Avoiding bad genes: oxidatively damaged DNA in germ line and mate choice

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Summary

August Weismann proposed that genetic changes in somatic cells cannot pass to germ cells and hence to next generations. Nevertheless, evidence is accumulating that some environmental effects can promote heritable changes in the DNA of germ cells, which implies that some somatic influence on germ line is possible. This influence is mostly detrimental and related to the presence of oxidative stress, which induces mutations and epigenetic changes. This effect should be stronger in males due to the particular characteristics of sperm. Here, we propose the hypothesis that females are able to avoid males with oxidatively damaged DNA in the germ line by using oxidative-dependent (pre- and post-mating) signals. This new hypothesis may shed light on unsolved questions in evolutionary biology, such as the benefits of polyandry, the lek paradox, or the role of sexual selection on the evolution of aging. ***BioEssays*** **30:1–8, 2008.**

Introduction

Since maintaining the integrity of the genome is of vital importance, organisms have evolved a range of mechanisms to overcome the mutagenic and lethal effects of damage in the DNA. August Weismann[(1)] proposed that genetic changes in somatic cells cannot pass to germ cells and hence to next generations (sequestration of gem-cell lineage, i.e. "the Weissmann barrier"). His hypothesis would rule out the inheritance of acquired characteristics as proposed by Jean-Baptiste Lamarck,[(2)] thus constituting a cornerstone in the modern evolutionary theory. In recent years, evidence is accumulating that some environmental effects can promote heritable changes in the DNA of germ cells, which implies that some somatic influence on germ line is possible. Here, we argue that this influence is frequent and mostly detrimental on male germ line, being an important selective agent shaping mate choice in animals.

The genetic constitution of the offspring depends on the integrity of sperm and egg DNA, and this integrity will exert a large influence on individual fitness. We propose the hypothesis that oxidation of DNA in the germ line is a prominent force in the evolution of mate choice and sexual signalling. The hypothesis assumes that by avoiding oxidatively damaged sperm the choosy sex avoids heritable effects derived from DNA damages. We predict that females are able to avoid males with oxidatively induced DNA damages in the germ line by means of oxidative dependent (pre- and post-mating) signals. The hypothesis also predicts that traits that have evolved in this context should signal the level of oxidative damage in germ cell DNA independently from other condition-related parameters (i.e. they should be specific).

The hypothesis expands earlier ideas[(3–6)] taking the advantage of recent studies on evolutionary biology, genetics and reproductive biology, which highlight the role of oxidative stress as an important agent involved in DNA damage, transgenerational congenital diseases and a key role in sexual signals. We have focused on females avoiding damaged sperm because they are commonly the choosy sex and because DNA damage is higher in the male germ line than in the ovum (see below), although selection on males avoiding females with damaged DNA in the ovum is also possible.

Sperm is particularly prone to oxidative-induced DNA damage

In the chapter VIII of his book "The descent of man, and selection in relation to sex" Charles Darwin[(7)] noted that "the greatest number of abnormalities is found in the males". This suggestion was expanded by Haldane,[(8)] reporting that the rate of mutation to haemophilia is about ten times higher in

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males than females. Since then, "male-driven evolution", the idea that male-originating mutations drive the mutational component of evolution, is well supported in a variety of organisms.[9,10] Thus, several studies in vertebrates[11–13] indicate that the mutation rate is higher in spermatogenesis than in oogenesis.

The occurrence of higher mutation rate in males is commonly attributed to the greater number of germ cell divisions in male germ line.[10] Moreover, since male germ line is constantly produced during adulthood (in vertebrates, females commonly produce all their gametes before maturity), probabilities for mutation in males would also increase due to the accumulation of somatic mutations in genes involved in the replication machinery.[10] Also, all DNA repair processes are inactivated at the late stages of spermatogenesis; thus, DNA lesions induced at these stages remaining non-repaired.[14] In humans, spermatozoa frequently possess high levels of nuclear DNA damage,[15] which is associated with cancer and genetic abnormalities (e.g. achondroplasia, multiple endocrine neoplasia and Apert's syndrome) in the embryo and offspring.[16–18]

