Bulletin of Regional Natural History
Formerly Bollettino della Società dei Naturalisti in Napoli

Death, sex, and immortality

Roberto Ligrone

DOI https://doi.org/10.6093/2724-4393/8315

***Correspondence:**
roberto.ligrone@gmail.com

Affiliation:
Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta, Italy

Conflict of Interest: The author declares that he has no conflict of interest.

Financial Disclosure Statement: The Author declares that no specific funding was received for this work.

Accepted: 14 September 2021

Abstract

DNA is intrinsically unstable due to spontaneous mutation and degradation. Yet, life has thrived for about four billion years, adapting to most diverse environmental conditions. The ultimate reason for the striking resilience and versatility of life is sex, here defined as any mechanism that recombines DNA from separate organisms. Sex is a universal property of life that originally emerged as a spontaneous by-product of the machinery for gene duplication and repair. Sex counteracts genetic erosion (Muller's ratchet), thus stabilizing biological information across time. Concurrently, sex builds novel genes and novel genomes, thus fostering genetic innovation and evolution. Bacterial sex is independent of reproduction, generally involves short DNA sequences, and encompasses a relatively high frequency of horizontal gene transfer between distantly related taxa. Because of this, bacterial sex produces large pangenomes, fosters population ecological flexibility, and blurs species demarcation. Sex in eukaryotes is associated with reproduction and involves an alternance of cellular fusion and meiosis, each cycle setting whole-genome recombination. Sexual reproduction involves major additional costs relative to bacterial sex and is probably an ancestral trait of eukaryotes, but its origin is a matter of speculation. Sexual reproduction maintains sharp inter-species boundaries, prevents the development of pangenomes, and favours ecological specialization. Except for gene transfer from endosymbionts,

horizontal gene transfer has had a marginal role in genome evolution in eukaryotes. Eukaryotes lacking sexual reproduction might use a bacterial sort of sex as demonstrated for bdelloid rotifers. The soma of complex multicellular eukaryotes has three hierarchical levels of organization (systemic, organ and cellular) and three related states of death.

Keywords: Biological information, Genetic recombination, Horizontal gene transfer, Pangenome, Sexual reproduction.

Riassunto

Il DNA è intrinsecamente instabile a causa di una spontanea tendenza a mutare e degradarsi. Ciò nonostante, la vita esiste sulla Terra da circa quattro miliardi di anni e si è adattata alle più diverse condizioni ambientali. La ragione ultima della straordinaria resilienza e versatilità della vita è il sesso, termine che comprende qualsiasi meccanismo che ricombina DNA proveniente da organismi distinti. Probabilmente nato come uno spontaneo sottoprodotto del macchinario biochimico preposto alla duplicazione e riparazione del materiale genetico, il sesso è una proprietà universale della vita. Il sesso conserva l'informazione biologica nel tempo, contrastando l'erosione genetica dovuta all'accumulo di mutazioni (Muller's ratchet). In parallelo, il sesso promuove l'innovazione genetica e l'evoluzione attraverso la creazione di nuovi geni e nuovi genomi. Il sesso nei batteri non è associato alla riproduzione, di solito coinvolge simultaneamente uno o pochi geni, e comporta il trasferimento orizzontale di geni anche fra taxa filogeneticamente lontani. Nei batteri, perciò, il sesso tende a produrre pangenomi di grandi dimensioni, amplifica la flessibilità ecologica delle popolazioni, e confonde la separazione fra specie. Il sesso negli eucarioti è associato alla riproduzione, comporta l'alternanza di fusione cellulare e meiosi, e ciascun ciclo ricombina l'intero genoma. La riproduzione sessuale comporta notevoli costi aggiuntivi rispetto al sesso nei batteri ed è probabilmente un tratto ancestrale degli eucarioti, ma le sue origini sono incerte. La riproduzione sessuale mantiene una netta separazione fra le specie, previene lo sviluppo di pangenomi e favorisce la specializzazione ecologica. Con l'eccezione dell'acquisizione di geni da endosimbionti, il trasferimento orizzontale ha avuto un ruolo marginale nell'evoluzione dei genomi negli eucarioti. La perdita della riproduzione sessuale in alcuni eucarioti potrebbe essere compensata da meccanismi sessuali di tipo batterico, come dimostrato nei rotiferi bdelloidi. Il soma degli eucarioti multicellulari complessi ha tre livelli gerarchici di organizzazione (sistemico, di organo, cellulare) e tre corrispondenti stati di morte.

Parole chiave: Informazione biologica, Pangenoma, Ricombinazione genetica, Riproduzione sessuale, Trasferimento orizzontale di geni.

How to cite
Roberto Ligrone (2021). Death, sex, and immortality. Bulletin of Regional Natural History (BORNH), Bollettino della Società dei Naturalisti in Napoli. Vol.1, n. 3, pp. 71 - 89 ISSN: 2724-4393.

1. Introduction

Living organisms get old and die. This is a most obvious consideration for multicellular organisms like humans or oaks, less so for unicellular organisms.

A multicellular organization has evolved multiple times both in bacteria and eukaryotes under selection pressure for labour division and cellular specialization (Nicklas and Newman 2013; West et al. 2015). In eukaryotic multicellular organisms (henceforth referred to as "multicells"), the ability to produce offspring is restricted to germ cells, the rest (somatic cells) only performing ancillary functions and eventually dying. In weismannist multicells, the germ line separates from the somatic line early in development. The somatic line produces the body tissues and organs, whereas the germ line produces the reproductive cells (either gametes or meiospores). Weismannist multicells are not common: some animal clades (nematodes, arthropods, and vertebrates) and volvocine algae, a green algal line including *Volvox* and its relatives (Gilbert 2006; Hallmann 2011). In nematodes, arthropods and frogs, the germ line is pre-determined by cytoplasmic inducers at a specific position in the egg cell, whereas in mammals the germ line is positionally induced during early embryo development (Gilbert 2006). Separation of the germ line in *Volvox* is associated with genetically determined asymmetrical division during embryo development (Kirk 2001). In most multicells, including many invertebrates and land plants, there is no clear-cut separation between a soma and germ line, new germ cells continuously developing from stem cells in the adult individuals. In both weismannist and non-weismannist multicells, however, the germ line is potentially immortal, in the sense that it perpetuates itself across generations. In contrast, the soma remains alive only for a limited time varying with the species and environmental conditions. Most animals are short-lived, with a life cycle lasting a few months or less. Others can live for over a year, and some for over a century.

