Article ID: WMC003275 ISSN 2046-1690

The Germ-soma Conflict Theory of Aging and Death: Obituary to the "Evolutionary Theories of Aging"

Corresponding Author:
Prof. Kurt Heininger,
Professor, Department of Neurology, Heinrich Heine University Duesseldorf - Germany

Submitting Author:
Prof. Kurt Heininger,
Professor, Department of Neurology, Heinrich Heine University Duesseldorf - Germany

Article ID: WMC003275

Article Type: Original Articles

Submitted on:20-Apr-2012, 01:51:59 PM GMT **Published on:** 20-Apr-2012, 05:38:59 PM GMT

Article URL: http://www.webmedcentral.com/article_view/3275

Subject Categories:AGING

Keywords: Aging, Reproduction, Evolution, Darwinian demon, Resources

How to cite the article: Heininger K. The Germ-soma Conflict Theory of Aging and Death: Obituary to the "Evolutionary Theories of Aging" . WebmedCentral AGING 2012;3(4):WMC003275

Source(s) of Funding:

No funding

Competing Interests:

No competing interests

Additional Files:

References

The Germ-soma Conflict Theory of Aging and Death: Obituary to the "Evolutionary Theories of Aging"

Author(s): Heininger K

Abstract

Regarding aging, the scientific community persists in a state of collective schizophrenia. This state is owed to the so-called “evolutionary theories of aging”. According to these theories, aging, following the peri-/postreproductive decline of selection pressure, is posited to be haphazard, not selected for, not adaptive, and not programmed. And yet, defying these concepts, aging is phylogenetically conserved and subject to regulation by classical signaling pathways and transcription factors. While some blatantly deny this genetic control, others (particularly geneticists) appear to accommodate with the obvious incompatibilities.

First it is shown that the “evolutionary theories of aging” are based on circular reasoning and fallacious post hoc, ergo propter hoc argumentations and that their basic assumptions are flawed. Second, by a simple change of perspective, replacing a soma-centered (as in the “evolutionary theories of aging”) by a germline-centered point of view and based on compelling evidence from more than 400,000 scientific publications (of which more than 4,000 have been referenced in this work) a non-group-selective, evolutionary mechanism is elaborated that explains the co-selection and programming of reproduction and aging/death.

A gedankenexperiment reveals: A Darwinian demon that exhibits both immortality and infinite reproduction is not viable in a world of limited resources. According to the phylogenetic record and deep genetic homologies, death of the soma is identified as the ultimate cost of reproduction. At the evolutionary base of the unicellular/multicellular transition, microbial organisms have a “feast and famine” lifestyle. Following the exhaustion of a resource, they build multicellular structures that generate spores that can resist the nutrient-depleted environment. The metamorphosis to spores is fuelled by energy and building blocks provided by the – not altruistic, but rather spore-induced cytocidal – death of clonal siblings. Their phenotypes identify these processes as ancient reproductive events creating distinct generations: germs (that can germinate later under more favorable conditions) and fruiting bodies (that nurse and disseminate the spores and die). In contrast, “cheater” phenotypes that are defective to carry the cost of death at reproductive events are less fit under conditions of feast and famine cycles and have a high risk to go extinct. Tracing both the genomic and physiological “fossil record” and the phenotypic pattern throughout phylogenesis demonstrates that evolution succeeded to enforce this general principle in multicellular unitary organisms by creating a dichotomy between soma and germline cells, making the former the mortal breeder and the latter the virtually immortal genetic vector. Importantly, germline cells control the longevity of the soma from ‘within’ by a variety of signals, e.g. sexual hormones. These signals, on the one hand, make the mature organism vital and attractive for mates and, on the other hand, limit the reproductive potential of the parent organism and drive a variety of aging pacemakers, particularly the senescene of the immune system.

The transgenerational conflict between germline cells and soma over utilization of limited resources is the evolutionary rationale of postreproductive aging/death, semelparous organisms being a particularly drastic witness of the link between reproduction and death. Semelparity and iteroparity are threshold traits and as such opposite ends of a continuum of life history variation rather than representing fundamentally different life history strategies. Although the cost of reproduction, e.g. in terms of impaired immunocompetence and survival, still shapes the life history trade-offs of iteroparous organisms, the temporal uncoupling of reproduction and death concealed their evolutionary co-selection. Death in the natural habitat due to environmental hazards such as predation, infection, accident, or starvation often predates the germ signaling-dependent demise and may hinder the individuals to exploit their full longevity potential. A protected environment, however, like in human societies, laboratories and captivity unmasks the process of aging.

In contrast to unitary organisms, modular organisms (e.g. plants, benthic aquatic invertebrates) that have no segregated germline and in which the adult body itself is a reproductive unit, may evade senescence. However, they are subject to territorial, density-dependent mortality patterns, due to e.g. self-thinning or chemical warfare, and density-limited seed recruitment driven by interindividual competition for resources. Thus, the different lifestyle of sessile

organisms (being unable to move away from local resource competition) and mobile organisms (that, even when dispersing take their internal “competitor” with them) determined their transgenerational resource management systems. The germ-soma conflict shaped the different bauplans of unitary and modular organisms, is the motor driving animal coevolutionary “Red Queen” dynamics and possibly fuelled the Cambrian explosion of animals.

This update of the evidence-based germ-soma conflict theory (Heininger, 2002a) integrates the wealth of data in a holistic eco-evo-devo approach. The germ-soma conflict theory takes into account and explains the known facts related to aging and postreproductive death: the near-universality, the genetic programming, and the reproduction-, resource-utilization-, and stress-resistance-dependent modulation (including such phenomena as late-life mortality plateaus and gender dimorphic life expectancies). This change of paradigm resolves the inconsistencies of the pseudoevolutionary and unjustifiably so-called “evolutionary theories of aging” and roots the phylogenetically conserved programming of aging and death solidly in the evolutionary theories of Darwin, Wallace and Weismann

Table of Contents

1. Introduction

It is a capital mistake to theorise before one has data. Insensibly one begins to twist facts to suit theories instead of theories to suit facts.
Arthur Conan Doyle (1859-1930)

Summary

For more than 50 years, the so-called “evolutionary theories of aging” (ETAs) shaped modern thinking in evolutionary biology and gerontology. According to them, aging is due to the age-related decline of the force of natural selection in parallel to the reproductive value of the organism. Consequently, aging is considered not selected for, maladaptive, and haphazard. Rooted in population genetics, the hypothetico-deductive ETAs were deeply entrenched in theory some time before there was evidence to back them up and were adopted largely by default (due to the lack of alternatives) rather than for any compelling evidential reasons (Milewski, 2010). However, at odds with the ETAs, evidence has now accumulated that aging is regulated by phylogenetically conserved, canonical signaling pathways and transcription factors.

Darwin’s theory of natural selection requires that evolutionary changes be adaptive that is, that they be useful to the organism; in Darwin’s words: “..natural selection can act only through and for the good of each being” (Darwin, 1859, p. 113). It was already a central element of Darwin’s evolution theory that populations tend to outgrow the resources available to them and the ensuing competition among individuals is the driving force leading to the struggle for survival (Darwin, 1859, chapters III and IV). In fact, the insight (gained from the lecture of Malthus’s An Essay on the Principle of Population) that population growth will tend to outstrip supplies of food and space, led Darwin to the conclusion that this pressure, analogous to breeder’s artificial selection, was a natural form of selection (Ruse, 2009).

Redundant postreproductive organisms compete with their progeny for limited resources. Thus, in a world of finite resources there should be a strong selection pressure against the survival of the reproductively no longer active, postreproductive individuals. The problem of how aging evolved was first debated by August Weismann (1889) who developed the theory of programmed death which proposed that aging evolved to the advantage of the species and that there must be an evolutionary advantage to having only a limited lifespan. According to his theory, older members of a species are expected to die of old age by specific death mechanisms established by natural selection meant to eliminate old members of a population so that they would no longer compete with the progeny for food and other resources. A similar idea was already put forward earlier by A.R. Wallace, the co-discoverer of natural selection (Wallace, 1858), who was the first to give an evolutionary explanation of aging (written between 1865 and 1870, cited by the editor E. B. Poulton in Weismann (1891), pp. 23-24). Weismann believed that there existed an internal control mechanism in charge of the aging process. However, these theories interpreted as advocating the action of group selection (but see Rose, 1990), were rejected following the insight that group selection

forces are weak and that only in special circumstances an advantage to the group can outweigh a disadvantage to the individual (Williams 1966; Maynard Smith, 1976; Dawkins 1989; Leigh, 2010). It has been claimed that virtually any theory that posits an advantage to aging will fall in the group-selective "good-of-the-species" category (Austad, 1997 p.56). And any such theory was denounced as circular and flawed (Austad, 1997 p. 55; Arking, 1998 p. 112; Kirkwood, 1999 p. 59; Kirkwood, 2005) in that "it assumes what it ostensibly tries to explain (that is, the decrease in the probability of survival with the increasing age of individuals) and then denies its own premise (by suggesting that older and less fit...individuals can outcompete younger and fitter ... individuals)" (Arking, 1998 p. 112). In 1932, Bidder reasoned that In the 1940's and 1950's J.B.S. Haldane, P.B. Medawar, and G.C. Williams hypothesized that aging might evolve because the force of natural selection declines with age and might thus be inefficient at maintaining function at old age (Haldane, 1941; Medawar, 1952; Williams, 1957). For Haldane (1941) the critical clue to the cause of ageing was the human genetic disease then known as Huntington's Chorea, now called Huntington's disease. Haldane was struck by the age at which victims of Huntington's disease first show symptoms, usually their thirties, or later. He argued that a disease this devastating could only occur because, in our ancestral conditions before agriculture and civilization, such a disease would have had no selective effect. Few people would have survived into their forties anyway, before the advent of civilization, and their late-life medical genetic problems simply would not have mattered to evolution by natural selection (Rose et al., 2008).

Data on age-related mortality patterns in wild animal populations reveal that, in many species, individuals rarely survive to ages when senescent deterioration becomes apparent (Medawar, 1952, Lack, 1954; Finch, 1990). For example, animals living in harsh conditions in their natural habitat survive for only relatively short period of time compared to those living in the protected conditions of captivity. For instance, 9 out of 10 mice living in the wild will die before the age of 10 months, whereas the same mice raised in captivity have an average lifespan of 24 months. The median lifespan of chimpanzees living in captivity is between 23 years (males) and 30 years (females), and almost 20% of captive female chimpanzees survive to age 50. On the other hand, in wild conditions, the median lifespan of chimpanzees is only 8 years and almost none survive to age 50 (Ljubuncic and Reznick, 2009). For most natural populations, extrinsic mortality (due to accidents, predation, infection, starvation, disease, cold, etc.) is such that death occurs well before "old age". This means that a) there is no requirement for aging to weed out "worn-out individuals"; b) there is no evidence that aging in fact serves as a significant mortality force in the wild; and c) there can have been scant opportunity to evolve genes specifically for aging, even if they were beneficial, since natural selection would not normally "see" them in action (Kirkwood, 2005).

In his 1952 essay, 'An unsolved problem of biology', Medawar laid out the basic evolutionary problem of aging. His argument went that, since all organisms eventually die from different causes (e.g. diseases, accidents, predation), genes beneficial early in life are favored by natural selection over genes beneficial late in life (Medawar, 1952). Hamilton (1966) formalized the theory that senescence is an inevitable consequence of the progressive weakening of selection with age. Thus, the power of natural selection fades with age, making it possible for hazardous late-acting genes to exist (Charlesworth, 2000). The mutation accumulation theory of aging of Medawar considers aging as a byproduct of natural selection. Old age is not under selective pressure per se, and there is no evolutionary mechanism to rid a population of mutations that cause detrimental effects only in old animals (Medawar, 1952). The probability of an individual reproducing is age dependent. It is zero at birth and reaches a peak in young adults. Then it decreases due to the increased probability of death linked to various external (predators, illnesses, accidents) and internal (senescence) causes. Under such conditions, deleterious mutations expressed at a young age are severely selected against because of their highly negative impact on fitness (number of offspring produced). On the other hand, deleterious mutations expressed later in life are relatively neutral to selection because their bearers have already transmitted their genes to the next generation.

The antagonistic pleiotropy model by Williams ('pay later' theory) proposed that some genes are beneficial at earlier ages but harmful at later ages. The genes with age-related opposite effects are called pleiotropic genes (Williams, 1957). Accordingly, the theory of antagonistic pleiotropy is based on 2 assumptions. First, it is assumed that a particular gene may have an effect not only on one feature but on several traits of an organism (pleiotropy). The second assumption is that these pleiotropic effects may affect individual fitness in antagonistic ways. For example, a gene that increases survival to reproductive age or reproductive output will be favored by natural selection if it decreases the chances of dying at age 10 or 20. Thus, harmful late-acting genes can remain in a population if

they have a beneficial effect early in life such as increasing fitness at early ages or increasing reproductive success. Natural selection will frequently maximize vigor in youth at the expense of vigor later on and thereby produce a declining vigor during adult life (Williams, 1957).

There were attempts to find a better name for the antagonistic pleiotropy theory and to specify in more detail how one and the same gene could have both deleterious and beneficial effects. The disposable soma theory (DST) predicts that aging occurs due to the accumulation of damage during life and that failures of defensive or repair mechanisms contribute to aging (Kirkwood, 1977; Kirkwood and Holliday, 1979; Kirkwood and Austad, 2000). It postulated a special class of gene mutations with antagonistic pleiotropic effects in which hypothetical mutations save energy for reproduction (positive effect) by partially disabling molecular proofreading and other accuracy promoting devices in somatic cells (negative effect). In other words, given finite resources, the more resources an animal spends on bodily maintenance, the less it can expend on reproduction, and vice versa. The key feature of the DST is its emphasis on the optimal balance between somatic maintenance and repair versus reproduction. Fisher (1930) was the first who had called attention to the significance of determining how natural selection adjusts the partitioning of the energy budgets of organisms among reproduction, growth, and maintenance (Hirshfield and Tinkle, 1975; Pianka, 1976). Moreover, life history theory had put forward these ideas emphasizing the role of resource availability and allocation for life history decisions (MacArthur and Wilson, 1967; Stearns, 1977).

The three theories are based on the consistent concept of the declining force of natural selection and are more complementary than mutually exclusive (Kirkwood and Rose 1991) and hence, in this paper are dubbed “evolutionary theories of aging” (ETAs). The ETAs dominate the thinking in evolutionary biology and gerontology for more than half a century. Evidently, it is the merit of these theories that they have tied the study of senescence to the central theory of modern biology (although, both Wallace and Weismann were the first to accomplish this).

The recent decades have witnessed significant inconsistencies between the ETAs and the available evidence. Hence, the ETAs have faced staunch opposition. Sacher (1978) considered that “the implication that… organisms are mortal only because of the accumulation of adventitious senescence genes is more easily reconciled with a cosmology of special creation than with current scientific conceptions”. Lints (1983, 1985) strongly criticized, not the ETAs, but rather the first experiments claiming to confirm them. The most recent and most sophisticated challenge comes from demographers and mathematicians, who argue that a change in certain assumptions, made by Hamilton’s quantitative formulation of the patterns of decline in the force of natural selection, can lead to quite different outcomes (Vaupel et al., 2004; Baudisch, 2005; Martin, 2007; Wachter et al., 2008). Demographers have also emphasized the counterintuitive observation that the age-specific mortality rates for exceedingly old members of a cohort begin to decline (Vaupel et al., 1998) (see chapter 14). Various findings contradict the assumptions of the DST (Mitteldorf, 2001; Blagosklonny, 2007; Speakman and Król, 2010). According to Martin (2007), perhaps the severest challenges have come from the very old observations that a single simple environmental manipulation, dietary restriction, can provide substantial enhancements in longevity of a wide range of organisms (Mitteldorf, 2001) and the more recent discovery that loss-of-function mutations in a canonical signal transduction pathway can provide substantial increases in longevity for worms, fruit flies, and mice, the first “public” modulation of aging (Partridge and Gems, 2002). Moreover, evidence that costs of reproduction and longevity trade-offs in Caenorhabditis elegans, a model nematode (Riddle et al., 1997), appear to arise from molecular signals and are not resource based (Barnes and Partridge, 2003; Baumeister et al., 2006) has questioned the validity of the DST. Repeatedly, it has been attempted to align these findings with the ETAs (Leroi, 2001; Harshman and Zera, 2007; Mukhopadhyay and Tissenbaum 2007). However, these attempts were bound to fail since they did not question the principal validity of the ETAs and their pseudo-evolutionary assumptions. In addition, the theory of mutation accumulation has lost favor in recent years, as such phenomena are difficult to reconcile with the hypothesis that aging derives from chance mutations, recently acquired, which selection has had insufficient time to eliminate (Mitteldorf, 2004).

Various authors proposed theories implicating that aging has been the direct object of selection and is adaptive and programmed: the evolvability concept (Skulachev, 1997; Libertini, 2006; Goldsmith, 2008; 2010), various group/kin selection theories (Bowles, 2000; Longo et al., 2005; Libertini, 2006; Bourke, 2007; Mitteldorf and Pepper, 2007), the telomere-cell senescence theory (Milewski, 2010), theories based on the analogy of cellular and organismal programmed death (Skulachev, 1999; 2002; Bredesen, 2004) and in

a spatially structured model (Travis, 2004; Dytham and Travis, 2006). Mitteldorf (2006) seized on Weismann's idea and showed theoretically that aging may have evolved to prevent populations from outgrowing their habitat and food supply, which in turn protects populations from the threat of extinction. However, the shortcoming of these adaptive theories is that they attempt to explain the programming of aging in the context of the fundamental assumption of the ETAs that aging is due to the declining force of natural selection in postreproductive individuals. Basing their arguments on the inconsistencies inherent to the ETAs, they have to use group-level selection arguments for their justification and present no convincing evolutionary mechanism underlying the programming. Hence these theories were unable to challenge and question the ETAs and did not gain popularity.

The late G.C. Williams emphasized that the necessity for 'improvement is long overdue' (Williams 1992). To quote Williams (1992) further: 'a fuller theory of the evolution of senescence should be a 'fitness-maximization model with realistic genetic, development and demographic constraints. It must be able to predict the effects of age on measures of adaptive performance in a diversity of populations subject to senescence'. In a recent review that looks like a desperate uphill battle stemming against the wave of genetic evidence that is threatening to sweep aside the untenable ETAs, the closing sentence was: "Only by stronger efforts to couple the discovery of genetic features associated with ageing with the rigorous framework of evolutionary life history theory will the necessary links be forged." (Kirkwood and Melov, 2011). This is exactly what is done here.

A propos the circularity of reasoning: Circular reasoning is a formal logical fallacy in which the proposition to be proven is assumed implicitly or explicitly in one of the basic assumptions. Essentially, the argument assumes that its central point is already proven, and uses this in support of itself. Thus, the circularity of an argument stands or falls with the verification of its principal assumption. According to the principles of the Modern Synthesis, the force of selection should decline postreproductively. But is this the cause of aging? The argumentations of the ETAs may be replete of circular reasonings if their basic assumption is refuted that aging is due to the declining force of selection pressure with aging. To illustrate this point I give you another quote from the a.m. recent review (Kirkwood and Melov, 2011): "Finally, a simple yet forceful observation speaks against the existence of an active set of gene-controlled mechanisms that serve specifically to cause ageing and death: if such mechanisms existed, they would be susceptible to inactivation by mutation. Yet among the many gene mutations that have been discovered that affect lifespan, often increasing it significantly, none has yet been found that abolishes ageing altogether." I ask you to return to this quote after the lecture of this paper and decide yourself where the circularity of reasoning lies. Finally, in this review (after denouncing that the programmed-aging concept is "commonly expressed by newcomers to the field") the authors write: "But to hold to the idea that ageing is programmed, in the face of the evolutionary logic and experimental evidence to the contrary [sic! KH], is as unpromising a scientific stance as to continue to assert that the sun orbits the earth." To remain in the scenario of the historic controversy over the geocentric or heliocentric systems, I am afraid that the writers of these lines were not aware who, in the end, may be casted for the role of Galileo Galilei and who for the role of the Catholic Church.

In the following, I will refute the ETAs. The assumptions of my evidence-based theory are based on a robust body of evidence that was accumulated over the last 20 years, evaluating more than 400,000 scientific publications and a plethora of books. The result of this comprehensive study is an ecological-evolutionary scenario in which the omnipresent selective pressure exerted by limited resources shaped the evolutionary necessity of aging/death, not as a group-selective outcome but resulting from an individual germ-soma conflict over the utilization of resources. The complexity of aging/death causation and regulation is reflected by the argumentation and hence the paper is no easy reading. I have tried to assist the reader by providing short summaries at the beginning of the chapters. This assistance may abet a rather saltatory style of reading with intermittent full text reading depending on the reader's focus of interest. Taking the potentially saltatory style of reading and the complexity of the topic into account there is some inbuilt redundancy of argumentation both to keep track and stress the consistency and consilience of data.

2. The "Structure of Scientific Revolutions"

If the parts of a theory do not hang together without contradiction, the theory should be discarded.
Ruse, 1999

Summary
According to T. Kuhn, scientific revolutions have a certain inbuilt logic and course of events.

Intriguingly, Kuhn predicted that almost always those who achieved fundamental inventions of a new paradigm have been outsiders to the field whose paradigm they changed. According to Vester, from inside a system the intellectual approach is constructivistic, deterministic, production-oriented, technocratic, fraught with linear thinking; from outside it is holistic, function-oriented, cybernetic, tackling issues with interconnected thinking. The Law of Causality states: Every event must have a cause. The concept of causation must be carefully distinguished from the concept of correlation. A non-causal correlation can be spuriously created by an antecedent "confounding factor" which causes both events. Therefore, experimentally identified correlations should not be mistaken as causal relationships unless spurious relationships can be ruled out.

2.1 The Structure of Scientific Revolutions

In his seminal book "The structure of scientific revolutions", T. Kuhn (1962, 1970 2nd edn.) sketched the sequence of events leading to a scientific change of paradigm. In his second phase of normal science, puzzles are solved within the context of the dominant paradigm. As long as there is consensus within the discipline, normal science continues. Over time, progress in normal science may reveal anomalies, facts that are difficult to explain within the context of the existing paradigm. While usually these anomalies are resolved, in some cases they may accumulate to the point where normal science becomes difficult and where weaknesses in the old paradigm are revealed. Kuhn refers to this as the phase of crisis. Alluding to the discovery of oxygen, Kuhn wrote: "By the time Lavoisier began his experiments on airs in the early 1770's, there were almost as many versions of the phlogiston theory as there were pneumatic chemists. That proliferation of versions of a theory is a very usual symptom of crisis. In his preface (to De Revolutionibus, KH) Copernicus complained of it as well." Nowadays, reviewers of the theories of aging routinely deplore a similar situation. Accordig to an estimate in 1990, more than 300 theories have been put forward (Medvedev, 1990) and the number has risen since (Rattan, 2006).

After significant efforts of normal science within a paradigm fail, science may enter the third phase, that of revolutionary science, in which the underlying assumptions of the field are reexamined and a new paradigm is established. Intriguingly, Kuhn predicted that "almost always those who achieve fundamental inventions of a new paradigm have been either very young or very new (i.e. outsiders, KH) to the field whose paradigm they changed. Obviously these are the men who, being little committed by prior practice to the traditional rules of normal science, are particularly likely to see that those rules no longer define a playable game and to conceive another set that can replace them." (p. 90). Kuhn anticipated that it will be possible to reconstruct the chronology of the theories on the basis of the theories' scope and content, because the more recent a theory is, the better it will be as an instrument for solving the kinds of puzzle that scientists aim to solve.

2.2 Interconnected thinking

One of Kuhn's thoughts I find particularly unorthodox. Why should it be that outsiders may have a better chance to delineate the functioning of a complex system? Vester (2007, p. 98) provides an explanation similar to Kuhn's: from inside a system, the intellectual approach is constructivistic, deterministic, production-oriented, technocratic. Climbing out (or being out) of a system gives a perspective leading to a holistic, function-oriented, cybernetic model. A first step is to realize the complexity of the system through data mining, interconnected instead of linear thinking and pattern recognition. In the context of an evolutionary explanation of aging, linear thinking is epitomized by the "waning of the force of selection → aging" causality while the interconnected thinking places aging in the dynamic, biocybernetic context of its ecological, evolutionary and developmental network.

Thinking in linear pathways, characterized by a single input and a single output, is replaced by network concepts that have multiple inputs and outputs, respectively a single, highly plastic output, shaped by a multitude of modulators.

The systems thinking approach incorporates several tenets (Skyttner, 2006):

* Interdependence of objects and their attributes - independent elements can never constitute a system
* Holism - emergent properties not possible to detect by analysis should be possible to define by a holistic approach
* Goal seeking - systemic interaction must result in some goal or final state
* Inputs and Outputs - in a closed system inputs are determined once and constant; in an open system additional inputs are admitted from the environment
* Transformation of inputs into outputs - this is the process by which the goals are obtained
* Entropy - the amount of disorder or randomness present in any system
* Regulation - a method of feedback is necessary for the system to operate predictably

* Hierarchy - complex wholes are made up of smaller subsystems
* Differentiation - specialized units perform specialized functions
* Equifinality - alternative ways of attaining the same objectives (convergence)
* Multifinality - attaining alternative objectives from the same inputs (divergence)

I would like to add consilience (Wilson, 1998) - the unification of knowledge - to this list. Systems thinking is the unifying principle, targeted at augmenting the internal consistency and coherence of concepts in ecology, ontology and evolution.

2.3 The "Law of Causality"

Since Aristotle's time, scientists have maintained that to understand a phenomenon in a scientific way we must know its cause(s) (Ruse, 2003). The idea of causality is based on our belief that events in the universe are interconnected. Classical (linear/monocausal) sciences use the term "cause" for the first of two interconnected events and the term "effect" for the other. So the idea is that events occur in a special temporal order. This conception is hardened by our perception and experience, and it seems that the conception of causality is crucial for creating models of the world and hence for practising sciences at all (Brunner and Klauninger, 2003). The Law of Causality states: Every event must have a cause (Hughes and Lavery, 2004). Therefore we explain particular events and general patterns by identifying the causal factors involved. According to Mayr (1961) causality,..., is believed to contain three elements: (i) an explanation of past events; (ii) prediction of future events; (iii) interpretation of teleological – that is "goal-directed"- phenomena. Ordering two or more events in a causal order is crucial for a scientific understanding. Another order of events is their temporal order. While the temporal order is observable, outside of a controlled scientific experiment the causal order is not. This is because a complete causal account specifies the necessary and sufficient conditions for something to occur and both of these conditions involve counterfactual statements (Damer, 19995; Hughes and Lavery, 2004). Counterfactual statements are about what would have happened had the purported necessary and sufficient conditions not been satisfied. These possibilities are, by definition, not observable. For these reasons, the concept of causation must be carefully distinguished from the concept of correlation. Two events that regularly occur at the same time or in the same sequence may be both correlated and related as cause and effect or they may be correlated without being in a direct causal relationship. A correlation is observed when different events occur at the same time or occur regularly in the same sequence. With causation, one event (the cause) is responsible for, or brings about, another event (the effect). We can see the need for this distinction by considering one of the causal fallacies, common cause. Someone might notice that a sore throat is always accompanied by sinus congestion (a correlation). On the basis of this observed correlation, the sick person might fallaciously believe that the sore throat causes the sinus congestion. But really, the sore throat and the sinus congestion are both caused by a third factor, namely, a cold virus. So while the sore throat and sinus congestion have a common cause, neither causes the other (Hughes and Lavery, 2004). There is a correlation but no causal relationship between the sore throat and the sinus congestion, while both are epiphenomena of their common cause, the cold virus. Hence, a spurious relationship is fallaciously assumed when two occurrences have no causal connection, yet it may be inferred that they do, due to a certain third, unseen factor (referred to as a "confounding factor").

Hence, causality evaluations should be based on three criteria: (i) Correlation: cause and effect must vary together; (ii) Time sequence: the cause must come before the effect; (iii) Non-spuriousness: the relationship between two events cannot be explained by any third variable.

The literature on the ultimate cause of aging is replete with deductions from phenotypes of aging trying to infer what evolutionary processes may have led to the phenomenon that organisms suffer the loss of fitness allegedly related to aging. So far, the scientific approaches to the enigma of aging consistently have been focussed on creating correlations. But correlations may be corrupted and invalidated by spuriousness. Causal relationships are dependent on temporal cause-effect relationships. It is a simplistic approach to assume that the temporal cause-effect relationships of aging become evident through observations during the lifetime of an organism. This approach only provides correlations, the real causes of aging as an almost ubiquitary phenomenon are hidden deep in evolutionary time. As the temporal order of evolutionary events is rarely observable in real time mode, evolutionary biology has to resort to the observation of the sequence of events on a phylogenetic and ontogenetic time scale. This approach, tapping the "fossil record" in genome (Buss, 1988 p. 90), physiology and develoment has already provided deep insights for phylogenetic and ontogenetic events (Gould, 1977; Buss, 1988; Heininger, 2001; 2002a). This systemic approach has

also been realized in this work.

3. Aging is due to the declining force of natural selection: A fata morgana

…that an opinion has been widely held is no evidence whatever that it is not utterly absurd….
Bertrand Russell (1929)

Summary

The basic assumptions of the ETAs are flawed and replete with circular reasoning. Natural selection is an outcome that is brought about by the joint selective forces of the biotic and abiotic environment. Obviously, not the selective forces decline postreproductively since they continue to act indiscriminately upon the individual organisms without regard of their reproductive status. It is the reproductive value of the organism that declines. In contrast to the ETAs' assumption, evolution "sees" the postreproductive organisms, being one of the most important selective forces that determine the fitness of the progeny, either by procuring resources and protection during altriciality or competing for limited resources thereafter. It is a fundamental property of coevolutionary systems that the conflict between the interests of the interactors, not the transfer of genes, drives the coevolutionary dynamics. Moreover, the assumptions are flawed that organisms are unable to maintain a youthful, vigorous state; that in the wild, animals do not live long enough to experience aging; and that under the threat of extrinsic death organisms would no longer invest sufficiently into somatic maintenance and repair. The essential fallacy of the ETAs is based on the fact that the outcome of natural selection (i.e. the representation of the organism's genes in future generations) that obviously lies in the future is linked to a phenomenon, the postreproductive aging of the parent that, from the perspective of the offspring as the carrier of the genetic information, lies in the evolutionary past. Even worse, some Lamarckian-type inheritance (in its original sense of genetic heritability of somatic change) is implied in the ETAs. A simple change of perspective from a soma-centered to a germline-centered point of view helps to understand that somatic aging co-selected with reproduction, is selected for, adaptive, and fitness-enhancing.

According to a recent review of Milewski (2010) the ETAs "are hypothetico-deductive in nature (Rose, 1991) meaning that when first conceived they were deduced from assumed laws or premises rather than from empirical observations (Achinstein, 2000). Hypothetico-deductive theories (by definition) have strong theoretical backing, and indeed the aforementioned theories of aging were rooted in population genetics several decades ago (Hamilton, 1966; Charlesworth, 1980)." (As Lewontin (1974, p. 267) put it: ..population genetics is not an empirically sufficient theory …. Built into both deterministic and stochastic theory are parameters and combinations of parameters that are not measurable to the degree of accuracy required.) Milewski (2010) further argued: "However, these decades witnessed a paucity of research into the biochemistry of aging. The two mainstream evolutionary accounts of aging, then, were deeply entrenched in sophisticated theory some time before there was evidence to back them up. This situation is not problematic as long as theories such as those above function solely as stimuli for research. It is only when such theories are not treated tentatively, but instead prematurely accepted, that problems arise. The reason for this is that when theories are accepted by the scientific community, they become the standard paradigm for the discipline in question. Once a paradigm is established, it begins to dictate how empirical data are interpreted (Kuhn, 1962; 1970 2nd ed.; Ladyman, 2002). Since hypothetico-deductive theories do not arise from empirical observations, but from assumed laws or premises, it is thus crucially important that they receive empirical verification before being used as the standard explanatory tool of the discipline. With regard to the mutation accumulation theory and the antagonistic pleiotropy theory, it seems that they have been adopted as gerontology's paradigm largely by default (due to the lack of alternatives) rather than for any compelling evidential reasons. All empirical data are now first and foremost, and almost always, interpreted in the light of one or the other of them. This acceptance has been premature (Mitteldorf, 2004)."

The ETAs are a paragon for paralogic reasoning and to understand their cognitive basis please envision the state of knowledge of the founders of the ETAs, Medawar, Williams and Hamilton. In the 1950ies and 60ies, aging was perceived as a phenomenon particularly in humans and a variety of captive animals. It was thought not to exist in the wild due to the action of extrinsic death. Thus, it was inferred that aging has no phylogenetic history.

Natural selection is the holy grail of evolutionary biology. There is some controversy how natural selection is related to fitness (e.g. see Rosenberg and Bouchard, 2008). Without entering this debate, fitness can be defined in terms of relative reproductive success (Ariew and Lewontin, 2004). The biotic and abiotic environment is the context that gives rise to the relationship between phenotype and fitness (selection). The analysis of the causes of selection is in essence a problem in ecology (Wade and Kalisz, 1990). Importantly, fitness is a probabilistic quantity since it cannot be equated with actual reproductive success. According to Darwin's dictum (1859), that "natural selection acts solely by and for the good of each", full force natural selection was held not involved in the fitness-eroding action of aging. It was only a small cognitive step to assuming that the declining force of natural selection in postreproductive organisms (since it was assumed that postreproductive individuals are not "seen" by evolution) would enhance either accumulation of mutations or genes with age-related opposite effects.

To recognize the fallacies of argumentation the nature of natural selection has to be clarified. Natural selection is a metaphor (and as such also seen by Darwin) born from the analogy with breeder's artificial selection (Ruse, 2003). It was conceived by Darwin and Wallace (Kutschera, 2003) to explain the workings of nature. A highly plausible and therefore successful metaphor. But unlike breeder's selection, natural selection has no (omni)potent or metaphysical decider that decides who is allowed to breed and who not. Natural selection is brought about by the joint selective forces of the biotic and abiotic environment. In fact, the underlying mechanism is that organisms fail to reproduce (or reproduce less than their competitors) because they are not fit enough (e.g. because they are not fast enough to escape a predator, not immunocompetent enough to withstand an infection, not cold-resistant enough to endure winter, not efficient enough to exploit resources or not dominant enough to secure a territorium that attracts mates) their genes are not (or less) represented in the next generation. It was even suggested that a scaling-down of the meaning of natural selection to "the elimination of the unfit," as originally intended by AR Wallace, might ultimately prove a more effective means of relating it to larger-scale, longer-term, evolutionary processes (Smith, 2012). The generally accepted modern definition of natural selection is that it is an outcome (stemming from Fisher 1930; Endler, 1986; Bock, 2010) due to "nonrandom (differential) reproduction of genotypes" (Ehrlich and Holm, 1963) or "nonrandom differential survival or reproduction of classes of phenotypically different entities" (Futuyma, 1986). Most importantly, natural selection is no physical force and does not act. (Darwin himself is not a good reference for this fact. In his On the Origin of Species I quit counting after 35 expressions like "natural selection acts" or similar expressions in the first third of the book). In fact, the interactors in the scenario of nature are the respective individual and its biotic and abiotic environment (e.g. predators, infectious agents, helpful kin, beneficial or deleterious mutations, competitors, droughts, or limited resources leading to starvation) that determine whether the individual may survive to reproduce.

After these necessary clarifications I discuss the assumptions of the ETAs:

1. Aging is due to the declining force of natural selection. In the words of Medawar (1952): "In the post-reproductive period of life, the direct influence of natural selection has been reduced to zero...".

The fallacy of the above statement consists of two components. The first is that it uses the limitation of the reproductive period that is an essential manifestation of somatic aging (e.g. Packer et al., 1998) and regulated by the same signaling pathways (see chapter 14) as one of the basic assumptions. Later versions scheduled the declining force of natural selection at the time of reproductive maturation to take the finding into account that the first signs of senescence are detected at that time. However, this does remedy the circularity of the argument just as little as an alternative formulation: "The force of natural selection [thus] declines with age" (Rose, 1984), since both imply the declining reproductive value of the aging organism. Immortal organisms, virtually all of them sessile organisms (the importance of this fact will become evident later), have an unlimited reproductive potential and hence no declining reproductive value and no postreproductive period (see chapters 6.1.4 and 15.1). Even a theoretically immortal organism would suffice to prove the circularity of this argument. As discussed in chapter 1, assuming the proposition to be proven implicitly or explicitly in one of the basic assumptions is a key feature of circular reasoning. Essentially, the argument assumes that its central point (here the finiteness of the reproductive period) is already proven, and uses this in support of itself.

The second component refers to one of the principles of population genetics that the force of selection should decline postreproductively (Fisher, 1930; Haldane, 1941; Medawar, 1946; 1952; Williams, 1957; Hamilton, 1966). The fallacies of this concept are multifacetted. The issue of the putative declining force of natural selection has to be assessed from two perspectives, both the perspectives of the parent(s)

and the offspring. In the light of the insight that natural selection does not live an independent, virtually metaphysical, existence but is dependent on a variety of biotic and abiotic forces, the question arises which of these forces are expected to decline? Obviously, not the forces decline since selective forces like predators, infectious agents and famine continue to act indiscriminately upon the individual organisms without regard of their reproductive status. When selective forces act on a postreproductive organism, however, the outcome of this interaction has no effect in terms of the representation of the organism's genes in the next generation. Hence, the outcome of natural selection is affected but the reason for this fact is that the reproductive potential of the organism is zero, not that the force of natural selection is zero. Thus, the term "declining force of natural selection" is a misnomer. In fact, this term confounds the force of natural selection that does not decline with age and reproductive value that is decreasing with age. Fisher (1930) showed that in organisms with a senescent life history, mortality rate tends to closely follow the reproductive value. Although it has been argued that reproductive value is an inappropriate measure of the force of selection against age-specific mutations (Hamilton, 1966; Vaupel et al., 2004; Rose et al., 2007), reproductive value as a proxy to the force of natural selection features prominently in various versions of the ETAs (Partridge and Barton, 1996; Kern et al., 2001; Ossa et al., 2006). Differentiating between reproductive value and strength of selective forces reveals a paradox that immediately questions the validity of the "declining force of natural selection" paradigm. Obviously, the reproductive potential of an organism is dependent on the harshness of ecological conditions: the harsher the conditions the lower will be the organism's reproductive potential. Conversely, future reproductive success may even increase in more benign conditions with low-intensity selective forces e.g. more abundant resources. Thus, reproductive potential and intensity of natural selection often have an inverse relationship. In fact, declining selective forces with age would boost reproductive potential, counterregulating the waning of selective forces. As a result of this self-contradictory circular logic similar to a Catch-22-type situation, the status quo of immortality as given in basal metazoa like Porifera, Cnidaria and Platyhelminthes should have been maintained.

It may be argued that although the concept of a declining force of natural selection with age may be fallacious, the fact cannot be refuted that the outcome of natural selection is affected. As a consequence there are late-acting mutations such as the ones causing M. Huntington, M. Parkinson, or M. Alzheimer that are not selected against since they manifest only in postreproductive individuals, representing a model case for the evolutionary mechanisms operating in the causation of aging. Again, this objection is compromized by circular reasoning: the finiteness of the reproductive period as manifestation of aging is used as argument in support of the evolution of aging. I emphasize the distinction between the "force of natural selection" (of which, as has been argued, reproductive value is an inappropriate measure [Hamilton, 1966; Vaupel et al., 2004; Rose et al., 2007]) and the "outcome of natural selection". The latter is clearly dependent on the declining reproductive value which is a manifestation of aging (see chapters 11.4 and 14), establishing the logical fallacy of circular reasoning.

Another fallacy of the ETAs is the assertion, often used but highly fallacious, that postreproductive organisms are not "seen" by evolution. This notion tacitly implies that organisms have to transmit genes to future generations to be evolutionarily relevant. This argument is as flawed as would be the conjecture that predators, parasites, competitors, resource shortage, weather, or catastrophes are not "seen" by, or are irrelevant to, evolution only because these biotic and abiotic factors are not connected to the target individual via a germline. In fact, it has its fitness consequences for the offspring, both positive and negative, that the postreproductive parents are still physically present with all their effects on resource availability and brood care. Resource availability is one of the strongest selective forces as will be discussed in chapter 5. There are positive fitness consequences of the parents in terms of brood care and transfer of resources during the period of altriciality and beyond (Lee, 2003; Bourke, 2007). But there are also negative consequences in terms of competition for limited resources. Importantly, these fitness consequences are not related to group selection; it is the individual offspring that benefits, respectively suffers from the positive and negative consequences of the ongoing presence of the parents. Thus, the ancestors are a selective force with opposing effects on the fitness of the progeny. The evolution of brood care is conclusive evidence that postreproductive individuals are a strong selective force. For instance, female Octopus cyanea survive for a month after spawning, during which time they brood their eggs (which are otherwise unprotected) and generally die a few days after the eggs hatch (Wodinsky, 1977; Van Heukelem, 1983). Strangely enough, the fitness-enhancing and selective force of intergenerational resource transfer has been

appreciated (Lee, 2003; Bourke, 2007), while the fitness-eroding effect of intergenerational resource competiton was ignored. There is compelling evidence that selective forces unfailingly act in postreproductive individuals: a) the public mechanisms of aging as epitomized by the canonical signaling pathways from yeast to man do not decay (see chapter 17.1.4); b) natural selection could evolve brood care; c) hormesis, including dietary restriction, as lifespan-extending mechanism is also operative in postreproductive individuals (see chapter 17.3.4).

Apart from the misnomer and the non-realization of the evolutionary impact of postreproductive organisms, the essential fallacy of the ETAs is based on the fact that the outcome of natural selection that obviously lies in the future is linked to a phenomenon, the postreproductive aging of the parent that, from the vectorial, unidirectional, perspective of the germline (and that is the only relevant perspective in terms of evolutionary processes and outcomes) lies in the past. Thus, this fallacy has both a temporally and logically fallacious component. 'Backwards (or reverse) causation', by which some future state or event influences ('causes') an action in the present or past, is often characteristic of teleological arguments (Gross, 2006). And it is related to the "fallacy of affirming the consequent" (also called post hoc, ergo propter hoc argumentation) which is logically inadmissible in the natural sciences (MacNeill, 2009). That fallacy, expressed informally, takes the following form: "If A is the cause, then B follows from it. B has occurred. Therefore, A is the cause." In the admittedly simplified version of the ETAs: "If the declining force of natural selection is the cause, then aging follows from it. Aging has occurred. Therefore, the declining force of natural selection is the cause of aging."

Even worse, the ETAs imply some type of Lamarckian-type inheritance: Even the most staunch proponents of the ETAs, although they deny that aging is programmed, now have to agree that longevity is "regulated by genes" (see chapter 17.3). Since the ETAs infer that "declining selective forces" that act during the parent's postmaturational lifetime leave their heritable footprints in the genome of the progeny some Lamarckian-type inheritance (in its original sense of genetic heritability of somatic change, Lamarck, 1809) must be involved (but see the issue of soft [epigenetic] inheritance; Gissis and Jablonka, 2011).

2. According to the ETAs, selection continues to operate with full force until reproduction begins. The forces of natural selection are decreasing gradually, parallel to the decline of the reproductive potential of the organism. Once reproduction is complete the force of selection is reduced to zero (Kirkwood and Cremer, 1982).

Asexually reproducing basal metazoa are virtually immortal and, given sufficient resources, may reproduce indefinitely (see chapter 6.1.4). How could aging have evolved in the phylogenetic descendants of these "perfect" organisms? The ETAs assume that aging evolved in iteroparous organisms. Since selection is operating with full force at the onset of reproductive activity, the first clutch of these animals should benefit from the full force of natural selection. Later clutches should be less privileged and under the relaxed selection regime may suffer incremental fitness losses. In fact, as a manifestation of reproductive aging, quality of later offspring has been shown to decline in a variety of taxa (see chapter 14). This decline extends to various features of viability and fitness including longevity. According to evolutionary theory, the fitter first clutch with its indefinite reproductive potential (at least as is assumed in the idealized world of the ETAs) should always have been able to outcompete the less fit later clutches and aging would never have had a chance to evolve.

3. The antagonistic pleiotropy model by Williams ('pay later' theory) proposed that some genes are beneficial at earlier ages but harmful at later ages. Natural selection will frequently maximize vigor in youth at the expense of vigor later on and thereby produce a declining vigor (aging) during adult life.

Time is a scalar. Time has magnitude, but is not considered to have direction as such. Time is moving "forward" and generally cannot move in another direction. Evolution is without foresight. Something that occurred in the evolutionary past can affect the present, but something that will occur in the future cannot. Genes are like time, they move only in one direction, are transmitted in a time-dependent manner and cannot look forward. As Fodor and Piattelli-Palmarini put it (2010 p. 11): "The general principle is straightforward: according to evolutionary theory, nothing can affect selection except actual causal transactions between a creature and its actual ecology." How could something that will affect the parent organism in the future (e.g. aging) shape the genes that it transmits before this event occurs?

As many authors, including Williams (1957) have noticed, antagonistic pleiotropy to arise requires opposing selective forces. Antagonistic pleiotropy is a reality (see chapter 13) and is caused by the opposing selective forces exerted in the coevolutionary conflict of soma and germline cells/offspring. Full blown selective forces on the side of the juvenile organism opposed by declining selective forces on the side of the aging organism (as assumed by the ETAs),

however, may result in a runaway process (Fisher, 1930), as discussed in chapter 13.

4. The ETAs imply that postreproductive aging and mortality are evolutionary default states that, by some inherent properties (caused by the declining force of natural selection and e.g. antagonistic pleiotropic genes), organisms are unable to maintain a youthful, vigorous state.

There are a variety of organisms that, as far as we know to date, do not age and are virtually immortal. For instance, given sufficient resources, a variety of plants and sessile animals, organisms without a segregated germline in which the body itself is the reproductive unit, can reproduce indefinitely and do not age. Some of them even retain the potential for ontogeny reversal due to the occurrence of cell-transdifferentiation processes and are able to cycle between the state of mortality and immortality (see chapter 6.1.4). There are no physical reasons (other than the ones I will elaborate in this paper) why organisms should not be able to maintain their soma indefinitely (see chapter 16). In fact, relatively "primitive" metazoa, sponges or Hydra, are able to maintain their soma in good health virtually indefinitely and simultaneously invest ample resources into asexual reproduction. Why should these "primitive" creatures succeed where allegedly much fitter birds and mammals fail? Living systems are defined by their capacity to gather order from their environment, concentrate it, and shed entropy with their waste. Organisms in their growth phase become stronger and more robust; no physical law prohibits this progress from continuing indefinitely. The same conclusion is underscored by experimental findings that various insults and challenges that directly damage the body or increase the rate of wear and tear have the paradoxical effect (paradoxical at least from the perspective of the ETAs; Forbes, 2000) of extending lifespan (see chapter 10.1). A fundamental understanding of aging must proceed not from physics but from an evolutionary perspective: The body is decaying because systems of repair and regeneration that are perfectly adequate to build and rebuild a body of ever-increasing resilience are being held back (Mitteldorf, 2010a).

5. In the wild, animals do not live long enough to experience aging. This means that a) there is no requirement for aging to weed out "worn-out individuals"; b) there is no evidence that aging in fact serves as a significant mortality force in the wild; and c) there can have been scant opportunity to evolve genes specifically for aging, even if they were beneficial, since natural selection would not normally "see" them in action (Kirkwood, 2005).

There is cumulative evidence that in the wild intrinsic aging shapes the vulnerability to extrinsic death in a variety of species (Fuller and Keith 1980; Bjorge and Gunson 1989; Boyd et al. 1994; Mech et al. 1995; Ricklefs 1998; 2000; 2008; 2010a; Ashman et al., 1999; Møller and de Lope 1999; Ricklefs and Scheuerlein, 2001; 2002; Doums et al., 2002; Williams and Day, 2003; Smith et al., 2004). Thus, it is highly circular to argue that extrinsic mortality, e.g. due to predation and infection (that is often resulting from aging-related sensory and motor deficits or immunosenescence), made the evolution of programmed aging, e.g. as resource competition regulator, redundant. Until recently, it was assumed that individuals in the wild were highly unlikely to show signs of senescence (Holmes and Austad, 1995; Kirkwood and Austad, 2000). This assumption has been refuted (e.g. Jones et al., 2008) (see chapter 17.1.5). Definitely, senescence has evolved outside of a "selection shadow" (Turbill and Ruf, 2010). Already Williams (1957) acknowledged that "No one would consider a man in his thirties senile, yet, according to athletic records and life tables senescence is rampant during this decade". Even if animals are not 'dying of old age', senescence may be causing many deaths. Nature is such a competitive place that even a little bit of senescence can be fatal (Williams, 1957; Mitteldorf, 2010b).

Long-lived species are fitter and more competitive, e.g. efficiently can avoid death due to predation (e.g. by being top predators themselves) or other environmental hazards thanks to evolutionary achievements such as wings (birds), shells (turtles), large body size (whales, elephants), homeothermy (birds, mammals), and large brain size that facilitates learning and problem solving (primates). Obviously, evolution of ever fitter organisms is inherent to evolutionary processes. But why should the cellular and organismal processes that confer longevity be limited? Why not extend life indefinitely (Mitteldorf, 2010a)?

6. Under the threat of extrinsic death organisms would no longer invest sufficiently into somatic maintenance and repair.

After billion-years of evolution, organisms are survival machines (Darwin, 1859; Dawkins, 1989). Why should these organisms that have survived the harshest selection regimes succumb to the threat of death in a type of "anticipatory obedience"? What led evolutionary biologists to attribute these survival machines this anthropomorphic loser image? Organisms are selected to fight (or run) for their life to the very end. There is strong evidence that under a variety of stressors, including dietary restriction, both

reproductive and postreproductive organisms upregulate stress resistance resulting in the longevity-extending action of hormesis (see chapters 9.2 and 10.1). The somatic stress response phenotype of aging (see chapter 10) is further evidence that aging somas are not ready to succumb without resistance. It has been argued that given that resources are finite, the more an animal spends on bodily maintenance, the less it can expend on reproduction (Kirkwood, 1977; Kirkwood and Holliday, 1979). If this is the reason for less investment into somatic maintenance: why then do organisms after their reproductive phase, when all resources could be invested into somatic maintenance, not upregulate somatic repair?

Evolution by natural selection is a quite simple and straightforward (and therefore highly attractive) concept. And has one major topic: fitness, the issue that unifies the concepts of natural selection (the summary of forces that act upon an organism leading to the outcome of differential reproduction) and adaptation (both the phenotypic response of the organism to these forces and the result of differential reproduction). What benefits this fitness (within the given constraints) is kept, what erodes it will be deleted. The fundamental question finally is: whose fitness is evolution's main concern, the fitness of the offspring/germline cells or of the ancestors/soma? According to the current paradigm it is the soma. This has led to the multiple inconsistencies and incompatibilities of the ETAs. A simple change of perspective radically changes the whole picture. I will show that from the perspective of the germline cells aging/death of the soma becomes selected for, adaptive, fitness-enhancing and thus has been ultimately programmed. In fact, the ancestors may be fitness-enhancing for the progeny – e.g. when they provide brood care – but at any rate are fitness-eroding when they are around for a too long time. This highly plausible concept, meanwhile older than 120 years, was discredited following the unfortunate controversy over group selection. I will show that group selection is an often overlooked reality and, in fact, is behind the lack of germline segregation in sessile, modular organisms (see chapter 19.2). But evolution did not require group selection to enforce somatic aging and death. True to Orgel's second rule: "Evolution is cleverer than you are" it "relied" on the time-honored fundamental principles of natural selection and the selective forces of resource limitation that each individual has to endure and that makes any evolutionary system vulnerable to takeover by innovators that succeed to economize these resources.

4. Gedankenexperiment: Darwinian demon

The elder Geoffroy and Goethe propounded, at about the same time, their law of compensation or balancement of growth; or, as Goethe expressed it, "In order to spend on one side, nature is forced to economise on the other side."
Charles Darwin, On the Origin of Species, 1859

Summary
A Darwinian demon can produce infinitely many offspring and live indefinitely. High reproduction and survival, however, cannot be attained simultaneously – they compete for limited resources and entail direct and indirect costs to each other. However, in the absence of costs, it is assumed that natural selection would favor the evolution of a Darwinian demon, should evolution not have been able to constrain it. An organism that may be able to excel at resource acquisition, being able to pay all incurred costs would have the potential to evolve as Darwinian demon exploiting all resources and extinguishing biodiversity.

As discussed in chapter 2.3, counterfactual statements are, by definition, not observable. But they are amenable to thought experiments. A Darwinian demon is a hypothetical organism that can maximize all aspects of fitness simultaneously and would exist if evolution was entirely unconstrained (Law, 1979; Silvertown, 2005). Such organisms would reproduce directly after being born, produce infinitely many offspring, and live indefinitely. If such a demon existed, this highly successful organism would populate the entire world with its own kind, beating out other species and eventually extinguishing biodiversity (Dieckmann and Ferrière, 2004). Please note that exactly this Darwinian demon is the organism underlying the evolutionary concepts of the ETAs: "The anomalous nature of ageing as a putative adaptation is that it is bad for the individual in which the process is exhibited. An animal that grows to maturity and thereafter reproduces indefinitely has, other things being equal, a greater Darwinian fitness than one that grows to maturity and then survives and reproduces for only a fixed period of time." (Kirkwood and Melov, 2011). But a Darwinian demon that exhibits immortality and infinite reproduction cannot exist, because high reproduction and survival cannot be attained simultaneously – they compete for limited resources and entail direct and indirect costs to each

other. This “Partition of Resources Model”, in which resource allocation to one trait means deprivation of these resources to another one has been proposed as the main explanation for trade-offs between life-history traits (Roff, 2002; Roff and Fairbairn, 2007). From this gedankenexperiment it may be concluded that a finite lifespan should be an obligate companion of reproduction in a world of limited resources.

This is how the classical argumentation goes, rehearsed in a multitude of publications about life history theory. But can the fact that no Darwinian demon has evolved so far, sufficiently explain the costs of reproduction and the life history trade-offs? The evolutionary impossibility of a Darwinian demon was challenged theoretically, involving the ability of a Darwinian demon to influence evolutionary change (Leimar, 2001) is getting increasing support (Rosenberg, 2001; Heininger, 2001, Jablonka and Lamb, 2005). Pleiotropy and fitness interactions among traits greatly increase the possibilities for a Darwinian demon. Within this framework it was shown that combination of pleiotropic mutations and shifting fitness landscapes can evidently have a strong impact on the range of possible adaptive change allowing a Darwinian demon to control the long-term outcome of evolution and to relegate natural selection to the position of a relatively uninteresting background process (Leimar, 2001).

In the absence of costs, it is assumed that natural selection would favor the evolution of the Darwinian demon (Reznick et al., 2000). The intuitive concept of costs of reproduction was questioned both experimentally and theoretically (van Noordwijk and de Jong, 1986; Spitze 1991; 1995; Spitze et al., 1991; de Jong and van Noordwijk, 1992; Lynch and Spitze, 1994; Roff, 2000; Vorburger, 2005). Daphnia populations that experienced predation evolved such that they were younger and larger at maturity, and had higher fecundity. This combination of traits suggested that predation had caused the evolution of a ‘better’ waterflea because it increased reproductive output with no apparent cost (Spitze, 1991). Clones that developed more quickly also grew faster and had higher fecundity throughout life (Spitze et al., 1991; Lynch and Spitze, 1994). Reznick et al. (2000) stressed the challenge this poses to life-history theory: why has this ‘superflea’ failed to dominate its intraspecific competitors in the wild? These ‘superfleas’ however, are only super when resource availability is high (Reznick et al., 2000; Tessier et al., 2000). Intriguingly, a similar life history response was recently described in a vertebrate, the guppy, that lived and reproduced longer in high-predation streams than in low-predation streams (Reznick et al., 2004). This time, predation had caused the evolution of a “superfish” because it increased reproductive output with no apparent cost to longevity. The study, however, gives no data on resource availability/acquisition that may have moderated the longevity-fecundity set point. Lifespan and reproductive investment can be uncoupled in benign circumstances in a variety of invertebrates and vertebrates (Messina and Fry, 2003; Tatar et al., 2003; Ernande et al., 2004; Partridge et al., 2005; Ricklefs and Cadena, 2007; Lee et al., 2008; Flatt, 2011) and thus appears to be a general phenomenon.

In this context, the amount of resources in the environment is no invariable predictor of the amount of resources an individual can procure. Resource availability to an organism is also a function of its ability to acquire resources (Rogers, 1992). An organism that may be able to maximize resource acquisition, a fitness trait under directional selection (Rowe and Houle, 1996; Hunt et al., 2004), evidently would have the potential to evolve as Darwinian demon exploiting all resources of its planet host, should other constraints not apply (Houle, 2001). Apparently, biodiversity may have been at stake would evolution by natural selection have been “indifferent” enough to allow cost-free reproduction, “abstain” from the establishment of reproduction-dependent, intrinsic death or some other constraint and rather rely on the action of extrinsic death to keep any potential Darwinian demon at bay. Theoretical evaluations have shown that a Darwinian demon can be prevented and population stability can evolve as a consequence of selection on individuals (Zeineddine and Jansen, 2005).

A word on the teleological phenotype of some wording. Of course, evolution is not teleological in the sense that its processes or actions are for the sake of an end, i.e., the Greek “telo” or final cause. Evolution is undesigned and without foresight. Only in hindsight the human observer is tempted to describe its processes or outcomes with words like opt, select, determine etc. or is tempted to see some purpose behind its actions which, of course, is not the case. Even ‘adaptedness’ (as a posteriori result rather than an a priori goal-seeking; Mayr, 1992) or ‘natural selection’ are, sensu stricto, teleological outcomes. Accordingly, there is no “force of selection” or “selective force” as this would be an intentional, teleological force. For the sake of a vivid style of writing, I often resort to teleological-type wordings (like thousands of other authors in evolutionary biology); otherwise the “politically correct” word ‘extinction’ would have to “adorn” every second sentence. In the words of S. H. P. Madrell "the proper but cumbersome

way of describing change by evolutionary adaptation [may be] substituted by shorter overtly teleological statements" for the sake of saving space, but that this "should not be taken to imply that evolution proceeds by anything other than from mutations arising by chance, with those that impart an advantage being retained by natural selection (Madrell, 1998). And F. Ayala (1998) has argued that all statements about processes can be trivially translated into teleological statements, and vice versa, but that teleological statements are more explanatory and cannot be disposed of. Finally, according to Hull (1982), Haldane (in the 1930ies) remarked that “teleology is like a mistress to a biologist: he cannot live without her but he’s unwilling to be seen with her in public.”

5. The limited resources paradigm and the "tragedy of the commons"

...evolution may lay claim to be considered the most central and the most important of the problems of biology. For an attack upon it we need facts and methods from every branch of the science – ecology, genetics, paleontology, geographical distribution, embryology, systematics, comparative anatomy – not to mention reinforcements from other disciplines such as geology, geography and mathematics.
Huxley 1942, p. 13

Summary
Aging/death can only be understood from a joint ecological and evolutionary, an eco-evo, perspective. The limitation of resources is a pervasive feature in ecological communities. Feast/famine cycles, intraspecific self-thinning phenomena, interspecific physical and chemical warfare for space and food, density-body size/biomass scaling, overexploitation of common resources (the tragedy of the commons) and “prudent predator” phenomena are the phenotypic and molecular footprints of the ubiquitous competition for limited resources in plant and animal communities. It has to be stressed that the ecological “limited resources paradigm” is no group-selective argument but, similar to the fitness concept, is a transgenerational concept: the resource-limited environment exerts its selective force on the individual organism that is struggling for its existence in age-structured populations.

In hindsight it is one of the huge enigmas why the plausible concept of the limited resources that was forwarded by Darwin and Wallace as the driving force of natural selection (Kutschera, 2003), and was advocated by Wallace and Weismann as evolutionary cause of aging and death, was abandoned in the 1950ies (even before group selection was discredited as a significant evolutionary force). In fact, limited resources is the fundamental raison d’être of natural selection. It may only be speculated why for the scientists of the mid 20th century, resource limitation was not such a pervasive evolutionary force as the Founding Fathers of the evolution theory had perceived. In the following, I will present compelling evidence that resource limitation is one of the strongest if not the strongest selective force. I do this to counter the neglect committed by the founding fathers of the ETAs.

As Smith (2011) put it: “In some respects natural selection is a quite simple theory, arrived at through the logical integration of three propositions (the presence of variation within natural populations, an absolutely limited resources base, and procreation capacities exceeding mere replacement numbers) whose individual truths can hardly be denied.” The resulting struggle for existence is the engine that drives evolution. On the other hand, Haeckel (1866) defined ecology as the science of the struggle for existence (Cooper, 2003). Thus, from early on, ecology and evolution have been intertwined. In this vein of thought Van Valen (1973a) described evolution as “the control of development by ecology”. Calls for an ‘integrative’ understanding of biological processes keep being repeated in the literature, from Dobzhansky’s (1973) famous quote “Nothing in biology makes sense except in the light of evolution” to current, more focused statements that evolution itself only makes sense when viewed in its ecological context (Coulson et al., 2006; Saccheri and Hanski, 2006; Johnson and Stinchcombe, 2007; Metcalf and Pavard, 2007; Pelletier et al., 2007; 2009; Kokko and López-Sepulcre, 2007; Blute, 2008; Bassar et al., 2010; Matthews et al., 2011; Schoener, 2011).

