Title: Selection for mitochondrial quality drives the evolution of sexes with a dedicated germline

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Abstract: The origin of the germline-soma distinction in metazoans is a fundamental unsolved question. Somatic gametogenesis in sessile sponges and corals contrasts starkly with early germline sequestration in bilaterians with higher energy requirements, elaborate body plans and fast evolution of small mitochondrial genomes. We develop an evolutionary model to investigate whether selection for mitochondrial quality can drive germline evolution. In basal metazoans with low mutation rates, somatic gametogenesis optimizes gamete quality through segregation of mitochondrial mutations in multiple cell divisions. The need to maintain mitochondrial quality in somatic tissues explains the evolution of oogamy and male–female gamete specialization. Higher mitochondrial mutation rates promote early sequestration of a dedicated germline, permitting complex developmental processes. Rising oxygen drove germline evolution in motile bilaterians, igniting the Cambrian explosion.

One Sentence Summary: Selection for high mitochondrial fitness drives the evolution of male–female gamete dimorphism and a dedicated germline in metazoans.

Keywords: evolution, germline, mitochondria, oogamy

Main Text: Bilaterian animals generate reproductive cells through sequestration of a dedicated germline, separating primordial germ cells from somatic and stem cell populations early in embryogenesis (*1*). Germline cells alone retain the capacity to provide genetic information for future generations. The strict distinction between reproductive and somatic cell lineages is generally interpreted as resolving evolutionary conflict due to competition between cells within an individual that threatens the stability of multicellular cooperation (*2, 3*). Yet neither plants nor basal metazoans sequester a dedicated germline, and several metazoan groups (e.g. Ectoprocta, Entoprocta) have secondarily derived somatic gametogenesis (*4)*, implying that selfish conflict might not be the principal reason for the early sequestration of gamete precursor cells. In addition, selfish conflict between cells does not address the gamete specialization found in bilaterians, with large oocytes sequestered early in females and tiny sperm produced throughout life in males.

There is a striking association across animal groups between increasing complexity of development, high metabolic rates in somatic tissues with correspondingly greater energetic requirements, and an early germline, suggesting mitochondrial involvement. Contrast sessile sponges and corals, characterized by limited tissue differentiation, somatic gametogenesis and extremely low metabolic and mitochondrial mutation rates (*5-7*) with bilaterians, which display strong somatic differentiation linked to high metabolic rates, early germline sequestration, and rapid evolution of tiny mitochondrial genomes (*8, 9*). The potential role of mitochondrial inheritance in driving germline evolution has never been formally explored. Here we analyse an evolutionary model to explore the role of mitochondrial fitness in (i) stabilizing the state of somatic gametogenesis, (ii) the evolution of oogamy and uniparental inheritance in multicellular organisms with tissue differentiation; and (iii) the evolution of early germline sequestration in bilaterians and other metazoans with higher metabolic rate.

The model considers an ancestral multicellular metazoan with variable tissue-level differentiation (Figure 1). Gametes are formed either from adult somatic tissues, or early in embryogenesis. At each mitotic cell division, the mitochondrial population is doubled and then randomly segregated between daughter cells, with a probability of copying errors in mitochondrial genes of μ_S per cell division. We also consider mutations resulting from 'background' damage, μ_B (e.g. caused by oxidative damage or UV radiation). Background mutations can accumulate even in the absence of DNA replication, as is known in immature oocytes (*10*) and aging epithelial cells (*11*). Deletions generated during the repair of damaged mtDNA may also represent a substantial contribution to the background mutation rate μ_B (*11*, *12*). The relative contribution of μ_S and μ_B has been measured as the ratio of transitions (which occur largely through copying errors) to transversions (arising from oxidative damage caused by UV radiation or ROS) (*13*). In basal metazoans such as sponges, corals (and indeed angiosperms) μ_B dominates (*6*), whereas in mammals, the ratio is about even (*14)*. The resulting population of mitochondria is subject to a mutational load that impacts on cell fitness

$$\omega(m) = 1 - \left(m/M \right)^2$$

where m is the number of mutant mitochondria and M is the total size of the mitochondrial population. This concave fitness function assumes that a relatively high number of mutants must accumulate before cell function is significantly undermined, as is the case in mitochondrial diseases *(15)*. The fitness of each somatic tissue is taken to be the mean fitness of its constituent

cells. In the case of multiple tissues, we assume that adult fitness is determined by the quality of the worst tissue, as each has a vital organismal function and any tissue failure results in severe organismal impairment.