Why does the soma of multicells die whereas the germ line is potentially immortal? Arguably, remaining alive indefinitely would be highly adaptive for an organism fit enough to have reached reproductive maturity. The ultimate reason why this is not possible is that genomes tend to mutate with time, accumulating errors that reduce their functionality. Mutations may be as small as the replacement of a single nucleotide or as large as the deletion of million nucleotides. Mutations may occur at any time, either spontaneously or because of exposition to mutagens such as high-energy radiations or chemicals. Among chemical mutagens, of special importance are reactive oxygen species (e.g., the superoxide radical, hydroxyl radical and hydrogen peroxide) that are spontaneous by-products of the aerobic metabolism (Dizdaroglu and Jaruga 2012). Estimates vary, but it is possible that as many as tens of thousands genetic lesions occur in each cell of a multicellular organism

daily (Lindahl 1993). A particularly critical phase in the cell cycle is genome replication, during which mutations arise from errors in DNA duplication. In other words, the very mechanism that perpetuates biological information also contributes to its degradation. Cells have a diversity of DNA repair mechanisms that correct at least a part of the damages from mutation (Vijg 2014). With time, however, mutations unavoidably impair cellular viability. It is known, for example, that human fetal cells can divide between 40 and 60 times, after which they enter a senescence phase and die. This is known as the Hayflick limit from the name of the senior researcher who discovered it (Hayflick and Moorhead 1961). Many unicellular eukaryotes have life cycles that recall the separation of a soma and a germ line as observed in multicells, so what said for multicells also applies to them.

Bacteria appear to be virtually immortal because they keep proliferating as long as there are favourable environmental conditions. Some bacteria can even produce special resting cells that survive unfavourable conditions and return active when conditions are again permissive. If, however, we could follow every single cell in a bacterial population, we would see that at a point most cells die out. This can easily be proved in culture by maintaining the concentration of essential metabolites at a low constant level. The bacterial population will grow up to a certain size and then will remain stable although cells continue dividing, indicating that the number of newly formed cells equals the number of cells that die per unit time. In this condition, known to microbiologists as the "stationary phase", the population size depends on the level of essential nutrients, provided that all other relevant parameters such as temperature, pH or redox potential are adequate (Prescott 2005). Thus, despite the lack of a separation between a soma and a germ line, healthy cells continuously replace aged cells, and bacterial populations may remain viable indefinitely.

2. Sex is essential for genome maintenance

What ensures the potential immortality of the germ line in multicells and of populations of bacteria and unicellular eukaryotes despite the intrinsic instability of genetic information?

The answer is *sex*, here defined as any mechanism that produces novel genomes by combining DNA from separate organisms.

Sexual mechanisms in bacteria encompass *transformation*, *transduction*, and *conjugation* (Redfield 2001; de La Cruz et al. 2010; Borgeaud 2015), with transformation likely playing a predominant role (Takeuchi et al. 2014). Analogous mechanisms are documented in the archaea but are still poorly known (van Wolferen 2015). Because sexual processes in prokaryotes do not involve cellular fusion or meiosis as is the case in eukaryotes, they are here collectively referred to as *non-meiotic sex*. DNA acquired by non-meiotic sex may be recognized as foreign DNA and degraded, or may be retained, expressed, and transmitted to next generations. In the latter case, newly acquired DNA may be conserved as an independent plasmid or be inserted in the main chromosome, either as an additional sequence (a process called *illegitimate* or *non-homologous recombination*) or in lieu of a pre-existing homologue sequence (*legitimate* or *homologous recombination,*

Fig 1). These processes are under the control of the same enzymatic machinery responsible for DNA replication and repair, notably RecA and RecBCD enzymes (Redfield 2001; Hoff et al. 2018). A detailed description of the molecular mechanisms underlying sexual processes in bacteria is accessible in molecular biology textbooks such as Krebs et al. (2017). It suffices here to note that random sequence shuffling by non meiotic sex permits the recovery of functional genes and functional genomes in bacterial populations (Takeuchi et al. 2014).

therefore more commonly referred to as *sexual reproduction*. A distinctive property of meiotic sex is that the zygote receives a complete chromosome set from each gamete. For the process to be reiterated over time it is necessary that, after each syngamy and before the formation of new gametes, *meiosis* re-establishes the haploid chromosome number.

Either random and usually limited to short DNA sequences in prokaryotes or involving the whole genome at each round in eukaryotes, sex is essential for the

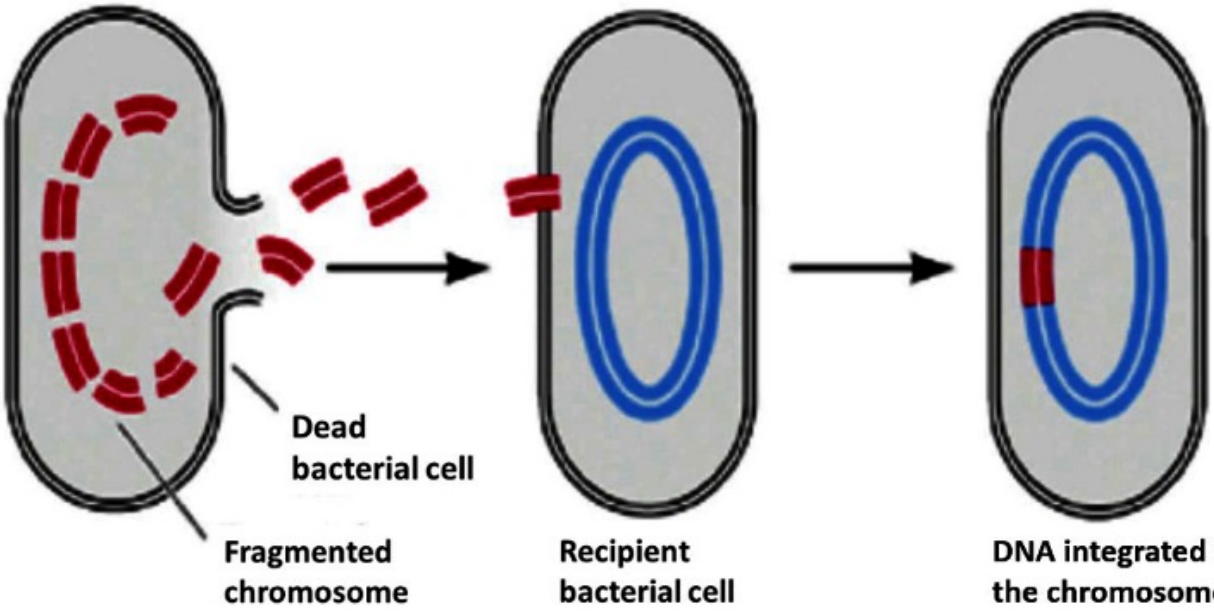

Figure 1: Bacterial transformation. A DNA fragment from a dead cell is captured by a recipient cell and incorporated into its chromosome by legitimate recombination. If the original DNA segment contained a genetic lesion and the novel one (in red) was intact, recombination repristinates the function impaired. As shown in this figure, bacterial sex usually involves relatively short DNA segments.