The larger number of germ cell divisions, the longer period of germ cell production and the lack of repair mechanisms in the late stages of germ cell formation implies that male germ line should be more exposed to damages produced by environmental factors than female germ line. The most-important environmental factor by which DNA damage is induced in the male germ line is oxidative stress,[14,19] which is commonly defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant molecules in favour of the former.[20] Spermatozoa are particularly vulnerable to oxidative stress due to the requirements for energy by the sperm-motility apparatus, which demands a high level of respiratory activity, leading to ROS production.[21] Moreover, plasma membranes of spermatozoa, whose physical properties and functional integrity determine in part motility and fertilising ability, hold large amounts of polyunsaturated fatty acids, prone to oxidative injury,[22] whereas the cytoplasm contains low concentrations of antioxidant enzymes.[23] On the other hand, sperm DNA is protected from oxidative stress by its chromathin compact organization (the DNA strands are tightly wrapped around the protamine molecules) and by enzymatic and no-enzymatic antioxidants in the array of the seminal plasma.[24–25] Antioxidants obtained from diet would also contribute to prevent damage in sperm DNA.[26] For instance, in humans, when the level of dietary antioxidants are insufficient to maintain seminal antioxidants, the oxidative lesions in sperm DNA are more than doubled.[27] Additionally, in the semen, the antioxidant defence system and lipid peroxidation can vary among individuals of the same species.[28] Thus, if variation among males is high, selection should favor females that avoid males with greater amounts of oxidatively damaged DNA in the germ line.

Heritable effects of oxidative stress on sperm DNA

In spermatozoa, it has been shown that ROS can be generated both at the mitochondria (NADH-dependent oxido-reductase)[29] and at the plasma membrane (NADPH-oxidase system).[30] It is known that spermatozoa experimentally exposed to free radicals suffer a large number of DNA abnormalities (Fig. 1), including chromosomal rearrangements, histone modifications, cross-links, deletions, base modification, base-free sites and frame shifts.[31–35] Thus, for example in humans, smoking produces oxidative DNA lesions in sperm, such as 7,8-dihydro-8-oxoguanine (8-oxoG; Fig. 1),[36] and increases the risk of cancer in the offspring.[37]

Oxidative-induced primary lesions on the DNA structure may directly promote mutations, or serve as permutation damage sites, some of which after repair in the embryo may also result in mutations.[38] Moreover, oxidative stress is also involved in the accumulation of structural DNA modification in germ cells, which after fertilization may have a negative effect on genomic stability of the developing embryo[39] leading to epigenetic heritance. Epigenetic information—differences in the programmes of gene expression without changes in the DNA sequence—is encoded by DNA methylation, histone modifications and other chromatin proteins.[40,41] Alterations in the epigenetic programming of the germ line [such as produced by oxidative stress, particularly in DNA methylation[42] and histone modifications[43]] can induce an epigenetic transgenerational disease state.[44] In humans, epigenetic abnormalities have been linked to some congenital diseases (e.g. Beckwith/Wiedemann and Angelman's syndromes).[17]

Methylation of DNA (Fig. 1) is a critical epigenetic factor in the regulation of gene expression and genome stability in many organisms.[45] Such methylation, generating 5-methylcytosine, results in gene silencing. Although the vast of DNA methylation marks are erased after fertilization,[46] a small subset of imprinted genes maintains a defined DNA methylation pattern that is transmitted through the male or female germ line, resulting in allelic expression differences.[47] Oxidative insults produce aberrant DNA methylation patterns[48] and modification of the DNA methylation pattern of imprinted genes has been shown to induce transgenerational diseases.[49]

The impact of oxidative stress on the sperm DNA and its potential transgenerational effects on the offspring open a field for sexual selection forces to act. From the perspective of current evolutionary theory, females should try to optimize mate choice by coupling with best-fitted males that will provide them with direct benefits (non-heritable benefits that increase female fitness) and/or indirect benefits (heritable benefits accrued by the offspring).[50] Sexual selection would thus promote the evolution of signals of the intrinsic quality of the male. In the present hypothesis, we propose that some of

