What can we infer about the origin of sex in early eukaryotes?

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Current analysis shows that the last eukaryotic common ancestor (LECA) was capable of full meiotic sex. The original eukaryotic life cycle can probably be described as clonal, interrupted by episodic sex triggered by external or internal stressors. The cycle could have started in a highly flexible form, with the interruption of either diploid or haploid clonal growth determined by stress signals only. Eukaryotic sex most likely evolved in response to a high mutation rate, arising from the uptake of the endosymbiont, as this (proto) mitochondrion generated internal reactive oxygen species. This is consistent with the likely development of full meiotic sex from a diverse set of existing archaeal (the host of the endosymbiont) repair and signalling mechanisms. Meiotic sex could thus have been one of the fruits of symbiogenesis at the basis of eukaryotic origins: a product of the merger by which eukaryotic cells arose. Symbiogenesis also explains the large-scale migration of organellar DNA to the nucleus. I also discuss aspects of uniparental mitochondrial inheritance and mitonuclear interactions in the light of the previous analysis.

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1. Introduction

When looking at the enormous diversity of present-day meiotic eukaryotic sexual systems and how they are integrated in highly complex life cycles, it is hard to imagine them being ultimately derived from the combination of a set of diverse unicellular, prokaryotic, repair mechanisms. However, recent insights make it more and more likely that the basic meiotic cycle appeared upon the formation of the first eukaryote [1]. A highly insightful hypothetical reconstruction of the origins of eukaryotic sexual reproduction from humble (archaeal) beginnings is given by Goodenough and Heitman [2]. Complete meiotic sex is characterized by four separate, but interlocking, mechanisms involved are as follows: (i) Ploidy alterations: increases via cellular fusion processes and reductions via meiosis; (ii) cell–cell fusion regulation, which started out with the fusion of similar cell types (isogamous fusion) and later became anisogamous; (iii and iv) coupling mating-type allele regulation to (differential) organelle transmission and to (adaptive) spore formation [2]. In (archezoan) theories that postulated eukaryotic evolution prior to endosymbiont entry, organelle transmission was seen as a later addition to a fully operational meiotic mechanism, but nowadays meiotic sex is seen as one of the set of processes that probably arose in reaction to endosymbiont entry [3]. To be precise, in reaction to reactive oxygen species (ROS) stress (see [4–7] and references therein). Thus, (the regulation of) organelle transmission had to be around from the get-go.

For a lot of pertinent mechanisms, we can readily envisage how they probably evolved from prokaryotic forerunners [2]. A hyperthermophilic archaeon species displays cell–cell fusion [8]. Gene exchange upon cell fusion has been observed in
some Archaea, occasionally triggered by conditions of stress [4,9]. Sequential steps characterizing meiosis clearly evolved from mechanisms catalyzed by (homologous) DNA repair enzymes, again induced by ROS. A prime example of such a protein is Spo11. This is an archaeal topoisomerase VI homologue that retained its ability to introduce the crucial double-stranded breaks, while losing its ligase ability. Also, proteins of the RecA recombinase protein family, such as DMC1, which is involved in finding allelic DNA sequences on homologous chromatids during meiosis, share a common prokaryotic ancestor. DMC1 arose very early in eu- karyotic evolution [10]. Thus, meiotic recombination (essential in many explanations for the retention of sex, in spite of its costs—see below—though not as crucial for theories regarding the origin of sex) must have evolved from more simple repair mechanisms of damaged DNA strands [11]. As a last example, archaeal/bacterial changes in the expression of receptor molecules upon encountering stress conditions possibly evolved into a system of regulated mating-type molecule expression [2]. In conclusion, although many specific pathways remain to be elucidated, it is not difficult to imagine how the complete meiotic sex machinery evolved from the integration of a set of diverse archaeal and bacterial precursor proteins. This set became available upon merging a lokiarchaeon-like host [12] with a bacterium related to present-day Caenorhabditis elegans [3]. So we can envisage the ‘how’ for the evolution of sex; what about the (combination of) ‘why’s’?

2. Sex: who needs a reason?

It is almost a cliché to state that sex implies relatively high costs. However, one should critically look at the assumptions implicit in such a statement, as elegantly explained in Lehtonen et al. [13]. Of note, the first step from asexuality to meiotic isogamous sex might not have represented a high cost at all [13], allowing the ‘smooth’ development from repair mechanisms described above. Still, meiosis, the key step, is capable of breaking up unfavourable gene combinations, and sexual reproduction normally requires mating partners, which are not always easy to find. Even in ancestral isogamous sex, the possible exploitation of the opposite mating type can translate into less efficient conversion of environmental resources into offspring, and given two sexes (anisogamy), their divergent evolutionary interests can reduce the efficiency of resource allocation even further [13–15]. The first cost, breaking up good combinations, is probably less problematic than imagined, because the population of sexually derived offspring will in most cases also contain individuals close to the local optimum of favourable gene combinations. Even so, meiotic sex clearly is a complex, time consuming and in many instances costly process that has to have ‘compensating’ benefits.