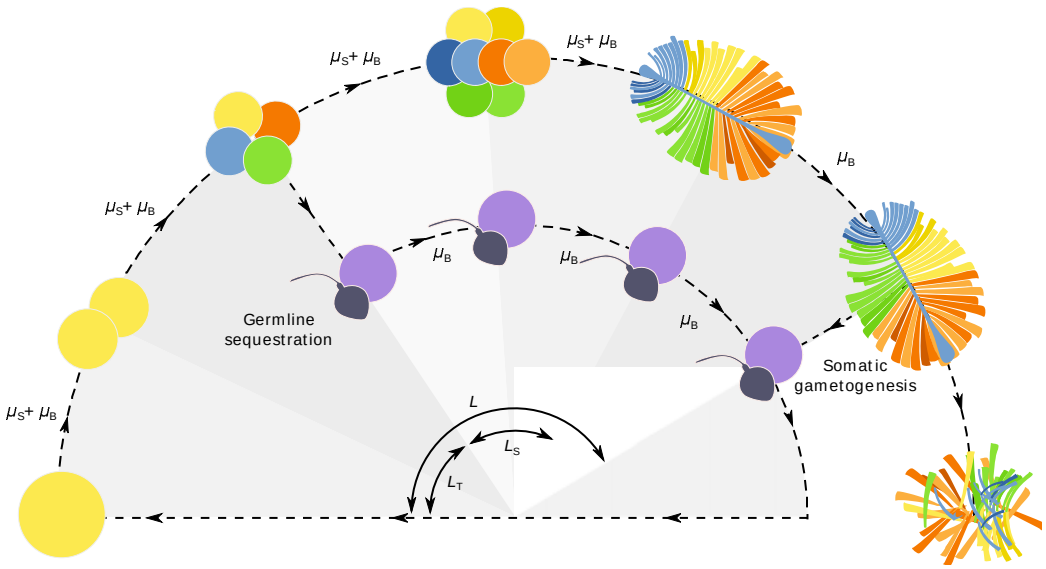

Fig. 1. **Life cycle of the model organism.** Starting with a zygote, the embryo undergoes L_T cell divisions before cells commit to one of 2^{LT} somatic tissues. Each tissue therefore originates from a single precursor cell (color-coded). Differentiation is followed by an additional L_S divisions in somatic tissues. The total lifespan of an organism L can exceed L_T+L_S, meaning that an organism can accumulate background mutations even after development is complete. The adult reproduces at the end of its lifespan L, and then dies. Gamete precursor cells are cloned from randomly chosen terminally differentiated somatic cells (ancestral state). If there is a germline, gamete precursor cells are cloned from embryonic stem cells after L_g cell divisions (for convenience $L_g = L_T$ in the figure; and we set $L_g \leq L_T$). Mitochondrial replication is associated with mutation probability μ_S. Background mutations (μ_B) accumulate over time even in the absence of mitochondrial replication.

We initiate the model by considering the simplest developmental state: an organism without tissue-level differentiation or germline–soma distinction, with isogamous gametes that have biparental inheritance of mitochondria and two mating types. Mathematical modeling (*16, 17*) suggests that some degree of biparental inheritance may be widespread, especially in simple organisms with small numbers of mitochondria and low mutation rates. Recent deep sequencing shows that mitochondrial heteroplasmy (mixtures of genetically distinct mitochondrial populations) and biparental inheritance of mitochondria are more common than once thought (*18, 19*), even in relatively complex metazoans (*18, 20*). Basal metazoans were probably sessile filter feeders with relatively limited metabolic capabilities, resembling modern sponges (*7*), and generally fit these assumptions of low developmental complexity and low mutation rates.