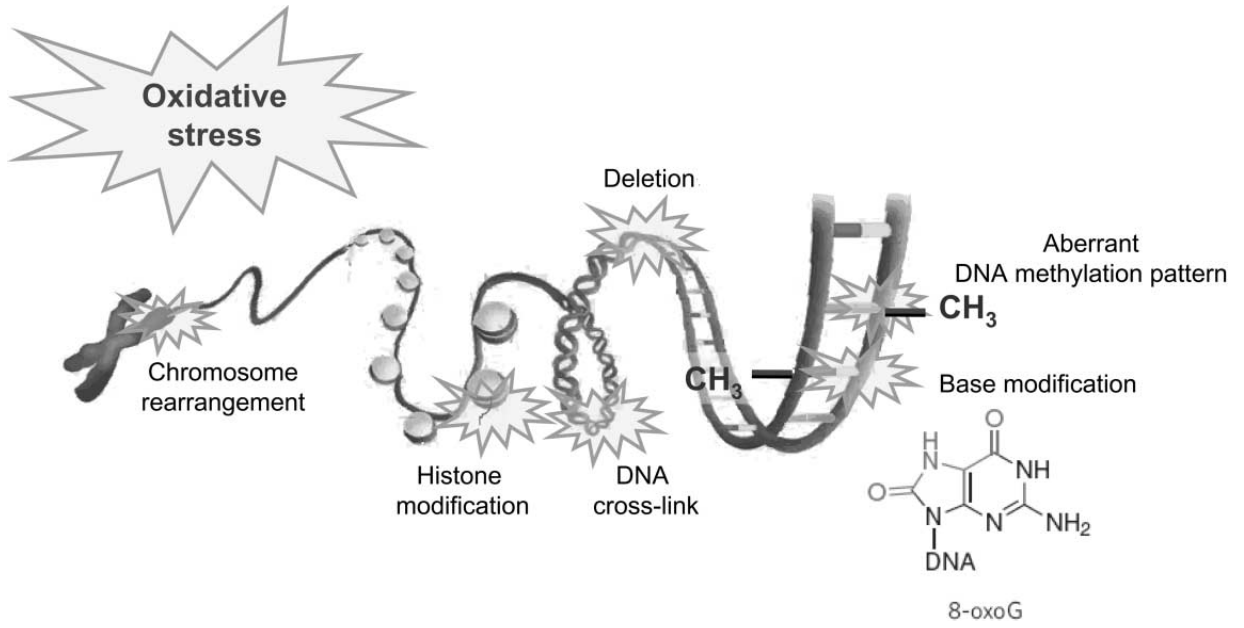

Figure 1. Some sperm DNA abnormalities associated with oxidative stress.

these signals would be used by females to assess the efficiency of the holder in preventing oxidative damage of sperm DNA. Females could also evolve passive or active mechanisms to discriminate sperm carrying oxidized DNA after copulation. Thus, females could select individuals (or sperm) with enhanced DNA integrity to fertilize their ova.

Precopulatory choice: sexual signals as markers of DNA damage in male germ line

The idea that females can use sexual signals to avoid aberrant males was first argued by Charles Darwin:[7] "females may accept not the male which is the most attractive to her, but the one which is the least distasteful". There are some examples that females are using sexual signals to avoid incompatible genes (e.g. Ref. 51 and references therein). Further, females may be also able to avoid "bad" genes arising from oxidatively damaged DNA if the amount of DNA damage correlates with the expression of secondary sexual characters (Fig. 2).

It has been suggested that oxidative damage is the proximal cause of the genuine information revealed to prospective females through male sexual secondary traits.[3] Coloured sexual signals could be excellent candidates to play a role in this context. Carotenoids are pigments with antioxidant properties[52] responsible for many yellow-red coloured traits involved in sexual signalling. Recent works suggest that carotenoid-dependent coloration honestly reflects the antioxidant status of the signaller.[53,54] At present, there are no studies relating antioxidant-dependent sexual signals and sperm DNA damage, but several lines of evidence suggest that this relationship is highly probable. First, recent studies indicate that secondary sexual traits mirror somatic antioxidants and oxidative stress,[53–55] and sperm DNA damage is highly affected by somatic oxidative stress.[43,56] Second, secondary sexual traits reflect sperm characteristics, such as motility,[55,57] which correlate with oxidative-induced damage

