Current Biology
Review

The Expensive Germline and the Evolution of Ageing

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http://dx.doi.org/10.1016/j.cub.2016.04.012

The trade-off between survival and reproduction is the bedrock of the evolutionary theory of ageing. The reproductive system regulates ageing of the soma, and removal of germ cells extends somatic lifespan and increases resistance to a broad variety of abiotic and biotic stresses. The general explanation for this somatic response is that reduced reproduction frees up resources for survival. Remarkably, however, the disruption of molecular signaling pathways that regulate ageing increases lifespan without the obligatory reduction in fecundity, thus challenging the key role of the survival–reproduction trade-off. Here, we review the diverse literature on the costs of lifespan extension and suggest that the current paradigm is overly centered on the trade-off between lifespan and fecundity, often neglecting key aspects of fitness, such as development time, defense against parasites and, in particular, the high costs of germline maintenance. Compromised germline maintenance increases germline mutation rate, which reduces offspring fitness and ultimately can terminate germline proliferation across generations. We propose that future work should incorporate the costs of germline maintenance in the study of ageing evolution, as well as in applied biomedical research, by assessing offspring fitness.

Introduction

Ageing, or senescence, is a progressive physiological deterioration of an organism, which reduces reproduction and increases the probability of death [1]. Given the existence of cell repair mechanisms [2,3], and the fact that ageing reduces Darwinian fitness [4,5], the evolution of ageing requires an explanation. Organisms undergo a constant wear-and-tear and natural selection optimizes allocation to somatic maintenance and reproduction to maximize fitness rather than longevity. Because of extrinsic (non-ageing) mortality due to predation, disease and abiotic hazards, current reproduction is worth more than future reproduction and the force of natural selection late in life is weak [6–8]. Therefore, deleterious mutations whose effects are concentrated in late-life can accumulate in the population [6,9,10]. However, it is also theoretically likely that some mutations will be antagonistically pleiotropic with respect to early-life and late-life fitness, and when such mutations increase overall fitness, they rapidly can become fixed. Thus, ageing can evolve through a combination of mutation load (mutation accumulation theory) and the trade-off between early- and late-life fitness (antagonistic pleiotropy theory) [6,7]. While natural selection can increase longevity by purging deleterious mutations [10,11], the trade-off between survival and reproduction plays a key role in the evolution of ageing. Indeed, today we know that mutations conferring increased stress resistance and longevity to the soma can result in reduced reproductive performance [12–17]. Thus, the underlying mechanism, at least in part, concerns reallocation of resources between two competing functions: reproduction and somatic maintenance. However, recent findings have called the inevitability of this trade-off into question.

The most accomplished account of the trade-off between reproduction and survival to date is the 'disposable soma' theory of ageing [8,18,19]. This theory focuses on the fact that somatic maintenance requires a complex machinery to orchestrate a vast number of operations, which maintain the functional integrity of cells and tissues. These operations are likely to result in errors [20,21]. High-fidelity quality control that would reduce such errors to a negligible level is costly and these costs will compete with the costs of reproduction. Because resources are limited, and because extrinsic mortality will destroy even intrinsically immortal organisms, investing into error-proof somatic maintenance is wasteful and not an evolutionarily stable strategy [8]. In this sense, the soma is disposable and investment into somatic maintenance has to be optimized to allow error-prone repair in order to invest the rest of the limited resources into reproduction [8].

A considerable body of literature provides empirical support for the disposable soma theory, using both laboratory [22–25] and natural populations [26–30]. However, in recent years, there has been a surge in the number of studies from diverse fields of research, such as molecular genetics, population genetics and dietary ecology, that challenged the ubiquity of the energy trade-off between reproduction and longevity, and in particular, its importance for the evolution of ageing [15,17,31–38]. In fact, it is becoming increasingly common to suggest that reproduction and lifespan are not constrained by direct competition for limited resources but are rather connected by common molecular signaling networks optimizing metabolism differently for growth and fecundity versus somatic maintenance and longevity [17,35,36,39,40]. In this view, the effects of genetic or phenotypic experimental interventions that extend lifespan can be uncoupled from the corresponding costs of reproduction [17,35,36,39,41]. Here, we review the diverse literature related to this question and suggest that uncoupling of reproduction and survival may be more difficult than it appears and that energy trade-offs may well underlie the regulation of somatic lifespan by molecular signaling pathways that regulate ageing. In particular,

we focus on one key aspect of reproduction — the cost of germline maintenance. Despite the fact that this cost is explicit in the original formulation of the disposable soma theory, it has been largely neglected in empirical studies. Our goal is threefold: first, we want to emphasize that germline maintenance is costly and this cost needs accounting for when considering the evolution of life-history trade-offs; second, we argue that the reduction in germline maintenance can constitute the 'missing cost' of lifespan extension; and third, we suggest that comprehensive measurement of the costs of lifespan extension by genetic, dietary or pharmacological means should include competitive assays of offspring fitness and/or direct estimates of mutation rates in offspring.