Assuming that the background mutation rate μ_B is relatively high compared with the frequency of copying errors μ_S, as in sponges (*6*), there is little selection for the early sequestration of germ cells. This is because multiple rounds of cell division from the zygote to the adult allow segregation of mitochondrial mutations between cells. Late differentiation of germ cells (i.e. somatic gametogenesis) results in greater variance in mutation load between gametes compared with early sequestration (Fig. 2A). Increasing variance in the load of mitochondrial mutations

amongst gametes facilitates selection, and so improves fitness over generations. At low μ_S, the benefit of increased variance between gametes outweighs the benefit of sequestering an early germline (Fig. 3A) – curtailing cell division in the germline prevents the accumulation of copying errors, but hinders selection as it lowers variance in mutation load between gametes. When μ_B is relatively high, mutations continue to accumulate, even in the absence of copying errors. Somatic gametogenesis allows these mutations to be segregated over cell divisions, but this is not possible within the non-dividing early germline. Thus segregation of mutations explains why basal metazoans do not benefit from having a germline (Fig. 3A).

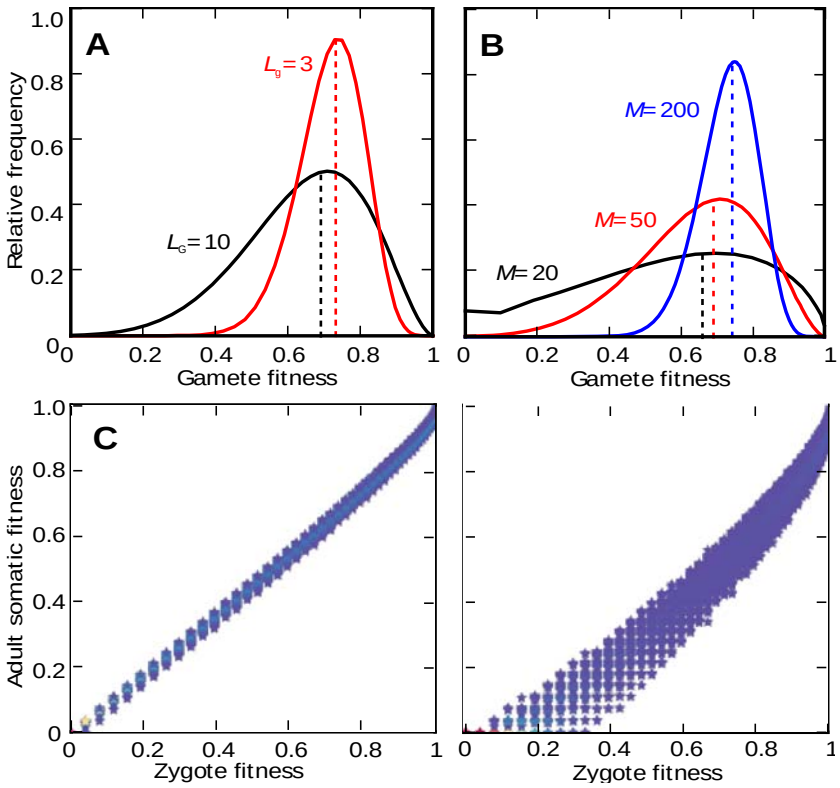

Fig. 2. **Segregation of mitochondrial mutations determines gamete quality and adult fitness. (A)** Fitness variation of gametes is higher with late differentiation of gametes from somatic cells (L_G = 10) compared with early germline sequestration (L_g = 3); mean fitness falls slightly, but late differentiation produces more high-fitness gametes. **(B)** With late gamete differentiation (L_G = 10), increasing the number of mitochondria per cell improves mean fitness but lowers fitness variation between gametes, producing fewer high-fitness gametes. **(C)** In organisms lacking tissues, adult fitness corresponds closely to zygote fitness. **(D)** In contrast, in organisms with 8 tissues, adult fitness depends on the segregation of mitochondria; increasing variance between tissues undermines mean adult fitness as organismal fitness depends on the function of the worst tissue. (Parameter values μ_S = 0.002, μ_B = 0.005, M = 50.)

