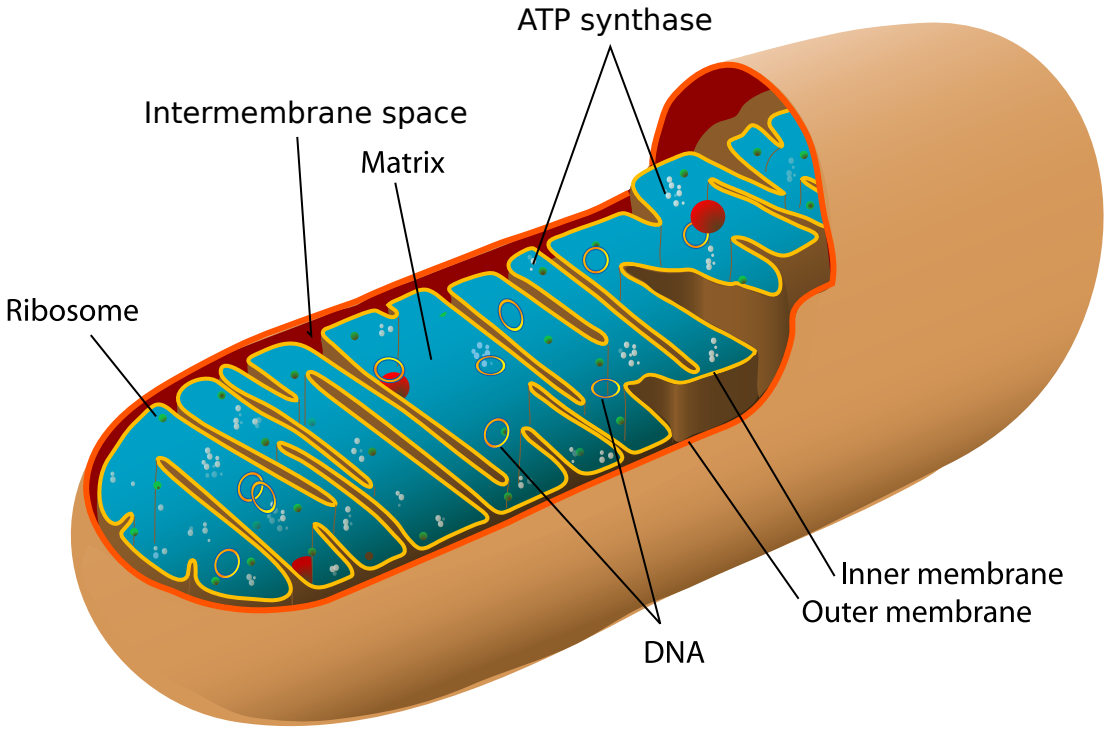

Figure 2: Structure of a typical mitochondrion.

See Supplement 2 for the software used for the analysis[49].

Appendices

A: Mitochondria

The mitochondrion is an organelle present in the vast majority of eukaryotic cells. The mitochondrion contains a double membrane. Each membrane is a phospholipid bilayer with embedded proteins. The space between the inner and outer membranes is termed the intermembrane space. The space within the inner membrane is termed the matrix.

Somewhere in the range of 100 to 500 mitochondria are found in a typical cell[50]. The mitochondria in the cell are capable of undergoing processes of fission (splitting) and fusion (joining). Mitochondria may engage in these dynamics as part of a quality control mechanism that also involves autophagy[51].

The structure of a typical mitochondrion is illustrated in Figure 2.

Mitochondria nearly always have their own circular double-stranded DNA genome, commonly re-

CYTB	99.6%
COX1	99.5%
COX3	99.4%
ND5	99.0%
ND4	99.0%
ND2	99.0%
COX2	99.0%
ND6	98.9%
ATP6	98.9%
ND3	98.9%
ND1	98.9%
ND4L	98.7%
ATP8	95.1%
ATP9	9.7%
RPS3	7.5%
RPS12	5.4%
RPL16	4.9%
ND9	4.6%
ND7	4.5%
RPS4	4.5%

Table 2: Estimated frequency of the top 20 mtDNA protein coding genes from an analysis of 13,959 RefSeq mitochondrial genomes. Due to vagaries in the names used for orthologous genes, frequencies are likely to be slight under-estimates. For further details see materials and methods.

ferred to as mtDNA. This genome is much smaller than the nuclear genome both in terms of the number of genes encoded, and in terms of the number of base pairs.

mtDNA differs far more between species than within species. Indeed, it is hypothesized that all the mitochondrial genomes found in humans today descended from a single mitochondrial "Eve" that existed perhaps 200,000 years ago[52].

