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REVIEW

A combinational theory for maintenance of sex

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Sexual reproduction implies high costs, but it is difficult to give evidence for evolutionary advantages that would explain the predominance of meiotic sex in eukaryotes. A combinational theory discussing evolution, maintenance and loss of sex may resolve the problem. The main function of sex is the restoration of DNA and consequently a higher quality of offspring. Recombination at meiosis evolved, perhaps, as a repair mechanism of DNA strand damages. This mechanism is most efficient for DNA restoration in multicellular eukaryotes, because the initial cell starts with a re-optimized genome, which is passed to all the daughter cells. Meiosis acts also as creator of variation in haploid stages, in which selection can purge most efficiently deleterious mutations. A prolonged diploid phase buffers the effects of deleterious recessive alleles as well as epigenetic defects and is thus optimal for prolonged growth periods. For complex multicellular organisms, the main advantage of sexuality is thus the *alternation* of diploid and haploid stages, combining advantages of both. A loss of sex is constrained by several, partly group-specific, developmental features. Hybridization may trigger shifts from sexual to asexual reproduction, but crossing barriers of the parental sexual species limit this process. For the concerted break-up of meiosis-outcrossing cycles plus silencing of secondary features, various group-specific changes in the regulatory system may be required. An establishment of asexuals requires special functional modifications and environmental opportunities. Costs for maintenance of meiotic sex are consequently lower than a shift to asexual reproduction.
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The paradox of sex

Why do most eukaryotic organisms reproduce sexually? The evolution of sex has been considered the 'Queen' of evolutionary biology (Williams, 1975; Bell, 1982) and is still under intensive debate. Sex is understood here in a broad sense as 'a process in which the genomes of two parents are brought together in a common cytoplasm to produce progeny, which may then contain re-assorted portions of the parental genome' (Bernstein *et al*., 1984; Birdsell and Wills, 2003). In a broad definition, sex occurs in viruses, prokaryotes and eukaryotes. In prokaryotes, sex is not directly linked to reproduction, which is understood here as a process of forming new individuals. In eukaryotes, sex is predominantly directly linked to reproduction in the combined processes of meiosis and outcrossing.

The paradox of maintenance of sex in eukaryotes arises from the fact that it has a lot of immediate individual costs, whereas it is difficult to give evidence for any advantages compared with asexual reproduction. The basic assumption suggests two costs of sex: the cost of meiosis (which breaks up co-adapted gene combinations) and that of male individuals not producing offspring (Maynard Smith, 1978; Bell, 1982). The latter point is strongly orientated towards sex in bisexual organisms, for example most animals. Gender separation, however, is no ubiquitous feature of eukaryotic sex. In hermaphroditic organisms, costs of males are reduced to that of a male function, but have still been estimated as being around 1.5-fold (Lloyd, 1988). A more comprehensive inventory over all groups of organisms showed five potential costs of sex (Lewis, 1987): (1) cellular–mechanical costs of meiosis and syngamy (the time needed for meiosis, syngamy and karyogamy, which confers an inactive stage and a delay of synthetic processes); (2) recombination (breakup of favourable combinations of alleles); (3) fertilization (risk exposure and density dependence); (4) cost of males and 'genome dilution' *sensu* Lewis, 1987 (a sexual parent transmits only 50% of its genes to the offspring); and (5) costs of sexual selection. Whereas (1) and (2) are costs of meiosis, (3)–(5) are costs of outcrossing; a discussion of costs of sex in this sense restricts the topic to sex of eukaryotic organisms.

Short-term advantages of asexuality are manifested in the taxonomically widespread phenomenon that asexual animals and plants often have much larger distribution areas than their sexual relatives, and tend to higher altitudes and latitudes and previously glaciated areas (Kearney, 2005; Hörandl, 2006, 2009; Hörandl *et al*., 2008). Such observations suggest a higher general fitness for asexual reproduction. Nevertheless, this phenomenon is restricted to a few genera and certain geographical areas, where environmental changes may have provided opportunities for shifts to asexuality (Hörandl, 2009). The taxonomic distribution of meiotic sex, however, is predominant among all groups of eukaryotes and not restricted to certain environmental conditions.

A large number of theories have been developed for the long-term success and maintenance of sexuality, but none of them have provided unequivocal support for a

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single hypothesis. In general, hypotheses for the maintenance of sex can be grouped into three main categories (Birdsell and Wills, 2003): (1) sex as an effective way to create genetic variation in the offspring, which allows for a faster adaptation to environmental variability (for example, Burt, 2000); these models rely mostly on the effects of recombination. (2) Sex as a restoration mechanism for damage of DNA strands, or mutational or epimutational change of the genome; here the main arguments are that meiosis would provide a tool for repair of double-strand breaks (for example, Bernstein *et al*., 1988; Bernstein, 1998) or would restore DNA methylation patterns (for example, Holliday, 1988). Recombination would also avoid a long-term accumulation of disadvantageous mutations; an asexual lineage cannot produce offspring with a lower mutational load than any previous generation (Muller's ratchet). Moreover, recombination can break up negative epistasis and thus allows for a more efficient purging of deleterious mutations (Kondrashov, 1988, 1993). (3) A third group of hypotheses regards sex as a consequence of phylogenetic fixation, that is, a feature inherited from ancestors that we cannot get rid of. This model does not seek an advantage of sexual reproduction *per se*, but regards it as a 'imperative relic' inherited from eukaryotic ancestry (Margulis and Sagan, 1986). It exceeds the topic of this paper to review all previous hypotheses. The comprehensive review by Birdsell and Wills (2003) outlines pros and cons of all contemporary models. In general, it is difficult to give evidence for ubiquitous advantages of single factors that would compensate the costs of sex. Therefore, many modern authors argue for pluralistic approaches and combinations of mutation- and recombination-based models (West *et al*., 1999; Pound *et al*., 2004; Ben-Ami and Heller, 2005; Cooper *et al*., 2005).

Empirical data from extant asexual organisms draw attention to some hitherto overlooked factors, such as the importance of shifts of ploidy levels. In this study, I wish to integrate these factors into new theory, which combines previous ideas in a framework of evolutionary history and increasing complexity of organisms. Origin, maintenance and constraints of loss of sex have different reasons and selective forces (Figure 1). Meiosis-outcrossing cycle may have evolved as a DNA restoration mechanism of double-strand damage, disadvantageous mutations and epimutations. This restoration mechanism became a main advantage for multicellular organisms, which start their development from a single cell. The main advantage of sex for complex organisms with high levels of cell differentiation is probably the alternation of a haploid selection phase and a diploid duration period, which is buffered against effects of deleterious recessive mutations. The buffered diploid period becomes predominant for growth and cell differentiation in eukaryotes with complex bodies (Figure 2). Secondary features that ensure mating compatibility help to establish sexual reproduction. Once sex is functionally and phylogenetically fixed, the loss of sex requires a combination of individually deleterious changes (Figure 3). Constraints of losses of sex are reviewed for animals and seed plants as evolutionarily advanced groups. Maintenance of meiotic sex is easier than the shift to asexuality and various mechanisms have evolved to balance costs of sex in eukaryotes. Sexual reproduction is consequently seen as a strategy to improve the quality of nuclear DNA in the offspring, which is crucial for the viability of complex organisms. Some suggestions for future research will hopefully stimulate thoughts and research on hitherto overlooked factors for explaining the 'paradox of sex'.