5.1 Reproduction outgrows resources

Populations have the potential to grow exponentially, but this is confronted with the limited nature of resources, and natural selection subsequently favors those individuals who compete best for the scarce resources and can use them most economically (Grover, 1997). In fact, that populations outgrow resources is the central idea of Malthus’s An Essay on the Principle of Population (1798), that led Darwin and Wallace to the conclusion that this pressure,

analogous to breeder's artificial selection, was a natural form of selection (Ruse, 2009). The pervasive nature of the limited resources paradigm can be easily illustrated. If an organism produces two offspring, and each of them produces two offspring, and so on, then the total number grows at an increasingly rapid rate: 1 $\rightarrow$ 2 $\rightarrow$ 4 $\rightarrow$ 8 $\rightarrow$ 16 $\rightarrow$ 32 $\rightarrow$ 64... to 2^n after n rounds of reproduction (Gregory, 2009). The unrestrained growth of a bacterial colony for 14 days would yield a biomass exceeding the actual biomass on earth. After 200 divisions, a symmetrically dividing microorganism would generate 2^{200} cells, a number of organisms that would even exceed the total number of atoms in the universe (Dawkins, 1995; Takagi, 1999). Resource availability is arguably one of the largest selective forces acting upon populations of microbes (Lenski et al., 1991; Bohannan and Lenski, 1997). Reproduction itself drives the organisms to constantly outgrow the available resources and thus is the imperative force behind the persistent shortage. Hence, the natural lifestyle of microbes is characterized by an inherent "feast and famine" cycle, limiting amounts of nutrients being rather the rule than the exception, long periods of nutritional deprivation being punctuated by short periods that allow fast growth (Kolter et al. 1993, Msadek 1999; Navarro Llorens et al., 2010). The genetic signature of this microbial lifestyle is highlighted by the presence of feast/famine regulatory transcription factors that are systematically distributed throughout archaea and eubacteria (Kawashima et al., 2008). Moreover, the number of rRNA genes is selected to allow microbes in natural bacterial assemblages to respond quickly and grow rapidly in unpredictable environments characterized by fluctuations in resource availability (Koch, 1971; Klappenbach et al., 2000; Stevenson and Schmidt, 2004; Weider et al., 2005).

Populations that grow large enough to deplete the supply of such resources are said to be resource limited. In such populations, an individual's ability to survive or reproduce may depend on its success in competition with others for scarce resources. The larger a population grows, the more likely it is to deplete its resources (Rogers, 1992). Feast/famine cycles are not only characteristic to microbial lifestyles but are also at least intermittent features of predator/prey, herbivore/plant and competitor interactions (Schaffer and Rosenzweig, 1978; Goulden and Hornig, 1980; Rogers, 1992; Holland, 1995, Kendall et al., 1999; Abrams, 2000; Sinervo et al., 2000; Gilg et al., 2003; Vandermeer, 2006; Wang et al., 2009; Getz and Owen-Smith, 2011). In fact, the classical model of predator-prey dynamics, the Lotka-Volterra equation, predicts that under most conditions predator populations, like prey populations, go through a series of oscillations between feast and famine, at each cycle approaching the brink of extinction (Holland, 1995; Mitteldorf, 2010b).

5.2 Resource limitation is a pervasive ecological phenomenon

Over the millions of years since the Cambrian explosion, living beings have accumulated an enormous biomass. Terrestrial plants, as the main source of biomass only evolved in the mid-Palaeozoic era, between about 480 and 360 million years ago (Kenrick and Crane, 1997; Pires and Dolan, 2012). It can be assumed that at the time of the Cambrian radiation, that was a radiation of aquatic organisms, the earth was much more resource-limited than it is today due to generally low rates of productivity for the deep and open oceans (Woodward, 2007) and a much reduced coast line in supercontinents Pannotia, later Gondwana and Laurasia. In fact, Cambrian-to-Devonian seas were characterized by extremely low nutrient (super-oligotrophic) conditions (Martin, 1996). Resource management was an evolutionary necessity. Thus, as has been shown theoretically, somatic aging - and death - is a constitutive element of life in a resource-limited system (Wallace, 1967; Theodoridis et al., 1996; Travis, 2004).

A huge amount of data indicates that limitation of nutrients and building blocks, particularly phosphorus and nitrogen in aquatic and terrestrial ecosystems, is a pervasive phenomenon in the natural wild habitat and shapes food webs (e.g. Sinclair, 1974; 1975; 1989; Coe et al., 1976; White, 1978; Dempster and Pollard, 1981; Saether, 1985; Skogland, 1985; Fowler, 1987; Fenchel, 1988; Boutin, 1990; Owen-Smith, 1990; Merrill and Boyce, 1991; White, 1993; Fritz and Duncan, 1994; Elser et al., 1996; Gaillard et al., 1996; Koerselman and Meuleman, 1996; Osenberg and Mittelbach, 1996; Polis and Strong, 1996; Bilde and Toft, 1998; Loison and Langvatn, 1998; Bronikowski and Arnold, 1999; Mduma et al., 1999; Bourgarel et al., 2002; Gaedke et al., 2002; Miller et al., 2002b; Olsen et al., 2002; Güsewell, 2004; Hood et al., 2005; Thingstad et al., 2005; Elser et al., 2007; Hoekman, 2007; LeBauer and Treseder, 2008; Molleman et al., 2008). Nitrogen is generally considered to be the "proximate limiting nutrient" in marine systems, representing local limitation while phosphate supplied by continental weathering and fluvial discharge is viewed as the "ultimate limiting nutrient" influencing system productivity on longtime scales (Tyrell 1999; Hessen et al., 2004) (see chapter 9.4 for a further discussion of this issue).

Any component of the environment of a plant, by varying the amount of adequately nutritious plant tissue available to herbivores, may affect the abundance of food through all subsequent trophic levels (White, 1978). Bottom-up proponents have argued that populations are limited primarily by nutrients and that this resource shortage is the major force structuring communities (White, 1978; Polis and Strong, 1996), whereas top-down proponents have argued that predators are the main determinants of food web dynamics (Hairston et al., 1960; Hairston, 1993). It is broadly recognized that top-down and bottom-up effects are not mutually exclusive, but that these forces in combination determine food web structure and dynamics (Power, 1992; Denno et al., 2003; Nyström et al., 2003; Hoekman, 2007). The concept of density-dependent natural selection was proposed in the original r/K-selection model (MacArthur and Wilson, 1967, Pianka 1970; Boyce, 1984). The terms refer to the suggestion that r-selection will increase r (the instantaneous growth rate), whereas K-selection will increase K (the equilibrium size, usually called the carrying capacity) (Armstrong and Gilpin 1977). This classification has been of enormous heuristic value in ecology, although it is of only limited value in describing nature (Begon and Mortimer 1986; Rogers, 1992). (Dawkins [1982] observed that “ecologists enjoy a curious love/hate relationship with the r/K concept, often pretending to disapprove of it while finding it indispensable”). A resource-rich, noncompetitive, r-environment selects for traits that enhance population growth rate, including early maturity, small body size, high reproductive effort, and high fecundity. Conversely, resource-limited, competitive, K-environments select for traits that enhance persistence of individuals, including delayed maturity, large body size, high investment in individual maintenance at the cost of low reproductive effort, low fecundity with a large investment in each offspring, and longer lifespan. These alternative constellations of life-history traits became known as life-history strategies (Pianka 1970; 1974; Reznick et al., 2002a). Later life history theories and ecological data (Kawasaki, 1980; Sibly et al., 2005; Winemiller, 2005; Brook and Bradshaw, 2006) confirmed the selective force of chronic resource limitation. Accordingly, a variety of density-related responses are linked to the underlying limited resources paradigm (Stearns, 1977; 1992; Clutton-Brock and Harvey, 1978, Boyce, 1984; Roff, 1992; Mylius and Diekmann, 1995; Brook and Bradshaw, 2006; Johnson, 2007; Bonenfant et al., 2009). In fact, models show that many mammals, birds, fishes and insects are found living at densities at the carrying capacity of their environments (Sibly et al., 2005; Brook and Bradshaw, 2006). Thus, in most animal species a population's growth rate is a decreasing function of density (Sibly et al., 2005) which explains the relative stability of animal populations that do not increase at rates their fertility would allow. Populations of vertebrate and invertebrate species are in general regulated by the production of adult individuals being a decreasing function of population density (Klomp, 1964; Tanner, 1966; Harrison, 1995; Myers et al., 1995; Kuang et al., 2003; Sibly et al., 2005; Bassar et al., 2010). Moreover, senescence and mortality are dependent on density (Graves and Mueller, 1993; Carey et al., 1995; Coulson et al., 2001; Seymour and Doncaster, 2007; Reznick et al., 2012).

5.3 Competion for food and space in plants and sessile animals

Self-thinning refers to a pattern of density-dependent growth and survival commonly observed in plants (Westoby 1984; Sackville Hamilton et al., 1995; Bégin et al., 2001) and animals (Begon et al., 1986; Fréchette and Lefaivre, 1995; Latto 1994). As individuals in a cohort grow over time, increasing per capita resource demands lead to a shortage of resources. This shortage leads to mortality and/or emigration, and population density declines accordingly. Since the original studies (Tadaki and Shidei, 1959; Yoda et al., 1963), the rule has been studied many times in both forestry and agriculture and has been observed to apply for many even-aged and age-structured plant populations ranging from mosses and herbaceous plants to large trees (White and Harper 1970; Bazzaz and Harper 1976; Westoby 1977; 1984; Mithen et al., 1984; Weller 1985; 1987; 1990; White 1985; Lonsdale 1990; Oliver and Larson, 1990; Eid and Tuhus, 2001; Yang et al., 2003; Monserud et al., 2004; Temesgen and Mitchell, 2005; Bravo-Oviedo et al., 2006; Getzin et al., 2006; Deng et al., 2008) and growing within different conditions as expressed by different ecological regions (Bégin et al., 2001). Resource allocation is the prime driver of self-thinning trajectories (Morris, 1999, 2002; 2003; Morris and Myerscough, 1991). In plants and sessile animals, self-thinning relationships represent an upper limit above which combinations of population density and biomass cannot occur because of intraspecific and interspecific competition for resources (Yoda et al., 1963; Franklin et al., 1987; Norberg, 1988, Fréchette and Lefaivre, 1990; Ayre and Grosberg, 1995). Both above- and below-ground competition has to be taken into account (Becker et al., 1988; Sanford, 1989; 1990; Gerhardt, 1996; Ostertag, 1998; Coomes

and Grubb, 2000; Lewis and Tanner, 2000; Barberis and Tanner, 2005; Schnitzer et al., 2005; Bartelheimer et al., 2010). In suspension-feeding benthic organisms flow-mediated resource competition is pervasive (Kim and Lasker, 1997). Both competition for space and seasonally variable food availability elicit stress responses in benthic marine animals (Coma et al. 2000; Rossi and Snyder, 2001; Coma and Ribes 2003; Rossi et al., 2006a; b). Thus, self-thinning and biomass-density relationships in benthic animals follow the same rules as in plant populations (Hughes and Griffiths, 1988; Norberg, 1988; Fréchette and Lefaivre, 1990; 1995; Fréchette et al., 1992; Guiñez and Castilla, 2001; Linares et al., 2008; O'Gorman and Emmerson, 2011). In addition, interspecific competition substantially reduces plant fecundity and seed recruitment (Miriti et al., 2001; Silvertown et al., 2002; Fréville and Silvertown, 2005).

For mobile animals, “self-thinning” is generally thought to occur through food-regulated growth and mortality (Begon et al. 1986; Bohlin et al., 1994), but competition for space may also lead to self-thinning (Grant et al., 1998). If resource availability is constant over time, this leads to an inverse linear relationship (thinning line) between population density and mean body size on a logarithmic scale. Generally, organisms with indeterminate growth, little parental care and a sessile (or passively dispersed) life history (e.g. plants, benthic animals) appear to exhibit regular patterns in the local size–density relationship (Marquet et al., 1990; Enquist et al., 1998; 2009; Schmid et al., 2000; Kerr and Dickie, 2001; Cyr, 2000; White et al., 2007; Deng et al., 2008; Price et al., 2010), whereas organisms with determinate growth, significant parental care and highly directed mobility tend to exhibit less regular local-scale patterns (Damuth, 1981; LaBarbera, 1989; Silva and Downing, 1995; Gaston and Blackburn, 2000; Ackerman et al., 2004; Ernest, 2005; Reuman et al., 2008; Yvon-Durocher et al., 2011). There are many reported cases where relationships between population density and body size show patterns consistent with food-regulated self-thinning (Begon et al., 1986; Elliot, 1993; Bohlin et al., 1994; Latto, 1994; Armstrong, 1997; Dunham and Vinyard, 1997; Dunham et al., 2000). Food-regulated self thinning and biomass/body size-density scaling are footprints of intra- and interspecific competition for limited resources in ecological communities. Intriguingly, while competition prevails in more benign habitats, when abiotic stress is high interactions among plants appear to become more positive (Callaway et al., 2002; Callaway, 2007; Brooker et al., 2008; Maestre et al., 2009).

Terrestrial and aquatic plants and sessile aquatic animals frequently compete for space and food, adopting mechanisms to minimize fouling or overgrowth by epibionts and maximize their own space-capture abilities. Exploitative competitive ability in any organism consists of two distinct aspects: competitive effect, which is the ability of an organism to reduce the performance of other organisms, and competitive response or tolerance, which is the ability of an organism to continue to perform relatively well in the presence of competitors (Lang and Chornesky, 1990; Silvertown and Dale, 1991; Aarssen and Epp, 1990; Goldberg and Barton, 1992; Gurevitch et al., 1992; Bengtsson et al., 1994; Silvertown and Charlesworth, 2001; Chadwick and Morrow, 2011). Several biological mechanisms that mediate ecologically significant interactions among reef organisms have been described (Rützler 1970; Glynn 1976; Vicente 1978, 1990; Wellington and Trench 1985; Paul, 1992; Chadwick and Morrow, 2011). For example, sponges overgrow and suffocate each other and corals (Rützler, 1970; Arnold and Steneck, 2011), anemones use acrorhagi and catch tentacles to sting, incapacitate or kill competitors (Williams, 1991; Ayre and Grosberg, 2005), scleractinian corals effect extracoelenteric damage to neighbors via extended mesenterial filaments and long sweeper tentacles (Francis, 1973; Lang, 1973; Richardson et al., 1979; Wellington, 1980; Sheppard, 1982; Williams, 1991; Chadwick and Morrow, 2011). Likewise, hydrocorals and octocorals can move onto and spread across scleractinians, and thereby compete successfully for space with reef-building corals (Wahle, 1980; La Barre and Coll, 1982; Tursch and Tursch, 1982) and also use sweeper tentacles for agonistic behavior (Sebens and Miles, 1988). Bryozoans employ sweeper appendages which are effective in competition and in prevention of fouling (Jackson, 1977). Corals and sponges commonly compete for space on many reefs, with up to 12 interactions per square meter. Sponges, gorgonians and corals were found overgrowing aggressively each other depending on water depth (Suchanek et al., 1983), species identity and cover (Aerts, 1998). Studies have indicated that sponges are the superior competitor in 80% of these encounters (Bryan, 1973; Vicente, 1978; 1990; Suchanek et al., 1983; Plucer-Rosario, 1987; Hill, 1998; Arnold and Steneck, 2011).

Chemical effectors mediating interspecific aggression and defense have also been identified. These allelopathic mechanisms have demonstrable effects on species distributions and individual survivorship in terrestrial plant communities (Fraenkel, 1969; Whittaker and Feeny, 1971; Fay and Duke, 1977; Rosenthal and Janzen, 1979; Meinwald, 1982; Targett

and Isman, 1986; Barnes and Putnam, 1987; Callaway and Aschehoug, 2000; Bais et al., 2003; 2006; Baldwin, 2003; Bertin et al., 2003; Inderjit and Duke, 2003; Callaway et al., 2005; Lambers et al., 2008; Thorpe et al., 2009; 2011; Barney et al., 2009; Kegge and Pierik, 2010), and aquatic benthic communities (Jackson and Buss, 1975; Sheppard, 1979; Barbier, 1981; Bak et al., 1982; Fenical, 1982; Norris and Fenical, 1982; Palumbi and Jackson, 1982; Coll and Sammarco, 1983; Colwell, 1983; Dyrynda, 1983; Faulkner and Ghiselin, 1983; Sullivan et al., 1983; Webb and Coll, 1983; Steinberg, 1984; Scheuer, 1985; Bakus et al., 1986; La Barre et al., 1986a; b; Porter and Targett, 1988; Thacker et al., 1998; Nishiyama and Bakus, 1999; Paul and Puglisi, 2004; Chadwick and Morrow, 2011).

Unusual secondary metabolites that often proved to be toxic in bioassay examinations have been isolated from numerous sessile solitary and colonial coral reef organisms (Tursch et al., 1978; Bakus and Thun, 1979; Bakus, 1981; Coll et al.,1982a; b; Cimino et al., 1983; Sullivan et al., 1983; Targett et al., 1983; Coval et al., 1984; Gerhart, 1984; Bandurraga and Fenical, 1985; Kashman et al.,1985; Coll et al., 1985; McCaffrey and Endean, 1985; Thompson, 1985; Thompson et al., 1985; LaBarre et al., 1986a, b). In addition to their role in organism defense and aggression, secondary metabolites are also implicated in the maintenance of living space (Jackson and Buss, 1975; Green, 1977; Jackson,1977; Sheppard,1979; 1982; Sammarco et al., 1983). Sponges are remarkable because they lack specialized organs and behaviors, and yet are successful in an environment where such adaptations are common. However, sponges do contain a variety of bioactive secondary metabolites. A multitude of compounds with lethal or growth inhibitory properties have been described from tropical sponges (Russell and Saunders, 1967; Martin and Padilla, 1973; Baker and Murphy, 1976; Faulkner, 1977; Cimino, 1977; Minale, 1978; Kaul and Sinderman, 1978; Hashimoto, 1979; Carmely et al., 1983; Braekman et al., 1985; Manes et al., 1985; McCaffrey and Endean, 1985; Mayol et al., 1986; Engel and Pawlik, 2000).

5.4 The Tragedy of the Commons

Public goods allow groups composed largely of cooperators to outperform groups composed mainly of non-cooperators. However, public goods also provide an incentive for individuals to be selfish by benefiting from the public good without contributing to it. This is the essential paradox of cooperation—known variously as the “tragedy of the commons”, “multi-person prisoner’s dilemma” or “social dilemma” (Killingback et al., 2006). The tragedy of the commons (a situation where individual competition reduces the resource over which individuals compete, resulting in lower overall fitness for all members of a group or population) provides a useful analogy allowing to understand why shared resources tend to become overexploited (Hardin, 1968). The analogy, which dates back over a century prior to Hardin’s original paper (Lloyd, 1833), describes the consequences of individuals selfishly overexploiting a common resource. The tragedy of the commons was originally applied to a group of herders grazing cattle on a common land. Each herder only gains a benefit from his own flock, but when a herder adds more cattle to the land to graze everyone shares the cost, which comes from reducing the amount of forage per cattle. If the herders are driven only by economic self-interest, they will each realize that it is to their advantage to always add another animal to the common: they sacrifice the good of the group (by forgoing sustainable use of the resource) for their own selfish gain. Thus, herders will continue to add animals, eventually leading to a “tragedy” where the pasture is destroyed by overgrazing (Hardin, 1968; Rankin et al., 2007a). The difficulties inherent in protecting shared common resources, such as marine stocks or clean air, are well known: while everyone benefits from an intact resource, there is an individual-level temptation to cheat (e.g. to overexploit or pollute) because cheating brings economic advantages to the individual while costs are distributed among all individuals (Rankin et al., 2007a). In humans, solving the dilemma often requires negotiation and sanctions on disobedient individuals. This changes the payoffs, so that group-beneficial behaviour also becomes optimal for the individual. If the tragedy can only be avoided when higher-level incentives are invoked, as in the case of legal incentives, this raises the question of how non-human organisms can avoid overexploiting the resources they depend on (Rankin et al., 2007a). During the group selection debate of the 1960s (Williams, 1966) it became clear that this question is not trivial: natural selection acts primarily at the level of the gene or individual, and therefore favors individuals which serve their own selfish interests (Williams, 1966; Dawkins, 1976). A tragedy of the commons in evolutionary biology refers to a situation where individual competition over a resource reduces the resource itself, which can in turn reduce the fitness of the whole group (Leigh, 1977). The tragedies can apply to a range of levels: individuals, groups, populations or species. Thus, even individual organisms may suffer from selfish exploitation of their commons e.g. by root competition in plants (Gersani et al., 2001) or selfish cancerogenesis (Hardin, 1974). The concept has been used in a diversity of fields in

biology, ranging from plant-competition for resources (Gersani et al. 2001) to the evolution of cooperation and conflict in insect societies (Wenseleers and Ratnieks, 2004). What the tragedies have in common is that individuals are selfishly maximizing their own fitness at the expense of the productivity of the group or population. The tragedy of the commons analogy has become increasingly used to explain why selfish individuals in animal and plant populations do not evolve to destroy the limited collective resources (Frank, 1995; Gersani et al. 2001; Falster and Westoby, 2003; Foster, 2004; Wenseleers and Ratnieks, 2004; Rankin and López-Sepulcre, 2005; Kerr et al. 2006; Rankin and Kokko, 2006). Factors such as high relatedness in social groups (Wenseleers and Ratnieks, 2004), diminishing returns (Foster, 2004), policing and repression of competition (Frank, 1995; 1996; Hartmann et al., 2003; Ratnieks and Wenseleers, 2005), pleiotropy (Foster et al., 2004) or control of population density (Suzuki and Akiyama, 2005; Hauert et al., 2006; Kokko and Rankin, 2006; Rankin, 2007; Frank, 2010) may all act to constrain the evolution of harmful traits, and thus reduce the potential for a tragedy of the commons to arise in such populations. At low population densities, one should expect lower encounter rates and more per capita resources, which may result in less intense conflicts. Therefore a feedback between density and conflict can be expected, which may act to resolve a tragedy that might have occurred in the absence of such a feedback (Kokko and Rankin, 2006; Rankin, 2007). On the other hand, cheaters, mutants who contribute less or nil to the effort but fully enjoy the benefits, may impair bacterial, amoebae or plant population productivity and fitness and potentially drive populations extinct (Hilson et al., 1994; Pál and Papp, 2000; Ennis et al., 2003; Fiegna and Velicer, 2003; Rainey and Rainey, 2003; Castillo et al., 2005; Rankin et al., 2007a; Kuzdzal-Fick et al., 2011). This may result in selection at the species level (Rankin and López-Sepulcre, 2005; Okasha, 2006; Rankin et al., 2007b).

The “prudent predator” concept (Slobodkin, 1961; 1974; Goodnight et al., 2008) has revealed evolutionary outcomes of predator-prey interactions and provided evolutionary mechanisms to resolve the tragedy of the commons dilemma. Original studies by Wynne-Edwards (1962), suggesting that predators executed restraint in reproduction in order to avoid overexploitation of resources were later dismissed as inadequate since any such restraint appears to require group selection (Maynard-Smith, 1964; Williams, 1966). However, there is theoretical justification for a self-consistent limitation of reproduction by predators (Mitteldorf et al., 2002). The rapidly reproducing types modify their local environment, depleting resources in a way that is detrimental to their survival, but this environmental modification and its feedback to population growth may require many generations (Mitteldorf et al., 2002; Goodnight et al., 2008). When a predator evolves, the evolutionarily stable type is outcompeted in the short term by seemingly fitter mutants, which have the highest numbers of offspring for many generations but go extinct in the long term (e.g., after 200 generations) (Rauch et al., 2002; 2003). The benefit of restraint is that better resource management may prolong the persistence of the group. One way to dodge the problem of defection is for altruists to interact disproportionately with other altruists. With limited dispersal, restrained individuals persist because of interaction with like types, whereas it is the unrestrained individuals that must face the negative long-term consequences of their rapacity (Nahum et al., 2011). Theoretical studies have confirmed the role of spatial structure in promoting restraint in victim–exploiter interactions (Mitteldorf et al., 2002; Killingback et al., 2006). Intriguingly, like the prudent predator concept, introduction of an aging factor into the predator-prey dynamics attenuates the oscillations predicted by the Lotka-Volterra equation and stabilizes populations (Holland, 1995; Mitteldorf, 2006; 2010b).

Strangely enough, although the original essay of Hardin (1968) dealt with the threat of human overpopulation, the tragedy of commons argument has not been used in gerontology to justify any evolutionary mechanism to limit population growth to preserve limited resources for the generations to come. Possibly, this deficit may have been caused by self-censoring, dreading the label “group-selective”; but on the other hand, any argument related to Tragedy of the Commons, Multi-person Prisoner’s Dilemma or Social Dilemma concepts that proved to be highly successful in ecology and social sciences could be denounced as group-selective.

Mankind managed to exploit an increasing amount of resources thereby repeatedly extending its habitat carrying capacity. Archeological evidence (Klein 1989) shows that new kinds of stone tools appeared and spread out from an African origin some 40,000 years ago, and genetic evidence (Rogers and Harpending 1992) suggests that a dramatic burst of population growth occurred at roughly the same time. Archeological data suggest that another burst of population growth followed the origin of agriculture some 6000 years ago. More recently, the industrial revolution allowed another spurt of population growth that still continues (Rogers, 1992). Only through

access to these additional resources mankind escaped “Malthusian catastrophe” so far. Mankind is now the greatest threat to biodiversity (Wilson, 1992; May, 2010) claiming an unprecedented share of the resources on this planet (Meadows et al., 1972; Turner, 2008). Is it a surprise that this species is sort of negligent in acknowledging the finiteness of resources? But even the species, coming closer to the theoretical Darwinian demon than any other species before (Silvertown, 2005), is about to experience the economic burden and intergenerational conflicts in an ever-graying world (Altman, 2002; Rice and Fineman, 2004; Stanton and Rutherford, 2006; OECD, 2007; Fishman, 2010), witnessing the pervasive nature of the limited resources paradigm.

5.5 Limited resources and mortality

In a world of limited resources, immortality of an organism would curb its reproductive success since the immortal ancestors would compete with the offspring for the available resources. Therefore, elimination of the ancestors and their competition for resources should confer an evolutionary advantage to the genes of the progeny and consequently also to the genes of the ancestors.

I would like to emphasize that the limited resources paradigm is a transgenerational economic concept (allocating the available resources to the overall headcount of competing individuals) that should not be mistaken as a group-selection argument. It is the individual organism that experiences the shortage, if e.g. a certain amount of prey (or plants for herbivores) has to be shared between several generations of predators instead of the progeny alone. For instance, resource overlap between competing species has its fitness costs at the individual level (Minot 1981; Tilman 1982; Gustafsson 1987; Sasvári et al. 1987; Wedin and Tilman, 1993; Löfgren, 1995; Martin, 1996; Grover, 1997; Newton, 1998; Martin and Martin, 2001; Eccard and Ylönen, 2002; 2007; Koivisto et al., 2007). When individuals experience resource depletion it matters little whether the consumption responsible was carried out by members of the same or of other species (Grover, 1997). Field experiments have shown that both growth and survival rates are depressed by competition, particularly when competition occurs between very similar phenotypes (Schluter, 1994; 1995; 2003; Hatfield and Schluter, 1999; Rundle et al., 2000; Vamosi et al., 2000; Pritchard and Schluter, 2001). Intraspecific competition for resources (Klomp, 1964; Tanner, 1966; Harrison, 1995; Myers et al., 1995; Kuang et al., 2003) certainly would be intensified between immortal ancestors and their offspring. Resource acquisition, metabolic efficiency and economic utilization of resources (at least in resource-limited environments) have rate/yield trade-offs, are fitness traits under directional selection and a source of social conflict (Fitter, 1986; Boggs, 1992; Rowe and Houle, 1996; Stelling et al., 2002; Hunt et al., 2004; Kreft, 2004; Kreft and Bonhoefer, 2005; MacLean, 2007; Frank, 2010). For instance, if two amino acids at a given position on the protein can do the same job, then selection might favour the retention of the one for which synthesis requires less energy. The amino-acid compositions in the proteomes of Escherichia coli and Bacillus subtilis reflect the action of such selection pressure (Akashi and Gojobori, 2002). According to MacArthur and Wilson (1967, p. 149): “Evolution … favours efficiency of conversion of food into offspring”. How resources affect individual survival and reproductive success can be described by the fitness function, w(x), whose value is the expected number of offspring born to individuals with x units of resource (Rogers, 1992). In a similar ecological context, an energetic definition of fitness was put forward. According to the formulation of Brown et al. (1993; 2004), reproductive power is composed of two component processes: acquisition (acquiring resources and storing them in reproductive biomass) and conversion (converting reproductive biomass into offspring) (Loreau, 1998; Allen et al., 2006). Together with the density-body mass relationship (see chapters 5.1 and 5.2) these scaling relationships set the ecological framework for resource-limited fitness parameters.

Competition for resources is a source of conflict (MacLean and Gudelj, 2006) and strong coevolutionary force (Lawlor and Maynard Smith 1976; Stephens and Krebs, 1986; Grover, 1997; Svanbäck and Bolnick, 2005; Araújo et al., 2011) that can have a variety of outcomes including co-existence, specialization, character displacement, niche shifts, speciation, and extinction (MacArthur and Levins, 1964; Tilman, 1982; Helling et al., 1987; Schluter, 1994; 2000; 2001; Grover, 1997; Rainey and Travisano, 1998; Dieckmann and Doebeli, 1999; Bolnick, 2004; 2011; Pfennig and Pfennig, 2005; Hall and Colegrave, 2007; Svanbäck and Bolnick, 2007).

Like the limited resources paradigm, the fitness concept is a transgenerational concept and, to my knowledge, no one has ever denounced the latter as flawed and advocating a group selection theory: Fitness describes the ability of a phenotype to both survive and reproduce, and is dependent on the average contribution to the gene pool of the next generation that is made by an average individual of the specified genotype or phenotype. Moreover, defining fitness in terms of immediate offspring is not

mandatory (Okasha, 2006). According to Fisher (1930), as noted in his treatment of sex ratio evolution, some evolutionary phenomena require the consideration of offspring counts over more than one generation. Thus, there are short-term and a long-term aspects to fitness (Beatty and Finsen, 1989; Sober, 2001). This distinction is not trivial. In fact, short-term reproductive success may threaten the evolutionary success of a geno-/phenotype, by placing too great a demand on available resources (Beatty and Finsen, 1989). Long-term concepts of fitness have been forwarded by Thoday (1953; 1958) and Cooper (1984). Thoday suggested that fitness should be defined as the probability of leaving descendants in the very long run; he proposed 108 years as an appropriate time scale. Cooper argued that the "expected time to extinction" of a particular genotypic or phenotypic subpopulation may be the adequate measure of fitness. There is an ongoing controversy over the time scale of fitness concepts which I will not touch here. However, failure to realize the intergenerational, long-term dimensions of the

fitness concept may have led to the misgivings that aging and death are maladaptive.

In the preceding chapters we have seen that limited resources may qualify as the pervasive selective force underlying aging/death. Thus we have a cause (resource limitation) in search of one, possibly several, mechanisms to an end (aging/death). In fact, for plants and sessile aquatic animals we have already identified a mechanism (but, as we shall later see, not the only one): intraspecific self-thinning and both intraspecific and interspecific physical and chemical (allelochemical) warfare, driven by density-dependent competition for resources. Besides growth, reproduction is the driving force behind the pervasive resource limitation for plants. Reproduction could also be the driving force for death in mobile animals. Wouldn't it make evolutionary sense to establish the constraints (preventing the Darwinian demon and the tragedy of the commons) in the very process that causes the scarcity of resources? Due to their mobile lifestyle, however, the strictly territorial death mechanisms of self-thinning and allelopathy are no evolutionary option in animals.

In the DST (Kirkwood, 1977), the limited resources paradigm already plays a critical role in that the organisms have to allocate their limited resources to both reproduction and tissue maintenance. However, it was not taken into account that the limited resources paradigm is not only intraindividual but also transgenerational, that there are conflicting interests between soma and germline cells and that the latter, residing within the soma, may be endowed to shape the conflict in their sense. Therefore, the intraindividual partition of resources between reproductive and non-reproductive tasks may pay off to be extended transgenerationally to reproductive and non-reproductive (aged) individuals. The question arises, how this conflict may be mediated. Since negotiation over the utilization of resources is no option for non-primate organisms, altruism is no evolutionary stable strategy (ESS) in non-kin interactions, thus policing and violent enforcement of interests appears to be a viable and particularly cheater-resistant ESS. That colonial cells forcefully exploit their siblings to their own advantage is common practice throughout phylogenesis (Heininger, 2001; González-Pastor et al., 2003; Rozen et al., 2009). For instance, cannibalism as phenotype of intraspecific conflict and regulation of population density has been recorded for thousands of species (Fox, 1975; Polis, 1981; Elgar and Crespi, 1992; Wise, 2006; Alabi et al., 2009).

6. Evolution linked reproduction and aging/death

Knowledge is the object of our inquiry, and men do not think they know a thing till they have grasped the 'why' of it (which is to grasp its primary cause).
Aristotle

Summary

Each organism has evolved its own solution to the challenge of reproduction in a world of limited resources but there appear to be common underlying principles from which generalizations can be inferred. Reproduction by assymetric division evolved as response to a conflict of cells over resource allocation. The phylogenetically most ancient reproductive events involve unicellular organisms that socialize under metabolic stress following the exhaustion of their resources. Both social bacteria like Streptomyces and Myxococcus and eukaryotes like Dictyostelium aggregate under nutrient deprivation in a facultatively multicellular behavior to differentiate into reproductive structures (e.g. fruiting bodies or aerial mycelia) which serve to nurse and disseminate highly resistant germ cell-like spores. The death of stalk cells forming the fruiting body fuels the metamorphosis of vegetative cells to highly resistant spores/germ cells. At the transition to multicellularity, these stress responses to environmental cues (e.g. nutrient deprivation, stress) were co-opted into a developmental context in which a variety of

morphogens orchestrates the ontogenetic events. The linked developmental differentiation/apoptosis pathways and their stress- and antagonistic pleiotropy- phenotype are the legacy of the cellular survival/death decisions in primordial stress responses. Aging/death is a universal companion of asymmetric reproduction. Aging/death as a "collateral damage" of reproductive events has evolved in several unicellular organisms and at transitions to multicellularity which argues for a strong, ubiquitous, selective motif for its evolution.

In the major theories about the evolutionary causes of aging and death, reproduction plays a more or less central role. Wallace and Weismann regarded reproduction as main culprit that doomed the postreproductive individual as a dispensable competitor for limited resources. The ETAs consider reproduction as both "innocent" bystander that takes the selection pressure from the postreproductive individuals and as diverter of internal resources that are then missing for tissue maintenance. At any rate, a link between reproduction and aging/death has been implied by the major theories of aging. Life history theory very early linked reproduction and survival in a trade-off (MacArthur and Wilson, 1967; Stearns, 1977): reproductive effort compromises the survival of the parent organism (Roff, 1992, Stearns, 1992, Charlesworth, 1994; see chapter 8).

Evolutionary theory treats reproduction as falling into two categories: sexual and asexual. Sexuality is generally considered to be equivalent to meiosis or prokaryotic conjugation, and asexuality, to represent binary fission, mitosis, or ramet production (i.e., whole organism cloning through budding, spore formation, microcysts, gemmules, statoblasts, rhizomatrv, etc.). These are all treated as replicatory events that occur at the level of the individual (Buss, 1983). After more than a century of debate, the major factors of the evolution of reproduction are still obscure (Kondrashov, 1993). Reproduction is the biological process by which a "parent" organism produces an individual "offspring" organism. In many taxa, reproduction proceeds through an unicellular bottleneck. Spores, seeds, eggs and sperm are the germ cells, the unicellular carriers of the genetic information to the next generation.

Could there be a causal nexus between reproduction and aging/death? I argue that it is our ignorance of the evolutionary roots and rationale of asymmetric reproduction (reproduction with a germline/soma segregation) that underlies the vagueness of our concept concerning aging and death.

The ETAs, in the first place looked at the phenomenon and made unfounded assumptions (predictions) on its evolutionary role. That some of these predictions were later found to be correct (most were found to be incorrect) does not vindicate the theories but may be owed to the spuriousness of correlations (i.e. a third, unknown cause underlying the correlation). I have a fundamentally different approach: to go back in evolution and explore the conditions under which death occurs in ancient organisms and thereby learn more about the basic conditions that led to the manifestation of the phenomenon and its evolutionary rationale. Please note that I make no premature assumptions, taking the reductionist stance to answer holistic questions.

Let me be your guide on this paleobiological journey to the roots of the life/death dichotomy.

6.1 The phylogenetic record of reproduction and death: from bacteria to bilateria

6.1.1 Gram-positive Bacteria

Clostridia apparently appeared as a separate class ~2.7 billion years ago, before the initial rise in oxygen (the 'great oxidation' event) (Battistuzzi et al., 2004), whereas bacilli (which contain the genus Bacillus) diverged as a class ~2.3 billion years ago, around the time of the great oxidation event. The different environmental conditions at the time of divergence might explain the differences between the bacilli and the clostridia in the upper part of their differentiation (sporulation) programmes (Paredes et al., 2005). Studies of sporulating cultures of bacilli and clostridia have revealed the very similar sequence of morphological changes that lead to the creation, maturation and eventual release of spores in both genera. However, much less investigation of the sporulation process in the obligately anaerobic clostridia has been undertaken due to their oxygen sensitivity.

Bacillus subtilis

The ability to differentiate into many distinct cell types is a hallmark of the soil-dwelling, grampositive bacterium Bacillus subtilis. B. subtilis divides symmetrically under abundant resources. In nutrient limiting conditions a subpopulation of cells differentiates into spores (Errington, 2003, Eichenberger et al., 2004; Piggot and Hilbert, 2004). Spore development is energy intensive and, once committed, cells may not exit this state for prolonged periods. Thus, this bacterium has evolved mechanisms to delay entry into sporulation as long as possible. It accomplishes this by having the capacity to direct a subpopulation of cells down a differentiation pathway that gives rise to so-called cannibals (Gonzalez-Pastor et al., 2003; Ellermeier et al., 2006; Claverys and Havarstein, 2007). Cannibal cells are

resistant to two toxins that they secrete to kill a fraction of their siblings. Cells that have entered the pathway to sporulate produce and export a killing factor and a signaling protein that act cooperatively to block sister cells from sporulating and to cause them to lyse. The sporulating cells feed on the nutrients thereby released which allows them to keep growing rather than to complete morphogenesis (González-Pastor et al., 2003). Once initiated under ongoing metabolic stress, however, the process of sporulation is kept under stringent control by an expanded two-component signal transduction system called a phosphorelay (Stephenson and Lewis, 2005). In sporulation, the cell division septum forms not at mid-cell as during vegetative growth but instead at a polar location dividing the cell into two compartments of unequal size: a larger mother cell and a smaller forespore (Meisner et al., 2008). The two cells follow different patterns of gene expression orchestrated by cell type- and stage-specific RNA polymerase sigma factors. The mother cell goes on to engulf the forespore and nurture the latter as it matures into a resistant spore that is released upon mother cell death due to lysis. Propagation of B. subtilis for less than 2,000 generations in a nutrient-rich environment where sporulation is suppressed led to rapid initiation of genomic erosion including biosynthetic pathways, sporulation, competence, and DNA repair (Brown et al., 2011).

In addition to spores and cannibals, B. subtilis, in a bet-hedging strategy (Veening et al., 2008a), can undergo several other developmental processes. For instance, within multicellular aggregates known as biofilms a subpopulation of cells differentiates to produce an extracellular matrix that encases the community (Branda et al., 2006, Chai et al., 2008, Chu et al., 2006, Vlamakis et al., 2008). The subpopulation of cells that differentiates into cannibals is the one that produces the extracellular matrix. Nutrients released by the cannibalized cells are preferentially used by matrix-producing cells, as they are the only cells expressing resistance to the toxins. As a result this subpopulation increases in number and matrix production is enhanced when cannibalism toxins are produced. The cannibal/matrix producing subpopulation is also generated in response to antimicrobials produced by other microorganisms and may thus constitute a defense mechanism to protect B. subtilis from the action of antibiotics in natural settings (Lopez et al., 2009; Liu et al., 2010). Cannibalism functions as a mechanism of programmed cell death (PCD) to modulate diverse developmental processes in bacteria (Engelberg-Kulka et al., 2006). Recently, B. subtilis sporulation was shown to involve preferential development of spores at the tips of aerial structures. The B. subtilis fruiting-body-like structures that serve as preferential sites for sporulation were recognized in the context of biofilms formed by wild isolates of this bacterium and appear to be a developmental feature that has been lost in many laboratory strains (Branda et al., 2001). Cannibalism in B. subtilis delays not only unicellular endospore formation but also the formation of the aerial fruiting body-like structures where spores localize (Lopez et al., 2009). Thus, for the generation of spores that are dispersed by aerial structures multiple death events of siblings take place: for biofilm formation and buildup of fruiting bodies that consist of dead cells.

Clostridium perfringens

Clostridium perfringens is a Gram-positive, spore-forming anaerobic bacterium. It is ubiquitous in nature, including soil, insects, and the intestinal tract of animals and humans (McClane, 2001). C. perfringens undergoes sporulation under nutritionally deprived conditions. During sporulation growth it produces C. perfringens enterotoxin, an important virulence factor for food poisoning and nonfood-borne gastrointestinal diseases in humans. At the final stage of sporulation, mother cells lyse to release the enterotoxin and metabolically dormant endospores which are resistant to high heat and can survive in the environment for long periods of time (Rood, 1997; Paredes-Sabja and Sarker, 2009). The sporulation network itself remained conserved since several billion years, whereas the ability to adapt efficiently to new environments evolved (de Hoon et al., 2010).

Epulopiscium

The Gram-positive bacteria Epulopiscium spp. colonize the intestinal tracts of certain species of herbivorous and detritivorous surgeonfishes. These bacteria produce multiple active (rather than dormant) intracellular offspring. Small internal offspring are observed near the tips of the mother cell early in the morning, at the end of the fish's night fast (Flint et al., 2005). Usually two viviparous offspring are produced, although, in a newly discovered Epulopiscium morphotype, up to 12 offspring per mother cell have been observed (Angert, 2005). Eventually, the mother cell is lysed, releasing the offspring (Montgomery and Pollak, 1988; Ward et al., 2009).

6.1.2 Gram-negative Bacteria

Myxococcus

Myxobacteria are Gram-negative gliding bacteria and comprise a number of genera of the Proteobacteria (Dworkin, 1996). Myxobacterial cells are single-celled

but social; they swarm by gliding on surfaces as they feed cooperatively. Upon nutrient deprivation, populations of the bacterium M. xanthus migrate towards high-density focal points and cluster into aggregates of approx. 100,000 rod-shaped individuals (Shimkets, 1999). These aggregates form a multicellular fruiting body in which a fraction of the cells develop into myxospores. During the process of aggregation, early mound and fruiting body formation, 20 to 90% of the cells lyse, depending on the particular conditions of development. Spore development includes the differentiation from the rod-shaped vegetative cell to a spherical, nonmotile, environmentally resistant myxospore. Cannibalism is considered to provide essential nutrients for the conversion of rod-shaped cells to mature spores (Wireman and Dworkin, 1977). One pathway governs aggregation and sporulation of some cells in the starving population and requires so-called C-signaling, whereas another pathway causes programmed cell death and requires the MazF toxin (Mittal and Kroos, 2009).

Streptomyces

Streptomycetes are Gram-positive, mycelium-forming, soil bacteria that play an important role in mineralization processes in nature and are abundant producers of secondary metabolites. On nutrient depletion, aerial hyphae are erected on top of the substrate mycelium and these eventually septate into chains of unigenomic spores (Chater and Losick, 1997). Substrate hyphae undergo extensive cell death, liberating nutrients on which aerial hyphae and spores develop (Miguélez et al., 1999; Fernandez and Sanchez, 2002; Manteca et al, 2006). Antibiotic production is switched on at a time that corresponds to the early stages of development (Rigali et al., 2008). Pro-apoptotic protein domains, including caspase domains (Aravind et al. 1999), are associated with nutrient stress, antibiotic production, and spore differentiation in Streptomyces (Bibb, 2005; Rigali et al., 2008).

6.1.3 Early Eukaryotes

Volvocine algae

The spheroidal green alga Volvox and its close relatives, the volvocine algae, span the full range of organizational complexity, from unicellular and colonial genera to multicellular genera with a full germ-soma division of labor and male-female dichotomy; thus, these algae are ideal model organisms for addressing fundamental issues related to the transition to multicellularity and for discovering universal rules that characterize this transition. Although Volvox appears to have diverged from unicellular ancestors approximately 200 million years ago (Herron et al., 2009) “the Volvocinae do represent a didactic model for the evolution of multicellularity and the germline. As used by Weismann, this model still well symbolizes the emergence of the germline with meiosis reducing the doubled genetic informational content into a haploid genome. Further, it resembles the evolution of gamete dimorphism to maintain sex in males and females, and is an instructive model for the first metazoan capable of dying and producing a true carcass” (Lankenau, 2008).

Volvocine algae are flagellated photosynthetic organisms that range from unicellular (i.e. Chlamydomonas) and multicellular forms with no cell differentiation (e.g., Gonium and Eudorina; 8–32 cells) or incomplete germ-soma differentiation (Pleodorina; 64–128 cells) to multicellular forms with complete germ-soma separation (i.e. Volvox; 500–50,000 cells) (Kirk, 1998; 1999). Of all living species, Volvox carteri represents the simplest version of an immortal germline producing specialized somatic cells. This cellular specialization involved the emergence of mortality and the production of the first dead ancestors in the evolution of this lineage (Hallmann, 2011). In multicellular volvocine colonies the number of cells is determined by the number of cleavage divisions that take place during their initial formation, and cell number is not augmented by additional cell divisions (Kirk, 1997). In colonies without germ-soma separation (i.e., Gonium, Eudorina), each cell gives rise to a daughter colony. The life cycle corresponds to one of discrete generations as the parent colony dies as soon as the daughter colonies hatch. In an asexual life cycle, daughter cells of C. reinhardtii hatch from their mother cell walls within several hours after asymmetric cell division (Harris, 1989). Sporangin of the unicellular green alga Chlamydomonas reinhardtii that mediates breakdown of the sporangial cell wall to liberate the daughter cells after cell division is characterized as a subtilase-like serine protease. The sporangin gene is specifically transcribed during S/M phase in a synchronized vegetative cell cycle. In immunoblot analyses using a polyclonal antibody raised against the sporangin polypeptide, the enzyme is synthesized after mitotic cell division and accumulated in the daughter cells before hatching (Kubo et al., 2009). Immunofluorescence analyses showed that sporangin is localized to the flagella of the daughter cells within the sporangial cell wall, and released into the culture medium. The data suggest that sporangin is released from flagella concurrently with the digestion of sporangial cell wall, and then the daughter cells are hatched from the sporangia in the Chlamydomonas

vegetative cell cycle. Unicellular C. reinhardtii secretes a vegetative lytic enzyme to digest the cell walls of mother cells, allowing daughter cells to be released after mitosis (Harris, 1989; Matsuda et al.,1995). Remarkably, the hatching enzyme acts only on the mothers' cell walls and not on those of daughter cells (Matsuda et al.,1995). Of all living species the multicellular green alga, Volvox carteri, represents the simplest version of an immortal germline producing specialized somatic cells. Volvox carteri possesses only two cell types, 16 reproductive cells (gonidia) in the interior of a sphere whose surface is formed by about 2000 biflagellate somatic cells (Kirk, 1997). Intriguingly, asymmetric divisions that set aside germ and somatic cell precursors during embryogenesis in Volvox carteri is regulated by stress responsible chaperones (Cheng et al., 2005). When mature, each gonidium divides to form a juvenile with this same cellular composition. Half-way through their maturation, juveniles hatch out of the parenteral spheroid, whereupon parental somatic cells undergo programmed death while juvenile gonidia prepare for a new round of reproduction.

The unicellular alga, Acetabularia mediterranea is a giant cell that, when mature, has a cap containing thousands of spores. Maturation requires several months and virtually all the contents of the cell are donated to the progeny. The cell dies after the hatching of the spores (Hämmerling, 1963; Runft and Mandoli, 1996).

Dictyostelium discoideum

The interspecies comparison of thousands of individual protein sequences confirmed that the ancestor of the eukaryote Dictyostelium diverged from the ancestors of animals and fungi at some time after the divergence of ancestral plants (Loomis and Smith 1990; Baldauf and Doolittle, 1997; Eichinger et al., 2005). Thus Dictyostelium is more closely related to present-day animals than are plants. Although orthologous animal and yeast proteins will generally show a higher degree of sequence similarity to each other than to the Dictyostelium counterpart, Dictyostelium has many more genes held in common with animals than do the yeasts. The simple interpretation of these facts is that, at some time after the divergence of fungi from animals, there was massive gene loss during fungal evolution. What this means in practice is that Dictyostelium offers access to many protein classes that are not represented in the yeasts (Williams, 2010). At the crossroads of uni- and multicellularity, Dictyostelium is a model organism for the study of differentiation and development (Janssens and Van Haastert, 1987; Heininger, 2001; Williams, 2006; 2010; Bonner, 2009; Kawabe et al., 2009; Kessin, 2010), apoptosis/programmed cell death (Cornillon et al., 1994; Heininger, 2001; Bonner, 2009; Kessin, 2010), asexual and sexual reproduction (Godfrey and Sussman, 1982; Urushihara and Muramoto 2006; Bonner, 2009; Flowers et al., 2010; Amagai, 2011), autophagy (Otto et al., 2003; 2004), cell motility (Annesley and Fisher, 2009), mitochondrial disease (Annesley and Fisher, 2009), and innate immunity (Chen et al., 2007). In this faculty, a variety of functional equivalences between proteins of Dictyostelium and other eukaryotic species were found (Annesley and Fisher, 2009).

Following nutrient deprivation, between 104 and 106 of the social amoebae Dictyostelium discoideum aggregate into mobile slugs that are spatially heterogeneous with respect to cell fate. Upon extended starvation, slugs transform into stationary fruiting structures that consist of a round, spore-bearing sorus at the top of a long, thin stalk (Mohanty and Firtel, 1999; Williams, 2006). Differentiation to spores and apoptosis of the stalk cells are closely linked in a social stress response (Christensen et al., 1998). The stress response not only represents a primordial differentiation/apoptosis event but is also regarded as primordial reproduction event (Heininger, 2001; 2002a; Foster et al., 2004; Angert, 2005; Jack et al., 2008). In this ancient amoebozoan stress response, cyclic adenosine monophosphate (cAMP) plays a key role in controlling morphogenesis and cell differentiation. As a secreted chemoattractant cAMP coordinates cell movement during aggregation and fruiting body morphogenesis and controls gene expression at different developmental stages, while intracellular cAMP is extensively used to transduce the effect of other stimuli (Reymond et al., 1995; Schaap, 2011). Prestalk and prespore cells retain the option of transdifferentiating throughout the period of slug migration, so that if a slug is bisected into a front and a back portion, each part has the potential to form a correctly proportioned fruiting body (Raper, 1940). There has been a longstanding controversy as to how a cell makes the initial choice of becoming a spore or stalk cell. A consensus may have developed around a model in which initial cell type choice in Dictyostelium is dependent on the cell cycle phase that a cell happens to be in at the time that it starves (Jang and Gomer, 2011). Recent findings, however, suggest that the first cells to starve become reproductive spores outcompeting late starvers to become prespores first (Huang et al., 1997; Kuzdzal-Fick et al., 2010). Approx. 20% of aggregating populations die while contributing to formation of the stalk and basal disk, the relative

proportion of differentiating spore and apoptotic stalk cells being determined by nutrient availability, quorum sensing and secreted factors, so-called morphogens such as differentiation-inducing factors (DIFs), chlorinated alkylphenones (Kay and Jermyn 1983, Kay et al. 1999, Brown and Firtel 2000) and other polyketide factors such as a glycoprotein psi factor (psi, prespore-inducing factor) (Kawata et al., 2004; Serafimidis and Kay, 2005; Saito et al., 2006; Yamada et al., 2010). Although it would be virtually impossible to identify parent-offspring lineages, the life cycle of Dictyostelium alternates between unicellular bottlenecks (spores) and multicellular stages (building the dying fruiting body).

Sexual reproduction of D. discoideum (Raper, 1984) which is rarely observed in the laboratory, appears to be widespread in the wild (Flowers et al., 2010). In the sexual phase that is induced e.g. by starvation and moist conditions, two haploid cells from distinct (or sometimes similar) mating types fuse to form a diploid zygote. This large and motile cell attracts, mediated by cAMP, haploid solitary cells of either mating type and becomes intensely phagocytic, cannibalizing those cells that did not fuse. This structure is known as giant cell and it can consume hundreds of amoebae for nutrition. As it digests the victims, it constructs three walls of cellulose and becomes a macrocyst (Erdos et al., 1973; Wallace and Raper, 1979; Saga and Yanagisawa, 1982; 1983; Okada et al., 1986; Lewis and Jamieson, 1997; Shaulsky and Kessin, 2007). When cannibalism is complete, the macrocyst eventually undergoes recombination and meiosis (Flowers et al., 2010).

6.1.4 Basal Metazoa

Placozoa, Porifera, Cnidaria, and Ctenophora

Four phyla (Porifera, Ctenophora, Placozoa, Cnidaria) are typically thought to have an ancient origin within Metazoa (Collins et al., 2005). Many adult Cnidaria (e.g. Hydra), Ctenophora and sponges contain endodermally derived pluripotent stem cells (sponge archaeocytes, Cnidarian interstitial cells) that can give rise to both somatic cell types and germline cells throughout adulthood (Bosch and David, 1987; Agata et al. 2006; Müller, 2006; Extavour, 2007; Juliano and Wessel, 2010; Alié et al., 2011). The vasa-related genes encode an ATP-dependent RNA helicase from the DEAD-box family and are involved in germ cell determination and formation in metazoans (Extavour and Akam, 2003; Gustafson and Wessel, 2010). In sponge and Hydra the vasa-related genes are expressed in germline cells and in multipotent interstitial stem cells and ectodermal epithelial cells (Mochizuki et al., 2000; 2001; Rebscher et al., 2008). In these animals, as well as in plants that reproduce by agametic cloning, the adult body is itself a reproductive unit that increases its fitness as a function of genet size. As discussed in chapter 5.3 the sessile zoophyte (Hughes, 2005) are subject to territorial intra- and interspecies resource competition that determines their colonial population dynamics. Given the apparent longevity of many of these clonal organisms, species undergoing agametic cloning are often assumed to be non-aging and even potentially immortal (Nilsson Sköld and Obst, 2011). The mortality rates of several species of coral may decrease with age and colony size (Babcook 1991).

Sponges (phylum Porifera) appear to be the phylogenetically oldest, extant metazoa (Müller, 2003, Erpenbeck and Wörheide, 2007). Sponge cells can proliferate indefinitely due to their stem cell-like pluri-/totipotency (Koziol et al., 1998; Schröder et al., 2003). Almost all cells are telomerase-positive and presumably provided with an unlimited potency for cell proliferation and differentiation (Custodio et al., 1998; Koziol et al., 1998; Müller, 2006). Telomerase-positive cells can be triggered to telomerase-negative cells by dissociating them into single cells. Multicellular aggregates from dissociated single cells of the marine sponge Suberites domuncula, termed primmorphs, turn from the telomerase-negative state into the telomerase-positive state (Custodio et al., 1998). One gene, SDLAGL, has high sequence similarity to the longevity assurance genes from other metazoa. While in single cells no transcripts of SDLAGL could be identified, high expression was seen after re-aggregation of single cells and in proliferating cells of primmorphs (Schröder et al., 2000). In addition to sexual reproduction, sponges have two asexual forms of reproductioon: fragmentation and building of gemmulae. The gemmules are formed by many sponges in response to adverse physiological or ecological factors (Simpson, 1984). For example, gemmulation occurs in response to starvation and an increased bacterial load (Rasmont, 1963; Simpson, 1984; Böhm et al., 2001). Marine sponges have the machinery for apoptosis, which they execute during the formation of gemmules (Wagner et al., 1998; Wiens et al., 2003; Wiens and Müller, 2006; Adamska et al., 2011) rendering immortal cells mortal.

The life cycle of the Hydrozoa is typically characterized by the alternation of three life stages: the planula larva, the postlarval benthic polyp stage (mainly colonial), and the mobile adult stage, i.e., the medusa. Hydra polyps grow continuously due to proliferation of epithelial and interstitial stem cells throughout the body column. However, polyps do not increase in size since cells are continuously

transferred to asexual buds, which form on the lower body column, and lost at the tentacle tips and in the basal disk. Budding is dependent on feeding: well-fed polyps produce roughly one bud per day; starved polyps cease to form buds after 1–2 days. This striking dependence of budding on feeding is not due to a change in cell proliferation but rather to apoptosis (Bosch and David, 1984; David et al., 2005). The increase in cell numbers, however, is dramatically different: cell numbers increase exponentially in fed animals but do not change in starved animals. This difference is due to an increased rate of apoptosis in starving polyps. Bosch and David (1984) observed a 7-fold increase in epithelial cells containing phagocytosed apoptotic bodies in starving polyps compared to well-fed polyps. Asexual budding and fission as ontogenetic events are also associated with cellular apoptosis in Hydra and anemones (Mire and Venable, 1999; Berking, 2003; Genikhovich et al., 2006). Sessile Hydra vulgaris polyps have been described to lack senescence as measured by a lack of increasing age-specific mortality rate over at least 4 years. A declining reproductive rate, however, may suggest reproductive aging over this time period (Martinez, 1998). Polyp colonies can last for several years, annually producing large batches of sexually competent medusae, followed by periodic colony shrinkage and recurrent cycles of regenerative processes, without any obvious sign of senescence (Forrest 1963; Müller 1996). On the other hand, some Hydra species show increasing mortality with age when induced to undergo sexual differentiation (Brien, 1966; Tardent, 1974), surviving for 4 months on average with deterioration in multiple physiological functions (Yoshida et al., 2006). Both spermatogenesis and oogenesis in Hydra involve apoptosis (Honegger et al., 1989; Miller et al., 2000; Kuznetsov et al., 2001). Stress factors such as low temperature, crowding and starvation are effective in stimulating sexuality (Burnett and Diehl 1963; Bell and Wolfe, 1985). Medusae, the mobile jellyfishes, which represent the sexual form of many Hydrozoans and Scyphozoans, are known to die soon after gametogenesis but rarely may also life up to two years and spawn twice (Spangenberg, 1965; Yasuda, 1969; Hamner and Jenssen, 1974; Miyake et al., 1997; Lucas, 2001; Watanabe et al., 2009; Ojimi and Hidaka, 2010). When exposed to environmental stressors, e.g. starvation, several Cnidarian medusae retain the potential for ontogeny reversal (Bavestrello et al. 1992; Piraino et al. 1996; 2004, De Vito et al. 2006; Schmich et al., 2007) due not only to regeneration by proliferation of interstitial cells, but also to the occurrence of cell-transdifferentiation processes (Schmid 1972; 1974; 1992; Schmid et al. 1982; Schmid and Alder 1984; Alder and Schmid 1987; Seipel et al., 2004) together with the activation of both degenerative and apoptotic processes (Carlà et al. 2003; Valentini, 2006). Transdifferentiation could enable the medusae to evade death and attain potential immortality. Turritopsis medusae can go back to polyp at least three times, each time producing new medusae that go back to polyps (Boero et al., 2005). However, although medusae of several species have the ability to transdifferentiate into polyp structures before or even after initiation of processes of sex-cell determination (Boero et al. 2002; Piraino et al. 2004), this potential is curtailed after spawning (Piraino et al. 2004). Intriguingly, FoxO that is anti-aging in C. elegans (see chapter 10) is highly expressed in Hydra pluripotent, virtually immortal, interstitial cells and, like in C.elegans, Drosophila and mammals is involved in stress response regulation, indicating that basic mechanisms of FoxO and mortality control arose before the evolution of bilaterians (Bridge et al., 2010).