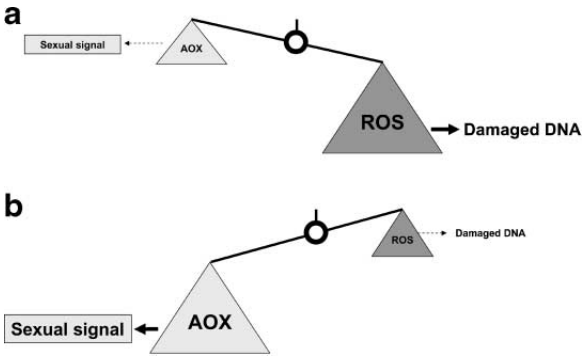

Figure 2. Oxidative stress can be defined as an imbalance between the availability of antioxidant resources (AOX) and production of reactive oxygen species (ROS) by the organism. **a:** When the balance is tilted to ROS, sperm DNA can be damaged and the expression of sexual signals is attenuated. **b:** When the balance is tilted to AOX, DNA damaged is attenuated and the full expression of sexual signals is favoured. Note that low levels of ROS have also important physiological functions.

to sperm DNA.[58] Third, the exposure to some pro-oxidant contaminants (i.e. polychlorinated biphenyls; PCBs) increases the probability of sperm DNA damage[59] but also induces a decrease in body antioxidants to scavenge oxygen reactive substances,[60] which could be mirrored in sexual signalling. In fact, American kestrels (*Falco sparverius*) experimentally exposed to PCBs decreased plasma carotenoids and coloration.[60]

However, the best evidence of a link between sexual signalling and sperm DNA damage comes from the recent Crews et al. study.[61] These authors found that female rats detect and avoid transgenerational epigenetic defects in males, but not in females, triggered by a fungicide. This fungicide, vinclozolin, promotes an epigenetic reprogramming of the germ line, induction of erroneous imprinted genes (by DNA methylation) that stand across subsequent generations, which in turn provokes disease states or tissue abnormalities.[62] As far as we know, this is a unique study showing that females can use a signal (probably a sexual signal such as some odours) to detect and avoid males with damaged DNA in the gem line. However, the mechanism underlying the effect of vinclozolin in both sexual signals and DNA damage is unknown. The vinclozolin is an androgen receptor antagonist that disturbs the epigenetic profile of male germ cells but also promotes oxidative stress.[63] Crews et al.[61] suggested that vinclozolin was able to induce changes in the expression of the Major Histocompatibility Complex (MHC) genes in three generations of vinclozolin rats. Rodents can discriminate the odours of individuals that differ genetically only at a single MHC locus, which indicates that MHC genes influence individual odours.[61] This example illustrates that heritable defects in the germ line may be mirrored by sexual signals; this should be specially expected when oxidative stress is the mechanism underlying both sexual signalling and sperm DNA damage.

Finally, when the oxidative damage in the germ line tends to increase in males as they age,[18,43,56] females might also avoid older males as mates using antioxidant-dependent sexual signals.[54,64] Senescence, the progressive decline with age in performance of somatic maintenance as well as reproductive activities, is believed to be universal in the life history of age-structured animals;[65] oxidative stress is thought to be one of the key mechanisms responsible for most of the degenerative processes associated with aging,[65] including damages in the germ line.[6,18] Interestingly, although early models of sexual selection suggested that females should prefer old males as mates because merely by surviving a male with a long lifespan has proven his high genetic quality for viability,[66] when age-related increase in male mutation load is incorporated in models, females evolve a preference for younger males.[67] These results supports the idea that potential benefits of mating with older males[66] could be mitigated by the deleterious effects of de novo germ-line mutations, stressing the importance of germ-line quality in the evolution of female preference. The age-dependent patterns of both male sexual attractiveness and female preference found in some species,[54,64] support the idea that females may use antioxidant-dependent sexual signals to assess variation in the efficiency of mates in preventing oxidative damage to sperm DNA.