The Missing Trade-off

The problem of the missing trade-off between reproduction and lifespan is not new and has been discussed extensively because of its central importance to researchers in many different fields and many issues have been raised [12,17,35,36,40,42–45]. First, the costs of increased lifespan could be potentially offset by trade-offs with traits other than fecundity [17], such as longer development time and decreased protection against pathogens. Some of the long-lived mutants suffer from slow growth rate, while pharmacological treatments can lower immune response. These considerations are supported by the studies showing reduced fitness of long-lived mutants of yeast, nematodes, fruit flies and mice under more natural conditions [12–14,46]. Another possibility is that lifespan extension has negligible or even positive effects on reproduction in one sex, but has negative effects in the other sex [47]. Such sexual antagonism can maintain genetic variation for lifespan in the population [47–49]. Here, we briefly discuss the possibility that lifespan extension comes at the cost of reduced growth, development and immunity, and then focus on the cost of maintaining the genome and the proteome of the germline.

Growing Fast and Dying Young

The negative association between growth rate and lifespan is well known [50] and early studies found that artificial selection for increased growth rate results in reduced longevity in the fruit fly *Drosophila melanogaster* [51] and in the house mouse *Mus musculus* [52]. Furthermore, comparative work showed that increased embryo growth rates are associated with reduced longevity in birds and mammals [53] and increased growth may explain why large dog breeds age faster than small breeds [54]. Similarly, lizards (*Niveoscincus mircolepidotus*) with experimentally increased growth rate had lower survival in nature [55], while experimentally induced compensatory growth in the stickleback (*Gasterosteus aculeatus*) results in reduced longevity [56]. Because growth rate (mass per unit time) and development rate (cell differentiation per unit time) are two different yet closely linked traits, it is not clear which one is the main contributor to the negative correlation between growth rate and lifespan. For example, selection for early age at reproduction results in the evolution of short lifespan and rapid growth in fruit flies [57].

The patterns described above fit well with findings in biogerontology that identified the connection of nutrient-sensing signaling networks, such as insulin/insulin-like (IIS) and target-of-rapamycin (TOR) pathways, with growth, reproduction and ageing [45]. Subsequent research strongly focused on the connection between nutrient-sensing signaling, reproduction and ageing [39], while somewhat ignoring growth, but this is now changing rapidly [45]. However, the trade-offs between development time or growth rate and adult lifespan cannot account for the costs of longevity when lifespan extension treatments are applied to fully grown, sexually mature animals [37].

Long-lived and Vulnerable to Disease

While pathogen resistance is vital for survival, there is evidence for a trade-off between immune response and longevity [58,59]. Activation of the immune system prior to infection increases pathogen resistance but reduces longevity in the absence of infection in *Drosophila melanogaster*, suggesting that activation *per se* is costly and can accelerate ageing [58]. In fact, both phenotypic induction of immune response [60] and evolution of increased immune function [61] reduce survival in the absence of pathogen challenge in insects. Immunity is costly in humans [62] and the genetic trade-off between innate immunity to infectious diseases (e.g. cholera and tuberculosis) and fertility has been suggested as a potential reason for the relatively high prevalence of infertility in humans (one in seven of heterosexual couples in the industrialized world) [63]. Interestingly, rapamycin, the drug that inhibits TOR and increases longevity in animal models, is approved for clinical use in humans as immunosuppressant [64]. Therefore, it is possible that down-regulation of IIS/TOR signaling decelerates ageing and increases longevity in model organisms only in the absence of pathogens. However, germline ablation increases lifespan and upregulates immune function, rendering animals lacking a germline more resistant to bacterial pathogens [65–67]. Furthermore, recent work in *Caenorhabditis elegans* nematodes showed that damaging the germline triggers the induction of innate immunity, resulting in increased resistance to pathogen infection, as well as to abiotic stresses, such as heat and UV radiation [68]. It is thus possible to induce lifespan extension accompanied by, and in part supported by, increased immune function.