Planarians

Planarians are free-living members (class Turbellaria) of the phylum Platyhelminthes, the flatworms. These animals are among the simplest organisms that have three tissue layers, bilateral symmetry and tissues with distinct organs. These traits assign the Platyhelminthes an important position in the evolution of the metazoa as basal members of the bilateria (Brusca and Brusca, 1990; Barnes et al., 1993). Thus, planarians have key anatomical features (mesoderm, central nervous system and excretory system) that might have been platforms for the evolution of the complex and highly organized tissues and organs found in higher organisms. Planarian flatworms are an exception among bilaterians in that they possess a large pool of adult totipotent stem cells, the neoblasts (approximately 20–30% of the cells in the parenchyma), that enables them to promptly regenerate any part of their body and produce daughter cells that will go on to differentiate into all known cell types found in the animal, including the germline (Baguñà et al., 1989; Agata and Watanabe, 1999; Brøndsted, 1969; Newmark and Sánchez Alvarado, 2002; Shibata et al., 2010). vasa-related genes are also expressed in neoblasts and germline cells of planarians (Shibata et al., 1999; Pfister et al., 2008). Their robust regenerative ability is a constituent of asexual reproductive capacity, in which complete animals develop from tiny body fragments within a week (Shibata et al., 2010). Clonal reproduction in the freshwater planarian Schmidtea mediterranea that is an important model for regeneration and stem cell research, comprises both asymmetric binary fission

and fragmentation (generation of multiple offspring during a reproduction cycle) (Quinodoz et al., 2011). Apoptosis probably takes place to a large extent in normal intact worms and following fission as well as during their regeneration (Hwang et al., 2004). Unlike most planarians, which undergo fission and then regenerate the missing structures (architomy) (Newmark and Sánchez Alvarado, 2002), Stenostomum first differentiates the new structures and then undergoes fission (paratomy). Sonneborn (1930) showed that lines generated from successive anterior fragments ultimately senesced and were unable to propagate further, whereas lines generated from successive posterior fragments could be propagated without senescence. Given that asexual cultures of Stenostomum have been maintained for more than a thousand generations in the laboratory (Nuttycombe and Waters, 1938) it might be concluded that these posterior fragments are immortal. It seems as if the process of forming a brain de novo somehow revitalizes the posteriorly derived fission fragments, rendering them refractory to the effects of aging and senescence. In contrast, the free-living flatworm Macrostomum lignano, an obligatory sexually reproducing planarian, has a median lifespan of about 205 days (Mouton et al., 2009). Some planarians switch between asexual and sexual reproduction in response to environmental signals (Curtis, 1902, Hyman, 1939). In the sexually reproducing planarian species Dugesia lugubris (O. Schmidt), a drastic decrease in tissue neoblast density as the planarian increases in size and a shortage of stem cells for regeneration and repair has been considered the critical factor in planarian senescence (Lange, 1968; Balász and Burg, 1974). Asexual S. mediterranea display indefinite somatic telomerase activity in proliferating stem cells and maintain telomere length somatically during reproduction by fission or when regeneration is induced by amputation. In contrast, sexual animals only achieve telomere elongation by passage through a germline stage (Tan et al., 2012).

Some triclads may exert cannibalism as density-regulatory behavior when crowding is high or resources are low (Hull, 1947; Froehlich, 1955; Armstrong, 1964; Hartry et al., 1964; Davies and Reynoldson, 1969; Davison, 1973). A mortalin-like heat shock protein-related gene is essential for neoblast viability. It possibly serves to keep a p53-like protein (see chapter 10) signaling under control, thus allowing neoblasts to escape cell death programs (Conte et al., 2009). Inactivation of the planarian homolog to the mammalian tumor suppressor PTEN disrupts regeneration, and leads to abnormal outgrowths in both cut and uncut animals followed soon after by death (lysis). Further findings supported the notion that planarian PTEN homologs regulate stem cells and regeneration through target of rapamycin (see chapter 9) signaling (Oviedo et al., 2008). In C. elegans, PTEN (daf-18) has been associated with regulation of metabolic rates, longevity and dauer formation (Ogg and Ruvkun, 1998; Gil et al., 1999; Mihaylova et al., 1999). Thus planarian stem cell viability and reproduction is regulated by signaling pathways controlling metabolism, stress responses and longevity in higher metazoans.

Botryllus schlosseri

The colonial urochordate Botryllus schlosseri (Tunicata, Ascidiacea) is a ubiquitous, filter-feeding inhabitant of shallow waters and harbors worldwide. It is the modern-day descendent of the phylum that made the transition between invertebrates and vertebrates. Due to its highly conserved molecular mechanisms it is a valuable model for vertebrate allorecognition and immune tolerance (McKitrick and De Tomaso, 2010), apoptosis (Ballarin et al., 2008; Cima et al., 2010), differentiation (Degasperi et al., 2009), and soma-germline conflict (Stoner et al., 1999; Laird et al., 2005). Intriguingly, the organism`s life history traits are at the crossroads of asexual/sexual reproduction, semelarity/iteroparity and apoptosis/phenoptosis. Each colony arises from a sexually produced, chordate larva that attaches itself to a substratum and undergoes metamorphosis. The resulting oozooid immediately begins to produce buds that originate as outgrowths from its lateral wall and are genetically identical to the parent. The entire parental generation of zooids in a colony synchronously dies every week as the asexually derived generation of buds reaches functional maturity. This process, called takeover, involves massive programmed cell death of zooid organs via apoptosis followed by programmed removal of cell corpses by blood phagocytes within approximately one day (Ballarin et al., 2008; Cima et al., 2010). Developing buds in conjunction with circulating phagocytes are key effectors of zooid resorption and macromolecular recycling during takeover, and as such engineer the reconstitution of a functional asexual generation every week (Lauzon et al., 2002). Zooid lifespan during cyclic blastogenesis is regulated by two independent signals: a bud-dependent and a bud-independent signal (Lauzon et al., 2007). Blocking phagocyte ingestion of senescent cells leads to an interruption of zooid degeneration (Voskoboynik et al., 2004), suggesting that removal signals on apoptotic cells, clearance of cell corpses by phagocytes, nutrient and macromolecular recycling by the developing buds, and completion of the blastogenetic cycle are closely

linked. Bud maturation is also related to and conditioned by the regressing zooids: when, in conditions of stress, takeover occurs earlier, maturation of primary buds is anticipated as the result of the sudden availability of great quantities of nutritive material deriving from the clearance of senescent cells and tissues from the old zooids (Manni et al., 2007). In this case, colonies frequently have only two asexual generations, as the buds cannot yet yield new budlets. Conversely, extirpation of all buds except one from adult zooids significantly increases the lifespan of the adult generation and of its buds, as well as the size of the buds and their blastogenetic potential, so that colonies with up to five generations can be obtained (Manni et al., 2007); zooids can reach giant sizes when derived from a single bud left in a colony (Lauzon et al., 2002). This finding indicates that sustaining bud growth is an arduous task for zooids, which cannot support the development of their buds to adulthood, except through their death and dissolution and recycling of their tissue components (Manni et al., 2007). In addition to this weekly death process, individual colonies have finite lifespans. After a period of 5-10 cycles of asexual growth, mature ovaries and testes appear in the blastozooids and the colonies enter sexual reproduction. In a single population of colonies both semelparous (reproducing once) and iteroparous (reproducing repeatedly) sexual reproduction strategies (see chapter 7) are observed (Grosberg, 1988; Harvell and Grosberg, 1988). Semelparous colonies are characterized by a) rapid growth to first reproduction, b) early age at first reproduction, c) high reproductive effort and d) death immediately following the production of a single clutch. Iteroparous colonies a) grow at about half the rate of semelparous colonies, b) postpone sexual reproduction until they are nearly twice the age of semelparous colonies, c) invest roughly 75% less in reproductive effort than semelparous colonies, and d) produce at least three clutches before dying (Grosberg, 1988). Most zooids in each iteroparous colony produce eggs continuously throughout the period of sexual reproduction. The duration of reproduction extends for 7-70 days (1-10 cycles), with one clutch of eggs produced during each cycle. Each zooid contains up to five eggs per cycle, although most zooids produce only one to two eggs per cycle (Chadwick-Furman and Weissman, 1995). After the period of sexual reproduction, colonies pass through four stages of degeneration. First, blood vessels narrow and blood flow slows. Then, the zooids shrink and become densely pigmented. In the third stage, circular systems (groups) of zooids are disconnected and become disorganized. In the fourth and final stage, the protective tunic softens and disintegrates, and all of the tissue dies. In all cases, the initial stages of senescence lead to the death of the entire colony within 1-2 weeks (Chadwick-Furman and Weissman, 1995). Semelparous colonies numerically dominate the population through midsummer. Later in the summer, iteroparous colonies are most numerous. The reproductive strategy appears to be genetically determined and probably evolved as adaptive response to a seasonally changing environment.

A quite peculiar type of senescence and death occurs in laboratory-maintained colonies of Botryllus schlosseri. Colonies grown under field conditions attain large sizes, form compact structures, reproduce rapidly, and senesce soon after reaching maturity, whereas laboratory-maintained colonies grow slowly, often fragment into subcolonies, and may not undergo sexual reproduction (Brunetti, 1974; Brunetti and Copello, 1978; Chadwick-Furman and Weissman, 1995). Laboratory colonies (genets) and the clonal replicates (ramets) derived from these genets undergo regressive changes at the end of their lifespan that are unlinked to sexual reproductive effort (Rinkevich et al., 1992) and which are distinct from those occurring during takeover. These changes often lead to the simultaneous death within the same blastogenic cycle of previously separated ramets, months following their separation from a given genet. The nonrandom nature of this process strongly suggests that senescence is mediated by a heritable component (Rinkevich et al., 1992; Lauzon et al., 2000). This type of senescence is reproducibly associated with characteristic morphological changes that are conserved from colony to colony and appear identical to those reported in sexually reproducing field colonies (Lauzon et al., 2000). Comparing old (7–12 years) asexual strains of a colonial ascidian, Diplosoma listerianum, with their recent sexually produced progeny, evidence for long-term molecular senescence in the asexual lineage was obtained (Nilsson Sköld et al., 2011). However, aging is also observed in the sexually reproducing colonies (Ryland and Bishop, 1990) which, as sessile animals, are also subject to territorial competition that may curtail their longevity in the wild (see chapter 5.3).

Colonial tunicates have hemoblasts, which are undifferentiated coelomic cells that give rise to terminally differentiated cells, accessory cells and germ cells (Kawamura and Sunanaga, 2010). In adults, vasa expression was observed in the gonads, as well as in a population of mobile cells scattered throughout the open circulatory system. vasa expression was dynamic during asexual development in both fertile and infertile adults, and was also enriched in a

population of stem cells (Brown and Swalla, 2007; Brown et al., 2009; Rosner et al., 2009). When a colony is naturally or experimentally depleted of vasa -expressing cells, vasa and vasa-expressing germ cells can reappear in the colony (Brown et al., 2009). Highly potent stem cells which regulate the activities of asexual blastogenesis and sexual gametogenesis and eventually cause soma-germ conflict may exist in colonial tunicates (Kawamura and Sunanaga, 2010).

6.2 Sexual reproduction as stress response

To keep the number of examples concise, I focused on asexual reproductive events. However, sexual reproduction is another routine response of unicellular and multicellular organisms to stress. The frequency of sexual reproduction generally depends on the condition of an individual, called fitness-associated sex, a pattern found broadly across both facultatively sexual prokaryotes and eukaryotes (Bell, 1982; Hadany and Beker, 2003; Hadany and Otto, 2007; 2009). Individuals that are starved undergo sex in a wide variety of organisms, including bacteria (Dubnau, 1991; Redfield, 1888; 1993; Jarmer et al., 2002; Foster, 2005), yeast (Kassir et al., 1988; Mochizuki and Yamamoto, 1992; Mai and Breeden, 2000), Chlamydomonas reinhardtii (Harris, 1989; Merchán et al., 2001) and Daphnia (Kleiven et al., 1992). In lower eukaryotes such as yeasts (Davey 1998; Kassi et al. 2003), Aspergillus (Skromne et al. 1995), Neurospora (Nelson and Metzenberg 1992), and C. reinhardtii (Matsuda et al., 1992; Abe et al., 2004) deprivation of nitrogen that is a limiting resource throughout phylogenesis (see chapter 5.2) induces the morphogenesis of asexual or sexual spores. In V. carteri a brief exposure to elevated temperatures generates egg-bearing sexual daughters (Kirk and Kirk, 1986). Sex in the nematode, Strongyloides ratti, is induced in response to a host immune response (Gemmill et al., 1997; West et al., 2001). Cells with DNA damage have also been shown to undergo sex in viruses (Bernstein, 1987), bacteria (Wojciechowski et al., 1989), and yeast (Bernstein and Johns, 1989). Mating behavior is favored by unfavorable and fluctuating environmental conditions (Frank and Swingland 1988, Nelson, 1996; Robson et al. 1999; Becks and Agrawal, 2010; Schoustra et al., 2010). In soil microfungi, sex is more common under stressful environmental conditions associated with drought severity and high salinity (Grishkan et al., 2003). In four of five plant studies reviewed by van Kleunen et al. (2001), there was relatively greater allocation to sexual than to vegetative reproduction at high density (competitive stress). Competitive stress also favors sexual reproduction in Daphnia and a rotifer (Berg et al., 2001; Gilbert, 2004). As the common final pathway of a variety of stress responses (Lindquist, 1986; Sanchez et al., 1992; Finkel and Holbrook, 2000; Heininger, 2001; Mittler, 2002; Mikkelsen and Wardman, 2003; Sørensen et al., 2003; Apel and Hirt, 2004) oxidative stress is an inducer of sexual reproductive activity (Bernstein and Johns, 1989; Nedelcu and Michod, 2003; Nedelcu et al., 2004). Death is a concomitant of sexual reproduction. Links between sexual reproduction and death have been inferred repeatedly (Ruffié, 1986; Clark, 1996, Reznick and Ghalambor, 1999; Partridge et al., 2005). I woud like to emphasize, however, that it is reproduction per se, both aseual and sexual, the rise of a new generation of offspring that underlies the evolutionary rationale of aging.

6.3 Semelparity: somatic death following reproduction

The evolutionarily most ancient asymmetric reproductive events occurred in unicellular, facultatively multicellular, organisms. These reproductive events involved the death of clonemates that provided the energy and building blocks for the metamorphosis of their siblings to spores. At the transition to obligate multicellularity, a division of labor separated reproductive and somatic functions. The soma became the mortal breeder that in a source and sink relationship provides the resources to disperse the germ cells or nurse and hatch the brood. Semelparous organisms reproduce once and die afterwords. In this sense, B.subtilis and the volvocine algae can be regarded as semelparous organisms. Likewise, multicellular semelparous organisms in which the reproductive phase determines the timing and execution of somatic catastrophic death (Finch, 1990; Rose, 1991; Arking, 1998) are best suited to demonstrate the link between reproduction and aging/death.

Semelparous animals include many insects (Fritz et al., 1982), including some species of butterflies, cicadas (Karban, 1997), and mayflies, some molluscs such as squid and octopus (Hanlon and Messenger, 1996), and many arachnids (reviewed by Finch, 1990). Semelparity is much rarer in vertebrates, but in addition to fishes, like Pacific salmon, European eel (Frost, 1950; Aarestrup et al., 2009), Cynolebias nigripinnis and Nothobranchius rachovii (Herrera and Jagadeeswaran, 2004), capelin (Burton and Flynn, 1998) and lampreys (Larsen, 1980; Finch, 1990), examples include smelt, a few lizards, snakes (Shine, 2003), and marsupial mammals (Lee and Cockburn, 1985). Annual plants, including rice, grain crops and most domestic vegetables, are semelparous.

Long-lived semelparous plants include agave, yuccas, and some species of bamboo (Janzen, 1976).

Maternal death (endotokia matricida) is a bizarre feature in some semelparous species. Finch (1990, p.102) provided several examples of matricidal hatching. Quite instructive is the reproductive behavior of Adactylidium and Acarophenax tribolii, two closely related mites (Gould, 1980). In Adactylidium, the pregnant female mite feeds upon a single egg of a thrips, growing five to eight female offspring and one male in her body. The hatched offspring devour their mother from the inside out and the single male mite mates with all its sisters. The females, now impregnated, cut holes in their mother's body so that they can emerge to find new thrips eggs. The male emerges as well, but does not look for food or new mates, and dies after a few hours. In Acarophenax tribolii, the single male even dies before birth. The extreme resource limitation appears to have shaped the reproductive behavior in these species. In a variety of spiders juveniles eat their mother before dispersing from the communal nest (Wise, 2006). Females of the Japanese foliage spider, Chiracanthium japonicum, are eaten by their offspring at the end of the maternal care period (Toyama, 2003). Oogonia of the annelid Polygordius epitocus phagocytose the mother until nothing is left of the last segments of the maternal organism but a thin cuticle. That cuticle breaks open and liberates the ova (Beklemishev 1964). Inside the female nematode Ascaris nigrovenosa, the embryos devour the undeveloped ova, as well as prey upon the internal organs of the mother, in consequence of which they grow very rapidly. At the end of a few hours nothing remains of the maternal body except the cuticle (Mecznikow, 1866). Similarly, when C. elegans fourth-stage larvae or fecund adults are starved the developing eggs hatch within, killing their mother organism (Johnson et al., 1984; Chen and Caswell-Chen, 2004; Angelo and Van Gilst, 2009). Like the reproduction of B. subtilis, and other examples of endotokia matricida, this reproductive event in its drastic type of resource sparing and recycling, highlights in pure form the general pattern in reproduction: the parent generation after dispersing the germ cells or nursing and hatching the brood is eliminated as potential competitor for resources.

In semelparous life histories an intimate relationship exists between reproduction on one hand and senescence and death on the other. These phenomena are often timed with great precision. For example, the cuttlefish (Sepietta oweniana), which lays eggs with protective capsules, may die within a day after mating and spawning (Bergstrom and Summers, 1983). In contrast, female Octopus cyanea survive for a month after spawning, during which time they brood their eggs (which are otherwise unprotected) and generally die a few days after the eggs hatch (Wodinsky, 1977; Van Heukelem, 1983).

A strange case of convergent evolution concerns the phenomenon that some mature semelparous organisms such as Pacific salmon, lamprey, Octopus or mayflies do not feed. In many insects the short-lived reproductive stage even has defective mouthparts or digestive organs; however, aphagy may also occur without rapid senescence (Finch, 1990). Convergent evolution is a potent indicator of optimal design and strong selective forces (Conant and Wagner, 2003; Tishkoff et al., 2007; Wilkens, 2010). The ETAs argue that postreproductive death of Pacific salmon or Octopus is due to “reproductive exhaustion” and invoke antagonistic pleiotropy as underlying mechanism (e.g. Rose, 1991 pp. 103-106). The adaptive value of this behavior, however, only becomes tangible within the framework of the germ-soma conflict theory.

6.4 Co-option: conservation and innovation

It is not possible to identify what is new in evolution without understanding the old. This is a reflection of the way evolution works, with some novelties being traceable as modifications of primitive conditions and others having origins that are much less obvious.
Shubin et al., 2009

Co-option, exaptation, and preadaptation are related terms referring to shifts in the function of a trait during evolution. For example, a trait can evolve because it served one particular function, but subsequently it may come to serve another. Exaptations are common in both anatomy and behavior. A classic example is how feathers which initially evolved for heat regulation, were co-opted for display, and later for use in bird flight (Shipman, 1998).

Advances in developmental genetics, palaeontology and evolutionary developmental biology have shed light on the origins of some of the structures that most intrigued Charles Darwin, including animal eyes, tetrapod limbs and giant beetle horns. Studies of deep homology are showing that new structures need not arise from scratch, genetically speaking, but can evolve by deploying regulatory circuits that were first established in early metazoans. The deep homology and homoplasy of generative processes and cell-type specification mechanisms in animal development has provided the foundation for the independent evolution of a great variety of structures (Shubin et al., 2009; Wake et al., 2011). Embryonal development of higher metazoans appears to recapitulate phylogenesis

(Northcutt 1990, Ohno 1995). This phenomenon, although it is no longer accepted in the sense of Haeckels original doctrine (Gould, 1977) is now exploited in evo-devo biology to the study of the evolution of body plans and designation of phylogenetic maps (Goodman and Coughlin 2000; Shubin et al., 2009).

Several starvation and stress responses were co-opted for evolutionary novelties (Heininger, 2001). In green alga, an environmental stress response was co-opted into a developmental response segregating germline cells and soma (Nedelcu and Michod, 2006; Nedelcu, 2009). The formation of teeth-like structures that are associated with bacteriovorous feeding and predatory behavior on fungi and other worms is an evolutionary novelty, which is restricted to some members of the nematode family Diplogastridae. These teeth-like denticles represent a polyphenism that is controlled by starvation and the co-option of an endocrine switch mechanism that is strongly influenced by mutations in the nuclear hormone receptor DAF-12 and its ligand, the sterol hormone dafachronic acid that control the formation of arrested dauer larvae (Bento et al., 2010). The dauer larvae`s ability to attach to other animals to facilitate dispersal appears to have been co-opted for nematode parasitism. The dafachronic acid-DAF-12 system as the core endocrine module for dauer formation (see chapter 9.1) has a conserved role in the mammalian parasite Strongyloides papillosus by controlling entry into the infective stage. Application of Delta7-dafachronic acid blocks formation of infective larvae and results in free-living animals (Ogawa et al., 2009; Wang et al., 2009).

6.5 The evolutionary nexus of differentiation, apoptosis, reproduction, and post- reproductive death

Man still bears in his bodily frame the indelible stamp of his lowly origin.

Charles Darwin. The Descent of Man and Selection in Relation to Sex (1871)

For approximately 3 billion years unicellular organisms were the only life form on earth. Unable to both store significant amounts of nutrients and/or to economize the use of nutrients during abundance, replication of bacteria in their natural habitat inevitably cycles between exponential growth in log phase and growth arrest in stationary phase (Nyström 1998). Thus, the natural lifestyle of these microbes is characterized by an inherent “feast and famine” cycle, limiting amounts of nutrients being rather the rule than the exception and long periods of nutritional deprivation punctuated by short periods that allow fast growth (Kolter et al. 1993, Msadek 1999). The unicellular starvation response in stationary phase brought about the asocial “resist or mutate” phenomena that were an unicellular dead end street (Storz and Hengge-Aronis, 2000; Heininger, 2001). Social unicellular organisms like the prokaryotes B. subtilis and M. xanthus and the eukaryote D. discoideum, however, respond to nutrient deprivation with multicellular programs in which differentiation to highly resistant spores is energetically costly and was linked to death of their clonemates (Heininger, 2001). Thus, both differentiation and reproduction share the same evolutionary roots in the starvation/stress response of unicellular, facultatively multicellular, organisms. Intriguingly, the cells and structures involved in these ancient differentiation events were dubbed “mother” and “daughter” cells, or fruiting “bodies” reflecting their source/sink relationship and reproduction and germ cell/soma phenotypes. Thus, the metamorphosis of vegetative cells to spores represents the first phylogenetically detectable multicellular reproductive event.

Embryology and developmental genetics played no part in forging the Modern Synthesis (Mayr, 1982; Gilbert et al., 1996; Müller, 2007). However, nothing could demonstrate more convincingly the role of ontogeny for our understanding of phylogeny than the books of Gould (1977) and Buss (1987) on this subject. Although Haeckels doctrine that ontogeny recapitulates phylogeny has been abandoned by science (Gould, 1977), ontogenetic events are a window into the evolutionary past (Gould, 1977, Buss, 1987; Heininger, 2001). Model organisms like D. discoideum, Porifera, Hydra, and B. schlosseri have deepened our insights into the evolutionary roots of signaling pathways regulating ontogenetic processes (Heininger, 2001). A similar integrative and coherent view of reproduction, both asexual and sexual, is missing. Otherwise, it should not have escaped the attention of evolutionary biologists that death both cellular and/or organismal is an invariant concomitant of asymmetric reproduction. A comprehensive account, similar in scope to this one, on the evolutionary roots of sexual reproduction, its relationship to asexual reproduction and the multifaceted causes that determine the evolutionary success of sexual reproduction is underway (Heininger, in preparation).

Following the transition to multicellularity, environmental cue-dependent, stress-elicited morphogens such as cAMP or DIFs evolved into intercellular signaling factors that regulate morphogenesis in the internal milieu of multicellular organisms. Cumulative evidence argues for the phylogenetic conservation of these processes.

Dictyostelium-derived DIFs elicit evolutionarily highly conserved, dose-dependent and context-specific effects. The conservation and ambiguity of the signals has been ascertained through their effects on various mammalian cell lines causing both growth arrest, differentiation and apoptosis (Asahi et al., 1995; Kubohara et al., 1995a; b: 1998; Kubohara, 1997; 1999: Kubohara and Hosaka, 1999; Miwa et al. 2000; Takahashi-Yanaga et al., 2003). Moreover, DIF-1 inhibits progesterone-induced oocyte maturation in Xenopus laevis (Kubohara et al., 2003), mimicking its action on Dictyostelium prespore cells (Kubohara and Maeda, 1997). Conversely, mammalian morphogens induce cell differentiation in D. discoideum (Kubohara et al., 1993). Like in Dictyostelium, cAMP plays a central role in cell differentiation and apoptosis of virtually every cell type (Chen et al., 1998; Andreatta et al., 2004; Wang et al., 2005; Ichiki, 2006; Chera et al., 2007; Peltier et al., 2007; Chen et al., 2011; Zhang et al., 2011), including gametes (Maller, 1985; Quarmby, 1994; Sassone-Corsi, 1998; Don and Stelzer, 2002; Kang and Han, 2011). Throughout phylogenesis this capacity is executed in an antagonistic pleiotropic action. Even downstream effector mechanisms including regulation by glycogen synthase kinase-3 (GSK3) appear conserved (Harwood et al., 1995; Aubry and Firtel, 1999; Takahashi-Yanaga et al., 2003; 2006). GSK3, a serine/threonine protein kinase, was discovered as a metabolic enzyme, that regulates the response of glycogen synthase to insulin; however, it is now known to be active as a component of the Wnt signaling pathway (derived from the Drosophila Wingless (Wg) and the mouse Int-1 genes), (Li F et al., 2006; Speese & Budnik, 2007; Maiese et al., 2008), in a wide range of cellular processes, ranging from apoptosis to embryonic development (Cohen and Frame, 2001; Frame and Cohen, 2001; Harwood, 2001). Although no homologs of the antagonistic pleiotropic canonical Wnt signaling pathway (see chapter 13) have been identified in the Dictyostelium genome, key components of the pathway, like GSK and β-catenin, are present and functionally linked (Grimson et al., 2000; Croce and McClay, 2008). GSK3 is present in all eukaryotes and shows strong sequence conservation within its catalytic core (Plyte et al., 1992). GSK3 links metabolism with fundamental processes including cell fate determination, transcriptional control, and, in mammals, oncogenesis and neurological diseases. In Dictyostelium, mediating the action of DIFs and cAMP, GSK controls spore and stalk cell fate decisions (Harwood et al., 1995; Plyte et al., 1999; Schilde et al., 2004; Harwood, 2008). During development of organisms as diverse as Drosophila, C.elegans, Xenopus and mammals, GSK3 is required in increasingly complex signal transduction pathways for cell fate specifications (Bourious et al., 1990; Siegfried et al., 1992; He et al., 1995; Woodgett, 2001; Sato et al., 2004; Shirayama et al., 2006; Welham et al., 2011) including germline specification (Yamanaka, 2008; Adhikari and Liu, 2009; Arur et al., 2009; Lluis and Cosma, 2009).

The roots of the events in starvation/stress responses are also witnessed by the oxidative stress that drive both ontogenetic processes/cellular differentiation (Allen and Balin, 1989; Sohal and Allen, 1990; Heininger, 2001; Zs.-Nagy, 2001; Martindale and Holbrook, 2002) and asexual and sexual reproductive events (Heininger, 2001; Agarwal et al., 2005; Nedelcu, 2005; Singh et al., 2008). Intriguingly, the pattern of heat shock protein expression in yeast sporulation and Drosophila oogenesis is similar (Kurtz et al., 1986). Simulating the stress signal (i.e. a change in cellular redox status) in a developmental rather than environmental context, the stress response was co-opted into a developmental response segregating germline cells and soma (Heininger, 2001; Nedelcu and Michod, 2006; Nedelcu, 2009). Evolved as a stress response, however, reproduction is highly sensitive to additional stressors (Hales and Robaire, 2010). Stress both promotes (Maeda and Tsukamura, 2006) and inhibits reproduction. Intriguingly, as a legacy to these shared evolutionary roots, similar neurotransmitters and nuclei within the hypothalamus control stress and reproduction (Dobson et al., 2003).
The link between developmental differentiation and apoptosis is so strong that even in a metazoan organism with its abundant resources, developmental differentiation is not feasible without apoptosis, the latter providing the energy and building blocks (Heininger, 2001). In analogy, reproduction without concomitant death has a fitness cost in facultatively multicellular organisms. Clonal amoebae and bacterial colonies with a cheater phenotype (i.e. defective to carry the cost of death at reproductive events) have a high risk to get extinct under conditions of feast and famine cycles (Hilson et al., 1994; Pál and Papp, 2000; Ennis et al., 2003; Fiegna and Velicer, 2003; Rainey and Rainey, 2003; Castillo et al., 2005; Kuzdzal-Fick et al., 2011). Likewise, yeast apoptosis mutants are outcompeted by wild type strains that are able to pay the price of programmed cell death of reproduction and aging (Fabrizio et al., 2004a; b; Herker et al., 2004). In primordial reproductive events, fratricide and cannibalism were essential requisites to provide the resources for the energy-costly metamorphosis of vegetative cells into resistant germ cells (Heininger, 2001; Gonzalez-Pastor et al., 2003; Guiral et al., 2005;

Engelberg-Kulka et al., 2006; Claverys et al., 2007; Nariya and Inouye, 2008). The death of the clonemates/soma was co-opted in multicellular organisms as a means to rein in the resource utilization of the progenitors and increase the chances of the germ cells' evolutionary success in a world of limited resources. The evolution of many apoptotic protein domains can be traced back to unicellular eukaryotes and even bacteria (Aravind et al., 1999). Microbial processes like sporulation, stationary-phase cell death, and fruiting body formation carry features of apoptotic cell death (Cornillon et al., 1994; Chaloupka and Vinter, 1996; Hochman, 1997). Particularly, a multitude of molecular and phenotypic features characteristic for invertebrate and vertebrate developmental apoptosis, including the ambiguousness in concert with cell differentiation (the dual survival/death decision), are already established in D. discoideum (Heininger, 2001).

Proteins that are related to the retinoblastoma tumor suppressor RB and the E2F transcription factor are conserved in most eukaryotic lineages, including animals and plants. RB family proteins and E2F family proteins function in a wide range of biological processes, including cycle controlling, differentiation, development and apoptosis and hence, are central to cellular life-death decisions (Iaquinta and Lees, 2007; Polager and Ginsberg, 2008; van den Heuvel and Dyson, 2008; Cao et al., 2010). A retinoblastoma ortholog controls stalk/spore preference in Dictyostelium and was increased about 200 times when D. discoideum transited from unicellular to multicellular form (MacWilliams et al., 2006). In most animals, the specification of germ cells occurs very early during ontogeny by asymmetric distribution of germ plasm. A multi-level selection theory predicted that a reproductive division of labor is required to evolve prior to subsequent functional specialization. The frequency distribution of division of labor and the sequence of its acquisition confirm that reproductive specialization evolves prior to functional specialization (Simpson, 2012). The whole range of differentiation/apoptosis features that segregates immortal germ cells and mortal somatic cells is phylogenetically conserved from Dictyostelium (Machesky et al., 1998), Botryllus (Brown et al., 2009), Xenopus (Hensey and Gautier, 1998), and avian (Sanders et al., 1997) to mammalian embryogenesis (Tam and Behringer, 1997; Manova et al., 1998; Pesce and Scholer, 2000; Extavour and Akam, 2003). Direct and indirect evidence (Sanders et al., 1997; Heyer et al., 2000; Pampfer, 2000; Artal Sanz et al., 2003) suggests the effector role of oxidative stress and thus the stress legacy of the germ cell specification event (Heininger, 2001). Asymmetric division is a prerequisite of germ cell specification in the multicellular alga Volvox (Miller and Kirk, 1999). A variety of genetic markers of germline specification are conserved throughout the animal kingdom (Gavis, 1997; Saffman and Lasko, 1999; Wylie, 1999) witnessing the inherited totipotency-mortality regulation (see chapter 17.3.5). From the various markers of germline specification (e.g. piwi, oskar, nanos), I pick the vasa gene family to demonstrate the phylogenetic conservation of germline specification. The vasa-like genes regulate reproductive stem cells, but not lineage-specific stem cells, which may be regulated by gcm genes (Agata et al., 2006). The vasa-related genes encode an ATP-dependent RNA helicase from the DEAD-box family and are involved in germ cell determination and formation in metazoans (Extavour and Akam, 2003; Gustafson and Wessel, 2010). RNA helicases have been found to be essential for translation initiation, sexual development and G1 arrest in yeast (Maekawa et al., 1994; Linder, 2003), social development in Dictyostelium resulting in spore/fruiting body formation (Machesky et al., 1998) and germ cell determination in nematodes (Roussell et al., 1994). In Dictyostelium, disruption of the helC gene, which encodes helicase C, a member of the DEAD-box family of RNA helicases, led to developmental asynchrony, failure to differentiate and aberrant morphogenesis (Machesky et al., 1998). In Botryllus, vasa expression was observed in the gonads, as well as in a population of stem cells. vasa expression is correlated with the potential for gamete formation, which suggests that it is a marker for embryonically specified, long-lived germline progenitors and might play a homeostatic role in asexual development (Sunanaga et al., 2006; 2007; Brown et al., 2009). In sponge and Hydra the vasa-related genes are expressed in germline cells and in multipotent interstitial stem cells and ectodermal epithelial cells (Mochizuki et al., 2000; 2001; Rebscher et al., 2008). vasa-related genes are also expressed in neoblasts and germline cells of planarians (Shibata et al., 1999; Pfister et al., 2008). vasa-related genes are expressed in germline cells of sea anemones (Extavour et al., 2005), C. elegans, Drosophila, Xenopus (Ikenishi, 1998), fishes (Knaut et al., 2000), birds (Tsunekawa et al., 2000), rodents (Toyooka et al., 2000) and humans (Castrillon et al., 2000). This suggests a conserved genetic control mechanism in germline differentiation of all animals (Extavour and Akam, 2003; Extavour, 2007). In fact, one strong argument for the putative monophyletic origin of the germline is the ubiquitous conservation of vasa gene-encoded RNA helicase and its orthologs in

metazoans (Schüpbach and Wieschaus 1987; Lankenau, 2008).

Nurse cell apoptosis is a necessary event during gametogenesis (in a source-sink relationship) from Porifera and Hydra to mammals (Fell, 1969; King, 1970; Spradling, 1993; Cavaliere et al., 1998; McCall and Steller, 1998; Gumienny et al., 1999; Kuznetsov et al., 2001; Matova and Cooley, 2001; Technau et al., 2003; Alexandrova et al., 2005), reminiscent of the primordial reproductive events requiring fratricide to fuel the spore metamorphosis. Mitochondrial ribosomal RNA is an indispensable factor of germ cell determination (Iida and Kobayashi, 1998; Kashikawa et al., 1999). Thus, mitochondrial translation machinery (Kashikawa et al., 1999) and respiratory activity (Akiyama and Okada, 1992) appear to be phylogenetically conserved determinants of spore formation in protozoa (Maekawa et al., 1994; Machesky et al., 1998) and primordial germ cell specification in metazoa (Iida and Kobayashi, 1998; Kashikawa et al., 1999; Isaeva and Reunov, 2001). Remarkably, mitochondria undergo a prespore-specific functional and structural transformation in Dictyostelium (Matsuyama and Maeda, 1998; Inazu et al., 1999) which may be a predecessor of the metazoan primordial germ cell-defining nuage/sponge-like formation (Gavis, 1997; Wilsch-Brauninger et al., 1997).

7. Semelparous and iteroparous life-history strategies

There is simply no denying the breathtaking brilliance of the designs to be found in nature. Time and again, biologists baffled by some apparently futile or maladroit bit of bad design in nature have eventually come to see that they have underestimated the ingenuity, the sheer brilliance, the depth of insight to be discovered in one of Mother Nature's creations. Francis Crick……baptized this trend in the name of his colleague Leslie Orgel, speaking of what he calls "Orgel's Second Rule: Evolution is cleverer than you are."

Daniel Dennett, Darwin's Dangerous Idea, 1995

Summary

A variety of features related to available resources, reproductive investment, extrinsic mortality, environmental hazards and predictability shape the reproductive strategy of organisms. Intriguingly, both semelparous or iteroparous reproduction strategies are often followed by closely related species and even populations within the same species. Thus, semelparity and iteroparity, within their constraints, are threshold traits with plastic responses to a variety of environmental cues and can be considered as opposite ends of a continuum of life history variation.

7.1 Aging is a corollary of iteroparous reproduction

There are two fundamental choices of life-history strategies in plants and animals – semelparity/monocarpy and iteroparity/polycarpy (Charlesworth, 1980). Semelparity is sometimes referred to as 'big-bang reproductive strategy', as semelparous species devote most of their energy and resources to maximizing the number of offspring in a single cycle of reproduction, and die soon after reproducing. The most intriguing aspect of this behavior is that death appears programmed, even to be actively pursued by the organism as if under the influence of "death hormones" (Denckla, 1975; Young and Augspurger, 1991; Wilson, 1997). Throughout phylogeny, a myriad of species follows this reproductive track. In his epic book, Finch (1990) devoted 76 pages to this life history trait. Iteroparous species, in contrast, reproduce multiple times. Although there appears to be a fundamental dichotomy between these two strategies, these life history traits are numerically continuous (reproducing once, twice, etc.). A variety of features related to reproductive investment, available resources, extrinsic mortality, environmental hazards and predictability shape the reproductive strategy of species or populations within species (Stearns, 1992; Charlesworth, 1994). Thus, in very unpredictable environments, it may be a successful strategy to produce offspring continually during the reproductive season until accidental death of the individual (Harper, 1977).

Aging trajectories differ between semelparous and iteroparous reproduction modes. In semelparous organisms, aging is often described as catastrophic or rapid with ensuing "sudden" death (Finch, 1990), while in iteroparous organisms senescence is gradual or even negligible (Finch, 1990). All proponents of the ETAs agree that aging in semelparous organisms is fundamentally different from aging in iteroparous organisms. Therefore, in publications concerned with the ETAs, aging, in a tacit agreement, is understood as aging in iteroparous organisms. Given that aging-related changes manifest as soon as reproductive maturity is reached (Arking, 1998 p. 12),

the iteroparous reproduction strategy is routinely associated with the aging phenotype (Hautekèete et al., 2001). Taking the immediate postreproductive, the semelparous, death as worst case scenario for the soma, iteroparous strategy-related delay of death means a substantial gain of fitness for the soma. Hence aging, in accord with evolutionary theory but in contrast to the assumptions of the ETAs, is highly fitness-promoting for the soma. This change is accomplished by a simple change of the point of reference. Although the ETAs made the prediction that immortality is unlikely to be the end result of evolution by selection (Tuljapurkar, 1997), the point of reference of the ETAs is immortality. In the words of Kirkwood and Melov (2011): “The anomalous nature of ageing as a putative adaptation is that it is bad for the individual in which the process is exhibited. An animal that grows to maturity and thereafter reproduces indefinitely has, other things being equal, a greater Darwinian fitness than one that grows to maturity and then survives and reproduces for only a fixed period of time.” This is a totally unrealistic assumption in a world of limited resources as a simple gedankenexperiment has shown.

The germline is well maintained so that it is vitually immortal connecting humans through an unbroken sequence of passed genes to the origin of life some 4 billion years ago. In contrast, the soma that nurtures and disseminates the germ cells is discarded. Hence, somatic aging is regarded as maladaptive, leading progressively to cumulative functional deficits and ultimately resulting in death (see definitions of aging in Arking, 1998, p. 9). The tacit implication of this concept is that the default state of somatic life capacity is an infinite lifespan which, however, is shortened by the inability of somatic maintenance (Partridge and Barton, 1993).

The interplay between investment into reproduction and availability of resources on one hand and somatic fitness and environmental threats on the other hand determines the adaptive value of reproductive (r or K) strategy and hence the evolution of life history traits (Heininger, 2002a). Stress resistance, like reproductive success, is a feature of fitness. Stress resistance is consistently linked to a delay of post-reproductive death and, conversely, this delay is selected in a phenotypic cluster with stress resistance characters (Parsons, 1995; 2002; Lin et al., 1998; Longo, 1999; Johnson et al., 2000). Since at death the fitness of the individual has declined to zero, it can be assumed that any delay of death means a gain of fitness and is advantageous to the individual. Importantly, mild-to-moderate stressors like reduced dietary intake or physical activity increase stress resistance (the hormesis phenomenon, see chapter 10.1) and can induce a delay of death (Heininger, 2002a).

7.2 Plasticity of semelparous and iteroparous life-history strategies

Any organism has a limited amount of resources at its disposal, and these have to be partitioned between reproductive and nonreproductive activities. A larger share of resources allocated to reproductive activities, that is, a higher reproductive effort at any age, leads to a better reproductive performance at that age; this may be considered as a profit function. This reproductive effort also leads to a reduction in survival and growth (see chapter 8) and consequent diminution of the reproductive contribution of the succeeding stages in the life history; this may be considered as a cost function. Natural selection would tend to an adjustment of the reproductive effort at every age such that the overall fitness of the life history would be maximized. A model of life history processes has been developed on the basis of these considerations (Gadgil and Bossert, 1970; Stearns, 1992, Roff, 1992). It leads to the following predictions:

1. If the form of the profit function is convex, or that of the cost function concave, the optimal strategy may be to breed repeatedly. Otherwise, the optimal strategy is to breed only once in a suicidal effort like a salmon (big-bang reproduction).
2. The value of reproductive effort continuously increases with age in the case of repeated reproducers.
3. If all the stages in the life history following a certain age are adversely affected, the age of reproduction will tend to be lowered in the case of big-bang reproducers, and the reproductive effort at all ages preceding that stage will tend to increase in the case of repeated reproducers.
4. As the reproductive potential increases with size at a slower rate, reproductive effort will be lower at maturity, reproductive effort will increase at a higher rate with age, and growth will continue beyond maturity.
5. A uniform change in the probability of survival from one age to the next at all ages would have no effect by itself, on the age of reproduction in big-bang breeders or on the distribution of reproductive effort with age in the repeated reproducers.
6. Such a change in survivorship would lead to a change in the equilibrium density of a population. If the population is resource limited, this would affect the availability of resources to the members, of the population in such a way that an increase in mortality would increase the availability of the resources.

7. For a resource-limited organism a greater availability of resources would lead to a lowering of the age of reproduction in the case of the big-bang breeders, and to a greater reproductive effort at all ages for the repeated breeders.

The ETAs have a hard time to explain the existence of semelparity. The mere existence violates some of the basic assumptions of the ETAs. The first incompatibility is related to the declining force of natural selection. In the words of Kirkwood and Cremer (1982): “For a semelparous organism, the Medawar-Williams argument of a declining force of natural selection with age does not apply. This is because selection continues to operate with full force until reproduction begins. Once reproduction is complete the force of selection is reduced to zero unless the adult plays an active part in promoting survival of the young. Thus, for semelparous species, the pattern of selection corresponds exactly with that envisaged more generally by Weismann, and his panmixia argument directly applies.” And further: “Ageing in such organisms is therefore very different from ageing in repeatedly reproducing, or iteroparous, species in which the lifespan could, in principle, extend indefinitely.” The other two assumptions are related to the normative force of extrinsic death (which semelparous organisms obviously do not experience). The one is the “it does not pay to maintain the soma indefinitely if you have to die anyway” argument. It can hardly be assumed that semelparous organisms succumb to death in a sort of “anticipatory obedience”. The last assumption is that extrinsic death should have prevented the evolution of genes related to aging and death. According to this vein of thought, for most natural populations, extrinsic mortality (due to accidents, predation, starvation, disease, cold, etc.) is such that death occurs well before “old age”. This means that a) there is no requirement for aging to weed out “worn-out individuals”; b) there is no evidence that aging in fact serves as a significant mortality force in the wild; and c) there can have been scant opportunity to evolve genes specifically for aging, even if they were beneficial, since natural selection would not normally “see” them in action (Kirkwood, 2005). Caleb Finch (1994) argued: “The latent capacity of certain semelparous species for much longer adult life phases might be considered to challenge the deduction from mathematical population genetics theory that "senescence will always creep in" (Hamilton, 1966), because there will be little or no selection against the accumulation of genes with adverse effects that are delayed until later in life when individuals are contributing less progeny (Hamilton, 1966; Rose, 1991). However, as Rose points out (1991, pp. 167-168), the evolutionary theory of aging does not require that the leading causes of death during senescence be synchronized with other aging processes.” After consulting the referenced pages in Michael Rose’s book, I understand this to mean that semelparous death, in this terminology “the leading cause of death” is considered just another, although acute, physiological mechanism that happens to precede other physiological causes of death like atherosclerosis or cancer. No discussion e.g. of the fact that this does not fit into the pattern of extrinsic death-triggered frustration of the efforts for somatic maintenance or that the acuity of death is not compatible with the slow workings of postreproductively relaxed selection.

Most semelparous species die immediately after reproduction without any clear environmental cause, e.g. long-lived semelparous species, such as Agaves (Young and Augspurger, 1991), some Yuccas (Schaffer and Schaffer, 1979) and bamboos (Keeley and Bond, 1999), cicadas (Karban, 1997) or Pacific salmon (Finch, 1990). This suggests programmed death (Young and Augspurger, 1991; Wilson, 1997). Intriguingly, both semelparous and iteroparous reproduction strategies are often followed by closely related species and even populations within the same species. Semelparity and iteroparity can occur among species within a genus (Schaffer and Elton 1974; Schaffer and Schaffer 1979; Woolhead and Calow 1979; Young 1990), in closely related species (Law, Bradshaw and Putwain 1977; Pitelka, 1977; Primack, 1979; Sano and Morishima 1982; Marshall et al., 1985; van Groenendael and Slim 1988; Robichaux et al., 1990; Sainte-Marie, 1991; Lesica and Shelly 1995, Brenchley et al., 1996; Olive et al., 1997; Conti et al., 1999; Crespi and Teo, 2002; Rocha et al., 2005; Bonser and Aarssen, 2006; Karsten et al., 2008; Vilela et al., 2008), subspecies (Spira and Pollak, 1986; Huxman and Loik, 1997; Hautekèete et al., 2001), males and females within a species (Braithwaite and Lee, 1979; Hölldobler and Wilson, 1990; Burton and Flynn, 1998; Oakwood et al. 2001; Lourdais et al., 2002), populations within the same species (Leggett and Carscadden 1978; Maltby and Calow, 1986; Till-Bottraud et al., 1990; Letschert, 1993; Stump, 1994; Hautekèete et al., 2001; Lesica and Young, 2005; White and Robertson 2009; Williams, 2009; Seamons and Quinn, 2010; Kim and Donohue, 2011), genotypes within one single population (Grosberg, 1988; Oakwood et al., 2001) or individuals of a population depending on size (Iguchi and Tsukamoto, 2001), resource availability (Johnson et al., 1984; Baird et al., 1986; Davies and Dratnal, 1996; Gems et al., 1998; Hautekèete et al., 2001; Stelzer, 2001;

Bonnet et al., 2002; Chen and Caswell-Chen, 2004; Wolfe et al., 2004; Ruf et al., 2006; Shapira and Tan, 2008; Angelo and Van Gilst, 2009; Fisher and Blomberg, 2011) or pollinator abundance (Paige and Whitham, 1987). Medusae, the mobile jellyfishes, which represent the sexual form of many Hydrozoans and Scyphozoans, are known to die soon after gametogenesis and spawning but rarely may also life up to two years and spawn twice (Spangenberg, 1965; Yasuda, 1969; Hamner and Jenssen, 1974; Miyake et al., 1997; Lucas, 2001; Watanabe et al., 2009; Ojimi and Hidaka, 2010). Under favorable conditions, chinook salmon can exhibit some iteroparous traits (Unwin et al., 1999). Male capelin fishes (Mallotus villosus Müller) die after an intense spawning period and appear to be semelparous, while females are inherently iteroparous (Flynn and Burton, 2003; Huse, 1998). In other capelin populations both female and male fishes may be either semelparous or iteroparous (Christiansen et al., 2008). Likewise male marsupial mice of the genus Antechinus, die after an intense mating period and are semelparous, while females are inherently iteroparous (Braithwaite and Lee, 1979; Fisher and Blomberg, 2011). However, high adult female mortality rates between mating and weaning of the offspring may select for a 'bet-hedging' mating strategy in semelparous males (Kraaijeveld et al., 2003). Closely related dasyurids are polyestrous and iteroparous. The semelparous Pacific salmon is thought to have evolved fairly recently from iteroparous relatives in the Atlantic (Crespi and Teo, 2002). In ayu Plecoglossus altivelis, an annual osmeroid fish with a single breeding season, large females spawn once, while small females spawn twice with an interval of some 2 weeks (Iguchi and Tsukamoto, 2001). In a variety of eusocial ants, males mate once and then die while the female queen has an extremely long lifespan (Hölldobler and Wilson, 1990). Semelparity and iteroparity existing within the same species has been observed in separate populations of the leech Erpobdella octoculata (Maltby and Calow, 1986) residing in two Scottish lakes. The authors suggested a genetic basis for the variation in fecundity and post reproduction mortality, and thus investment in reproduction, after this variation persisted in laboratory cultures of the wild populations. The erpobdellid leech Nephelopsis obscura drawn from a single population in southern Alberta, Canada had a broad range of post-reproductive mortality responses dependent on the balance of resource availability and reproductive investment indicating an adaptation to habitat unpredictability by adopting a flexible life history, rather than being strictly either semelparous or iteroparous (Baird et al., 1986).

Extensive evidence suggests that spiders frequently are food limited, i.e., a relative shortage of prey limits their growth, development, reproduction, and/or survival (Wise, 2006). Females of the semelparous crab spider Lysiteles coronatus guard their egg mass against predators. L. coronatus females do not consume food during the 40-d guarding period; this results in a 30.2% loss in their weight. Dissection of guarding females indicated that their ovaries developed temporarily during egg guarding and that the developed ovaries were subsequently reabsorbed. These results suggest that the females maintain the potential to produce a second egg mass in case of egg loss, but that this potential declines towards the end of the guarding period. A few females produce a second egg mass after they had lost the first one. Thus, facultative second oviposition and iteroparity in L. coronatus females has evolved as an adaptation to egg loss (Futami and Akimoto, 2005). In the matriphagous black lace-weaver spider, Amaurobius ferox (Amaurobiidae), there is a positive relationship between female body-mass and the number of offspring. The transfer of maternal body-mass to the offspring is via a batch of inviable trophic eggs which is immediately eaten by the spiderlings (Kim and Roland 2000), and later via matriphagy (Kim and Horel 1998). The tropic eggs and matriphagy improve development and survival of the offspring (Kim and Roland 2000, Kim et al. 2000). The brood-caring mothers are physiologically capable of producing a second clutch, but their net reproductive output, calculated as the number of surviving midinstar juveniles, is maximized by matriphagy versus the alternative strategy of abandoning the offspring early in order to lay a second clutch (Kim et al. 2000; Kim, 2009). In the semelparous spider Stegodyphus lineatus, few females that had their broods experimentally reduced shortly after hatching, re-laid broods. However, producing a new clutch did not prevent females from feeding the remaining offspring from the first brood. Matriphagy occurred only in broods that were reduced shortly before the young normally consume the mother. The timing of the mother's death was a function of the resources the female had left after brood reduction, namely her body mass. Thus, the spiders are generally plastic in that they possess the potential to invest in a second brood (Schneider et al., 2003). In the hump earwig Anechura harmandi that differs from closely related iteroparous earwig species with regard to its unfavorable habitat, cannibalism of the female parent (that guarded the eggs) by her offspring after hatching and semelparity seems to have evolved in unfavorable environmental conditions (Kohno, 1997). A controlled breeding

experiment reported full mating compatibility among offspring from both semelparous and iteroparous females of the European earwig Forficula auricularia, confirming that both types of females belong to one single species (Meunier et al., 2011). When iteroparous C. elegans are starved, the organisms may become facultatively semelparous. The developing eggs hatch within, consuming the parent's body contents and killing their mother organism, a reproductive strategy called endotokia matricida (Trent et al., 1983; Johnson et al., 1984; Gems et al., 1998; Chen and Caswell-Chen, 2004; Shapira and Tan, 2008; Angelo and Van Gilst, 2009). Some of those larvae use the resources to reach the resistant, long-lived dauer stage. If starved under similarly extreme conditions, larvae from eggs laid outside of the body are unable to develop into dauers (Chen and Caswell-Chen, 2004). Alternatively, the starved adults may enter adult reproductive diapause that enables sexually mature adults to delay reproductive onset 15-fold and extend total adult lifespan at least threefold (Angelo and Van Gilst, 2009). C. elegans particularly highlights the importance of resource availability for iteroparity-semelparity transitions. Facultative endotokia matricida has been observed in a variety of parasitic and free-living oviparous nematodes as a response to food limitation (Ayalew and Murphy, 1986; Johnigk and Ehlers, 1999; Baliadi et al., 2001; Hirao and Ehlers, 2010). A mammal, the edible dormouse can switch between functional semelparity and iteroparity depending on resource availability (Ruf et al., 2006). Partial male semelparity was also observed in a South American marsupial (Martins et al., 2006). Studies have suggested that semelparity is associated with environmental stress. Likelihood of semelparity decreased with soil nutrient enhancement in Picris, Scabiosa (Verkaar and Schenkeveldt 1984) and bamboo (Janzen 1976).

In a given environment, differences in life-history strategies between populations or species may have a genetic basis (Stearns, 1992; Roff, 1992; 2002; Charlesworth, 1994) and are due to natural selection (Schaffer, 1974a; b; Schaffer and Rosenzweig, 1977; Schaffer and Schaffer, 1979; Bell, 1980; Ranta et al., 2000; Rieseberg et al., 2002) while changes in environmental parameters may affect life-history traits of a given population/species within their respective constraints (Johnson et al., 1984; Orzack and Tuljapurkar, 1989; Orzack, 1993; Gems et al., 1998; Chen and Caswell-Chen, 2004; Sgro and Hoffmann, 2004; Wolfe et al., 2004; Ruf et al., 2006; Shapira and Tan, 2008; Angelo and Van Gilst, 2009; Fisher and Blomberg, 2011). The plasticity of life history traits in response to environmental changes (Gotthard and Nylin, 1995; Olive et al., 1997; Nylin and Gotthard, 1998) is also observed with regard to semelparity-iteroparity transitions (Johnson et al., 1984; Baird et al., 1986; Paige and Whitham, 1987; Grosberg, 1988; Stump, 1994; Gems et al., 1998; Willson, 1999; Hautekèete et al., 2001; Iguchi and Tsukamoto, 2001; Oakwood et al. 2001; Bonnet et al., 2002; Chen and Caswell-Chen, 2004; Wolfe et al., 2004; Ruf et al., 2006; Shapira and Tan, 2008; Angelo and Van Gilst, 2009; Williams, 2009; Fisher and Blomberg, 2011).

We may actually witness evolution in action. Tasmanian devil facial tumor disease, a recently emerged infectious cancer, has caused an abrupt transition from iteroparity towards virtual semelparity in the largest extant carnivorous marsupial, the Tasmanian devil (Sarcophilus harrisii). Devils have shown their capacity to respond to this disease-induced increased adult mortality with a 16-fold increase in the proportion of individuals exhibiting precocious sexual maturity (Jones et al., 2008). Other species approaching semelparity are the sea star Acanthaster planci (Stump, 1994), the snake Vipera aspis (Bonnet, 2011), tenrec and patas monkeys (Stephenson and Racey, 1995; Dewar and Richard, 2007; Isbell et al., 2009) and several insect species (Young, 2010). Fir waves, a set of alternating bands of fir trees in sequential stages of development, develop by wave-regeneration following wind disturbance. Wind disturbance occurs predictably in time and space in sub-alpine forests and leaves a narrow window for opportunity of offspring recruitment. Available evidence supports an ongoing change to semelparity in sub-alpine stands of fir trees in the genus Abies that exhibit wave regeneration (Silvertown, 1996). Demographic responses can, over time, evolve into new, genetically mediated life-history parameters (Reznick et al., 1990; Kokko and López-Sepulcre, 2007; Jones et al., 2008). The case of V. aspis shows that the transformation from iteroparity to semelparity does not involve a rearrangement of alleles with the creation of a genetic barrier between semelparity and iteroparity, but rather differential environmental constraints (Bonnet, 2011). Other studies have reached similar conclusions for both ectotherms and endotherms (Schmidt et al., 2006; Mayor et al., 2009). For instance different capelin populations exhibit absolute semelparity versus iteroparity strategies; such facultative semelparity results from interactions between spawning habitat, physical forcing, and predatory pressure (Christiansen et al., 2008). In a copepod, the probability of death following a massive reproductive investment depends upon food availability (Mayor et al., 2009).

Most nereid polychaetes are strictly semelparous. Mature adults of the segmented worm Nereis stop to feed and die soon after spawning. Endocrine manipulation in Nereis diversicolor by the regular implantation of cerebral ganglia from immature donors unveiled characteristics associated with a capacity to engage in repeated gametogenic cycling. The authors concluded that these latent capacities are reminiscent of features of iteroparous life histories, characterized by repeated breeding, and are postulated to be vestiges of an iteroparous ancestry. They also constitute a preadaptation for iteroparity and reveal how readily a reversal to this condition could occur. The study suggests that reproductive strategies may be unexpectedly labile in even their most fundamental aspects (Golding and Yuwono, 1994).

All plant meristems (the plant pluripotent stem cells) are semelparous because their growth is terminated when they differentiate into reproduction organs. Since plants are constructed by iterative meristem growth, the fact that reproduction is a terminal act for meristems ought to make the evolutionary transition from iteroparity to semelparity in the whole plant a morphologically simple one (Silvertown, 1996). Shifts between monocarpic and polycarpic strategies can be realized in evolution through relatively minor shifts in developmental processes without the need for dramatic genetic innovation (Thomas et al., 2000). In isolated situations, such as islands, woody perennials have evolved repeatedly from annual ancestors (Carlquist, 1974). For example, in various annual herbaceous lineages, such as Sonchus and Echium, woody perennial species evolved on isolated islands from their continental annual ancestors (Böhle et al., 1996; Kim et al., 1996; Groover, 2005). In several taxa of rosette-producing plants, e.g. Yucca-Agave (Schaffer and Schaffer, 1979), Espeletia (Smith, 1980; 1981) and Lobelia spp. (Young, 1984), the iteroparous species are found in the more mesic sites, while the semelparous species grow in the drier, rockier, i.e. more resource limited, sites (Young, 1981). Within the grass tribe Triticeae, the ancestral grass was a polycarpic perennial and the monocarpic annual habit is a derived trait (Chapman, 1996). Modern cultivars of domesticated Triticeae grains such as wheat (Triticum aestivum and Triticum durum), rye (Secale cereale), and barley (Hordeum vulgare), exhibit an extreme form of an annual monocarpic habit (Lammer et al., 2004). The wild progenitor of monocapic Oryza sativa is the Asian common wild rice, O. rufipogon, which shows a range of variation from perennial to annual types (Khush, 1997). The native monocarpic plant Common Evening Primrose (Oenothera biennis L., Onagraceae) exhibits phenotypic variation for annual vs. biennial flowering strategies. This variation in flowering strategy is correlated with genetic variation in relative fitness, and phenotypic and genotypic selection analyses revealed that environmental variation resulted in variable directional selection on annual vs. biennial strategies. Specifically, a biennial strategy was favoured in moderately productive environments, whereas an annual strategy was favoured in low-productivity environments (Johnson, 2007). The scarlet gilia (Ipomopsis aggregata) is normally semelparous but high-elevation plants may also be iteroparous. The facultative switch to iteroparity became more likely as the season progressed and as the abundance of pollinators declined. The probability of iteroparity increased significantly by excluding pollinators or removing flower buds early in the season (Paige and Whitham, 1987). In three of five sympatric Agave littaea (Agavaceae) species there was evidence of selection pressures towards semelparity because pollinators are selecting for taller inflorescences requiring a higher reproductive investment (Rocha et al., 2005). Individuals of the semelparous Yucca whipplei var. whipplei produce larger inflorescences, with more flowers and more seed-bearing fruits than do individuals of the iteroparous Y. whipplei var. caespitosa (Huxman and Loik, 1997). In Erysimum capitatum (Brassicaceae), plants in alpine environments are iteroparous perennials, but those below tree line are semelparous perennials (Kim and Donohue, 2011). Iteroparous plants produce more rosettes at the juvenile stage than do semelparous plants; those rosettes perennate, enabling subsequent reproductive episodes. Thus, the number of rosettes produced before reproduction is a strong determinant of parity. Under drought conditions, typical of the field sites of semelparous E. capitatum populations, juvenile plants with fewer rosettes had higher survival, and the physical excision of rosettes improved survival under drought stress in five of six natural populations. Because rosette production at the juvenile stage is necessary for iteroparity, these results demonstrate that drought-induced selection on traits that determine early survival has significant potential to influence the evolution of adult life-history expression (Kim and Donohue, 2012). In Arabidopsis thaliana the MADS box proteins SOC1 and FUL not only control flowering time, but also affect determinacy of all meristems. Downregulation of both proteins caused monocarpic Arabidopsis to assume a perennial lifestyle with asexual reproduction (Melzer et al., 2008). Aspects of the soc1 ful double mutants, such as vegetative buds, recurrent growth cycles, longevity and extensive woodiness, were reminiscent of plants with a perennial

life style. Similar phenotypic traits have also been acquired in Sy-0, a naturally occurring Arabidopsis accession from the Isle of Skye in which the expression of SOC1 and FUL is strongly reduced (Poduska et al., 2003; Wang Q et al., 2007). On the other hand, a mutation of a gene in a close relative of Arabidopsis, polycarpic Arabis alpina, caused A. alpina to phenocopy some but not all of the monocarpic habits of Arabidopsis (Wang et al., 2009). A single chromosome addition from a wild perennial relative in the genus Thinopyrum, Thinopyrum elongatum, confers a polycarpic, perennial habit to monocarpic wheat (Lammer et al., 2004). Given that there are typically both monocarpic and polycarpic species within the same plant family, and that their relationships indicate that transitions between monocarpy and polycarpy are common, the genetic differences between monocarpic and polycarpic species in a particular family are not extensive (Amasino, 2009).

Intriguingly, paleobiological evidence suggests that adaptations to an increasingly harsh environment in the Death Valley during the early Holocene (roughly 10,000 to 8000 years B.P.) favored a switch from iteroparity to semelparity for a small rodent, Neotoma lepida, the desert woodrat. This change of reproductive strategy was proposed based on spatial, temporal and physiological relationships between body size and temperature and on calculations using life tables and body size production allometries (Smith and Charnov, 2001).