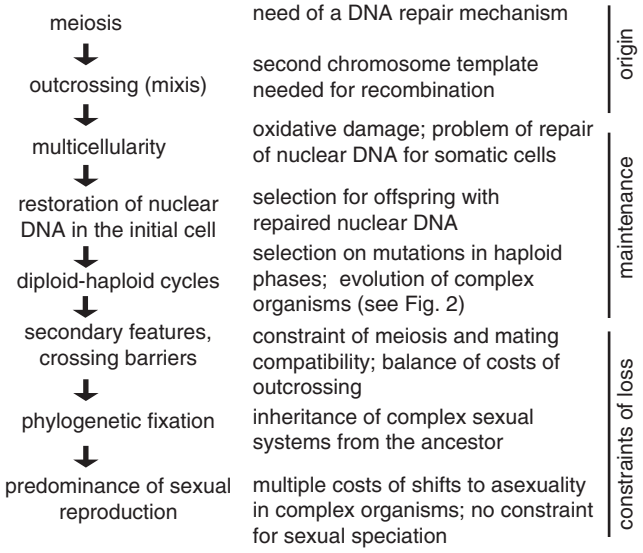

Figure 1 Hypothetical outline of evolutionary history of sexual reproduction in eukaryotes. Steps towards obligate sexual reproduction (left column). Functional constraints and selective forces driving evolution from one step to the next (right column).

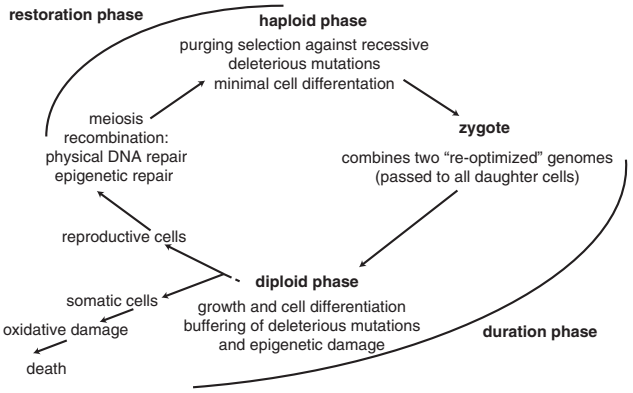

Figure 2 Scheme of potential advantages of sex in complex multicellular organisms. A haploid restoration phase of the genome alternates with a diploid duration phase, which is optimal for growth and cell differentiation. The advantage of regular sex is, thus, a qualitatively improved genome in the diploid phase, which is important for organisms with a high organic complexity (animals, vascular plants).

Evolutionary strategies of extant asexuals

Both DNA restoration models and environmental models postulate an advantage of sexuality because of a certain feature, assuming that the individual response to selection remains constant. In fact, this is not necessarily the case because asexuality is frequently connected to hybridity and polyploidy (at least in those groups in which the concept of ploidy can be rigorously applied). In Simon *et al*., 2003 survey of asexual metazoa, polyploidy is reported for 19 of the 36 listed taxa; in animals, asexual taxa can be diploid or polyploid, but

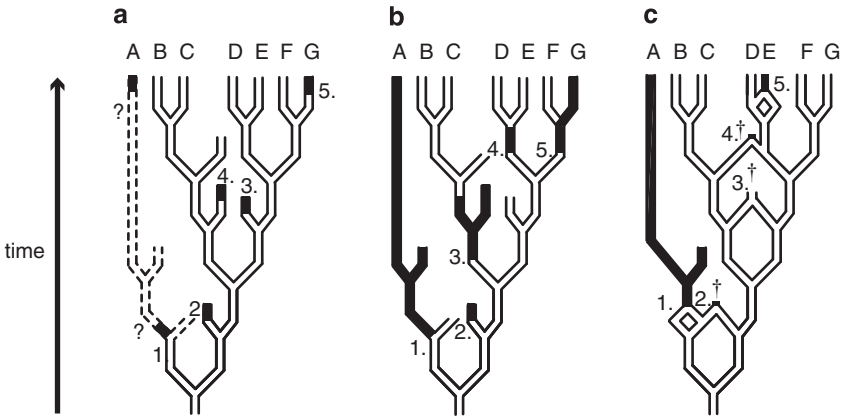

Figure 3 Three explanations for the observed phylogenetic distribution that asexuality is rare and scattered in the phylogeny of multicellular eukaryotes. White, sexual lineages; black, asexual lineages; A–G, extant taxa; 1–5, shifts from sexuality to asexuality. (**a**) Asexuals are short lived because of the accumulation of deleterious mutations and/or lower genetic variation; the model is contradicted by the existence of ancient asexuals (lineage A). (**b**) Asexuality is unstable because the shift back to obligate sexuality happens frequently (note that this model assumes equal extinction rates of asexuals and sexuals); empirical evidence for this model is scarce. (**c**) Shifts from sexuality to asexuality are most frequently triggered by interspecific hybridization (unsuccessful shifts marked by '†'); shifts to asexuality are rarely successful because of the divergence of sexual parents and functional constraints. Owing to unblocked speciation of sexuals, frequencies of asexuality remain low.

almost all polyploids have asexual reproduction. As in plants, asexuals are frequently of hybrid origin (Kearney, 2005). In seed plants and ferns, almost all apomictic taxa are polyploid (Asker and Jerling, 1992). However, seed plants have also considerable frequencies of sexual polyploids, which allows for a disentangling of polyploidy and mode of reproduction.

Polyploidy and hybridity alter genotype and phenotype in many different ways, which violates the basic assumptions of current hypotheses that lower genotypic variation or higher mutational load of asexual lineages would be disadvantageous, 'all else being equal'. Polyploidy can mask effects of deleterious mutations because of multiple chromosome sets. Allopolyploidy can induce a wide range of gene expression patterns: gene redundancy can diversify gene function or result in silencing, activation or subfunctionalization of genes. These processes increase genetic variation, which allows for a more efficient response to various selection pressures (for example, Soltis *et al*., 2003). Polyploids, especially those of hybrid origin, may have increased fitness because of heterosis effects (Hochholdinger and Hoecker, 2007). Genes may have greater expression in heterozygous conditions (overdominance) or gene redundancy may cause a dominant complementation of slightly deleterious recessives (dominance) (Comai, 2005; Hochholdinger and Hoecker, 2007). Expression of homoeologous genes is reprogrammed through epigenetic mechanisms (Comai, 2005). Polyploids may also maintain epigenetic patterns in homologues and minimize effects of demethylation patterns in gene expression; genetic and epigenetic effects may also interact and result in altered phenotypic traits (Chen, 2007). Genome duplications have occurred in all groups of eukaryotes and are an important factor for evolution and selection by increasing genetic diversity and complexity (Crow and Wagner, 2006; Meyer and Schartl, 1999). Re-evaluation of phylogenies of extant and extinct vertebrates suggests that genome duplications may have reduced considerably the risk of extinction (Crow and Wagner, 2006). The extensive literature on sexual polyploid plants reports increased tolerance of polyploids to nutrient-deficient soils, drought and cold temperatures (Levin, 2002). Polyploidy may alter response to herbivores dramatically (for example, Nuismer and Thompson, 2001). The background for altered biotic interactions are probably conspicuous changes in secondary metabolism, which may be because of chromosome doubling (Levin, 2002), or even because of hybrid origin (Orians, 2000; Kirk *et al*., 2005). A higher resistance to pests and pathogens of polyploids compared with their diploid relatives is known in several sexual plants (Levin, 2002). Polyploid plants of hybrid origin might show a considerable differentiation in ecological niches (Levin, 2002), and different cytotypes may show a differentiation in habitats (Soltis *et al*., 2003). In animals, the distributional success of asexual organisms has been often referred to effects of polyploidy rather than to asexuality itself (for example Lundmark and Saura, 2006). To summarize, the response to selection of a polyploid or hybrid asexual organism may differ considerably from that of a diploid sexual relative.