The Cost of Germline Maintenance

The cost of reproduction is commonly understood in terms of gamete production, cost of mating and parental care (e.g. pregnancy and post-natal care). It is routinely assessed as the number of eggs laid or progeny produced. Therefore, the absence of a negative correlation between egg or offspring production and increased lifespan in organisms where molecular signaling pathways that regulate lifespan have been blocked using gene-knockouts or RNAi knockdown often causes researchers to suggest that longevity can be relatively cost-free, or at least does not depend directly on the reallocation of limited resources from reproduction [41,69,70]. Moreover, germline ablation extends lifespan compared to simply sterile or non-reproducing organisms, further suggesting that germline-soma signaling, rather than costly trade-offs between reproductive and somatic functions, are responsible for longevity. In *C. elegans*, germline ablation extends lifespan but this effect is negated if both the germline and the somatic part of the gonad are ablated [69,71]. Because in both cases the animals are fully sterile, it has been suggested that the reduced cost of reproduction is not vital for increased lifespan in animals without a germline;

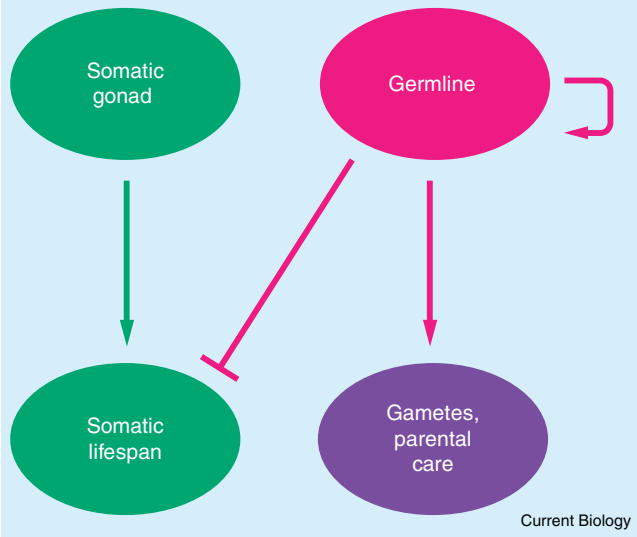

Figure 1. The simplified model for regulation of somatic lifespan via signaling from the germline and the somatic gonad.
The somatic gonad produces a signal that increases genome surveillance and repair, as well as clearance of damaged proteins via ubiquitin/proteasome system (UPS) and autophagy in somatic cells, and extends lifespan. This signal is partially blocked by the germline, which allocates resources to reproduction and self-maintenance. In sterile mutants that have a germline but cannot produce eggs or sperm, the germline will continue to allocate resources to self-maintenance, and may continue to block the signal from the somatic gonad because of the lack of feedback regarding sterility. Removal of the germline will allow the somatic gonad to channel more resources to somatic maintenance. Removal of both the germline and the somatic gonad will remove all relevant signaling and will not increase lifespan despite the availability of resources that are not invested into reproduction or germline maintenance.

instead, the somatic gonad generates signals that promote longevity, while germline signaling reduces longevity [69] (but see Figure 1).

The idea that signaling networks responsible for the frequently observed negative correlations between reproduction and lifespan are not directly dependent on costly energy trade-offs, and, therefore, increased lifespan and reduced fecundity can be decoupled, is relatively mainstream in contemporary biogerontology [17,35,36,39]. However, the presence of the germline is likely to necessitate substantial energy expenditure on germline maintenance even in the absence of actual gamete production. Germline stem cells (GSCs) are essentially immortal in the sense that all living cells are descendants of the same original lineage, but this immortality comes at a cost. All cells, including GSCs, are experiencing high levels of genome and proteome damage that require costly quality control, repair and degradation of toxic waste products. For example, germline mutations that reduce proper DNA repair in the germ cells result in the death of the otherwise immortal germline in just a few generations [72,73]. In order to maintain their functionality and reproductive potential across generations, GSCs have to protect their genome and their proteome; otherwise, the germline would not be able to proliferate indefinitely [74]. The corollary is that, in the absence of the germline, the resources used for expensive germline maintenance can instead be allocated to the maintenance of the soma (Figures 1 and 2).