Meiotic sex is a process unique to eukaryotes, consisting in the formation of a *zygote* by fusion (*syngamy*) of two specialized cells, the *gametes*. Unlike sexual processes in bacteria and archaea, meiotic sex is associated with reproduction and is

preservation of biological information. In both cases, legitimate recombination permits the replacement of altered sequences with correct sequences from other cells (Fig. 1). In the absence of homologous recombination, genomes

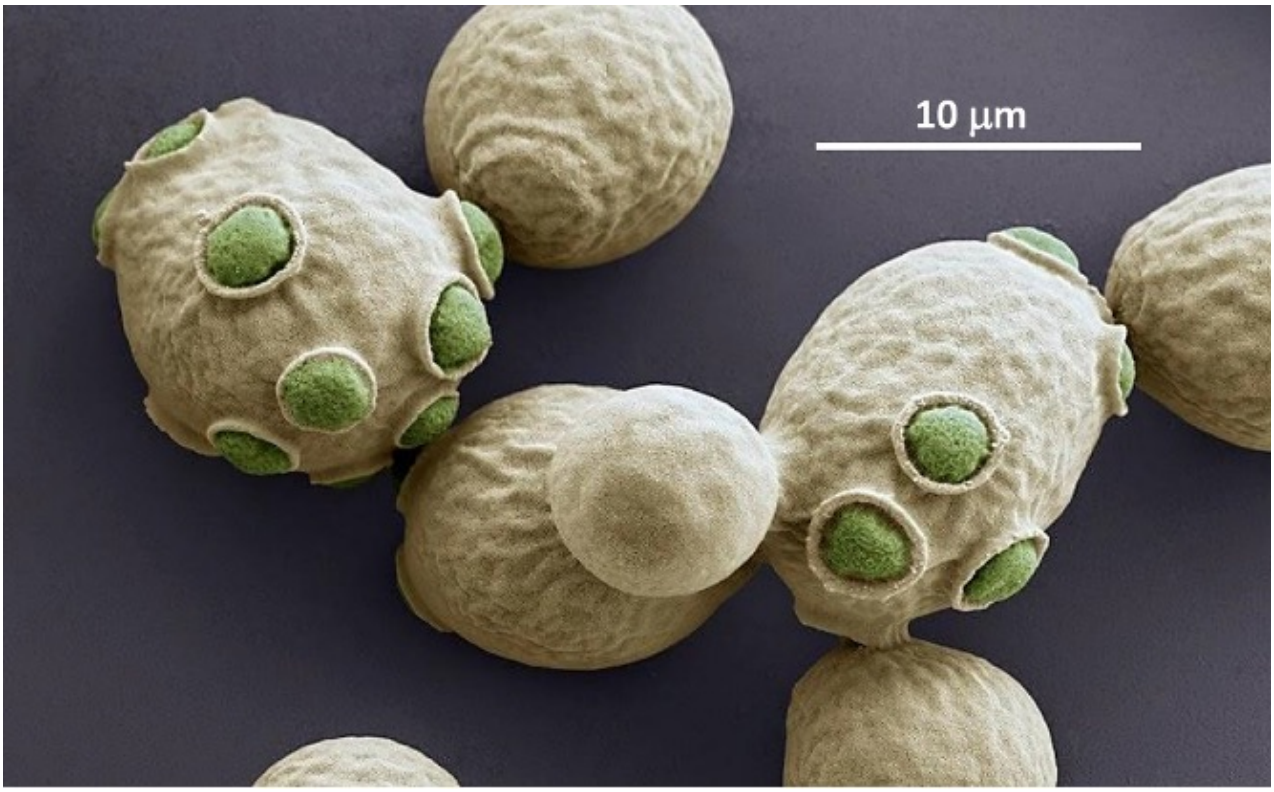

Figure 2: Cells of *Saccharomyces cerevisiae*, an ascomycetous yeast with a haplo-diplobiontic life cycle. Both haploid and diploid cells reproduce asexually by gemmation. The cup-shaped structures visible on the surface of cells are scars left by past gemmation events; when their whole surface is covered with scars, the cells stop dividing and die. Vegetative cells are therefore equivalent to the soma of a multicellular organism. Cells with a full reproductive potential are re-formed by meiotic sex.

would accumulate a growing load of deleterious mutations, an effect known in evolutionary genetics as Muller's ratchet from the name of the researcher who first recognized its relevance in evolution (Muller 1932). Under the control of natural selection, sex ensures the persistence of functional genomes across generations (Maynard Smith and Szathmàry 1995; Narra and Ochman 2006; Szöllősi et al. 2007; Vos 2009; Takeuchi et al. 2014; Rocha 2016).

Somatic cells in multicells cannot use sex for repairing genetic damage. The accumulation of genetic lesions translates into morphological and functional aging, eventually causing the death of the soma (Ren et al. 2017). Germ cells in weismannist organisms are less prone to genetic damage than somatic cells because they are protected within the organism body and have low metabolic activity until they engage in reproduction. Germ cells in non-Weismannist organisms develop from the same stem cells that produce the soma, and do not benefit from protective mechanisms present in weismannist organisms. The evolutionary significance of the two strategies is not clear. A recent model suggests that selection for mitochondrial quality drives either early or late germline separation, depending on high or low mutation rate of mitochondrial DNA, respectively (Radzvilavičius et al. 2016). In either case, germs cells are subject to genetic damage that on the long term would stop perpetuation if there were not sex.

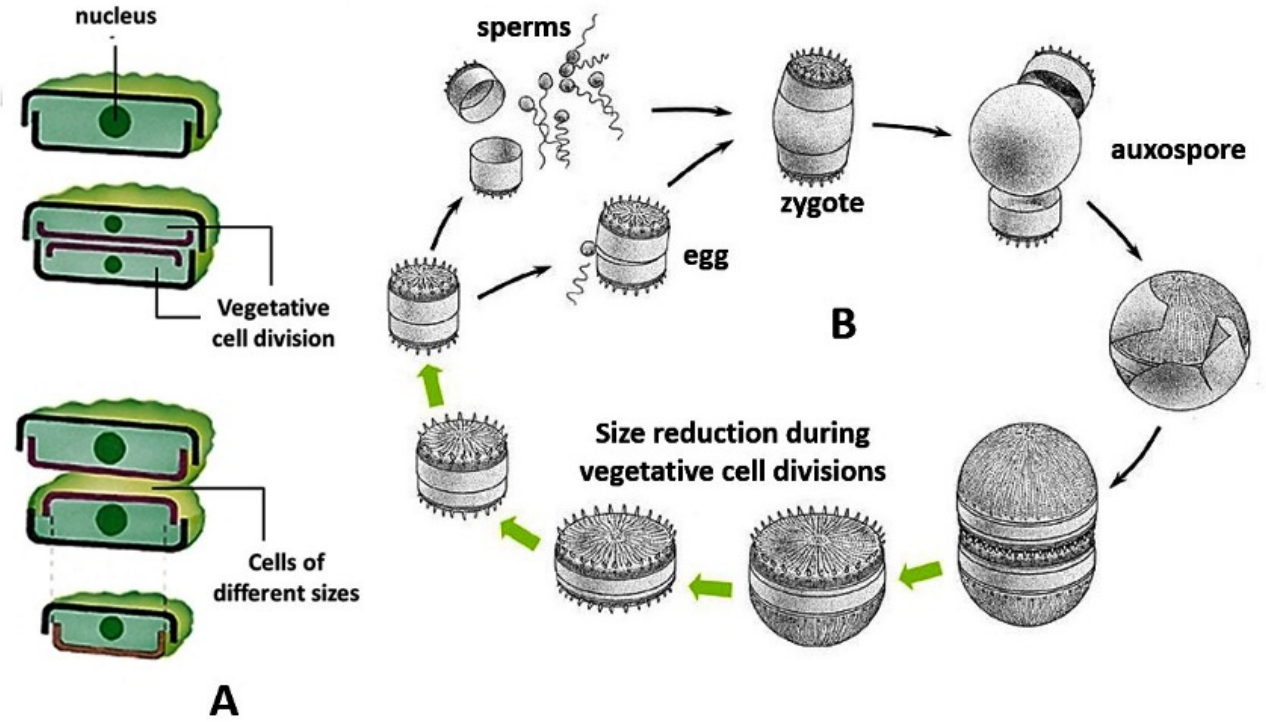

Figure 3: Life cycle of centric diatoms. (A) Vegetative cells (equivalent to the soma of a multicellular organism) are diploid; cell sizes progressively reduce during cell division. When size attains a minimum, the cells divide by meiosis and produce gametes (either sperms or an egg). The zygote gets rid of the rigid siliceous cell wall and expands to form an auxospore, which starts a new sequence of vegetative cell divisions.