Multiple copies of the same mtDNA genome exist within a single eukaryotic cell. A typical cell might have around 5,000 mtDNA copies contained within its mitochondria[53].

The mtDNA genome almost invariably includes genes for the large and small subunits of the mitochondrial ribosome and usually all of the corresponding mitochondrial tRNA genes. The remaining mtDNA genes vary to some extent from species to species. These remaining genes nearly always include genes for components of the electron transport chain (COX, ND, and CYTB genes) and ATP synthase (ATP genes). Occasionally genes for mitochondrial ribosomal proteins (RPL and RPS genes) are also present. This is shown in Table 2. Most species' mitochondria comprise the same 13 protein coding genes, but the sequence making up each gene will vary between species.

The vast majority of mitochondrial proteins are not encoded by the mtDNA, but by the nuclear genome[54], and are directed to the mitochondria by the presence of a mitochondrial targeting presequence that is cleaved off.

The mitochondria are the locations of the energy producing reactions of the cell. The citric acid

cycle turns pyruvate and water into CO_2 and in so doing produces the cofactors NADH, $FADH_2$, and GTP. The electron transport chain oxidizes NADH and $FADH_2$ releasing energy which is used to pump H^+ from the matrix to the intermembrane space. ATP synthase then uses the resulting H^+ electrochemical gradient to produce ATP from ADP.

mtDNA is normally maternally inherited. Various mechanisms exist to prevent the paternal inheritance of mtDNA in most species[55].

Base pairs in mitochondrial genes evolve 10 times more rapidly than base pairs in nuclear genes, but because the mtDNA coding regions are roughly $\frac{1}{2{,}000}$th the length of nuclear DNA coding regions, the mitochondrial genome effectively evolves 200 times more slowly than the nuclear genome. To be precise, humans and chimpanzees are estimated to have diverged $T = 6.7{\times}10^6$ years ago[56]. Comparing human and chimpanzee mtDNA, the non-synonymous substitution rate of protein coding genes is $2{\times}10^{-9}$ substitutions per site per year[57]. The rate of substitution for the mtDNA rRNA genes is somewhat higher. For synonymous sites the substitution rate is $3{\times}10^{-8}$[57]. These substitution rates should be compared to the nuclear DNA non-synonymous and synonymous substitution rates of protein coding genes of around $2{\times}10^{-10}$ and $9{\times}10^{-10}$ substitutions per site per year respectively[58][Supplement S23, site weighted K_a and K_s values divided by $2T$].

The somatic mutation rate of the mitochondrial genome is around $2{\times}10^{-7}$ mutations per base pair per year based on mutational accumulation in aged humans[59][$1.9{\times}10^{-5}$ mutations divided by a mean age of 83 years]. It is hypothesized that the female germ line contains quiescent template mitochondria that are protected from this high rate of mutation[60].

The human mitochondrial genome contains 16,568 base pairs. In addition to genes for the large and small subunits of the mitochondrial ribosome, it contains the standard 22 tRNA genes, 11 electron transport chain genes, and 2 ATP synthase genes. The gene content of the human mitochondrial genome is identical to that of most other organisms, although in terms of base pairs fungal and plant mitochondrial genomes are substantially larger.

Acknowledgments

I am very grateful for the time Steven Greidinger, Hina Zain, Michael Klüppel, and Martin Predavec spent reviewing early versions of this manuscript and providing me with feedback.

Conflict of interest disclosure

The author declares they have no financial conflicts of interest in relation to the content of this manuscript.

Supplements

Supplement 1 - Mitochondrial genome sequence data.
https://doi.org/10.5281/zenodo.7901579

Supplement 2 - Mitochondrial genome analysis software and results.
https://doi.org/10.5281/zenodo.7901623

References

[1] John Maynard Smith. *The evolution of sex*. Cambridge University Press, 1978.

[2] Richard Dawkins. *The extended phenotype*. Oxford University Press, 1982.

[3] Laura Eme, Anja Spang, Jonathan Lombard, Courtney W Stairs, and Thijs JG Ettema. Archaea and the origin of eukaryotes. *Nature Reviews Microbiology*, 15(12):711–723, 2017.