The Costs of DNA Replication Fidelity

Several lines of evidence suggest that the costs of germline maintenance can be substantial. We start with indirect but highly relevant evidence for the cost of replication fidelity in bacteria [75], bacteriophages [76] and viruses [77–79]. Replication fidelity depends in part on the maintenance of genome integrity. It is often suggested that high mutation rates are beneficial for microorganisms, especially under stressful conditions, because it increases their evolvability and speeds up adaptation to novel or changing environments. However, increased mutation rate under stress could also reflect limited resources being available for high replication fidelity [75,80]. Strains of *E. coli* bacteria with larger genomes, for example, grow more slowly under suboptimal conditions [75]. More direct empirical evidence comes from a T4 bacteriophage where high replication fidelity has been shown to impose time and energy costs when comparing polymerase enzymes obtained from a strain with increased fidelity to their counterparts from wild-type strains [76].

The evolution of DNA replication fidelity is thus predicted to balance the benefits of high repair against time and energy constraints. In line with this reasoning, experimental work showed that mutation rate in a stomatitis RNA virus evolves as a result of the trade-off between selection for reduced mutation rate and high biochemical costs of replication fidelity [77]. Similarly, data on the human HIV-1 virus supported the trade-off between enzymatic accuracy that is necessary for high fidelity replication and the maximum rate of polymerization [78]. Recent work on the evolution of HIV-1 under anti-viral treatments provides further evidence for the biochemical cost of high fidelity replication. The human HIV-1 virus has very low replication fidelity, which has been used in biomedical research to disrupt viral proliferation altogether using replication inhibitors that can incorporate themselves into the viral genome [79]. This treatment, however, results in the rapid evolution of drug-resistant strains that have increased replication fidelity. Interestingly, increased replication fidelity reduces fitness of the virus manifesting itself in reduced replication rates. Moreover, when antiretroviral treatment is discontinued, viral strains rapidly evolve lower replication fidelity, strongly supporting the hypothesis that increased fidelity reduces fitness. There could be a number of reasons for reduced fitness of high fidelity HIV-1 strains but many high fidelity mutants take more time to polymerize DNA and also have lower processivity, i.e. the time the enzyme remains associated with the nucleic acids [79].

In higher organisms, some experimental support for the costs of high replication fidelity comes from an experimental evolution study in *D. melanogaster* [81]. Populations exposed to X-ray irradiation for over 600 generations evolved increased resistance to radiation damage. However, when the selection for radiation resistance was discontinued, one population lost part of the radiation resistance after 220 generations, supporting the idea that DNA repair is too costly in the absence of a strong environmental mutagen. In mammals, males are hemizygous for the X chromosome, such that recessive deleterious X-linked mutations will be expressed in this sex, suggesting that the benefits of increased fidelity of the X chromosome could outweigh the costs of high repair. Indeed, in mice, the mutation rate on the X chromosome seems to be lower compared to autosomes [82], providing

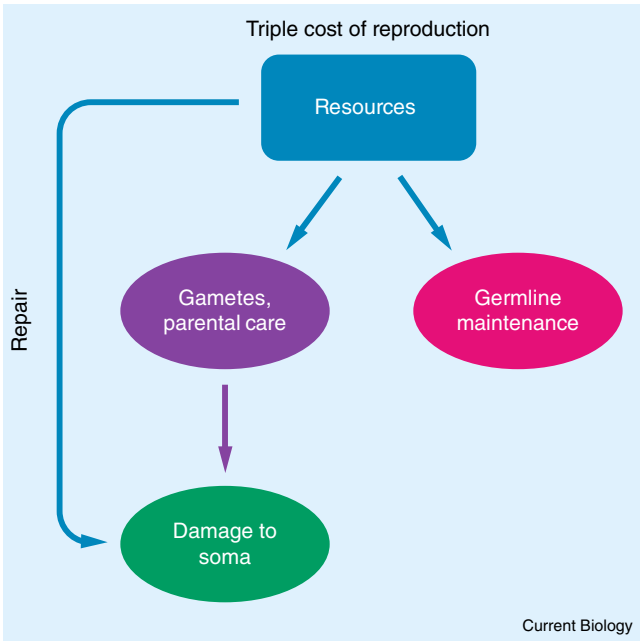

Figure 2. Triple cost of reproduction.
Reproduction necessitates resources (blue rectangle and arrows) to be allocated to: gamete production (eggs or sperm) and parental care; repair of somatic damage caused by gamete production and parental care; and germline maintenance. Blocking reproduction removes two costs — direct energy costs of reproduction and damage caused by reproduction but leaves out the cost of germline maintenance.

evidence in support of the costs associated with high fidelity repair. The cost of germline maintenance can also be inferred from the observations that in nature, birds [83] and mammals [84] reduce their testis size outside the breeding season and whenever they experience environmental stress.