Reduced genetic diversity has often been seen as a major disadvantage of asexuality. Recent research in population genetics, however, has shown that asexual organisms do not lack genetic diversity. They just harbour it in another way. Allelic diversity is often maintained in highly heterozygous, hybrid or polyploid individuals. Heterozygosity is an effect of hybrid origin of asexuals and measures of heterozygosity of asexuals usually exceed that of sexual relatives (Hörandl and Paun, 2007). Heterozygosity can considerably increase fitness of asexuals because of hybrid vigour and high phenotypic plasticity (Hochholdinger and Hoecker, 2007).

Genotypic diversity is present in asexual populations through a diversity of different genotypes or clones (Loxdale and Lushai, 2003; Lushai *et al*., 2003; Hörandl and Paun, 2007). Multilocus genotypic variation can arise from multiple hybrid origin of asexuals, by facultative sexuality, backcrossing to sexuals and mutations (Hörandl and Paun, 2007). Therefore, genotypic variation

may be reduced within populations, but is distributed among asexual, locally distributed clones (Hörandl and Paun, 2007). Empirical data suggest that even asexual organisms may efficiently respond to selection through clonal diversity (for example, Jokela *et al*., 2003). Genetic diversity distributed among clones probably enables asexual organisms to colonize better variable environments than sexuals because of exploiting the whole resource space, as suggested by the frozen niche variation model (Vrijenhoek, 1984), which gets increasing support from recent empirical studies (Hörandl, 2006). Asexual organisms can be successful in spatial dimensions despite a reduced genotypic variability, whereby better founder abilities, heterozygosity and clonal diversity may be the key factors (Kearney, 2005; Hörandl, 2006, 2009; Hörandl *et al*., 2008). Moreover, genotypic variation created by recombination alone infers no ubiquitous advantage. Theoretical work suggests that recombination is advantageous only under certain conditions and selection models (for example, Brooks, 1989; Otto and Nuismer, 2004). In models of antagonistic co-evolution, recombination increases the response to directional selection, but the differences in population fitness fail to overcome the costs of sex (Peters and Lively, 2007).

If asexuality has the same efficiency in the response to selection because of effects of polyploidy and clonal diversity, and sex has a twofold cost in each generation, then selection should favour asexuality with rare recombination events over obligate sex. It would be expected that eukaryotes are predominantly polyploid asexuals with occasional or intermittent sexuality. The question arises, why do organisms not reproduce with 'a little bit of sex' (Green and Noakes, 1995)?

A further challenge for theoretical considerations is the existence of 'ancient asexual scandals' (Judson and Normark, 1996; Martens *et al*., 2002), such as the bdelloid rotifers (at least 40 myr old, ~363 asexual species) and the Darwinulid ostracods, which have been shown to be exclusively asexual since 208 myr (25 asexual species). Under the assumptions of both mutation-based and environmental models, asexual lineages should have short live spans, either because of the accumulation of deleterious mutations over time or because the competition with genetically variable sexuals drives them faster to extinction. Interestingly, bdelloid rotifers seem to be free of deleterious retrotransposons, which may help for a reduction in mutational load (Arkhipova and Meselson, 2005). Furthermore, bdelloid rotifers represent most probably ancient polyploids or diploids with ancient duplications of genes (Welch and Meselson, 2000). The question arises—how important is it for eukaryotes to have haploid, diploid or polyploid genomes, and what is the role of genetic diversity? The following theory presents answers to these questions.

A combinational theory for the evolution of sex in eukaryotes

If sex requires a short-term, individual selective advantage to be maintained then hypotheses based on the function of DNA are more convincing than environmental models. The individual's interest is to maintain its identity (autopoiesis) and to provide genetic continuity through reproduction (Margulis and Sagan, 1986). According to this aspect, selection for viability should be stronger than selection for evolvability (Redfield, 2001). As an undamaged genome is a basic requirement for autopoiesis and viability, individual-level selection should primarily act for processes restoring the genome in the next generation.

Origin of sex

Reasons for origins of sex are not necessarily the same as for the maintenance of sex (Birdsell and Wills, 2003). In the first living organisms, exchange of genetic material could have arisen as a DNA repair mechanism, which was necessary because of exposure to an intense UV radiation before an atmosphere with oxygen and a protective ozone layer was formed (Levin, 1988; Birdsell and Wills, 2003). Redfield (2001) suggested that transformation, the uptake of free DNA by competent bacteria, could have had also a nutritive function. The transfer of genes through transduction and conjugation could be side-effects of activities of genetic parasites; whether natural selection favours sex in bacteria because of the creation of new genetic combinations, is under dispute (for example, Redfield, 2001). Some forms of recombination may have evolved already in the earliest forms of life. Egel and Penny (2007) suggest that meiosis may have originated before the last common eukaryotic ancestor, and evolved in parallel to mitosis. Many proteins involved in meiosis have homologues in prokaryotes, and some activities may have been a preadaptation for meiosis (Egel and Penny, 2007): The cut-and-paste activities of topoisomerases, recombinational break repair by RecA-type recombinases and mismatch repair-related proteins are relevant for crossing over during meiosis, whereas clustering of telomeres may have led to homologue synapsis. Margulis and Sagan (1984, 1986) developed a comprehensive hypothesis for the evolution of sexual reproduction in the context of the endosymbiotic origin of eukaryotic cells. Organization of the nuclear genome in chromosomes is coupled with the evolution of mitosis. Starvation and cannibalism are seen as the main triggers for merging of cells and the evolution of outcrossing. Meiosis evolved out of mitosis through tardy kinetochores, consequently segregating chromosomes rather than chromatids. Meiosis was maintained as a mechanism to sort better the genetic diversity that has resulted from the merging of genomes.