Mathematical models concerning semelparity and iteroparity and their transitions (Cole, 1954; Charnov and Schaffer, 1973; Young, 1981; Bulmer, 1985; Benton and Grant, 1999; Ranta et al., 2002; Wilbur and Rudolf, 2006; Zeineddine and Jansen, 2009) consider these life history strategies as on a continuum of variables such as population growth rate, juvenile and adult survivorship, prereproductive development time, time between reproductive episodes, senescence and ecological variables. Obviously, both ecological conditions in the respective niches and the organisms' intrinsic constraints shape this multidimensional parameter space.

Cole compared the intrinsic growth rates of semel- and iteroparous species and concluded: "for an annual species, the absolute gain in intrinsic population growth which could be achieved by changing to the perennial reproductive habit would be exactly equalent to adding one individual to the average litter size" (p. 118, Cole 1954). Considering the ease at which an organism could increase offspring number by one, Cole reasoned that selection should favor semelparity. In the model of Young (1981), increasing values of population growth rate and juvenile survival favored semelparity, increasing values of adult survival and age of senescence favored iteroparity.

Bonnet (2011) proposed that if the costs independent of fecundity represent a major proportion of the total costs of reproduction compared to the costs that increase with offspring number, then it is profitable to maximize offspring number (fecundity) per reproductive event, resulting in semelparity. On the other hand, a high likelihood of survival is crucial for iteroparity to evolve. The stochasticity of the environment itself may suffice, under conditions of high adult survival, for iteroparity to evolve (Fischer et al., 2011). The model predicted that semelparity is particularly common in unstable, temporary habitats in which adult survival chances from one year to the next are low. The model also shows how properties of a stochastic environment critically influence whether organisms should be semelparous or whether, and to which degree, iteroparity is expected to evolve (Fischer et al., 2011).

Many traits are phenotypically discrete but may be polygenically or polyetiologically determined. Such traits can be understood using the threshold model of quantitative genetics that posits a continuously distributed underlying trait, called the liability, and a threshold of response: individuals above the threshold display one morph and individuals below the threshold display the alternate morph. Reproductive mode is a threshold trait (Roff 1996; 1998; Lesica and Young, 2005). The discontinuous variation in life history depends on both the genotype and the environment. The plasticity of semelparous and iteroparous reproduction strategies suggests semelparity and iteroparity as opposite ends of a continuum of life history variation rather than representing a simple dichotomy (Woolhouse, 1983; Pontier et al., 1993; Silvertown, 1996; Benton and Grant, 1999; Thomas et al., 2000; Boonstra et al. 2001; 2007; Hautekèete et al., 2001; Iguchi and Tsukamoto, 2001; Woods and Hellgren, 2003; Boonstra, 2005; Bonnet, 2011).

8. The costs of reproduction

…integrating an understanding of mechanisms into life history theory will be one of the most exciting tasks facing evolutionary biologists in the 21st century.
Barnes and Partridge (2003)

Summary

Trade-offs between reproduction and growth/survival are central to life history theory. The costs of reproduction shape such diverse life

history traits as reproductive strategies, e.g. semelparity and iteroparity, clutch size, brood care, mating behavior, and longevity. The costs are, at least in part, paid by a variety of progeriatric processes: increased resource utilization, stress susceptibility and accelerated immunosenescence and are orchestrated by antagonistic pleiotropic actions of reactive oxygen species as the final common pathway of life-death decisions. The costs of reproduction may be highly variable and modulated by resource availability and overridden by heterogeneity in individual quality. Germ cells and their signaling mediate at least part of the costs possibly independent of the energetic costs of reproductive activity.

Reproduction and survival compete for limited resources and entail direct and indirect costs to each other, constraining the evolution of a Darwinian demon. Life history theory very early linked reproduction and survival in a trade-off (MacArthur and Wilson, 1967; Pianka, 1970; Stearns, 1977; 1991): reproductive effort compromises the survival of the parent organism (Roff, 1992, Stearns, 1992; Charlesworth, 1994). In this context, I like the verb “to compromise” for its connotation. Unlike its thesaurus alternatives “impair, harm, hurt, endamage, endanger” it also has the connotation of “strike a balance, make an arrangement”. In fact, the implications of a trade-off are similar, that an advantage is traded against a disadvantage. However, the “balance” in this trade-off is highly biased: evolution “arranged” that the germline cells are the beneficiaries and, most often, the soma is the one who pays the costs (see chapter 11).

The DST of aging (Kirkwood, 1977; Kirkwood and Holliday, 1979) introduced the concept that aging is driven by a conflict concerning the allocation of resources between reproductive activity and the maintenance of the soma. This theory already (although later than life history theorists) perceived a conflict between reproduction and the maintenance of the soma. However, it put this conflict into the context of the ETAs, linking the highly programmed process of reproduction with an allegedly stochastic process of somatic maintenance (Kirkwood, 1999; Finch and Kirkwood, 2000).

However, that reproduction by far exceeds the more passive role, assigned by the ETAs by merely taking the force of selection pressure from the soma, is supported by multiple lines of evidence.

* Inhibiting the signaling of germ cells by gonadectomy prolongs the lifespan of the soma in both semelparous and iteroparous organisms by a mechanism that is not resource related (Barnes and Partridge, 2003; Baumeister et al., 2006). The latter notion is based on proximate mechanisms, particularly the finding that lifespan extension is not a simple consequence of sterility but is due to germline signaling (Barnes and Partridge, 2003; Baumeister et al., 2006) contradicting the DST, but does not contradict ultimate evolutionary rationales of aging/death as elaborated in this paper.

* The reproductive event causes somatic death in semelparous organisms (see chapter 7).

* There is a wealth of data on the cost of reproduction in iteropaous organisms in terms of trade-offs. Authors often, although they agree that there are costs, disagree on the size of costs (Reznick, 1985; Harshman and Zera, 2007). For iteroparous organisms, the costs of reproduction for the soma may manifest in a, compared to semelparous organisms, protracted course of aging. In the short or long run reproductive activity, however, is fatal for the soma. Demonstrating that semelparity and iteroparity are no unrelated reproduction strategies (as is maintained by proponents of the ETAs), but threshold traits above a continuum of underlying “liabilities” (see chapter 7.2), supports the notion of shared physiological processes causing aging and death.

So far, fitness is often understood in terms of its two basic components: survival and reproduction (but see chapter 19.1). Investment in one component has costs for the other, leading to trade-offs in fitness components. A wealth of findings show that fitness trade-offs underlie the evolution of diverse life-history traits in extant organisms (Charlesworth, 1980; 1994; Roff, 1992; Stearns, 1992; Flatt and Heyland, 2011). The costs of reproduction shape such diverse life history traits as reproductive strategies, e.g. between semelparity and iteroparity, clutch size, brood care, mating behavior, and longevity. Clearly the cost of reproduction in semelparous organisms is death. For iteroparous organisms, the costs are less evident. Selection experiments revealed that there is an energetic basis underlying the trade-off between fecundity and longevity (Service, 1987; Djawdan et al., 1996). In birds, for instance, reproduction is associated with significant metabolic costs. Because most avian species maintain atrophied reproductive organs when not active, reproduction requires major tissue remodeling in preparation for breeding. Females undergo rapid (days) recrudescence and regression of their reproductive organs at each breeding attempt, while males grow their organs ahead of time at a much slower rate (weeks) and may maintain them at maximal size throughout the breeding season. Egg production leads to a 22%–27% increase in resting metabolic rate over non-reproductive values. In male birds, gonadal recrudescence may lead to a 30% increase in resting metabolic rate (Vézina and

Salvante, 2010). Quite obviously, for non-avian taxa reproduction also affords a high investment (for the joint cost of gamete production, gestation/brood care and the behavioral activities associated with reproduction) in terms of energy and resource utilization, restricting, on the other hand, somatic growth (Williams, 1966; Calow 1979; Bell, 1980; Reznick, 1983; Bell and Koufopanou, 1986; Berglund and Rosenqvist, 1986; Loudon and Racey, 1987; Gittleman and Thompson, 1988; Reiss, 1989; Geber et al., 1999; Rocheleau and Houle, 2001; Barnes and Partridge, 2003; Lester et al., 2004; Wheelwright and Logan, 2004; Roff et al., 2006; Speakman, 2008; Szymanski et al., 2009; Bergeron et al., 2011). Less palpable are costs in terms of immunocompetence (Gustafsson et al., 1994; Richner et al. 1995; Ots and Hõrak, 1996; Sheldon and Verhulst, 1996; Deerenberg et al., 1997; Nordling et al., 1998; Siva-Jothy et al., 1998; Klein and Nelson, 1999; Norris and Evans, 2000; Adamo et al., 2001; Hasselquist et al., 2001; McKean and Nunney, 2001; 2008; Herms, 2002; Rolff and Siva-Jothy, 2002; Zuk and Stoehr, 2002; Ardia et al., 2003; Hanssen et al., 2003; 2005; Fedorka et al., 2004; 2007; Ardia, 2005; Greenman et al., 2005; Gwynn et al., 2005; Bourgeon and Raclot, 2006; Greives et al., 2006; Carney, 2007; French et al., 2007; Harshman and Zera, 2007; Miyata et al., 2008; Speakman, 2008; Knowles et al., 2009; 2010; Cox et al., 2010; Mills et al., 2010; Short and Lazzaro, 2010; Fleury and Huvet, 2012), susceptibility to stress and oxidative stress (Boonstra et al., 2001; Salmon et al., 2001, Wang et al., 2001; Wingfield and Sapolsky, 2003; Alonso-Alvarez et al., 2004; 2006; Koochmeshgi et al., 2004; Wiersma et al., 2004; Bertrand et al., 2006; Klose et al., 2006; Delaporte et al., 2007; Harshman and Zera, 2007; Rush et al., 2007; Samain et al., 2007; Bizé et al., 2008; Monaghan et al., 2009; Bergeron et al., 2011), locomotor performance (Miles et al., 2000; Speakman, 2008), and predation risk (Tuttle and Ryan, 1981; Berglund and Rosenqvist, 1986; Svensson, 1988; Magnhagen, 1991, Pavlová et al., 2010; Bonnet, 2011). These different costs may add up to decrease survival in iteroparous animals (Snell and King, 1977; Bell, 1980; Partridge and Farquhar, 1981; Clutton-Brock et al., 1982; Tuomi et al., 1983; Bell and Koufopanou, 1986; Gunderson and Dygert, 1988; Svensson, 1988; Martin, 1995; Sinervo and DeNardo, 1996; Gunderson, 1997; Sgró and Partridge, 1999; Miles et al., 2000; Hunt et al., 2002; Barnes and Partridge, 2003; Koivula et al., 2003; Lester et al., 2004; Stjernman et al., 2004; Davies et al., 2005; Liker and Székely, 2005; Partridge et al., 2005; Paukku and Kotiaho, 2005; Beauplet et al., 2006; Michod et al., 2006; Roff et al., 2006; Delaporte et al., 2007; Hadley et al., 2007; Proaktor et al., 2007; Townsend and Anderson, 2007; Creighton et al., 2009; Descamps et al., 2009; Buoro et al., 2010; Cox et al., 2010; Cox and Calsbeek, 2010; Dao et al., 2010; Hamel et al., 2010, Papadopoulos et al., 2010; Edward and Chapman, 2011; Fisher and Blomberg, 2011; Flatt, 2011). However, in humans the data are not consistent (Le Bourg et al., 1993; Westendorp and Kirkwood, 1998; Lycett et al., 2000; Korpelainen, 2000; Thomas et al., 2000; Lummaa, 2001; Muller et al., 2002; Doblhammer and Oeppen, 2003; Dribe, 2004; Hurt et al., 2006; McArdle et al., 2006; Le Bourg et al., 2007; Penn and Smith, 2007; Rickard et al., 2007; Gagnon et al., 2009; Kuningas et al., 2011).

The costs of reproduction may be highly variable and modulated by resource availability (Lycett et al., 2000; Dribe 2004; McKean and Nunney, 2005), those breeding in favorable circumstances, at least at face value, being 'immune' to reproductive costs (Chippindale et al., 1993; Tatar and Carey, 1995; Carey et al., 2002; Messina and Fry, 2003; McKean and Nunney, 2005). Moreover, its detection may be overridden by heterogeneity in individual quality (Ardia, 2005; Beauplet et al., 2006; Le Bohec et al., 2007; Risch et al., 2007; Weladji et al., 2008; Hamel et al., 2009; Papadopoulos et al., 2010), local environmental effects (Sinervo and DeNardo, 1996), and transfer of the costs to offspring (Martin and Festa-Bianchet, 2010). Similarly, in laboratory animal populations, lifespan and reproductive investment can be decoupled in benign circumstances (Reznick et al., 2000; Tatar et al., 2003; Partridge et al., 2005; Ricklefs and Cadena, 2007; Lee et al., 2008; Flatt, 2011). Moreover, wild invertebrate populations may be able to avoid the costs of reproduction (Spitze 1991; 1995; Spitze et al., 1991; Lynch and Spitze, 1994; Roff, 2000; Vorburger, 2005) depending on resource availability. The route to a Darwinian demon is paved by resource abundance (see chapter 4), should evolution not have "taken precautions".

As will be discussed in the following chapters, the growth/survival costs of reproduction are, at least in part, caused by a variety of progeriatric processes: increased resource utilization (chapter 9), stress susceptibility (chapter 10) and accelerated immunosenescence (chapter 11).

Interpretation of any costs of reproduction is difficult in plants because of (1) potentially low or restricted resource investment in reproduction (Jennersten, 1991; Hemborg and Karlsson, 1998); (2) relative to the expense for single reproduction, possession of a large resource budget (Primack and Hall, 1990; Ehrlén and van Groenendael, 2001) that can be provided from source organs (e.g., mature leaves or roots) according

to the demands of sink organs (e.g., developing seeds) (Marshall 1990, Wardlaw 1990); (3) formation of physiologically integrated modular structures (Sprugel et al., 1991; Obeso, 1998); (4) compensative acceleration of photosynthetic abilities during reproduction (Hansen 1970; Herold, 1980; Fujii and Kennedy 1985; Lehtilä and Syrjänen, 1995); and/or (5) photosynthetic function of reproductive organs (Bazzaz et al., 1979; Jurik, 1985; Brenchley et al., 1996; Antlfinger and Wendel, 1997; Guido and Hardy, 2003; Horibata et al., 2007). Thus, reproduction may have little negative effect on growth and may even enhance growth in favorable environments (Reekie and Bazzaz 1987; Lehtilä and Syrjänen 1995). Increased probability of detecting a cost of reproduction with increasing environmental stress has been confirmed at least in some cases (Primack and Antonovics, 1982; Biere, 1995; Sandvik, 2001; Rose et al., 2009), but not in others (Reekie, 1991; Cheplick, 1995; Saikkonen et al., 1998). Possibly, there is some threshold level of reproductive effort vs. available resources above which costs are realized (Tuomi et al., 1983). Reproductive allocation is higher in annual than in perennial plants (Karlsson and Méndez, 2005) and higher in herbs than in woody plants (Cheplick, 2005). A demographic classification of life histories also indicates differences between short-lived herbs, forest herbs, shrubs, and trees in the relative importance of fecundity and survival for lifetime fitness (Franco and Silvertown, 1997). Thus, long-lived species might show costs of reproduction in terms of growth or fecundity but not in survival, which would be prioritized in this life strategy. On the other hand, short-lived species would be more prone to survival costs due to high reproductive investment and priority of fecundity and growth in this life history strategy (Aragón et al., 2009). Hence, only inconsistently, reproductive costs on growth (Freeman et al., 1976; Harper, 1977; Lloyd and Webb, 1977; Samson and Werk, 1986; Ågren, 1988; Krischik and Denno, 1990a, b; Elmqvist et al., 1991; Allen and Antos, 1993; Silvertown and Dodd, 1999) and survival in iteroparous plants have been demonstrated (Sarukhán, 1976; Harper, 1977; Piñero et al., 1982; Bierzychudek and Eckhart, 1988; Krischik and Denno, 1990; Éscarre and Houssard, 1991; Rameau and Gouyon, 1991; Allen and Antos, 1993; Dawson and Ehleringer, 1993; Silvertown and Dodd, 1999; Obeso, 2002; Espírito-Santo et al., 2003; Sletvold and Ågren, 2011). In 65 species of iteroparous perennial plants a positive relationship between rate of senescence and reproductive lifespan has been demonstrated, suggesting increasing risk of death with successive reproductive events (Silvertown et al., 2001). Clonal growth is a necessary but not sufficient condition to prevent the evolution of senescence as cost of reproduction. Clones that fragment are more likely to escape the evolution of senescence at the genet level than clones that remain physiologically integrated (Silvertown et al., 2001).

Oxidative stress is a central mediator in the reproductive events of B. subtilis (Hecker and Völker, 1990; Storz and Hengge-Aronis, 2000; Smits et al., 2005; Ryu et al., 2006; Tam le et al., 2006), Myxococcus (Storz and Hengge-Aronis, 2000; Otani et al., 2001; Viswanathan et al., 2006), Streptomyces (Cho et al., 2000; Storz and Hengge-Aronis, 2000; Lee et al., 2005), yeast and fungi (Hohmannn and Mager, 2003; Aguirre et al., 2005; Takemoto et al., 2007; Scott and Eaton, 2008), Dictyostelium (Bloomfield and Pears, 2003; Garcia et al., 2003), and Botryllus schlosseri (Lauzon et al., 2002 ; Rosner et al., 2007). There is multifaceted evidence that, as a legacy to the primordial reproductive events, the trade-offs between life-history traits are orchestrated by antagonistic pleiotropic actions of reactive oxygen species (Alonso-Alvarez et al., 2006; Costantini, 2008; Dowling and Simmons, 2009; Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010; Isaksson et al., 2011) as the final common pathway of life-death decisions (Heininger, 2001).

8.1 Germ cells exact at least part of the costs of reproduction

There are multiple examples that removal of germline cells or inhibition of their signaling extends the somatic lifespan in a variety of plants (Wareing and Seth, 1967; Noodén, 1980) and animals (Guarente and Kenyon, 2000). If the cap of the unicellular giant algae, Acetabularia mediterranea and acetabulum, containing the spores is amputated, cell death can be prevented. The nucleus residing in the base of the cell regenerates a new cap. By repetitive amputations of the cap the lifespan of the giant algae can be prolonged substantially (Hämmerling, 1963; Runft and Mandoli, 1996). Extirpation of all buds except one from adult zooids of Botryllus schlosseri significantly increases the lifespan of the adult generation and of its buds, as well as the size of the buds and their blastogenetic potential (Manni et al., 2007). As early as 1821, Reichart found that when flower buds were removed from the Vienna wall flower, normally an annual plant, this plant would continue vegetative growth for two or more years. When flowers were allowed to develop, the plant would rapidly set fruit and die. Numerous workers have substantiated and extended these results with other species: Mattirolo (1899) with leguminous plants; Mason (1922) with Sea

Island cotton; Murneek (1926) with tomato (Lycopersicon esculentum, Mill.); Leopold et al. (1959), Lindoo and Noodén (1977) with soybean; Leopold et al. (1959) with spinach; Wareing and Seth (1967) with annual bean plants; Malik and Berrie (1975) with peas (Pisum sativum L.); and many other authors. Noodén and Murray (1982) depodded (deseeded) a soybean plant except for a single pod cluster in the center of the plant. The pod cluster induced yellowing of the nearest leaf even if the petiole contained a zone of dead phloem (that was inactivated with a jet of steam), whereas most of the rest of the plant remained green. A similar treatment of a single leaf on a fully depodded plant (leaves stay green) did not cause that leaf to turn yellow. Since nutrients would have to be withdrawn from the leaves via the phloem, the pods do not induce yellowing by pulling nutrients out of the leaf and must be able to exert their influence via the xylem. This indicates that pods induce senescence by producing a dead signal or a poison killing leaves (Noodén and Murray, 1982; Skulachev, 2011) (see chapter 11.2.3). Sterile mutants of the monocarpic plant Arabidopsis thaliana are also longer-lived (Noodén and Penney, 2001). Murneek (1932) proposed that the developing embryos induce senescence in the parent plant due to a competition by the embryos for food reserves. Delay of sexual maturation or reproductive phase in Nereis and C. elegans slows aging (Johnson et al., 1984). Sterile mutants of S. cerevisiae (Kennedy et al., 1995) and C. elegans (Van Voorhies, 1992) have an increased lifespan. This lifespan extension is not a simple consequence of sterility but is due to germline signaling, as removing the entire gonad (the germ cells as well as the somatic reproductive tissues) does not extend lifespan (Hsin and Kenyon, 1999). Thus, the costs of reproduction in C. elegans have been considered not resource-based (Barnes and Partridge, 2003). Starvation of a mature specimen of a flatworm leads to regression to an immature stage and extension of lifespan (Kozloff et al., 2000). Lifespan in lubber grasshoppers has been shown to be increased by ovariectomy (Drewry et al., 2011). Lifespan extension was observed in virgin (Maynard Smith, 1958; Fowler and Partridge, 1989; Chapman et al., 1995) or mated Drosophila females that lay eggs at a reduced rate (Partridge et al., 1987), and sterile Drosophila mutants (Maynard Smith, 1958; Sgró and Partridge, 1999). Similarly, unmated adult male and female stink bugs lived 6 and 2 times longer, respectively, than mated. The average lifespan for virgin females was 60 days compared with 30 days for mated females (Mitchell and Mau, 1969). Delayed reproductive maturation and reduced fecundity due to reduction-of-function mutants in the insulin/IGF signaling pathway extend the lifespan of C. elegans (Friedman and Johnson, 1988) and Drosophila (Clancy et al., 2001; Tatar et al., 2001). Pharmacological acceleration of reproductive maturation, on the other hand, normalizes longevity in these mutants (Tatar et al., 2001b). The removal of the corpora allata, source of the juvenile hormone that controls ovary maturation, doubles adult insect longevity (Tatar and Yin, 2001). Ovariectomized female grasshoppers lived significantly longer than sham treated females (Hatle et al., 2008). The female Octopus hummelincki lays eggs, broods them, reduces its food intake, and dies after the young hatch. Removal of both optic glands (which promote gonad development) in Octopus after spawning results in cessation of broodiness, resumption of feeding, increased growth, and greatly extended lifespan (Wodinsky, 1977). In Anolis lizards, elimination of reproductive investment via surgical ovariectomy and/or removal of oviductal eggs increased breeding-season survival by 56%, overwinter survival by 96%, and interannual survival by 200% relative to reproductive controls (Cox and Calsbeek, 2010). Robertson (1961) showed that the maturation of gonadal tissue and not the effort related to actual spawning leads to degeneration and death and that if sexual development is prevented by castration kokanee salmon can survive well beyond the normal lifespan of this species. Wild eel usually spawn and die at about ten years and never more than twice this age (Frost, 1950). European eel in captivity, the spawning migration to the Sargasso Sea being evidently impossible, may live up to 55 years (Comfort, 1956). Importantly, the energetic cost of the 6,000-km migration is actually quite low (van Ginneken and van den Thillart, 2000; van Ginneken and Maes, 2005) and cannot be assumed to be the cause of the postreproductive death. When castrated, semelparous marsupial male mice survive more than one breeding season (Lee and Cockburn, 1985). Likewise, prevention of the frenzied sexual activity of male marsupial mice delays death (Woolley, 1966). Following castration, both inbred male rats (Talbert and Hamilton, 1974; Drori and Folman, 1976), female and male cats and dogs live several years longer than their intact counterparts (Hamilton, 1974; Bronson, 1981) all of which argues for the phylogenetic conservation of these mechanisms (but see Comfort, 1956). Castration may also extend life in male humans (Hamilton and Mestler, 1969) although evidence for this effect is inconsistent (Heriot, 1956; Nieschlag et al., 1993; Jenkins, 1998). Obesity which is a common phenomenon in human and pet castrates (Hausberger

and Hausberger, 1966; Root Kustritz, 2007) has a progeroid action (see chapter 9.3) and may counterbalance the life-extending action of castration in humans and pets.

9. Reproduction and aging/death: modulated by resource availability and utilization

We are killing ourselves with knife and fork -Popular wisdom

Summary

Rooted in a metabolic stress response, the dual reproduction/death trajectories are subject to sensing of resource availability and utilization. A phylogenetically ancient response to resource limitation, metabolic dormancy, delays reproduction and slows or even halts aging. Dietary restriction mimicks the metabolic dormancy response to resource limitation in laboratory animals and is the most robust lifespan-extending method. Target of rapamycin (TOR) and insulin-like signaling are eukaryotic and metazoan monitors of resource utilization. Both signaling pathways antagonistically regulate reproduction and somatic maintenance: increased signaling advances reproductive activity and accelerates somatic aging. Loss-of function mutations, on the other hand, slow aging. Moreover, other signaling pathways such as AMP kinase and sirtuins link metabolic and stress response pathways to reproductive activities and aging trajectories. In addition to caloric nutrients, another limited resource, phosphate, is an essential modulator of lifespan.

From yeast, nematodes and insects to mammals, utilization of resources has a profound impact both on reproductive maturation, reproductive events and aging trajectories. Max Rubner (1908) in his exploration of the relationship of metabolic rate (oxygen consumption) and body mass, first noted the now well-known inverse correlation between the rate of oxygen consumption and the longevity in eutherian mammals. This observation was expanded further by Pearl (1928), in the so-called rate-of-living hypothesis, which proposed that longevity is inversely proportional to metabolic rate. Although disproved in its narrow sense (Sohal, 1986; Arking, p. 417 ff), the theory pioneered the concept of an association between metabolism and aging. Extending these concepts, the Dynamic Energy Budget theory linked energy metabolism and oxidative damage to organismal aging (Kooijman, 2000; van Leeuwen et al., 2002; 2010; Sousa et al., 2010). Evolution 'established' a link between reproduction and aging/death via the sensing of resource utilization of the individual organism. The metabolic control of aging by nutrient sensing pathways is the genetic signature of the evolutionary rationale of aging/death that evolved as a transgenerational conflict over resource allocation between germ cells and soma. Not only nutrients are resources at short supply. As discussed in chapter 9.4, phosphate as a limited resoure is an equally potent progeroid agent. These links make perfect sense in a world of limited resources where the death of the ancestors aimed at conserving the vital resources for the progeny, and the evolutionary value and eventual "redundancy" of the ancestor is judged by the amount of resources it has utilized during its lifetime.

9.1 Diapause and hibernation: metabolic dormancy as response to environmental dearth

Starvation is a key environmental factor for bacteria to divert investment from replication toward formation of metabolically dormant spores. Dormancy is a bet-hedging strategy used by a wide range of taxa, including microorganisms. It refers to an organism's ability to enter a reversible state of low metabolic activity when faced with unfavorable environmental conditions. Dormant microorganisms generate a seed bank, which comprises individuals that are capable of being resuscitated following environmental change (Lennon and Jones, 2011). Bacterial spores allow long-term survival, as they are the most resistant cell type known so far (Nicholson et al., 2000). Two hundred and fifty million years of dormant state in a kind of 'bacterial hibernation', followed by successful germination, has been claimed for spores of a Bacillus species (Vreeland et al., 2000). Similarly, as response to harsh environmental conditions multicellular organisms may exhibit developmental arrest and metabolic inhibition during diapause, dauer formation, anaerobiosis, torpor, hibernation or estivation (Storey and Storey, 1990; MacRae, 2005). The response may be either behavioral in response to, or genetically predetermined in, anticipation of adversity. Facultative metabolic rate depression is the common adaptive strategy of anaerobiosis, hibernation, and estivation, as well as a number of other arrested states. By reducing metabolic rate by a factor ranging from 5 to 100 fold or more within months or even years of dormancy, animals gain an extension of survival time. Thus, some ectotherm animals are able to halt reproduction and aging, at least temporarily, during

diapause (Tatar et al., 2001a; Baumeister et al., 2006; Allen, 2007; Fielenbach and Antebi, 2008; Schmidt and Paaby, 2008; Sim and Denlinger, 2008). Reproductive diapause is a response to metabolic stress in nematodes and insects (Riddle and Albert, 1997; Renfree and Shaw, 2000; Tatar and Yin, 2001; Lopes et al., 2004) and slows aging in molluscs, nematodes and a variety of arthropods (Riddle and Albert, 1997; Tatar and Yin, 2001; Tatar et al., 2001a; MacRae, 2005; Hahn and Denlinger, 2007; Schmidt and Paaby, 2008; Abele et al., 2009). In endotherms, metabolic dormancy during hibernation and estivation also delays aging (Heininger, 2000b; Brunet-Rossinni and Austad, 2004; Bieber and Ruf, 2009; Golub, 2010; Storey, 2010; Turbill et al. 2011).

9.2 Dietary restriction, aging and reproduction

A strong support for the relationship between resource utilization and aging came from dietary restriction (DR) studies (Weindruch and Walford, 1988; Heininger, 2002a; Masoro, 2002; Fontana et al., 2010). DR mimicks in the laboratory the hypometabolic or dormant state that nature programmed in response to harsh environmental conditions: diapause, dauer formation, anaerobiosis, torpor, hibernation or estivation (Storey and Storey, 1990). Reduced dietary intake consistently retards aging and increases lifespan in yeast (Lin et al., 2000; Reverter-Branchat et al., 2004; Fabrizio et al., 2003; 2005; Jiang et al., 2000; Powers et al., 2006; Kaeberlein et al., 2007; Smith et al., 2007), rotifers (Kirk, 1997; Yoshinaga et al., 2000; Weithoff, 2007; Ozdemir, 2009; Kaneko et al., 2011), daphnia (Ingle et al., 1937), nematodes (Hosono et al., 1989; Gerhard; 2001; Houthoofd et al., 2002; Sutphin and Kaeberlein, 2008), arthropods (Austad, 1989; Chapman and Partridge, 1996; Sohal and Weindruch, 1996; Gerhard; 2001; Mair et al., 2003; Partridge et al., 2005; Bass et al., 2007; Grandison et al., 2009a), fishes (Comfort, 1963; Weindruch and Walford, 1988; Patnaik et al., 1994; Gerhard; 2001) and mammals (Weindruch and Walford, 1988; Masoro, 1998; Wanagat and Weindruch, 2000).

DR results in vertebrate animals that are mostly infertile, although there is some variation depending on the sex, the degree of restriction, and the age at which restriction is first applied (Weindruch and Walford 1988; Masoro, 2002; Martin et al., 2008; Selesniemi et al., 2008). For example, in mice DR by 40% arrests follicular cycles in females (Nelson et al. 1985), whereas in female rats subjected to the same level of restriction, some reproductive activity continues (McShane and Wise 1996). Puberty in female rodents is delayed and reproductive senescence occurs later (Holehan and Merry, 1985; Nelson et al. 1985). On refeeding, female rodents previously kept on restricted diets are able to reproduce at much later ages than fully fed controls (Holehan and Merry 1986; Selesniemi et al., 2008). Spermatogenesis in male rats is shut down if moderate DR is experienced early in life but this does not happen if DR is experienced in late life (Graves, 1993). DR also reduced fecundity, although inconsistently, in flies (Chippindale et al., 1993; Graves, 1993; Chapman and Partridge, 1996; Carey et al., 1998; Grandison et al., 2009b) and C. elegans (Klass, 1977; Kozloff et al., 2000; Mair et al., 2009). With the opportunity for mating and reproduction, fly reproduction continued across all diet levels but carried costs in terms of increased mortality (Carey et al., 2002).

The DR effect is the oldest, most robust, and best-known intervention to increase lifespan. Its life-extending effect, however, is irreconcilable with the DST (Mitteldorf, 2001; Blagosklonny, 2007). A modification of the DST that was designed to comply with the experimental DR data (Shanley and Kirkwood, 2000) also could not stand up to scrutiny (Mitteldorf, 2001). It has been shown experimentally that reallocation of nutrients to the soma does not explain the responses to DR (Grandison et al., 2009b; Tatar, 2011). Drosophila on lifespan-extending DR, although having greater allocation of ingested nutrients to the soma relative to the allocation to reproduction (Min et al., 2006; O'Brien et al., 2008), have a reduced absolute allocation to the soma in comparison to flies on full diets (O'Brien et al., 2008). Moreover, relative resource allocation to somatic tissues was not affected by ovariectomy in grasshoppers arguing against the prediction that enhanced storage in ovariectomized females results from a physiological shift in allocation of ingested nutrients (Judd et al., 2011). If aging was a matter of insufficient energy allocation to both reproduction and tissue repair then more food energy should lessen the need for compromise, and, as predicted by the DST, the body should be able to both live longer and increase fertility. However, contrary to this prediction abundant utilization of resources, particularly glucose, shortens life expectancy in such diverse organisms as yeast (Longo and Fabrizio, 2002; Fabrizio et al., 2003; 2005; Kaeberlein et al., 2005; Powers et al., 2006; Roux et al., 2009; Ruckenstuhl et al., 2010), Daphnia (Lynch and Ennis, 1983), C. elegans (Schulz et al., 2007; Lee et al., 2009; Porter Abate and Blackwell, 2009; Schlotterer et al., 2009), flies (Foley and Luckinbill, 2001; Bross et al., 2005; Carey et al., 2008; Skorupa et al., 2008), and mammals (Cerami, 1985; Mlekusch et al., 1996; Weindruch and Sohal, 1997; Archer, 2003; Kassi and

Papavassiliou, 2008; Huffman and Barzilai, 2009; Nunn et al., 2009; Pi-Sunyer, 2009). High glucose induces activation of a signaling cascade that mediates premature senescence in mammalian skin fibroblasts and endothelial cells (Blazer et al., 2002; Yokoi et al., 2006), demonstrating the evolutionary conservation of this abundance signal. Even food-associated olfaction has been able to shorten lifespan, while loss-of-function mutations in olfactory and gustatory neurons extended lifespan (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Libert et al., 2007). Although not consistently (see Masoro, 2006), adult- and late-life-onset DR is able to delay aging-related changes, morbidity and longevity in worms, flies and mammals (Yu et al., 1985; Pugh et al., 1999; Lane et al., 2000; Cao et al., 2001; Berrigan et al., 2002; Goto et al., 2002; Mair et al., 2003; Dhahbi et al., 2004; Magwere et al., 2004; Rae, 2004; Lenaerts et al., 2007; Smith et al., 2008; Sharma et al., 2010; Wang et al., 2010; Cameron et al., 2011). Thus, it is incompatible with the DST that: (i) reallocation of nutrients to the soma does not explain the responses to DR and ovariectomy; (ii) when resources are abundant enough to satisfy the energetic requirements of both reproduction and survival, longevity is reduced, i.e. tissue maintenance and repair fail prematurely. (iii) Even postreproductive organisms that are no longer required to invest resources into reproduction, live longer when exposed to depleted resources.

9.3 Nutrient and energy-sensing pathways, reproduction and aging

Reproduction is an energetically costly process (Szymanski et al., 2009) and its linkage to nutrient sensing is evolutionarily conserved (Lindsley and Rutter, 2004; Walker et al., 2005; Narbonne and Roy, 2006a; Hietakangas and Cohen, 2009; Jasper and Jones, 2010). Insulin-like and TOR signaling are the bodies' monitors of resource utilization. Insulin signaling is a conserved feature in all metazoans (Barbieri et al., 2003; Piper et al., 2008). Insulin, insulin-like growth factor (IGF) and insulin/IGF-like signaling (IIS) evolved with the appearance of multicellularity, allowing primordial metazoans to respond to a greater diversity of environmental signals. The IIS pathway is split into two complementary and interacting subsystems. The functional separation of IGF and insulin signaling that is seen in mammals dates to approximately 600 million years ago, as the two distinct types of molecules are already present in the lower metazoan tunicate phylum (McRory and Sherwood, 1997). Insects have a single insulin/IGF system that may correspond to the ancestor of the dual insulin/IGF system. The pathway has diverse functions in multicellular organisms, and mutations in IIS can affect growth, development, metabolic homoeostasis, fecundity and stress resistance, as well as lifespan (Broughton and Partridge, 2009). Insulin and insulin-like messengers signal nutritional status to the reproductive axis and thus regulate reproductive functions such as maturation and gametogenesis in nematodes (Tissenbaum and Ruvkun, 1998; Dillin et al., 2002; Narbonne and Roy, 2006a; Edmonds et al., 2010; Korta et al., 2010; Luo et al., 2010; Michaelson et al., 2010), insects (Riehle and Brown, 1999; Drummond-Barbosa and Spradling, 2001; Garofalo, 2002; Drummond-Barbosa and Spradling, 2004; Manière et al., 2004; 2009; LaFever and Drummond-Barbosa, 2005; Richard et al., 2005; Tu et al., 2005; Narbonne and Roy, 2006a; Hsu et al., 2008; Arik et al., 2009; Hsu and Drummond-Barbosa, 2009), fishes (Sang et al., 2008; Reinecke, 2010), amphibians (El-Etr et al., 1979; Chuang et al., 1993; Liu et al., 1995; Sadler et al., 2010), reptiles (Sparkman et al., 2009; 2010), birds (McMurtry et al., 1997; Yun et al., 2005; Li ZH et al., 2006; Ou et al., 2009; Tang et al., 2010), and mammals (Zhou and Bondy, 1993; Adashi et al., 1997; Zulu et al., 2002; Todd et al., 2007; Velazquez et al., 2008; Ivell and Anand-Ivell, 2009). Both at the gonadal and CNS/hypothalamic level, the insulin/IGF system is involved in germ cell maturation and reproductive functions in all invertebrate and vertebrate taxa (Hsin and Kenyon, 1999; Wang and Chard, 1999; Brüning et al., 2000; Burks et al., 2000; Lackey et al., 2000; Narbonne and Roy, 2006a; Jasper and Jones, 2010).

A conserved phosphatidylinositol 3-kinase pathway (AGE-1/PI3K) acts downstream of the insulin/insulin-like growth factor (IGF)-1 receptor homolog DAF-2 in C. elegans (Morris et al. 1996). First evidence that aging is regulated by canonical signaling pathways came from age-1 mutants of C. elegans that increase C. elegans lifespan greater than twofold (Klass 1983; Friedman and Johnson, 1988; Larsen et al. 1995; Morris et al. 1996). Thereafter, it was demonstrated that a variety of loss-of-function mutations in the growth hormone (GH), insulin and IGF-like signaling pathways or inhibitors of the pathways extend the lifespan in rotifers (Yoshinaga et al., 2005), nematodes (Kenyon et al., 1993; Vanfleteren and De Vreese, 1995; Kimura et al., 1997; Lin et al., 1997; Ogg et al., 1997; Tissenbaum and Ruvkun, 1998; Vanfleteren and Braeckman, 1999; Guarente and Kenyon, 2000; Wolkow et al., 2000; Cowen, 2001; Gems and Partridge, 2001; Kenyon, 2001; Ayyadevara et al., 2008; Kwon et al., 2010), flies (Clancy et al., 2001; Tatar et al., 2001; 2003; Bartke, 2001; Broughton et al., 2005; Fridell et al., 2009;

Toivonen and Partridge, 2009), and mice (Brown-Borg et al. 1996; Flurkey et al. 2001; 2002; Blüher et al., 2003; Coschigano et al., 2003; Holzenberger et al., 2003; Taguchi et al., 2007; Selman et al., 2008; 2011). In human populations, genetic polymorphisms in IIS pathways are associated with longevity (Bonafe et al., 2003; Kojima et al., 2004; Lunetta et al., 2007; Hong et al., 2008; Suh et al., 2008; Willcox et al., 2008; Anselmi et al., 2009; Flachsbart et al., 2009; Li et al., 2009; Pawlikowska et al., 2009; Chung et al., 2010; Soerensen et al., 2010; Guevara-Aguirre et al., 2011). A comprehensive screen of aging/longevity signaling pathways in C. elegans revealed a germline-dependent and a germline-independent IIS affecting longevity (Hansen et al., 2005). IIS signaling controls aging not only at the systemic level but also at the level of the individual organ function such as the heart (Wessells et al., 2004).

The TOR signaling pathway is a serine/threonine kinase of the phosphatidylinositol kinase-related kinase family and is phylogentically even older than the IIS pathway, being evolutionarily conserved in eukaryotes from unicellular algae to plants and from yeast to man (Bögre et al., 2003; Crespo et al., 2005; Jacinto and Lorberg, 2008; Wang and Proud, 2009). In the resource-utilization dependent regulation of reproductive activity, TOR has a role in reproductive functions of yeast (Weisman et al., 2001), plants (Menand et al., 2002; 2004; Deprost et al., 2005; 2007; Guiboileau et al., 2010; Ren et al., 2011), arthropods (Hansen et al., 2004; Zhang et al., 2006; Arsic and Guerin, 2008; Maestro et al., 2009; LaFever et al., 2010; Roy and Raikhel, 2011), amphibians (Schwab et al., 1999), and mammals (Sananes et al., 1998; Walensky et al., 1998; Skrzypek and Krause, 2007; Chen et al., 2009; Roos et al., 2009; Roa and Tena-Sempere, 2010). TOR regulates the maintenance of germline stem cells and their differentiating progeny in the Drosophila ovary via insulin-dependent and -independent mechanisms (LaFever et al., 2010). Signaling through the TOR pathway controls senescence in yeast (Fabrizio et al., 2001; Kaeberlein et al., 2005; 2007; Powers et al., 2006; Pan and Shadel, 2009; Wei et al., 2009; McCormick et al., 2011), plants (Liu and Bassham, 2010; Deprost et al., 2007; Ren et al., 2011), C. elegans (Vellai et al. 2003; Jia et al. 2004; Hansen et al., 2007; Soukas et al., 2009; McCormick et al., 2011), Drosophila (Kapahi et al., 2004; Luong et al., 2006; Bjedov et al., 2010) and mammals (Harrison et al., 2009; Selman et al., 2009). Inhibition of TOR signaling activity consistently can delay the aging process in a variety of model organisms (Vellai et al., 2003; Kapahi et al., 2004; Luong et al., 2006; Hansen et al., 2007; Kaeberlein et al., 2007; Syntichaki et al., 2007; Kaeberlein and Kennedy, 2008; Selman et al., 2009). Remarkably, somatic life-extending rapamycin induces oocyte loss in Drosophila, mice and humans (Thomson and Johnson, 2010; Thomson et al., 2010; McLaughlin et al., 2011; Yu et al., 2011). Through the regulation of chemotaxis and signal relay, a TOR homologue plays an essential role in controlling aggregation in Dictyostelium by coordinating the two essential arms of the developmental pathway that leads to multicellularity (Lee et al., 2005; Kölsch et al., 2008) thus controlling life/death decisions.

Although yeast do not have an insulin-signaling pathway, they appear to have precursors of such a metabolic control pathway that function in the glucose/nutrient-signaling cascade and are homologous to the serine/threonine kinase Akt/PKB of insulin-signaling pathways in C. elegans and mammals (Barbieri et al., 2003; Parrella and Longo, 2010). Genetic studies suggest that changes in glucose metabolism affect the replicative lifespan of S. cerevisiae. In particular, increased longevity occurs with mutations affecting the glucose-responsive cAMP-dependent protein kinase A (PKA) pathway, hexokinase (catalyzing the first step in the glycolytic pathway), or the SNF1 pathway (SNF1p) (Ashrafi et al., 2000; Lin et al., 2000; Bitterman et al., 2003). In yeast, the SNF1 serine/threonine kinase plays an essential role in metabolic adaptation to different carbon sources, in response to environmental stress such as salt stress and heat shock and in developmental processes such as sporulation, lifespan and aging (Ashrafi et al., 1998, 2000; Honigberg and Lee, 1998). Loss of the SNF1p activator SNF4p produces a 20% increase in lifespan, whereas forced expression of SNF1 produces rapid aging (Ashrafi et al., 2000). SNF1p is the yeast homolog of AMP kinase (AMPK), a mammalian cellular energy sensor and “fuel gauge” (Hardie et al., 1998; Kemp et al., 1999; Hardie and Hawley, 2001; Heininger, 2001). In mammals, AMPK controls metabolic enzymes in response to stresses that affect cellular energy supply, including nutrient limitation, hypoxia, heat shock and exercise. AMPK is activated by AMP and inhibited by ATP via an allosteric mechanism. Thus, AMPK is a sensor of low energy levels and becomes active when the AMP:ATP ratio is high. For example, when energy availability is limited in rodents, AMPK functions to restore normal energy levels by stimulating glucose uptake in skeletal muscle and glycolysis in the heart and promoting feeding by regulating a hypothalamic circuit (Marsin et al., 2000; Mu et al., 2001; Andersson et al., 2004; Minokoshi et al., 2004). Notably, AMPK that is activated by the lower energy levels that occur

following ischemia or anoxia is antagonized by IIS (Beauloye et al. 2001; Apfeld et al., 2004). SNF1A/dAMPKalpha is a single orthologue for its mammalian counterparts in Drosophila melanogaster. Adult-onset inhibition of dAMPKalpha shortens lifespan and enhances sensitivity to oxidative stress and starvation stress (Tohyama and Yamaguchi, 2010). The C. elegans AMPK α subunit AAK-2 is activated by AMP and functions to extend lifespan and to lower fertility (Apfeld et al., 2004; Mair et al., 2011). AMPK and DAF-16/FOXO function in parallel to mediate the lifespan extension of DAF-2/insulin-like receptor mutants (Apfeld et al., 2004) and DR (Greer et al., 2007).

In yeast, the response to glucose is also mediated by two protein kinases, PKA and SCH9. Mutations in the CYR1/PKA and SCH9 genes can extend the longevity of replicative and nondividing cells up to threefold (Thevelein and de Winde, 1999, Fabrizio et al., 2001; 2003; 2004a; Kaeberlein et al., 2005). Both SCH9 (homologous to the serine/threonine kinase Akt/PKB of insulin-signaling pathways in C. elegans, Drosophila, and mammals) and CYR1 function in pathways that mediate glucose-dependent signaling, stimulate growth and glycolysis, and decrease stress resistance, glycogen accumulation, and gluconeogenesis (Thevelein and de Winde, 1999; Fabrizio et al., 2001).

Adipose tissue is not only a triglyceride storage organ. White adipose tissue is also an endocrine organ and producer of certain bioactive substances called adipokines among which are found plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-α), resistin, leptin, and adiponectin (Fonseca-Alaniz et al., 2007; Fernández-Sánchez et al., 2011). Adipose tissue has a key role in the regulation of reproductive activity and lifespan. The adipokine leptin is produced in adipose tissue, circulates in proportion to energy stores, and as part of the adipose tissue-hypothalamic regulatory loop acts in a negative feedback manner in the hypothalamus and other regions of the brain to control feeding, energy expenditure, and neuroendocrine systems (Ahima et al., 2000; Hill et al., 2008; Ahima, 2011). Coupling the state of body energy stores to reproductive functions, leptin also promotes puberty in animals (Barash et al. 1996; Cheung et al., 1997; Chehab et al., 1997; Cunningham et al., 1999; Watanobe, 2002; Tena-Sempere, 2007; Roa et al., 2010). In a variety of these functions, leptin cooperates with insulin (Hill et al., 2008). Because adipose tissue is the organ that secretes adipokines and these in turn generate reactive oxygen species (ROS), adipose tissue is considered an independent factor for the generation of systemic oxidative stress (Fernández-Sánchez et al., 2011). Thus, adipose tissue and leptin levels also play a regulatory role in aging, low leptin levels in DR being lifespan extending, high levels in obesity being progeriatric (Barzilai and Gupta, 1999; Shimokawa and Higami, 2001; Das et al., 2004; Smith et al., 2004; Klöting and Blüher, 2005; Picard and Guarente, 2005; Dixit, 2008). Aging- and obesity-induced leptin resistance with its loss of feedback control appear to even accelerate aging-related morbidity and mortality (Li et al., 1997; Barzilai et al., 1998; Scarpace et al., 2000; 2001; 2002; Scarpace and Tumer, 2001; Wang et al., 2001; Gabriely et al., 2002; Scarpace and Zhang, 2007; 2009). The neuroendocrine germline-brain-adipose tissue feedback loops are phylogenetically highly conserved and regulate reproduction and aging in worms, flies, mice and humans (Hwangbo et al., 2004; Blüher et al., 2005; Broughton et al., 2005; Flatt et al., 2005; Kenyon, 2005; Mukhopadhyay et al., 2005; Baumeister et al., 2006; Beckstead and Thummel, 2006; Berman and Kenyon, 2006; Mak et al., 2006; Bishop and Guarente, 2007a; 2007b; Mukhopadhyay and Tissenbaum, 2007; Wang et al., 2008; Narbonne and Roy, 2009; Panowski and Dillin, 2009). The progeriatric actions of adipose tissue and leptin support the notion that higher somatic energy levels accelerate aging which is incompatible with the DST. There is only one interpretation to the progeriatric effect of overutilization of resources: evolution allocated a more or less fixed resource budget to each individual within which it has to fulfill its most important biological function, reproduction. And evolution “appointed” censors to watch over this budget, the resource utilization sensors IIS and TOR. These censors can be corrupted by simple one-step mutations as a variety of mutagenesis experiments have shown. In long-term experiments with E. coli it was shown that during that time, each population experienced billions of mutations, far more than the number of possible point mutations in the ~ 4.6-million-bp genome (Lenski, 2004). Thus, each population should have tested every typical one-step mutation many times (Blount et al., 2008). The obvious question is not whether organisms throughout phylogenesis may not have found the lifespan-extending mutants in the wild, but why these mutations that increase the survival of the soma (often, provided that abundant resources are available, without compromising the fitness of the offspring) did not succeed. It has been argued that “ if a genetic programme existed solely to cause ageing, any mutation that inactivated such a programme would confer a selection advantage and the genes responsible would become extinct.” (Kirkwood and

Melov, 2011). This sentence betrays a fundamental misunderstanding due the focus of the ETAs on somatic aging. In fact, no “genetic programme existed solely to cause ageing”. Aging and death are inextricably linked to reproduction and reproduction/death are jointly programmed. And due to this linkage, “any mutation that inactivated such a programme” would confer not a selection advantage but a disadvantage and is selected against. Thus, I think there is only one plausible explanation for the evolutionary failure of mutations that change the optimal wild-type reproduction/aging balance (see chapter 12.1): 1. The evolutionary value of these mutations can only be appreciated in the context of the limited resources paradigm. 2. Mutations that delay aging beyond the optimum established by evolution in the wild habitat of an organism are deleterious for the reproductive fitness of the organism and are selected against. 3. Evolution appointed as guardian of the compliance of the soma with the budget of limited resources (that is measured by the nutrient utilization sensors) the conflict partner that has the most vested interest in this compliance, the germline cells (see chapter 11).

Diapausal/dauer metabolic dormancy is regulated by IIS (Baugh and Sternberg, 2006; Baumeister et al., 2006; Williams et al., 2006; Allen, 2008; Fielenbach and Antebi, 2008; Honda et al., 2008; Martin, 2008; Sim and Denlinger, 2008), TOR (Jia et al., 2004; MacRae, 2005) and AMPK (Narbonne and Roy, 2009; Ramnanan et al., 2010). Inhibition of germline proliferation during C. elegans dauer development requires AMPK signaling (Narbonne and Roy, 2006b) or interference with IIS (Baugh and Sternberg, 2006) revealing an antagonistic regulation of reproduction and longevity. DR, that mimicks some of the phenotypes of diapause, dauer formation and hibernation, is also controlled by the IIS and TOR pathways in a variety of animal models (Cornils et al., 2011). The Indy mutation in Drosophila carries a mutation in a sodium dicarboxylate cotransporter, a membrane protein that transports Krebs cycle intermediates and is thought to modulate nutrient utilization or to lower intermediate metabolism, thus extending lifespan through a mechanism similar to that of DR (Rogina et al., 2000).

Sirtuins are a family of nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylases widely distributed in all phyla. The requirement for NAD+ as a co-substrate suggests that, as the cellular redox balance of NAD+ and NADH is highly related to catabolic fluxes and the energy homeostasis, the sirtuins might have evolved as sensors of cellular energy and redox states coupled to the metabolic status of the cell (Michan and Sinclair, 2007; Canto and Auwerx, 2009). In this faculty, sirtuins, together with AMPK and their control of mitochondrial biogenesis are vital links in a regulatory network for metabolic homeostasis (Canto and Auwerx, 2009). Sirtuins are important regulators of organism longevity. In yeast and multicellular organisms, sirtuins catalyse NAD+-dependent deacetylation of histones and transcription factors that regulate stress, metabolism, and survival pathways (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001; Lin and Guarente, 2003; Rogina and Helfand, 2004; Leibiger and Berggren 2006; Longo and Kennedy, 2006; Bishop and Guarente, 2007a; Michan and Sinclair, 2007; Herranz et al., 2010). Evidence has accumulated implicating sirtuins also in the lifespan-extending effects of DR (Lin et al., 2000; Cohen et al., 2004; Wood et al., 2004; Leibiger and Berggren, 2006; Medvedik et al., 2007) and the regulation of longevity-promoting autophagy, either directly or indirectly through a downstream signaling network (Lee et al., 2008; Salminen and Kaarniranta, 2009).

Mitochondria are integrated into a variety of metabolic and oxidative signals. There is a huge literature on the role of mitochondria in aging including mechanistic theories of aging (Harman, 1972; Bratic and Trifunovic, 2010). Approximately 0.2–2% of the oxygen taken up by the cells are converted to ROS by mitochondria (Harper et al., 2004; Orrenius et al., 2007). Thus, approx. 90% of ROS arise in the mitochondria as byproducts of aerobic metabolism—the process that is fuelled by nutrients and is responsible for the formation of ATP. IIS and TOR control mitochondrial functions including the mitochondrial retrograde signaling pathway (Lambert et al., 2004; Schieke et al., 2006; Narasimhan et al., 2009; Ramanathan and Schreiber, 2009). A systematic RNA interference (RNAi) screen in C. elegans identified a mutation in the mitochondrial leucyl-tRNA synthetase gene (lrs-2) that impaired mitochondrial function and was associated with longer-lifespan. The long-lived worms with impaired mitochondria had lower ATP content and oxygen consumption (Lee SS et al., 2003). Mitochondrial components that influence lifespan acted downstream of DAF-16 or parallel to IIS and could not simply be assigned to lower free radical production (Lee SS et al., 2003).

The evolutionary roots of postreproductive aging and death as part of a dual life-death decision in a metabolic stress response are reflected by its intricate regulation through signaling pathways such as TOR, IIS, AMPK, sirtuins and mitochondrial metabolic capacity that link the organism’s resource utilization to its reproductive activity and aging trajectory.

9.4 Phosphate and aging

The germ-soma conflict theory of aging argues that the conflict over the future use of limited resources underlies the evolutionary rationale of somatic death. A prerequisite of this concept is the identification of resource limitations. To understand the physiological processes that drive eukaryotic/metazoan aging we have to look at the ecological conditions that prevailed at the time when the genetic foundations for postreproductive aging/death were laid. The Precambrian and Cambrian-to-Devonian seas were characterized by extremely low nutrient (super-oligotrophic) conditions (Martin, 1996). Both organic C, nitrate and phosphate were at very short supply. There was a very significant and widespread phosphogenic event around the Proterozoic-Phanerozoic transition that had a profound effect on the biota, and the elevated availability of phosphate may have been a driving mechanism for the Cambrian faunal 'explosion' and the initiation of biomineralization (Cook, 1992). However, the reason why calcium phosphate was never used on a large scale by protists and most invertebrate groups may reflect the scarcity of phosphorus in the environment. Thus, even at the start of the Phanerozoic, phosphorus supply was probably very limited, compared to Ca, C and Si. This would have put organisms building phosphatic hard parts in a competitive disadvantage, compared to those opting for silica or calcium carbonate. The disadvantage would have been most severe for organisms at the lowest trophic levels, which dominate the production of biomass. In the end, only higher organisms, starting with fishes in the Silurian, have been producing phosphatic body parts to any considerable extent (Van Cappellen, 2003).

There is a long-standing controversy between the 'geochemists' view (phosphorus regulation) and the 'biologists' view (nitrogen regulation) how primary production in most of the world's oceans is controlled (Tyrell, 1999). It appears somewhat enigmatic that nitrogen can be a major limiting element in the biosphere given the enormous amounts of N2 in the atmosphere and the existence of nitrogen-fixing cyanobacteria and plants. When nitrate is scarce relative to phosphate (low $[NO3^{-}]$:$[PO4^{-3}]$) then those organisms which can obtain their nitrogen from the super-available, atmosphere-derived dinitrogen (N2), will become more numerous (Schindler et al., 2008). As this N2-fuelled algal growth is eaten and decomposed, its nitrogen returns to dissolved inorganic ammonium and nitrate in the water, increasing $[NO3^{-}]$:$[PO4^{-3}]$ in a negative feedback. However, there is no atmospheric reservoir of phosphorus and so there is no alternative source once phosphate runs out. According to this view that has been confirmed in whole-ecosystem experiments nitrate gets topped up when scarce relative to phosphate. Nitrate concentrations therefore slavishly follow phosphate concentrations, and so it is phosphate dynamics that control ocean primary production and fertility (Tyrell 1999; Carpenter, 2008; Schindler et al., 2008). Nitrogen is generally considered to be the "proximate limiting nutrient" in marine systems, representing local limitation while phosphate supplied by continental weathering and fluvial discharge is viewed as the "ultimate limiting nutrient" influencing system productivity on long-term scales (Tyrell, 1999; Hessen et al., 2004). Shortfalls of nitrogen can be compensated for in the long term through N2 fixation, although availability of trace elements, notably iron, and light limitation may limit this process (Martin, 1990).

Phosphate is not only an essential component of cell structure (DNA and membrane phospholipids) but also a key mediator of numerous cellular activities, including energy metabolism (ATP production), translation, and kinase-mediated signal transduction. Phosphate availability is a major factor limiting growth, development, and productivity of plants and animals. Empirical evidence shows that the specific growth rate is positively related to RNA concentration both between and within taxa in both unicellular and multicellular organisms. Ribosomes are present in very large numbers: ribosomal protein accounts for approximately 50% of the total protein and rRNA represents approximately 80% of the total RNA in a cell (Warner, 1999). To meet this huge demand, eukaryotic cells contain more than 100 copies of the gene for rRNA as a large cluster(s) on the genome (rDNA) and each cell produces approximately 2000 ribosomes per minute. rDNA is the most abundant gene in the cell. For these reasons, rDNA is considered as the 'king of the housekeeping genes' in terms of function and quantity (Kobayashi, 2011). Since RNA is rich in P and constitutes a substantial part of the total P in invertebrate organisms, a high growth rate is also connected with a high P content, a relationship that is modulated in vertebrates by bone content (Sterner and George 2000, Vanni et al. 2002). The reason for this pattern is that the growth of all biota is closely linked with their protein synthesis rate, and thus with the concentration of ribosomal RNA (Elser et al., 1996; Vrede et al., 2004; Gillooly et al., 2005). Cumulative evidence suggests that throughout phylogenesis phosphate in itself represents a signal that is sensed by and entertains a complex homeostatic network regulating multiple factors

necessary for diverse biological processes (Silver and Dranitzki-Elhalel, 2003; Lin et al., 2009; Prié et al., 2009; Rouached et al., 2010; Chiou and Lin, 2011; Khoshniat et al., 2011).

According to the evolutionary logic of the germ-soma conflict theory, utilization of limited resources is not for free but is sanctioned by longevity limitation. Lifespan control of translation may not only clock phosphate but also amino acid utilization. Accordingly, there is multiple crosstalk between phosphate and other nutrient sensing pathways. Evidence suggests that TOR and its inhibitor rapamycin have multiple effects on phosphate balance (Schwarz et al., 2001; Shojaiefard and Lang F, 2006; Kempe et al., 2010; Tataranni et al., 2011). In Saccharomyces cerevisiae, the TOR pathway controls Go entry in response to carbon and/or nitrogen availability and in close collaboration with a phosphate-sensing pathway (Wanke et al., 2005). A systematic lifespan analysis of conditions that inhibit protein synthesis revealed that reducing the levels of ribosomal proteins, ribosomal-protein S6 kinase or translation-initiation factors increases the lifespan of C. elegans (Hansen et al., 2007). In many organisms, nutrient limitation has been found to inhibit translation, at least in part, by down-regulating the highly conserved kinase TOR (Wullschleger et al., 2006). Inhibition of TOR decreases ribosome biogenesis as well as the process of translation itself. For example, when TOR activity falls, phosphorylation of ribosomal-protein S6 kinase (S6K) is reduced, which, in turn, leads to the dephosphorylation and inactivation of translation elongation factor 2 (eEF2) kinase, thereby inhibiting global translation (Wang et al., 2001).

Although low phosphate diet does not reduce blood insulin levels, it indeed alters expression of insulin-responsive genes in a way similar to that induced by diet restriction (Xie et al., 1999; Xie et al., 2000), resulting in increased gluconeogenesis and decreased glycolysis. This may be owed to the fact that low phosphate diet induces moderate insulin resistance (Paula et al., 1998; Haap et al., 2006). Thus, moderate insulin resistance induced by phosphate restriction, as well as hypoinsulinemia induced by diet restriction, attenuate intracellular insulin signaling activity and induce similar changes in insulin-responsive gene expression, resulting in a similar metabolic state (Kuro-o, 2010).