Perhaps some of the most compelling evidence for the cost of high fidelity repair in multicellular organisms comes from experimental studies investigating condition dependence of mutation rates. One study used a particularly elegant design to separate the rate of germline DNA damage from DNA repair ability in *D. melanogaster* [85]. *D. melanogaster* females can repair lesions in damaged male sperm. This maternal repair system allows manipulating female condition and germline mutation rates independently. Female flies can be raised on high- and low-quality food to produce high- and low-condition offspring, which are subsequently presented with mutagenized male sperm that need repairing. Low-condition flies had higher mutation rates, suggesting they were less able to apply costly conservative DNA repair and instead used cheaper, more error-prone pathways. In a follow-up study, where phenotypic manipulation of condition was replaced by constructing experimental genotypes carrying deleterious alleles, genetically inferior strains had higher mutation rates, again suggesting that low-quality genotypes are unable to provide sufficiently conservative repair [86]. Interestingly, when females were raised on high-quality food but were subsequently stressed as adults by exposing them to high levels of male harassment, such females had very low reproductive output but were better able to repair mutagenized male sperm [87]. It is possible that when high-condition females are severely stressed as adults, they shrink their germline and allocate most of their remaining resources to more expensive DNA repair in both the germline and the soma (see below).

While germline maintenance is costly, the germline needs to be better protected than somatic cells because progressive accumulation of damage in germ cells would abolish germline immortality [73,74]. In line with this idea, DNA repair was highest in the germline compared to other tissues across different genetic strains of house mice [88]. Correspondingly, direct estimates of tissue-specific mutation rates in mice suggest that germ cells had lower mutation rates than all other tissues (including brain and liver) across all ages [89]. Interestingly, mammalian embryonic stem cells have superior stress resistance compared to differentiated cells and it appears that stress resistance is downregulated following differentiation in mouse [90] and human [91] cells. The authors suggest that their findings support increased allocation of resources into the germline at the cost of downregulating maintenance and repair in the somatic cells. While the costs of replication fidelity are particularly important for the germline, there are many other aspects of macromolecular proofreading, maintenance and repair that are energetically costly [92]. Consideration of the costs of preserving macromolecular integrity was at the core of the original proposal of the 'disposable soma' theory [8] and we are going to discuss this further in the following section. To summarize, genome maintenance is costly and the germline needs more protection to sustain itself through generations than disposable somatic cells.

The Costs of Proteome Protection

Germline immortality depends not only on the protection of the germline genome but also on the protection of its proteome — the set of proteins produced, folded and transported within individual cells. Because the functionality of the proteome is key for the functionality and survival of the cells, organisms invest considerable energy into protein quality control. During a cell's life, molecular chaperones and different proteolytic systems, in particular the ubiquitin-proteasome system (UPS) and autophagy, detect and repair damaged proteins, or fully degrade the ones that are beyond repair [93]. The protective capacity of these systems declines with age, resulting in loss of proteostasis and accumulation of damaged proteins in ageing tissues [93,94]. Tellingly, germline cells enjoy elevated levels of protein quality control compared to somatic cells throughout life, as highlighted by recent experimental work in *C. elegans* and *D. melanogaster*.

In *D. melanogaster*, eggs of young and aged flies exhibit lower amounts of protein damage and higher activity of cell surveillance and quality control systems than in age-matched somatic cells. While fly eggs do show accumulated protein damage with age, consistent with the general observation of germline ageing, at least some of the key parts of the protein quality control machinery, which show age-specific decline in soma, do not seem to decline with age in eggs [95]. The most common active proteasome, 26S, had much higher activity in young eggs than in young soma, and while its activity declined in the soma of aged flies, it maintained its original level in age-matched eggs [95]. Subsequent work confirmed that gonads of aged flies maintain higher levels of proteostasis compared to the soma, show lower

levels of oxidative damage and possess the ability to activate high proteasome activity in response to oxidative stress similar to that of the young gonads [96]. Taken together, these findings suggest that increased investment into the protection of the germline proteome contributes to germline immortality, while the somatic cells pay the ultimate price of energy-saving protein quality control. This suggests that switching off investment into the germline proteome should increase somatic protein quality control and help maintain proteostasis in somatic cells and, consequently, increase the lifespan of the soma.