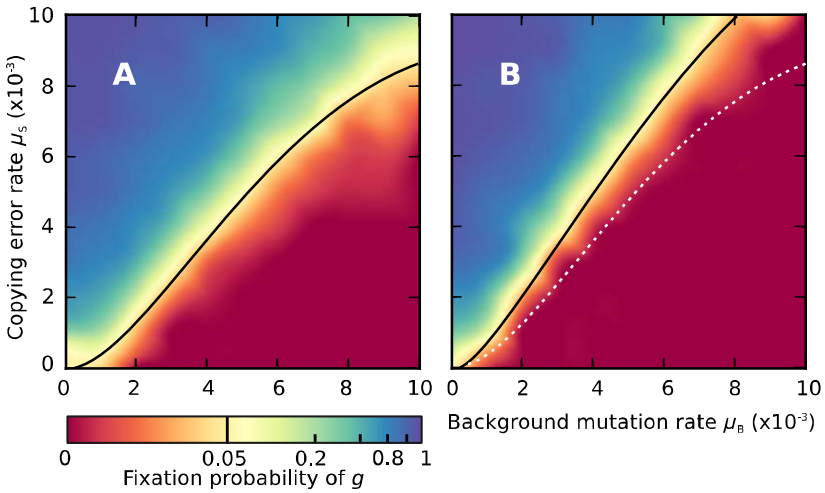

Fig. 3. Evolutionary stability of the germline. (A) Fixation of an allele (g) encoding early germline sequestration ($L_g = 3$) is favored at high μ_S and relatively low μ_B (blue region). At low μ_S and high μ_B (red region) late gamete differentiation ($L_G = 10$) is stable, because mitochondrial segregation increases variance and the number of high-fitness gametes (see Fig. 2A), opposing selection for the invading g allele. The black line depicts fixation probability of a neutral g allele introduced at a frequency 0.05. **(B)** Fixation of early germline ($L_g = 3$) becomes even less likely in organisms with 8 tissues; dotted line shows neutrality in (A). $M = 50$.

Many multicellular organisms, even simple ones like hydroids, have multiple cell types and tissues. Adding tissue-level differentiation changes this minimal picture. Imagine an organism of the same size, but now with differentiation into distinct somatic tissues. Random segregation of mitochondrial mutations inherited by the zygote (plus *de novo* mutations occurring during development) results in tissue-precursor cells carrying variable numbers of mutants. Some tissue-precursor cells segregate with higher and others with lower mutation loads, producing variation in tissue quality within an individual (Fig. 2C-D). While increased variance between gametes is beneficial, it has the opposite effect on cells comprising adult somatic tissues, as the fitness of an organism depends on the mutational state of the worst tissue. The model shows that selection for a sequestered germline is actually weaker in organisms with multiple tissues (Fig. 3B), even though adult fitness declines (Fig. 2D). The simplest way to improve adult fitness is to lower variance in mitochondrial fitness between tissue-precursor cells. Increasing the number of mitochondria decreases variance generated by random segregation (see *Supplementary Methods*) and improves somatic fitness; but having more mitochondria also decreases variance between gametes (Fig. 2B). The loss in variation between gametes is exacerbated by early germline sequestration, as this strongly limits the effectiveness of mitotic segregation in generating mitochondrial variance between gametes. This deficit overwhelms any benefit to somatic tissue fitness, and militates against selection for an early germline (Fig. S1A). Thus, the evolution of greater complexity, with some tissue-level differentiation and more mitochondria per cell, in fact stabilizes gametogenesis to the end of somatic development (Fig. S1A-C), accounting for why even quite complex metazoans with multiple tissues (as well as plants) do not have a germline or have secondarily evolved somatic gametogenesis.

The tradeoff between adult fitness and gamete quality can be obviated through the asymmetric inheritance of mitochondria from the two parents. Imagine that gametes in one of the two mating types undergo Q additional rounds of mitochondrial replication without cell division, producing

a large oocyte with more mitochondria. This form of anisogamy through oogamy is universal in extant metazoan groups and is common to multicellular organisms in general (*21*). It has important consequences because it modifies the effect of segregation, and hence the distribution of mutations in somatic cells and gametes. First, oogamy means that the size of the mitochondrial population rises in the zygote (but not in somatic cells). The first few rounds of cell division in the growing embryo therefore partition the large mitochondrial population between daughter cells, without the need for further replication. This dampens the effect of mutation segregation in early cell divisions before differentiation of tissue-progenitor cells (see *Supplementary Methods*). But it still allows mutation segregation by the time gametes are produced from somatic cells at the end of development. With multiple tissues, a larger zygotic mitochondrial number lowers the risk of one tissue inheriting a disproportionate share of mitochondrial mutants; the fitness of individual tissues becomes more equal, improving adult fitness. Note that the development of an oocyte does not alter the total input of mutations per generation due to replication errors (μ_S). Nor does it impact on the net input of background mutations (μ_B), which is simply a function of lifespan unchanged by oogamy.