Inorganic phosphate functions also as a key regulator of oxidative phosphorylation. Mitochondrial membrane potential, NADH concentration, and oxygen consumption in isolated mitochondria increase in a hyperbolic manner as extra-mitochondrial phosphate concentration increases (Bose et al., 2003). In addition, high phosphate concentration enhances delivery of reducing equivalent to cytochrome c in Complex III in the electron transport chain, which also increases ROS production (Bose et al., 2003). Furthermore, inorganic phosphate induces mitochondrial permeability transition (the inner membrane becomes non-selectively permeable to small solutes) in Ca2+-loaded mitochondria, which occurs under pathological settings including ischemia/reperfusion and triggers cell death (Kowaltowski et al., 2001). Thus, inorganic phosphate plays multiple roles in the regulation of oxidative stress and mitochondrial function in health and disease (Kuro-o, 2008; 2010).

The most cogent evidence for a role of phosphate in aging trajectories comes from mutant mice with loss-of-function mutations in the klotho (klotho$^{-/-}$) and fibroblast growth factor 23 (FGF23$^{-/-}$) genes, both involved in phosphate homeostasis, that have similar progeroid phenotypes (see chapter 10.3.1).

Phosphatopathies are universally observed in patients with chronic kidney disease (CKD). Hyperphosphatemia was identified as a potent mortality risk in CKD and coronary disease patients (Block et al., 1998; 2004; Goodman et al., 2000; Ganesh et al., 2001; Kestenbaum et al., 2005; Tonelli et al., 2005). Vascular calcification is recognized as a major contributor to cardiovascular disease in end stage renal disease patients. Vascular smooth muscle cells respond to elevated phosphate levels by undergoing an osteochondrogenic phenotype change and mineralizing their extracellular matrix (Giachelli, 2009). Cardiovascular mortality of a 35-year-old patient on dialysis is equivalent to that of an 80-year-old “healthy” individual, making CKD the most potent accelerator of vascular senescence (Meyer and Levey, 1998; Goodman et al., 2004). The vast majority of patients with CKD die prematurely not due to renal failure, but due to early onset of common age-related diseases such as cardiovascular disease, cancer, and infection (Sarnak et al., 2003; Go et al., 2004). Consequently, the spectrum of death causes in patients with CKD is similar to that of the general population. Patients with CKD also prematurely suffer many aging-like symptoms, including hypogonadism, skin atrophy, osteopenia, and cognitive impairment. Thus, CKD may be viewed as a progeroid syndrome (Kuro-o, 2010; 2011).

10. Stress and aging

The key remaining questions of evolutionary biology are more ecological than genetic in nature.
Edward O. Wilson, 1987

Summary

Increased stress resistance has been documented as a correlated response to selection for extended longevity. Low-dose stressors create stress tolerances and cause lifespan extension (the hormesis phenomenon). Phylogenetically conserved gerontogenes have a role in stress responses and, when overexpressed, extend longevity. Aging organisms exhibit various systemic and cellular stress response phenomena that identify the stressor that causes aging as extrinsic and distinct to the aging soma. The control of aging by stress sensing pathways is the genetic signature of the evolutionary roots of aging/death that evolved as part of the survival/death decisions during nutrient stress-elicited reproductive events. As a legacy of, and witness to, these evolutionary roots both reproduction and aging are controlled by oxidative and metabolic stress signaling pathways.

10.1 Stress resistance, hormesis and aging/death

In a further level of hierarchy, the reproduction-aging network is modulated by stress susceptibility/resistance and, conversely, feeds back on the stress response axis. Increased stress resistance has been documented as a correlated response to selection for extended longevity (Service, 1987; Zera and Harshman, 2001; Scannapieco et al., 2009). Both oxidative and/or metabolic stress resistance are affected (Luckinbill et al. 1984, Rose, 1984; Service et al. 1985, Service, 1987; Rose et al. 1992; Force et al. 1995; Harshman and Haberer, 2000). Selection for stress resistance, on the other hand, slows the course of aging and extends lifespan in a variety of organisms (Parsons, 1995; Bubliy and Loeschcke 2005; Pijpe et al., 2008; Burke and Rose, 2009). Moreover, lifespan extension mutants of a variety of taxa have an increased resistance to a variety of stressors including oxidative stress, heat shock, and UV radiation (Lin et al., 1998; Longo, 1999; Johnson et al., 2000; 2001; Fabrizio et al., 2001; Longo and Fabrizio, 2002; Holzenberger et al., 2003; Ayyadevara et al., 2008).

Postreproductive death being the evolutionary default setting, a variety of organisms succeeded to delay death stress-resistence-dependently. Stress resistance is a component of fitness and delays postreproductive death and increases lifespan. Many types of stressors, when experienced at mild-to-moderate preconditioning intensities create tolerances, e.g. thermotolerance, radiation tolerance, ischemic tolerance (Morimoto and Santoro, 1998; Heininger, 2000b; 2002a; Cypser and Johnson, 2002). The tolerogenic mechanisms include increase of antioxidant defences, upregulation of heat shock protein (hsp) expression and DNA repair. The common denominator of tolerance induction in eukaryotes appears to be oxidative stress. Following the multitude of correlations between oxidative stress, stress resistance and longevity, both an oxidative stress theory of aging (Harman, 1956; Muller et al., 2007; Buttemer et al., 2010) and stress theory of aging have been put forward (Parsons, 1995). From yeast to mammals, susceptibility to stress and oxidative stress is inversely correlated to lifespan (Larsen, 1993; Sohal and Weindruch, 1996; Kapahi et al., 1999; Golden and Melov, 2001; Jazwinski, 2001; Buttemer et al., 2010). Mitochondria epitomize the ambiguous, “double-edged sword” relationship of stress, stress resistance and aging (Heininger, 2001; 2002a). Mitochondria as major source and target of oxidative stress play a key role in aging and the progeroid action of a variety of environmental stressors (Sohal and Weindruch, 1996; Ozawa, 1999; Kowald, 1999; Golden and Melov, 2001; Kregel and Zhang, 2007). On the other hand, genetically modified mice that have extra mitochondria live and remain reproductively active approx. 2 years longer than wild-type mice (Hanson and Hakimi, 2008). Across phylogeny, animals which have been exposed to or are resistant against a variety of low-dose stressors (e.g. radiation, methanol, heat, cold, gravity, pH abnormality, exercise, repetitive injury) including DR exhibit lifespan extension (the hormesis phenomenon) (Parsons, 1995; Martinez, 1996; Masoro, 1998; 2006; Lin YJ et al., 1998; Morimoto and Santoro, 1998; Longo, 1999; Forbes, 2000; Johnson et al., 2000; Minois, 2000; Turturro et al., 2000; Jazwinski, 2001; Yu and Chung, 2001; Sinclair, 2005; Olsen et al., 2006; Rattan, 2008; Le Bourg, 2009; Gómez et al., 2009; Mattson and Calabrese, 2009; Mitteldorf, 2010b). The key role of mitochondrial increased formation of reactive oxygen species in hormesis has been appreciated (Tapia, 2006; Ristow and Schmeisser, 2011). In line with the association of stress resilience and longevity, long-lived animals are more resistant to stress/oxidative stress (Lin YJ et al., 1998; Kirkwood et al., 2000; Braeckman et al., 2001; Jazwinski, 2001). On the other hand, increased stress resistance has its cost, inhibiting reproductive activity in plants (Heil et al., 2000; Heil and Baldwin, 2002; Durrant and Dong, 2004) and animals (Krebs and Loeschcke 1994; Silbermann and Tatar 2000; Kenyon, 2001; Samain et al., 2007; Kwan et al., 2008; Burke and Rose, 2009; Monaghan et al., 2009; Marshall and Sinclair, 2010).

A multitude of phylogenetically conserved gerontogenes (Johnson et al., 2000; 2001; Rattan and

Singh, 2009) have been identified such as daf-16/FOXO family (Johnson et al., 2000; 2001; Zhang et al., 2009; Bridge et al., 2010), SKN-1/Nrf (Sykiotis and Bohmann, 2008; Oliveira et al., 2009; Zhang et al., 2009; Alavez et al., 2011; Li et al., 2011), HSP/HSF (Tatar, 1999; Cherkasova et al., 2000; Morley and Morimoto, 2004; Singh et al., 2007; Sakurai and Enoki, 2010; Alavez et al., 2011), SLR-2 (Kirienko and Fay, 2010), and sirtuins (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004) all of which when overexpressed extend longevity and all have a role in stress responses. Knocking in of hsps extends the lifespan of C. elegans (Yokoyama et al., 2002; Walker and Lithgow, 2003) while knockdown of hsps or HSF-1, a master regulator of heat-shock response shortens the lifespan of the worm (Garigan et al., 2002; Morley and Morimoto, 2004; Kimura et al., 2007). Gerontogenes play also key roles in cellular life/death decisions during development linking the programming of development and aging, cellular and somatic death (Heininger, 2001; Hoogeboom and Burgering, 2009).

The intimate interdependence of metabolic and stress-related signals has its roots in the pervasive nature of metabolic and oxidative stress during the 'feast and famine' cycles of primordial organisms (Heininger, 2001). These counterregulations are part of the global antagonistic pleiotropic network that inversely controls stress competence and resource utilization and is operative from bacteria to plants and mammals (Ferenci, 2005; Baena-González and Sheen, 2008; Managbanag et al., 2008). Thus, resource utilization monitored both by IIS and TOR signaling affects the stress resistance of organisms (Holzenberger et al., 2003; Tatar et al., 2003; Deprost et al., 2007) and this effect may be mediated by IIS-dependent modulation of e.g.hsp expression (Walker and Lithgow, 2003; Baba et al., 2005). In addition, IIS regulates other gerontogene action through phosphorylation and inhibition of the longevity-promoting transcription factors SKN-1/Nrf (Baumeister et al., 2006; Tullet et al., 2008; Okuyama et al., 2010) and DAF-16/FOXO family (Kimura et al., 1997; Paradis and Ruvkun, 1998; Lee et al., 2001; Lin et al., 2001; Hertweck et al., 2004; Baumeister et al., 2006). IIS also impairs C. elegans immune defense (Garsin et al., 2003; Murphy et al., 2003; Evans et al., 2008a; b). Moreover, IIS modulates various stress response pathways (Murphy et al., 2003) and IIS loss-of-function mutations increase stress resistance (and longevity) in various organisms (Riddle et al., 1997; Holzenberger et al. 2003; Neumann-Haefelin et al., 2008; Henis-Korenblit et al., 2010). On the other hand, stress response pathways like the FOXO and JNK pathways can antagonize IIS and its progeroid action (Wang MC et al., 2005).

10.2 The general and oxidative stress of aging

Thus, stress resistance helps to resist aging and (keeping in mind that an abiotic or biotic stressor is a factor that is extrinsic to the stressed organism) whatever causes somatic aging can be identified as stressor that is forced upon the soma (see chapter 10.4). In accord with this interpretation, aging organisms exhibit multiple both systemic and cellular features of basal stress exposure and declining stress responsivity (Frolkis, 1993; Haigis and Yankner, 2010). Aging is associated with an elevated basal sympathetic nervous activity. In contrast, the reactivity of the sympathetic and the parasympathetic nervous activity are reduced (Wichi et al., 2009). In animals, aging enhanced the basal activity of the HPA axis and perturbed the HPA responsiveness (De Kosky et al., 1984; Oxenkrug et al., 1984; Reul et al., 1991; Meaney et al., 1992; Hatzinger et al., 1996). Furthermore, glucocorticoids (GCs) in response to a stressor is increased and the recovery of GC levels is impaired (Sapolsky et al., 1983, Sapolsky and Donnelly, 1985, Issa et al., 1990, van Eekelen et al., 1992), accompanied by a decrease in the concentration of GC binding proteins, thus augmenting the amount of free GC (Meaney et al. 1992, van Eekelen et al. 1992, Fleshner et al., 1995). A causal relationship of the increased activity of the HPA axis with aging has been suggested. The "glucocorticoid cascade hypothesis" of stress and aging conceptualized the findings that 1) plasma GC levels correlate with hippocampal aging; 2) adrenalectomy protects against features of hippocampal aging; 3) and long-term stress and GC administration elicit premature development of hipppocampal aging (Landfield 1978, Sapolsky et al., 1986; Landfield and Eldridge, 1994, Smith 1996; McEwen, 2002; Landfield et al., 2007). Increased CSF, particularly ventricular, cortisol levels have been demonstrated in humans (Swaab et al., 1994; Murakami et al., 1999). Due to a mild concomitant increase of serum cortisol levels, the cortisol CSF/serum ratio is elevated (Murakami et al., 1999; Heininger, 1999) suggesting an intrathecal accumulation of glucocorticoids. Conversion of inactive precursors into active agents at the level of CNS target structures appears to be the underlying principle (Heininger, 2000b). These findings are in line with the fulminant, rapid senescence-related increase of GC observed in semelparous animals like male marsupials and spawning salmon (see chapter 11.2.4). These general stress response-related alterations are also reflected at the level of gene

expression profiles in aging C. elegans (McCarroll et al., 2004; Golden and Melov, 2007; Bell et al., 2009; Park et al., 2009), Drosophila (Landis et al., 2004; McCarroll et al., 2004; Sørensen et al., 2007; Sarup et al., 2011), Anopheles gambiae (Wang M-H et al., 2010) and various organs of aging mammals, including heat shock response genes, oxidative stress inducible genes, and DNA damage inducible genes (Papaconstantinou et al., 1996; Lee et al., 1999; Zou et al., 2000; Kayo et al., 2001; Weindruch et al., 2001; Park and Prolla, 2005; Bell et al., 2009; de Magalhães et al., 2009). Overall, as evidence of a stress response phenotype, aging is associated with an increased HPA axis activity exerting particularly CNS-targeted effects and cellular stress markers.

Cellular stress markers in an aging organism include the expression of heat shock proteins (Beere, 2004), nuclear factor-kappaB (NF-κB) (Kriete and Mayo, 2009), ApoD (de Magalhães et al., 2009) and sestrins (Lee et al., 2010a; b). Stress proteins, including the heat shock proteins (HSPs), the most ancient defense system in all living organisms (Sreedhar and Csermely, 2004), function in combating the stress-induced damage. Experiments have shown that transgenic worms which are genetically engineered to have certain types of HSP upregulated, such as Hsp16 or Hsp70F, live much longer (Morrow and Tanguay, 2003; Yokoyama et al., 2002). Moreover, transcriptional activator heat shock factor HSF-1, which regulates the heat-shock response, also influences aging in C. elegans. Hsu et al. (2003) have shown that hsf-1 overexpression extends lifespan. They found that HSF-1, like the transcription factor DAF-16, is required for daf-2-insulin/IGF-1 receptor mutations to extend lifespan. The metazoan transcription factor NF-κB signaling pathway, that shows some homology to the yeast mitochondrial retrograde response (Srinivasan et al., 2010) is a master regulator for inflammatory responses, mediating cellular defense against infectious agents and environmental and cellular stress (Mercurio and Manning, 1999, Wang and Zhang, 2002; Piva et al., 2006) and is highly conserved in innate immunity (Silverman and Maniatis, 2001). Numerous studies have demonstrated that in cells subjected to oxidative stress there is a potent NF-κB response. As such, NF-κB is often referred to as the cellular ‘sensor’ for oxidative stress (Li and Karin, 1999; Mercurio and Manning, 1999; Storz et al., 2003). Activation of the NF-κB signaling pathways is central to the cell's survival response countering the induction of apoptosis by a variety of challenges (Barger and Mattson, 1996; Beg and Baltimore, 1996; Liu et al., 1996; van Antwerp et al., 1996; Wang et al., 1996; Wu et al., 1996; Baichwal and Baeuerle, 1997; Glazner et al., 2000; Manna et al., 2000; Castagné et al., 2001). In a further legacy of the ambiguous survival/death pathways inherited from ancient reproduction/apoptosis events, NF-κB activation also promotes apoptosis (Grilli et al., 1996; Grimm et al., 1996; Lin et al., 1995; Qin et al., 1998; Schneider et al., 1999; Castagné et al., 2001). NF-κB is the motif that is most strongly associated with aging being identified as a candidate activator of aging-related transcriptional changes in multiple tissues (Salminen et al., 2008a; Ljubuncic and Reznick, 2009; Salminen and Kaarniranta, 2010a; b). Recent studies have demonstrated that constitutive activation of NF-κB is a ubiquitous phenomenon among various cell types in the aging phenotype, contributing to deleterious effects. Expression of NF-κB with age is consistent with elevated levels of inflammatory markers and a pro-inflammatory phenotype, manifested in many age-associated diseases (Salminen et al., 2008a; Kriete and Mayo, 2009). Inducible genetic blockade of NF-κB for 2 weeks in the epidermis of chronologically aged mice by adenovirus-mediated expression of dominantly active IκBα reverted the tissue characteristics and global gene expression programs to those of young mice (Adler et al., 2007). Also, age-specific NF-κB blockade and orthogonal cell cycle interventions revealed that NF-κB controls cell cycle exit and gene expression signature of aging in parallel, but not sequential pathways. Age-associated genes whose expression was inhibited by NF-κB blockade included those related to chromatin/transcriptional regulation, protein modification/signal transduction, cell cycle/growth control and mitochondria. These results identify a conserved network of regulatory pathways underlying mammalian aging and show that NF-κB is continually required to enforce many features of aging in a tissue specific manner.

It is one of the paradoxes of aging that the stress response capacity is impaired in aging when it is vitally needed. However, this paradox is not a consequence of some haphazard wear and tear or mutation accumulation but is evolutionarily intended. Thus, responses of the aging organisms to stressors tend to be blunted and prolonged (Heininger, 1999a; Sørensen and Loeschcke, 2002; Grotewiel et al., 2005; Calderwood et al., 2009) and stress resistance attenuated (Drapeau et al., 2000; Przybysz et al., 2009; Sykiotis and Bohmann, 2010). The life-sustaining antioxidative systems shut down during normal aging in yeast (Kale and Jazwinski, 1996), worms (Larsen et al., 1995), flies (Arking, 1998), and mammals (Petropoulos et al., 2001; Golden et al., 2002), increasing the vulnerability to stressors (for aging-related downregulation of klotho expression see

chapter 10.3.1). Hence, oxidative stress elicits an upregulation of antioxidant defences in young but not aged C. elegans (Darr and Fridovich, 1995). Likewise, HSP expression following a variety of stressors is blunted in aged mammalian and human cells (Liu et al., 1989; Blake et al., 1991; Heydari et al., 1993; 2000; Fawcett et al., 1994; Holbrook and Udelsman 1994; Lee et al., 1996; Rao et al., 1999; Volloch and Rits, 1999; Njemini et al., 2002; 2006; Jurivich et al., 2005; Calderwood et al., 2009). Survival- and stress resistance-enhancing autophagy becomes impaired with aging (Bergamini et al., 2004; Rajawat and Bossis, 2008), possibly due to TOR and IIS signaling. TOR inactivation activates autophagy (Crespo et al., 2005; Díaz-Troya et al., 2008; Chang et al., 2009; Jung et al., 2010; Neufeld, 2010; Pérez-Pérez et al., 2010). Likewise, reduced IIS (Florez-McClure et al., 2007) increases autophagic activity while insulin inhibits cellular autophagy (Pfeifer and Warmuth-Metz, 1983). The deficiency of stress pathways is exemplified by the NF-κB stress transduction pathway. NF-κB is constitutively activated in senescence but its stimulation by stressors is attenuated (Ponnappan, 1998; Helenius et al., 1999; Ponnappan et al., 2004). These data indicate that the functional reserves are strained and exhausted by the aging-associated adaptations leaving the organism defenceless and more vulnerable to additional stressors (Azhar et al., 1999; Volloch and Rits, 1999). The aging-related loss of stress competence is programmed. Inhibition of the gerontogenes by germ cell derived signals and resource utilization-sensing pathways (see chapters 10.3 and 11) impair the stress response competence in aging model organisms and in humans (Sykiotis and Bohmann, 2008).

10.3 The metabolic stress of aging

Too small to accumulate substantial energy depots during phases of nutrient abundance, microbial populations enter stationary phases when facing nutrient shortage. The characteristic pattern of metabolic adaptations to deprivation was established as early as in stationary phase bacteria and protozoa: hypometabolism and downregulation of oxidative respiration, upregulation of glycogen synthesis, and storage, and oxidation of lipids from endogenous polyhydroxybutyrate/alkanoate reserves, membranes, and exogenous sources (Hand and Hardewig, 1996; Heininger, 2001). Thus, the cell takes advantage of the higher energy yield from fatty acid β-oxidation while glucose, on the other hand, the precursor of amino acids and nucleotides, is saved for anabolic purposes (Heininger, 2000b; 2001; 2002a; b). These adaptations subserve the dual purpose to reduce the generation of toxic oxygen species and to minimize the utilization of endogenous reserves (Heininger, 2000b; 2001; 2002a; b). Displaying the pattern of a prototypic deprivation response, aging prokaryotic and eukaryotic microorganisms engage in both hypometabolism and increased energy storage (Heininger, 2001; Lin et al., 2001). Likewise, in metazoa the adaptive reponse to metabolic stress is regulated via the reciprocal glucose-fatty acid cycle (Heininger, 2000b; 2001; 2002a; b) und is characterized by hypometabolism and an altered energy consumption/storage balance. The cells of an aging metazoan organism are also deprived of nutrients due to hormone and growth factor deficiencies/resistances (Heininger, 1999a). Responding to the cellular nutrient stress, aging organisms downregulate metabolic rate and fat-free mass (Keys et al., 1973; Fukagawa et al., 1990; Visser et al., 1995; Poehlman et al., 1993; Klausen et al., 1996; Piers et al., 1998; Greenberg et al., 2000; Lovejoy et al., 2008; Bergeron et al., 2011) and favor nutrient storage over nutrient utilization (Calle-Sescandon et al., 1995; Blaak et al., 2001; Lin et al., 2001; Roberts and Rosenberg, 2006), causing the increased propensity to obesity during aging (Chien et al., 1975; Noppa et al., 1980; Cohen, 2001; Carr, 2003; Lee et al., 2003; Gardner et al., 2005; Lovejoy et al., 2008). In a highly conserved hypometabolic adaptation (Storey and Storey, 2007), accumulation of fat stores before, and consumption of these stores during, environmental nutrient shortage is a common feature of bacterial stationary phase (Heininger, 2001), C. elegans dauer formation (Larsen et al., 1995; Van Voorhies, 2001; Luedtke et al., 2010), insect diapause (Ohtsu et al., 1993; Hahn and Denlinger, 2007; 2011) and vertebrate hypometabolism and hibernation (Kenagy, 1989; Heininger, 2000b; Geiser, 2004; Storey, 2010). Programmed for the metabolic stress response, aging ad libitum-fed organisms store the fuel surplus and the median-age individual may become obese (Andres, 1984; Vardi and Pinhas-Hamiel, 2000). It has been proposed that (excluding pregnancy), “not gaining weight after early adulthood” may serve as an epidemiological paradigm of caloric restriction in humans (Lee et al., 2003; Gardner et al., 2005). The visceral fat mass that is regulated by neuroendocrine signals (Dallman et al., 1995; Carrascosa et al., 2009) appears to play an important role in the generation of the metabolic syndrome (Kissebah and Krakower, 1994; Gabriely and Barzilai, 2001; Carr, 2003; Rabe et al., 2008). The aging-related metabolic alterations, including insulin resistance, are similar to what is observed in hypometabolic states throughout

phylogenesis (Storey and Storey, 2004; 2007), such as mammalian hibernation, and likely provides a survival pathway during a period of perceived cellular metabolic stress (Heininger, 2000b; 2002a; Martin, 2008; Storey, 2010). Evidence from C. elegans involves signals from the germline cells (Wang et al., 2008; Panowski and Dillin, 2009) in the metabolic reprogramming. In rats, epigenetic dysregulation with age is a highly tissue-dependent phenomenon. Changes in methylation occur consistently near genes that are involved in metabolism and metabolic regulation, implicating their potential role in the pathogenesis of age-related diseases (Thompson et al., 2010).

The aging organism defends itself against the molecular damage and functional decline that is imposed upon it. These supposedly detrimental changes accompanying old age are in fact evolutionary adaptations to prolong life after reproduction (Heininger, 2002a; Le Couteur and Simpson, 2011). For instance, insulin resistance appears to be an evolutionarily conserved physiological mechanism activated at the cellular level in response to conditions stimulating ROS production, thus leading to the prevention of oxidative stress, and extension of life (Erol, 2007; Nunn et al., 2009).

10.3.1 Klotho, key pleiotropic node of the resource utilization-stress-aging network

Klotho (named in reference to the goddess of Greek mythology who spins the thread of life) is a single-pass transmembrane protein with structural homology to glycosidases. It is phylogenetically conserved from C. elegans to mammals (Matsumura et al., 1998; Château et al., 2010; Solari, 2010). Klotho interacts with various fibroblast growth factor (FGF) receptors (FGFRs) and is an obligatory co-receptor of FGF23 (Kurosu et al., 2006; Long and Kharitonenkov, 2011), a bone-derived hormone that suppresses phosphate reabsorption and vitamin D biosynthesis in the kidney. Klotho thus plays a major role in calcium and phosphate balance (Torres et al., 2007; Razzaque, 2009; Civitelli and Ziambaras K, 2011). The FGFR-klotho complex also has an evolutionarily conserved function in the development of excretory organs such as the worm excretory canal and the mammalian kidney (Polanska et al., 2011). In mammals, klotho is expressed primarily in tissues that function in the regulation of calcium and phosphate homeostasis such as the distal convoluted tubules of the kidney and in lesser amounts in the choriod plexus of the brain and parathyroid glands, but also in mature germ cells (Li et al., 2004). Inorganic phosphorus and calcium play a fundamental physiological role in energy production, membrane transport, and signal transduction (Weisinger and Bellorin-Font, 1998; Heininger, 1999a).

Mutant mice with a loss-of-function mutation in the klotho gene (klotho$^{-/-}$) develop normally to approx. 3 weeks of age, but then express a progeroid phenotype, including arteriosclerosis, thymic atrophy, pulmonary emphysema, sarcopenia, osteoporosis, and cognitive decline, and die prematurely at about 8-9 weeks of age (Kuro-o et al., 1997; Nabeshima, 2002; Nagai et al., 2003; Kuro-o, 2009), a lifespan only about 5-6% of that of wild-type mice (Nabeshima, 2006). Local expression of the klotho gene retards or partially improves pathological abnormalities in several organs of klotho$^{-/-}$ mice after onset of the phenotypes (Shiraki-Iida et al., 2000). Since Klotho may be involved in the regulation of gonadotropin and gonadotropin releasing hormone production/secretion in the pituitary gland and hypothalamus the mice never become sexually mature and are infertile. Intriguingly, the mice display a decreased insulin production and increased insulin sensitivity (Utsugi et al. 2000) which may also be involved in the gonadal immaturity (see chapter 9). Phosphate retention and multiple symptoms of premature aging can be ameliorated by correcting hyperphosphatemia by a low phosphorus diet (Morishita et al., 2001; Kuro-o, 2010).

Mice deficient in fibroblast growth factor FGF23 (FGF23$^{-/-}$) have a similar progeroid phenotype as klotho deficient mice (Razzaque et al., 2006), including hyperphosphatemia, hypercalcemia, and increased levels of vitamin D, suggesting a common signaling pathway that regulates vitamin D, serum calcium and phosphorus levels (Tsujikawa et al., 2003; Kuro-o, 2006; 2010; Stubbs et al., 2007). Normalization of vitamin D activity in either klotho$^{-/-}$ or FGF23$^{-/-}$ either by feeding a vitamin D deficient diet or knockout of 1α -hydroxylase (involved in the activation of vitamin D) substantially rescued the progeroid phenotype (Tsujikawa et al., 2003; Nabeshima, 2006; Stubbs et al., 2007). Increased renal activity of sodium-phosphate cotransporters (NaPi2a) leads to severe hyperphosphatemia in klotho$^{-/-}$ mice. Genetically reducing serum phosphate levels in klotho$^{-/-}$ mice by generating a NaPi2a and klotho double-knockout (NaPi2a$^{-/-}$/klotho$^{-/-}$) strain resulted in amelioration of premature aging-like features. The double-knockout mice gained reproductive ability, recovered their body weight, reduced their organ atrophy, and suppressed ectopic calcifications, with the resulting effect being prolonged survival. More importantly, following feeding with a high-phosphate diet, premature aging-like features reappeared, clearly suggesting that phosphate toxicity is the main cause of

premature aging in klotho$^{-/-}$ mice (Ohnishi and Razzaque, 2010). Importantly, low phosphate diet rescued multiple phenotypes of FGF23$^{-/-}$ mice despite the fact that it further increased already high serum calcium and vitamin D levels (Stubbs et al., 2007), suggesting that phosphate, but not calcium or vitamin D, is primarily responsible for the aging-like phenotype.

Shedding of the extracellular domain, comprising about 94% of the entire klotho protein, produces a circulating factor (secreted Klotho) which acts like a hormone on surfaces of cells to modulate function of channels (Huang, 2010) and growth factors including IGF-1, insulin, TOR and Wnt (Liu H et al., 2007; Kuro-o, 2009). Insulin appears to stimulate the cleavage and release of the extracellular domain of Klotho (Chen et al., 2007). The secreted klotho protein functions as a putative β-glucuronidase that removes terminal sialic acids in the glycans of several ion channels, including a calcium channel, transient receptor potential vanilloid type isoform 5 (TRPV5) (Chang et al., 2005; Cha et al., 2008), and a potassium channel, renal outer medullary potassium channel-1 (ROMK1) (Cha et al., 2009). Removal of sialic acids by secreted klotho protein on the cell surface prevents internalization of these ion channels, resulting in increase in transepithelial calcium absorption and potassium secretion in distal nephrons, respectively. By genetic perturbation of insulin/IGF-1 signaling the progeroid phenotype of Kl$^{-/-}$ mice was attenuated, indicating that the activity of Klotho that inhibits insulin/IGF-1 signaling accounts, at least in part, for its anti-aging properties (Kurosu et al. 2005). Transgenic mice in which klotho is overexpressed live significantly longer than wild type controls (Kurosu et al., 2005). In α-klotho transgenic mice, the blood glucose level is normal but the insulin level is higher than in wild-type mice, and a hypoglycemic response to injected insulin and IGF1 is shown. The anti-aging action of klotho appears to be conserved from C. elegans to humans (Arking et al., 2002; 2005; de Oliveira, 2006; Château et al., 2010).

The Klotho gene product plays a key role in the regulation of endogenous oxidative stress (Kurosu et al., 2005). Tissues from klotho$^{-/-}$ mice exhibit elevated oxidative stress and Klotho transgenics are resistant to oxidative stress (Kurosu et al., 2005; Kuro-o, 2008; 2009; Hsieh et al., 2010). Klotho-overexpressing transgenic mice have higher SOD2 expression in muscles and less phosphorylated FOXOs than wild-type mice, associated with less oxidative stress as evidenced by lower levels of urinary 8-OHdG, a marker of oxidative damage to DNA (Yamamoto et al. 2005). Treatment of mammalian cells with the secreted klotho protein inhibited insulin/IGF-1 signaling, activated FOXO, increased expression of manganese superoxide dismutase (MnSOD) and reduced oxidative damages and apoptosis induced by paraquat (Yamamoto et al. 2005; Rakugi et al., 2007). Moreover, overexpression of klotho upregulates mitochondrial complex I (Sato et al., 2005) and IV activities, along with decreases in superoxide anion generation, lipid peroxidation and mitochondrial DNA fragmentation (Haruna et al., 2007). Klotho gene infusion into the tail vein of mice and rats suppressed ROS formation and upregulated MnSOD expression and total SOD activity (Ohta et al., 2007). Klotho acted as a humoral factor to reduce H2O2-induced apoptosis and cellular senescence in vascular cells (Ikushima et al., 2006). Klotho overexpression in a mouse model of glomerulonephritis that develops nephrotic syndrome and progressive renal injury resulted in dramatic improvement of renal functions and morphological lesions in both the tubular and glomerular compartments, suggesting that the klotho gene product has a remarkable renoprotective effect by potentially serving as a circulating hormone (Haruna et al., 2007). The renoprotective effect may be, at least in part, mediated by upregulation of heat shock protein 70 expression (Sugiura et al., 2010). Klotho suppressed tumor necrosis factor (TNF)-α -induced expression of adhesion molecules (that exert potent proatherogenic effects) and NF-κB activation (the molecular culprit of inflamm-aging). These actions identified klotho as anti-oxidant and anti-inflammatory agent (Maekawa et al., 2009; 2011).

On the other hand, klotho expression is suppressed in several in vivo and in vitro animal models of stress and disease (Aizawa et al., 1998; Ohyama et al., 1998; Mitani et al., 2002; Mitobe et al., 2005; Thurston et al., 2010). In vivo and in vitro, TNF inhibited klotho expression through an NF-κB-dependent mechanism (Moreno et al., 2011), an effect potentiated by interferon (IFN)-γ (Thurston et al., 2010). The reduced expression of klotho is mediated by oxidative stress (Saito et al., 2003).

Thus, klotho and oxidative stress are linked in a double-negative feedback loop (Ferrell, 2002). In this circuit, klotho (A) inhibits or represses oxidative stress (B) and B inhibits or represses A. Thus, there could be a stable steady state with A on and B off, or one with B on and A off, but there cannot be a stable steady with both A and B on or both A and B off. Such a circuit could toggle between an A-on state and a B-on state in response to trigger stimuli that impinge upon the feedback circuit (Ferrell, 2002).

In humans, higher serum phosphorus levels are associated with increased morbidity or mortality from cardiovascular disease (CVD) in patients with chronic

kidney disease (CKD) or prior CVD (Braun et al., 1996; Block et al., 1998; Goodman et al., 2000; Ganesh et al., 2001; Oh et al., 2002; Ishimura et al., 2005; Kestenbaum et al., 2005) and with an increased CVD risk in individuals free of CKD in the community (Tonelli et al., 2005; Dhingra et al., 2007). Klotho RNAi induces premature senescence of human cells via a p53/p21 dependent pathway (de Oliveira, 2006). With aging, klotho expression decreases in a variety of human tissues (Shih and Yen, 2007; Witkowski et al., 2007; Takumida et al., 2009; Wang and Sun, 2009). In the white matter of the rhesus monkey, klotho protein expression is also significantly decreased with age (Duce et al., 2008; King et al., 2011). In older community-dwelling adults, plasma klotho is an independent predictor of all-cause mortality (Semba et al., 2011a) and cardiovascular disease (Semba et al., 2011b). Older adults with lower plasma klotho have poor skeletal muscle strength that is a predictor of poor outcomes (Semba et al., 2012). The levels of kidney klotho mRNA expression, protein production and circulating klotho are also greatly reduced in various acute and chronic renal diseases (Koh et al., 2001; Hu et al., 2010; Moreno et al., 2011; Asai et al., 2012; Pavik et al., 2012). Likewise, klotho mRNA expression is significantly decreased in some animal disease models. Because of sustained circulatory and/or oxidant stress, klotho expression is decreased in spontaneously hypertensive rats, deoxycorticosterone acetate–salt hypertensive rats, 5/6 nephrectomized rats, noninsulin-dependent diabetes mellitus rats (Aizawa et al., 1998; Nagai et al., 2000), ischemia–reperfusion injury models (Vonend et al., 2004), and rats with acute myocardial infarction (Aizawa et al., 1998).

Single nucleotide polymorphisms in the human klotho gene were found associated with lipid metabolism, glucose metabolism, bone mineral density and systolic blood pressure (Rhee et al., 2006b; Riancho et al., 2007; Shimoyama et al., 2009), longevity (Arking et al., 2002; 2005) and common age-related diseases including essential hypertension (Wang et al., 2010), metabolic syndrome (Majumdar and Christopher, 2011), coronary artery disease (Arking et al., 2003; Rhee et al., 2006a; Oguro et al., 2011), severity of non-diabetic end-stage renal disease (Bostrom et al., 2010), osteoporosis (Kawano et al., 2002; Ogata et al, 2002; Yamada et al., 2005), and stroke (Arking et al., 2005; Majumdar et al., 2010).

In summary, the klotho/FGF23 complex is a pleiotropic key player at the crossroads of the limited resources utilization/metabolism-stress/oxidative stress axes of aging/death control (for klotho's role in immunosenescence and link to the reproductive axis see chapter 11.2.4).

10.4 The organism, its stress response and aging

Physiologists define stress as how an organism responds to a stressor, a stimulus that causes stress. A stressor is a chemical or biological agent, environmental condition, an external stimulus or event that causes stress to an organism, deranges its homeostasis. Most importantly, according to Hoffmann and Parsons (1991), an extrinsic stressor is an environmental factor, a biotic or abiotic condition, event or organism that is distinct from the individual upon which it acts. Stress responses, on the other hand are directed against these stressors and try to overcome, at least attenuate, the perturbation of the homeostasis caused by them. In organisms with highly developed neuronal systems, stressors may also be imagined, and hence rather autochthonous. However, in the context of aging and its evolutionary roots in primordial organisms with absent or primitive neuroendocrine systems such a discrimination can be neglected. In addition, however, intrinsic cellular stress due to genetic stress caused by e.g. inbreeding (Bijlsma et al., 1997; 2000; Kristensen et al., 2002) and deleterious mutations (Trotter et al., 2002; Zhao et al., 2002; Bijlsma and Loeschcke, 2003) has to be taken into account.

The ETAs are unable to provide a sound explanation for the control of aging by the stress response network and the stress response phenotype of aging. It would be hard to find an evolutionary rationale for the evidence that organisms that have a billion-year-long evolutionary history of economic use and optimal allocation of resources should invest less resources into tissue maintenance (as the DST suggests) only to invest resources later to mitigate the sequelae of ensuing tissue failure.

Selye (1936) was the first to recognize the relative uniformity of the general stress response (GSR) (that he dubbed "general adaptation syndrome") to diverse stressors. The evolutionarily highly conserved GSR has been studied extensively in yeast, animals and plants (Kültz, 2003; 2005; Fujita et al., 2006; Walley et al., 2007). At the cellular level, the GSR results in oxidative and nitrosative stress as the final common pathway and a response pattern including hsp expression (Lindquist, 1986; Sanchez et al., 1992; Finkel and Holbrook, 2000; Mikkelsen and Wardman, 2003; Sørensen et al., 2003). Both intrinsic and extrinsic stress (Jiménez et al., 1994; Dahlgaard et al., 1995; Bijlsma et al., 2000; Keller et al., 2002) and various extrinsic stressors (Kiesecker and Blaustein, 1995; Folt et al., 1999; Xiong et al., 1999; Williams et al., 2007) may often strongly interact, and there is

cumulative evidence that this may lead to a strong synergism between the two stresses causing normally nonsevere stresses to become harmful when combined. The shared molecular pathways may account for a general phenomenon of GSR and hormesis: low to moderate intensity stressors elicit also cross-tolerances to stressors other than the conditioning stressor (Sanchez et al., 1992; Folt et al., 1999; Pichereau et al., 2000; Bowler and Fluhr, 2000; Pastori and Foyer, 2002; Treinin et al., 2003; Kültz, 2005; Baena-González and Sheen, 2008; Zhang et al., 2009). Importantly, hormesis is a unique feature of extrinsic stressors. Hence, that hormesis, the cross-tolerance to a variety of extrinsic stressors confers tolerance to aging (Parsons, 1995; Martinez, 1996; Masoro, 1998; 2006; Lin YJ et al., 1998; Morimoto and Santoro, 1998; Longo, 1999; Johnson et al., 2000; Minois, 2000; Turturro et al., 2000; Jazwinski, 2001; Yu and Chung, 2001; Sinclair, 2005; Rattan, 2008; Le Bourg, 2009; Mattson and Calabrese, 2009; Sørensen et al., 2010) defines aging as another GSR phenotype and strongly indicates that its causative factor is a stressor that is distinct from the aging soma. In bacteria, aging appears to require the harmful effects of an extrinsic factor (Rang et al., 2012). Hence, the mature soma and the germ cells causing the aging/death of the soma (see chapter 11) should be considered as distinct units of selection (Okasha, 2006, see chapter 19.2).

11. Germline cells engender somatic aging and death

Natural selection will never produce in a being any structure more injurious than beneficial to that being, for natural selection acts solely by and for the good of each. No organ will be formed …for the purpose of causing pain or for doing an injury to its possessor.
Charles Darwin, On the Origin of Species, 1859

Summary

Cumulative evidence obtained in taxa as diverse as prokaryotic bacteria, unicellular eukaryotes, plants, invertebrates, and vertebrates indicate that somatic death as "collateral damage" of reproductive events is triggered by signals from the germline cells. Most compellingly, signals from the germline cells in C. elegans and Drosophila control somatic longevity. The germline signals are part of a highly integrated signaling network that include metabolic and stress response pathways. The immune system is a major target of these signals. In invertebrates and vertebrates gonadal signal-mediated immunosuppression is a short-term concomitant of reproductive events. Moreover, gonadal hormones entail a long-term degeneration of immunocompetence, e.g. by lymphoid organ atrophy that leads to immunosenescence, a prime pacemaker of organismal aging. Importantly, semelparous and iteroparous organisms share the same pattern of neuroendocrine changes, in a continuum of temporal sequences that range from catastrophic to gradual.

Science is a part of human culture (Sober, 1994). As such, to what extent is science subject to cultural values? The key debate is that between objectivity and subjectivity: Does science obey certain disinterested norms or rules, designed or guaranteed to tell us something about the real world (as Popper holds), or is it a reflection of personal preference, the things in culture that people hold dear (as Kuhn asserts)? According to Ruse (1999) import of cultural values into science, and particularly evolutionary biology, has an essential role. But should we let cultural values come in the way of the scientific quest for truth?

Evolution does not "care" for man-made cultural norms and desirable behavior. The behavioral blueprint of cells is egoistic survival and reproduction. Without doubt, the most efficient way that people – or organisms – could survive was by banding together against the elements. In unicellular organisms multicellular behavior (e.g. biofilms, molds, slugs) is a response to environmental stressors (Heininger, 2001). Clearly, both cooperation and altruism are among the foundations of human societies and hence are highly desirable social behaviors. In humans, asocial behavior often requires negotiation and sanctions on disobedient individuals. This changes the payoffs, so that group-beneficial behavior also becomes optimal for the individual. If asocial behavior can only be avoided when higher-level incentives are invoked, as in the case of legal incentives, this raises the question of how lower taxa can avoid egoistic behavior and e.g. overexploitation of the resources they depend on (Rankin et al., 2007a). The evolutionary value of cooperation is self-evident for behavior that improves the chances of survival for the individual, e.g.in biofilm formation. But what about altruistic behavior that involves the death of an organism? Cells are not willing to forego their genetic programming for survival and reproduction deliberately. Cells are not able to negotiate and "convince" other cells that something like "altruistic suicide" is beneficial for the group. Group selection, even kin selection, is not strong enough to elicit such behavior (Williams, 1966). Quite rightfully, the limited-resources-based

Weismannian aging/death theory was dismissed due to its group selection core. Darwin (1859) and Dawkins (1989), among others, told us that all living organisms are programmed as survival machines. Very strong selection pressures are required to coerce an organism into death. I argue that only violent measures are adequate to enforce such a behavior. Earlier, I presented compelling evidence (which is going to be extended soon, Heininger, in preparation) that both compulsory measures and deceit succeeded to coerce cells into, and trapp them in, stress pathways with “dead-ends” (Heininger, 2001). Apoptosis is no “altruistic suicide” but programmed cytocide (Heininger, 2001). Scientists have difficulties to accommodate with the obvious enforced nature of programmed cell death (PCD). The scientific literature on PCD is still replete with wordings like “altruistic suicide”. Thus, the concept of kin/group selection that was dismissed as underlying the cause of aging/death at the multicellular level is resurrected as an argument for the programming of altruistic suicide at the unicellular level (Nedelcu, 2011). On the other hand, cellular fratricide is widely documented and readily accepted in multicellular (Green et al., 2003; Leisegang et al., 2010; Safirstein, 2011), and unicellular organisms (Gilmore and Haas, 2005; Claverys and Håvarstein, 2007; Be'er et al., 2009; Eldholm et al, 2009; Thomas and Hancock, 2009; Pérez-Dorado et al., 2010). It may be the developmental, obligate nature of fratricide and cannibalism during PCD (Heininger, 2001) that causes the culturally biased discomfort. I would like to illustrate what I call cultural pre-occupation in the interpretation of the evolutionary cause of the germ-soma dichotomy and the cause of somatic death. In her 2009 paper A. Nedelcu wrote: “The terminal differentiation of somatic cells in V. carteri is dependent on the expression of regA—a master regulatory gene that encodes a transcription factor thought to repress several nuclear genes coding for chloroplast proteins (Kirk et al. 1999; Meissner et al. 1999). As a result, the growth (dependent on photosynthesis) and reproduction (dependent on growth) of somatic cells are suppressed. Repression of their reproductive potential is costly for somatic cells, but this behaviour is beneficial for the group as a whole, since it ensures its motility. In other words, somatic cells are altruistic, and regA is directly responsible for this behaviour”. What the author does not mention is that regA is under control of the gonidia that thereby suppress all germ cell functions in somatic cells and determine the apoptotic fate of somatic cells (Kirk et al. 1999, Meissner et al. 1999). So far, cultural bias obscured the impartial view on the evolutionary causation of cellular and organismal death.

11.1 Reproductive maturity and lifespan: a temporal relationship

Among invertebrates and vertebrates, maximum longevity has a consistent relationship to age at sexual maturity (Lindstedt and Calder, 1981; Prothero, 1993; de Magalhães et al., 2007; Ricklefs, 2010b; Ridgway et al., 2010). Similarly, the median adult lifespan correlates with age at sexual maturity in a variety of taxa (Williams, 1966; Tinkle, et al., 1970; Markofsky and Perlmutter, 1972; Charlesworth, 1980; Stearns and Crandall, 1981;Charnov and Berrigan, 1990; Charnov, 1993; Gemmill et al., 1999; Francis et al., 2001; Kilborn et al., 2002; Guarino et al., 2003; Metcalfe and Monaghan, 2003; Nee et al., 2005). Demographic data led Baudisch and Vaupel (2010) to hypothesize that the shape of aging (i.e. how strongly senescence is favored) is determined not by the absolute age-independent hazard of death but by two relative quantities: the ratio of age-independent mortality to mortality at maturity and the ratio of the intrinsic rate of deterioration to mortality.

The ETAs proposed that aging starts with sexual maturity (Medawar 1955; Williams, 1957; Libertini, 1988; 2006; 2008; Arking, 1998 p. 12; Loison et al., 1999; Harper and Crews, 2000). Initially they provided no mechanistic clue other than the declining force of natural selection and Williams’s opposing selective forces (see chapter 13). But natural selection should not yet decline at the time of maturity when the reproductive potential of the organism is at its peak. According to Kirkwood and Cremer (1982), selection continues to operate with full force until reproduction begins. The DST later posited that reproductive activity, in an intrasomatic competition, drains the resources from somatic maintenance setting the stage for gradual functional decline. According to this logic, abundant resources should allow allocation of sufficient resources to both somatic maintenance and repair and prevent aging. However, they accelerate aging (see chapter 9).

The erosion of vertebrate immunocompetence related to thymic involution starts with gonadal maturation (see chapters 11.2.4 and 11.3). In semelparous species like Pacific salmon, changes related to aging, e.g. senile plaques in the brain (Maldonado et al. 2000; 2002), are already full blown at spawning (see chapter 11.2.4). In plants, the primary biological signal that initiates the senescence process is active at or near the time of flowering (Burke et al., 1984). At that time none of the conditions considered by the ETAs necessary for the onset of aging (declining natural

selection due to decreased “visibility”, threat of death (at least not more than during immaturity) apply, organisms are in their prime and should not yet have to pay the “price” (Williams, 1957) as suggested by the antagonistic pleiotropy ‘pay later’ theory.

I argue that the timing of the onset of aging is compelling evidence for the germ-soma conflict. Evolution “equipped” germ cells with the faculty to determine the aging/death of the soma (see chapter 11.2). From the standpoint of germ cells it would be optimal to start the aging/death process at the time when reproduction is ongoing or has been completed. Evolution “found” a solution to this problem by linking reproduction and aging through resource utilization. If reproduction is not possible in harsh environments, both resource utilization, reproduction and aging can be stalled through the processes of diapause, dauer formation or hibernation (see chapter 9.1). Other environmental stressors are also able to delay reproduction (Wingfield and Sapolsky, 2003). Since reproduction and aging/death are regulated by the same signaling pathways, evolution could not ‘allow’ to uncouple aging from reproduction and stall the aging process temporarily independent from resource availability and stress occurrence, easing the time constraints e.g. for finding a mating partner. Such an uncoupling eventually may have endowed the soma to evade the germ cell-dependent longevity regulation with all hazards of cheating and evasion to immortality. The latter obviously had to be prevented. Hence, evolution closely linked aging to reproductive maturity and resource utilization and, importantly, this link had to operate irrespective of reproductive success. This even may have the consequence that, if conditions are adverse for reproduction, single individuals or even species may have to skip reproduction with the extreme consequence of extinction. Obviously, evolution ‘opted’ for the latter consequence before risking the advent of Darwinian demons.

11.2 Germline signals drive somatic aging

One of the arguments against the genetic programming of aging is as follows: Aging cannot be programmed “because a gene that promotes aging would most likely decrease reproductive fitness and therefore would be subject to negative selection” (Butler et al., 2003). Such an argument obviously would not hold if the germline cells themselves would control aging/death of the soma to increase the chances of survival of the progeny.

One of the central tenets of the germ-soma conflict theory is that the death/aging of the soma is mediated by actions of the germ cells. Phenotypically, the germline cell-dependent aging/death is actuated by two mechanisms. In some once-reproducing organisms such as Bacillus subtilis, Myxococcus xanthus, Volvox carteri, Botryllus schlosseri, Adactylidium and Acarophenax tribolii, the offspring hatch from inside the mother and kill the mother organism during their hatching. In other semelparous and all iteroparous organisms, the germline cells effect the aging/death of the soma by signaling pathways, e.g. by hormones. Circumstantial evidence for this signaling has been obtained by the fact that death/aging is delayed by removal of the germline cells (see chapter 8.1). Endotokia matricida (see chapters 6.3 and 7.2) is no freak behavior from the horror cabinet of nature but a phenotype (evolved in extreme resource-limited habitats in which the parental organism serves well to nurture the progeny), that drastically illustrates the general principle of a source-sink relationship between parents and germs/offspring and that germline cells are programmed to eliminate their parental resource competitors. That the two mechanisms of endotokia matricida and iteroparous reproduction are interchangeable is shown in C. elegans where, when fourth-stage larvae or fecund adults are starved or otherwise stressed, the developing eggs hatch within (called bagging), killing their mother organism (Horvitz and Sulston, 1980; Trent et al., 1983; Johnson et al., 1984; Gems et al., 1998; Chen and Caswell-Chen, 2004; Shapira and Tan, 2008; Angelo and Van Gilst, 2009). Given that larvae of C. elegans cannot become dauers under complete starvation, a possible benefit of bagging is that the parental body provides nutrition and so allows larvae to become dauers under extremely food-limited conditions (Chen and Caswell-Chen, 2004).

11.2.1 Prokaryotes

Bacillus subtilis

Although synthesized prior to septation and partitioned into both the forespore and the mother cell, the transcription factor σF becomes active only in the forespore, therein initiating a genetic program that culminates in the formation of the dormant spore, launching by intercellular signaling the developmental program of the mother cell (Levin and Losick, 2000). Throughout, the temporally and spatially controlled cascade of RNA polymerase sigma factors is driven by the endospore and leads to the development and release of the spore from within the terminally differentiated, apoptotic mother cell (Hosoya et al., 2007). A screen for B. subtilis mutants in which sporulation is blocked after the formation of the polar septum detected the spoIISAB operon (that controls a toxin-antitoxin complex). Inactivation of spoIISB

decreases the sporulation efficiency of wild type cells by 4 orders of magnitude, whereas inactivation of spoIISA in the same background has no effect on sporulation. In contrast, spoIISA inactivation in a spoIISB null mutant background fully restores sporulation, indicating that SpoIISB is required only to counteract the negative effect of SpoIISA on sporulation (Adler et al., 2001). Inappropriate spoIISA (toxin) expression causes lysis of vegetatively growing B. subtilis cells and, when expressed heterologously, Escherichia coli cells (Florek et al., 2008), effects that are countered by co-expression of spoIISB (antitoxin), identifying SpoIISA-SpoIISB as a toxin-antitoxin system (Rešetárová et al., 2010; Florek et al., 2011) that is controlled by the developing spore.

Myxococcus

During M. xanthus fruiting body formation, approximately 80% of the cells undergo cell lysis, while the remaining 20% are converted to myxospores. In response to starvation, programmed cell death in the cells building the fruiting body is effected by a toxin-antitoxin system that is subdued in the myxospores (Nariya and Inouye, 2008). The levels of the bifunctional transcription factor/antitoxin MrpC and its related proteolytic fragment MrpC2 are increased, inhibiting the cell death pathway in the spores via direct interaction of MrpC with MazF, a toxin (Mittal and Kroos, 2009a). In its role as an antitoxin, binding of MrpC to the MazF toxin prevents programmed cell death in cells destined to form spores (Nariya and Inouye, 2008). In cells destined to undergo programmed cell death, binding of MrpC to the mazF promoter region would activate transcription, leading to increased MazF. Accordingly, MrpC is a key determinant of cell fate (Mittal and Kroos, 2009b). Deletion of the toxin results in elimination of the obligatory cell death during fruiting body development causing dramatic reduction in spore formation (Nariya and Inouye, 2008).

11.2.2 Basal eukaryotes

Yeast

By asymmetric division, some yeast and fungi strains generate daughter cells which, in contrast to symmetrically dividing bacteria, leave the mother cell intact (Horvitz and Herskowitz, 1992). The yeast Saccharomyces cerevisiae reproduces by asymmetric cell division, or budding (Hartwell and Unger, 1977). Mother cells have a limited lifespan, as defined by the number of divisions (approx. 20 to 35) (Jazwinski, 1993; Steffen et al., 2009) that it can potentially complete before it dies (Mortimer and Johnston, 1959). Evidence indicates that this is caused by some unequally distributed material between mother and daughter cell. A mechanism underlying the replicative clock and, hence, lifespan determination may depend on the asymmetric distribution of mitochondria between yeast mother and daughter cells which is caused by a cytoskeleton-dependent polarized movement of mitochondria (Simon et al., 1997; Yang et al., 1999). Moreover, the daughter cell does not inherit extrachromosomal ribosomal DNA circles (ERCs), which accumulate in mother cells during growth and have been suggested to cause replicative senescence (Sinclair and Guarente, 1997). Mutants lacking the phylogenetically conserved silent information regulator, Sir2p, an NAD (nicotinamide adenine dinucleotide)–dependent histone deacetylase (Imai et al., 2000; Smith et al., 2000), that is involved in transcriptional silencing and the control of genomic stability, contain more ERCs than their wild-type counterparts and have a reduced replicative potential (Kaeberlein et al., 1999). The finding that segregation of active mitochondria to daughter cells is important for maintenance of age asymmetry (Lai et al., 2002) raised the possibility that mitochondrial dysfunction may be a normal cause of aging and suggested that age asymmetry depends on partition of active and undamaged cellular components to the progeny. In fact, carbonylated proteins accumulate with replicative age in the mother cells but are not inherited by daughter cells during cytokinesis (Aguilaniu et al., 2003). Carbonylated proteins are associated with Hsp104p-containing protein aggregates and these aggregates, like oxidized proteins, are retained in the progenitor cell during cytokinesis by a Sir2p-dependent process. Deletion of HSP104 resulted in a breakdown of damage asymmetry, and overproduction of Hsp104p partially restored damage retention in sir2Delta cells, suggesting that functional chaperones associated with protein aggregates are required for the establishment of damage asymmetry (Erjavec et al., 2007). Daughter cells clear themselves of damaged proteins by a polarisome- and tropomyosin-dependent polarized flow of aggregates into the mother cell compartment establishing an aging (soma-like) and rejuvenated (germ-like) lineage (Liu et al., 2010).

Dictyostelium discoideum

So-called morphogens such as differentiation-inducing factors (DIFs), chlorinated alkylphenones (Kay and Jermyn 1983, Anjard et al. 1998, Brown and Firtel 2000, Nadin et al. 2000; Williams, 2006), and a glycoprotein psi factor (psi, prespore-inducing factor) (Kawata et al., 2004; Yamada et al., 2010) regulate the reproductive event in D. discoideum. Prespore cells determine the fate of apoptosing prestalk cells by DIFs and other factors (Early and Williams, 1988; Maruo et al., 2004). The DIFs are synthesized in

prespore cells and are inactivated in prestalk cells (Brookman et al. 1987, Kay et al., 1999; Thompson and Kay, 2000a; b; Kay and Thompson, 2001), establishing a feedback loop controlling DIF-1 levels (Insall et al., 1992). The inactivation, however, is inhibited by cAMP (Insall et al., 1992). At low doses DIFs may induce prespore differentiation (Oohata, 1995) but at higher doses are requisite for prestalk cell development (Kopachik et al. 1983, Sobolewski and Weeks 1988; Maruo et al., 2004). Prespore-produced DIF-1 inhibits redifferentiation of prestalk cells into prespores, i.e. transition into the dispersing and perennial germline (Firtel, 1995; Hudson et al., 2002). DIF-1 is rather a poison than a signal (Atzmony et al., 1997; Parkinson et al., 2011; Strassmann and Queller, 2011a) and acts antagonistically to cAMP by repressing prespore differentiation and directing a proportion of the cell population to differentiate as prestalk cells (Kay and Jermyn, 1983; Early and Williams, 1988; Kawata et al., 1996; Strassmann and Queller, 2011b). Mitochondrial malate dehydrogenase (mMDH) may be one of the target molecules of DIF-1 that inhibits the enzymatic activity of mMDH and cell energy production, probably leading to the inhibition of proliferation (Matsuda et al., 2010) and cell death (Luciani et al., 2009). Thus, prespore cells inhibit the conversion of prestalk to prespore cells (Inouye 1989, Shaulsky and Loomis 1993; Kawata et al., 1996; Söderbom and Loomis, 1998; Maruo et al., 2004; Yamada et al., 2010) and ensure the provision of nutrient supply by apoptotic stalk cells. Intriguingly, prespore cells become apoptotic stalk cells when prestalk cells are removed from the equilibrium (Nadin et al. 2000; Ràfols et al., 2001) and prestalk cells become prespore cells when prespore cells are removed (Shaulsky and Loomis 1993; Ràfols et al., 2001; Maruo et al., 2004). It can be inferred that survival and death of cells depends on environmental cues, the proportion of dying cells being regulated by the nutritive needs of the germ cells.

Volvocales

Sporangin of the unicellular green alga Chlamydomonas reinhardtii is a subtilase-like serine protease. The enzyme is synthesized and accumulates in the daughter cells before hatching. Sporangin is localized to the flagella of the daughter cells within the sporangial cell wall, and released from flagella concurrently with the digestion of mother's sporangial cell wall, and then the daughter cells are hatched (Kubo et al., 2009).

Volvox carteri somatic development and somatic death during hatching are controlled by germ cells and juveniles. V. carteri juveniles hatch from their parental spheroid by digesting the parental extracellular matrix (Jaenicke and Waffenschmidt, 1979; Kirk, 1998). The hatching enzyme, a serine protease that is orthologous to Chlamydomonas sporangin, is synthesized and accumulates in juveniles as an inactive precursor with prodomain and is cleaved to yield the active enzyme at hatching. The enzyme degrades only the portions of the parental somatic sheet just above the juveniles to make openings from which juveniles are liberated (Fukada et al., 2006). After outside release of the protease the whole parental somatic sheet is degraded (Fukada et al., 2006). A locus, regA, controls germ-soma differentiation and is strongly expressed in germ cells, but not somatic cells. RegA blocks reproductive development in somatic cells by preventing chloroplast biogenesis, thereby making it impossible for the cells to grow enough to reproduce. Importantly, gonidia suppress all germ cell functions in somatic cells and determine the apoptotic fate of somatic cells (Kirk et al. 1999, Meissner et al. 1999). This behavior is also susceptible to defection and selfish mutants; indeed, mutations in regA result in somatic cells regaining reproductive abilities, which in turn results in them losing their flagellar capabilities (e.g., Kirk et al. 1987). As motility is very important for these algae (e.g., flagellar activity is required to maintain themselves in the water column at an optimum position relative to sun light intensity) (Kirk 1998, p. 70), the survival and reproduction of V. carteri individuals in which such mutant somatic cells occur is negatively affected (Solari, Kessler, and Michod 2006); this is supported by the absence of Reg mutants as established populations in nature, although they occur spontaneously at a rather high rate (Kurn et al. 1978). Misinterpreting the control of regA expression, reg A was dubbed an altruistic gene (Nedelcu and Michod, 2006; Nedelcu, 2009). However, regA is a repressive, fratricidal gene allowing the gonidia to repress the reproductive abilities of somatic cells and defining their apoptotic fate. Remarkably, some of the late somatic genes in V. carteri are related to senescence-associated genes in higher plants (Shimizu et al., 2002).