[4] Shona A Mookerjee, Akos A Gerencser, David G Nicholls, and Martin D Brand. Quantifying intracellular rates of glycolytic and oxidative ATP production and consumption using extracellular flux measurements. *Journal of Biological Chemistry*, 292(17):7189–7207, 2017.

[5] Katarzyna Zaremba-Niedzwiedzka, Eva F Caceres, Jimmy H Saw, Disa Bäckström, Lina Juzokaite, Emmelien Vancaester, Kiley W Seitz, Karthik Anantharaman, Piotr Starnawski, Kasper U Kjeldsen, et al. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature*, 541(7637):353–358, 2017.

[6] Chunxin Wang and Richard J Youle. The role of mitochondria in apoptosis. *Annual Review of Genetics*, 43:95, 2009.

[7] Maria Van Gurp, Nele Festjens, Geert Van Loo, Xavier Saelens, and Peter Vandenabeele. Mitochondrial intermembrane proteins in cell death. *Biochemical and Biophysical Research Communications*, 304(3):487–497, 2003.

[8] Natalia A Belikova, Yury A Vladimirov, Anatoly N Osipov, Alexandr A Kapralov, Vladimir A Tyurin, Maksim V Potapovich, Liana V Basova, Jim Peterson, Igor V Kurnikov, and Valerian E Kagan. Peroxidase activity and structural transitions of cytochrome c bound to cardiolipin-containing membranes. *Biochemistry*, 45(15):4998–5009, 2006.

[9] Giuseppe Petrosillo, Giacoma Casanova, Mariagiuseppa Matera, Francesca Maria Ruggiero, and Giuseppe Paradies. Interaction of peroxidized cardiolipin with rat-heart mitochondrial membranes: induction of permeability transition and cytochrome c release. *FEBS Letters*, 580(27):6311–6316, 2006.

[10] Kambiz N Alavian, Gisela Beutner, Emma Lazrove, Silvio Sacchetti, Han-A Park, Pawel Licznerski, Hongmei Li, Panah Nabili, Kathryn Hockensmith, Morven Graham, et al. An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. *Proceedings of the National Academy of Sciences*, 111(29):10580–10585, 2014.

[11] Alicia J Kowaltowski, Roger F Castilho, and Anibal E Vercesi. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca2+ is dependent on mitochondrial-generated reactive oxygen species. *FEBS Letters*, 378(2):150–152, 1996.

[12] Martin Ott, John D Robertson, Vladimir Gogvadze, Boris Zhivotovsky, and Sten Orrenius. Cytochrome c release from mitochondria proceeds by a two-step process. *Proceedings of the National Academy of Sciences*, 99(3):1259–1263, 2002.

[13] Hélène Martini and João F Passos. Cellular senescence: all roads lead to mitochondria. *The FEBS Journal*, 2022.

[14] Clara Correia-Melo, Francisco DM Marques, Rhys Anderson, Graeme Hewitt, Rachael Hewitt, John Cole, Bernadette M Carroll, Satomi Miwa, Jodie Birch, Alina Merz, et al. Mitochondria are required for pro-ageing features of the senescent phenotype. *The EMBO Journal*, 35(7):724–742, 2016.

[15] Philip John Livingstone Bell. Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus? *Journal of Molecular Evolution*, 53(3):251–256, 2001.

[16] Ann-Christin Lindås, Karin Valegård, and Thijs JG Ettema. Archaeal actin-family filament systems. In *Prokaryotic Cytoskeletons*, pages 379–392. Springer, 2017.

[17] Thijs JG Ettema, Ann-Christin Lindås, and Rolf Bernander. An actin-based cytoskeleton in archaea. *Molecular Microbiology*, 80(4):1052–1061, 2011.

[18] Rolf Bernander, Anders E Lind, and Thijs JG Ettema. An archaeal origin for the actin cytoskeleton: implications for eukaryogenesis. *Communicative & Integrative Biology*, 4(6):664–667, 2011.

[19] Natalya Yutin, Maxim Y Wolf, Yuri I Wolf, and Eugene V Koonin. The origins of phagocytosis and eukaryogenesis. *Biology Direct*, 4(1):9, 2009.