The three most general descriptions of evolutionary advantages of recombinatorial meiotic sex are as follows [14,16]:

(i) it breaks up unfavourable allele combinations and thus allows rapid adjustments under fluctuating selection;
(ii) it allows quick searching for better combinations of alleles; tapping the ‘unlimited’ hidden genetic potential of genes in finite, ‘limited’, populations (compare general considerations in [17]);
(iii) it protects the genome against mutational meltdown: i.e. it allows the organism to fight off Muller’s ratchet [18].

First of all, it cannot be stressed enough that the explanations proffered are not mutually exclusive, and one or the other can be dominant depending on the specific instance studied (see below). The first two explanations can be seen as two sides of the same coin; sexual recombination upon meiotic crossover allows rapid gene reshuffling, quickly both getting rid of bad combinations (i) and finding good ones (ii). Even the last explanation (iii) can be described as such: quickly finding combinations of good (meaning still unharmed by mutation) genes. More efficiently purging deleterious combinations (i) was demonstrated using (a)sexual forms of the water flea Daphnia pulex [19,20]. The ratio of non-synonymous to synonymous nucleotide substitution (Kω/Kσ; which should go up in the absence of efficient purging) in the 13 mitochondrial (t) protein genes was indeed much higher in asexual lineages. Because we are looking at mitochondrial genomes here, it should be clear that this improved ratio is of course not obtained via recombination processes in mitochondrial DNA, but via more efficient mitochondrial purging in sexual lineages. Modelling shows that enhanced genetic robustness (relative strength to cope with mutations) and negative epistasis (deleterious mutations reinforcing each other, allowing efficient sexual purging) coevolve in organisms capable of meiotic recombination [20,21].

‘Other’ explanations include the famous ‘Red Queen’ hypothesis, which states that sexual recombination allows organisms to keep up with each other in the arms race between hosts and parasites, imagining organisms to be doomed without it [22,23]. As mentioned, a lot of the confusion regarding the validity of this theory (or any theory, for that matter) in explanations for the retention of costly meiotic sex comes from the unfortunate tendency to single out one hypothesis, excluding others, while in fact different models might be more or less important depending on the example studied. Quite a few studies support the arms race model. Using Caenorhabditis elegans as a host and the bacterium Serratia marcescens as parasite, the Red Queen hypothesis was tested. Caenorhabditis elegans populations, propagating either sexually, by self-fertilization, or by using both strategies, were exposed to the bacterium. Self-fertilizing populations were indeed rapidly driven extinct by parasites while outcrossing sexual populations were not. Thus, in this case, coevolving pathogens selected for biparental sex [24]. Studies using freshwater snails (Potamogeton pygmaeus) had already demonstrated clonal populations to be much more sensitive to coevolving parasitic challenges than sexual populations [25]. Earlier work with the same organism had also demonstrated the cost of sex, the clonal population outcompeting the sexual one under ‘ideal’ laboratory conditions [26]. In a very recent study, Drosophila melanogaster was even shown to increase the relative amount of recombinant offspring upon infection (using gram-negative bacteria such as S. marcescens or Providencia rettgeri as well as the parasitic wasp Leptopilina clavipes). This response seems to be caused by transmission distortion rather than by increased recombination [27]. However, studies not supportive of the Red Queen hypothesis are also often referred to. Hanley et al. [28] compared the amount of mites in a parthenogenetic gecko species with amounts in two related sexual ancestral...
species. Mite infestations were significantly higher in the sexual species in this habitat. Of course, this can be due to the spread of mites by sexual encounters. In humans, a lot of very nasty parasites and pathogens spread sexually; as such I do not consider these instances as disproof of the Red Queen hypothesis. Interesting population genetics models try to analyze the impact of species interactions (such as host–pathogen interactions) on the evolution of sex. Surprisingly, most species interactions seem to select against sex [29]. The authors conclude ‘…although the Red Queen favours sex under certain circumstances, it alone does not account for the ubiquity of sex’. In conclusion, some examples of the hypothesis in action are convincing, but it is clear that it cannot explain the ubiquitous presence of sex on its own, which seems only logical as it represents only a specific subset of general theories (i) and (ii).