11.2.3 Plants

The continuous generation of organ systems by the plant's meristems is countered by the programmed senescence and/or abscission (shedding) of existing organs throughout the life of the plant. Ethylene regulates many aspects of the plant life cycle, including seed germination, root initiation, root hair development, flower development, sex determination, pollination, fertilization, fruit ripening, senescence, and responses to biotic (such as pathogen attack) and abiotic (such as wounding, hypoxia, and chilling)

stresses (Bleecker and Kende, 2000; Lin et al., 2009). Intriguingly, ethylene is also a trigger for Dictyostelium sexual reproduction (Amagai, 2011). Unlike unitary organisms, plants have no segregated germline cells and no germline-specific hormone. Hence, in addition to reproductive activity ethylene regulates stress responses and a number of vegetative growth processes, most notably induction of asymmetric stem and petiole growth. Due to the higher integration of somatic and germline tissue and shared hormone biosynthesis and signaling pathways, plant germline signaling effects are less obvious than in unitary organisms. Ethylene regulates many aspects of plant developmental processes interacting with other phytohormones like jasmonic acid, salicylic acid (Wang et al., 2002), auxin (indole-3-acetic acid), gibberellic acid, cytokinins (Lin et al., 2009; Yoo et al., 2009), and abscisic acid (Beaudoin et al., 2000). Plant germ cell-derived ethylene operates during flowering, ovary development, pollination and ripening of fruit (O'Neill, 1997; Lin et al., 2009; Lozano et al., 2009) and senescence of some petals when ethylene production is autocatalytic. Climacteric and non-climacteric fruits have traditionally been viewed as representing two distinct programmes of ripening associated with differential respiration and ethylene hormone effects. In climacteric fruits, for example, tomato (Solanum lycopersicum), banana (Musa acuminata), apple (Malus domestica) and melon (Cucumis melo), the onset of ripening is marked by increased ethylene synthesis and respiration. In non-climacteric fruits, for example, strawberry (Fragaria×ananassa), grape (Vitis vinifera), and citrus, these changes are not apparent (Seymour et al., 1993; Giovannoni, 2004). However, recently a good correlation has resulted between the expression of the genes in non-climacteric plants and the data of ethylene production. In particular, similarly to what occurs during climacteric fruit ripening, there is an increased synthesis of receptors concomitant with the increased synthesis of ethylene in non-climacteric plants as well. Thus, even the little ethylene produced by ripening non-climacteric plants is sufficient to trigger ripening-related physiological responses (Trainotti et al., 2005; Chervin et al., 2006; Lin et al., 2009; Wang et al., 2010, Seymour et al., 2011). In climacteric fruits, ripening usually commences in one region of a fruit, spreading to neighbouring regions as ethylene diffuses freely from cell to cell and integrates the ripening process throughout the fruit (Pech et al., 1987, Lelièvre et al., 1997; Alexander and Grierson, 2002; Vriezen et al., 2008; Wang et al., 2009; Costa et al., 2010). In many species, the onset of petal, leaf or plant senescence is regulated by endogenous ethylene (Grbic and Bleecker, 1995) while in other species there is no such ethylene control. In the first group, a sharp rise in ethylene production precedes visible wilting, and any stress that increases the rise in endogenous ethylene production hastens the time to senescence. In still other species, flower life is terminated by petal abscission, before visible senescence symptoms and its onset appears to be regulated by ethylene (van Doorn and Stead, 1997; van Doorn, 2001). Expression of ethylene biosynthetic genes suggests that the ovary or associated tissues (ovules, placenta) are the initial site(s) of ethylene production during natural carnation (Dianthus caryophyllus L.) flower senescence (ten Have and Woltering, 1997). Breakdown of macromolecules into mobile compound is important in plant senescence (Buchanan-Wollaston et al., 2003). Degradation of polysaccharides, proteins, lipids, and nucleic acids results in mobilization of sugars and nitrogenous compounds, before visible senescence. These mobile molecules are transported, through the phloem, to the ripening fruit, establishing a source-sink relationship (van Doorn, 2004; Baldet et al., 2006). Leaf senescence is related to fruit production in different Arabidopsis ecotypes and it was concluded that the close correlation of bolting and senescence either indicates that leaf senescence and reproduction are regulated by common exogenous (e.g. light) and/ or endogenous (e.g. sugars) factors, or that senescence is triggered by reproductive development (Levey and Wingler, 2005). Consistent with the latter notion, sink demand of the inflorescence does not control senescence of Arabidopsis leaves (Hensel et al., 1993; Noodén and Penney, 2001). At high nutrient supply, delayed senescence does not affect flowering or fruit formation. Nutrient deficiency, however, reduced flowering and fruit formation in wild-type plants and, more severely, in transgenic plants with delayed senescence (Wingler et al., 2005). At low nutrient supply, production of buds was delayed in transgenic plants with delayed senescence compared with the wild type, and fewer buds developed into flowers and fruits (Wingler et al., 2005). On the other hand, sucrose treatment delays the time to visible senescence. It is also known to delay the large rise in ethylene production, mainly by decreasing ethylene sensitivity (Nichols, 1973; Mayak and Dilley, 1976). The delay of visible senescence after sucrose treatment is associated with preventing the up-regulation of numerous genes. Since these senescence-associated genes are shown to be regulated by ethylene, the data indicate that sucrose regulates ethylene signaling (Hoeberichts et al., 2007) (see also chapter 15.3.1). Taken together, these

findings demonstrate that germ cell-derived ethylene triggers recycling of photosynthates from senescing leaves and plants that is, at least in semelparous plants and under nutrient stress, necessary for seed and fruit development and ripening. Provision of sucrose can delay senescence in this source-sink relationship.

11.2.4 Metazoa

Reproductive behavior is a highly complex behavior that is driven by a multitude of environmental and intrinsic cues. One of the most essential cues has to come from the storage organs signaling that sufficient calorie-rich resources are available to initiate the energetically demanding reproductive activity (Schneider, 2004; Hill et al., 2008). Another signal concerning the timing of reproduction comes from the environment. Unicellular organisms and plants are able to deposit highly resistant spores and seeds in a seed bank (Templeton and Levin, 1979; Nunney, 2002; Lennon and Jones, 2011) that can come to life when conditions are favorable enough, sometimes million of years later (Vreeland et al., 2000). With some notable exceptions among invertebrates, e.g. Daphnia (Cáceres, 1998), metazoan fertilized eggs are less resistant. Hence, reproduction timing has to take into account the more or less predictable future resource availability to ensure maximum possible survival of the offspring. To this end, photoperiod cues are used. (Plants use these cues as well to complete their cycle of vegetation during a season). In sexually reproducing organisms, olfactory cues are used to find mates. Thus, a variety of sensory inputs are required to optimize the timing of reproduction. The germline cells, mostly buried deep within the organisms, have no access to these informations. Almost from the beginning of metazoan evolution, neuronal structures evolved to sense and process these informations. Marvelling at the economy and "sophistication" of evolved processes it should not come as surprise that evolution "exploited" the same structures and processes that serve the purpose of reproduction to ensure the future fitness of the offspring by eliminating the postreproductive individuals.

At a variety of functional levels, the intimate coordination of functions promoted the evolutionary integration of structures and transfer of functions from one structural entity to the other. Thus, mitochondrial and plastid genes have been transferred to the nucleus (Perna and Kocher, 1996; Mourier et al., 2001; Leister, 2005; Kleine et al., 2009). Similarly, structures and functions intimately related to reproductive activity were integrated into an organism-wide network regulating metabolism, stress, reproduction and aging. This integration of reproductive functions is highlighted by brain structures such as corpus allatum in Drosophila, optic glands in Octopus and hypothalamus, particularly its arcuate nucleus, in mammals. Signals from the brain regulate reproductive maturation and aging in animals (Golding and Yuwono, 1994; Boulianne, 2001; Braeckman et al., 2001; Finch and Ruvkun, 2001). Thus, an interplay between gonads and brain drives the dynamics of both reproductive phase and aging (Nelson et al., 1995; Wise et al., 1997) and elicits reproductive cessation as aging-related feature (Packer et al., 1998). In higher metazoa, the reproductive phase/aging balance is controlled by hormones of the hypothalamic-pituitary-gonadal (HPG) axis (Kalra et al., 1993; Nelson et al., 1995; Wise et al., 1997). In these intricately tuned feedback cycles it is sometimes difficult to discriminate between cause and effect. The fundamental question finally is: who is in the driving seat, germline cells or soma? According to the current paradigm it is the soma. This has led to the multiple inconsistencies and incompatibilities of the ETAs. A simple change of perspective radically changes the whole picture. From the perspective of the germline cells, aging/death of the soma becomes selected for, adaptive, and fitness-enhancing and thus evolution that, by default, has an offspring-biased perspective programmed aging and death.

The vast majority of metazoa reproduces sexually. In addition to their reproductive actions, gonadal hormones mediate both somatic maintenance and aging, dependent on intricately balanced, Janus-faced modes of action (Nathan and Chaudhuri, 1998). To avoid any misunderstanding: the primary action of gonadal hormones during the reproductive phase of sexually reproducing organisms is to render the soma vital and attractive to enhance its chances of mating and reproductive success. The progeroid actions of gonadal hormones have to be seen in the context of their antagonistic pleiotropic actions (see chapter 13) that mediate the germ-soma conflict over the future utilization of resources.

Nematodes

In C. elegans, a variety of ground-breaking discoveries concerning the regulation of somatic aging by germline-derived signals have been made (Mukhopadhyay and Tissenbaum, 2007; Panowski and Dillin, 2009; Kenyon, 2010). Signals from the reproductive system control the lifespan of the nematode. When the germline cells of C. elegans are removed, the animals live approximately 60% longer than normal (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002). This lifespan extension is not a simple consequence of sterility but is due to signaling of the somatic gonad, as removing the entire gonad (the

germ cells as well as the somatic reproductive tissues) does not extend lifespan (Hsin and Kenyon, 1999). Thus, the costs of reproduction in C. elegans have been considered not resource-based (Barnes and Partridge, 2003; Baumeister et al., 2006). Moreover, laser-microbeam ablation of cells that give rise to the germline causes adults to become giant. Ablation of these cells in self-sterile mutant worms also causes gigantism, suggesting that the germline represses growth because it is the source of a growth-antagonizing signal rather than because of a sink of resources required for reproduction (Patel et al., 2002). The two parts of the C. elegans reproductive system, the germline cells and the somatic reproductive tissues, antagonistically influence the lifespan of the animal. Removing the germ stem cells (GSCs) increases longevity. This lifespan extension requires the somatic gonad (Arantes-Oliveira et al., 2002; Hsin and Kenyon, 1999; Berman and Kenyon, 2006) that signals through dafachronic acid to the DAF-12-sterol signaling pathway (Yamawaki et al., 2010). The somatic reproductive tissue appears to counterbalance the germline-exerted influence on the IIS pathway (Hsin and Kenyon, 1999; Gerisch et al., 2005). Consistent with these observations, GSC overproliferation has deleterious consequences for longevity that can be inhibited by longevity-promoting mutations (Pinkston et al., 2006). Similarly, ectopic germline proliferation that caused germ cells to accumulate in the body cavity substantially shortened the lifespan of C. elegans (Garigan et al., 2003). Numerous studies in C. elegans suggest the existence of a complex network of endocrine interactions between GSC and the soma that coordinate reproduction and somatic maintenance in response to nutritional conditions (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002; Pinkston et al., 2006; Wang et al., 2008; Yamawaki et al., 2008; Jasper and Jones, 2010). Interestingly, GSCs can influence metabolic homeostasis in worms directly through endocrine mechanisms (Wang et al., 2008). Worms in which GSCs have been laser ablated, or that carry mutations that disrupt GSC maintenance or proliferation, exhibit a significant decrease in overall body lipid storage. This decrease is caused by transcriptional upregulation of a triglyceride lipase in the worm intestine, resulting in leaner, long-lived animals (Wang et al., 2008). The lifespan increase produced by loss of the germ cells requires the DAF-16/FOXO transcription factor, the product of a gerontogene (Johnson et al., 2000; 2001). In response to germ-cell removal, DAF-16 accumulates in nuclei of adult intestinal cells. The somatic gonad is not required for DAF-16 nuclear accumulation or for the increased stress resistance that is produced by germ-cell removal. The somatic gonad is required, however, for expression of specific DAF-16 target genes. DAF-16 is known to be activated by reduced insulin/IGF-1 signaling in C. elegans. In certain insulin/IGF-1-pathway mutants, the somatic gonad is not required for germ-cell removal to extend lifespan. These mutations reduce IIS below a critical threshold level. At these low levels of IIS, factors normally provided by the somatic gonad are no longer needed for germ-cell removal to increase the expression of DAF-16 target genes (Yamawaki et al., 2008). The germline daf-16-dependent signal to regulate lifespan acts independently of the upstream IIS/daf-2 gene (Lin et al. 2001). The reproductive pathway acts in parallel to IIS, as loss of germline almost doubles the lifespan of daf-2 mutants (Hsin and Kenyon, 1999). Endocrine steroid hormone signaling from the germline to intestine, which is also the animal's site of fat storage (adipose tissue), was identified as an essential element in the control of C. elegans longevity (Baumeister et al., 2006; Beckstead and Thummel, 2006; Berman and Kenyon, 2006; Wang et al., 2008). In C. elegans lacking a proper function of the insulin receptor and the intestinal peptide transporter, downregulation of this signaling led to extreme longevity (Spanier et al., 2010). When germ cells are removed, mRNA and protein levels of nhr-80, a nuclear receptor, increase. This promotes the mono-desaturation of stearic acid to oleic acid by inducing the transcription of the stearoyl-CoA-desaturase, fat-6/SCD1. Both nhr-80 and the SCD activity are required to augment the lifespan of germline-depleted animals. Furthermore, a lack of SCD activity can be bypassed by addition of exogenous oleic acid. This lifespan extension can occur in the absence of daf-16/FOXO but requires the presence of the nuclear receptor DAF-12/VDR (Goudeau et al., 2011).

The germline controls the innate immune response of C. elegans somatic cells to two different Gram-negative bacteria. Loss of germline cells enhanced nematode survival in the presence of pathogens (Aballay and Ausubel, 2001; Kim et al., 2002; Alper et al., 2010). The germ cell-mediated impairment of immune competence results in increased susceptibility to bacterial infections that contributes to mortality in C. elegans (Aballay and Ausubel, 2001; Garigan et al., 2002). In contrast to the IIS pathway, the germline acts via distinct signaling pathways to control lifespan and innate immunity. Thus, a complex regulatory network integrates inputs from IIS, p38 MAPK signaling, and germline stem cells to control innate immunity that is an important

determinant of longevity in C. elegans (Troemel et al., 2006; Alper, 2010; Alper et al., 2010; Kriete et al., 2010).

Arthropods

Two hormones coordinately orchestrate the stereotyped program of insect growth and development. Juvenile hormone (JH) and 20-hydroxy-ecdysone (20E) are highly versatile hormones, coordinating development, growth, reproduction and aging in insects. Ecdysteroids, steroid hormones that are produced within the adult gonads (Garen et al., 1977; Riddiford, 1993; Freeman et al., 1999; Gilbert et al., 2002; Terashima et al., 2005; Bodin et al., 2007; Ogihara et al., 2007; Maeda et al., 2008; Casas et al., 2009; Parthasarathy et al., 2010; Yamazaki et al., 2011), and structurally related to mammalian sex steroids (Ables and Drummond-Barbosa, 2010), are responsible for initiating each developmental and physiological transition and JH is critical for determining the nature of the transition in a stage-specific manner. Pulses of the hormonally active 20E orchestrate the development of Drosophila from the embryo through the three larval instars into the reproductively mature adult, in particular developmental timing and metamorphosis (Riddiford, 1993; Thummel, 1996; Kozlova and Thummel, 2000; Truman, 2005). 20E regulates a wide range of developmental and cellular processes such as cell polarity (Romani et al., 2009), cell cycle (Fallon and Gerenday, 2010), and cell migration (Bai et al., 2000), as well as cell and tissue proliferation, differentiation, histolysis, and apoptosis (Thummel, 1996, 2001a; b). In the adult, 20E affects, among other things, courtship behavior (Ishimoto et al., 2009), vitellogenesis and egg production (Bownes, 1982; Richard et al., 1998, 2001; Buszczak et al., 1999; Carney and Bender, 2000), adult reproductive diapause (Richard et al., 2001), and innate immunity (Flatt et al., 2008b).

Several lines of evidence suggest that the gonads by means of 20E/ecdysone receptor (EcR) signaling affect fly lifespan. Longevity is extended in mutants deficient for ecdysone synthesis or mutants of the EcR (Simon et al., 2003). A mild ubiquitous inactivation of EcR during adulthood is sufficient to slow the aging of male flies, whereas a stronger EcR inactivation decreased longevity (Tricoire et al., 2009). Surprisingly, ubiquitous inactivation of EcR strongly decreased female lifespan. This deleterious effect was suppressed in sterile mutant females, suggesting that EcR represses a negative signal for lifespan produced in ovaries (Tricoire et al., 2009).

Delayed reproductive maturation and reduced fecundity due to reduction-of-function mutants in the IIS pathway extend the lifespan of Drosophila (Clancy et al., 2001; Tatar et al., 2001). Pharmacological acceleration of reproductive maturation, on the other hand, normalizes longevity in these mutants (Tatar et al., 2001). Ablation of the germline by forced differentiation of GSCs was found to extend lifespan in males and females (Flatt et al., 2008a). GSC loss influenced metabolic homeostasis, resulting in hypoglycemia, while at the same time causing changes in IIS/dFOXO activity that are reminiscent of insulin resistance (Flatt et al., 2008a). However, ablation of the germline by use of maternal effect mutations that result in progeny with a precociously absent or significantly reduced germline does not affect lifespan significantly, indicating that, as in the worm, lifespan extension by loss of GSCs is dependent on complex variables, such as timing of germline loss, presence of somatic cells in the gonad, genetic background, and diet (Barnes et al., 2006; Lee et al., 2008; Jasper and Jones, 2010).

20E increases DNA synthesis in the corpus allatum, the gland responsible for JH production (Chiang et al., 1997) and JH synthesis is regulated by ecdysteroids (Dubrovsky, 2005; Kaneko et al., 2011). In Drosophila, exposure to JH in the larval medium (and up to 24 h posteclosion) increased early life fecundity but reduced lifespan of normal flies. Selection for resistance to JH induced changes in JH metabolism or signaling, which led to longer lifespan as a correlated response (Flatt and Kawecki, 2007). Reproductive diapause has been well studied in monarch butterflies, several grasshoppers, and several Diptera, including Drosophila and Phormia. In monarchs and in grasshoppers, reproductive diapause physiology has been experimentally induced by the surgical removal of the corpora allata; allatectomy in each case was found to double adult longevity. Among Drosophila, the endemic D. triauraria of Japan, and D. littoralis of Finland over-winter as adults in reproductive diapause. How D. melanogaster over-winters is poorly understood, but reproductive diapause can be cued by cool temperature.

In laboratory studies, the mortality rates of post-diapause D. melanogaster are similar to rates of newly enclosed, young flies. This implies that senescence during diapause is slow or negligible. Slow aging during the diapause period may involve elevated somatic stress resistance as well as reallocation of resources to somatic maintenance. Adult Drosophila females undergoing reproductive diapause exhibit ovarian (vitellogenic) arrest, increased stress resistance, and greatly improved adult survival (Tatar and Yin, 2001; Schmidt and Paaby, 2008). Diapause is associated with reduced

ovarian ecdysteroid levels, a condition that can be rescued by 20E application (Richard et al., 1998, 2001). In addition, natural variation in diapause incidence has been mapped to a gene encoding an ecdysteroid-responsive RNA binding protein that is expressed in the peripheral nervous system and the ring gland, a composite larval endocrine organ that contains the ecdysteroidogenic prothoracic gland (Schmidt et al., 2008).

Germline-less, long-lived flies exhibit increased expression of the secreted fly insulin/IGF-binding protein IMP-L2 (Flatt et al., 2008a) that is an IIS antagonist (Honegger et al., 2008). Therefore, fly germline ablation possibly extends lifespan by interacting with IIS and 20E/EcR signaling, as is the case in the nematode. Moreover, sterile, long-lived insulin receptor mutants exhibit ovarian ecdysteroid deficiency – and 20E deficiency might thus contribute to lifespan extension upon reduced IIS (Tu et al., 2002). Reproductive diapause in Drosophila is proximally controlled by downregulation of juvenile hormone, a phenotype that is also produced by mutants of the insulin-like receptor InR, homologue of C. elegans daf-2 (Tatar and Yin, 2001). Eliminating germ cells in D. melanogaster increases lifespan and modulates insulin signaling. Long-lived germline-less flies show increased production of Drosophila insulin-like peptides (dilps) and hypoglycemia but simultaneously exhibit several characteristics of IIS impedance, as indicated by up-regulation of the Drosophila FOXO (dFOXO) target genes 4E-BP and l (2)efl and the insulin/IGF-binding protein IMP-L2. These findings suggest that signals from the gonads regulate lifespan and modulate insulin sensitivity in the fly and that the gonadal regulation of aging is evolutionarily conserved (Flatt et al., 2008; Gáliková et al., 2011).

The similar nature and range of developmental, physiological, and organism life history traits influenced by 20E/EcR and dafachronic acid/DAF-9/DAF-12 suggests that there are striking parallels between the steroid hormone pathways of worms and flies. It is thus tempting to speculate that 20E/EcR and dafachronic acid/DAF-9/DAF-12 might in fact represent functionally homologous endocrine systems (Gáliková et al., 2011) and may work analogously to mammalian estrogen receptor by coupling nutrient cues to maturation (Magner and Antebi, 2008).

Eusocial insects

In eusocial insects, according to the super-organism concept (Hölldobler and Wilson, 2008), the functionally sterile workers can be compared to somatic tissue and the reproductive queens to the germline (Oster and Wilson, 1978; Moritz and Fuchs, 1998; Gordon, 2010). Colony longevity (approximated by queen lifespan) and metabolic rate scale with the total biomass of the colony (Keller and Genoud, 1997; Gillooly et al., 2010; Hou et al., 2010) supporting the superorganism concept. The germ-soma conflict theory, interpreting the reproductive queens as the germline, offers insight into the fundamentally different longevities of queens and workers that hardly can be explained by the ETAs. The “battlefield” of the germ-soma conflict that is the soma in unitary organisms, becomes the colony in eusocial colonies. Like multicellular organisms, social insect colonies display trade-offs in resource allocation between growth, defense, and reproduction (Sudd and Franks, 1987; Bourke and Franks, 1995; Kaspari and Byrne, 1995). The longevity of the queen(s) is determined by the ecological necessities of the colony. The death of the colony is equivalent to the death of the soma. Under environmental stress, the ant colony as a super-organism is expected to sacrifice somatic tissue (brood and workers) to ensure queen germline and hence colony survival (Wilson, 1971; Taylor, 1978; Sorensen et al., 1983; Chapuisat et al., 1997). The individuals are relatively autonomous components, and considerable losses of workers can be tolerated without the risk of colony extinction.

Understanding of ovarian signaling in sterile worker honey bees is supported by several recent studies that tie social behaviour, endocrine feedback sensitivity, and longevity to variation in ovary size (Amdam et al. 2006, 2007; Page and Amdam 2007; Münch et al., 2008). Larger ovaries are linked to an earlier onset of foraging behaviour and thus a shorter lifespan. In contrast, smaller ovaries are associated with later foraging onset, and a longer life (Page and Amdam 2007). Larger ovaries and thereby shorter lifespans are also linked to a preference for pollen hoarding, which despite worker sterility represents an ancestral hallmark of reproductive behavior (Amdam et al. 2006). Thus, the associations between ovary size, foraging onset and foraging preference fit well to data from Drosophila and C. elegans, in that they support an evolutionary conserved link between fertility and lifespan (Leroi 2001; Hsu et al., 2003; Partridge, Gems and Withers, 2005; Flatt and Kawecki, 2007; Münch et al., 2008). In C. elegans aging, the progeroid germline signal is antagonized by a lifespan-extending signal from the somatic gonad. Similarly, in worker honey bees an antigeroid somatic signal is antagonized by a signal from brood. Vitellogenin, a nutritive yolk protein that is produced and secreted by the fat body (corresponding to the mammal liver), lowers susceptibility to oxidative stress (Seehuus et al. 2006), improves innate immunity (Amdam et al., 2004) and

extends lifespan (Amdam et al., 2004; Nelson et al., 2007; Smedal et al., 2009). Exposure to a pheromone secreted by larval brood reduces the amount of stored vitellogenin in worker bee fat body, their lifespan and the long-term survival of colonies (Smedal et al., 2009). Brood pheromone has a positive effect on vitellogenin stores in 3–4-day-old bees and a negative effect in later life (Smedal et al., 2009) (see chapter 15.2 for an interpretation).

Mollusks

Assuming that semelparity and iteroparity are the opposite ends of a continuum of reproductive strategies implies that similar signaling pathway should operate in both of them, albeit with an accelerated mode of action in semelparity that may facilitate to identify causal relationships. In Octopus, semelparous reproduction and postreproductive death are controlled by a gonad-brain-gonad feedback loop. The paired optic glands, which are analogous to the anterior pituitary in the context of gonadal maturation, are found on the upper posterior edge of the optic tract of the cephalopod Octopus vulgaris. Sexual maturation results from the increased secretion of a gonadotropin (GnRH) by the optic glands (Wells and Wells, 1959; 1972; 1975; Iwakoshi et al., 2002; Minakata et al., 2009). Both structure and functions of the GnRH family are, at least partially, evolutionarily conserved between cephalopods and vertebrates (Kah et al., 2007; Minakata et al., 2009). Optic gland secretion of GnRH suppresses protein synthesis in the muscles. This is associated with an increase in the total amino acid pool in the muscles and with a considerable increase in the concentration of free amino acids circulating in the blood. These events are associated with a rapid growth of the ovary and its ducts, and a loss of weight elsewhere (O'Dor and Wells, 1978). O. vulgaris stop feeding two or more weeks before the single spawning period but resume feeding after removal of both optic glands (Wodinsky, 1977). Starvation, per se, does not lead to the apparently inevitable degenerative changes seen in mature Octopuses (O'Dor and Wells, 1978). O'Dor and Wells (1978) had suspected the existence of gonadal hormones based on their finding that the ratio of blood to muscle amino acid levels was markedly different in animals maturing with and without ovaries; the animals with ovaries intact showed a high blood to muscle ratio that suggests that the ovary produces a hormone that increases the release of muscle amino acid into the blood from the pool in the muscles. In fact, Oct-GnRH was shown to induce steroidogenesis of testosterone, progesterone and 17β-estradiol in Octopus ovary and testis, where oct-GnRH receptors are abundantly expressed (Kanda et al., 2006). Both 17β-estradiol and progesterone have been detected in the ovary of O. vulgaris, and their concentrations changed in correlation with ovarian development. The 3β-hydroxysteroid dehydrogenase activity in the ovary indicates that in O. vulgaris the reproductive system is the source of sex steroid hormones (D'Aniello et al., 1996; Di Cosmo et al., 2001). Estrogen receptor (ER) orthologs have been identified in various mollusks (Matsumoto et al., 1997; Thornton et al., 2003; Kajiwara et al., 2006; Bannister et al., 2007), including the cephalopod O. vulgaris (Di Cosmo et al., 2002; Keay et al., 2006). Secretion from the optic glands is held in check pre-maturationally by an inhibitory nerve supply from the subpedunculate area, the olfactory and optic lobes (Wells and Wells, 1959; Froesch, 1974; Di Cosmo and Di Cristo, 1998). The action of this region is in turn dependent upon the integrity of the optic nerves and thus, presumably, upon light (Wells and Wells, 1959). Castration led to swelling of the male and female reproductive ducts, phenotypes that are also induced by optic gland activation. The findings have been interpreted as due to the gonadectomy-related elimination of an inhibitory feedback acting upon the optic glands (Richard and Lemaire, 1975). Estradiol up-regulated Oct-GnRH and O. vulgaris estrogen receptor (Oct-ER) mRNA levels in the olfactory lobes (De Lisa et al., 2012). Estradiol binds Oct-ER causing conformational modifications and nuclear translocation consistent with the classical genomic mechanism of the ER. Moreover, estradiol triggered a calcium influx and cyclic AMP response element binding protein phosphorylation via membrane receptors, providing evidence for a rapid nongenomic action of estradiol in O. vulgaris olfactory lobes (De Lisa et al., 2012) that project to the optic glands. To my knowledge, the functional role of gonadal hormone signaling on GnRH inhibition (Mayer et al., 2010; García-Galiano et al., 2012; Wolfe and Wu, 2012) has not yet been tested in cephalopods in vivo. However, at least in other mollusks, estrogens and endocrine-disrupting chemicals (EDCs) that mimic the action of estrogens have been found to initiate reproductive activity (Mori, 1969; Varaksina et al., 1992; Jobling et al., 2004; Wang and Croll, 2003; 2004; 2006; Ketata et al., 2008; Matthiessen, 2008); whether these effects have been accomplished via disinhibition of GnRH secretion has not been reported. The immature gonads of mollusks exhibit very early expression of steroidogenic enzymes and aromatase at the onset of reproductive maturation (Matsumoto et al., 1997; Osada et al., 2004), arguing for the initiation of maturation by gonadal hormone signaling. Both the prematurational inhibition and maturational disinhibition of GnRH signaling by gonadal hormones

as signal for the onset of maturation (Dungan et al., 2006; Kauffman et al., 2007; Biran et al., 2008; Clarkson et al., 2009; Lindemans et al., 2009; Mayer et al., 2010; García-Galiano et al., 2012; Kenealy and Terasawa, 2012; Wolfe and Wu, 2012) and the role of gonadal hormones in reproductive aging (Hung et al., 2003) are evolutionarily conserved from invertebrates to mammals. The key role of the optic gland-gonadal axis not only for reproduction but also for postreproductive death has been established. Removal of both optic glands in female Octopus after spawning resulted in cessation of broodiness, resumption of feeding, increased growth, and greatly extended lifespan (Wodinsky, 1977). Senescent changes include heavy infection by Aggregata octopiana of the digestive tract of wild and cultured post-spawned females as well as poorly healing, infected injuries, arguing for the occurrence of rapid immunosenescence (Van Heukelem, 1983; Reimschuessel and Stoskopf, 1990; Anderson et al., 2002; Pasual et al., 2010).

In Pacific oysters, hemocyte activities (phagocytosis, adhesion), the main cellular mediators of the innate defence system in bivalves, decrease during gametogenesis, especially when gonads approach ripeness with adverse effects on susceptibility to pathogens and survival during the spawning season (Delaporte et al., 2006; 2007; Gagnaire et al., 2006; Duchemin et al., 2007; Samain et al., 2007; Huvet et al., 2010; Li et al., 2010; De Decker et al., 2011; Fleury and Huvet, 2012). Moreover, the humoral immune response was also found attenuated by spawning (Li et al., 2009; 2010). Oyster lines, either resistant or susceptible to summer mortality differ in their reproductive effort. Strains susceptible and resistant to summer reproduction-related mortality (Huvet et al., 2010; Samain, 2011) represent varieties on the semelparity-iteroparity continuum that we encountered in chapter 7.2. The resistant lines showed a significantly higher resistance to oxidative stress, lower reproductive effort and smaller gonad area than the susceptible lines (Delaporte et al., 2007; Samain et al., 2007; Fleury et al., 2010; Huvet et al., 2010; Samain, 2011). Moreover, increased resource availability, in contrast to the predictions of the DST, increased mortality of both susceptible and resistant oyster lines (Delaporte et al., 2007; Samain et al., 2007). The cellular and humoral innate immune competence is also deteriorated by spawning in European abalone (Travers et al., 2008). Estradiol and EDCs had dose-dependent effects on parameters of bivalve innate immunity, activating immune responses at lower levels and inhibiting at higher levels (Canesi et al., 2007).

Fishes

Fish reproductive hormone levels are dependent on photoperiod and melatonin, a known mediator of the effects of external factors on reproductive function in vertebrates. Photoperiod is perceived by pineal photoreceptors and/or eyes and transduced into rhythmic melatonin signals (Migaud et al., 2007; Falcón et al., 2010). In eel, melatonin stimulates the dopaminergic system of the preoptic area, which is involved in the inhibitory control of gonadotropin [luteinising hormone and follicle-stimulating hormone] synthesis and release (Sébert et al., 2008). Concerning the pituitary–gonadal axis, melatonin treatment decreased gonadotropin and reduced sexual steroid (11-ketotestosterone, oestradiol) plasma levels in Pacific salmon and eel (Amano et al., 2004; Sébert et al., 2008). Puberty should occur only when body size and energy stores are sufficient enough to allow the success of reproduction. Thus, metabolic signals such as insulin-growth factors or leptin and ghrelin are involved in the triggering of teleost puberty (Okuzawa, 2002). Common features are the transient and large activation of brain-pituitary-peripheral neuroendocrine axes (“neuroendocrine crises”) during which classical regulations such as homeostatic maintenance of hormones levels and negative feedbacks may be overruled. Thus, in teleosts, strong positive feedbacks by sex steroids on the brain and pituitary have been evidenced that are largely amplifying the activation of the gonadotropic axis at puberty (Dufour and Rousseau, 2007). In teleost fishes, gonad-derived sex steroids not only regulate reproduction, sexual behaviour and secondary sexual characters but also gonadotropin secretion (de Vlaming, 1974). The stimulatory effect of exogenous sex steroids on the gonadotropic activity in immature male fishes has been demonstrated in various species, both at the brain and/or pituitary and/or blood and gonadal levels in rainbow trout (Atteke et al., 2003; Magri et al., 1985), platyfish (Schreibman et al., 1989), African catfish (Cavaco et al., 1998), seabass (Zanuy et al., 1999), and salmonids (Okuzawa, 2002). This mode of control of gonadotropin release by blood sex steroid levels is known as feedback mechanism. There are examples of positive and negative feedback actions (Peter, 1973; Peter and Crim, 1979). In the Atlantic salmon parr, testosterone has a positive feedback action on gonadotropin secretion, since testosterone implants in brain nucleus lateralis tuberis or pituitary result in a rapid increase in gonadotropin levels in the pituitary (Crim and Peter, 1978; Crim and Evans, 1979, 1980). Evidence points to a dichotomy in the hormonal regulation of oocyte maturation in teleost fishes. In one

group of fishes exemplified by the rainbow trout, the medaka and others, the ovary seems to be the source of maturation-inducing steroids, whereas in the other group exemplified by the catfish, salmon and goldfish, an extra-ovarian relay via the interrenal (the equivalent to the mammal adrenal gland) seems to be the major source of maturation-inducing steroids (Sundararaj et al., 1985). In the latter species, oocyte maturation is induced by a variety of 11-deoxygenated corticosteroids (Sundararaj and Goswami, 1971; Sufi et al., 1982). The peak of seasonal activity of the interrenal tissue reportedly corresponds to the reproductive period (Verma and Misra, 1992). These authors have noted hyperplasia of the interrenal tissue at the time of breeding in different fishes. In sockey salmon, Oncorhynchus kisutch, the development of interrenal hypertrophy is directly related to ovarian maturation and suggested the existence of a pituitary-interrenal-ovarian axis in the maturation of at least few teleostean oocytes (McBride and Fagerlund, 1976). Sundararaj and Goswami (1971) have shown that the concentration of plasma cortisol increased approximately four fold after injection of either ovine luteinizing hormone (LH) or salmon gonadotropin in the sexually regressed as well as gravid catfish. Ovariectomy in the sexually regressed catfish does not prevent the LH induced rise in plasma cortisol levels. It therefore appears highly probable that the plasma cortisol in the fishes studied by the above authors is of interrenal origin and its synthesis and release are stimulated by gonadotropins secreted by the pituitary gland. Thus, in teleosts, gamete maturation is a complex phenomenon involving interaction between gonadotropin and steroid hormones, the latter being synthesized either in the oocyte follicles or in the interrenal tissue (Sundararaj et al., 1985). Corticosteroids and sex steroids impair the immune competence in teleosts (Slater and Schreck 1993; Schreck, 1996; Sapolsky, 1998; Harris et al., 2000; Law et al., 2001; Kurtz et al., 2007; Buchtíkováa et al., 2011). Reproductive activity-related immunosuppression (Nakanishi, 1986; Iida et al., 1989; Hou et al. 1999; Buchtíkováa et al., 2011) and thymic involution (Tamura and Honma, 1974; Nakanishi, 1986; Fishelson, 1995) appear to be involved in the spawning-associated susceptibility to parasite infections (Robertson 1979; Schreck 1996; Sapolsky, 1998) and tumors (Kortet et al., 2002).

The reproduction-related events can be best demonstrated in the semelparous Pacific salmon where these changes occur in a time lapse mode. In juvenile salmon, 17 alpha-methyltestosterone administration increases the number of cells expressing gonadotropin-releasing hormone mRNA in the preoptic area of future precocious males and elevates pituitary gonadotropin content in both immature males and females (Amano et al., 1994). The steroid 17a,20β-dihydroxy-4-prengnen-3-one (17,20-P) is produced by the gonads of salmonid fishes in response to a pre-spawning rise in gonadotropin, and regulates final gamete maturation in both sexes (Scott and Canario, 1987; Nagahama, 1987; Nagahama et al., 1993). In salmonids, blood levels of 17,20-P rise markedly prior to spawning, and in some species remain elevated for several days thereafter (Scott et al. 1983). Only very low concentrations of 17,20-P are required to stimulate final gamete maturation (100-fold less than peak plasma concentrations) in both sexes, and thus it has been postulated that 17,20-P plays other physiological roles at spawning (Scott and Canario 1987). Under normal physiological conditions, even in the face of elevated stress-induced cortisol levels, peripheral targets are protected from cortisol excess by cortisol-metabolizing enzymes, which inactivate cortisol before it can bind to cellular receptors and initiate a biological response. High physiological concentrations of 17,20-P, however, almost completely inhibited cortisol catabolism by the coho salmon kidney, exposing, in a positive feedback mechanism, tissues to high local concentrations of cortisol (Barry et al., 2010). 17,20-P had no effect on cortisol metabolism by the closely related iteroparous rainbow trout (Oncorhynchus mykiss) kidney. In addition, 17,20-P competes with cortisol for binding to corticosteroid binding protein and increases the concentrations of biologically active ‘‘free” cortisol in chinook salmon (Barry et al., 2001). 17,20-P can also serve as a substrate for cortisol biosynthesis, further contributing to systemic cortisol excess (Barry et al., 1997). These events could be part of the mechanism that leads to the symptoms of cortisol excess associated with the post-spawning mortality of semelparous Pacific salmon (Barry et al., 2010). In contrast, under normal physiological conditions, even in the face of elevated stress-induced cortisol levels, peripheral targets are protected from cortisol excess by cortisol-metabolizing enzymes, which inactivate cortisol before it can bind to cellular receptors and initiate a biological response (Westring et al., 2008; Barry et al., 2010). Both, the regulation of hypercorticism at the effector level and impairment of cortisol negative feedback on the hypothalamus–pituitary–interrenal axis argue for the active, neuroendocrine regulation of semelparity-related death. Moreover, evidence that gonadectomy blocks the normal increase of cortisol in pre-spawning salmon suggests that a gonadal factor

regulates cortisol excess (Fagerlund and Donaldson, 1970; Donaldson and Fagerlund, 1972). In vitro experiments with interrenals of coho salmon (Oncorhynchus kisutch) indicate that salmon gonadotropin is extremely corticotropic and both ACTH and gonadotropin stimulate the secretion of large quantities of androstenedione from the interrenal (Schreck et al., 1989). In Pacific salmon, the increase in the activity of the pituitary–gonadal axis components has already started in the maturing adults while they are still in the Gulf of Alaska in winter. In the homing adults, the pituitary contents and the plasma levels of gonadotropins and plasma sex steroid hormones peak during upstream migration from the coast to the natal hatchery. The seasonal increase in the activity of the pituitary–gonadal axis is an important endocrine event that is inseparable from initiation of spawning migration of Pacific salmon (Crossin et al. 2009; Onuma et al., 2009; Hruska et al., 2010). Marked hyperplasia of the interrenal tissue was found to be present in the sexually mature fishes of both sexes of Pacific salmon (Robertson and Wexler, 1957). Beginning interrenal hyperplasia was observed in immature salmon taken on their spawning migration several months before they were due to spawn. This change became more pronounced with progressive development of the gonads (Hane and Robertson, 1959). Studies on the anadromous form of the rainbow trout, the steelhead, which suffers a considerable mortality after spawning, also revealed the presence of marked hyperplasia of adrenal cortical tissue at full sexual maturity, whereas the nonmigratory rainbow trouts (hatchery reared) which usually survive their initial reproductive effort showed very little hyperplasia of the adrenal tissue (Hane and Robertson, 1959; Robertson and Wexler, 1959). In addition to maturation, corticosteroids play an important role in osmoregulation during salt-fresh water migration (McCormick and Bradshaw 2006) and olfactory recognition of the home-stream olfactory bouquet during homing (Carruth et al., 2002). Pacific salmon demonstrate marked somatic degeneration of all major organ systems including the central nervous system during home-stream migration and at the spawning grounds (Robertson and Wexler, 1960, 1962a; Robertson et al., 1961a,b; Sapolsky, 1998) and die within 2 to 3 weeks after spawning. During this period, the salmon develops ulcers, muscle atrophy, thymic involution and immune collapse, opportunistic infections, and a collection of diseases all accompanied by excessive glucocorticoid levels. Senile plaques resembling those associated with human aging and Alzheimer's disease, as well as dead neurons and neurites, have been detected in the brains of spawning kokanee salmon (Maldonado et al., 2000; 2002). The immunosuppressive action of cortisol and sex steroids (Sufi et al., 1982; Slater and Schreck 1993; Maule et al. 1996; Schreck, 1996; Sapolsky, 1998; Harris et al., 2000; Law et al., 2001; Miller et al., 2009) appears to be involved in the spawning-related susceptibility to parasite infections (Robertson 1979; Schreck 1996; Sapolsky, 1998; Jones et al., 2004; Wagner et al., 2005; Crossin, 2008). Cortisol has been implicated in the senescence and ultimately the death of adult Pacific salmon after spawning (Dickhoff, 1989; Stein-Behrens and Sapolsky, 1992; Sapolsky, 1998). If the adrenal glands are removed after spawning, the salmon does not die but lives for up to one more year (Sapolsky, 1992). Although the activation of the HPA axis during sexual maturation of Pacific salmon is similar to the axis activation during stress, the high levels of cortisol in salmon were suggested to be genetically programmed and not related to exposure to stressors (Dickhoff, 1989). Further studies revealed that the hypothalamic–pituitary–interrenal axis activation and increase in plasma cortisol concentration is not due to stressors previously considered to cause the cortisol increase, such as long-distance migration, starvation and changes in salinity (Sufi et al., 1982; Carruth et al., 2000) since the senescent changes and death occur both in land-locked as well as ocean-going Pacific salmon (Robertson and Wexler, 1962b; Carruth et al., 2000; 2002).

In summmary, in a cascade of events that are initiated by germline cell-derived signals and effected by gonadotropin and, in some species such as the Pacific salmon, relayed by interrenal corticosteroid hormones, reproductive events promote teleost aging and, in the semelparous Pacific salmon, death. At least in part, reproduction effects progeria and death via immunosuppressive mechanisms.

Reptiles

Interactions between reproductive activity and immunocompetence have been noted in several species of lizards and turtles (Lutton and Callard, 2006; Zimmerman et al., 2010). Both the seasonal and age-related thymic involution of reptiles are believed to be a result of interactions between the lymphoid tissue and the neuroendocrine system (El Ridi et al., 1988; Kruman, 1992; Zimmerman et al., 2010). Increased testosterone levels and testosterone administration caused immunosuppression (Saad et al., 1990; el Masri et al., 1995; Veiga et al., 1998; Hareramadas and Rai, 2001; Belliure et al., 2004; Oppliger et al., 2004) and increased the susceptibility of male lizards to parasitic infestation (Salvador et al., 1996; 1997; Veiga et al., 1998; Olsson et al., 2000; Klukowski and

Nelson, 2001; Cox and John-Alder, 2007). The increased parasitic load was prevented by castration (Cox and John-Alder, 2007). Thymic involution, inhibition of thymocyte proliferation and accelerated caspase-dependent thymocyte apoptosis were caused by female and male gonadal hormones (Hareramadas and Rai, 2005; 2006) and male and female sex hormones inhibited cytotoxic activity of macrophages (Mondal and Rai, 2002). Castration prevented gonadal hormone-caused thymic atrophy (Hareramadas and Rai, 2005; 2006). Thymic regression and immunosuppression during the breeding phase and thymic recovery in the non-breeding phase were related to testicular activity and gonadal hormone levels (Saad et al., 1990; Lal et al., 2009).

In turtles, testosterone caused immunosuppression and thymic involution and spontaneous immunodeficiency covaried with seasonal reproductive activity in both genders (Leceta et al., 1989; Saad et al., 1991; Varas et al., 1992; Muñoz et al., 2000; Muñoz and De la Fuente, 2001).

Birds

The elucidation of germline cell-derived signals in birds is complicated by the fact that most avian species maintain atrophied reproductive organs when not reproductively active (Vézina and Salvante, 2010). Numerous studies, by elevating testosterone experimentally in non-breeding birds, have induced suppression of the immune system (Folstad and Karter, 1992; Duffy et al., 2000; Peters 2000; Casto et al., 2001; Duckworth et al., 2001; Buchanan et al., 2003; Mougeot et al., 2004; 2006; Owen-Ashley et al., 2004; Alonso-Alvarez et al., 2007) and reduced survival (Nordling et al., 1998; Casto et al., 2001; Reed et al., 2006; Zysling et al., 2006). However, the literature regarding whether elevations of endogenous testosterone are immunosuppressive is conflicting (Saino et al., 1995; Nordling et al., 1998; Hasselquist et al., 1999; Roberts et al., 2004; Ardia, 2005; Greenman et al., 2005), possibly because most studies investigated acquired immune functions (Greives et al., 2006). Moreover, the detection of reproductive costs in terms of immunosuppression may be overridden by heterogeneity in individual quality and condition (Peters, 2000; Duckworth et al., 2001; Ardia, 2005). In a wild population of breeding birds, elevated testosterone levels were found to compromise innate as well as acquired immune functions (Greives et al., 2006).

An inverse relationship between gonadal development and size of bursa of Fabricius, a bird lymphoid organ, was reported for a number of bird species (Kirkpatrick 1944, Davis 1947, Lewin 1963; Eerola et al., 1987; Mercer-Oltjens and Woodard, 1987; Broughton, 1994) and immunosenescence was demonstrated in free-living swallows (Palacios et al., 2007; 2011). Regression of the bursa of Fabricius and thymus in intact quail was prevented by castration and induced by gonadal hormones (Mase and Oishi, 1991).

Mammals

Like in teleosts, semelparous mammalian species epitomize the signaling events that orchestrate the reproduction-related death. The carnivore dasyurid marsupials are found in Australia and South America. The life history of all species is characterized by a highly synchronized life cycle in both sexes, culminating in a brief mating period followed by total male mortality (Woolley, 1966; McDonald et al, 1981; Naylor et al., 2008). The synchronous life cycle includes pronounced endocrinological changes. In males, the mating period is preceded by a rapid rise in plasma testosterone concentration and accompanied by a marked androgen-induced fall in plasma corticosteroid-binding globulin concentration, to less than the total glucocorticoid concentration, which itself rises. As a consequence plasma free (biologically active) glucocorticoid concentration rise approximately 10-fold just before the males disappear from the populations (Bradley et al, 1975; 1980; McDonald et al., 1981; Kerr and Hedger, 1983). Moreover, the usual adaptive negative feedback on adrenocorticotropin secretion is suppressed (Bradley et al. 1975, 1980; McDonald et al. 1986; Bradley 1987, 1990; Oakwood et al., 2001). This highlights that, like in Pacific salmon (Barry et al., 2001; 2010; Westring et al., 2008), the mating-induced stress phenotype is regulated by the neuroendocrine reproductive axes and not due to “normal stress” where this downregulation of corticosteroid-binding globulin and feedback on adrenocorticotropin secretion does not occur (Oakwood et al., 2001; Breuner and Orchinik, 2002; Bradley, 2003). The stressful mating period results in death from adrenocortical suppression of the immune and inflammatory responses, severe hemorrhagic ulceration of the upper digestive tract, anemia, glomerulonephritis and tubular necrosis (Bradley, 2003). Occasionally, males in the wild survive longer than this immediate post-mating period, and post ‘die-off’ survivorship for a second year has also been documented in captivity. Intriguingly, these males experience a ‘reproductive senescence’ since the seminiferous tubular collapse, seen before the mating period in their first year of life is irreversible and complete, although the cycling of other sexual characteristics suggests that the hypothalamic-pituitary-gonadal pathways and function of the interstitial cells of the testes are retrievable (McAllan, 2009). The females survive to rear their

young and may mate again in their second year (Fisher and Blomberg, 2011). Gestation lasts from 26 to 34 days, depending on the species. However, developmental arrest can occur at several stages during embryogenesis, elongating the apparent gestation duration by several days (Oakwood et al., 2001). A general pattern becomes visible from marsupial male die-off: the causal role of gonadal hormone-related immunosuppression, mediated by glucocorticosteroids.

In iteroparous mammals, both estrogens and testosterone are cytotrophic/-protective in a variety of tissues, e.g. heart, skin and central nervous system (CNS) (Heininger, 1999a), supporting the maintenance of organismal functionality during the reproductive period. On the other hand, the involution of the thymus and degeneration of various brain structures (e.g. distinct hypothalamic nuclei and pineal gland), tissues that have a pacemaker function in aging, is advanced by cytotoxic actions of estrogens and testosterone. Both pro-oxidant and anti-oxidant effects of estrogens might involve different estrogen receptors that can have either genomic or non-genomic action to manifest further hormonal response (Kumar et al., 2010). The progeroid actions of estrogens appear to depend on neuronal cell types, ratio of different types of estrogen receptors present in a particular cell and context specificity of the estrogen hormone responses (Nilsen, 2008; Kumar et al., 2010). Specifically, estrogens, although neuroprotective at short term (Heininger, 1999a), induce degenerative changes in cholinergic basal forebrain neurons upon chronic replacement (Gibbs, 1997). The neurotrophic/neuroinhibitory dualism of estrogens has also been described in hypothalamic slices and cell cultures (Bueno and Pfaff, 1976; Rasmussen et al., 1990); the neurotrophic actions presumably operate in cells regulating reproductive behaviour while aging-pacemaker neurons may be targets of the toxic actions. Aging is associated with a disruption of hypothalamic catecholaminergic networks which engender the aging of the somatotropic, thyrotropic and gonadotropic axes (Meites, 1990; Wise et al., 1997). Evidence indicates that estrogens contribute to the derangement of hypothalamic catecholaminergic rhythmicity and function (Wise et al., 1997; Legan and Callahan, 1999). Moreover, gonadal steroids are degenerative and cytotoxic in a variety of hypothalamic nuclei (Schipper et al., 1981; Brawer et al., 1993; Yang et al., 1993), incite loss of arcuate nucleus synapses (Leedom et al., 1994), actuate the oxidative stress-mediated degeneration of β-endorphin neurons in the arcuate nucleus (Brawer et al., 1993; Desjardins et al., 1995) and elicit neuronal and glial stress reactions (Day et al., 1993; Seifer et al., 1994; Mydlarski et al., 1995; Krebs et al., 1999). As a result, estrogens exert a self-limiting feedback control of reproductive activity by degenerating the HPG axis (see chapter 11.4). The degeneration of the HPG axis by gonadal hormones not only promotes reproductive aging but has also systemic progeroid effects. Prepubertally ovariectomized mice that received young transplanted ovaries at 11 months showed a 40% increase in life expectancy, relative to intact controls. The 11-month-old recipient females resumed estrus and continued to cycle for several months past the normal point of reproductive senescence (Cargill et al., 2003). Estrogens and other ovarian factor(s) may also play a role in aging-related pituitary changes (Pasqualini et al., 1986; Telford et al., 1987).

Thymic involution and immunosuppressive actions of sex hormones

A major underlying cause for aging of the immune system is the structural and functional atrophy of the thymus, and associated decline in T-cell genesis (Makinodan and Kay, 1980; Bodey et al., 1997; Lynch et al., 2009). The thymus gland is a central lymphoid organ in which bone marrow-derived T-cell precursors undergo differentiation, eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs (Savino and Dardenne, 2000). Thymic involution is the most dramatic age-associated change in the immune system and it reflects the loss of organ mass and cellularity leading to a decrease in T-cell output, i.e. the number of lymphoid emigrants entering the peripheral T-cell pool (Stutman, 1978; Thoman, 1995; Berzins et al., 2002). This reduction in T-cell output from the thymus initiates the homeostatic expansion of pre-existing memory cells (Ernst et al., 1990; Utsuyama et al., 1992; Kurashima et al., 1995), and is thought to be responsible for narrowing of the T-cell receptor repertoire (Mosley et al., 1998; LeMaoult et al., 2000) leading to less efficient responses of elderly to newly encountered antigens (Miller, 2000). Whilst multiple mechanisms may contribute to this process, the temporal alliance of thymic decline with puberty has implicated a causative role for sex steroids. Thymus involution as marker of immune senescence and “aging clock” (Makinodan and Kay, 1980) is initiated and perpetuated following sexual maturation (Goya, 1992; Hirokawa et al., 1992; Bodey et al., 1997). The causal role of sex steroids is firmly established and supported by findings that peripubertal gonadectomy or chemical castration can considerably postpone age-related thymic atrophy and consequently functional deterioration of the immune system in rats and mice, while later in life it produces reversal of aging-induced changes in the thymus in

both sexes (Fitzpatrick et al., 1985; Grossman, 1985; Utsuyama and Hirokawa, 1989; Utsuyama et al., 1989; Kendall et al., 1990; Blacker et al., 1991; Olsen et al., 1991; Windmill et al., 1993; Rao et al., 1996; Leposavic et al., 1996; 1999; Windmill and Lee, 1998; Roden et al., 2004; Heng et al., 2005; Pešic et al., 2007; Hince et al., 2008; Kelly et al., 2008; Perišic et al., 2009). Similarly, immunodeficiency following cytoreductive treatments such as chemotherapy can be reversed by gonadectomy or sex steroid ablation (Heng et al., 2005; Sutherland et al., 2005; Dudakov et al., 2009; Goldberg et al., 2010). On the other hand, administration of sex steroids either accelerates thymic atrophy in intact (Kuhl et al., 1983; Luster et al., 1984; Dulos and Bagchus, 2001; Yellayi et al., 2002) or inhibits thymic (re)growth in gonadectomized rodents (Greenstein et al., 1986; Fitzpatrick and Greenstein, 1987; Olsen et al., 1991; Windmill et al., 1993; Öner and Ozan, 2002). Chimeric mice with androgen receptor-defective stroma but wild-type thymocytes did not undergo thymic atrophy, suggesting that the stroma is the target of androgen-induced regression (Olsen et al., 2001). Cumulative evidence indicates that gonadectomy-induced thymic hypercellularity is due to the following mechanisms: i) reduced thymocyte apoptosis, ii) increased thymocyte proliferation, and iii) enhanced T-cell generation.

Evidence has accumulated that both testosterone, the major circulating androgen in men, and progesterone, the hormone associated with the maintenance of pregnancy, are immunosuppressive (Grossman, 1985; Van Vollenhoven and McGuire, 1994; Zera and Harshman, 2001; Williams, 2005; Snider et al. 2009). Both hormones reduce NK cell activity (Furukawa et al., 1984; Toder et al., 1984; Baley and Schacter, 1985; Hou and Zheng, 1988; Page et al., 2006), impair macrophage production of TNF and nitric oxygen (Miller et al., 1996; Miller and Hunt, 1998; D'Agostino et al., 1999), and suppress NF-κB signal transduction, which is involved in mediating proinflammatory cytokine production (Li and Verma, 2002). Both hormones can also promote the production of Th2-associated anti-inflammatory cytokines such as IL-10 (D'Agostino et al., 1999) and IL-4 (Piccinni et al., 1995). The intrathymic aromatization of testosterone, thus conversion to estradiol, appears to be a central pathomechanistic process since aromatase inhibitors also restore the thymus in aging rats (Greenstein et al., 1992).

Estrogens, possibly dose-, target-, age- and time-dependently (Van Vollenhoven and McGuire, 1994), and estrogen receptor subtype-selectively (Li and McMurray, 2006; Stygar et al., 2007) can both stimulate and inhibit immune functions. Importantly, the immunosuppressive action of E is more pronounced in aged compared to young mice (Smith and Holladay, 1997; Utsuyama et al., 2002). Similarly, estrogen is anti-inflammatory in young, but pro-inflammatory in aged rodents (Nordell et al., 2003; Johnson and Sohrabji, 2005; Sohrabji, 2005). Multiple mechanisms have been put forward to describe how estrogens cause/accelerate thymic atrophy (Perišic et al., 2009). First, an accumulating body of evidence indicates that estrogens act either directly, at multiple developmental steps on developing T-cells, or indirectly on thymic epithelial cells to inhibit generation of signaling important for thymocyte survival, may enhance thymocyte apoptosis and thereby induce thymic atrophy (Leceta et al., 1988; Aboussaouira et al., 1991; Gilbody et al., 1992; Kincade et al., 1994; Martin et al., 1994a; b; Mor et al., 2001; Hoffman-Goetz et al., 2001; Okasha et al., 2001; Yao and Hou, 2004; Wang et al., 2008). Second, it has been shown that estrogens have an inhibitory effect on thymocyte proliferation in vitro (Gulino et al., 1985). Third, it has been strongly suggested that elevated peripheral blood estrogen levels may inhibit T-cell development at multiple stages at both pre-thymic and thymic level, and consequently induce thymic atrophy (Screpanti et al., 1989; 1991; Silverstone et al., 1994; Rijhsinghani et al., 1996; Leposavic et al., 2001; Zoller and Kersh, 2006; Wang et al., 2008).

Several lines of evidence suggest that sex steroids are also physiological regulators of bone marrow lymphopoiesis (Frey-Wettstein and Craddock, 1970; Medina et al., 1993; Medina and Kincade, 1994; Smithson et al., 1994; 1995; 1998; Dudakov et al., 2009; 2010; Goldberg et al., 2010). For example, increases in systemic levels of estrogen, or normal pregnancy, result in preferential reduction of lymphocytes (Medina et al., 1993; Medina and Kincade, 1994; Smithson et al., 1995). Treatment of mice with dihydrotestosterone (DHT)3, a form of testosterone that cannot be metabolized to estrogen, also reduced numbers of IL-7-responsive B-cell precursors (Medina and Kincade, 1994). This bone marrow functional decline further exacerbates thymic involution (Dudakov et al., 2009; 2010). Castration causes dramatic, long-lived enhancement of B lymphopoiesis in bone marrow and increased numbers of mature B-cells in the periphery (Masuzawa et al., 1994; Wilson et al., 1995; Erben et al., 1998).

As discussed in chapter 10.3.1, klotho is a key pleiotropic node of the resource utilization-stress-aging network. Klotho knockout mice develop premature thymic atrophy and disruption of thymic microenvironmental organization due to a defect in

thymic epithelial cells that support proliferation and survival of thymocytes (Kuro-o et al., 1997; Min et al., 2007). This pathophysiology is similar to that observed in thymic involution associated with the normal aging process. Injection of KGF (keratinocyte growth factor, FGF7), which induces proliferation of thymic epithelial cells, improves thymopoiesis and restores thymic degeneration not only in Klotho-deficient mice but also in aged wild-type mice (Min et al., 2007). Klotho knockout mice, in addition to thymic involution, also exhibit an impaired B-cell lymphopoiesis in bone marrow (Okada et al., 2000). In the aromatase-deficient mouse, a model of estrogen deficiency, kidney klotho mRNA and protein levels were elevated. Estradiol treatment of aromatase-deficient mice decreased klotho expression in the kidney at both the mRNA and protein levels (Öz et al., 2007). Thus in both mice and humans, estrogens promote tubular reabsorption of calcium (Nordin et al., 1991), presumably via the inhibition of klotho expression and action (Öz et al., 2007). Kidney tubular klotho appears to be the main source of circulating klotho. Circulating klotho levels in children are significantly higher (mean ± SD; 952 ± 282 pg/mL) than those in healthy adults (562 ± 146 pg/mL; $P < 0.001$) (Yamazaki et al., 2010).

Certain changes, observed in the CD4+ cells of rheumatoid arthritis (RA) patients, resemble those observed during physiological aging, but occur at an earlier age. Klotho is down-regulated at the mRNA, protein, and enzymatic (β-glucuronidase) activity levels both in the healthy elderly and especially in RA CD4+ lymphocytes. The reduction of klotho expression and activity in both elderly and patients' lymphocytes occurs in concert with the down-regulation of T-cell costimulatory molecule CD28, the latter known to be dependent on increased levels of TNF-α. Thus, a common mechanism of klotho down-regulation, but executed at various times in life, may underlie both physiological and disease-related T-cell aging (Witkowski et al., 2007). I conjecture that estrogen's immunocompromizing and immunosenescence-advancing action is mediated, at least in part, by inhibition of renal klotho expression causing klotho systemic deficiency.