Because sex is a random process, a (conspicuous) part of reproductive cells do fail to repristinate functional genomes and are eliminated by natural selection.

Much the same occurs in populations of unicellular eukaryotes, in which vegetative cells can divide a limited number of times, and cells with a full reproductive potential are periodically regenerated by meiotic sex (Figs 2 and 3).

A most dramatic example of clonal aging and sexual rejuvenation occurs in ciliates, a group of unicellular aquatic eukaryotes known for their nuclear dualism. Ciliate cells possess two sorts of nucleus, a tiny diploid micronucleus and a large "ampliploid" macronucleus containing only a part of the genome, amplified many times. The micronucleus is inactive in genetic expression, its only function being genome storage and duplication. The macronucleus takes care of cell functioning, expressing the organism phenotype. Monoclonal cultures of ciliates gradually lose vitality and expire after a number of divisions (200-350 in *Paramecium aurelia*, and up to 1,500 in *Tetrahymena*) because they are not able to perform meiotic sex (which requires genetically distinct mating types). When macronuclei of clonally young cells were injected into aged cells, the vitality of the recipient cell (expressed as the number of subsequent clonal fissions) was increased, indicating that DNA damage in the macronucleus is the cause of aging (Aufderheide 1986; Holmes and Holmes 1986). Meiotic sex, based on the exchange of a micronucleus between cells of

compatible mating types, re-starts the cycle. It is pertinent to note that, because recombination is a random process, only a fraction of the cells resulting from meiotic sex receives a functional genome, the rest being swiftly eliminated by natural selection.

As in unicellular eukaryotes, most bacterial cells die after several rounds of cell division because of the accumulation of genetic lesions, but some manage to maintain a functional genome by randomly incorporating DNA segments from other cells, thus ensuring survival. The bacteria can incorporate DNA not only from genetically related cells but also from taxonomically distant donors. Sex between distantly related taxa is referred to as *horizontal gene transfer* (HGT), although the underlying mechanisms are the same as for genome maintenance within homogeneous populations. HGT enables bacteria to acquire novel metabolic pathways and colonize novel niches. Events of HGT have been responsible, for example, for the spread of photosynthesis and aerobic respiration across a wide taxonomic spectrum in bacteria (Hohmann-Marriott and Blankenship 2011; Schoepp-Cothenet et al. 2013). HGT is the main mechanism underpinning the expansion of genomes and protein diversity in prokaryotes (Treangen and Rocha 2011; Takeuchi et al. 2014; Vos et al. 2015), whereas gene duplication and neofunctionalization of gene copies is the prevalent mechanism in eukaryotes (see section 3). HGT in eukaryotes is much less frequent than in prokaryotes and does not contribute to long-term genome evolution, except for gene transfer from endosymbionts (Ku et al. 2015), notably those that generated the mitochondrion and the chloroplast (Timmis et al. 2004).

3. Sex drives genetic innovation

If legitimate recombination is essential for the conservation of biological information, illegitimate recombination permits genes to move from a genotype[1] to another, thus "experimenting" interaction with novel genes and novel environments. From the perspective of genes, it does not matter that most gene copies dispersed in the environment are lost or enter unfavourable genotypes: a single copy that happens to benefit from above-average fitness will rapidly spread. A fitness-improving mutation is a most rare event, and even more unlikely is the appearance of multiple favourable mutations in the same cell. Sex-mediated illegitimate recombination permits favourable mutations appeared separately to associate in the same genotype and sum their effects. Widening the perspective, illegitimate recombination potentially enables genes to interact with all other genes in the surrounding environment. Indeed, the notion of *selfish gene* by Richard Dawkins (1976) is rooted in gene shuffling among genomes. Illegitimate recombination not only creates new genomes, but also makes new, chimeric genes. A particularly effective mechanism for making new genes is random re-arrangement of sequences encoding for domains of separate proteins, a process known as "domain shuffling" (Long et al. 2003). Sex is not the only mechanism capable of producing novel genes, yet its contribution in expanding the genetic repertoire is biologically important (Rocha 2003).

By reducing selection pressure against large genome sizes (Section 5), sexual reproduction has given a dramatic contribution to genetic innovation in

eukaryotes. The mechanism involved is gene duplication and neofunctionalization of redundant gene copies (Van de Peers et al. 2009; Holland et al. 2017). Many eukaryotic lineages went through whole-genome duplications that have created thousands of new genes. Early in their evolutionary history, the vertebrates underwent two whole-genome duplications that probably underpinned their outstanding biological success (Bertrand and Escriva 2011). Nothing of this would have been possible without sexual reproduction.

That said, one should not assume that sex is always beneficial. Quite the opposite (Goodenough and Heitman 2014). Extant genomes are the product of million or billion years of evolution. As for mutations, sex-mediated alterations in genomic structure are much more likely to be for the worse than for the better. Sex, however, is an essential biological mechanism because it works over large numbers.

[1]The term genotype indicates the set of genes present in a single cell or individual, whereas a genome is the set of genes that characterizes a whole species (including allelic variants). For many bacteria it is necessary to distinguish between genome and pangenome (see section 4).

4. Sex blurs interspecific boundaries in bacteria but fosters species isolation in eukaryotes

Sex has opposite collateral consequences in bacteria and eukaryotes. Bacteria can acquire DNA not only from relatives but also from distantly related sources. Because of this, natural bacterial populations usually present greater genetic diversity than single strains or isolates. For bacteria, therefore, it is necessary to distinguish between genome and *pangenome*, the first referring to the set of genes in a single isolate or strain, the latter to the global gene repertoire present in all strains and isolates attributed to the same species (Medini et al. 2005; Tettelin 2008; Lapierre and Gogarten 2009; Mira et al. 2010). The pangenome usually encompasses a set of genes common to all strains (*core* or *backbone genome*), plus a vast pool of genes specific to some strains (*accessory genome*). The core genome consists of genes controlling essential functions such as gene duplication/expression and fundamental metabolic pathways. The accessory genome consists of genes involved in facultative functions such as the use of certain metabolites or antibiotic resistance. The fact that bacterial pangenomes are usually much larger than the genome of single isolates or strains greatly enhances the chance of gene recombination through sex. On the other hand, genetic promiscuity makes species demarcation a most difficult task in bacterial taxonomy. By convention, bacterial strains or isolates are currently assigned to the same species if sequence divergence in their 16S rRNAs is below 1% (Cohan and Perry 2007). Apparently low, this is the average level of divergence found in 18 S rRNA (the eukaryotic homologue of bacterial 16S rRNA) of mammals belonging to different orders, for example a goat and a dog.