Other explanations for the retention of meiotic sex can be subsumed under the heading of ‘promotion of genetic diversity’. It has been proposed that as the environment is unpredictable, creating diverse offspring enhances the chances of having some successful individual organisms among them [30] or that sex allows more efficient energy extraction from the environment as there will be a range of slight differences in sexual populations [31]. All these aspects can of course play a role and might occasionally be involved in the maintenance of sex in populations; however, these ideas are at the same time rather vague and obvious. In this context, the term ‘evolvability’ is apt: a sexual diverse population has a much better chance of coming up with adaptive combinations when confronted with a (rapidly) changing environment; thus, sex would be retained over longer evolutionary time scales, because organisms opting out would pay the ultimate prize for temporary success (see [1,14,15] and references therein). However, all these theories smack of teleology. These models might explain retention but not the origin of the eukaryotic meiotic system. In that regard, countering mutational meltdown (iii), with mechanisms directly evolving from DNA repair mechanisms, is by far our best bet. Before explaining that combatting mutational meltdown was the driving force at the ‘origin’ of meiotic sex, I will try to show that efficiently purging mutational load is still a very powerful raison d’être for the ‘retention’ of sex as well. Surprisingly, we need a rather late development in the sexual saga, the process of sexual selection, to demonstrate that efficiently purging mutational load is what sex is all about. Let us briefly look at a recent study of a ‘recent’ mechanism [32].

3. Intermezzo: sexual selection shows up mutational load

Sexual selection operates mostly via male–male competition and/or female choice (though in much more exciting and interesting forms than previously thought, see e.g. examples of rapid evolution and speciation or of sexual antagonistic coevolution, the arms races of sexual organs [35]). It could work as an important filter against mutational load provided that, as stated by Darwin, ‘sexual selection will have given its aid to ordinary selection’ [34, p. 127]. This extra, intraspecific, level of selection can of course only work if enough of the genetic make-up of the organism influences its sexual success, i.e. when many traits influence the relative success when confronted with competition and choice, a situation known as ‘genic capture’ (success depends on overall condition, which in turn depends on many genes [35]). A beautiful new study uses differences in sexual selection to show that sex indeed can counteract mutational meltdown [32]. Lumley et al. use populations of the flour beetle Tribolium castaneum bred for 7 years, equivalent to about 50 generations, under the following conditions: males/females 10:10 (male biased); 10:90 (female biased); 5:1 (polyandry); 1:1 (forced monogamy). Male–male competition is considered a stronger selection force than female choice, and females have larger male populations to choose from in the first set-up, so male-biased populations are expected to show the strongest effect. Upon completion of these enforced regimens, flour beetles were challenged with continuous inbreeding, in a 3-year extinction assay. The conclusions were as clear as they were stunning: when comparing, on average, male-biased families with female-biased families, the former survived 44% longer; when comparing polyandrous families with monogamous families, the former survived 37% longer. Reproductive fitness (the number of offspring per generation of inbreeding) told the same story. One can conclude that in this model system, mutational meltdown is prevented by the meiotic system of reshuffling followed by constant weeding out of mutations using sexual selection, before such mutations can get to fixation in the population. This is of course only shown via the indirect measures of resistance to extinction and reproductive fitness, and a large-scale sequencing effort might throw up some surprises with regard to the actual mutational hotspots responsible. In conclusion, we have seen that complex animals use meiotic sex to prevent Muller’s ratchet, explaining its retention in this example. Could it also have been the driving force for the evolution of meiotic sex in the first place, as suggested above? Let us return to the dawn of the eukaryotes.