Bernstein *et al*. (1988) see the main function of meiosis in repair of 'physical' damages, that is, double-strand breaks of the DNA. The authors argue that meiotic recombination is mostly 'cryptic', that is without an exchange of alleles in flanking regions. Meiosis is rather designed for repair of double-strand breaks of chromosomes, for which a second, undamaged template is necessary. Creation of allelic variation would be just a by-product of the repair mechanism, but not the target of meiosis. Oxidative damage would be the main subject for recombinational repair (Bernstein, 1998). Advance in the research on DNA repair has indeed shown that double-strand breaks are initiating events of recombination, and homologous recombination is an important DNA repair mechanism (for example, Bleuyard *et al*., 2006). Recombination as a repair mechanism, as suggested by Bernstein *et al*. (1988), would provide a ubiquitous selective advantage by improving the DNA in the meiotic products. The enzymes involved in DNA repair

through homologous recombination are rather conserved among eukaryotes (Bleuyard *et al*., 2006), suggesting an early origin of the mechanism.

Another advantage would be gained from meiosis as a repair mechanism of epimutations (Holliday, 1988). Holliday argues that a certain methylation pattern must be maintained in the germline for the controlled activity of genes and normal development. Non-methylated sites initiate recombination at meiosis, for which a methylated homologue is required. Recombination at meiosis is seen as an active, *targeted* repair mechanism. Meiosis would maximise the removal of heterozygotic epigenetic damage and guarantee the maintenance of methylation patterns in the germline, whereas somatic cells have less efficient means to recognize and remove epigenetic defects. Epigenetic effects may be environmentally induced and may be important for phenotypic response to selection and ecological interactions (for example, Richards, 2006; Bossdorf *et al*., 2008). Epigenetic information may be also transmitted to the next generation (Bond and Finnegan, 2007). The repair of disadvantageous epigenetic changes, as suggested by Holliday (1988), would infer an important short-time advantage of meiosis. Repair of strand damages of DNA and of epigenetic effects would both provide explanations for the need of a second chromosome set and thus for diploidy.

Meiosis–mixis cycles may have occurred in primitive eukaryotes initially only occasionally, but must have arisen early in the evolution of eukaryotes. The lack of meiosis in some extant protists can be also explained by secondary loss. Recent phylogenomic studies on meiosis genes suggest that even 'candidates' for ancient asexual eukaryotes, the parasitic protists *Giardia* and *Trichomonas vaginalis*, possess meiosis genes and are potentially capable of meiosis (Ramesh *et al*., 2005; Malik *et al*., 2008). The rather conserved machinery of meiosis supports an early origin in the phylogeny of eukaryotes (Ramesh *et al*., 2005; Malik *et al*., 2008) or even before the common ancestor of eukaryotes (Egel and Penny, 2007).

Despite the advantages of physical or epigenetic repair, it is questionable whether this would suffice to explain maintenance of regular sexual reproduction. Permanent diploidy or polyploidy could also serve for this purpose; the elaborated cycles of meiosis, karyogamy and syngamy would not be needed for repair (for example, Kondrashov, 1993). Repair of DNA strand breaks could be also carried out through the sister chromatid, as it occurs during mitosis. The breakthrough and establishment of sexual reproduction must have occurred together with multicellular organization. Multicellularity and cell differentiation infer a couple of selective advantages and may have evolved multiple times in eukaryotes, as early as 1.2 byr ago (Rokas, 2008). Margulis and Sagan (1986) argue that sex might be maintained rather because of advantages of multicellularity and specialization of cells than of sex itself. Here I would like to expand this argumentation by discussing that sex as a restoration mechanism of nuclear DNA infers a big advantage for multicellular eukaryotes.

Maintenance of sex

A complex multicellular organism develops from mitotic divisions of a single initial cell, which passes its genome to all daughter cells. If the initial cell has a thoroughly 'repaired' nuclear genome, all daughter cells will benefit from that because they are a monophyletic group, which has arisen from a single initial cell. In contrast, any later DNA restoration on differentiated cells is less efficient because it has to be done multiple times in specialized tissues. Cell differentiation is connected to an increase of genome size and number of genes (Rokas, 2008), which infers that targeted repair mechanisms become more complex. Mutations and, perhaps, even epigenetic damage accumulate during the life span of the organism in the nuclear DNA. Cellular selection within the organism can erase inviable cells, but cannot act efficiently on mildly disadvantageous mutations or epimutations as long as the whole organism is alive; cellular selection may act in differentiated tissues not efficiently because only a part of the genome is actually expressed. Any restoration of the nuclear genome is most efficient in the initial cell.

For eukaryotic organisms, oxidative stress accelerates ageing of somatic cells. Mitochondria and even plastids are involved in complex electron transfer systems during respiration and photosynthesis, respectively; incorrect transfers produce free radicals that cause mutations. Oxidative damage to the DNA of mitochondria and chloroplasts, and later on even of nuclear DNA, may limit life span of somatic cells (Allen, 1996). Somatic cells undergo senescence and programmed cell death. Therefore, for nuclear DNA of somatic cells, repair mechanisms at mitosis may suffice for keeping up autopoiesis during the life span of that organism; a more thorough and costly restoration mechanism of nuclear DNA, such as recombination and genetic exchange at meiosis plus repair of epigenetic damage, is unnecessary and also inefficient because cell death is inevitable. In contrast, if nuclear DNA is repaired during meiosis, then the improved DNA can be passed to the products of meiosis and consequently to the initial cell of the new organism.

The first multicellular organisms were probably haploid. If the meiospore has a repaired DNA, then it will be directly passed to the growing organism. In diplontic organisms (for example, animals), the initial cell is the diploid zygote, which receives the repaired DNA directly from haploid gametes. In haplodiplonts (plants), either the spore or a zygote is the initial cell of the multicellular organism. In any event, the repaired nuclear genome of the initial cell will be passed to *all* daughter cells of the growing organism.

In metazoans, the early separation of the somatic line and the germ line implies a direct and faithful transmission of the genetic/epigenetic information from the haploid gametes to the diploid zygote (Lankenau, 2007). Oxidative damage in the germline is minimized by repression of mitochondrial oxidative phosphorylation in the female gametes and maternal inheritance of mitochondria (Allen, 1996). Nevertheless, a repair during meiosis will be efficient even if the reproductive cells differentiate later in adult multicellular organisms (for example, in plants). In ferns and seed plants, the spores develop into short-lived female and male gametophytes producing the gametes. In flowering plants, only few cell divisions occur between meiosis and the fusion of egg and sperm nuclei to form the new initial cell, the zygote. The point is, meiosis as a DNA repair mechanism is most efficient shortly *before* a new initial cell is formed. Any asexual initial cell, in contrast, starts with the same

physical damages and the same epimutational load of nuclear DNA as the parental organism.

In small, unicellular organisms, the costs of meiosis are high because of the time needed for this process and because of the inability of division of labour among cells (Lewis, 1987). Moreover, meiosis is a process vulnerable to errors (Margulis and Sagan, 1986) and requires compatibility of chromosome sets. Karyogamy of gametes with uneven chromosome numbers may cause later meiotic disturbances, laggard chromosomes, aneuploid gametes or, in the case of polyploidy, multiple spindles during mitosis (Comai, 2005). In a single cell, coupling recombination directly with reproduction infers that any erroneous meiosis or outcrossing will strongly decrease fitness of the progeny or would even lead to a loss of a fit parental organism. For unicellular organisms, meiotic sex is therefore extremely costly. It is assumed that in early unicellular eukaryotes sex was perhaps initially only a facultative process aside predominant asexual reproduction. In protists, sexual reproduction is mainly a strategy for survival during periods with low nutritient supply by forming diploid dormant stages (e.g., hardy cysts). Meiosis just re-establishes the haploid, active stage (Egel and Penny, 2007). Asexual reproduction is predominant in favourable environmental conditions. In fact, frequencies of asexuality are highest in protists compared with other eukaryotes (Burt, 2000).