Postcopulatory choice

Sexual selection may not only act through behaviours leading to differential copulation with the preferred mate, but also across postcopulatory mechanisms after mating with several mates.[68] In that scenario, females should choose among sperm from different males simultaneously (or sequentially) present at their genital tract.[68–70] Postcopulatory mechanisms potentially used by females to discriminate sperm carrying oxidatively damaged DNA would include behavioural and physiological mechanisms operating in the female reproductive tract, which may act as a passive barrier.

Behavioural postcopulatory mechanisms

Mating with more than one male is the norm for females of many species.[68,69] In addition to generating competition between the ejaculates of different males,[69] multiple mating may allow females to dilute any damaged sperm and to bias sperm use.[68] Similarly, mating repeatedly with the same partner, a common behaviour in many animals,[71] may benefit the female by avoiding aged and oxidized sperm.

Females may reduce the chances of fertilization by oxidized DNA by directly ejecting the sperm: a wide spread behaviour among insects, birds and mammals.[68] The time the sperm is retained in the male or female reproductive tracts before fertilization strongly influence sperm aging and viability, in part due to a longer exposure to free radicals (reviews e.g. in Refs 4 and 72). Accordingly, in *Drosophila melanogaster*, females eject stored sperm out of the seminal receptacle, and this does not depend on the receipt of either sperm or seminal fluids from a new mate.[73] In the black-legged kittiwake *Rissa tridactyla*, a monogamous seabird, females eject inseminations performed with the same mate several weeks before egg laying, retaining those inseminations that occurred soon before laying.[74] Furthermore, ejecting sperm from early copulations had a positive effect on hatching success and chick condition.[74] Thus, both studies suggest that females may be dumping aged and oxidized sperm, and that this behaviour may have fitness consequences.

Physiological postcopulatory mechanisms

In species with internal fertilization, spermatozoa should travel a tortuous path toward the ovum after its deposition in the female tract. Female reproductive tract represents a formidable barrier for spermatozoa and, for example, in humans, it

is estimated that only one of every 25,000 spermatozoa inseminated reach the fallopian tubes.[75] This drastic reduction of sperm numbers suggests a strong selection within the female tract.[68,76] Indeed, the female tract is an environment where numerous interactions take place and many proximate mechanisms among several taxa have been proposed to explain how the female's egg and reproductive tract may control which sperm is allowed to fertilize (e.g. reviews in Refs 77 and 78). Here, we suggest that oxidative damage to sperm DNA could be translated into changes in spermatozoa characteristics that could allow postcopulatory discrimination by females. In fact, we know that sperm carrying oxidized DNA shows reduced fecundity rates at least in humans.[19] In this context, although active mechanisms should be not discarded, current evidences (see below) suggest that an important mechanism of postcopulatory sperm discrimination is the female reproductive tract as passive filter of damaged sperm.

In this line, the first potential mechanism of filtering would be the length of the female reproductive tract. The distance that spermatozoids must travel to find the egg can be an important filter because the energetic expense of sperm motility will increase ROS production by mitochondria respiration.[21] Such ROS may react with the high concentrations of polyunsaturated fatty acids in the sperm plasma membrane leading to a loss of membrane fluidity and integrity.[79] Therefore, sperm carrying oxidised DNA should be more vulnerable to the further attack of free radicals, being discarded in long female tracts. Reduced fecundity of sperm with oxidized DNA[19] is likely due to detrimental ROS effects on the motility of spermatozoa, peroxidation of membrane lipids leading to compromised sperm–ovum fusion and decreased chromatin quality.[80,81] Female could therefore exert a passive filtering of sperm carrying oxidized DNA due to correlated damages on the sperm capacity to move, reach and fertilize the ovum.