4. Did ‘heavy breathing’ lead to sex?

It seems clear that the recombinatorial sexual system is derived from repair systems induced by stress (see above). When we try to reconstruct the sequence of events at the basis of eukaryotic development, a few important aspects have to be taken into account. First of all, all extant eukaryotes are derived from an endosymbiotic organism (the last eukaryotic common ancestor, LECA) which arrived relatively late on the evolutionary scene. Eukaryotes only arose approximately 1.6–2.1 billion years ago [36], decidedly later than the Great Oxygenation Event (GOE) 2.3 billion years ago [37]. They took their time, especially compared with the ‘instant’ evolution of prokaryotes which are at least 3.5 billion years old [38,39]. Eukaryotes are the result of a merger between a lokiarchaeon-like host [12] and an α-proteobacterium-like endosymbiont, becoming the mitochondrion. However, I should mention again that there are two competing models for the origin of the eukaryotes, the ‘symbiogenesis’ (merger) model described here and the ‘archezoa’ hypothesis [40]. In essence, the latter hypothesis states that a complex amitochondriate eukaryote was able to take up and accommodate a bacterium, while symbiogenesis proposes that converting an archaean to a eukaryote was made possible by, and thus resulted from, this uptake. The latest analyses fully support the eukaryote as a ‘recent’ partnership between an archaeon and a bacterium [41]. Thus, symbiogenetic theories are more widely
accepted [42], but gradualist archean models still permeate recent publications [43,44]. Practically all the eukaryotic hallmarks (e.g. the nucleus, endoplasmatic reticulum, peroxisomes and meiotic sex systems) can be explained by evolutionary forces unleashed by the merger of the two founding cells [3]. In this context, it should be stressed that further organelle formation, even that of the chloroplast, and the, much later, eukaryotic uptake of eukaryotes by phagocytosis (for ongoing examples, see [43]) would develop along pathways set during the primordial bacterium to mitochondrion conversion (see below). Secondly, as mentioned, this occurred after the GOE, which means that oxygen, both as the final acceptor for oxidation processes and as a source of high amounts of internal ROS coming from the endosymbiont, enters the picture. ROS, such as singlet oxygen, $^1\text{O}_2$, superoxide anions, $\text{O}_2^-$, hydroxyl radicals, $^\cdot\text{OH}$, and hydrogen peroxide, $\text{H}_2\text{O}_2$, are highly toxic metabolic oxidation by-products, which can give rise to very high levels of lipid, protein and DNA damage. It should be stressed that due to the pleiotropic effects of oxidative modifications on all major groups of macromolecules, even more oxidative damage might ensue, giving rise to a vicious circle possibly leading to even higher mutation levels [45]. Thirdly, symbiogenesis allowed a quantum leap in opportunities in respect to ATP generation, both in regards to substrate versatility (the different metabolic pathways of the two founding cells being combined) and in respect to efficiency (internal oxidative phosphorylation by ‘mitochondria’). The momentous endosymbiont to organelle conversion and many other ‘expensive’ cellular adaptations are paid for by the fact that ATP generation is no longer limiting. Yes, meiotic sex is very costly, but eukaryotes are able to pay.

5. A ‘double whammy’: more information to protect against more mutation

It becomes clear that under these circumstances a meiotic system almost had to evolve when we consider simple basic aspects of Muller’s ratchet: it depends on only two variables, i.e. total information content and mutation levels [18]. Both go up upon the merger of the two founding organisms. The metabolic versatility referred to is of course dependent upon the presence of two mostly (?) non-redundant genomes. Of note, ‘information content’ is a better description than DNA content as the total amount of DNA does not (have to) correlate with amount of information stored. In the case of diploid cells (at the basis of heterosis, i.e. hybrid vigour, already described by Darwin [46] when discussing the benefits of outcrossing), the ‘double’ DNA content can be efficient in masking the effects of mutations precisely because of informational redundancy. This is of course the reason why so many organisms have dominant diploid stages in their life cycles. The extra DNA in diploid cells most likely originated in the context of (meiotic) recombinatorial repair induced by the presence of mutations. This brings us to the second and probably much more important variable: mutation level. With the advent of the oxidizing pre-mitochondrion, the archaean finds itself with an endogenous ROS (mutation) generator. Not only will there be endogenous ROS formation, but integrating metabolic pathways will lead to enhanced internal ROS formation. Why? The archaenal host possibly metabolized carbohydrates, and the endosymbiont most likely encoded (aerobic) catabolic pathways using carbohydrates, lipids, glycerol and amino acids, with at least a few of the citric acid cycle enzymes, a fatty acid (β) oxidation pathway and a respiratory chain. Interestingly, the currently cytoplasmic glycolysis pathway seems also partially (?) derived from the endosymbiont and not from the host, which might have been (related to) a hydrogen-metabolizing archaean [47]. Be that as it may, different catabolic substrates (oxidized using the pathways mentioned) generate different relative amounts of the electron carriers FADH$_2$ and NADH (different $F/N$ ratios) to be oxidized by the respiratory chain (glucose at $F/N = 0.2$, long-chain fatty acid at $F/N$ approaching 0.5) [48]. This simple observation elegantly explains why eukaryotes evolved peroxisomes early on (as an alternative β-oxidation performing organelle, without the generation of FADH$_2$ to be burned in the mitochondrion) and why long-living eukaryotic cells (such as neurons) minimize β-oxidation to lower endogenous ROS formation [48]. It might be instructive to list the other adaptations explained by enhanced endogenous ROS formation. The almost complete bacterial (mitochondrial) genome reduction as a result of migration of genes to the host genome can be understood as an ‘escape’ from the area flooded by ROS (the mitochondrial genome is attached to its internal membrane [49]) to a more distant area protected by the nuclear membrane. Thus, nuclear membrane formation itself could have come about under pressure of pre-mitochondrial ROS formation (but see also [50]). Large cellular size increase to dilute ROS and the advent of diploid chromosomes, as well as ongoing improvements in DNA repair and replication in the nucleus, would also make sense. The evolution of autophagy, together with the cycles of mitochondrial fusion and fission, can be seen as mechanisms selected to specifically rid the cell of mitochondrial (DNA) damaged by ROS [49]. These mechanisms could even be behind the development of uniparental mitochondrial inheritance (UPI) as well (see below). Interestingly, fusion/fission balance is also important for mitochondrial adaptation to different substrates, such as glucose or fatty acids [51]. However, it is not clear when the fusion/fission cycle became part of the eukaryotic repertoire [49,52]. As stated previously, full meiotic sex can be interpreted and understood in the light of high local ROS production (figure 1). (For a more detailed description of the issues involved, see [48,53] and references therein.) Thus, theoretically, more information that has to be protected against higher levels of mutation would give the ideal combination of driving forces to allow meiotic sex to develop. Did it indeed evolve like this?