[20] Nadine Teysseire, JA Boudier, and Didier Raoult. Rickettsia conorii entry into Vero cells. *Infection and Immunity*, 63(1):366–374, 1995.

[21] T Hackstadt. The biology of rickettsiae. *Infectious Agents and Disease*, 5(3):127–143, 1996.

[22] PR Rich. The molecular machinery of Keilin's respiratory chain. *Biochemical Society Transactions*, 31(6):1095–1105, 2003.

[23] Matthew Hartfield and Peter D Keightley. Current hypotheses for the evolution of sex and recombination. *Integrative Zoology*, 7(2):192–209, 2012.

[24] Richard Dawkins. *The selfish gene*. Oxford University Press, 1976.

[25] Jussi Lehtonen, Michael D Jennions, and Hanna Kokko. The many costs of sex. *Trends in Ecology & Evolution*, 27(3):172–178, 2012.

[26] Robert A Heinzen, Stanley F Hayes, Marius G Peacock, and T Hackstadt. Directional actin polymerization associated with spotted fever group Rickettsia infection of vero cells. *Infection and Immunity*, 61(5):1926–1935, 1993.

[27] Yvonne Gar-Yun Chan, Sean Phillip Riley, and Juan Jose Martinez. Adherence to and invasion of host cells by spotted fever group Rickettsia species. *Frontiers in Microbiology*, 1:139, 2010.

[28] Zhenguo Lin, Hongzhi Kong, Masatoshi Nei, and Hong Ma. Origins and evolution of the recA/RAD51 gene family: evidence for ancient gene duplication and endosymbiotic gene transfer. *Proceedings of the National Academy of Sciences*, 103(27):10328–10333, 2006.

[29] T Martin Embley. Multiple secondary origins of the anaerobic lifestyle in eukaryotes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1470):1055–1067, 2006.

[30] Justin C Havird, Matthew D Hall, and Damian K Dowling. The evolution of sex: a new hypothesis based on mitochondrial mutational erosion: mitochondrial mutational erosion in ancestral eukaryotes would favor the evolution of sex, harnessing nuclear recombination to optimize compensatory nuclear coadaptation. *BioEssays*, 37(9):951–958, 2015.

[31] John Maynard Smith. Contemplating life without sex. *Nature*, 324:300–301, 1986.

[32] Benjamin B Normark, Olivia P Judson, and Nancy A Moran. Genomic signatures of ancient asexual lineages. *Biological Journal of the Linnean Society*, 79(1):69–84, 2003.

[33] Eugene A Gladyshev, Matthew Meselson, and Irina R Arkhipova. Massive horizontal gene transfer in bdelloid rotifers. *Science*, 320(5880):1210–1213, 2008.

[34] Nicolas Debortoli, Xiang Li, Isobel Eyres, Diego Fontaneto, Boris Hespeels, Cuong Q Tang, Jean-François Flot, and Karine Van Doninck. Genetic exchange among bdelloid rotifers is more likely due to horizontal gene transfer than to meiotic sex. *Current Biology*, 26(6):723–732, 2016.

[35] Paul Simion, Jitendra Narayan, Antoine Houtain, Alessandro Derzelle, Lyam Baudry, Emilien Nicolas, Rohan Arora, Marie Cariou, Corinne Cruaud, Florence Rodriguez Gaudray, et al. Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer adineta vaga. *Science Advances*, 7(41):eabg4216, 2021.

[36] Roger L Blackman, Emma De Boise, and Andrzej Czylok. Occurrence of sexual morphs in Trama troglodytes von Heyden, 1837 (Hemiptera, Aphididae). *Journal of Natural History*, 35(5):779–785, 2001.

[37] Isa Schön, Giampaolo Rossetti, and Koen Martens. Darwinulid ostracods: ancient asexual scandals or scandalous gossip? In *Lost sex*, pages 217–240. Springer, 2009.

[38] Gerrit Kuhn, Mohamed Hijri, and Ian R Sanders. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature*, 414(6865):745–748, 2001.

[39] Jirí Vávra. "Polar vesicles" of microsporidia are mitochondrial remnants ("mitosomes")? *Folia Parasitologica*, 52(1/2):193, 2005.

[40] Joseph E Ironside. Multiple losses of sex within a single genus of Microsporidia. *BMC Evolutionary Biology*, 7(1):1–16, 2007.