Meiotic sex in eukaryotes depends on mechanisms of gamete recognition for preventing syngamy between incompatible cells. This is the reason why, for example, bull sperm cannot fertilize a mare. Moreover, chromosome sorting during meiosis requires high levels of synteny[2] and sequence similarity in homologs. If homologs are not

sufficiently similar, they do not pair and meiosis is disrupted. Because of this, only closely related individuals can interbreed. Under unnatural conditions, for example in captivity, individuals belonging to different species may overcome pre-zygotic barriers and interbreed, yet the hybrids are usually sterile because of meiotic failure. Sexual reproduction, therefore, establishes strong boundaries between eukaryotic species. The biological notion of species currently applied to multicellular eukaryotes defines the species as *a cohesive, monophyletic group irreversibly isolated from other populations by reproductive and ecological barriers*.

Because of rampant HGT and the lack of meiotic sex, the above definition is not appliable to prokaryotes.

[2]Synteny is the physical co-localization of homologous sequences (or genetic loci) along homologs in an individual or a species.

5. Why did eukaryotes evolve sexual reproduction?

Sexual reproduction is almost universal in eukaryotes, there being very few asexual eukaryotic lineages, most of which are unicellular. In several cases, closer scrutiny of eukaryotic organisms originally reported as asexual has revealed signs of sexual reproduction. Notably, *Giardia* and *Trichomonas*, two taxa placed at the base of the eukaryote tree and apparently lacking meiotic sex, were found to have genes involved in meiosis, suggesting that the lack of meiotic sex is a derived condition in extant eukaryotes (Rallenh et al. 2005; Malik et al. 2007; Speijer et al. 2015).

Sexual reproduction requires two organisms (or two cells) for making a new one. This is the so-called two-for-one cost (Maynard Smith and Szathmàry 1995). Additional costs include gamete loss, the need for flagella and chemo-sensorial mechanisms, the production of sexual attractors and inductors, the involvement of vectors (e.g., in flowering plants) or courtship (in many vertebrates) and the transmission of parasites (Lehtonen et al. 2012). A further cost of sexual reproduction is the dissolution of well-adapted genotypes, a negative effect exacerbated by cross-fertilization and often mitigated in nature by regular reliance on asexual reproduction (Goodenough and Heitman 2014). This is probably the main reason why numerous eukaryotes, either uni- or multicellular, use sexual reproduction only in response to stress, whilst they stick to asexual reproduction under favourable conditions.

Why did eukaryotes evolve sexual reproduction despite heavy cost? The question has been addressed several times and given a diversity of tentative explanations, all stemming from the recognition that the maintenance of eukaryotic genomes is particularly problematic. Indeed, eukaryotic genomes are on average three orders of magnitude larger than bacterial genomes (10^9 *vs* 10^6 base pairs) and have expanded considerably in number of protein-coding genes, size of genes, number of gene families, regulatory DNA content, and extent of non-coding repetitive sequences (Elliott and Gregory 2015). This large amount of DNA is distributed in several chromosomes, probably because it could not be handled in a single chromosome as is generally the case in bacteria. For a discussion of the possible

adaptive significance of eukaryote genome expansion, see Cavalier-Smith 2005.

Cavalier-Smith (2002, 2010) suggests that meiosis evolved in parallel with mitosis in ancestral eukaryotes, initially to correct errors in chromosome segregation during mitosis, and subsequently to shift from diploidy to haploidy in response to environmental signals. In Cavalier-Smith's narrative, eukaryotes diverged from a prokaryotic ancestor as phagotrophic predators of other cells. Ancestral eukaryotes lived predominantly as haploid cells under favourable conditions, whereas they shifted to diploidy by endoreduplication (genome duplication not followed by cellular division) and formed dormant cysts to survive food scarcity or other types of stress. Doubling the chromosome set helped dormant cells to retain a functional genome after prolonged exposition to ultraviolet or other damaging agents. Meiosis not only repristinated fast-growing haploid cells but also recovered functional genomes by chromosome sorting. Endoreduplication was later replaced by cell fusion, which greatly enhanced genetic polymorphism and recombination.

Several alternative models are linked to mitochondrial evolution. The mitochondrion, one of the most distinctive traits of extant eukaryotes, derived from an alpha-proteobacterial endosymbiont (Martijn et al. 2018). Under selection pressure for better integration, a large chunk of the endosymbiont genome was transferred to the host genome and a part was completely lost. Mitochondria, however, retain a small genome that is essential for their functioning (Allen 2003).

Tilquin et al. (2018) propose that sexual reproduction evolved in eukaryotes to permit mitochondrial complementation by fusion of non-clonal cells (i.e., gametes from different parents). This avoided the decay of the mitochondrial genome, which can no longer be rescued by HGT because of isolation from free-living bacterial populations. The hypothesis implies by-parental mitochondrial inheritance, thus contrasting with almost universal uniparental inheritance in extant eukaryotes (Greiner et al. 2014; Radzvilavičius et al. 2017).

Hörandl and Speijer (2018) propose that increased production of ROS from mitochondrial metabolism set the stage for meiotic sex under selection pressure to reduce host genome erosion. Theoretical modelling suggests that the benefits from HGT in contrasting the Muller's ratchet decline with increasing genome size (Colnaghi et al. 2020). Based on this result, Colnaghi et al. (2020) reason that sexual reproduction replaced HGT in ancestral eukaryotes to ensure the maintenance of expanding genomes. Considering that larger genomes are at greater risk from ROS, the two models fit well with each other and could be nicely combined.

Brandeis (2021) suggests that meiosis evolved in parallel with the mitochondrion to "purify" the host genome from random insertions of mitochondrial sequences that altered pre-existing host genes. This is a questionable hypothesis because natural selection could have easily eliminated deleterious insertions without requiring a complex and expensive mechanism such as sexual reproduction. As a matter of fact, early eukaryotes had their genome massively invaded by group II introns of likely mitochondrial origin, and managed to avoid disaster by evolving the spliceosome, a molecular machine that removes the introns

from transcripts before translation (Martin and Koonin 2006).

In conclusion, the most convincing hypothesis is that sexual reproduction fixed in eukaryotes because it permitted the maintenance of large-sized multi-chromosome genomes. It is debated, however, if sexual reproduction emerged in the outcome of mitochondrial evolution or was an earlier event in eukaryogenesis (Ligrone 2018).