In addition to the length of the travel, there are other physiological barriers in the female reproductive tract that spermatozoa have to overcome to fertilize the ovum (Fig. 3). After released into the acidic environment of the vagina, sperm should pass into cervical mucus that selectively filters sperm.[82] The cervical mucus is enriched in leukocytes, and these leukocytes produce reactive oxygen molecules that have a deleterious influence on damaged sperm. Of those that penetrate cervical mucus, only sperm with vigorous motility and a plasma membrane resistant to oxidative conditions will be passing into the uterine environment.[83] Although the time sperm spend entering the uterus is brief, important changes in plasma membrane occur facilitated by the uterine environment.[84] The increased permeability to ions promoted by these changes depends on the membrane fluidity (negatively affected by lipid peroxidation) and it is essential to the dialogue between sperm and egg during the capacitation and chemotaxis processes.[85]

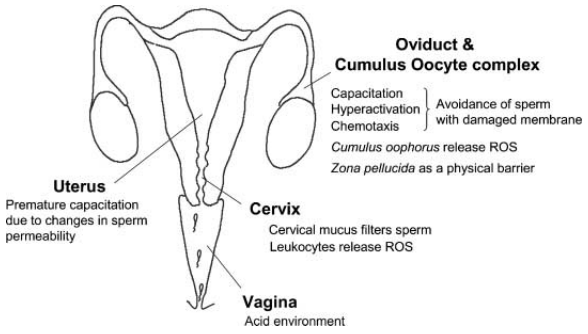

Figure 3. Female reproductive tract as a barrier to sperm carrying oxidized DNA. Spermatozoids must cross different barriers in the way to reach the ovum. In most of them, oxidative stress seems to be involved as a key mechanism in the filtering of damaged sperm.

Capacitation is a phenomenon only present in mammals where spermatozoa experience a process of ripening, which involves biochemical, biophysical and metabolic modifications of all parts of spermatozoon, necessary to fertilize the ovum.[86] The result is a more fluid membrane with an increased permeability to Ca^{2+}. An influx of Ca^{2+} produces increased intracellular cAMP levels and thus, an increase in motility[87] and hyperactivation, essential to overcome the resistance of the *zona pellucida*.[83] Capacitation must be timed precisely, because once spermatozoids are capacitated, they display increased metabolism and energy expenditure, which reduce their life expectancy due to increased oxidative stress.[88] A certain level of free radicals are however necessary to undergo capacitation.[86] Thus, it is probable that sperm with damaged membrane should be more sensitive to the attack of free radicals, being prematurely capacitated.

Another potential mechanism for the avoidance of oxidized sperm by females is related to chemotaxis, by which certain substances guide capacitated spermatozoa to the egg. It has been proposed that different spermatozoa might respond to different chemoattractants, resulting in sperm selection by females.[77] Receptors for chemoattractants such as guanylate cyclase are placed at the flagellum membrane.[77] Excessive amounts of activators for these receptors (i.e. guanylate cyclase-activating substances) may however exert opposite, anti-reproductive effects.[89] Such reverse effect is explained by the fact that some of these substances are in fact free radicals (e.g. nitric oxide) that also induce oxidative damages on the sperm membrane.[89] Those spermatozoids with an a priori oxidative damage to their membranes should be thus more susceptible to a negative action of such chemoattractants, allowing female filtering of damaged sperm.

At the end of the tract, spermatozoids reach the granulose cells accumulated around the egg cell (*cumulus oophorus*).

These cells also produce ROS that enhance the sperm capacitation.[90] It has been suggested that one of the functions of the cumulus may be the selectively filtering of prematurely capacitated spermatozoids, allowing crossing of only 10–20 spermatozoids.[83] Interestingly, covalent cross-links between structural DNA proteins in the sperm nucleus, protect nucleotides from oxidation but also allows the penetration of the spermatozoid into the next layer, i.e. the robust *zona pellucida*.[86]

In summary, only sperm with plasma membrane free of lipid peroxidation and protected by seminal antioxidants would be able to withstand increased oxidative stress[79] and to cross over the numerous barriers in female genital tract to reach the ovum.[91] Supporting this idea, it has been demonstrated in humans that the female tract facilitates the removal of sperm with DNA damage from the ejaculated sperm population.[92]