6. Is eukaryotic sex a symbiogenetic effect due to (proto) mitochondrial reactive oxygen species formation?

When trying to reconstitute whether ROS was the main driving force behind the development of sex, one encounters a severe problem. One of the effects of internal ROS formation at the basis of the eukaryotes was the further development of an extensive array of anti-oxidant measures, ranging from ROS scavenging substances [54] to many different dedicated anti-oxidant enzymatic systems, such as catalase, superoxide dismutases [55] and glutathione peroxidases [56], as well as redox state-regulating enzymes such as thioredoxins [57].
Not only that, but lower levels of ROS have morphed into essential signal molecules, giving rise to the concept of mitochondrial hormesis, in which low levels of ROS are essential in maintaining mitochondrial quality, by control of anti-oxidant pathways [58]. Did the mitochondrial mutation rate go down over time? Present-day eukaryotes vary strongly in mitochondrial mutation rates; thus the ancestral mutation accumulation rate is hard to infer. On the whole, evolution rates of organelle genomes are elevated [59], but their mutation rates can be low in plants [60], many unicellular eukaryotes [61,62] and the ‘oldest’ metazoa [63,64]. Mitochondrial evolution rates in higher animals and fungi tend to be substantially higher than their nuclear counterparts [65,66]. Thus, the initially high mitochondrial mutation rate at the dawn of the endosymbiotic merger might indeed have dwindled. Higher mutation rates in animals and fungi then probably came about later as a consequence of active lifestyles and their inherent high metabolic rates. Havird et al. [67], citing studies that identified high rates in unicellular and early-diverging eukaryotic lineages, maintain an ancestral high mutation rate with the low rate of many land plants as a derived condition. Importantly, in both scenarios, we start out with a high mutation rate however.

Bearing these complexities in mind, it is almost surprising that recent research can find some links between the occurrences of ROS-related stress and meiotic sex. But in 2013, Ho¨randl and Hadacek published ‘The oxidative damage initiation hypothesis for meiosis’ in which they also theorized that sex evolved (in part) from stress responses to the DNA-damaging effects of ROS [68]. Nedelcu [69], studying Volvox carteri, a multicellular green alga, found many sexual inducer (SI) and several SI-induced extracellular matrix protein genes to be induced under various stress conditions. Strikingly, in the presence of anti-oxidants, the alga is not able to start its sexual cycle. Further indirect evidence comes from incubations with iron chelators, as iron serves as an important cofactor in ROS formation, explaining why its metabolism is tightly regulated in ‘oxidative’ organisms, being both essential and toxic [70]. These chelators also inhibit sexual induction in this organism [71]. Recently, oxidative stress was shown to correlate with levels of (sexual) meiosis in a facultative apomictic (asexual) plant, Ranunculus auricomus [72]. In mammals, moderate increases of ROS were shown to trigger meiotic resumption in (rat) follicular oocytes [73].