This hypothesis has been directly tested in *C. elegans* by focusing on proteasome activity in germline-ablated animals. *C. elegans* is a particularly interesting and suitable model system for testing the costs of germline maintenance, because multiple studies have failed to find significant longevity costs of egg production in these animals. Indeed, proteasome activity and the levels of protein degradation were increased in long-lived GLP-1 mutants, which lack a germline [97]. On the other hand, sterile control animals had normal wild-type lifespan and did not show any increase in proteasome activity, suggesting that germline ablation rather than sterility is necessary for increased proteostasis of somatic cells [97]. Increased longevity of GLP-1 mutants depends on the activity of the worm FOXO transcription factor DAF-16, which also regulates proteasome activity, including the levels of RPN-6, a key subunit that is necessary for proteolytic activity. Overexpression of RPN-6 resulted in increased survival of worms exposed to oxidative stress and heat stress, and even improved motility of polyQ worms — a disease model that mimics protein misfolding leading to neurodegenerative diseases, such as Huntington's disease [97].

Similarly to the proteasome system, autophagy, the second main proteolytic system, is key to long life and is mediated by germline signaling [98]. Autophagy is increased upon germline ablation and mutations that interfere with autophagy abolish lifespan extension in animals without a germline [98]. Autophagy also appears to be functionally linked with fat metabolism, and in particular with lipid degradation. Overexpression of lipase LIPL-4 considerably increases lifespan even in wild-type animals but only in the presence of functional autophagy genes, showing that autophagy is necessary for lifespan extension [98].

The germline enjoys elevated protein quality control and the fact that individual organisms without a germline shift resources from maintenance of the germline proteome to the somatic proteome [99] provides strong support for the 'disposable soma' theory of ageing. In general, mutations that interfere with germline proliferation result in upregulation of several signaling pathways that increase proteolytic activity in the somatic cells [100]. Because germline removal upregulates both proteasomal activity and autophagy via different signaling pathways [97,98,100], it is possible that these two proteome protection systems increase longevity in complementary ways. Overall, the absence of the germline allows somatic cells to maintain more germline-like levels of proteome protection, and, therefore, become longer lived [94].

Collectively, the findings reviewed in the preceding sections suggest that protection of the germline genome and proteome is costly; that these costs need to be accounted for when considering life-history trade-offs, and that they may well constitute the missing cost of lifespan extension by interference with molecular signaling pathways that regulate ageing, when there is no corresponding reduction in gamete production (Figures 1 and 2).

Expensive Germline and Dietary Restriction

Dietary restriction, the reduction in food intake without malnutrition, is one of the most successful ways to extend lifespan and has been demonstrated in many different organisms ranging from yeast to worms to insects to mammals [39]. Dietary restriction commonly results in reduced reproduction (but see [34]), which prompted scientists to suggest that these two phenomena are functionally linked. Indeed, reduction in reproduction can increase lifespan in two non-mutually exclusive ways. First, resources that are saved from the investment into gamete production can be shuffled to somatic maintenance. Second, reduced gamete production alleviates the so-called direct costs of reproduction — the 'wear-and-tear' of tissues, DNA damage from free radicals and accumulation of toxic waste products in the cells [31,40,43]. Thus, reduced or abolished reproduction has a double benefit of increasing the amount of resources available for repair, while reducing the amount of damage that needs repairing (Figure 1). This double benefit can help explain some puzzling results. For example, in a landmark study of resource reallocation under dietary restriction, O'Brien *et al*. [33] showed that while *D. melanogaster* indeed allocate relatively more resources to somatic maintenance under low food treatment as predicted by resource reallocation models, the absolute amount of resources spent on the soma was still lower than in fully fed flies. This result seemingly contradicts the 'disposable soma' models promoting resource reallocation as the key functional mechanism behind lifespan extension under dietary restriction. Taking reduction in the amount of reproduction-driven 'direct damage' to the soma into account provides a potential explanation for this conundrum [33]. It would be interesting to directly estimate the amount of damage that necessitates repair in fully fed and restricted animals and compare it to the absolute amount of resources allocated to repair in both treatments.

While taking direct costs of reproduction into account can help explain some of the data that contradict resource reallocation models, some studies refute the possibility that reduced allocation to gamete production accompanied by reduced levels of direct damage can fully account for the observed lifespan extension. Most remarkably, dietary restriction extends lifespan in sterile and non-reproducing animals. For example, bacterial deprivation, an extreme form of dietary restriction where bacterial food is completely withheld from adult *C. elegans* worms, extends lifespan in non-reproducing nematodes [101], whereas classic dietary restriction extends lifespan in sterile *D. melanogaster* [102]. While these findings suggest that dietary restriction extends lifespan in the absence of resource reallocation from gamete production to somatic maintenance, they leave the possibility open that dietary restriction extends lifespan by reallocating resources from germline maintenance to somatic maintenance. This hypothesis is further supported by the fact that dietary restriction does not extend lifespan in *C. elegans* whose germline was surgically ablated [103].