Pineal gland

In seasonally breeding mammals that use changes in the photoperiod to time their reproductive cycles, temporal signals to the reproductive system are controlled by the daily rhythm in melatonin production (Tamarkin et al., 1985). The seasonality of reproduction represents the mechanism by which nature ensures the occurrence of births at a time of the year suitable for offspring survival, in relation to appropriate environmental conditions and food supply (Aleandri et al., 1996; Reiter et al., 2009). As a general principle, melatonin has negative effects on the reproductive axis (Martin et al., 2008). In short-day breeders such as the sheep, however, melatonin exerts a stimulatory effect on the reproductive axis (Karsch et al., 1984). Hence, the following discussion on the antagonistic control of melatonin and gonadal hormones applies to rodents and humans (Aleandri et al., 1996). The length of the nocturnal secretion of melatonin reflects the duration of the night and it regulates the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Changes in GnRH release induce corresponding changes in luteinising hormone secretion which are responsible for the alternating presence or absence of ovulation in the female, and varying sperm production in the male. The pineal gland hormone melatonin interacts with estrogen-signaling pathways through three different mechanisms: (a) the indirect neuroendocrine mechanism which includes the melatonin downregulation of the hypothalamic-pituitary-reproductive axis and the consequent reduction of circulating levels of gonadal estrogens, (b) direct melatonin actions by interacting with the activation of the estrogen receptor, thus behaving as a selective estrogen receptor modulator, and (c) the regulation of the enzymes involved in the biosynthesis of estrogens in peripheral tissues, thus behaving as a selective estrogen enzyme modulator (Cos et al., 2008). The role of melatonin in controlling sexual maturity, reproductive cycling, cancer, stress, and the immune response suggests that the pineal gland is one of the 'aging clocks' (Pierpaoli and Regelson, 1994). Grafting of pineal glands from young mice prolongs survival and delays reproductive senescence in aging mice (Lesnikov and Pierpaoli, 1994; Pierpaoli et al., 1997). Conversely, grafting of old pineal glands to young mice accelerates their aging (Lesnikov and Pierpaoli, 1994; Pierpaoli and Bulian, 2001). Gonadal hormones appear also to exert progeroid actions on the pineal-hypothalamic-pituitary axis. Estrogens and testosterone downregulate the secretory activity of the pineal gland, melatonin synthesis (Moujir et al., 1990; Pablos et al., 1993; Alonso-Solis et al., 1996; Okatani et al., 1998; Redins et al., 1999) and melatonin receptor-mediated effector functions (Seltzer et al., 1992; Recio et al., 1996; Clemens et al., 2001). These effects may be mediated both by catecholaminergic and opioidergic mechanisms (Yie and Brown, 1995; Alonso-Solis et al., 1996) and iron released by the action of estrogen (Pablos et al., 1993) and may underlie the

aging-related pineal calcification and loss of secretory activity (Schmid, 1993). Conversely, short day photoperiod and melatonin treatment delay reproductive aging in rodents, involving the central opioid system (Trentini et al., 1992; Pierpaoli et al., 1997; Place et al., 2004). Apart from melatonin, other, not yet specified, pineal factors appear to have a role in the geroprotective activity of the pineal gland (Anisimov et al., 2001). Pleiotropic actions define a pineal-gonadal-thymic signaling network. The effects of melatonin on the immune system are well established. Melatonin mediates seasonal changes in immune function (Nelson and Drazen, 2000; Martin et al., 2008). In general, melatonin is associated with enhanced immune function in most laboratory animals and non-domesticated taxa (Provinciali et al., 1996; Guerrero and Reiter 2002; Hotchkiss and Nelson 2002; Martin et al., 2008). However, its effects on the immune system are varied and depend on the specific arm of the immune system tested, the species studied and the timing of its delivery (Martin et al., 2008). The temporal pattern of effects of photoperiod and melatonin on the immune system is similar to the effects on the reproductive system. Photoperiodic rodents become refractory to short day lengths after prolonged exposure (approx. 20–24 weeks) and then 'spontaneously' regrow their testes. Melatonin does not affect immune activity in vivo or in vitro in refractory rodents (Prendergast and Nelson, 2001; Prendergast et al., 2002). Latitude of origin is related to responsiveness to photoperiod as deer mice from a high-latitude population, but not mice from a low-latitude population, responded reproductively to photoperiod and exogenous melatonin (Bronson, 1985). The same pattern emerged for immune responses; animals from the high-latitude population, but not the low-latitude population, increased in vitro cell-mediated immune function in response to both melatonin and short day lengths (Demas et al., 1996). Pinealectomy accelerates thymic involution that is prevented by melatonin supplementation (Csaba and Barath, 1975; Öner et al., 2004). Moreover, pinealectomy increases various indices of oxidative protein, lipid and DNA damage in aged rodents (Berker et al., 1996; Reiter et al., 1999) and accelerates collagen aging, a marker of biological age (Berker et al., 1996; Sell et al., 2000). Melatonin receptors are expressed and melatonin is synthetized locally in rodent testes (Tijmes et al., 1996; Stefulj et al., 2001; Frungieri et al., 2005; Izzo et al., 2010) but both testicular melatonin receptor expression and melatonin synthesis decline during rat aging (Sánchez-Hidalgo et al., 2009a; b). Intra-testicular injection of melatonin to the tropical rodent Funambulus pennanti decreased testicular androgen receptor expression, spermatogenesis and systemic testosterone levels but increased lymphatic tissue weight, leukocyte, lymphocyte count, lymphocyte proliferation in spleen and thymus, as well as general immunity in a duration-dependent manner (Ahmad and Haldar, 2010). Both estrogen exposure and melatonin decline may add up to generate the hypothalamic (Lin et al., 1990) and pineal (Lan et al., 2001) morphological changes during aging. In general, gonadal hormones and melatonin act antagonistically and bidirectionally on reproductive functions and somatic maintenance (Martin et al., 2008). Melatonin is a somatic trophic signal that is antagonized by gonadal hormones to optimize reproductive functions.

11.3 Immunocompetence, a key target of germ cell signaling

The immune system is the animal's defense mechanism to fight or control any parasitic or pathogenic infection and as such, is one of the major physiological mechanisms regulating host survival. Like reproduction, maintenance of an effective immune response is a nutritionally and energetically demanding process, requiring trade-off decisions (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Bonneaud et al., 2003; Martin et al., 2008). The vertebrate immune system has both adaptive and innate components while the invertebrate immune system is purely an innate one (DeVeale et al., 2004). Innate immunity is the first line of defense against infectious microorganisms (Hoffmann et al., 1999; Silverman and Maniatis, 2001; Kortet and Vainikka, 2008). An important class of innate immune effector molecules to fight pathogen infections are antimicrobial peptides (AMPs) that are produced in plants and animals.

11.3.1 Immunosuppressive action of reproductive activity

It is generally held that reproductive activity and immunocompetence compete for limited resources and impair each other. As discussed in chapter 8, reproductive activity exacts costs in terms of impaired immunocompetence. On the other hand, reduced reproductive rates were observed for immunocompetent individuals/strains e.g. in mosquitoes, fruitflies or birds (Ferdig et al., 1993; Yan et al., 1997; Schmid-Hempel, 2003; Bonneaud et al., 2003; Ahmed and Hurd, 2006; McKean et al., 2008). Likewise, activation of the immune system reduces reproductive output (Ilmonen et al., 2000; Lochmiller and Deerenberg, 2000; Ots et al., 2001; Colditz, 2002; Bonneaud et al., 2003; Kilpimaa et al., 2003; Jacot et

al., 2004). Immune activation has a strong testosterone-suppressive effect (Verhulst et al., 1999; Boonekamp et al., 2008). The beneficial effects of sterility on immunocompetence are conserved throughout phylogenesis (Miyata et al., 2008; TeKippe and Aballay, 2010).

However, the immune system-compromising actions of reproduction go beyond the competition for resources. The gonadal steroid-related impairment of immune functions is also observed in vitro and in vivo ecological systems that do not involve reproductive activity (Ansar Ahmed, 2000; Inadera, 2006; Casanova-Nakayama et al., 2011; Milla et al., 2011). Testosterone is immunosuppressive in a variety of vertebrates (chapter 11.2.4). Prevalence and intensity of parasitic infections is higher in males than females (Klein, 2000; 2004; Roberts et al., 2001; Zuk and McKean, 1996). Testosterone, although considered a male hormone, is also produced in females, and manipulation of testosterone level has immunosuppressive effects in females (Zysling et al., 2006; Schroderus et al., 2010). Although the effects of progesterone, testosterone and particularly estrogen on white blood cell functions are diverse, they have demonstrated an overall inhibitory effect on leukocyte proliferation (Beagley and Gockel 2003; Morale et al., 2003).

Invertebrates, due to their in comparison to vertebrates relatively simple immune system, are well suited to illustrate the interaction between reproduction and immunity and its seasonal adverse effects (Kortet and Vainikka, 2008). The germline controls the innate immune response of C. elegans' somatic cells to two different Gram-negative bacteria. Loss of germline cells enhanced nematode survival in the presence of pathogens (Aballay and Ausubel, 2001; Kim et al., 2002; Alper et al., 2010). The germ cell-mediated impairment of immune competence results in increased susceptibility to bacterial infections that contributes to mortality in C. elegans (Aballay and Ausubel, 2001; Garigan et al., 2002). Animals without a germline are resistant to P. aeruginosa and this resistance is suppressed by daf-16 mutation (Kurz and Tan, 2004). Long-lived worms with mutations in IIS pathways are resistant to Grampositive (En. faecalis and St. aureus) and Gramnegative (P. aeruginosa) bacteria (Garsin et al., 2003). In insects, 20E and JH signaling affects innate immunity (Meister and Richards, 1996; Dimarcq et al., 1997; Rolff and Siva-Jothy, 2002; Beckstead et al., 2005; Flatt et al., 2008b; Tian et al., 2010) and lifespan (Simon et al., 2003; Tricoire et al., 2009). In Drosophila, 20E activates innate immunity, while JH suppresses it (Silverman et al., 2000; Flatt et al., 2008). In contrast, the genome-wide microarray study by Beckstead et al. (2005) revealed that several AMP genes were down-regulated by 20E in EcR-dependent manners. In the silkworm, Bombyx mori, JH activates and 20E inhibits innate immunity (Tian et al., 2010).

Mating stimulates expression of innate immune system-related genes (McGraw et al., 2004; Lawniczak and Begun, 2004; Peng et al., 2005; Domanitskaya et al., 2007; Fedorka et al., 2007), but immune response and survival against infection is lowered. Thus, estimates of immunity based on gene expression do not appear to reflect an actual ability to defend against pathogens in the hours following copulation (Rolff and Siva-Jothy, 2002; Fedorka et al., 2007). Importantly, the trade-off between reproduction and immunocompetence does not appear to be resource-based (Fedorka et al., 2007).

11.3.2 Immunosenescence

Several immune functions are markers of biological age and predictors of longevity. Aging in vertebrates is characterized by an overall decline in immune function termed immunosenescence, which affects both the innate and adaptive immune systems (Aw et al., 2007; Gomez et al., 2008; Weiskopf et al., 2009). Immunosenescence is the cause of the increased vulnerability to infection (Louria et al., 1993; Pinner et al., 1996; Castle 2000; Schmader, 2001; Aspinall, 2003; De la Fuente, 2008; Weiskopf et al., 2009), cancer and autoimmune diseases of aged animals (Ershler and Longo, 1997; Pawelec, 1999; Castle, 2000; Burns and Leventhal, 2000; Malaguarnera et al., 2001; De la Fuente, 2008; Derhovanessian et al., 2008; Pawelec et al., 2010). In fact, immune responsiveness in birds (Tella et al., 2002), rodents (Doria and Frasca, 2000) and humans (Pawelec and Solana, 2001), MHC loci in mice (Goya, 1992; Dubey et al., 2000) and HLA haplotypes in humans (Goya, 1992; Caruso et al., 2001; De Benedictis et al., 2001) are associated with longevity. Aging humans display a less active cellular and humoral immune system (Oyeyinka, 1984; Huppert et al., 1998; Aspinall, 2000) which predicts senescent morbidity and mortality (Aspinall, 2000; Caruso et al., 2001). Immunological senescent decline proceeds in a coordinated fashion (Goya, 1992; Viveros et al., 2001) and is controlled by the hypothalamus and a variety of neuroendocrine axes (Hirokawa et al., 2001). The central role of the immune system for aging is highlighted by immune-modulating strategies to extend human lifespan (Aspinall and Mitchell, 2010).

Signaling pathways that act in the regulation of model invertebrate immunity are key to delineate the aging-related immunosenescent changes (DeVeale et

al., 2004; Kurz and Tan, 2004; Gravato-Nobre and Hodgkin, 2005). In C. elegans, the germline acts via signaling pathways distinct to the IIS to control lifespan and innate immunity. Thus, a complex regulatory network integrates inputs from IIS, DAF-16/FOXO, p38 MAPK signaling, and germline stem cells to control innate immunity that is an important determinant of longevity in C. elegans (Kurz and Tan, 2004; Gravato-Nobre and Hodgkin, 2005; Troemel et al., 2006; Alper, 2010; Alper et al., 2010; Kriete et al., 2010; TeKippe and Aballay, 2010). Murphy et al. (2003) compared daf-2 and age-1/PI(3)K long-lived mutants to wild-type and daf2/daf-16 double mutants and revealed that long-lived daf-2 mutants display an induction of genes involved in infection response. Intriguingly, exposure of C. elegans to S. enterica leads to a persistent infection in the worm intestine, stimulates programmed cell death in the germline and increases longevity. Conversely, loss-of-function mutations in the pro-apoptotic genes ced-3/-4 and egl-1 and gain-of-function mutations in the antiapoptotic gene ced-9 result in inhibition of the Salmonella-elicited programmed cell death and decrease longevity in the presence of the bacterium (Aballay et al., 2000; Aballay and Ausubel, 2001). Germline-deficient strains display increased resistance across a broad range of pathogens including Grampositive and Gramnegative bacteria, and the fungal pathogen Cryptococcus neoformans (TeKippe and Aballay, 2010).

Studies demonstrate that innate immune gene expression goes up at the middle of the Drosophila adult life at the same time that the mortality rate increases exponentially (Pletcher et al., 2002; Seroude et al., 2002; Landis et al., 2004; Libert et al., 2006; 2008; Sowell et al., 2007; Zhan et al., 2007; Sarup et al., 2011). In parallel, the function of the fly's innate immune system declines with age (Kim et al., 2001; Zerofsky et al., 2005; Grotewiel et al. 2005).

Ecdysteroids may have lost most of their reproductive functions in worker bees, but not in queen bees (Rachinsky and Engels, 1995), as higher levels of social organization were attained in the evolution of social insects (Hartfelder et al., 2002). An interplay between JH and vitellogenin affects the time-course of immunosenescence in functionally sterile worker honey bees (Amdam et al., 2005). The transition from nurse tasks inside the hive to exterior foraging activity is associated with a marked decline in immunity: an increase in the systemic JH titer induces hemocyte apoptosis (Rutz et al., 1974; Wille and Rutz, 1975; Amdam et al., 2004). This cascade results in a dramatic loss of hemocytes, which have several important immunological functions (Millar and Ratcliffe, 1994). JH injection is accompanied by decreased life expectancy and increased vulnerability to disease (Wille 1973). On the other hand, foragers that are forced to revert to hive-tasks show reversal of immunosenescence through proliferation of new cells that is triggered by a drop in the endogenous JH titer and an increase in the hemolymph vitellogenin level (Amadam et al., 2005). Diutinus bees, a stress-resistant temporal worker form that survives unfavorable periods, have higher numbers of hemocytes, lower levels of JH and higher vitellogenin titers (Fluri et al. 1977).

The thymus is the vertebrate pacemaker of immunosenescence and appears to integrate various aging-relevant modulatory factors. Gonadal hormones are the predominant drivers of thymic involution (chapter 11.2.4). Nonetheless, there is evidence to suggest that gonadal hormones are not the sole contributor to involution: thymic atrophy in hypogonadal mice with diminished sex steroid production presented no changes in cellularity or cellular distribution compared to wild-type littermates (Min et al., 2006).

Energy homeostasis is a key moderator of immunocompetence. Adipose tissue signaling, e.g. leptin, has an immunoregulatory, particularly immunoenhancing function (Demas et al., 2011). However, thymic involution is accelerated by excessive resource utilization and obesity (Dixit, 2008; Yang et al., 2009a; b). FOXO activates innate immunity in invertebrates (McElwee et al., 2003; Murphy et al., 2003; Becker et al., 2010). Since IIS downregulates FOXO activity (see chapter 10), IIS also impairs invertebrate immune defense (Garsin et al., 2003; Murphy et al., 2003; Evans et al., 2008a; b). The adverse impact of resource utilization on immune competence is phylogenetically conserved (Ewbank, 2003; Garsin et al., 2003; Dixit, 2008; Evans et al., 2008; Balistreri et al., 2010). In a feedback loop, the immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling (DiAngelo et al., 2009).

Chronic stress, reflected in long-term high levels of glucocorticoids, can depress the immune system (McEwen et al. 1997), although stress hormones may serve an immunostimulatory function in the short-term (Dhabhar and McEwen; 1999; Martin 2009). Chronic stress and its hormonal signals have been implicated in thymic involution (Maestroni et al., 1988; Gruver and Sempowski, 2008; Bjelakovic et al., 2009; Martin, 2009). The interaction of the endocrine axes mediating reproduction, resource utilization, and stress response to cause thymic atrophy is highlighted by the long-term, but ultimately transient, benefit of gonadectomy in

model animals (Min et al., 2006; Pešic et al., 2007; Perišic et al., 2009). Intriguingly, senescent gonadectomized and intact (then postreproductive) rodents exhibit the same extent of thymic involution, arguing for some catch-up atrophy in castrated animals mediated by the neuroendocrine axes (Pešic et al., 2007; Perišic et al., 2009).

In summary, sex steroid-induced immunosenescence is a major pacemaker of organismal aging. Rejuvenation of immunocompetence by sex steroid ablation (Holland and van den Brink, 2009; Aspinall and Mitchell, 2010) or melatonin supplementation (Karasek, 2007; Cardinali et al., 2008) is one of the most promising anti-aging strategies.

11.4 Germline-derived signals limit the soma's reproductive potential

Since Darwin, it has been one of the central tenets of evolutionary theory that organisms maximize their fitness (Darwin 1859; Dawkins 1976; Dennett 1995; Grafen, 1999). As can be expected, concepts related to the 'tragedy of the commons' concept like the 'prudent predator' (Slobodkin, 1961; 1974; Goodnight et al., 2008) have met staunch opposition and were denounced as group-selective (Maynard-Smith, 1964; Williams, 1966). However, the insight that self-limitation of reproduction attenuates the oscillations predicted by the Lotka-Volterra equation, stabilizes populations and prevents their extinction has supported these concepts (see chapter 5.4) (Mitteldorf et al., 2002; 2006; 2010b; Rauch et al., 2002; 2003; Killingback et al., 2006; Goodnight et al., 2008). Modern concepts advocate optimization of reproduction instead of maximization as "sound"evolutionary strategy (de Magalhães and Church, 2005; Grafen, 2006).

Both pro-oxidant and anti-oxidant effects of estrogens might involve different estrogen receptors that can have either genomic or non-genomic action to manifest further hormonal response (Kumar et al., 2010). The progeroid actions of estrogens appear to depend on neuronal cell types, ratio of different types of estrogen receptors present in a particular cell and context specificity of the estrogen hormone responses (Nilsen, 2008; Kumar et al., 2010). The pro-oxidant actions of estradiol appear to be conserved phylogenetically. In the marine sponge Geodia cydonium (Jameson), 17β-estradiol displayed its effect via the production of reactive oxygen species, caused increased expression of the cytoplasmic chaperone HSP70 and the nuclear chaperone thioredoxin, while at higher ROS levels the cells underwent apoptosis, lost telomerase activity and the number of DNA strand breaks increased (Wiens et al., 1999). Estradiol has been shown to have also a role in Cnidarian and planarian sexual reproduction (Atkinson and Atkinson, 1992; Tarrant et al., 2004; Twan et al., 2006; Miyashita et al., 2011).

The role of gonadal hormones in reproductive aging (Hung et al., 2003) has been studied in mammals. Estrogens induce aging-like dysfunctions in the regulation of estrous cycles and estrogens-induced LH surges (Mobbs and Finch, 1992; Desjardins et al., 1995; Tsai and Legan, 2001). These neurotoxic actions may be ultimately related to the loss of sexual functions (Brawer et al., 1993; Desjardins et al., 1995) and a self-limiting feedback control of reproductive activity as is evidenced by the restoration of sexual behavior by fetal hypothalamic transplants in aged (Huang et al., 1987; Hung et al., 1997) and medial preoptic area-lesioned rats (Giordano et al., 2001). Conversely, the reproductive senescence can be delayed by ovariectomy (Mobbs and Finch, 1992). Young ovary grafts partially restored cyclicity if old hosts were acutely ovariectomized but almost fully restored cyclic ovulatory function if the old hosts had been ovariectomized early in adulthood. With advancing age, however, the efficacy of the grafts declined progressively in both acute and long-term ovariectomized groups. These data show that both ovarian and hypothalamic-pituitary aging contribute to the etiology of anovulation. Although chronic withdrawal from ovarian secretions retards the age of onset of hypothalamic-pituitary aging, the duration of this ameliorative effect is limited by progressive neuroendocrine dysfunction (Felicio et al., 1983). The neurotoxic actions of gonadal hormones may depend on repetitive cycles of gonadotropin surges following excitatory/inhibitory imbalances due to estrogen-mediated increased glutamatergic transmission (Brann, 1995; Ping et al., 1997) and reduced inhibitory GABAergic input (Parducz et al., 1993). These imbalances may be causally related to the aging-associated decreased HPG responsiveness (Leedom et al., 1994; Zuo et al., 1996; Bonavera et al., 1998). In summary, although reproductive senescence is ultimately defined by ovarian follicular exhaustion, several lines of scientific evidence in humans and animals now suggest that estrogen-related dysregulation of estradiol feedback mechanisms and hypothalamic-pituitary dysfunction contribute to the onset and progression of reproductive senescence, independent of ovarian failure (Neal-Perry et al., 2010).

The female reproductive aging process is thought to be dominated by a gradual decrease in both the quantity and the quality of the oocytes residing within the follicles present in the ovarian cortex (te Velde and

Pearson, 2002; Broekmans et al. 2009). Oxidative stress, at least in part mediated by gonadal hormones, is a hallmark of gametogenesis and a variety of other sexual reproduction-related events (Riley and Behrman, 1991; Heininger, 2001; Agarwal et al., 2005; Fujii et al., 2005; Nedelcu, 2005; Metcalfe and Alonso-Alvarez, 2010; Shkolnik et al., 2011; Rizzo et al., 2012). Oxidative stress is involved in granulosa cell estrogen and progesterone production (LaPolt and Hong, 1995; Yacobi et al., 2007) and estrogen-mediated oocyte maturation (Tarin et al., 1998; Behrman et al., 2001) but, on the other hand, may contribute to ovarian senescence (Tarin et al., 1998; Behrman et al., 2001). Ovulation induces ROS generation in the ovaries that is an essential preovulatory signaling event for proper ovulation (Miyazaki et al., 1991; Shkolnik et al. 2011). Repeated exposure of stored oocytes to ROS at each ovulation results in oxidative damages, measured by 8-hydroxydeoxyguanosine levels in oocytes of the follicle pool (Chao et al. 2005; Miyamoto et al. 2010), declining oocyte quality and size of the ovarian follicle pool (Imai et al., 2012). The decrease in follicle numbers because of aging ensures aberrant hormonal regulation as a result of impaired negative feedback mechanisms of the hypothalamic-pituitary-ovarian axis. Decreasing numbers of follicles in the ovaries result in decreasing concentrations of circulating estrogens and inhibins during aging (Broekmans et al. 2009). With the loss of negative ovarian feedback at the hypothalamic-pituitary unit (MacNaughton et al., 1992; Burger et al., 1995; Landgren et al., 2004), follicle-stimulating hormone levels are elevated (Burger et al., 1995; Klein et al. 1996) which, in turn, accelerate the loss of follicles (McTavish et al. 2007).

In male reproductive senescence, Leydig cells, the testicular cells responsible for testosterone production, become steroidogenically hypofunctional (Zirkin et al., 1997). Oxidative stress which is requisite for steroidogenesis may play a causal role (Myers and Abney, 1988; Peltola et al., 1996; Zirkin et al., 1997) as evidenced by the prevention of Leydig cell aging following the suppression of Leydig cell steroidogenesis (Chen and Zirkin, 1999). Thus, testosterone-induced oxidative stress in the testes, although indispensable for spermatogenesis (Chainy et al., 1997), may advance the aging of the male reproductive organ. On the other hand, the increased length of male reproductive phase may, at least in part, depend on the protective action of testosterone against the neurotoxic action of estrogen on distinct hypothalamic neuron populations (Bloch and Gorski, 1988; Yang et al., 1993). As result of these actions, the reproductive phase is self-limited due to the multiple detrimental effects of gonadal steroids and reproductive activity-related oxidative stress on gonadal functioning and the HPG axis.

12. The germ-soma conflict

..it is to be emphasized that although patterns may underlie the rich and varied tapestry of the natural world, there is no single simple pattern. Theories must be pluralistic.
RM May (1974)

Summary
Conflicts may arise from competition among dividing cell lineages for opportunities to propagate or for access to the germline. Conflicts can also arise among the products of meiosis and between hosts and endosymbionts. A central conflict that has to be mediated in multicellular organisms with a germline-soma division revolves around the issue of resource allocation for both reproduction and somatic tissue maintenance and resolution of the transgenerational 'tragedy of commons' dilemma. From various biological levels of conflict it has become obvious that conflict breeds antagonistic coevolution that can be described by 'Red Queen' dynamics. Self-reinforcing adaptation/counteradaptation chain reactions may lead to both a higher mutational robustness and higher evolvability of the genetic network. The conflict between segregated germ cells and soma are posited to have fuelled the coevolutionary dynamics resulting in the Cambrian explosion and to play a role in the evolutionary success of sexual reproduction.

12.1 The physiognomy of conflict: yin and yang

Conflict is a pervasive feature of evolution. Conflicts are characterized by the conflict parties and the asset over which a conflict arises. Various types of limited resources are a particular intense matter of conflict between individuals competing for these resources. Often the conflict is quite obvious, sometimes it is less evident. In the latter cases, the conflict can be recognized from its physiognomy. Conflicts tend to result in balances. The two opposing forces behave like the weights of a scale that more or less counterbalance each other. Conversely, the balance phenotype is indicative of an underlying conflict of interests. The asset is the hinge around which the scale (conflict) revolves. The relative "weights" of the conflict parties and the length of the lever arms

determine the set point of the balance. The contested asset and its relative control by the conflict parties (the length of the lever arm) can be expected to tilt the balance one way or another. Balances are subject to disturbance dependent on ecological and intrinsic conditions. Balances that temporarily have come out of equilibrium may tend to return to set points or find another set point dependent on the change of relative "weights". Antagonistic pleiotropy is the phenotype of conflict mediation between the interacting individuals and between individuals and ecological factors (see chapter 13). Conflicting individuals attempt to change the balance to their advantage which may result in coevolutionary arms races and escalation. The most extreme outcome of such a conflict that has become unbalanced is the extinction of one of the conflict partners. Other potential outcomes are symbiosis, coexistence or various forms of exploitation such as parasitism. Importantly, coevolution is a feature of interacting biotic entities. Abiotic factors cannot coevolve. In this regard, resources have both a biotic and abiotic quality. Biotic resources such as plants, prey, hosts can respond to challenges and enter coevolutionary arms races. Dead resources cannot.

Formally, the soma is a resource for the germline cells. When it comes to identify phenotypes of competitive encounters it is essential to distinguish between the proper conflict parties and the contested assets. The ETAs only identified the asset over which the conflict arises but considered organisms as a homogeneous whole with identical interests. The workings of evolution teach us the lesson that they are not. Therefore the ETAs missed to identify the conflict parties and the transgenerational dimension of the conflict leading to the budgeting of resources. In fact, the budgeting of resources (leading at least in wild-type populations, in contrast to the predictions of the DST, throughout phylogenesis to a shortened lifespan with abundant resources) is key to the identification of the transgenerational dimension of the evolutionary programming of aging/death. Most importantly, conflicts are recognized by the trade-offs and costs that the interacting individuals have to pay for not being in total command of the resources necessary to meet their (selfish) needs. Conflicts due to predation (Lima and Dill, 1990; Brown and Kotler, 2004; Bolnick and Preisser, 2005; Lind and Cresswell, 2005), infection (Decaestecker et al., 2002; Linder and Promislow, 2009; Knowles et al., 2010), and mating/sexual antagonism (Arnqvist and Rowe, 2005; Chapman, 2006; Tregenza et al., 2006; Bonduriansky, 2009) have their costs. Reproduction has its cost for the soma (see chapter 8). Mutagenesis experiments demonstrate that it is easy to manipulate the reproduction/somatic survival balance by downregulating signaling pathways sensing resource utilization or upregulating stress resilience. The proponents of the ETAs never (at least to my knowledge) have asked a quite obvious question: assuming that aging is detrimental to somatic fitness, why did evolution not "find" these mutations itself, rendering the soma fitter? It can be objected that evolution may have found these mutations but since these mutant are routinely outcompeted by wild-type organisms (Gems et al., 1998; Hekimi et al., 1998; Walker et al. 2000; Chen et al., 2001; Marden et al., 2003; Jenkins et al., 2004; Van Voorhies et al., 2005; Mockett and Sohal, 2006) these mutants did not have a chance to be selected for. Thus, the fitness of the soma as determined by its longevity stands in conflict with its reproductive success. Reproductive success does not mean to produce as many offspring as possible, reproductive success clearly has a long-term component that is closely related to the fitness of the offspring. The compromise between somatic fitness and offspring fitness shapes the life history strategies of each individual in its given ecological habitat. A multitude of genetic and ecological factors affect this balance (see chapter 20). Obviously, the wild-type somatic aging trajectories are optimal for the reproductive success under the resource-limited conditions of the wild. Outside the laboratorium with its abundant resources, reproductive success is characterized by a set point that defines an optimum between the conflicting interests of the soma and the offspring. Thus, resource availability is probably the most essential factor that shifts the set point of the balance between somatic and offspring fitness. As discussed in chapters 4 and 8, longevity-fecundity set points and costs of reproduction are subject to moderation by resource availability.

While some may agree with the logic of the conflict between offspring and ancestors over the future utilization of resources (in fact, this concept featured prominently in a variety of group-selection versions since the days of Wallace and Weismann), the idea that this conflict is resolved by the germline-induced death of the parent(s) may make them shudder. However, both the "fossil record" of genome and physiology and phylogenetic phenotypic patterns leave no other interpretation. On the other hand, cannibalism (Fox, 1975; Polis, 1981; Elgar and Crespi, 1992; Wise, 2006; Alabi et al., 2009) , apoptotic cytocide (Heininger, 2001) and matricide (e.g. Finch, 1990) have been widely acknowledged. This is the "violent" legacy of the struggle for existence in a world of limited resources, inherited from unicellular and non-rational beings where negotiation over the access

to resources was no option.

Asian philosophies have realized the fundamental importance of opposites. Yin and yang are guided by the law of unity of the opposites that are in conflict but at the same time mutually dependent. Neither is able to exist in isolation. Life (of the offspring) and death (of the soma) are evolutionary necessities (Wallace, 1967; Theodoridis et al., 1996) and flip sides of the same coin.

12.2 Multicellularity and conflict

Evolutionary transitions in individuality occur when individuals combine to form new individuals, as occurred with the origin of cells, of eukaryotes, of multicellular organisms, and of integrated societies. During evolutionary transitions, preexisting individuals form groups, within which interactions occur that affect the fitnesses of both the individuals and the group. This transition has occurred multiple times independently, for example in the red algae, brown algae, land plants, fungi, and animals (Bonner, 1998; 2001; Kaiser, 2001; King, 2004; Grosberg and Strathmann, 2007; Rokas, 2008). For example, under certain conditions, bacteria associate to form a fruiting body, amoebae associate to form a slug, solitary cells form a colonial group, normally solitary wasps breed cooperatively, birds associate to form a colony, and some mammals form societies. During each of these evolutionary transitions in individuality, fitness was transferred from the individuals, making up the group, to the group itself, forming a new individual with a single evolutionary fate (Buss, 1987; Maynard Smith and Szathmáry, 1995; Michod, 1999a). According to multilevel selection theory the emergence and maintenance of higher units of selection from groups of lower-level individuals, e.g. multicellular organisms from groups of unicellular organisms (Maynard Smith and Szathmáry, 1995; Queller, 2000, Michod and Roze, 2001) requires the mediation of conflict among those individuals (Michod, 1996; 1999a).

Multicellular development (e.g. in M. xanthus and D. discoideum) represents an evolutionary bottleneck through which only a portion of starving populations can pass. Any group-limited fitness benefits conferred by fruiting body formation (which could include enhanced dispersal, protection from predators, starvation or caustic compounds and growth rate advantages upon germination) thus come at the cost of death for some individuals. In mixed populations, such a bottleneck encourages conflict between different genotypes over their relative representation among the limited number of spore slots available. Genotypes able to minimize their likelihood of developmental death will have a selective advantage at the individual level within fruiting bodies. Selfish genotypes that are disproportionately represented in spore populations have been identified (Dao et al., 2000; Velicer et al., 2000; 2002; Strassmann et al., 2000). Velicer et al. (2000, 2002) screened several developmentally defective genotypes of M. xanthus for frequency-dependent fitness relationships to the developmentally proficient wild-type that would constitute cheating. All eleven genotypes in these two studies were partially or completely defective at spore production (relative to the wild-type) during starvation as genotypically pure cultures. Being unable to cooperate fully with others of the same genotype, they are obligate defectors. However, when mixed as a 1% minority with the wild-type, six of these genotypes cheated during development by producing spores more efficiently than the wild-type. For at least two of these cheaters, their fitness superiority in mixed development was frequency dependent. The fitness of defectors was higher than that of cooperators when they were at low abundance but fell below cooperator fitness when defectors reached a critical abundance (Velicer, 2003; Kuzdzal-Fick et al., 2011) which in this case should occur routinely after repetitive rounds of dispersal since low-frequency cheaters outcompete cooperators. In game theory parlance this scenario is an instance of the chicken game (Velicer, 2003). Because both cooperation and defection are advantageous when rare, the chicken game predicts that cooperators and cheaters should be maintained in a balanced polymorphism over sequential cycles of development with oscillating relative frequencies of the two types (Fiegna and Velicer, 2003). The long-term coexistence of defector and cooperator genotypes also critically depends on the mode of dispersal and the dynamics of formation of social aggregation. If dispersal and colonisation happen by single cells, the survival of defectors is not possible since they are not able to build social groups alone (Jékely, 2007). A unicellular bottleneck can, at least partially, minimize these conflicts by periodically reducing genetic heterogeneity (and increasing kinship) among the cells that constitute multicellular organisms (Grosberg and Strathmann, 1998; Brockhurst, 2007). A common hypothesis is that the unicellular bottleneck of the germ cell acts as a conflict mediator, by increasing the kinship among cells in the organism, thereby aligning the interests of cells with the interests of the organism (Bell and Koufopanou, 1991; Maynard Smith and Szathmáry, 1995; Grosberg and Strathmann, 1998). A unicellular bottleneck initially makes the interest of all cells in a multicellular organism congruent (Maynard Smith, 1988; Frank, 1996), and stabilizes the organism as an individual evolutionary unit (Van Valen, 1988).

Hence, competition is usually limited by passage through a single-cell bottleneck (Maynard Smith, 1989). This means that each mutation, however effective a cheater, gets only one opportunity to cheat. This keeps it from being too much of a threat to multicellular individuality (Strassmann and Queller, 2004; Kuzdzal-Fick et al., 2011). However, cheating cellular defectors that reap the benefits of the common goods, like in cancerogenesis, remains a constant threat to multicellular life (Altenberg, 2005). To resolve the conflict over which cell is to be the germline, a stable, policed, germline/soma differentiation mechanism must have evolved at some point (Bryden, 2007). Since microbes can evolve many 'policing' mechanisms (Travisano and Velicer, 2004), it is not inconceivable that after several generations, the germ/soma differentiation may well have become established in the organism without the need for environmental cues (Bryden, 2007). It has been shown (Simpson, 2012) that at the transition to multicellularity a reproductive division of labor will evolve first in a majority of cases, and that the total extent of functional differentiation will be larger if there is a reproductive division of labor. Accordingly, specialization of cells in reproductive (germ cells) and vegetative functions (soma) is an almost universal feature of unitary multicellular life. Conflicts between units arise when the selection pressures on some of the units favor one outcome, whereas those on other units favor another. The most basic conflict is between units of the same species or organism when selection pressure on one of the units favors the survival of its own lineage over survival of the lineage of the other unit. Conflict can arise when two units have influence over a common feature (Lachmann et al., 2003). A central conflict that has to be mediated in multicellular organisms with a germline-soma division revolves around the issue of resource allocation for both reproduction and somatic tissue maintenance and resolution of the 'tragedy of commons' dilemma (Michod, 1999b).

The rather recent triassic evolutionary transition in individuality in the green alga Volvox, allowed to identify key innovations resulting from an early cycle of cooperation, conflict and conflict mediation that led to a rapid integration and radiation of multicellular forms in this group (Herron and Michod, 2008; Herron et al., 2009). Terminal differentiation of somatic cells in V. carteri involves the expression of regA—a master regulatory gene that encodes a transcriptional repressor (Kirk et al. 1999) that suppresses several nuclear genes coding for chloroplast proteins (Meissner et al. 1999). Consequently, cell growth (dependent on photosynthesis) and division (dependent on cell growth) of somatic cells are suppressed. Because they cannot divide, they do not participate directly in the offspring but contribute to the survival and reproduction of the colony (Kirk 1998, p 62–4; Solari, Kessler, and Michod 2006; Solari et al. 2006)—in the same way that sterile workers do in a social insect colony. The gene responsible for the permanent suppression of reproduction in the somatic cells evolved from a gene that in its unicellular relative, Chlamydomonas reinhardtii, is part of the general acclimation response to various environmental stress factors, which includes the temporary suppression of reproduction. In both plants and green algae, photosynthetic activities and chloroplast protein composition are adjusted in response to various environmental changes, as an adaptive mechanism (photosynthetic acclimation) that can enhance survival (Grossman 2000; Walters 2005) - especially in variable environments such as those in which volvocalean algae live (Kirk 1998). Dynamic changes in chloroplast composition are thought to ensure unnecessary investment in particular sets of proteins, thus releasing resources for use in other cellular processes (Grossman 2000; Walters 2005). Genes encoding chloroplast components can be regulated by factors like light and nutrient stress (Jain et al. 2005; Nedelcu, 2009). Simulating the stress signal (i.e. a change in cellular redox status) in a developmental rather than environmental context, the stress response was co-opted into a developmental response segregating germline cells and soma (Nedelcu and Michod, 2006; Nedelcu, 2009).

The specific details of this or any other evolutionary transition in individuality are bound to be unique, but there are basic principles of multilevel selection theory that are relevant to all such transitions (Maynard Smith and Szathmáry, 1995; Michod, 1999a). Because of the lack of extant intermediate forms, the primary source of evidence in these cases has been the fossil record. However, since fossil evidence of the early steps in these ancient transitions may be scant, comparative genomics may be a more fruitful approach (Herron and Michod, 2008; Rokas, 2008; Herron et al., 2009). Recent comparative functional analyses of microbial unicellular and multicellular genomes have begun to throw considerable light on the molecular commonalities exhibited by multicellular transitions. These have allowed to delineate the likely functional components of genetic toolkits required for multicellular existence. Surprising, several of these toolkit components have been identified in unicellular lineages. The study of these toolkit proteins in a unicellular context has begun yielding insights into their ancestral functions and how they were co-opted for multicellular development (Rokas, 2008).

Multicellular aggregations of microorganisms in response to environmentally induced resource depletion provide insights into the evolutionary "nurseries" of multicellularity. These multicellular aggregations "invented" the differentiation-apoptosis hybrid (Heininger, 2001), antagonistic pathways to reproduction and death (Heininger, 2002a) and germ-soma dichotomy (Weismann, 1889). The evolutionary pathways are still traceable by deep genetic homologies and homoplasies (Delsuc et al., 2005; Ciccarelli et al., 2006; Shubin et al., 2009; Wake et al., 2011), conserved signaling pathways and phenotypic footprints. The pervasive presence of oxidative stress during both reproductive events (Heininger, 2001; Agarwal et al., 2005; Nedelcu, 2005; Metcalfe and Alonso-Alvarez, 2010) and aging/death (Harman, 1956; Beckman and Ames, 1998; Dröge, 2002; Muller et al., 2007; Metcalfe and Alonso-Alvarez, 2010; Ristow and Schmeisser, 2011) are a legacy of ambiguous sporulation/cell death decisions during primordial reproductive events. Likewise, the antagonistic control of reproduction and aging/death by nutrient sensing (e.g. TOR and IIS) and stress response signaling pathways links them to primordial starvation responses with their life-death decisions.

Life history evolution is replete with examples of traits with antagonistic pleiotropic effects on fitness, traits that increase one component of fitness while decreasing another. For example, allocation of energy to reproduction (benefiting the germline cells) routinely decreases somatic survivorship (see chapter 8). Often, the phenomenon of antagonistic pleiotropy with its trade-offs is the signature of conflicting interests (Stearns and Magwene, 2003; see chapter 13). Thus, resource utilization that is monitored by the TOR and IIS signaling pathways is indispensable for germ cell maturation and dispersal but limits somatic longevity. Increased stress resistance enhances survival (Tatar, 1999) but has a cost to reproduction (Krebs and Loeschcke, 1994; Tatar, 1999; Silbermann and Tatar, 2000).

12.3 Mediation of germ-soma conflict by antagonistic Red Queen coevolution

Now here, you see, it takes all the running you can do, to keep in the same place -Lewis Carroll: Through the Looking Glass

The cited lines were the Red Queen's explanation to a confused Alice as to why she could run as fast as she could in Wonderland but never get anywhere, a situation analogous to the constant coevolutionary pressure exerted by a changing environment (Van Valen, 1973b). Species evolve in response to their biotic environment and this can lead to coevolution between species that interact in either a mutualistic or an antagonistic fashion (Futuyma and Slatkin 1983). From various biological levels of conflict it has become obvious that conflict breeds antagonistic coevolution (Rice and Holland, 1997; Zeh and Zeh, 2000; Arnqvist and Rowe, 2002; Rice and Chippindale, 2002; Eberhard, 2004; Lessells, 2006; Poulsen et al., 2007; Wilkins, 2010). As a result, there is selection on each interacting partner to manipulate the trait towards its own optimum and resist such manipulation by the other partner (Lessells, 2006). In this case, evidence for the operation of the coevolutionary process (i.e., antagonistic coevolution of a species with its enemies/conflict partners - predators, herbivores, competitors, mating partners, and pathogens) (Van Valen, 1973b; Levin, 1975; Jaenike, 1978; Hamilton, 1980; Bell, 1982; Lively, 2010), is well documented in both agriculture (Day 1974; Robinson 1976) and natural systems (Parker 1985; Lively 1989). The Red Queen is characterized by an interspecific evolutionary chain reaction: adaptation by species A $\rightarrow$ counter-adaptation by enemy species B $\rightarrow$ counter-adaptation by species A $\rightarrow$ ..., which can lead to a protracted period of coevolution (Ehrlich and Raven, 1964; Vermeij, 1983; 1987; 1994). Even intraspecies competition may lead to coevolutionary perpetual change (Wichman et al., 2005). Conflicts may be both disastrous and beneficial. The fitness-boosting value of coevolutionary systems has been demonstrated repeatedly (Spitze, 1991; Spitze et al., 1991; Lynch and Spitze, 1994; Reznick et al., 2004; Fisk et al., 2007). Guppies collected from stream environments lacking predators were found to be inferior in every aspect of their life history profile to those evolved in other, nearby sites with predators present (Reznick et al., 2004). Fitness gains appear to speed up under the challenge of moderate conflicts.

A parallel evolutionary chain reaction can occur between cell populations or non-allelic genes within the genome of a single organism. Mutation, fusion between different genotypes, and infection by symbionts are the key processes that can introduce genetic heterogeneity into the population of cells that constitutes a multicellular organism. The presence of genetically distinct replicating units in a multicellular organism can lead to several different kinds of conflict among cell lineages. The conflicts may arise from competition among dividing cell lineages for opportunities to propagate or for access to the germline (Buss, 1982; 1985; 1987). Conflicts can also arise among the products of meiosis (Haig and Grafen, 1991), and between hosts and endosymbionts (Bell and Koufopanou, 1991; Hurst and Hamilton, 1992; Frank, 1996). Thus, like host and parasite, the gene

products from the conflicting partners are part of the evolving, biotic environment of one another, and they can potentially coevolve in an antagonistic or correlational fashion, via a Red Queen process (Van Valen, 1973b; Van Valen, 1974; Stenseth and Smith, 1984; Rice and Holland 1997; Rice et al., 2005; Schulte et al., 2010). A key characteristic of antagonistic coevolution is that it can lead to a self-reinforcing adaptation/counteradaptation chain reaction that leads to both a higher mutational robustness and higher evolvability of the genetic network (Wagner, 2005; Lenski et al., 2006; Hintze and Adami, 2008; 2010; Yukilevich et al., 2008; Kartal and Ebenhöh, 2009; Whitacre and Bender, 2010; Fierst, 2011; Whitacre, 2011). That is, it can lead to recurrent, even perpetual, gene substitutions at antagonistically interacting loci and thereby continually drive genetic and phenotypic divergence among related species, isolated populations, cell populations and genetic loci. Displaying both the evolvability and robustness of coevolutionary systems, the aging/postreproductive death phenotype is extremely robust and resistant to invasion by cheater phenotypes. In general terms, antagonistic coevolution speeds the rate of evolution (Schmidt et al., 2000; Landry et al., 2005; Paaby and Schmidt, 2008; Schulte et al., 2010; Fox et al., 2011) whenever allelic substitution at one locus selects for a new allele at the interacting locus, and vice versa, so that no stable equilibrium can be achieved, or is achieved after many iterations. As a consequence, an increased rate of allelic substitution is a “footprint” left behind by the antagonistic coevolution process (Rice and Holland 1997; Schmidt et al., 2000; Landry et al., 2005; Pal et al., 2007; Paterson et al., 2010; Schulte et al., 2010; Fox et al., 2011).

By definition, heritable mutations arise only in the germline. In germline genetic mosaics, cell selection may affect the number of gametes of a given germ cell that appears in the next generation. Because of the dominant or dosis-dependent effects on cell behavior (Extavour and Garcia-Bellido, 2001), second-site mutations, arising in the germline and acting as suppressors or enhancers of preexisting inherited mutations, may be selected for in heterozygosis. Once these mutations (or combination of mutations) have been passed on to the gametes, they are subject to further selection during embryonic somatic morphogenesis in the progeny. This germline selection will affect a noticeable fraction of gene functions in both somatic and germline development, thus becoming a mechanism of selection for somatic development. This process may be involved in concerted evolution and molecular coevolution (Dover and Flavell, 1984). Any genes with direct and specific roles in reproduction are remarkable for their poor conservation even between closely related species (Wyckoff et al., 2000; Haag et al., 2002; Swanson and Vacquier, 2002; Haag and Doty, 2005; Clark et al., 2006; Panhuis et al., 2006; Haerty et al., 2007; Turner and Hoekstra, 2008). The long-standing mystery of sex determination and its extreme diversity (Haag and Doty, 2005) is most plausibly explained by the coevolutionary workings (Pazos and Valencia, 2008) of the germ-soma and sexual conflicts.

Parent-offspring conflict theory deals with the embryonal and postnatal conflicts about resource allocation (Mock and Parker, 1997). Data show that the assumptions of the parent-offspring conflict theory are met for relevant taxa: the high number of independent origins of viviparity, matrotrophy (direct maternal-fetal nutrient transfer), and hemochorial placentation (direct fetal access to the maternal bloodstream); the extreme diversity in physiological and morphological aspects of viviparity and placentation, which usually cannot be ascribed adaptive significance in terms of ecological factors; and divergent and convergent patterns in the diversification of placental structure, function, and developmental genetics (Crespi and Semeniuk, 2004). The theory is also supported by data demonstrating that embryos, fetuses and neonates actively manipulate their interaction with the mother, thereby garnishing increased maternal resources (Agrawal et al., 2001; De Jong et al., 2005; Kölliker et al., 2005; 2010; Apari and Rózsa, 2006; Grodzinski and Lotem, 2007; Qvarnström et al., 2007; Smiseth et al., 2008; von Engelhardt et al., 2009; Mas et al., 2009; Schrader and Travis, 2009; Banet et al., 2010; Hinde et al., 2010; House et al., 2010). The data indicate that selection favors adaptations of the mother, the fetus, or both in traits related to reproductive mode and investment (Crespi and Semeniuk, 2004). A comparative evolutionary analysis of the insulin-like growth factor II (IGF2) gene in teleost fishes revealed that genetic conflict is a general feature of adaptation to placental reproduction providing a rare example of natural selection acting in synchrony at the phenotypic and molecular level (O’Neill et al., 2007). In several members of the order Cyprinodontiformes, in which livebearing and placentation have evolved several times independently, IGF2 was found subject to positive Darwinian selection coincident with the evolution of placentation in fishes, with particularly strong selection among lineages that have evolved placentation recently. Positive selection was also detected along ancient lineages of placental livebearing fishes, suggesting that selection on IGF2

function is ongoing in placental species. These findings also constitute the first direct evidence of parent–offspring conflict driving gene evolution (O'Neill et al., 2007). Similarly, a cross-species comparison of transcript changes seen in long-lived nematodes, insects and mammals with lowered IIS, when compared to normal-lived controls, found that IIS-regulated genes are not conserved at the level of gene orthology or of paralogous gene groups. However, if IIS-regulated genes are compared across species at the level of gene category (in some cases, at a process level), cross-species similarities are visible. Thus, in contrast to the public role of IIS in aging, the set of genes regulated by IIS is largely lineage specific (McElwee et al., 2007). Likewise, the aging-related gene methuselah (mth) has experienced strong selection on expression level since D. melanogaster and D. simulans shared a common ancestor approximately two million years ago (Landry et al., 2005; Paaby and Schmidt, 2008). Over this same timescale, mth also shows a very high rate of protein evolution (Schmidt et al., 2000). These findings highlight two features of aging-related processes: 1) antagonistic coevolution, mediating the germ-soma conflict, led to a large diversity of lineage-specific, "private", molecular processes; 2) a strong selection pressure conserved the "public" nutrient sensing and stress-related phenotype of aging witnessing its rationale through all evolutionary ages.

Moreover, antagonistic coevolution stabilizes the phenotype (Rosenzweig and Schaffer, 1978). The conflict between germline cells and soma is reflected by a multitude of antagonistic pleiotropic effects. Importantly, these antagonistic pleiotropic effects are mediated by hormones in all metazoan species (Ketterson and Nolan 1999; Zera and Harshman, 2001; Ricklefs and Wikelski, 2002; Flatt et al., 2005; Harshman and Zera, 2007). Most importantly, germ-soma conflicts over resource allocation pervade all aspects of life. Resource allocation is clearly hierarchical: in laboratory and field experiments on bivalves, cladocerans, insects, and mammals, allocation to maintenance or storage was found to take precedence over allocation to reproduction under nutrient-poor or stressful conditions (Rogowitz 1996, Perrin et al. 1990, Boggs and Ross 1993, Jokela and Mutikainen 1995, Zera et al. 1998). However, evolution is not "interested" in the survival of the organism per se but in its survival to reproduce (see chapter 19.1). Thus, as soon as conditions allow, reproduction takes precedence over survival.

12.4 Coevolutionary germ-soma conflict and Cambrian Explosion

For reasons that have remained unclear, almost all of the modern animal phyla arrive in the fossil record during a geologically short period of about twenty million years, starting about 540-545 million years ago (Valentine et al., 1999; Baker, 2006; Marshall, 2006). The Cambrian explosion (CE) with its dramatic increase in both disparity and diversity has been a unique and troubling anomaly in the history of life. Various attempts have been made to explain the phenomenon. Several factors that are prerequisites for the explosion have been identified, necessary but not sufficient. Rapid change following a long period of quasi-stasis (due to "Snowball Earth"?) (Hoffman et al., 1998) suggests the existence of a triggering or enabling event, either external to organisms or incorporated in organisms (Butterfield, 2007). The time of onset is constrained by the evolution of the environment, whereas its duration appears to be controlled primarily by rates of developmental innovation (Marshall, 2006). It has been noted that the oxygen content of the atmosphere was slowly rising (Thomas, 1997). This was likely an enabler, and possibly a trigger (Nursall, 1959; Berkner and Marshall, 1965; Canfield and Teske, 1996; Ohno, 1997; Canfield et al., 2007). Most animals require molecular oxygen in order to produce their energy, and this has led to the widespread presumption that a rise in atmospheric oxygen was the essential precursor to the evolution of animals (Runnegar, 1991; Catling et al., 2005; Shields-Zhou and Och, 2011). Both a surge in seawater calcium and phosphate have been implied as capacitating environmental factors (Cook, 1992; Brennan et al., 2004; Porter, 2007; Fernàndez-Busquets et al., 2009). The animal phyla are divided into three groups. First are the sponges (Porifera) that do not have organized cell layers. Second are the diploblasts, which have two primary cell layers: an outer ectodermal layer and an inner endodermal layer. The Cnidaria (corals and jellyfish) and the jellyfish-like group, the Ctenophores, are the only diploblastic phyla. All of the remaining animal phyla are triploblasts, also collectively referred to as the bilateria. These grow from three primary cell layers: the outer ectoderm, the intermediate mesoderm (from which our skeleton and most of our muscles are derived), and the inner endoderm, which includes the gut. Over 99% of all living animals are triploblasts (Marshall, 2006). Both the geological fossil, ontogenetic and genetic records support the Ediacaran (c. 585–542 Myr ago) emergence of triploblasts (Aris-Brosou and Yang, 2002; 2003; Peterson et al., 2008; Rokas, 2008a; Xiao and Laflamme, 2008; Erwin, 2009). Explanations for the acceleration of the evolutionary tempo during CE

center on the invention of new trophic capacities, whether predation (Vermeij 1990, Bengtson 2002) or the related cropping (Stanley 1973, 1976). Many of these theories emphasize the role of predation, specifically in an effort to explain the massive skeletonization event that characterizes the fossil record of the explosion (Vermeij 1990). All of these have a common thread of coevolution, escalation, or arms races (Vermeij 1987, 2004; Butterfield, 2007). A fact that has received relatively little attention when trying to explain the causes of the CE: it is a phenomenon restricted to triploblasts and thus its causes should be sought in some evolutionary innovation unique to these organisms. Hence, cellular differentiation that was proposed as a candidate new technology associated with CE (Phoenix, 2009), is an improbable cause of CE since it is a property of all multicellular organisms. The fact that the developmental toolkit was unequivocally established in the last common protostome-deuterostome ancestor (Valentine et al., 1996; Martindale et al., 2004, Martindale, 2005; Couso, 2009; Erwin, 2009) and that the last common protostome-deuterostome ancestor probably lived prior to 555 million years ago strongly suggest that developmental innovation may have been necessary but cannot be a sufficient cause of the main Cambrian radiation. The developmental innovations were a precondition to the later events and may explain the extraordinary breadth of the radiation, but not the triggering of the event itself (Erwin, 2005).

Here, I suggest that the coevolutionary dynamics of the germ-soma conflict could make a perfect candidate for the driving force behind CE. In most animal phyla from insects to mammals, there is a clear division of somatic and germline cells. This is however not the case in plants and some animal phyla including tunicates, flatworms and the basal metazoan phyla Porifera and Cnidaria, where germ stem cells arise de novo from somatic cells. The latter contain a population of endodermally derived pluripotent stem cells (sponge archaeocytes, Cnidarian interstitial cells, and acoel neoblasts) that can give rise to both somatic cell types and gametes (Agata et al. 2006; Extavour, 2007). Recent phylogenetic analyses indicate that germline segregation appears to have evolved at or near the origin of the first bilaterians (Buss, 1987; Blackstone and Jasker, 2003; Sánchez Alvarado and Kang, 2005; Extavour, 2007). The protostome-deuterostome ancestor seems to have been endowed with a dedicated germline (Ross and Nelson, 2004). The remarkable discovery of what appears to be exquisitely preserved Neoproterozoic eggs and embryos (Xiao et al., 1998) has introduced a line of evidence into the early history of Neoproterozoic animals. The presence of oogenesis and cleavage suggests the establishment of adult metazoans with sequestered germ cell lines and differentiated somatic cell lineages. The conflict between segregated germ cells and soma may have fuelled the coevolutionary Red Queen dynamics resulting in the CE. This coevolutionary dynamic may also account for a putative acceleration of the molecular clock during the CE (Aris-Brosou and Yang, 2002; 2003; Bromham, 2003; Welch et al., 2005).

12.5 Coevolutionary germ-soma conflict and sexual reproduction

The Cambrian explosion occurred in bilaterian animals that are stage of a germ-soma conflict, irrespective of their asexual or sexual mode of reproduction. The evolutionary success of sexual reproduction is one of the great enigmas of evolutionary biology (Williams, 1975). The only direct effect of genetic recombination in sexual reproduction is the reduction of statistical associations among genes. However, linkage disequilibria (LD), certain combinations of alleles, are found together more often than expected by chance are pervasive. Any hypothesis that aims to explain the selective benefit of recombination must explain by which process such genetic associations are continually recreated and maintained (Salathé et al., 2009). Correlational selection builds favorable genetic correlations through the formation of LD at underlying loci governing the traits (Sinervo and Svensson, 2002). However, LD built up by correlational selection are expected to decay rapidly (ie, within a few generations) by recombination, unless correlational selection is strong and chronic that would be expected to generate an optimal trait combination (i.e. a single fitness peak). Biotic interactions that have 'Red Queen' dynamics often fuel chronic correlational selection, which is strong enough to maintain adaptive genetic correlations. So far, it has been thought that the Red Queen dynamics of parasite-host interactions may qualify as rationale for the evolutionary success of sexual reproduction (Salathé et al., 2008a, b). However, there has been considerable debate about whether parasites are generally virulent enough to cause enough selection on hosts for recombination to be beneficial (Peters and Lively, 1999; Schmid-Hempel and Jokela, 2002; Otto and Nuismer, 2004). Sexual reproduction owes at least part of its evolutionary success to the germ-soma conflict. In a parallel paper I posit that the pervasive germ-soma conflict provides the evolutionary framework for coevolutionary Red Queen dynamics and a correlational selection milieu in sexual reproduction (Heininger, in preparation).

13. Mutation accumulation and antagonistic pleiotropy

Hope you guess my name. But what's puzzling you is the nature of my game.
Rolling Stones. Sympathy for the Devil, 1968

Summary
Mutation accumulation and antagonistic pleiotropy are the mechanistic cornerstones of the ETAs. These two concepts, however, are mutually exclusive: mutation accumulation occurs in the absence of selective forces while antagonistic pleiotropy requires the action of opposing selective forces (as Williams [1957] himself mentioned). In the framework of the germ-soma conflict theory both mutation accumulation and antagonistic pleiotropy are features of aging, but they are epiphenomena and not its raison d'être. Antagonistic pleiotropy is the phenotypic result of the coevolutionary dynamics of the germ-soma conflict (see chapter 12). With regard to the concept of mutation accumulation, monogenic and polygenic human diseases of advanced age epitomize the fact that these diseases only manifest clinically at an age when they do no longer affect the reproductive value of the mutations' carrier and are not selected against. This phenomenon, however, is no evidence supporting the notion that evolution does not "see" postreproductive organisms. Postreproductive organisms are "seen" through their effects on the fitness of the progeny (see chapters 3 and 19.1).

Two distinct population genetic processes can produce ecological specialization—mutation accumulation and antagonistic pleiotropy (Rose and Charlesworth, 1980; Rose, 1991; Holt, 1996; Sgró and Partridge, 1999). In mutation accumulation (MA), mutations become fixed by genetic drift in genes that are not maintained by selection. Drift can contribute to divergence through the chance fixation of mutations in small effective population sizes. Antagonistic pleiotropy (AP) arises from trade-offs, such that the same mutations that are beneficial in one environment are detrimental in another (Cooper and Lenski, 2000; Elena and Lenski, 2003). There is a fundamental difference between MA and AP: MA is characterized by the absence of selection pressure while AP occurs in the presence of opposing selection. In the words of T. Prout (2000): "Antagonistic pleiotropy or fitness component trade-off is clearly a case of opposing selection." Hence, two opposing selective forces (e.g. exerted by different environments, parasite/host-, or sexual conflicts) effectuate an AP and associated trade-offs (Schluter et al., 1991). Conversely, life-history trade-offs are the phenotypic footprint of opposing selection (Nylin and Gotthard, 1998). According to Roff and Fairbairn (2007), "trade-offs are specified in quantitative genetics by a negative genetic covariance between traits, a covariance that could be caused by antagonistic pleiotropy or linkage disequilibrium (LD). In both cases a causal connection is inferred". AP is defined in terms of changes in fitness generated by pleiotropic phenotypic effects: an increase in fitness associated with the value of one trait is correlated with a decrease in fitness associated with the value of the other trait (Roff and Faibairn, 2007). This is indeed a form of correlational selection and it would be expected to generate an optimal trait combination (i.e. a single fitness peak). However, correlational selection is broader than this, encompassing fitness ridges or even saddles, with regions in which parallel changes in both traits (i.e. both increase or both decrease) have parallel rather than antagonistic effects on fitness (Phillips and Arnold, 1989; Roff and Fairbairn, 2007). Correlational selection can generate a suite of combinations that have equal fitnesses rather than a single fitness peak, and this may help to maintain variation in fitness trade-offs (Roff and Fairbairn, 2007). As will be discussed in chapter 17.1.2, reproduction and longevity are linked in a correlational selection pattern that determines their AP and trade-offs.