The second consequence of oogamy is that it induces partial uniparental inheritance, as more mitochondria derive from a single parent. Partial uniparental inheritance decreases mitochondrial variance *within* individuals, but increases variance *between* individuals *(22*), that is, cells within individuals become more clonal, but the progeny of a particular mating tend to differ more in the clones of mitochondria they harbor. That in turn improves adult fitness while facilitating selection between individuals (*22*). These two consequences make oogamy advantageous, with the benefit rising with the degree of gamete asymmetry (Fig. 4A-B). The benefit eventually plateaus or declines. With a very large oocyte and hence large population of mitochondria in the zygote, the suppression of segregational variance carries through to the production of gametes in the next generation and this weakens the response to selection, just as with an increase in mitochondrial number per cell (Fig. 2B, see also *Supplementary Material* eq. 8). This can overwhelm the advantage of oogamy in simple organisms with a single tissue (Fig. 4A), especially if the background mutation rate is relatively higher (Fig. 4B) or if the number of mitochondria per cell is higher (Fig.S2). In more complex organisms with multiple tissues, oogamy is more strongly favored, as it diminishes variation between tissues and more dramatically increases somatic fitness; this benefit outweighs the loss of variance between gametes at higher Q (Fig. 4A-B). Our hypothesis predicts that the optimal size of the female gamete should increase with increasing somatic complexity, i.e. larger soma and more tissues.