In line with this hypothesis, starvation indeed reduces germline proliferation and shrinks the pool of GSCs in fruit flies [104] and *C. elegans* [105]. Once the food becomes available again, the

few remaining GSCs start proliferating and restore the functioning germline [104]. In one study, Mair *et al*. [106] surprisingly found that aged male fruit flies under dietary restriction had more GSCs than control males. Intriguingly, however, their results suggest that, rather than increasing germline proliferation, dietary restriction increased germline maintenance and thus reduced age-specific loss of GSCs, resulting in a larger number of GSCs in aged flies. It appears that when the number of germline and somatic stem cells is substantially reduced under food shortage, the resources are diverted to the maintenance of the remaining germline and somatic stem cells, as well as the rest of the somatic cells, thereby prolonging the reproductive capability of the germline and the lifespan of the soma. Indeed, McLeod *et al*. [104] showed that both germline and somatic stem cells in *D. melanogaster* respond in the same way to dietary restriction by dramatically shrinking the pool of stem cells until conditions improve.

Further support for the longevity cost of germline maintenance comes from recent work in *C. elegans* showing that steroid signaling between the starved soma and the germline resulting in the reduction of the GSCs is a necessary prerequisite for lifespan extension under dietary restriction [107]. Starved adult worms produce a hormone (dafachronic acid, DA) which causes reduction in the number of germline nuclei [107]. Disruption of communication between the soma and the germline by mutations blocking this steroid signaling pathway (e.g. by interfering with the function of DA hormone receptor NHR-8) prevents reduction of the number of germ cells in starved worms and abolishes lifespan extension. Tellingly, when the effect of DA on the reduction of germ cell count is mimicked by using dietary restriction, the lack of steroid signaling is no longer a problem for dietary restriction-mediated lifespan extension [107]. These results strongly suggest that lifespan extension under dietary restriction is closely linked to the number of GSCs that require maintenance and the fewer GSCs are left in the germline, the more resources are available for maintaining the soma as well as for the remaining small pool of GSCs. As animals under dietary restriction reduce the number of their GSCs, they can enjoy a triple saving of energy: a reduction in gamete production (e.g. eggs or sperm), a reduction in direct damage to cells and tissues that need repairing and a reduction in the cost of germline maintenance (Figure 2).

Sex Differences in Lifespan

Sexes can be defined by the relative size of their gametes: males produce numerous small sperm, while females produce fewer, larger eggs. This difference in gamete size (anisogamy) leads to the evolution of sex-specific life history strategies, including sex differences in lifespan [48]. In many taxa, males live shorter than females, which is often attributed to higher male mortality due to risky reproductive strategies driven by sexual selection. We propose that in many taxa, males live shorter than females in part because they pay higher costs of germline maintenance, since males have to maintain the integrity of a large number of GSCs throughout life. The mechanisms of oogenesis vary much more across taxa than those of spermatogenesis as females may lay many thousands of eggs in each clutch in broadcast spawners, whereas mammals and birds produce relatively few eggs throughout their life [108]. Interestingly, species producing large numbers of eggs maintain a population of primordial germ cells throughout their life, which continues to produce oogonia, whereas in species producing fewer eggs, the oogonia typically divide to form a limited number of egg precursor cells. Arrested meiosis allows females to free energy to directly invest into the production of costly offspring. As a consequence, removal of the germline should increase lifespan much more in male mammals or birds than in their female counterparts. This idea is in line with an early report that neutering in domestic cats increased lifespan in males more than in females, resulting in similar lifespan in the sterilized animals of both sexes [109]. Furthermore, while castration increases lifespan in male mice and rats, the data on females are more ambiguous, with some studies reporting reduced lifespan in ovariectomized females [110]. Because the germline and the somatic gonad can produce opposing signals with respect to lifespan, future studies on the role of germline maintenance in sex differences in lifespan should aim to directly ablate germ cells.

Assessing the Costs of Lifespan Extension

Germline maintenance is energetically costly because of the need to protect, repair and maintain the genome and the proteome of the germline stem cells. This cost can be substantial, and, in theory, can rival the cost of gamete production *per se*. Therefore, we propose that reduced germline maintenance often constitutes the missing cost of lifespan extension. This hypothesis makes clear predictions that can be experimentally tested. Assessing the consequences of reduced germline maintenance can advance our understanding of the evolutionary biology of ageing and inform us about the potential risks involved in boosting the organismal investment into somatic maintenance.