7. Indirect evidence from ‘asexual’ eukaryotes

However, as becomes clear from these examples, the ‘evidence’ for the role of ROS in meiotic triggering is not abundant, as is to be expected given layers of later adaptations. Another way of looking at the presumed correlation between ROS and sex is to turn our attention to clonal species (i.e. species that really have lost the molecular machinery needed for sex, without paying the ultimate price of extinction). Real long-lived clonality turns out to be much more rare than once thought (see [1] and references therein) as clever studies looking for the presence of ‘sex genes’ using a meiosis detection toolkit pointed out [74]. Very few instances have been found where genome sequencing allowed us to remain convinced of a strictly asexual
nature for the eukaryote studied. One startling example of ‘hidden sex’ is found in *Ostreococcus tauri*, a planktonic coccoid green alga, the smallest free-living eukaryote we know of [75]. Sexual processes could not be found, whether studying *O. tauri* or its relatives. Meiotic genes, however, were present in its genome [75]. Population genetic studies, following eight nuclear markers on two chromosomes, pointed to genetic recombination [76]. The study states sexual encounters to be extremely infrequent, estimating the relative rate of meiosis/mitosis to be approximately $10^{-6}$! So even in populations that seem to be able to considerably lengthen their clonal stages in constant environments, the option of meiotic recombination is practically always retained. This again stresses the importance of fighting Muller’s ratchet. Real long-lived clonal eukaryotes, having irreversibly lost the option of meiotic sex, do exist however, and their characteristics strengthen the correlation between ROS and sex. Bdelloid rotifers, such as *Adineta vaga*, genome sequencing of which gave conclusive evidence for the absence of meiosis and sex, are the prime example [77]. These organisms have quite a lot to tell us. First of all, bdelloids seem to have reverted to more ‘prokaryotic’ ways to battle mutations. Genomic analysis shows the following: (i) the use of gene conversion, limiting the accumulation of mutations in the absence of meiosis; (ii) an increase of genes defending against transposons, with transposable elements being restricted to 3% of the genome; (iii) highly abundant horizontal gene transfer, 8% of genes probably having been obtained by this mechanism. An important conclusion in the light of the ‘ROS-sex connection’ proposed here: gene families involved in ROS resistance are strongly expanded as well [77]. To be able to survive desiccation, regularly occurring in their cyclically drying freshwater habitats, bdelloid rotifers evolved extraordinary resistance when confronted with ionizing radiation and concomitant ROS. This evolving capability (preventing extensive oxidative DNA damage) may have rendered meiotic sex repair mechanisms redundant [77]. These observations regarding bdelloids are in good agreement with the hypotheses regarding the benefits and origin of sex described above. They also predict that certain eukaryotes (e.g. those living in anoxic sediments or animal intestines, especially when habitat variation would be either of a predictable kind or absent) might show a more pronounced tendency to become asexual.

The interaction between diversifying environments and the plasticity of meiotic sex systems has given rise to enormous diversity in (sexual aspects of) living systems (e.g. [33] and the rest of this special issue). Thus, the ‘evolvability’ of the meiotic sex system, as well as the interactions of occasionally conflicting selection levels (genes, organelles, sexes), leads to a bewildering variety. Are there relative constants amidst all this diversity and do they fit with the model regarding the origin of meiotic sexual systems presented here?

8. The origin of uniparental mitochondrial DNA inheritance

Sexual diversity is endless. To give but a few examples: haploid stages can be the dominant life cycle (e.g. in algae such as *Chlamydomonas reinhardii* and fungi like *Schizosaccharomyces pombe*), males are (much) bigger or (much) smaller than females, the male seahorse ‘bears’ offspring, animals can change their sex, mammals determine sex internally (by their gene content, but in a more complicated way than was imagined [78]), while poikilothermic reptiles use an external clue, temperature [79], and even parthenogenesis can be sperm dependent [80]. Even the rule that seems rather strictly adhered to—mitochondria and their DNA come from only one parent (UPI), the ‘female’ in most cases—is not completely followed (exceptions are briefly discussed below and in [81]). But this ‘strict’ UPI in eukaryotes, even isogamous ones, needs explaining.

Let us look at some of the theories proposed in this context. First of all, uniparental organellar inheritance is hypothesized to come about because it stops the spread of selfish cytoplasmic DNA (but see below). However, because of the clonal nature of organellar DNA, this is interpreted as an evolutionarily unstable situation with mutations piling up over time. Reversal to biparental inheritance should then occur, according to Greiner et al. [82]. But uniparental (mostly maternal) mitochondrial DNA inheritance dominates the evolutionary landscape, and theoretical studies find mutations accumulating faster under biparental transmission of mitochondria. UPI decreases within-cell mitochondrial DNA variation but increases variation between cells, leading to efficient purifying selection of cells with higher levels of mutations [83]. In fact, the combination of UPI with multi-level transmission bottlenecks (so absent mitochondrial recombination) seems to give rise to purifying selection levels that are indistinguishable from those found for recombining nuclear genes [84]. Modelling studies even suggest that selection against heteroplasmy is enough to explain the evolution of UPI [85]. Heteroplasmy was indeed shown to be genetically unstable and physiologically detrimental in a mouse model [86]. Lastly, uniparental inheritance might favour mitochondrial-nuclear coadaptation [87], but see below.

There is no reason to think that sex did not evolve first in the most simple form possible. The initial mode of sexual reproduction during early eukaryotic evolution probably has been unisexual (allowing all gametes to mate) with biparental inheritance of mitochondria. Indeed this has been proposed recently [88]. However, LECA might already have had UPI, considering its universal distribution. I have alluded to the fact that autophagy, in conjunction with the cycles of mitochondrial fusion and fission, can be understood as a mechanism to specifically select ROS-damaged mitochondrial DNA for destruction (via so-called mitophagy) [49] and that these mechanisms could be involved in the development of UPI. If fusion and fission cycles were indeed early eukaryotic developments, then the high mutation rate of mitochondrial DNA located next to the ROS-producing respiratory chain would give rise to problems whenever biparental mixing upon gamete fusion occurred. Combination of mutations in genes encoding subunits of the mitochondrial molecular machines (ribosomes, the complexes involved in oxidative phosphorylation) would lead to negative epistasis and mostly impaired mitochondrial function upon organelle fusion. Another way of looking at this: UPI could facilitate the removal of deleterious mitochondrial mutations, while biparental mitochondrial mixing might ‘homogenize’ individuals and lower mitochondrial fitness. This might overwhelm efficient mitophagy, interfere with selection and give rise to accumulation of mutations. Compare, for example, the mathematical model in Hadjivasiliiou et al. [83].
9. Higher mutational loads in one gamete type and retention of uniparental mitochondrial inheritance