In a multicellular, complex organism, the risks of the combination of genetic exchange and reproduction are lowered because not the whole organism, but only a few cells have to undergo meiosis and only gametes have to merge; a mistake during meiosis or incompatible matings do not affect the whole parental organism, which is still able to reproduce with other gametes. Multicellular organisms can afford to 'risk' sex and 'waste' some gametes, and selection can act efficiently on gametes without inferring a big investment of the parental organism.

The time needed for meiosis is irrelevant in a multicellular organism because of the division of labour of cells. Moreover, multicellular organisms can develop better strategies to support successful outcrossing using higher motility and mate recognition systems. They can provide better nutritive support for juvenile periods of offspring. Division of labour and specialization of cells infer a general advantage not only for the exploitation of environments, but also for reproduction itself—multicellular organisms benefit best from the coupling of sex and reproduction.

Nevertheless, multicellularity alone would not suffice to explain the costs of regular sexual reproduction. *Volvocinae*, as models of simple, differentiated multicellular organisms alternate between sexual and asexual reproduction (Lankenau, 2007), a strategy also seen in many asexual animals (Schön *et al*., 2007); most asexual plants also have facultative sexuality (for example, Asker and Jerling, 1992). Still, the question remains: why regular sex?

It is striking that organism with a high organic complexity and predominant meiotic sex, such as animals and vascular plants have prolonged diploid life stages and short-lived, small haploid stages. The masking hypothesis states that diploidy will be favoured over haploidy under free recombination because of masking deleterious recessive mutations, but is less likely to be favoured when recombination rates are low (Otto and Goldstein, 1992). Diploidy implies in the long term an accumulation of heritable deleterious recessive mutations and mutation rates are in general higher in diploid than in haploid genomes. Even if somatic deleterious mutations are not inherited, they decrease the fitness of a population, and masking infers an advantage to diploidy compared to haploidy in both sexual and asexual organisms (Orr, 1995). Diploidy is thus optimal for an expanded, 'duration', period of a multicellular organism with a complex organization. Nevertheless, for the accumulation of mutations (and epimutations), diploidy is no infinite solution. Further outcrossing without reduction of ploidy level would lead to continuous polyploidization, which is disadvantageous because of problems at cell divisions and increase of DNA content (Comai, 2005). After a prolonged diploid stage, the return to haploidy leads to the expression of accumulated, but previously masked deleterious recessive alleles (Crow and Kimura, 1970). At the haploid phase, there will be a more efficient selection eliminating haploids with a high mutational load.

In organisms with a predominant diploid stage in the life cycle, selection on haploid phases is evident, but at quite different quantities (Joseph and Kirkpatrick, 2004): in pollen, up to 65% of the genome might be expressed and in spermatids of mammals just 1.3–3.8%. Nevertheless, selection may even act on unexpressed alleles. In animals, competition among actively moving spermatids, or other functions of the gametes, might be efficient enough for selection on haploid stages (Joseph and Kirkpatrick, 2004). Moreover, background effects may increase selection against deleterious mutations. With low levels of recombination, selection on individuals bearing deleterious mutations reduces the effective population size and thus genetic variance at other loci (Hill–Robertson effect). Recombination indirectly increases genetic variance in finite populations and improves response to selection (Keightley and Otto, 2006). This model was confirmed on haploid model organisms by Keightley and Otto (2006), and may consequently apply also to haploid gametes.

Theoretically, selection can act efficiently if haploid stages have a high variance and are produced in higher quantities than actually needed for reproduction. Meiosis creates variation in the haploid stages and the production of haploid stages outnumbers, usually by far, the amounts of diploid offspring. What should this 'waste' of gametes or spores, especially on the male side, be good for, if not an increased response to selection? It is not costly for a multicellular parental organism if purging selection acts on many 'cheap', small and short-lived haploid stages before the zygote is formed. In contrast, selection on the multicellular offspring becomes much more costly, especially if the parental organism has already invested its own resources on the offspring, for example, for providing nutritious support of juvenile periods. Selection on haploid stages is therefore an additional, highly efficient mechanism for improving the quality of DNA in the offspring and consequently for maintenance of sex.

Complex organisms with a predominant diploid stage can benefit best from the 'buffered' duration stage because of the time needed for growth and cell differentiation. This constraint may explain that animals

and seed plants with their complex bodies have a prolonged diploid growth phase and a reduced haploid phase in their life cycle. Land plants maintain the alternation of diploid sporophytes and haploid gametophytes, but show in their phylogeny a transition from a predominant haploid generation in bryophytes (mosses, hornworts and liverworts) to a prolonged diploid sporophyte generation in ferns and seed plants. In angiosperms, the haploid gametophytes are reduced to short-lived, few-celled 'organisms' without the capacity of autopoiesis. In animals, the stage of cell differentiation and growth is completely shifted to the diploid phase, which allows for the most complex organization among eukaryotes. Animals have up to 122 different cell types, flowering plants up to 44, fungi up to nine, slime molds three, green algae two; prokaryotic multicellular organisms have a maximum of three cell types (Rokas, 2008). Only animals and vascular plants have prolonged diploid phases. Within green plants, the progression from predominant haploid phases to predominant diploid phases may be also a consequence of an increase of tissue differentiation and physiological adaptations because of the demands of terrestrial life form. Prolonged diploid phases are a useful strategy for evolution and development of complex organisms. (This does not mean that they are evolutionarily more 'advanced' or more 'successful' than those with predominant haploid phases; fungi and green algae are evolutionary as successful as animals and seed plants—but they do not form such complex bodies).

Concluding, the *alternation* of haploid–diploid stages may have been a main reason for the fixation of regular cycles of meiosis-outcrossing cycles in complex multicellular eukaryotes, thus providing a trade-off for two contradicting evolutionary constraints: a buffered stable diploid duration period for growth and a restoration period for purging the genome before the formation of the new initial cell, the zygote. Therefore, it is highly efficient for a multicellular organism to combine DNA repair and purging selection on haploid stages *directly* or shortly before karyogamy. This strategy provides an 'optimized' DNA for all daughter cells of the zygote (Figure 1). It is an investment into increased quality of the offspring at the expense of quantity. If one wants to see a multicellular organism as a big cell colony (Margulis and Sagan, 1995), this strategy resembles in fact intermittent sexuality: a prolonged 'asexual' mitotic growth period alternates with a short 'sexual' repair and purging period which is restricted to the reproductive cells. The death of the rest of the organism, for example, all somatic cells, is inevitable. Coupling DNA restoration and reproduction is thus the optimal strategy of evolving and maintaining intra-organismal complexity.