Organisms evolve in a world of limited and unpredictable resources, suggesting that investment in germline-like maintenance of somatic cells is wasteful because the potentially immortal soma can be destroyed by external forces [8,18,19]. Nevertheless, there is considerable plasticity in germline regulation of somatic ageing with the germline receiving information from the soma and from the environment and mediating its signaling network in order to increase or decrease investment in the protection of the somatic cells. This plasticity may not have evolved to increase lifespan as such, but rather to increase the instantaneous resistance of the soma in the face of stress, with the by-product of lifespan extension, visible only under protected laboratory conditions. This logic suggests that using genetic and/or pharmacological manipulations of germline signaling to boost investment into somatic maintenance will reduce the resources available for growth, gamete production and germline maintenance when the total amount of available energy is limited (Figure 1). Thus, the diversion of resources to the soma is predicted to affect any or all of the following traits: total fecundity, timing of fecundity and offspring quality.

Research into the germline control of ageing has focused largely on total fecundity, and to a lesser extent on the timing of reproduction [37]. We would like to note here that even a modest reduction in early-life fecundity, without a reduction in net fecundity, can be detrimental for Darwinian fitness, and particularly so in organisms competing for an ephemeral

resource, such as classic laboratory model species. Nevertheless, even if timing and amount of progeny produced are completely unaffected by increased protection of the somatic cells, the reduction in the availability of resources for germline maintenance can increase mutation load in the resulting offspring. These predictions do not mean that biogerontological research aiming at extension of lifespan and healthspan (the length of time a person can maintain its full biological function, free from chronic age-related diseases) is doomed to encounter insurmountable problems for at least three reasons. First, it is possible that certain lifespan-extending interventions could reduce the quality and/or quantity of gametes but to an extent that, while detrimental for Darwinian fitness, would be considered negligible in terms of human health. Second, interventions that reduce germline maintenance could be aimed at the individuals refraining from future reproduction. Third, it is possible that artificially increasing investment into somatic maintenance in the presence of unlimited resources could offer some degree of increased stress resistance and prolonged lifespan without noticeable trade-offs with fecundity and offspring quality. This latter outcome is possible because organisms rarely evolve having uninterrupted access to unlimited resources in nature.

Nevertheless, these considerations call for increased emphasis on offspring health and reproductive potential in studies that manipulate signaling pathways to increase lifespan. For example, the drug metformin, which is currently being considered for the first-in-history clinical anti-ageing trial in humans [111], apparently reduced testicular size, sperm cell count and sperm motility and increased the number of abnormal sperm in adult male rabbits and reduced testicular size and number of Sertoli cells, which play a key role in the regulation of spermatogenesis, in male offspring of metformin-treated pregnant mice [112]. At the same time, metformin is a successful anti-diabetic drug associated with reduced rates of different cancers as well as overall mortality in mice [113] and humans [114], suggesting that it may be involved in reallocation of resources from germline maintenance to somatic maintenance, thereby potentially compromising reproductive performance of both adults and their offspring.

Conclusion

The cost of maintaining the integrity of the genomes and the proteomes of GSCs can explain why germline removal extends lifespan even in sterile or non-reproducing organisms. It also suggests that genetic and pharmacological interventions that manipulate germline signaling to boost investment into somatic maintenance can do so at the cost of germline maintenance, which in turn can result in increased mutation load in offspring. It would be particularly interesting to study the reproductive competitiveness of progeny resulting from experimental manipulation of molecular signaling pathways that regulate ageing in a variety of environmental conditions. Such experiments are certainly possible using a range of approaches, from RNAi knockdown of nutrient-sensing signaling to overexpression of genes controlling the proteolytic systems (ubiquitin–proteasome system and autophagy) to pharmacological interventions using drugs such as rapamycin, spermidine and metformin to experimental evolution. We predict that, all else being equal, forced investment into somatic cells will result in a reduced quality of offspring that will be particularly apparent in trials where animals have to compete for food or mates. At the same time, perhaps the most straightforward way to increase lifespan is to make the soma more germline-like by forcing the somatic cells to express the same level of genome and proteome surveillance and repair as their germline clones.

ACKNOWLEDGEMENTS

We thank Russell Bonduriansky for very helpful feedback and discussions. A.A.M. was supported by an ERC Starting Grant 2010 and Swedish Research Council. S.I. was supported by an ERC Starting Grant 2013, the Knut and Alice Wallenberg Foundation and the Swedish Research Council.

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