Challenges

Several issues need to be address to evaluate the relevance and generality of the hypothesis presented here. The first challenge will be to determine to what extent oxidative DNA damages in the sperm promote heritable changes in wild species. Artificial insemination with oxidatively damaged DNA in the sperm within the natural range is a promising approach. In terms of precopulatory choice, since oxidative stress could be frequently associated to a loss of overall condition, distinguishing between females choosing males with enhanced DNA integrity in the germ line and males carrying overall-condition-genes (probably genes at many loci; see Ref. 93) would represent empirical difficulties, but it should be not insurmountable. Studies demonstrating that experimentally induced oxidative damages in sperm DNA (avoiding collateral effects Ref. 94), are mirrored in sexual signals are required. In addition, comparative studies within a phylogenetic framework would allow testing whether the degree of DNA damage in the male germ line results in reliable sexual signals throughout the evolutionary changes. Indeed, in birds, germ-line mutation rates correlate with the intensity of sexual selection.[5]

In terms of postcopulatory mechanisms, the combination of artificial insemination experiments and the manipulation of oxidatively damaged DNA in the sperm, under sperm competition scenarios, may also help to understand whether the female reproductive tract is selecting sperm with enhanced DNA integrity. Indeed, in humans, sperm carrying damaged DNA have very limited fertilization rates, but when assisted reproductive techniques are used this sperm may fertilize an oocyte.[95] Thus, in support of our hypothesis, when the barriers that prevent sperm to reach the oocyte are being circumvented by assisted reproduction technologies, selection on sperm with DNA integrity disappears. Nevertheless, experiments about the underlying mechanisms (oxidative or other) are needed. Finally, further progress also requires tools for investigating oxidatively damaged DNA in the sperm of wild species. Fulfilling the potential role of sexual signals as markers of damaged DNA in the germ line will be best achieved through collaboration between reproductive physiologists, geneticists and evolutionary biologists.

Conclusions

The selective advantage of overcoming DNA damage is clear, and it has been argued that meiosis (i.e. sex) has been selected for the recombinational repair of DNA damage.[96,97] Accordingly, in facultative sexual lineages, recent evidence suggests that sex is an adaptive response to combat oxidatively damaged DNA.[98,99] Furthermore, the picture that emerges by incorporating results from current physiological and molecular studies into the evolutionary thinking strongly suggests that oxidation of DNA in the germ line can be a prominent force in the evolution of sexual selection. The two-fold cost of meiosis in females is added to the cost of DNA damage passed to progeny via the sperm, possibly resulting in selection of females that choose mates with low levels of damage in the germ line.

This perspective opens new avenues to solve recurrent problems in evolutionary biology. First, the lost of genetic variance of populations associated to sexual selection[100,101] could be counterbalanced by the incorporation of oxidative-induced de novo mutations in the gene pool. Such a process would contribute to reach a mutation-selection balance, which would help to explain the unresolved lek paradox (i.e. why do females in lek species continue to discriminate between males if the genetic benefits of choice are expected to be small;[101] e.g. Ref. 102). In addition, the avoidance of sperm with oxidized DNA would represent an important benefit of mating with several males, which would contribute an explanation of the evolution of polyandry across different taxa (see Refs. 6 and 103). On the other hand, since frequent ejaculation may reduce sperm DNA damage,[104] avoidance of damaged sperm could explain why some monogamous species copulate repeatedly with the same male. Furthermore, the age-dependent effect of oxidative stress on germline DNA supports the idea that sexual selection plays a key role in the evolution of lifespan. The avoidance of the oldest oxidized males would lead to discard those males carrying longevity-promoting genes that manage to survive. This would ultimately promote longevity shortening in the next generations. Accordingly, although irrespective of the presence of oxidative damage in sperm DNA, it has been suggested that female preference for intermediate-age males promote longevity shortening.[67,105] Finally, the hypothesis presented here may also apply for male mate choice. For instance, old oxidized males could prefer mating with young healthy females if their eggs are more efficient in repairing the DNA damage

brought into the oocyte by the fertilizing spermatozoon (the egg has some capacity to repair DNA damage during the first embryonic stages Ref. 106). Thus, future research on the mechanisms and consequences of gem-line selection in nature may have an impact on key topics on evolutionary biology, and may also shed light on the potential long-term consequences of assisted reproductive technologies.

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