Here, I will leave aside the speculations regarding the origin of UPI and focus on interesting present-day correlations between gamete differences and their organelar contributions to the next generation. We will not start from the perspective of genes and potential conflicts (e.g. [89]), but from the perspective of metabolism and physiology, though these do not have to be mutually exclusive. Clever insights of John F. Allen [90], using that vantage point, elucidate nicely what could be going on in multicellular organisms with clear anisogamy. After observing that ATP generation correlates with ROS and thus with mutations, Allen states (and I quote): ‘Motility of one gamete is required for fertilization, and requires ATP. It is proposed that male gametes maximize energy production for motility by sacrificing mitochondrial DNA to electron transfer and its mutagenic by-products, while female gametes, which are non-motile, repress mitochondrial oxidative phosphorylation’ [90, p. 135]. Actually, the Catch-22 situation for paternal mitochondrial DNA is even worse. Via direct competition, sperm is actively selected to be the fastest conveyance for the paternal chromosomes. This means that sperm should use mitochondrial β-oxidation (fatty acids containing 2-2 fold the amount of energy of sugars per gram). Although the occurrence of β-oxidation in sperm was doubted, recent studies of the human sperm tail [91] show it to be important. As mentioned above, this would lead to high ROS formation [48]. Outcompeting and even actively excluding the highly mutated mitochondrial DNA is thus strongly selected for. A few points should be stressed: (i) already early on in evolution, upon anisogamy, one of the two flagellated single-celled eukaryotes could take up a more active part in fertilization if it was the one that contributed less or even no mitochondrial DNA under the developing UPI regimen; (ii) on the whole chloroplasts are also maternally inherited, but the rule is somewhat less strict, probably because though they follow in the mitochondrion’s footsteps, these organelles generate less ROS; (iii) upon loss of the need for highly active sperm cells during the life cycle of an organism the strong purging of paternal mitochondria might on occasion be relaxed.

Do the exceptions to the rule of maternal mitochondrial DNA inheritance hold up in the light of the idea that high ROS generation makes for inferior mitochondrial DNA which has to be purged from the next generation? Two known exceptions are found in species of the Mytilidae (sea mussels) and Unionidae (fresh water mussels) which contain both maternal and paternal mitochondrial DNA [92], and some conifers, such as Sequoia sempervirens, maintaining paternal mitochondrial DNA [93]. Exceptions in seed-forming plants are not unexpected as the sperm cells have to be much less active than in animals. The presence of paternal mitochondrial DNA inheritance in animals such as mussels might seem to be more problematic until we take a look at the way fertilization takes place. Mussel sperm is taken along by water currents, and the energy for the final entry of the female is provided by the female incumbent siphon. Thus, high mitochondrial activity in mussel sperm is not selected for, which might allow ‘parent switching’ upon (viral) uptake of a selfish mitochondrial protein [94]. UPI is still completely retained in these instances, and it should be stressed that there are many examples of unicellular (isogamous) eukaryotes that do not have obvious differences in metabolic activity between their respective mating types and retain classical UPI, e.g. the biflagellate algae C. reinhardtii [95], the pennate diatom Haslea ostrearia [96] and the slime mold Polysphondylium pallidum [97]. All this is in line with the idea that very early on in eukaryotic evolution coexisting UPI defined the mating partner needing more energy (e.g. related to motility) as the ‘male’, excluding its mitochondrial DNA from the next generation. Absent checks of mitochondrial quality in males allowed further selection for optimal sperm motility as well as more cell divisions in the germline, further solidifying maternal inheritance of mitochondria. However, the last three examples make it likely that UPI came about in isogamous species without such differentiation.