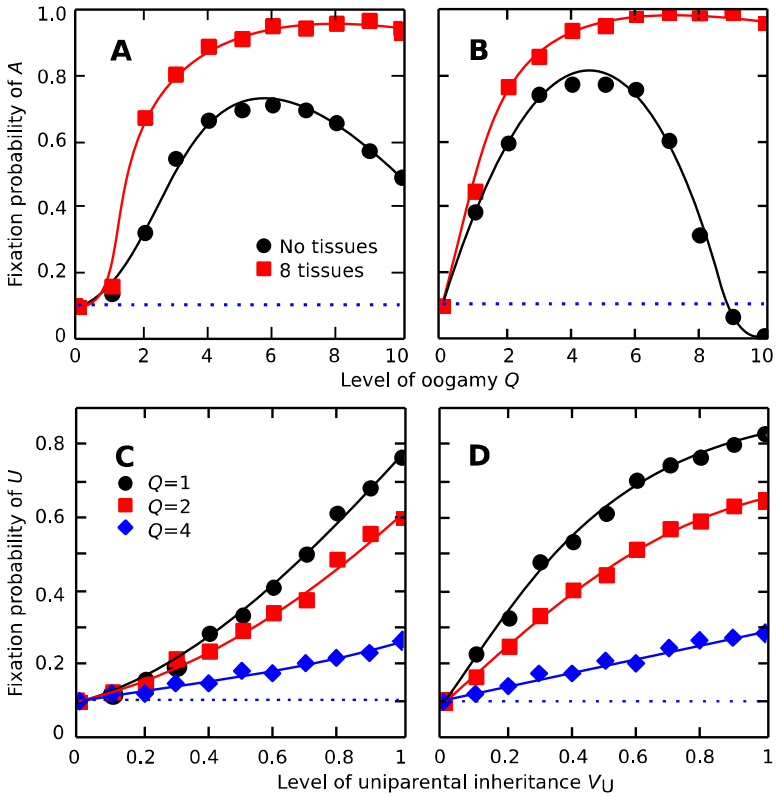

Fig. 4. Evolution of oogamy and uniparental inheritance of mitochondria. (A) Selection for oogamy (determined by the *A* allele) is stronger with multiple tissues (red line) than in organisms lacking tissue-level organization (black line). Oogamy increases gamete size in one mating type. The level of oogamy (Q) is the number of rounds of mitochondrial replication without cell division, forming a large zygote with $2^Q M$ mitochondria. The benefits of oogamy are greater with multiple tissues as lower variance between tissue-precursor cells has a greater effect on adult fitness than in organisms without tissues. Background mutation $\mu_B = 0.0005$. **(B)** Oogamy is favored over a lower range of Q when the background mutation rate is increased to $\mu_B = 0.005$, in particular in organisms without tissues. **(C)** The *U* allele destroys mitochondria in sperm; V_U is the fraction of male mitochondria excluded by the oocyte ($V_U = 1$ is full uniparental inheritance). Exclusion of male mitochondria (V_U) is evolutionarily advantageous, but selection is undermined by the prior evolution of larger oocytes (higher Q). Background mutation rate $\mu_B = 0.0005$. **(D)** A higher background mutation rate ($\mu_B = 0.005$) produces slightly stronger selection for uniparental inheritance, with higher Q again lessening selection for *U*. Neutral alleles fix with probability of 0.1 (dotted line). Other parameter values $M = 50$, $\mu_S = 0.001$.

Oogamy results in biased inheritance of mitochondria from the female gamete. To achieve complete uniparental inheritance in addition requires rejection of mitochondria from the opposite mating type (i.e. sperm), which is achieved in nature by a diverse range of mechanisms; for example via ubiquitination or physical exclusion (*23*). This is favored because a reduction in the number of mitochondria inherited from sperm enhances zygotic variance and the response to selection (Fig. 4C-D). However, selection for less sperm input is weaker if oogamy has already established an asymmetry in mitochondrial inheritance (i.e., with $Q > 0$, Fig. 4C-D). Oogamy may be a necessary precursor, as it releases gametes of the opposite mating type from a role in provisioning of the zygote, allowing "males" to specialize on small, numerous gametes that are designed to be good competitors for fertilization (*24*).

The model predicts that organisms with low μ_S and high μ_B will tend to evolve anisogamy, both oogamy and non-transmitting sperm, as seen in basal metazoans, including sponges, corals, and placozoa (*25*). Under these conditions there is little likelihood of selection for early sequestration of a germline, as maintenance of mitochondrial quality is readily achieved through segregation and gamete asymmetry. But if copying errors increase in frequency (i.e. μ_S rises), somatic gametogenesis is threatened by the faster accumulation of mitochondrial mutations. This provides the selective force favoring sequestration of a germline in which mitochondrial division is suspended, reducing the mutation rate to that caused solely by background physical disruption of mtDNA (μ_B). The model shows that increasing μ_S indeed favors early germline sequestration, although selection is considerably weaker if oogamy and uniparental inheritance of mitochondria (i.e. non-transmitting sperm) have already evolved (Fig. 5), as is generally the case. Importantly, selection for early germline sequestration is much stronger if μ_B is lowered specifically in female germ cells (Fig. 5), as low μ_B reduces mutation accumulation within a single generation, attenuating the need for segregation of mutants among gametes. Oocyte mitochondria are indeed transcriptionally suppressed and physically protected from background mutations in the ovaries of many bilaterians (*26-28*).