Loss of sex

As outlined before, polyploidy and intermittent sexuality may be equally efficient for maintaining extant asexual animals and seed plants. Why are these phenomena so rare? For this question, it is useful to consider phylogenetic hypotheses. Williams (1975) interpreted the scattered distribution of asexuality in the phylogenies of most eukaryotes in three different ways: either, sex changes irreversibly to asexuality, but increases the risk of extinction, which is the rationale of both the classical mutation-based models and environmental models (Figure 3a); in this case, ancient asexuals are indeed a 'scandal of evolution' because their mere existence contradicts the basic assumption of the model. Alternatively, sex changes occasionally to asexuality, but the reverse happens more readily (Figure 3b); in this case, ancient asexuals are no big problem, but here there is a lack of evidence to show that frequencies of shifts from asexuality to sexuality are higher than vice versa. The third scenario implies that shifts from sexuality to asexuality are rare because of functional constraints (Figure 3c). Here, ancient asexuals represent a rare, successful shift to asexuality in the past, but the age of asexual lineages infers no problem. In fact, empirical evidence gives strong arguments for the last scenario.

Five major constraints have to be considered for a loss of sex:

(1) Breakdown of existing pre- or postzygotic crossing barriers between the parental sexual species to form hybrids and/or polyploids for the origin of asexuality.
(2) Overcoming a reduced fertility of the first hybrid generation caused by meiotic disturbances.
(3) The *concerted* break-up of the meiosis-outcrossing cycle and establishment of an alternative way of embryo formation.
(4) An alternative for DNA restoration is needed.
(5) Silencing or modifying secondary features of sexuality
(6) Establishment of the newly arisen asexual lineage.

Hybridization and/or polyploidy are major triggers for parthenogenesis in animals (Simon *et al*., 2003; Kearney, 2005; Gomez-Zurita *et al*., 2006; Mable, 2007). Furthermore, in flowering plants, apomixis arises most probably from a combination of hybridization and polyploidy which may cause a spatial or temporal deregulation of gene expression in the regulatory system to establish apomixis (Grimanelli *et al*., 2001; Koltunow and Grossniklaus, 2003, Curtis and Grossniklaus, 2007). Hybrid origin of natural apomictic plants has been assessed by many cytogenetic studies (Asker and Jerling, 1992) and analyses based on molecular markers (for example, Koch *et al*., 2003; Paun *et al*., 2006; Palop-Esteban *et al*., 2007). Hybridization between distantly related lineages, however, is limited by divergence and incompatibility of genomes. The balance hypothesis by Moritz *et al*. (1989) suggests that genetic divergence of parental genomes has to be sufficiently large to cause a high proportion of unreduced gametes, but must not be too large to significantly decrease the viability or fertility of hybrids. This infers just a narrow window for losses of sex through hybridization.

The machinery of meiosis and recombination itself and even a higher complexity of organisms and genomes restrict mixis to 'compatible' individuals. Among sexual organisms, mating compatibility will be selected for because otherwise no or infertile offspring will be produced. Although bacteria can transfer genetic material even among distantly related lineages, multicellular eukaryotes cannot. Meiotic sex *requires* 'crossing barriers' to exclude incompatible mating partners. Therefore, all features promoting outcrossing with a genetically compatible, but not too divergent individual will be selected for. Mate recognition systems, reduction of density

dependence, compatibility systems, copulatory organs, sexual pheromones, sexual selection, behaviour, genomic imprinting, etc. will be selected for and will be inherited to the offspring. Such features limit hybridization between closely related species; divergence of genomes blocks hybridization between distantly related species. Consequently, frequencies of hybridization and consequently shifts to asexuality will be *a priori* limited. Moreover, asexuality will be always restricted to certain taxa, as groups with low predispositions to hybridization and polyploidy will hardly ever become apomictic. Even in flowering plants, interspecific hybridization is taxonomically unequally distributed despite a rather high general frequency (Arnold, 1997). In vertebrates, not only frequencies of hybridization, but also the tolerance to polyploidy may strongly limit actual frequencies of shifts to asexuality. Origins of asexuality may require special environmental opportunities for increased frequencies of hybridization. Dramatic environmental changes may cause fluctuations of distributional ranges of organisms and consequently a breakdown of prezygotic crossing barriers (Hörandl, 2009). The evolutionarily young age of most extant asexuals would be rather a consequence of rather recent opportunities for origins of asexuality during the Pleistocene epoch (Hörandl, 2009).

Meiosis and karyogamy cannot be easily uncoupled; the loss of meiosis, but maintenance of outcrossing, results in continuous polyploidization, which is disadvantageous and finally deleterious because of cellular constraints (Comai, 2005). Meiosis without restoring diploidy will maintain the haploid phase in which a masking of deleterious recessive mutations is missing. Most species of animals need a diploid chromosome set in the egg cell for normal development (Engelstädter, 2008). Some asexual animals reproduce through automixis. Here meiosis takes place and the products of meiosis either undergo gamete duplication or fuse again to form a diploid zygote. Automixis results in a loss of heterozygosity and the expression of recessive deleterious mutations. The resulting inbreeding depression and loss of fitness are probably major constraints for automixis (Engelstädter, 2008). In flowering plants, the shift from sexuality to apomixis in angiosperms is constrained by the coordination of three steps, each of it under a separate genetic control: (1) the skip or bypass of meiosis (apomeiosis); (2) the parthenogenetic development of an unreduced egg cell; and (3) modifications in the formation of nutritious tissues for the embryo, the endosperm. Single steps towards apomixis are deleterious and it is unlikely that apomixis arises through single mutations (Van Dijk and Vijverberg, 2005). Taken together, getting rid of a single component of sex is deleterious, which strongly decreases the likelihood of getting rid of both (Margulis and Sagan, 1986).

If the meiosis-outcrossing cycles are omitted, an alternative mechanism for restoration of DNA has to be found. As discussed above, this is not so easy for multicellular organisms with differentiated tissues. A possible alternative is the widespread polyploidy of asexual organisms. Polyploidy may simply increase the 'buffering' capacity of deleterious mutations. Nevertheless, even polyploidy has disadvantages, such as cellular constraints, disturbances of mitosis, and conflicts in signalling and regulatory systems from multiple chromosome sets (Comai, 2005; Chen, 2007). In vertebrates, polyploidy is stable in fish and frogs, rare in birds but does not occur in mammals (Comai, 2005). Possible reasons for the rarity or lack of polyploidy in some groups of animals are disruptions of sex-determination systems (for example, Mable, 2004). Polyploidy arises, most probably, in groups with high numbers of gametes, in which selection against unbalanced chromosome sets sorts out inviable gametes (Mable, 2004), or under unstable environmental conditions (Mable, 2004; Hörandl, 2009).