10. How important is mito-nuclear coevolution and adaptation?

The origin of meiotic sex at the basis of eukaryotic evolution, beginning with the merger of archaeal host and bacteria branching with the present-day α-proteobacteria, which I describe as resulting from the problems of defending a larger amount of information against mutation by internal ROS formation, has also given rise to alternative (not mutually exclusive) theories regarding the origin of sex. One such theory hypothesizes that recombination provided genetic variation to allow compensatory nuclear coadaptation in the face of mitochondrial mutation accumulation [67]. Sex, because of the need for rapid coadaptation of (originally separate) organisms, smacks of the Red Queen hypothesis, as has indeed been pointed out [98]. The importance of mitochondria for eukaryotic cell functioning can hardly be overstated and appreciation for its ever extending roster of metabolic functions is still growing [99–102]. Though there are of course indications for mito-nuclear coevolution [103,104], this in itself does not mean it has been instrumental in the origin of sexual recombination. The direct influence of internal ROS formation I consider more dominant, as already illustrated by the fact that the most important group of nuclear genes that have to coevolve are the ones encoding proteins that form a direct part of the mitochondrial multi-protein complexes or perform other essential organelar functions, which migrated to the nucleus, probably under ‘ROS pressure’, in the first place. Again, the two simple basic values defining Muller’s ratchet—total information content and mutation levels [18]—both increased upon the merger at the basis of the ‘eukaryotic revolution’. I would conclude that this constellation of driving forces seems sufficient to explain the advent of sex.

However, recent research into mito-nuclear interactions, using mice and fruit flies as model systems, has come up with intriguing results. In a pioneering study, mice with mismatched DNA genomes showed significantly decreased physical performance, compared with progenitors [105]. Coevolution of subunits of complex IV, cytochrome c oxidase, could be demonstrated in fruit flies, where crosses of mitochondrial and nuclear DNA from closely related but different species led to disrupted complex activity in males of interspecific genotypes [106]. That independently evolving genomes would become incompatible is not so surprising; however, the question of the relevance of mito-nuclear incompatibilities
within a population is a different matter. This came to the fore in response to recent discussions in the UK Parliament when it debated legislation to allow mitochondrial replacement (i.e. exchanging ‘mutated’ mitochondria within oocytes for healthy donor female mitochondria) to be used in the clinic. Some scientists raised valid concerns in the light of possible mito-nuclear interactions and asked for extra control experiments before implementing the technique [107,108]. Finally, I just want to raise a few theoretical points regarding the possible influence of mito-nuclear interactions on fitness. ‘Genic capture’ (success correlating with overall condition, resulting from the interactions of many genes [35]), as described above, as well as genetic robustness and negative epistasis [21] complicate the picture. Surprisingly, large mito-nuclear fitness effects on their own do not seem logically compatible with sexual selection. As this contradicts the ‘mito-nuclear compatibility hypothesis of sexual selection’ [109], I will discuss the issue in some detail. Large mito-nuclear effects would translate in strongly perceptible effects of certain combinations of mitochondrial and nuclear genes. A ‘winner’ male (either in direct male–male competition or as the ‘chosen one’ upon female choice) must have a good combination. But his nuclear DNA might be bad in combination with the mitochondrial DNA of the female, and there is no physiological indicator for this situation. Stating that half of the nuclear genome is still inherited from the mother improving the mito-nuclear match is another way of saying that mito-nuclear matching is not important in sexual selection, as this is independent of the choice of male. One might argue that a bad combination can also happen easily upon sexual combinations of nuclear genes only. The best example is two heterozygote parents getting homozygous recessive offspring. But here only one rare hidden danger lurks, while in the case of important mito-nuclear matching, a large multitude of nuclear genes are involved. Arguing that this multitude leads to an average phenotype is again stating that mito-nuclear matching is relatively unimportant. Thus, if there were large effects on phenotype, sexual selection would constitute a useless assessment, as the supposedly crucial mitochondrial DNA background will not come along in progeny. With large effects, the ‘perfect’ male nuclear genome selected might turn out to be ‘overruled’ in the new mito-nuclear combination, and, as mentioned, there is no way of knowing it from the phenotypes. Effects thus should be restricted to the post reprocatogenus lifespan. In the light of female choice, this would constitute another example of ‘men turning out to be rubbish’.

11. Conclusion

Here I have presented a model in which eukaryotic sex evolved in response to a (very) high mutation rate, due to the uptake of the endosymbiont, because this (proto) mitochondrion generated internal ROS. Catabolism of a larger array of different molecules became feasible upon the merger of the two metabolisms. This further increased internal ROS formation. Under these circumstances, a meiotic system evolved (from an array of host repair and signalling mechanisms) which could counter Muller’s ratchet. The clicks of the ratchet increased because (i) there is more to mutate and, even more importantly, (ii) mutation levels go up by a vicious circle of ROS formation. This led to many eukaryotic adjustments (such as gene migration to the nucleus, anti-oxidant measures, improved nuclear DNA repair and replication, as well as the arrival of peroxisomes), shaping a new domain of life [3]. Meiotic sex can also be seen as one of the outcomes of symbiogenesis: a stunning product of the merger by which the eukaryotes came into being [1]. It is surprising how ROS generation can even elegantly explain why mitochondrial DNA from the sperm that wins the race to the egg still does not end up in the next generation.

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