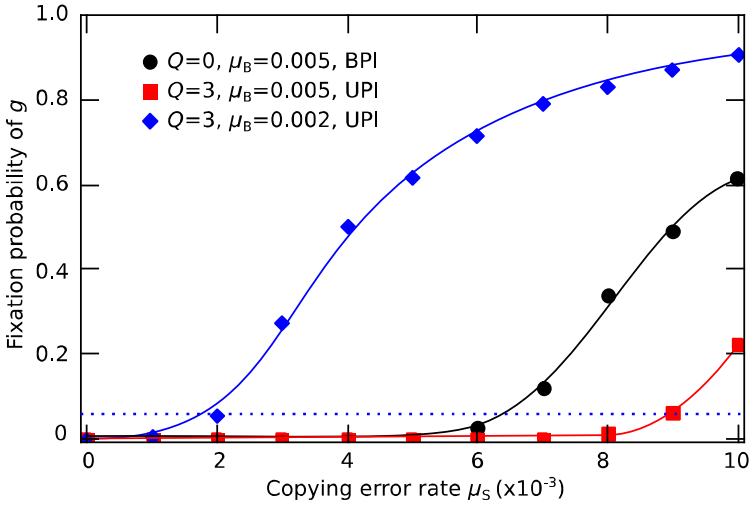

Fig. 5. Increasing copying error mutation rate drives germline evolution. The *g* allele codes for early germline sequestration in an organism with 8 tissues and 50 mitochondria per cell (L_g = 3; somatic gametogenesis has gamete differentiation at L_G = 10). Fixation of *g* is favored as the copying error rate (μ_S) rises. In the ancestral state of isogamy (Q = 0) and biparental inheritance (BPI, V_U = 0) *g* fixes readily with increasing μ_S (black line). However, fixation of *g* is depressed if anisogamy (Q = 3) and uniparental inheritance (UPI, V_U = 1) have already evolved (red line). In both these cases, the background mutation rate is high (μ_B = 0.005). Decreasing background mutation rate (μ_B = 0.002) increases the probability of early germline fixation (blue line) even in organisms with anisogamy (Q = 3) and uniparental inheritance (V_U = 1). Neutral allele fixes with probability of 0.05 (dotted line).

Why did the mitochondrial mutation rate, μ_S, increase in bilaterians? Rising oxygen levels in the late Neoproterozoic (*29*) enabled predation for the first time, because energy conservation from aerobic respiration approaches 40% compared with less than 10% for fermentation (*30,31*). Sustaining a viable population requires conservation of around 1% of the energy harvested by primary producers (*30*). Aerobic respiration therefore supports 5 or 6 trophic levels, and fermentation no more than two, making predation virtually impossible in anoxic worlds (*30*). In

the early Cambrian, predation drove an evolutionary arms race leading to greater size and physical activity in bilaterians (*31*,*32*). Greater activity necessarily increased rates of tissue turnover, protein synthesis and replication of mitochondrial genes, contributing to an elevated μ_S relative to sessile early metazoans. Accumulating mutations in nuclear genes associated with greater metabolic work can theoretically favor differentiation of the germline (*33*-*35*). Our model indicates that the germline–soma distinction may have arisen more specifically as the outcome of increasing mitochondrial mutation rate (μ_S), which directly reflects metabolic work, and which simultaneously explains the differentiation of the germline and the evolution of uniparental inheritance as well as male–female gamete dimorphism. Testes have a high metabolic rate, yet sperm pass on nuclear DNA, implying that mutations in nuclear genes induced by metabolic work have little to do with germline evolution in males. In contrast, sperm usually do not pass on mitochondrial DNA (*23*), which releases constraints on the male germline, permitting highly proliferative sperm production throughout life.

Intriguingly, if μ_S was initially high (which is not supported by the mitochondrial genomes of basal metazoans) then the model shows that an early germline and strict uniparental inheritance (rejection of male mitochondria) evolve rapidly (Fig. S1D) but in this case oogamy does not evolve (Fig. S3). No metazoans lack anisogamy, and this may be an accident of their evolution from ancestors with low μ_S. Other multicellular organisms such as slime molds do not have anisogamy, but do have strict uniparental inheritance, as predicted by the model if their mitochondrial mutation rate is high. This seems to be the case in *Dictyostelium discoideum*, in which the mitochondrial mutation rate is nearly as high as that of *Drosophila melanogaster*, despite an extremely low nuclear mutation rate (*36*). More tellingly, the early sequestration of a dedicated germline may have evolved independently in Ctenophores (*4*), which have extremely high mitochondrial evolution rates (*37*), and strong differentiation including muscle and neural tissues (*38*), again as predicted by the model.

For the last thirty years, the dominant explanation for the evolution of the germline has been that it was essential for the emergence of multicellular organisms through the suppression of selfish conflict between the cells that make up an individual. At its heart, this viewpoint lacks a rationale for the evolutionary stability of numerous organisms that lack a dedicated germline. It also sees the restricted mutational environment of the female germline as of secondary importance. Here we have turned this paradigm on its head, locating the key selective pressure as lying in the resistance to mutation accumulation amongst mitochondria. Selection for mitochondrial quality can explain the stability of somatic gametogenesis, as well as the evolution of oogamy, non-transmitting sperm and the germline, in the simplest terms. Germline sequestration in turn released constraints on mutation accumulation in somatic cells, facilitating tissue specialization, greater activity, and ultimately a disposable soma with emergent phenomena like ageing and death (*39*).

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