The importance of secondary features is highly group specific. For example, sex determination systems in bisexual animals require often the paternal contribution to the genome, otherwise only male offspring is produced (Engelstädter, 2008). All male offspring was observed, for example, in Komodo dragons in captivity, which reproduced through facultative automictic parthenogenesis (Watts *et al*., 2006). Moreover, most animals have a paternal inheritance of centrosomes. Centrosomes are, in animal cells, the major microtubule-organizing centres during mitosis. A shift to parthenogenesis requires an activation of spontaneous centrosome assembly (Engelstädter, 2008). In mammals, genomic imprinting in the embryo may be another major constraint blocking shifts to asexuality (Engelstädter, 2008). In angiosperms, genomic imprinting occurs in the nutritious tissue of seeds, the endosperm. The endosperm is in the majority of apomictic plants ($\sim$90%) still fertilized by at least one pollen nucleus, because the contribution of the paternal genome is needed for normal development (Koltunow and Grossniklaus, 2003; Spielmann *et al*., 2003; Vinkenoog *et al*., 2003; Talent and Dickinson, 2007). Seed abortion due to failure of endosperm formation has turned out to be a major problem for introducing apomixis into crops (for example, in maize and pearl millet) Curtis and Grossniklaus (2007). Beside these developmental constraints, even secondary features that have established to promote outcrossing have to be silenced or modified to avoid a transfer of gametes (for example, a hormone-driven mating behaviour or insect-attracting flowers in plants).

Finally, even if the step towards asexuality was successful, the newly arisen apomict has to establish in competition with the sexual parents. Hybrid vigour and heterosis effects may help in the establishment (for example, Kearney, 2005). Nevertheless, reduced fertility may be a major impediment for establishment of an asexual individual in direct competition to the sexual population (for example, Hörandl, 2008). Uniparental reproduction, that is, the ability to reproduce through a single (female or hermaphroditic) individual, may enhance rapid colonization and establishment of asexuals outside the range of the sexual species (for example, Hörandl *et al*., 2008). Nevertheless, in the case of sperm-dependent or pollen-dependent asexuality (pseudogamy), uniparental reproduction is not guaranteed. Most hermaphroditic pseudogamous animals still need two individuals for reproduction (Beukeboom and Vrijenhoek, 1998). Hermaphroditic pseudogamous plants have to evolve self-compatibility (that is, a breakdown of recognition systems of self-pollen on the stigma or in the style) for successful uniparental reproduction (Hörandl, 2008; Hörandl *et al*., 2008).

Once the loss of sexuality infers these multiple costs, it is irrelevant whether selection would favour theoretically asexuality under certain conditions: the individual has no choice any more, it has to reproduce sexually because of its genetic basis and features inherited from its ancestors. The alternative to sexuality is just sterility. The individual can shift to asexual reproduction only if the actual cost of this shift is lower than the cost for the maintenance of sexuality. This may also explain why cyclical or intermittent parthenogenesis is rare—from the regulatory system it might be simply too complicated to switch regularly between two modes of reproduction.

The multiple constraints discussed above make the successful shift to asexuality, an exceptional event. Asexuals will be rapidly outnumbered by sexuals, because in the same time period sexuals will speciate in various ways; they will not 'wait' until a new asexual lineage is formed and established. Actual frequencies of asexual individuals might be also increased by residual sexuality and transfer of genetic factors, controlling asexuality, to sexual populations. In flowering plants, pollen can potentially transfer apomixis into sexual populations (Van Dijk and Vijverberg, 2005). Nevertheless, different ploidy levels and other factors may limit actual frequencies of such an contagious origin of apomixis (Van Dijk, 2007; Hörandl and Temsch, 2009). This confirms the crucial problem of apomixis: it is dependent on frequencies of origins, and origins depend largely on unusual sexual events. Owing to the dependence on sexuality, the contagious origin of apomixis remains restricted to closely related species, in which hybridization is not yet blocked by high divergence or other interspecific crossing barriers.

Costs of shifts from asexuality to sexuality

This shift would imply only a high cost if organisms would have lost meiosis genes and any male function completely. Extant asexual organisms have usually remnants of sexuality in their reproductive pathways, which can be seen as modifications of the meiosis–syngamy cycle. In fact, facultative sexuality has been assessed in most apomictic plant groups studied so far (for example, Asker and Jerling, 1992; Hörandl and Paun, 2007), confirming empirically that the shift back to occasional sexuality confers only a low cost. Apomictic plants still produce the same reproductive organs as sexuals, including insect-attracting flowers and functional pollen (for example, Meirmans *et al*., 2006; Van Dijk, 2007). Similar patterns are observed in sporadically asexual animals, such as flatworms (*Schmidtea polychroa*): reproduction is occasionally asexual but still sperm dependent and depending on the main organs for sexual reproduction (Beukeboom, 2007). That is, the shift from apomixis back to sexuality confers usually only a low cost because most features and organs related to sexuality have been retained.

Moreover, asexuals have no need to evolve crossing barriers; their reproduction is not constrained by chromosomal, cytoplasmic or genomic incompatibilities of two individuals. There will be no selection for any special features ensuring mating compatibility. The lack of crossing barriers may explain why co-existing apomictic lineages with some residual sexuality can readily hybridize and form hundreds or thousands of new lineages, as known from many groups of flowering plants (for example, Fehrer *et al*., 2007; Hörandl *et al*., in press). Nevertheless, reports of origins of fully sexual species following crosses of apomicts are rare. Origin of sexual species from hybrids of apomicts have been reported in hawkweeds (*Hieracium*) introduced to New Zealand (Chapman *et al*., 2003) and was obtained from experimental crosses in buttercups (*Ranunculus*; Nogler, 1984): here reversals to sexuality can be explained by heterozygosity for genetic factors controlling apomixis in the mother plant. Occasional meiosis, segregation and merging of gametes with the wild-type allele for sexuality result in fully sexual offspring (Nogler, 1984). Full restoration of sexuality from asexuals was also postulated from phylogenetic patterns of orbatid mites (Domes *et al*., 2007). The rarity of such records may have two reasons: first, researchers may not have looked at it in the past; second, a newly formed diploid sexual may have difficulties to establish in a predominantly polyploid asexual population because of minority cytotype disadvantages (Levin, 2002). To what extent shifts from asexuality to full sexuality contribute to the observed phylogenetic patterns, needs to be studied (hypothesis of Figure 3b).

Balance of costs of sexual reproduction

Once sex is established in a phylogenetic lineage, its costs must only be lower than the costs of a shift back to asexuality. The comparison with asexuality becomes irrelevant. Asexuality will just increase if costs for shift to asexuality become low and costs for sex become high; dramatic climatic changes may provide such opportunities (Hörandl, 2009). In the case of obligate sex, multicellular organisms balance the costs of sex as follows:

(1) Cellular–mechanical costs can be overcome by multicellularity, which has a lot of additional extra advantages because of a specialization of tissues (see above).
(2) The costs of recombination within a sexual system are balanced by the advantage of repair mechanisms and alternating a buffered diploid period with a purging haploid period.
(3) The cost of fertilization, in the sense of Lewis (1987), covers two different aspects: first, risk exposure because of potential transfer of viruses, parasites etc., and second, density dependence of sexuality because of the requirement of a mating partner. The first disadvantage might be balanced by multicellularity (for example, development of an immune system or improved mate-recognition mechanisms). Density dependence can be considerably reduced by large and differentiated multicellular organism because of increased motility (Lewis, 1987). In sessile organisms, the density problem is severe, which explains why, for example, flowering plants invest so much into the development of insect-attracting flowers or into adaptations to wind pollination. Consequently, density dependence is balanced again by specialization of tissues.
(4) The cost of males, in the sense of Bell (1982), applies only to bisexual organisms. As sex is predominant in both bisexual and hermaphroditic systems, it is less