6. Are there exceptions to the universality of sex?

Sexual reproduction is the rule in eukaryotes, with a minority of taxa that lost it and seemingly reproduce only asexually. Bdelloid rotifers (Bdelloidea), minute animals living in freshwater habitats all over the world, are a most remarkable example. Unlike other rotifers, bdelloid rotifers reproduce exclusively by parthenogenesis, *viz.* by division of unfertilized diploid eggs formed without meiosis. A second remarkable trait of these animals is their ability to survive drought by activating desiccation-induced dormancy (Ricci and Fontaneto 2009; Boschetti et al. 2011). Despite the absence of sexual reproduction, bdelloid rotifers have evolved at a quick pace, producing over 450 species in about 35 MY from their divergence from a sexual progenitor. How did bdelloid rotifers manage to maintain functional genomes? The trick seems to be in part rooted in desiccation tolerance. To repair genetic damage after prolonged permanence in a dried condition, bdelloid rotifers have evolved a particularly effective DNA repair system (Hespeels et al. 2014). Crucially, however, these singular animals have a surprising ability to pick up foreign DNA and insert it in their genome, thus performing a bacterial sort of sex (Gladyshev et al. 2008; Eires et al. 2015). The loss of meiosis and of the associated requirement for homologs with high sequence homology probably facilitates the acquisition of DNA by HGT.

A possible reason for the loss of sexual reproduction in some eukaryotes is its heavy biological cost (Section 5). By analogy with bdelloid rotifers, it is likely that other eukaryotes that lost sexual reproduction also reverted to bacterial-like sex. Because asexual reproduction should lead to genetically homogeneous clonal populations, the impact of HGT in asexual eukaryotes might be assessed by evaluating genetic diversity within natural populations. Moreover, based on the apparent link between large genome size and sexual reproduction (Section 5), one might predict that asexual eukaryotes have reduced genome sizes compared with their sexual relatives.

The case of colonial anthozoa (Cnidaria) is somewhat different. These animals form extensive monoclonal colonies by asexual reproduction. Anthozoan colonies, but not single polyps, can live much longer than any known non-colonial animal, perhaps for thousand years. The reason is probably that novel polyps can develop from few stem cells of parent polyps (Sköld et al. 2009). Because the colonies lack the highly integrated homeostatic mechanisms present in the body of complex animals, genetic lesions may impair the survival of single polyps but not of the whole colony. This, for a while. No reef could survive for ever without sex. Indeed, although these simple animals largely rely on asexual reproduction for colony extension, they routinely use

meiotic sex for spreading. Moreover, genetic polymorphism in natural colonies suggests multiclonal composition (Maier 2012; Schweinsberg et al 2016) or fusion of non-clonal juvenile conspecifics during embryogenesis (Jiang et al. 2015). Intra-colony genetic diversity may contribute to elongating the lifetime of coral colonies.

The free-swimming sexual stage (medusa) of the hydrozoan *Turritopsis nutricola* (now named *T. dohrnii*) is able to revert to the asexual polyp stage, forming a new polyp colony. *Turritopsis* is one of the few instances of animals that revert to a sexually immature, colonial stage after having reached sexual maturity as a solitary individual (Piraino et al. 1996). Hydrozoans have a lifespan ranging from few hours to several months. Because of the ability to reiterate the medusa-to-polyp reversal multiple times, *Turritopsis* is considered to be potentially immortal. *Turritopsis*, however, also reproduces sexually and does so in response to the same unfavourable conditions that induce reversal to the asexual stage. The unique reproductive strategy of *Turritopsis*, therefore, appears to combine the two processes, with reversal to the asexual stage affording adult individuals a long subsistence, and sex ensuring genomic maintenance.

Land plants are typical non-weismannist multicells. They grow by the activity of apical meristems made of one to several initial cells and numerous derivatives. The derivative cells have high metabolic activity and high division rates. They divide a finite number of times and eventually differentiate into mature tissues. In contrast, the initial cells have low metabolic activity, low division rates and can divide indefinitely. Their function is to produce novel derivatives that replace those that differentiate and stop dividing. If for any reason an initial cell stops functioning, one of the other initials or an immediate derivative replaces it, re-establishing the correct geometry of the meristem. Theoretically, plants can keep growing indefinitely. By no means, however, this implies that they are immortal. Plants do die of old age, although their life span varies from few months or less to several hundred or thousand years (https://en.wikipedia.org/wiki/List_of_oldest_trees). Independently of the natural life span of single individuals, plants can be easily cloned, and many species do so spontaneously in nature. A colony of about 48,000 *Populus tremuloides* trees (nicknamed "Pando"), covering 43 ha in the Fishlake National Forest of Utah (USA), is considered one of the oldest and largest organisms in the world. Recent estimates set the colony's age at several thousand (up to 14,000) years (DeWoody et al. 2008). Pando's probably owes its existence to favourable conditions persisting locally from the end of the last glaciation. The recent increase in herbivore populations due to the elimination of predators by humans, and worsening climatic conditions due to global change are now seriously threatening its survival (Rogers and McAvoy 2018). Plants' ability to live for long times essentially depends on their extreme morphogenetic flexibility. Novel meristems may develop from stem cells in mature shoots, roots and leaves, and even mature cells can be induced to resume meristematic activity (Steeves and Sussex 1989). The Pando clone deploys a large reserve of stem cells stored in its root system. Significantly, individual stems rarely live over 130 years (Rogers and McAvoy 2018), probably because light, cold and other harmful agents cause more

genetic damage in aerial parts. Despite unusual morphogenetic flexibility, plants including "Pando" ultimately depend on sex for genome maintenance.

7. Complex multicells have three states of death

Death is the loss of homeostatic mechanisms that maintain living systems far from thermodynamic equilibrium. This transition coincides with cellular death in unicellular organisms, but not necessarily so in multicellular organisms. Here, in fact, death is the outcome of alterations of the supra-cellular organization that precede the loss of intracellular homeostasis. In other words, a multicellular organism may be dead whilst at least a part of its cells are still alive. A most remarkable example is HeLa cells, a cellular line derived from cervical cancer cells taken in 1951 from Henrietta Lacks, a patient who died the same year. Since then, HeLa cells have been kept in culture and multiplied, becoming an enormous boon to medical and biological research. HeLa cells developed by horizontal gene transfer from the papilloma virus 18 to human cervical cells, and their genome is different from Henrietta Lacks' genome in various ways, including the number of chromosomes. HeLa cells recall the hypothetical "cancer-like" cells devised by Levin (2021), which attain immortality by reversibly shifting from a unicellular to a multicellular life style. HeLa cells can live only in culture condition and do not form multicellular structures. Since their isolation, they have undergone numerous mutations, and most likely a vast number of lines have already extinguished.

Complex multicellular organisms have three hierarchically interlinked levels of organization, each depending on a specific set of homeostatic interactions. The first is the whole organism, whose existence in life depends on mechanisms controlling the interactions of organs and systems of organs (Levin 2021). The second is the single organs, which remain functional for a while after the first level of organization has been lost. The third is tissues and cells, which remain alive for some time after the loss of organ function and may even be isolated and kept in culture under appropriate conditions, as is the case for HeLa cells. We can therefore conclude that complex multicellular organisms die in three steps - systemic, organ and cellular - following each other in this order.