[41] Andrew J Roger, Staffan G Svärd, Jorge Tovar, C Graham Clark, Michael W Smith, Frances D Gillin, and Mitchell L Sogin. A mitochondrial-like chaperonin 60 gene in Giardia lamblia: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. *Proceedings of the National Academy of Sciences*, 95(1):229–234, 1998.

[42] Marilee A Ramesh, Shehre-Banoo Malik, and John M Logsdon Jr. A phylogenomic inventory of meiotic genes: evidence for sex in Giardia and an early eukaryotic origin of meiosis. *Current Biology*, 15(2):185–191, 2005.

[43] Esther Collántes-Fernández, Marcelo C Fort, Luis M Ortega-Mora, and Gereon Schares. Trichomonas. In *Parasitic Protozoa of Farm Animals and Pets*, pages 313–388. Springer, 2018.

[44] AV Gordeeva, Yu A Labas, and RA Zvyagilskaya. Apoptosis in unicellular organisms: mechanisms and evolution. *Biochemistry (Moscow)*, 69(10):1055–1066, 2004.

[45] Stan L Lindstedt and William A Calder. Body size and longevity in birds. *The Condor*, 78(1):91–94, 1976.

[46] Samir Okasha. Why won't the group selection controversy go away? *British Journal for the Philosophy of Science*, 52(1), 2001.

[47] Joan E Strassmann, Robert E Page, Gene E Robinson, and Thomas D Seeley. Kin selection and eusociality. *Nature*, 471(7339):E5–E6, 2011.

[48] Gordon Irlam. Hypotheses on the extended phenotype of the mitochondrion: sex, mortality, and aging: Supplement 1, 2023. doi:10.5281/zenodo.7901579.

[49] Gordon Irlam. Hypotheses on the extended phenotype of the mitochondrion: sex, mortality, and aging: Supplement 2, 2023. doi:10.5281/zenodo.7901623.

[50] Eugene D Robin and Ronald Wong. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *Journal of Cellular Physiology*, 136(3):507–513, 1988.

[51] Gilad Twig, Alvaro Elorza, Anthony JA Molina, Hibo Mohamed, Jakob D Wikstrom, Gil Walzer, Linsey Stiles, Sarah E Haigh, Steve Katz, Guy Las, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *The EMBO journal*, 27(2):433–446, 2008.

[52] Rebecca L Cann, Mark Stoneking, and Allan C Wilson. Mitochondrial DNA and human evolution. *Nature*, 325(6099):31–36, 1987.

[53] Francis J Miller, Franklin L Rosenfeldt, Chunfang Zhang, Anthony W Linnane, and Phillip Nagley. Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age. *Nucleic Acids Research*, 31(11):e61–e61, 2003.

[54] Panagiotis VS Vasileiou, Konstantinos Evangelou, Konstantinos Vlasis, Mihalis I Panayiotidis, Efstathios Chronopoulos, Panagiotis-Georgios Passias, Mirsini Kouloukoussa, Vassilis G Gorgoulis, and Sophia Havaki. Mitochondrial homeostasis and cellular senescence. *Cells*, 8(7):686, 2019.

[55] Miyuki Sato and Ken Sato. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1833(8):1979–1984, 2013.

[56] Sudhir Kumar, Glen Stecher, Michael Suleski, and S Blair Hedges. Timetree: a resource for timelines, timetrees, and divergence times. *Molecular Biology and Evolution*, 34(7):1812–1819, 2017.

[57] Graziano Pesole, Carmela Gissi, Anna De Chirico, and Cecilia Saccone. Nucleotide substitution rate of mammalian mitochondrial genomes. *Journal of Molecular Evolution*, 48(4):427–434, 1999.

[58] The Chimpanzee Sequencing and Analysis Consortium. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature*, 437(7055):69–87, 2005.

[59] Scott R Kennedy, Jesse J Salk, Michael W Schmitt, and Lawrence A Loeb. Ultra-sensitive sequencing reveals an age-related increase in somatic mitochondrial mutations that are inconsistent with oxidative damage. *PLoS Genetics*, 9(9):e1003794, 2013.

[60] Wilson BM de Paula, Cathy H Lucas, Ahmed-Noor A Agip, Gema Vizcay-Barrena, and John F Allen. Energy, ageing, fidelity and sex: oocyte mitochondrial DNA as a protected genetic template. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1622):20120263, 2013.