relevant whether genders are separated on different individuals or not. The evolution of anisogamy can still be explained by the theory of Weismann that there are two selective constraints for sexuality, that is, that of the need of mobility and high numbers of gametes for increasing the chance to find a mate, and nutritive support for the zygote, which would favour large, less motile and lower number of gametes (Hoekstra, 1987). Anisogamy and, consequently, the evolution of sexes and gender confer, thus, the advantage to meet two contradicting selective constraints by separating the functions on two types of reproductive phenotypes. For a sessile organism, mobility of gametes is not at all increased by separating male and female functions on different individuals, which explains, for example, low frequencies of dioecy in species of angiosperms ($\sim$4%); hermaphroditic flowers and investments into the use of vectors for pollen transfer are a more efficient strategy (see above). Alternative explanations are mating type evolution based on gamete recognition (Hoekstra, 1987), which increases compatibility. Compatibility might be also the main reason for the predominant limitation to two genders in more complex organisms—more genders would perhaps complicate mate recognition systems without inferring a selective advantage. A further explanation might be the difficulty to combine cell organelles (mitochondria, plastids) of two isogamous gametes in one zygote; it may be functionally easier that only one type of gametes, the egg cell, retains the cell organelles (Lane, 2002). Reduced oxidative stress of inactive egg cells may guarantee the transmission of undamaged mitochondria through maternal inheritance. Mitochondria of male, motile gametes have already been damaged by oxidative stress and are not inherited (Allen, 1996).

(5) The cost of sexual selection (for example, risk exposure, different phenotypes of males and females) is balanced mainly by improvement of mate recognition and thus by multicellularity and specialization of tissues.

Conclusion

The synthetic model presented here overcomes the flaws of deterministic thinking of early evolutionary biologists that sex is defined by its consequences ('sex is good for evolution, hence nature invented sex'). This thinking would give 'nature' an inappropriate role of a foresighted creator and 'evolution' the role of a targeted process. Sexual reproduction in eukaryotes as a DNA restoration mechanism is good for autopoiesis of the offspring, by transferring a qualitatively better nuclear DNA to the next generation. As such, meiotic sex gives an individual, short-term selective advantage. Especially for complex organisms, a higher quality of offspring would be more important than higher quantity (as achieved by asexual reproduction). Coupling sex and reproduction is the most efficient restoration mechanism for a multicellular eukaryotic organism with differentiated tissues. Prolonging the diploid phase and shifting selection to a 'cheap', short-lived haploid phase is most efficient for complex, long-lived organisms. Sexual reproduction is, thus, the best strategy for evolving tissue differentiation and intra-organismal complexity. The constraints of mating compatibility accelerate this process by the evolution of crossing barriers, which contribute to the fixation of sexual reproduction.

For complex organisms, genetic variation of gametes is probably more important for maintenance of sex than that of offspring. Genetic variation of offspring is rather a consequence, a by-product of sex increasing the variance in the response to natural selection; this is important for 'evolvability', but not the reason for the maintenance of the costly meiotic sex. Evolvability is neither dependent on sexual reproduction nor on a certain organization level. Prokaryotes have neither sexual reproduction nor meiosis, but have also evolved and still do so with enormous diversification, individual numbers and adaptive potentials (Margulis and Sagan, 1986). Furthermore, asexual eukaryotes do evolve and speciate, as seen in asexual protists and in ancient asexual metazoa (Welch and Meselson, 2000). In simple organisms, selection can act efficiently on high numbers of individuals and rapid generation turnover, and asexual reproduction provides quantitative selective advantages. Eukaryotes have evolved in terms of complex organization of organisms. This starts with eukaryotic cells and has led to organisms as complex as human beings. Here, selection acts not just on quantity of offspring, but also on quality of the genome to maintain complexity of the bauplan and fitness of the next generation. Meiotic sex, coupled with reproduction, is simply the most efficient and established way against decay of nuclear DNA in multicellular, complex organisms. Genetic variation in haploid phases contributes to the DNA restoration process after meiosis, but this is not the only cause for maintenance of sexual reproduction.

Proposals for future research

Many aspects in this proposed hypothetical framework need to be analysed further. Of special importance are (1) the role of meiosis for the repair of physical and epigenetic damage of DNA; (2) selection on haploid stages; (3) the role of diploidy and polyploidy in the buffering of effects of mutations; and (4) the functional constraints of silencing sex. The processes at recombination, especially for the restoration of epigenetic changes, need to be investigated. The roles of selection and gene expression on haploid, diploid and polyploid organisms or stages in the life cycle need further studies. Effects of mutation accumulation, detailed information on gene expression and epigenetic changes need to be studied in haploid, diploid and polyploid stages. The model further postulates that selection on haploid stages is more important for the maintenance of sex than selection on offspring. This assumption needs to be tested both on organisms with predominant haploid stages and on haploid gametes of predominant diploid organisms. Such analyses must be conducted on various groups of eukaryotes to get a clearer picture of how the predominance of diploid life stages in complex eukaryotes has evolved. The present evolutionary cost of sex hypotheses are almost exclusively based on observations on animals—which are by no means representative for understanding evolution of eukaryotes. A promising approach is the direct comparison of haploid and diploid stages with respect to gene expression and selection on

mutants. Detailed studies on mutation accumulation and selection mechanisms in ancient and in polyploid asexuals are needed to get insights into alternative 'buffering' mechanisms in prolonged periods without recombination. The effect and transmission of epimutations need to be studied and incorporated into mathematical modelling.

The consequences of the change from unicellular to multicellular organization of organisms need to be considered more explicitly in a 'cost of sex' modelling. A basic assumption of the presented model is that sexual reproduction becomes advantageous only for multicellular organisms. The advantage of a purged genome in the offspring after sexual reproduction has to be compared with the advantage of a higher quantity of offspring after asexual reproduction. Side effects of multicellularity, such as the advantage of a division of labour principle, have to be considered for the 'cost of sex' calculations. Here, it needs to be considered that neither extant protists nor highly advanced multicellular eukaryotes are necessarily good representatives of early eukaryotic history. Mathematical modelling, incorporating variables as discussed above, is a promising approach.

To further support the role of phylogenetic fixation, the features and genetic control systems of sexual and asexual systems need to be studied carefully in various groups of eukaryotes. Such data will be essential for the understanding of actual fixation of sex and the actual costs to shift from sex to asexuality. Finally, natural origins of asexual organisms from sexuals need to be examined more in detail and on various model systems—experimental work, studies on evolutionary history and genetic control are needed to fully understand how easily asexuality can actually arise. On the basis of such data, a predictive modelling can be developed for estimated frequencies of asexuality for a certain taxonomic group and tested against actual frequencies of asexuality in that group. This requires a highly group-specific research and 'costs' of loosing sex are with all likelihood different in various groups of eukaryotes. Nevertheless, the general pattern can be only understood by comparing different groups of organisms. Again, data from all the kingdoms of eukaryotes will be required. Environmental changes, as an opportunity for spread of asexuals, and frequencies of contagious origins of asexuality need to be investigated. To my opinion, overcoming the 'cost of sex' question through a combinational theory will not narrow, but rather broaden the research on evolution of sex and stimulate multidisciplinary approaches.

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