Legal determination of death in the developed world is made by medical professionals after checking irreversible cessation of heartbeat and breathing (*cardiopulmonary death*), or irreversible cessation of functions of the brain (*brain death*), both assumed to signal systemic death (Bernat et al. 2010; Goila and Pawar 2009). In the immediate aftermath of systemic death, organs such as the heart, liver or kidneys can be explanted and integrated into a recipient systemic network. Organ ability to survive transplant depends on their homeostatic interactions with the rest of the organism. The heart has an autonomous pacemaker and can resume beating after transplant although it has no connection with the recipient nervous system. Organs such as the kidneys, liver and lungs are mainly under the control of the endocrine system, which facilitates integration after transplant (Hill et al. 2016). Transplant of blood, skin, cornea, and bone marrow is even easier, as these tissues are under feeble system and organ homeostatic

control. Attempts at brain transplant in animals have met with significant success and might pave the way to exciting progress in bioengineering (Levin 2021). In humans, tissues may be recovered from donors up to 24 h after the cessation of heartbeat. Because of somatic aging, there are some general age guidelines for the different organs that can be donated, but in life-and-death instances there are no strict cut-off ages for donation.

8. Conclusions

Richard Dawkins (Dawkins 1976) provocatively described living organisms as ephemeral vehicles for immortal genes. What makes genes potentially immortal despite their intrinsic fragility is sex, which (a) permits the recovery of functional genes and functional genomes across generations, (b) creates new genes by recombining pre-existing sequences, and (c) enables genes to explore association with other genes present in the surrounding environment, thus creating novel genotypes and novel genomes. Sex, therefore, ensures the conservation of biological information and simultaneously drives genetic innovation. The emergence of new genotypes has an immediate positive impact in the ceaseless war against parasites in which all organisms are engaged (Lively 2010). Yet, sex is much more than this. Sex underpins life ability to perpetuate itself indefinitely and to adapt to changing conditions (Ligrone 2021).

It is important to correct the potential circularity of this reasoning. Genomes evolve thanks to sex (and occasional favourable mutations). Nevertheless, sex by itself is not a product of natural selection. Rather, sex emerged as a spontaneous by-product of the biochemical machinery for the duplication and repair of biological information at an early, probably pre-cellular stage of evolution. Only later did natural selection add the mechanisms specific to prokaryotic and eukaryotic sex.

The occurrence of meiotic sex in eukaryotes and non-meiotic sex in prokaryotes is not a mere difference in the mechanism of genome maintenance. Rather, it reflects a deep divergence in the life strategy of the two types of cellular organization. Prokaryotic sex leads to pangenomes and ecological flexibility, eukaryotic sex leads to vertical inheritance, species isolation and ecological specialization.

References

Allen JF (2003) Why chloroplasts and mitochondria contain genomes. Comparative and Functional Genomics 4: 31-36.

Aufderheide KJ (1986). Clonal aging in *Paramecium tetraurelia*. II. Evidence of functional changes in the macronucleus with age. Mechanisms of Ageing and Development. 37: 265-279.

Bernat JL et al (2010). The circulatory-respiratory determination of death in organ donation. Critical Care Medicine. 38: 963-70.

Bertrand S, Escriva H (2011) Evolutionary crossroads in developmental biology: amphioxus. Development 138: 4819-4830.

Borgeaud S et al (2015) The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer. Science 347: 63-67.

Boschetti C et al (2011) Foreign genes and novel hydrophilic protein genes participate in the desiccation response of the bdelloid rotifer *Adineta ricciae*. The Journal of Experimental Biology 214: 59-68.

Brandeis M. (2021) Were eukaryotes made by sex? BioEssays.43: 2000256. https://doi.org/10.1002/bies.202000256

Cavalier-Smith T (2002) Origins of the machinery of recombination and sex. Heredity 88: 125-141.

Cavalier-Smith T (2005) Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. Annals of Botany 95: 147-175.

Cavalier-Smith T (2010) Origin of the cell nucleus, mitosis and sex: roles of intracellular coevolution. Biology Direct 5: 6. http://www.biology-direct.com/content/5/1/7

ggCohan FM, Perry EB (2007) A systematics for discovering the fundamental units of bacterial diversity. Current Biology 17: R373-R386. Doi: 10.1016/j.cub.2007.03.032

Colnaghi M, Lane N, Pomiankowski A (2020). Genome expansion in early eukaryotes drove the transition from lateral gene transfer to meiotic sex. Elife 9: 9:e58873. Doi: https://doi.org/10.7554/eLife.58873

Dawkins R (1976) The selfish gene. Oxford University Press, Oxford.

de la Cruz F et al (2010) Conjugative DNA metabolism in Gram-negative bacteria. FEMS Microbiology Reviews 34: 18-40.

DeWoody J et al (2008). "Pando" lives: molecular genetic evidence of a giant aspen slone in central Utah". Western North American Naturalist. 68: 493-497.

Dizdaroglu M, Jaruga P. (2012). Mechanisms of free radical induced damage to DNA. Free Radical Research 46, 382-419.

Eires I et al (2015) Horizontal gene transfer in bdelloid rotifers is ancient, ongoing and more frequent in species from desiccating habitats. BMC Biology 13: 90. Doi: 10.1186/s12915-015-0202-9

Elliott TA, Gregory TR (2015) What's in a genome? The C-value enigma and the evolution of eukaryotic genome content. Philosophical Transactions of the Royal Society B 370: 20140331. http://dx.doi.org/10.1098/rstb.2014.0331

Gilbert SF (2006) Developmental biology. Sinauer, USA.

Gladyshev EA, Meselson M, Arkhipova IR (2008). Massive horizontal gene transfer in bdelloid rotifers. Science 320: 1210-1213.

Goila, A, Pawar M (2009). The diagnosis of brain death. Indian Journal of Critical Care Medicine. 13: 7-11.

Goodenough U, Heitman J (2014) Origins of eukaryotic sexual reproduction. Cold Spring Harbor Perspectives in Biology; 6:a016154. Doi: 10.1101/cshperspect.a016154

Greiner S, Sobanski J, Bock R (2014) Why are most organelle genomes transmitted maternally? Bioessays 37: 80-94

Hallmann A (2011) Evolution of reproductive development in the volvocine algae. Sexual Plant Reproduction 24:97-112.

Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Experimental Cell Research. 25: 585-621.

Hespeels B et al (2014) Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer *Adineta vaga* submitted to desiccation. Journal of Evolutionary Biology 27: 1334-1345.

Hill RW et al (2016). Animal physiology. Sinauer Associates, Sunderland.

Hoff G et al (2018) Genome plasticity is governed by double strand break DNA repair in *Streptomyces*. Scientific Reports 8: 5272. Doi: 10.1038/s41598-018-23622-w

Hohmann-Marriott MF, Blankenship RE (2011) Evolution of photosynthesis. Annual Review of Plant Biology 62: 515-548.

Holland PWH et al (2017) New genes from old: asymmetric divergence of gene duplicates and the evolution of development. Philosophical Transactions of the Royal Society B 372: 20150480.

Holmes GE, Holmes NR (1986). Accumulation of DNA damages in aging *Paramecium tetraurelia*. Molecular and General Genetics. 204: 108-114.

Hörandl E, Speijer D (2018). How oxygen gave rise to eukaryotic sex. Proceedings of the Royal Society B 285: 20172706. http://dx.doi.org/10.1098/rspb.2017.2706

Jiang L et al (2015) Fused embryos and pre-metamorphic conjoined larvae in a broadcast spawning reef coral [v2; ref status: indexed,http://f1000r.es/54p]. F1000Research 4: 44. Doi: 10.12688/f1000research.6136.2

Kirk DL (2001) Germ–soma differentiation in *Volvox*. Developmental Biology 238: 213-223.

Krebs JE, Goldstein ES, Kilpatrick, ST (2017) Lewin's Genes XII. Jones and Bartlett Publishers, USA.

Ku C et al (2015) Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524: 427-432.

Lapierre P, Gogarten JP (2009) Estimating the size of the bacterial pan-genome. Trends in Genetics 25: 107-110.

Lehtonen J, Jennions MD, Kokko H. (2012). The many costs of sex. Trends in Ecology & Evolution 27: 172-178.

Levin M (2021) Life, death, and self: Fundamental questions of primitive cognition viewed through the lens of body plasticity and synthetic organisms, Biochemical and Biophysical Research Communications 564: 114-133.

Ligrone R (2018) Biological innovations that built the world. A four-billion-year journey through life and Earth history. Springer.

Ligrone R (2021) The origins of life: novel perspectives over an old problem. Bulletin of Regional Natural History 1: 12-48.

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362: 709-15.

Lively CM (2010) Parasite virulence, host life history, and the costs and benefits of sex. Ecology 91: 3-6.

Long M et al (2003) The origin of new genes: glimpses from the young and old. Nature Reviews Genetics 4: 865-875.

Maier E et al (2012) Intracolonial genetic variation in the scleractinian coral *Seriatopora hystrix*. Coral Reefs 31: 505-517.

Malik SB et al (2007). An expanded inventory of conserved meiotic genes provides

evidence for sex in Trichomonas vaginalis. PLOS ONE. 3: e2879. doi: 10.1371/journal.pone.0002879

Martin W, Koonin EV (2006) Introns and the origin of nucleus-cytosol compartmentalization. Nature 440: 41-45.

Maynard Smith J, Szathmàry E (1995) The origins of life. From the birth of life to the origin of language. Oxford University Press, Oxford.

Martijn J et al. (2018) Deep mitochondrial origin outside the sampled alphaproteobacteria. Nature 557, 101-105.

Medini C et al (2005) The microbial pan-genome. Current Opinion in Genetics & Development 15: 589-594. Doi: 10.1016/j.gde.2005.09.006

Mira A et al (2010) The bacterial pan-genome: a new paradigm in microbiology. International Microbiology 13: 45-57.

Muller HJ (1932). Some genetic aspects of sex. American Naturalist 66: 118-138.

Narra HP, Ochman H (2006) Of what use is sex to bacteria? Current Biology 16: R705-R710.

Niklas KJ, Newman SA (2013) The origins of multicellular organisms. Evolution & Development 15: 41-52.

Piraino S et al (1996) Reversing the life cycle: medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). Biological Bulletin 190: 302-312.

Prescott et al. (2005). Microbiology. McGraw Hill, Boston.

Radzvilavičius AL et al (2016) Selection for mitochondrial quality drives evolution of the germline. PLoS Biology 14: e2000410. Doi:10.1371/journal.pbio. 2000410

Radzvilavičius AL, Kokko H, Christie JR 2017. Mitigating mitochondrial genome erosion without recombination. Genetics 207: 1079-1088.

Ramesh MA, Malik SB, Logsdon JMJr (2005) A phylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Current Biology 15: 185-191.

Redfield RJ (2001) Do bacteria have sex? Nature Reviews Genetics 2: 634-639.

Ren R et al (2017) Regulation of stem cell aging by metabolism and epigenetics. Cell Metabolism 26: 460-474.

Ricci C, Fontaneto D (2009) The importance of being a bdelloid: ecological and evolutionary consequences of dormancy. Italian Journal of Zoology 76: 240-249.

Rocha EPC (2003) Recombination in bacterial genomes and its consequences: from duplications to genome reduction. Genome Research 13: 1123-1132.

Rocha EPC (2016) Using sex to cure the genome. PLoS Biology 14: e1002417. Doi:10.1371/ journal.pbio.1002417

Rogers PC, McAvoy DJ. (2018). Mule deer impede Pando's recovery: Implications for aspen resilience from a single-genotype forest. PLoS ONE 13: e0203619.

Schoepp-Cothenet B et al (2013) On the universal core of bioenergetics. Biochimica et Biophysica Acta 1827: 79-93.

Schweinsberg M et al (2016) Inter- and intra-colonial genotypic diversity in hermatypic hydrozoans of the family

Milleporidae. Marine Ecology 38, https://doi.org/10.1111/maec.12388

Sköld et al (2009) Stem cells in asexual reproduction of marine invertebrates. In: (B. Rinkevich B, V. Matranga V, eds) Stem cells in marine organisms. Springer, p 105-137. Doi: 10.1007/978-90-481-2767-2

Speijer D, Lukeš J, Eliàš M (2015) Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proceedings of the National Academy of Sciences USA 112:8827-8834.

Steeves TA, Sussex IM (1989). Patterns in plant development. Cambridge University Press, Cambridge

Szöllősi GJ, Derényi I, Vellai T (2007) The maintenance of sex in bacteria is ensured by its potential to reload genes. Genetics 174: 2173-2180.

Takeuchi N, Kaneko K, Koonin EV (2014) Horizontal gene transfer can rescue prokaryotes from Muller's ratchet: benefit of DNA from dead cells and population subdivision. G3 (Bethesda) 4: 325–39.

Tettelin H (2008) Comparative genomics: the bacterial pan-genome. Current Opinion in Microbiology 12: 472-477.

Tilquin A, Christie JR, Kokko H. (2018). Mitochondrial complementation: a possible neglected factor behind early eukaryotic sex. Journal of Evolutionary Biology 31: 1152-1164.

Timmis JN et al (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nature Reviews Genetics 5: 123-135.

Treangen TJ, Rocha EPC (2011). Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. PLoS Genetics 7: e1001284

Van de Peer Y, Maere S, Meyer A (2009) The evolutionary significance of ancient genome duplications. Nature Reviews Genetics 10: 725-732.

van Wolferen M et al (2015) The archaeal Ced system imports DNA. Proceedings of the National Academy of Sciences of USA 113: 2496-2501.

Vijg J (2014) Aging genomes: a necessary evil in the logic of life. Bioessays 36: 282-292.

Vos M. 2009. Why do Bacteria engage in homologous recombination? Trends in Microbiology 17: 226-232.

Vos M et al 2015. Rates of lateral gene transfer in prokaryotes: high but why? Trends in Microbiology 23: 598-605.

West SA et al (2015) Major evolutionary transitions in individuality. Proceedings of the National Academy of Sciences USA 112: 10112-10119.

Bulletin of Regional Natural History (BORNH) ISSN 2724-4393.