It is the nature of aging that, so far, is puzzling both evolutionary biologists and geneticists: both MA and AP are features of aging, but they are epiphenomena and not its raison d'être. Reproduction that is indispensable for evolutionary processes is causative in programming death and aging. As such, the selective forces that determine aging/death should be very strong. In fact, aging/death is ubiquitous in asymmetrically reproducing organisms with age-structured populations. Organisms are always subject to several, often conflicting, selective forces in their natural habitat that shape their chances of survival and reproduction and the ecological structure of populations. MA in lineage-specific pathways may, at least in part, be shaping private aging mechanisms but, as discussed below, due to the stochasticity of its emergence, cannot be reconciled with the existence of public aging processes. In fact, there is little positive evidence (and much contrary evidence) concerning the reality of MA (Lamb and Maynard Smith, 1964; Kirkwood and Austad, 2000). As discussed in chapter 3, the declining reproductive value of an organism and its evolutionary visibility are different concepts and

should not be blended in a single flawed theory. The monogenic and polygenic human diseases of advanced age such as M. Huntington, M. Parkinson, M. Alzheimer, type 2 diabetes mellitus, have been taken as evidence for the declining force of natural selection in postreproductive organisms. However, the existence of these diseases epitomizes the fact that these diseases only manifest clinically at an age when they do no longer affect the reproductive value of the mutations' carrier (at least during most of the evolutionary history of mankind). Evolutionary visibility of a postreproductive organism, on the other hand, is as prominent as the presence of predators, competitors or helping kin. The epidemiology of these diseases also highlights the pattern of mutation accumulation and persistence: sporadic ('private') and far from being fixed throughout a population. Diseases like M. Alzheimer paradigmatically illustrate another issue of aging-related diseases. M. Alzheimer has monogenic and polygenic risk factors (Heininger, 2000a) and carries features of both normal and accelerated aging and it is quite a challenge to dissect the one from the other (Heininger, 2000b).

The theory of antagonistic pleiotropy (AP) is based on 2 assumptions. First, it is assumed that a particular gene may have an effect not only on one feature but on several traits of an organism (pleiotropy). Indeed, pleiotropy is one of the most common traits possessed by genes overall (He and Zhang, 2006) due to the highly integrated network structure of cellular signaling. In addition to that, pleiotropy is under strong stabilizing selection (Barton, 1990). The second assumption is that these pleiotropic effects may affect individual fitness in antagonistic ways, some beneficial but others detrimental to the organism. Although there are so many negative effects related to genes that are antagonistically pleiotropic, it is still present among most forms of life. As discussed in chapter 12, evolutionary conflicts lead to coevolution and AP. Species evolve in response to their biotic environment and this can lead to coevolution between species that interact in either a mutualistic or an antagonistic fashion (Futuyma and Slatkin, 1983) which results in compromises with trade-off phenotypes.

Antagonistically pleiotropic genes are the genetic basis for fitness trade-offs (Elena and Sanjuán, 2003). AP arises from trade-offs, such that the same mutations that are beneficial in one environment are detrimental in another (Cooper et al., 2001; Elena and Lenski, 2003). According to life history theory, the evolution of phenotypic traits is shaped by natural selection which tends to maximize fitness, and by trade-offs which constrain trait combinations (Roff, 2001). Three classes of trade-offs have been described: (1) allocation trade-offs resulting from the allocation of a resource with limited availability, (2) acquisition trade-offs between maximizing resource acquisition, a fitness trait (Hunt et al., 2004), and minimizing the risk of mortality and (3) specialist/generalist trade-offs resulting from specialization in a given environment (Angiletta et al. 2003). Thus, when organisms adapt genetically to one environment, they may lose fitness in other environments (Mills et al., 1967; Futuyma and Moreno, 1988; Fry, 1990; Bennett and Lenski, 1993; Cooper and Lenski, 2000). Moreover, adaptation to any one environmental condition limits diversification (Buckling et al., 2003). Several experiments with viruses that are able to infect more than one host have found that viruses that evolved on one host became less fit (or at least did not improve) on alternative hosts (Weaver et al., 1999; Crill et al., 2000; Turner and Elena, 2000; Cooper and Scott, 2001). In E. coli, nutrient-limited environments provide an ideal breeding ground for rpoS mutations while, in other settings, increased stress resistance selects for restoration of rpoS function. Hence extensive polymorphism in the mutS–rpoS region results from cycling between environments in which the functional or non-functional genes provide distinct fitness advantages (Ferenci, 2003). These counterregulatory adaptations are part of the global AP network that inversely controls stress competence and resource utilization and is operative from bacteria to mammals (Ferenci, 2005). In human populations, metabolic efficiency as provided by the 'thrifty genotype' is a survival advantage in harsh or less affluent environmental conditions (Coleman 1979, Rogers et al. 1986, Colagiuri and Brand Miller 1997). Exposed to abundant nutrients and a high-fat Western diet, however, this genetic outfit is associated with obesity and diabetes mellitus (Swinburn et al. 1991, King and Rewers 1993, Ravussin et al. 1994; Stoger, 2008) and an increased mortality due to cardiovascular diseases and diabetic nephropathy (Chukwuma and Tuomilehto 1998, Sievers et al. 1999a, b). At the cellular level, a multitude of signal transduction pathways including p53, NF-κB, E2F family members, tumor growth factor, or oxidative stress are involved in antagonistic pleiotropic cellular decisions like proliferation, differentiation, survival and apoptosis (Heininger, 2001; Dröge, 2002; Martindale and Holbrook, 2002; DeGregori and Johnson, 2006; Lambeth, 2007, Polager and Ginsberg, 2008; 2009; Hur and Walker, 2009; Metcalfe and Alonso-Alvarez, 2010; Mullineaux and Baker, 2010). The antagonistic pleiotropic canonical Wnt signaling pathway is one of a handful of powerful signaling pathways that play crucial roles in animal life by controlling the genetic

programs of embryonic development and adult homeostasis (DeCarolis et al., 2008; Grigoryan et al., 2008; Maiese et al., 2008). In Drosophila, Wnt signaling promotes maintenance of a variety of stem cells (Song and Xie, 2003; Lin et al., 2008; Takashima et al., 2008) while Wnt overexpression accelerates aging in the adult fly (Shen et al., 2009). Wnt signaling is implicated in embryonic development, tissue homeostasis and the maintenance of adult stem cell populations in younger mammals (DeCarolis et al., 2008; Grigoryan et al., 2008), while conversely Wnt signaling is implicated in promoting cellular senescence in aging mammals (Brack and Rando, 2007; Liu H et al., 2007). In these examples, opposing selective pressures originate from biotic or abiotic stresses or ontogenetic stages.

In fact, these AP actions reflect the evolutionary roots of survival and death decisions as programmed by primordial starvation/stress responses (Heininger, 2001; Monaghan et al., 2009). Importantly, in none of these examples AP was the cause of the phenomenon but its circumstantial epiphenomenon. While some AP effects are owed to the dynamics of environmental changes, AP is constitutive in organisms with a germ-soma dichotomy and is pervasive in genes involved in regulation of the gonad, the insulin-like growth factor pathway, free-radical scavenging, heat shock proteins and apoptosis (Leroi et al., 2005).

A comprehensive literature search (and the pure existence of this paper should be compelling evidence that when I write “comprehensive” I really mean “comprehensive”) did not identify a single AP (of course with the exception of the flawed ETAs) where AP was not the result of opposing selective forces. AP requires the action of opposing selective forces like in host/parasite interactions, sexual selection, competition between and within species, and different environmental pressures. These selective forces do not have to operate simultaneously but may operate sequentially like in the case of environmental selection pressures, one selecting for, the other against, a phenotypic trait: the AP only becomes manifest in the different environment that constitutes the opposing selective pressure (Lenski, 1988). AP is becoming manifest before and after maturation. Reproductive maturation is the defining feature separating the two ontogenetic stages and it is the mature germline with its gonadal hormones that exerts the opposing selective pressures. In free-ranging mute swans, age at first reproduction and age at last reproduction are under strong opposing directional selection as shown by quantitative genetic pedigree analyses (Charmantier et al., 2006), a relationship that is confirmed by artificial selection in Drosophila (Rose, 1991). Strangely enough, the conflicting interests of parasites/hosts, predators/prey, sexual mates, parents/offspring have been taken into account by evolutionary biologists to explain the Red Queen dynamics of these interactions. Solely the germ-soma conflict over the future utilization of resources, the most fundamental of conflicts (taking into account its pervasive nature and shaping of life history patterns of all multicellular organisms) escaped the attention of biologists, probably due to the censoring actions of the ETAs. Intriguingly, Williams (1957) wrote: “It is inconceivable in modern evolutionary theory that senescence such as operates in man between ages thirty and forty is selectively irrelevant” (p. 399). And further: “There are therefore two opposing selective forces with respect to the evolution of senescence. One is an indirect selective force that acts to increase the rate of senescence by favoring vigor in youth at the price of vigor later on. The other is the direct selection that acts to reduce or postpone the ‘price’ and thereby decrease the rate of senescence” (p. 402). However, Williams failed to identify the ‘opposing selective forces’. I am unaware of any extrinsic selective force (that should be a physical force such as predators, infectious agents, drought, or starvation) that acts in an opposite way on pre-reproductive and post-reproductive individuals. The only conceivable selective force is reproduction itself, generating offspring that, as is already a main topic in the parent-offspring conflict theory, have opposite interests concerning the allocation of resources. Realizing that he transgenerational conflict over resources is much more far-reaching than during the immediate peri-reproductive period and that a persistent, intrinsic, opposing selective force is required to bring about the phenomena of aging, the germ-soma conflict theory extends the period of conflict over the complete reproductive period. Moreover, I didn’t figure out how Williams’s “two opposing selective forces” relate to the declining force of natural selection that all ETAs have in common. This is a general fallacy of the ETAs that they refer to selective forces without identifying them (almost as being some higher, metaphysical force) and ignoring that natural selection is an outcome (chapter 3).

Fisher (1930) reasoned that when, as in the case of sexual selection, a strong selective force such as due to female mating preferences is more or less unchecked, it will lead to a runaway process. Such a runaway process is driven by highly asymmetric selective forces and may even result in extinction (Matsuda and Abrams, 1994). Fisher argued that often such a process must soon run against some check by counterselective forces (that are also in the best

interest of females) e.g. as determined by the viability of ornamented males. The shaping of AP as envisaged by Williams (1957) should also involve highly asymmetric selective forces: full blown selective forces on the side of the juvenile organism opposed by declining selective forces on the side of the aging organism. What is the expected outcome of the runaway process ensuing from this asymmetric selective contest? Theoretical life history modelling predicts that semelparity is favored over iteroparity (Cole, 1954). The model of Young (1981) predicted that increasing values of population growth rate and juvenile survival favor semelparity, increasing values of adult survival and age of senescence favor iteroparity. Thus, in a world of unlimited resources allowing unchecked population growth and a selective process favoring juvenile vigor over adult viability, semelparity would prevail. The world would be populated by semelparous organisms. But like in the case of sexual selection, there are counterselective forces (in the best interest of the germline cells) that are determined by the stress resistance and viability of the soma. Only these counterselective forces made somatic postreproductive survival, brood care, transfer of resources and experience and iteroparity evolutionarily feasible.

Integrating genetic models of aging into the genetic theory of adaptation, Moorad and Hall (2009) theoretically predicted that senescence per se reduces the probability that AP alleles arise by mutation. A direct result would be that AP mutations should arise with extremely low frequencies in already senescing populations (in which according to the ETAs natural selection is expected to decline). Hence, a mathematical model indicates that, under the framework of the ETAs, the MA and AP theories of aging should exclude each other. The germ-soma conflict theory provides a contextual framework in which both AP and MA, whose co-existence should be excluded by the Modern Synthesis, can coexist not as cause but as epiphenomena of aging.

Illustration 1 depicts the antagonistic pleiotropic network of signaling pathways. I am no mathematician but I invite any mathematician to calculate the probability that such a intricately tuned antagonistic pleiotropic network could have evolved independently in almost all phyla by chance (since according to the ETAs not enforced by natural selection). Without being a prophet, I predict that such a probability is converging to zero.

14. Germline signals and reproductive aging: late-life mortality plateaus and gender gap of life expectancies

Summary

Late life mortality plateaus occur generally in aging populations. Traditional ETAs fall short in explaining this phenomenon. In the germ-soma conflict theory the mortality plateau of old age is a logical consequence of the reproductive aging-related declining intensity of germline cell signaling. However, resource utilization and stress response signaling pathways continue to advance aging trajectories, although at a reduced rate. The human gender gap of life expectancies is readily explained from the gender dimorphic dynamics of gonadal hormone secretion in old age and their progeroid signaling pathways.

Aging appears to cease at late ages, when mortality rates roughly plateau in large-scale demographic studies. Carey et al. (1992) and Curtsinger et al. (1992) were the first to show the demographic leveling-off of late-life mortality in flies. Since the discovery of this postaging period of life, data from a variety of laboratories have suggested that late-life mortality plateaus occur generally in aging populations (Fukui et al., 1993; Tatar et al., 1993; Brooks et al., 1994; Kannisto et al., 1994; Charlesworth and Partridge, 1997; Vaupel et al., 1998; Drapeau et al. 2000; Mouton et al., 2009). Late-life mortality rates vary widely among species and the extent of this slow down is much more modest in humans than in invertebrates (Martin et al., 1996). But a reliable attribute of late-life is the switch from accelerating mortality to a relatively stable mortality, on average (Rauser et al., 2006). Traditional ETAs fall short in explaining this phenomenon: the postreproductive lifespan should be short because there is no selection against mutations that are not expressed until reproductive activity has ceased (Carey et al., 1992; Curtsinger et al., 1992; 1995; Pletcher and Curtsinger, 1998). The late-life mortality plateau has no presently agreed upon explanation. Two main theories have been offered. The first attempt to explain the apparent cessation of aging was extreme lifelong heterogeneity among groups with respect to frailty. Heterogeneity within aging cohorts may manifest itself such that only extremely robust individuals survive aging. This theory has received several experimental refutations. The second theory is that late-life plateaus in mortality reflect the inevitable late-life plateau in the force of

natural selection (Rose et al., 2006). Deceleration in hazard functions (mortality curves) and the resulting mortality plateaus are an intrinsic property of time-to-event traits that are affected by many underlying genetic and environmental factors. According to Williams (1957) higher extrinsic (environmentally imposed) mortality rates are predicted to result in the evolution of higher rates of intrinsic, or senescent, mortality (Edney and Gill 1968; Promislow 1991; Rose 1991; Stearns 1992). However, for many groups of organisms extrinsic mortality risk is a function of an organism's internal condition and hence susceptibility to such hazards (see chapter 17.1.5). For example, studies of wolf-ungulate predator-prey ecology have consistently demonstrated a condition-dependent component to predation risk, with very old, very young, and debilitated members of the prey species being differentially vulnerable (Fuller and Keith 1980; Bjorge and Gunson 1989; Boyd et al. 1994; Mech et al. 1995). Age-related immunological compromises have also been empirically investigated in both wild and captive populations. Old mice (Ashman et al., 1999) and barn swallows (Møller and de Lope 1999) exhibit the presence of age-related immunity decrements with high parasite loads. Beyond the direct mortality effect of pathogen infestation, higher parasite burdens might also impose additional costs through, for example, diminished foraging ability and/or increased risk of predation (Lafferty and Morris 1996; Bakker et al. 1997). An increase in interactive extrinsic mortality sources can select for slower senescent deterioration early in life but more rapid deterioration late in life (Williams and Day, 2003). Failure to account for these internal condition-dependent components of mortality risks compromizes the validity of the mathematical models of late-life mortality plateaus.

The germ-soma conflict theory provides a plausible biological mechanism for the demographic phenomenon of late-life mortality plateaus: reproductive senescence. Reproductive aging, the decline of late-life fecundity, is under genetic control (Austad et al., 1994) and occurs in yeast (Mortimer and Johnston, 1959; Steinkraus et al., 2008; Steffen et al., 2009), plants (Van Dijk, 2009; Ally et al., 2010), planarians (Balász and Burg, 1974), small freshwater invertebrates (Bell, 1984), nematodes (Hughes et al., 2007; Andux and Ellis, 2008; Tatar, 2010; Luo and Murphy, 2011), gastropods (Fretter and Graham, 1976; Rogers-Bennett et al., 2004), arthropods (David et al., 1975; Moya-Laraño, 2002; Rauser et al., 2006; Zhao et al., 2008; Maklakov et al., 2009; Zajitschek et al., 2009a; b; Carey and Molleman, 2010; Tatar, 2010), fishes (Comfort, 1961; Schreibman and Margolis-Nunno, 1989; Reznick et al., 2004; 2006), birds (Woodard and Abplanalp, 1971; Cherkin and Eckardt, 1977; Ottinger, 2001; Holmes and Ottinger, 2003; Catry et al., 2006; Charmantier et al., 2006; Lewis et al., 2006; McCleery et al., 2008; Reed et al., 2008; Aubry et al., 2009; Bouwhuis et al., 2010; 2012; Rebke et al., 2010; Kim et al., 2011), and mammals (Faddy et al., 1983; Gosden et al., 1983; Nelson and Felicio, 1985; Austad, 1993; VomSaal et al., 1994; Packer et al., 1998; Bérubé et al., 1999; Cohen, 2004; Wu et al., 2005; Beauplet et al., 2006; Dugdale et al., 2011; Bouwhuis et al., 2012) including humans (Austad, 1994; VomSaal et al., 1994; te Velde and Pearson, 2002). As a manifestation of reproductive aging with its deteriorating gamete quality, fitness of later offspring may decline in a variety of taxa (Lansing, 1947; Fleuriet and Vageille, 1982; Cadieu, 1983; Barnes, 1984; Rose 1984; Kerver and Rotman, 1987; Fox, 1993; Kennedy et al., 1994; Mohaghegh et al., 1998; Fox and Czesak, 2000; Hercus and Hoffmann, 2000; Kern et al., 2001; Priest et al., 2002; Tarin et al., 2005; Brommer et al., 2007; Wilson et al., 2007; Descamps et al., 2008; Bouwhuis et al., 2009; Torres et al., 2011).

Cumulative evidence indicates that somatic aging and reproductive aging are coupled in a variety of organisms (Rose and Charlesworth, 1981; Clare and Luckinbill, 1985; Wagner and Harper, 2003; Reznick et al., 2003; 2006; Valenzano et al., 2006; Basolo, 2008; Steinkraus et al., 2008; Ratcliff et al., 2009). Shared signaling pathways may account for this joint regulation. The joint regulation of somatic and reproductive aging by gonadal hormones is discussed in chapter 11. Both IIS (Larsen et al., 1995; Klein and Sauer, 2001; Garigan et al., 2002; Castrillon et al., 2003; Hughes et al., 2007; Shaw et al., 2007; Luo et al., 2010; Tatar, 2010), TOR signaling (Steinkraus et al., 2008; Reddy et al., 2009; Adhikari et al., 2010; LaFever et al., 2010) and the reduced activity of these pathways as accomplished under DR (Holehan and Merry, 1985; McShane and Wise, 1996; Chen et al., 2005; Hughes et al., 2007; Selesniemi et al., 2008; Angelo and Van Gilst, 2009) have been implicated in the antagonistic regulation of reproductive senescence. In humans, insulin resistance was predictive of time to final menstrual period (Sowers et al., 2010). The importance of nutrient sensing for lifespan and late-life aging trajectories was demonstrated by the extension of longevity by the lifelong and late-life administration of rapamycin in mice (Harrison et al., 2009; Anisimov et al., 2011; Miller et al., 2011).

As discussed in chapter 11, aging and death are forced upon the soma by germline signals. Moreover, reproductive aging, the limitation of the reproductive

potential of the organism is also brought about by these signals (see chapter 11.4). Actions of reproduction-related signals are causally involved in reproductive senescence. Reproductive aging is associated with the eventual loss of germline-dependent signaling molecules. The progeroid actions of these signals (as discussed in chapter 11) grind to a halt, resulting in a slowing of the aging process. The late-life mortality plateaus in postreproductive populations thus have a plausible mechanistic explanation. But why do postreproductive organisms then not recover and enjoy virtual immortality? In essence, there are three hierarchically organized somatic aging axes that, in higher taxa, are orchestrated by the neuroendocrine network. The reproduction-related axis is complemented by both the metabolism- and stress-signaling axes. And the latter axes are still functional and fuel, albeit at a reduced rate, the aging progress. Data from C. elegans suggest that the germline daf-16/FOXO-dependent signal may regulate lifespan independently of the upstream IIS pathway (Lin et al. 2001) and that a germline-dependent and a germline-independent IIS affects longevity (Hansen et al., 2005). Hence, in reproductive senescence the metabolic and stress axes are still intact to regulate the innate immune system and promote the expression of NF-KB (Salminen and Kaarniranta, 2010a; b; Salminen et al., 2011), the principal marker of tissue aging (Csiszar et al., 2008; Salminen et al., 2008a; Brink et al., 2009; Ljubuncic and Reznick, 2009; Rovillain et al., 2011). Thus, the IIS- and TOR-related compromise of gerontogenes, autophagic activity and stress response competence (see chapter 10) continue to promote aging trajectories, although at a reduced rate in the absence of germline-mediated progeroid signaling. That the reproductive phase is a self-limited life history trait underscores the self-consistent limitation of reproduction, attenuating the oscillations predicted by the Lotka-Volterra equation and avoiding extinction (Holland, 1995; Mitteldorf, 2006; 2010b) (see chapter 5.4). The germline signaling-effected limitation of the reproductive potential and longevity of the soma are the tribute of the organisms to evolution's long-term selective pressures exerted by limited resources.

The human gender gap of life expectancies, the higher female survival probability in the older age intervals (Guralnik et al., 2000) that stands in contrast to the earlier reproductive senescence of females (Velde et al., 1998) is another enigma that is not explained by the ETAs (Blagosklonny, 2010). The 2002 paper (Heininger, 2002a) introducing the germ-soma conflict theory, already showed that this phenomenon is readily explained from the gender dimorphic dynamics of gonadal hormone secretion in old age and their signaling pathways. The persistence of male gonadal hormone secretion in old age is an important causal factor for the, compared to females, shortened male lifespan. However, there is a complex interplay between progeroid germline signals and individual quality that deserves an in-depth discussion in another paper.

It should be emphasized that in the germ-soma conflict theory the mortality plateau of old age and the gender gap of life expectancy are logical consequences of the sexually dimorphic reproductive aging-related waning of germline cell signaling. In contrast, the ETAs required complex additional assumptions to explain the, in these theories, contra-intuitive existence of these phenomena.

15. Aging and death in modular and unitary organisms

Daring ideas are like chessmen moved forward; they may be beaten, but they may start a winning game.
Johann Wolfgang von Goethe

Summary

Reproduction and aging trajectories differ fundamentally in modular and unitary organisms. In modular organisms the adult body is itself the reproductive unit. Unitary organisms have segregated germline cells that lead to a postmaturational conflict over the future utilization of resources. In consequence, resource management of modular and unitary organisms differs fundamentally. Sessile modular organisms have a territorial competition for resources, while in unitary organisms the competitor from within is the transgenerational resource manager. Sexual reproduction of modular organisms requires the temporal segregation of germline cells which results in an intermediate type of resource management.

15.1 Reproduction and aging in modular and unitary organisms

Organisms may have modular or unitary body plans. Modular organisms are characterized by an indeterminate structure composed of iterated units or modules arrayed at various levels of complexity (such as leaves, twigs, and branches). Examples of modular organisms include plants and many sessile benthic invertebrates. In contrast, the body of unitary

organisms has a determinate structure consisting usually of a strictly defined number of parts (such as legs or wings) established only during embryogenesis. Mobile animals are examples. Unlike that of unitary creatures, the form of a modular organism derives from a characteristic pattern of branching or budding of modules, which may remain attached or become separated to live physiologically independent lives as parts of a clone. Clonal plants can reproduce sexually and vegetatively. Clonality means that a genetic individual (genet) consists of multiple, physiologically autonomous units (ramets) (Harper, 1977). As a result, a single plant may be considered an intermediate entity between an organism and a population. Physiologists and population biologists alike consider the individual plant as a 'population' of ramets and other repeated structures (Harper, 1977; Buss, 1987).

Plants, basal metazoans (sponges and Cnidaria) and basal bilaterians (flatworms) have asexual (using somatic stem cells) and sexual (using germ cells that arise from somatic multi/pluripotent stem cells) reproduction systems (see chapters 6.1.4 and 11.2.3). Plants with modular body plans (almost all of them) colonize space and time using two methods: first, by producing an expanding number of genetically distinct entities (genets) that are true independent individuals and second, by reiterating the body mitotically below or above ground through the production of stolons, runners, tillers, sprouts etc. (ramets). This latter means of colonization is not reproduction of an individual, but rather the expansion of an individual. Modular organisms tend to be sessile or passively mobile and, as genetic individuals, have the capacity for exponential increase in size (Andrews, 1998). The common feature and defining principle of all types of clonality is the asexual, vegetative production of offspring which are genetically identical (or at least extremely similar) to each other and to the parent organism. Clonality is characterized by the fact that offspring individuals are produced from somatic tissue without passing through regular meiotic cell cycles, thereby by-passing sexual recombination of the genetic material (Tuomi and Vuorisalo, 1989; Stuefer et al., 2002). In organisms that reproduce by agametic cloning, the adult body is itself a reproductive unit that increases its fitness as a function of genet size.

Plants and aquatic benthic animals on one hand and mobile animals on the other hand have different aging trajectories. Modular organisms do not necessarily undergo systemic senescence (Larson, 2001; Borges, 2009). In the Amazon forests millennium-old trees exist (Chambers et al., 1998) that may occur with a density of one millenarian in every 200 hectares (Williamson et al.,1999). Elsewhere, living conifer trees have been dated to more than 4000 years (Schulman, 1958; Johnson and Johnson, 1978) with the longevity record for individual trees being held by the non-clonal bristlecone pine Pinus longaeva living to 4900, 4770, and 4713 years, respectively (Currey, 1965; Lanner and Connor, 2001; Flanary and Kletetschka, 2005). On the other hand, familiar claims about the extreme maximum age of organisms (Wherry, 1972; Vasek, 1980) are mostly based on confusion of the lifespan of the ramet vs. the lifespan of the genet that produced it. For example, the exclusively asexual triploid clonal shrub Lomatia tasmanica (Proteaceae) has been dated to 43,600 years (Lynch et al 1998) with each individual ramet in the clone probably living for about 300 years. Some plants such as Larrea tridentata (creosote bush) (Vasek, 1980) or Gaylussacia brachycerium (huckleberry) (Wherry, 1972) with their >11,000 year old genets expand by the radial production of ramets that live for a mere 10-50 years. Some genets of benthic animals like sponges and Hydra are thought to be immortal (Forrest, 1963; Müller, 1996; Martinez, 1998; Schröder et al., 2003). If one allows for genet age (rather than ramet age) to be the criterion upon which we judge longevity, the longest-lived are probably some cyanobacteria, algae or fungi that evolved hundreds of millions of years ago and are still alive today as species that have expanded mitotically for this entire period (Larson, 2001).

Whereas genet senescence and death are coincident with shoot module death in semelparous plants, there is no evident relation between them in iteroparous plants. The lifespan of the genet reflects the birth and death rates of its modules and both aclonal and clonal plants that are iteroparous may achieve considerable longevity. The longevity of aclonal plants often seems to be restricted by the accumulation of dead material and the problems of being large. Clonal plants are, in contrast, potentially immortal. It is questionable whether the genets of iteroparous plants show senescence as defined for unitary organisms since there is no separation of germ plasm from soma and since apical meristems do not appear to senesce. Insofar as they retain the capacity for rejuvenescence from apical meristems, genets of modular organisms do not senesce; it is only the constituent organs that show senescence, death and decay (Watkinson and White, 1986). However, according to Dawkins (1982) the ramets, the units of clonal growth, are the individuals because they are the products of developmental cycles and so are potential sources of evolutionarily consequential, selectable variation. Plants can reproduce both sexually and asexually. On land, plant populations tend to be established by

sexual propagules and expand by asexual reproduction while in water the reverse pattern seems to be true since vegetative parts of aquatic plants are much more easily dispersed (Silvertown, 2008). Populations of sponges from a heterogeneous environment and under the influence of a strong upwelling had little clonality (7%), whereas the populations in a more homogeneous and temporally stable environment, had a five-fold larger (39%) proportion of asexually derived individuals (Zilberberg et al., 2006). Thus stressful, unstable environments appear to enhance sexual reproduction, a pattern that has generally been observed in organisms able to reproduce both sexually and asexually (Heininger, 2001). The rate of sexual reproduction determines whether a particular clone experiences senescence, so that high rates of clonal reproduction could slow down senescence (Gardner and Mangel, 1997; Laberge et al., 2000; Tanner, 2001).

15.2 Germline segregation and germ-soma conflict

Two basic factors appear to shape the evolutionary link between reproduction and the aging/mortality phenotype of an organism: (i) territoriality of the organism and (ii) germline segregation.

It has long been known that Weismann's doctrine (1892) of the separation of soma and germ is invalid in plants and sessile animals. Although plant germ cells ordinarily are produced from undifferentiated cell lineages, these cells are not set aside as in the sex gonads of many mammals (Babcock and Clausen, 1927). Many organisms such as plants, fungi and “lower” animals do not have a specialized germline (Buss, 1983). It has been shown (Simpson, 2012) that an early division of reproductive labor is essential for the evolution of a more complex functional differentiation in multicellular organisms. Separation of germline and somatic cells occurs early in development of most animals including insects, roundworms and vertebrates. In the entire plant kingdom and in several animal phyla including Porifera, Cnidaria, flatworms and tunicates, a clear division of somatic and germline cells is not established. Plant germ stem cells do not differentiate from a pool of primordial germ cells, but rather arise de novo from sub-epidermal somatic cells, the meristems. Moreover, most plant somatic cells can be isolated and give rise to a new individual organism (Buss, 1987; Chasan and Walbot, 1993). All the meristems of semelparous plants are involved in or die at reproduction and as a consequence death of the genet follows reproduction. For iteroparous plants, however, there are fundamental differences between the reproductive schedules of plants with a single shoot module and those with many shoot modules. The former demonstrate a relatively constant rate of reproduction from year to year following maturity whereas the latter show a continual increase in fecundity with size and age. The reproductive schedules of clonal plants are further characterized in relation to the allocation of meristems to either growth or reproduction (Watkinson and White, 1986).

Evolutionary conflict can occur at many levels (Rice and Chippindale, 2001). At the interspecific level, conflict may arise during the Red Queen process of antagonistic coevolution between a species and its enemies, parasites or competitors (Van Valen, 1973b; Jaenike, 1978; Hamilton, 1980). At the intraspecific level there are several major types of evolutionary conflict. Intraspecific competition and kin competition are key processes shaping the evolution of dispersal (Lambin et al., 2001), promoting niche variation (Svanbäck and Bolnick, 2007), may drive speciation (Rosenzweig, 1978; Dieckmann et al., 2004; Rice et al., 2005), but may also give rise to cannibalistic interactions (Polis, 1981; Elgar and Crespi, 1992; Alabi et al., 2009). Sexual conflict occurs when characteristics that enhance the fitness components of one sex reduce the fitness of the other sex. Numerous examples of sexual conflict resulting from the costs of mating, polyspermy, and sensory exploitation have been discussed in detail (Parker, 1979; Arnqvist and Rowe, 1995; Chapman and Partridge, 1996; Stockley, 1997; Howard et al., 1998; Partridge and Hurst, 1998; Holland and Rice, 1998; Parker and Partridge, 1998; Civetta and Clark, 2000; Stutt and Siva-Jothy, 2001; Knowles and Markow, 2001; Gavrilets and Waxman, 2002; Chapman et al., 2003; Wedell et al., 2006). Intergenomic conflict involves genes, generally located at different loci, that mediate contests (e.g. competition) between different individuals (Trivers, 1974; Parker, 1979; West-Eberhard, 1984; Rice and Holland, 1997). For example, seminal fluid proteins are known to increase a male's fertilization success although simultaneously reducing his mate's fitness by increasing her mortality (Chapman et al., 1995; Rice, 1996; Holland and Rice, 1998). When a new mutation evolves at a seminal fluid locus that increases male fitness at the expense of his mate, this selects for counter-adaptation by females at a second locus (e.g. a locus coding for a female receptor that interacts with the seminal fluid protein), which can lead to self-reinforcing, open-ended cycles of interlocus contest evolution (Rice and Holland, 1997). Intersexual ontogenetic conflict arises when alleles are expressed in both sexes but are selected diametrically between the sexes (i.e. sexually antagonistic alleles) (Rice and Chippindale, 2001). Intragenomic conflict

occurs between genes located in the same individual, such as ultraselfish nuclear genes or cytoplasmic genes that increase in frequency at the expense of other, nonallelic genes. For example, some cytoplasmic genes that are only transmitted through the matrilineage will feminize, sterilize or kill sons to promote reproduction through daughters (Hurst, 1992; Werren and Beukeboom, 1998).

A further type of intraindividual conflict is the germ-soma conflict. Various, but not all, features of this conflict have been recognized previously:

Studies in unicellular, facultatively multicellular, and basal metazoan taxa allow to estimate the conflict potential between closely related conspecifics about access to the germline. This type of conflict was demonstrated in chimeras of Myxobacteria (Buss, 1982; Pál and Papp, 2000; Velicer et al., 2000; Velicer and Vos, 2009; Vos and Velicer, 2009; Kraemer and Velicer, 2011), Dictyostelium (Buss, 1982; 1999; Ennis et al., 2000; Pál and Papp, 2000; Strassmann et al., 2000; Castillo et al., 2005; Khare and Shaulsky, 2010; Strassmann and Queller, 2011a) and B. schlosseri (Pancer et al., 1995; Stoner et al., 1999; Rinkevich et al., 2004; Rinkevich and Yankelevich, 2004; Rinkevich, 2005).

The conflict over resource allocation during the soma's lifetime. Two theories, the DST and parent-offspring conflict theory have taken this type of conflict into account.

The transgenerational aspect of the germ-soma conflict, however, that shaped the evolutionary rationale of somatic aging and death has not been recognized so far by the scientic community (but see Heininger, 2002a).

There are a variety of examples that show how evolution has selected for adaptive solutions to prevent the invasion of selfish individuals in animal and plant populations that may erode the limited collective resources (Leigh, 1977; 1991; Frank, 1995; Gersani et al. 2001; Falster and Westoby, 2003; Foster, 2004; Wenseleers and Ratnieks, 2004; Rankin and López-Sepulcre, 2005; Kerr et al. 2006; Rankin and Kokko, 2006). Factors such as high relatedness in social groups (Wenseleers and Ratnieks, 2004), diminishing returns (Foster, 2004), policing and repression of competition (Frank, 1995; 1996; Hartmann et al., 2003; Ratnieks and Wenseleers, 2005), pleiotropy (Foster et al., 2004) or control of population density (Suzuki and Akiyama, 2005; Hauert et al., 2006; Kokko and Rankin, 2006; Rankin, 2007; Frank, 2010) may all act to constrain the evolution of harmful traits, and thus reduce the potential for a tragedy of the commons to arise in such populations.

Cumulative evidences for the transgenerational germ-soma conflict are: (1) the phylogenetic legacy of life and death decisions (associated with germ-like spores and soma-like mother cells or fruiting “bodies”) related to reproductive events, rooted in metabolic stress responses (chapter 6); (2) the life history trade-offs between reproduction and somatic survival, most drastically demonstrated by semelparous organisms (chapter 8); (3) the adverse effects of germline signaling on somatic longevity (chapter 11); (3) the pleiotropy of signaling pathways monitoring resource utilization and mediating stress competence that antagonistically control reproductive success and somatic survival (chapter 13). Circumstantial evidence for the relevance of the germ-soma conflict that will need further elaboration comes from the Big Bang evolution of the Cambrian explosion and the evolutionary success of sexual reproduction (Heininger, in preparation).

Why did the transgenerational aspects of the germ-soma conflict escape the attention of biologists so far? I think this lack of recognition has several reasons. The presence of the germ-soma conflict can be best inferred and recognized from its signature in aging and postreproductive death. This recognition, however, was hampered by the flawed ETAs that suspected that the phenotypic manifestations of the germ-soma conflict were due to group selection effects. The lack of an integrated ecological-evolutionary approach may have contributed to this oversight. Further, as has been shown for sexual selection, the dynamics of the antagonistic coevolution may obscure the conflict itself (Rowe and Day, 2006). Moreover, the germ-soma conflict is not evident throughout the entire life. Obviously it does not play a role before reproductive maturation. Until maturity the interests of germline cells and soma are congruent: survive, grow, mature; a conflict over resources should not arise. With germ cell maturation things change. Then, an ontogenetic switch is flipped. It is suggested that DNA-methylation-dependent gene silencing in the germline cells (De Smet et al., 1999; Miranda and Jones, 2007) is the mechanisms by which the soma-germ conflict is delayed post maturationally. Interaction of DNA/histone methylation (Grishok, 2005; Law and Jacobsen, 2010) with heterochromatic silencing engaging the RNA interference machinery (Lippman and Martienssen, 2004; Pal-Bhadra et al., 2004; Saxe and Lin, 2011) together with Argonaute and Piwi-like proteins (Hutvagner and Simard, 2008; Saxe and Lin, 2011) introduces an additional level of complexity. During maturation mammalian germline cells undergo waves of demethylation and remethylation (Hajkova et al., 2002; 2008) and it is posited that during maturational epigenetic

reprogramming (Reik et al., 2001; Hajkova et al., 2002; 2008) the germ-soma conflict is programmed. For instance gametogenesis has to be de-repressed to take up reproductive activity after maturation (Casper and Van Doren, 2009; Hashiyama et al., 2011). Intriguingly, estradiol is able to decrease the binding activity of a repressor to methylated DNA and convert a specific gene from silent heterochromatin to active euchromatin (Jost et al., 1991; Jost and Saluz, 1993).

Decreasing IIS activity during C. elegans adulthood, particularly during the reproductive period, is required to extend lifespan, with IIS in the pre-adult, developmental period playing no role with regard to adult lifespan (Dillin et al., 2002; Piper et al., 2008). Accordingly, juvenile-only dietary restriction impairs reproductive activity but has no impact on adults (Tu and Tatar, 2003). Adult-specific and, particularly reproductive-period-specific, overexpression of dFOXO in flies was also demonstrated as effective to extend lifespan (Giannakou et al., 2004; 2007; Hwangbo et al., 2004), as was adult-specific overexpression of dPTEN (Hwangbo et al., 2004) as well as ablation of insulin producing neurons (median neurosecretory cells or mNSCs) in the last stage of preadult development (Broughton et al., 2005). Non-aging during larval developmental arrest in a variety of taxa is compatible with the assumption that programmed aging is only initiated after reproductive maturation (Miller and Hadfield, 1990; Tatar and Yin, 2001; Chen et al., 2007; Curran and Ruvkun, 2007; Strathmann and Strathmann, 2007). In sterile worker honey bees, exposure to a pheromone secreted by larval brood has a positive effect on lifespan-extending vitellogenin stores in 3–4-day-old bees and a negative effect in later life (Smedal et al., 2009). This is an intriguing example how the germ-soma (brood/worker bee) conflict may operate life-phase-selectively in the superorganism of a eusocial hymenoptera colony. Reproductive maturation affects a variety of neuroendocrinological axes (Döcke et al., 1981; Kawagoe and Hiroi, 1983; Belgorosky et al., 2009; Evuarherhe et al., 2009; Roa et al., 2010; Campbell, 2011). In C. elegans, germline proliferation produces a DAF-16 and KRI-1 mediated signal that negatively regulates lifespan while the somatic gonad promotes lifespan extension (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002; Berman and Kenyon, 2006). The regulation of lifespan by IIS is operative during adulthood (Dillin et al., 2002). Most importantly, puberty is associated with an increased insulin resistance (Amiel et al., 1986; Bloch and Clemons, 1987; Caprio et al., 1989; Caprio and Tamborlane, 1994; Travers et al., 1995; Moran et al., 1999; Goran and Gower, 2001; Roa et al., 2010) that may mark the activation of the germ-soma conflict over resources. The manifestation of the germ-soma conflict after maturity should, according to chapter 12.3, accelerate postmaturational coevolutionary dynamics. In fact, it has been shown in both C.elegans and Drosophila that genes expressed predominantly after reproductive maturity evolve more rapidly than genes expressed earlier in development (Cutter and Ward, 2005; Davis et al., 2005).

Thus, germline segregation and mode of reproduction are central to the evolutionary modulation of somatic aging and death in unitary and modular organisms. From chapters 15.1 and 15.2 the following general interrelationships can be inferred:

The developmental specification of germline cells in unitary organisms by either preformation or epigenesis (Extavour and Akam, 2003) determines the aging and mortality of the soma, irrespective of an asexual or sexual mode of reproduction. Examples for this trade-off lifestyle are birds or mammals for sexual, and rotifers for asexual organisms.

In modular organisms clonal reproduction may, but must not, lead to immortality at the genet level (Martinez and Levinton, 1992; Martinez, 1998). The population dynamics of clonal organisms is highly variable, and often characterized by the births and deaths of asexually produced ramets and high genet longevity (McFadden, 1991). Despite high rates of ramet turnover, ramet population densities may remain relatively stable over time. Crowding is known to be the signal for initiation of sexuality and diapause in a variety of clonally reproducing animals and plants (Gilbert, 2004). Sexual reproduction causes senescence as seen in Hydra (Tardent, 1974; Yoshida et al., 2006).

15.3 Modular and unitary organisms and their resource management

Long-lived species are fitter and more competitive, e.g. efficiently can avoid death due to predation or other environmental hazards thanks to evolutionary achievements such as wings (birds), shells (turtles), large body size (whales, elephants), homeothermy (birds, mammals), and large brain size that facilitates learning and problem solving (primates). Obviously, evolution of ever fitter organisms is inherent to evolutionary processes. But why should the cellular and organismal processes that confer longevity be limited (Mitteldorf, 2010a)? If evolution would have “trusted”, as the ETAs suggest, in the lifespan-limiting and resource-managing power of predation, infection and accidents, the planet, sooner or later, would be populated exclusively by Darwinian demon(s), huge, winged, armored, intelligent and finally immortal

organisms, excelling at resource acquisition and claiming all available resources. And finally, being forced to cannibalize themselves. Coevolutionary dynamics and mortality, both ensured by competition for limited resources are the evolutionary constraints to the rise of Darwinian demons.

Resource availability is variable both spatially and temporally. Coordination of resource management and reproductive activity are central to the evolutionary fate of organisms. Since reproduction leads to the generation of new organisms, control over the fate of the parent(s) was an evolutionary necessity in a world of limited resources. Territoriality of modular organisms and germline segregation of unitary organisms set the stage for fundamentally different resource management systems. I think, it is no coincidence, but has its adaptive rationale that immobile plants and benthic animals that cannot evade their local resource competition did not evolve segregated germline cells. They are bound in their coevolutionary web that keeps them from evolving as Darwinian demons. In contrast, mobile animals that can disperse and migrate evolved segregated germline cells and a germ-soma conflict. Thus, even when dispersing they take their "inner" competitor with them that keeps them from evolving as Darwinian demons.

15.3.1 Territorial resource management in modular organisms

Territorial modular organisms (Schenk et al., 1999; Trewavas, 2005) tend to compete with the other organisms of their community essentially for the same resources, i.e. space, light, water, and nutrients. Unable to evade the local competition, modular organisms are forced to have a territorial resource budget management. The territoriality of organisms subjects both reproduction and postreproductive survival to the resource-driven selection pressures at the plant community level (Goldberg et al., 2001). This includes an intense competition for light, space and food both inter- and intraspecifically (Grover, 1997; Schenk, 2006; Golubski et al., 2008; Novoplansky, 2009; Keuskamp et al., 2010) and inter- and intragenerationally (Rinkevich and Weissman, 1987; Williams and Briske, 1991; Huber et al., 2004). Importantly, space (which determines access to light and nutrients) is such a limited asset and competitive resource that it "allowed" evolution to ease the selective pressure of reproduction and rather put emphasis on space occupancy (Deussen et al., 1998; Prusinkiewicz, 2000; Casper et al., 2003; Deussen and Lintermann, 2005; Berger et al., 2008). Therefore modular organisms, in comparison to mobile animals, have a more loose coupling of reproduction and longevity: recruitment from the seed bank is highly dependent (at least in the wild) on space availability and the competitive properties of organisms. Following natural disturbance such as wildfire or windfalls, regrowth is viewed as largely a population process wherein four relatively distinct phases can be recognized: the establishment phase, the thinning phase (see chapter 5.3), the transition phase and the steady-state phase (Peet and Christensen, 1987). Means of contest are physical and allelochemical arms (see chapter 5.3) but also cooperation and alliances (Denison et al., 2003; Magori et al., 2003; Stuefer et al., 2004; Dudley and File, 2007). Thus, apart from environmental stressors limiting individual growth, competition for resources controls reproduction, recruitment (Eriksson and Ehrlén, 1992; Callaway and Walker, 1997; Hunt and Scheibling, 1997; Turnbull et al., 2000; Miriti et al., 2001; Rees et el., 2001; Silvertown et al., 2002; Marshall and Keough 2003; Tilman, 2004; Fréville and Silvertown, 2005; Marshall et al., 2006), senescence and death in natural plant and benthic aquatic animal communities (Strauss and Irwin, 2004; Morris et al., 2007).

Spatial pattern analyses suggest that while competition is often playing a significant role in tree mortality processes in old-growth stands (the steady-state phase), there is a substantial presence of biotic and abiotic mortality agents including insects and pathogens (Haack and Byler,1993; Slaughter and Parmeter, 1995; Garbelotto et al., 1997; Hansen and Goheen, 2000; Dobbertin et al., 2001; Wood et al., 2003; Rademacher et al., 2004; Rigg, 2005; Das et al., 2008; 2011). The impact of various factors that shape competition- and age-dependent vulnerability to biotic and abiotic factors, however, are difficult to decipher (Franklin et al., 1987; McDowell et al., 2008; Das et al., 2011).

15.3.2 Germline-controlled resource management in unitary organisms

The mobility of animals freed organisms from the confines of local competitive communities and made resource exploitation through dispersal and migration (e.g. in birds) a feasible alternative. Hence, territorial resource management plays a minor role in bilaterian taxa, compared to sessile organisms, but left its signature in body size-density scaling (see chapter 5.3). Overall, however, to save mobile animals from the tragedy of commons and ensure their evolutionary success, natural selection "had to replace" spatial selection pressure by an intergenerationally acting force of selection. Surely, it is no whim of nature that with the advent of large scale animal mobility,

segregated germ cells replaced the territorial resource management by an intergenerational resource management. Thus, in mobile animals, aging and mortality as transgenerational resource managers, constraining the evolution of a Darwininian demon and the tragedy of commons, are enforced by intraindividual germline signals (see chapter 11). Evolvability of these unitary organisms was tremendously accelerated by the Red Queen dynamics of the germ-soma conflict resulting in various organs with a sophisticated division of labor (Smith, 2012).

15.3.3 Intermediate resource management solutions

There are organisms following intermediate resource management strategies depending on the ecological conditions. As first prerequisite to these hybrid strategies, these organisms have no germline cells that are set aside during development. Hence, the adult organisms described in this chapter have toti-/pluripotent stem cells and almost all of them are sessile plants or animals. Intermediate solutions to the alternative territorial or germline control of resources are found in basic metazoans. Particularly intriguing in this respect are the planarians. Some of the species are mobile, reproduce asexually by fragmentation, and are immortal (sexually reproducing planarians have short lifespans, see chapter 6.1.4). Is this the dreaded Darwinian demon, exploiting all available resources and inundating the world with its organisms? Obviously not. Apart from being a lower-level member of the food chain (Davies and Reynoldson, 1969; Wright, 1975), some triclads may exert cannibalism as density-regulatory behavior when crowding is high or resources are low (Hull, 1947; Froehlich, 1955; Armstrong, 1964; Hartry et al., 1964; Davies and Reynoldson, 1969; Davison, 1973). Moreover, the organisms display an intriguing resource management system saving them from excessive feast and famine cycling: under increased resource availability asexually reproducing planarians show decreased fecundity and become obese (Davison, 1973; Sheiman et al., 2006; Dunkel et al., 2011). Some Cnidaria, like the Hydra, as sessile polyps have pluripotent stem cells that form asexual buds and their genets are immortal (Forrest, 1963; Müller, 1996; Martinez, 1998). When reproducing sexually, however, the polyps display segregated germline cells and have a finite lifespan (Brien, 1966; Tardent, 1974; Yoshida et al., 2006). Their mobile life forms, the medusae, reproduce sexually and are reported to die soon after spawning (Spangenberg, 1965; Yasuda, 1969; Hamner and Jenssen, 1974; Miyake et al., 1997; Lucas, 2001; Watanabe et al., 2009; Ojimi and Hidaka, 2010). Exposed to environmental stressors some medusae, before having reproduced sexually, may revert to polyps and resume asexual reproduction (see chapter 6.1.4).

Intriguingly, C. elegans longevity mutants exhibit a soma-to-germline transformation (mimicking, at least in part, the somatic stem cell phenotype encountered in basal animal phyla e.g. Porifera, Cnidaria) that contributes to their enhanced survival. The soma-to-germline transformation likely plays a role both during the pre-reproductive dauer stage, where C. elegans can survive for months, and during the post-reproductive adulthood to promote longevity (Curran et al., 2009).

Many plants are able to reproduce both asexually and sexually. The ability to reproduce asexually is widespread in plants, but less than 1% of the approximately 250,000 angiosperm species are thought to be substantially asexual (Asker and Jerling 1992; Whitton et al. 2008). Clonal growth of plants implies the asexual, vegetative production of genetically identical, potentially independent offspring individuals. Dedifferentiation of somatic cells in localized regions, the meristems (Nagata et al., 1994; Sugiyama, 1999), leads to clonal growth and potential immortalization. Most clonal plants are also capable of sexual reproduction by flowering and seed set. The relative importance of clonal growth vs. sexual reproduction, and the nature of internal and environmental controls on this balance remains rather unclear (Fagerström, 1992; Verburg, 1998). On the other hand, mounting evidence suggests the ecological control of these decisions and their life history consequences. Several studies have shown that within and between closely related species the importance of clonal and sexual reproduction can vary among habitats (Turkington, 1985; Sutherland and Vickery, 1988; Stöcklin, 1992, 1999; Eckert and Barrett, 1993; Krahulec, 1994) as a result of selection pressure on genetic trade–offs exerted by biotic and abiotic factors in contrasting habitats (Prati and Schmid, 2000). Moreover, in plants and benthic aquatic animals there is relatively greater allocation to sexual than to asexual reproduction at high density (competitive stress) (Harvell and Grosberg, 1988; van Kleunen et al., 2001) and environmental challenges (Romme et al., 1997). Gardner and Mangel (1997) have shown that the rate of sexual reproduction determines whether a particular clone experiences senescence, so that low rates may delay senescence. Experimental results with freshwater invertebrates and plants support this prediction (Bell, 1984; Laberge et al., 2000).

For 65 species of iteroparous perennial plants, a positive relationship was found between rate of senescence and reproductive lifespan, suggesting increasing risk of death with successive reproductive events and a positive correlation between age at first reproduction and mean reproductive lifespan. Clones that fragment are more likely to escape the evolution of senescence at the genet level than clones that remain physiologically integrated (Silvertown et al., 2001). The central role of meristems for the postreproductive survival of plants is illustrated in monocarpic/polycarpic plants. Monocarpic plants like Arabidopsis thaliana spend all their shoot apical meristems in a single big bang reproductive effort. A key feature of polycarpy is to maintain a supply of meristems that are capable of vegetative growth, i.e. shoot apical meristems that sustain growth of the plant in future growth cycles (Woolhouse, 1983; Amasino, 2009; Wang et al., 2009). Arabidopsis thaliana having mutations in both the soc1 and ful genes switched from sexual to vegetative reproduction and did not die after seed maturation like wild-type and single mutant plants but developed from a grass with a single basal rosette of small leaves to a highly branched shrub with many aerial rosettes. In all double mutants, the apical rosettes resulting from inflorescence meristem reversions, the aerial rosettes and the stems remained alive. Secondary growth appeared, being mediated by cambial activity absent from the wild type and formed a considerable wood cylinder (Melzer et al., 2008).

For sedentary plants and animals, the mode of resource management is territorial. However, modulation of this territorial resource management depends on the longevity of organisms. Long-lived organisms (e.g. trees, benthic animals) are subject to a territorial resource management that is characterized by strong interindividual competition for resources (see chapter 5.3). Long-lived herbaceous plants occur in environments where not only competition for resources is strong (e.g., mature forests), but also where few safe-sites for recruitment are available (e.g., rocky places). High survival of established individuals translates into a reduced dependence from fecundity and thus a higher probability of local persistence regardless of environmental variability (García et al., 2008). Longevity is an important life history trait for the persistence of populations at competitive, harsh or extreme environments or under frequent long periods of adverse conditions (Eriksson, 1996; Larson et al., 1999; García and Zamora, 2003; Forbis and Doak, 2004; von Arx et al., 2006). A long lifespan would therefore be a good strategy to avoid local extinctions caused by scarce and unpredictable recruitment as well as large population fluctuations (García et al., 2008). In short-lived gymnosperm and angiosperm plants, germline-dependent signals become more important as resource manager. There is a continuum of life history variation with, at one end, long-lived trees with little reproductive costs (relative to their biomass) and predominant territorial resource management and, at the other end, monocarpic annual plants with high reproductive investment (relative to their biomass) and lower territorial competition (particularly in agricultural systems with fertilizer use). Tree species with attributes typical of high capture and low conservation of resources, small seeds and short stature had faster population turnover rates (short longevity, and high mortality and recruitment rates) than species with opposite attributes (Easdale and Healey, 2009). In the wild, annual plant species may have strongly fluctuating population sizes due to poor survival or seed set and, to avoid extinction, have to rely on a persistent seed bank. A seed bank has the effect of overlapping the generations of the population integrating the effects of selection over long periods of time (Templeton and Levin, 1979; Eriksson, 1996; Ehrlén and Lehtilä, 2002; Nunney, 2002). When a population goes through a genetic bottleneck, recruitment of below-ground genotypes conserved in the soil can quickly restore above-ground genetic diversity as soon as the habitat conditions become suitable again (McCue and Holtsford 1998, Uesugi et al. 2007, Honnay et al., 2008). In terms of resource management, this evolutionary strategy is reminiscent of spore forming, bet-hedging organisms (Evans and Dennehy, 2005; Lennon and Jones, 2011).

16. Another gedankenexperiment

Summary

Basal metazoan organisms demonstrate that immortality and reproductive activity are no mutually exclusive phenomena. At this phylogenetic level the divergence of modular and unitary bauplans, sessile and mobile lifestyles, and fundamentally different life history strategies occurred. Competition for limited resources is the essence of the struggle for life and coevolutionary dynamics are the evolutionary conductor's baton to its rhythm. It can be assumed that through all evolutionary ages cheating organisms tried to play their own tune. The incentives to cheat should have been particularly attractive during the transition from modular to unitary organisms: evading the crippling territorial competition for

resources with a mobile lifestyle may have allowed to reap the benefits of both immortality and unrestrained reproduction due to the acquisition of sheer unlimited resources. With the hindsight of some 600 million years of evolution we can take stock: all the attempts to trick evolution failed. Only those organisms averted extinction that "modestly accepted" to carry their competitor for resources, their germ cells, within them constraining the evolution of Darwinian demons.

I would like to conceive another gedankenexperiment to elaborate on the interdependences of reproduction, mortality and mobility of organisms. Please follow me far back in evolutionary time to the Precambrian/Cambrian time when the divergence of Diploblasts and Triploblasts occurred. Paleontological and molecular data indicate that most bilaterian phyla appeared and diversified during the Cambrian explosion (Conway-Morris, 1993; 1998; Philippe et al., 2000). It is thought that during this time the evolutionary split between Cnidaria and Bilateria took place. Cnidaria and basal bilaterian Platyhelminthes have no segregated germline but somatic totipotent stem cells that can become germline cells. Imagine an urcnidarian or urbilaterian organism. Like modern Hydrozoa, the urcnidarian organisms can be considered to have been sessile and immortal (Forrest, 1963; Müller, 1996; Martinez, 1998). Sessile organisms are subject to a tight competitive pressure in their territorial community that restricts their reproductive activity and success (see chapter 5.3). Becoming mobile allowing active foraging to exploit patchy resources instead of to sit-and-wait greatly enhanced the fitness of these organisms, particularly in the superoligotrophic environment of the Cambrium (Martin, 1996). Cheaters (mutants that selfishly exploit all available resources leading into the tragedy of the commons) are widespread in unicellular and facultatively multicellular organisms (Hilson et al., 1994; Dao et al., 2000; Pál and Papp, 2000; Strassmann et al., 2000; Velicer et al., 2000; 2002; Ennis et al., 2003; Fiegna and Velicer, 2003; Rainey and Rainey, 2003; Castillo et al., 2005; Rankin et al., 2007a; Kuzdzal-Fick et al., 2011). Obviously, the cheaters in these organisms are easily obtained by simple one-step mutations. There can be little doubt that cheaters that, at short term, can exploit and outcompete cooperative genotypes, tried their evolutionary fate repeatedly.

Potential immortality, indefinite reproduction and mobility are not mutually exclusive as Hydrozoa and Platyhelminthes demonstrate (Sonneborn, 1930; Nuttycombe and Waters, 1938; Martinez, 1998) and can be conceived as promising starting point for the evolution of a Darwinian demon. While the mobile Platyhelminthes combine somatic totipotent stem cells and immortality, Hydrozoa evolved a mobile but mortal adult life stage, the medusa, with segregated germ cells that, however, can return repeatedly to immortal sessile forms by transdifferentiation cycles (see chapter 6.1.4). Resource competition has its fitness costs at the individual level (see chapter 5.5). Increased interspecific and intraspecific competition may slow down rates of adaptation substantially and fundamentally change patterns of adaptation to long-term environmental changes (Johansson, 2008; Urban et al., 2012). According to Bolnick (2004), density and frequency dependence of fitness results in a dynamic landscape: a fitness ''sphagnum bog'' (Rosenzweig, 1978). A key driver of frequency-dependent fitness is intraspecific competition (Milinski and Parker, 1991; Doebeli and Dieckmann, 2000). The fitness landscape shifts between stabilizing and directional selection at low density to disruptive selection at high density (Svanbäck and Persson, 2009). If the fitness of each phenotype depends on its frequency in the population — that is, if fitness is frequency dependent — then the fitness landscape will be dynamic and the mean phenotype will be kept in a fitness valley, allowing for persistent disruptive selection. Strong competition between similar phenotypes (e.g. parents and offspring) can disproportionately affect the most abundant (mean) phenotype (the fictitious one that has not been able to eliminate the parents) even though it may be adapted to the most abundant resource. Rarer consumer phenotypes (in our example the one that has been able to eliminate the parents) may have fewer resources available, but also have fewer competitors with which to share those resources, so their overall fitness is relatively high. The population is thus subject to disruptive selection; that is, its mean, abundant phenotype is at or near a minimum on the fitness landscape (Dieckmann and Doebeli, 1999; Doebeli and Dieckmann, 2000; Martin and Pfennig, 2009). Disruptive selection can be stable when fitness is negatively frequency dependent and this stability implies that disruptive selection could in fact be fairly common (Kingsolver et al. 2001; Bolnick, 2004; Martin and Pfennig, 2009). Competitive interactions may amplify changes in mean population sizes due to environmental changes and thereby increase extinction risks (Johansson, 2008). In competitive coevolution, evolution proceeds towards a so-called evolutionary branching point, where selection becomes disruptive and splits the population into two strategies. Coevolution of these strategies eventually leads to the extinction of one of them (Kisdi et al.,

2001). Disruptive selection due to frequency-dependent fitness may not only be the causal agent in the evolution of ecological variation and speciation but may equally have been operative in the selection of postreproductive aging and death.

Some 600 million years after the first animals became mobile, the potential ancient cheater phenotypes have not persisted. We only can speculate by how many "Malthusian catastrophes" evolution coerced taxa into obeying the "tragedy of the commons" principle (see chapter 5.4). Planarians, for instance, show decreased fecundity and become obese under increased resource availability (Davison, 1973; Sheiman et al., 2006; Dunkel et al., 2011). And, although medusae of several species have the ability to transdifferentiate into polyp structures before or even after initiation of processes of sex-cell determination (Boero et al. 2002; Piraino et al. 2004), this potential is lost after spawning (Piraino et al. 2004). Moreover, only those mobile organisms made it through evolutionary times that evolved a dedicated germline that "individualized" and "internalized" the community-level competition for resources of their sessile ancestors thereby curtailing the reproductive potential and longevity of their breeder "host".

By the way, the analogy between the early metazoan cheater phenotypes and human overexploitation of the planet is not fortuitous: seemingly unlimited resources made accessible by evolutionary or technological innovations, unrestrained reproduction, depletion of the resources, …. But after all this is only a fictitious gedankenexperiment that must not have occurred…However, similar recent scenarios that have occurred in human societies have been described by Jared Diamond (2005).

17. Aging is selected for, adaptive, and programmed

There must be no barriers for freedom of inquiry. There is no place for dogma in science. The scientist is free, and must be free to ask any question, to doubt any assertion, to seek for any evidence, to correct any errors. -J. Robert Oppenheimer, The Open Mind, p. 114 (1955)

Nevertheless, the idea of 'programmed' ageing, with its implicit (or occasionally explicit) reliance on an adaptive role, reappears regularly and is commonly expressed by newcomers to the field. -Kirkwood and Melov (2011)

Summary

Postreproductive organisms are a selective force, affecting the fitness of the progeny both positively and negatively. Thus, evolution could not be indifferent towards the postreproductive organisms. The pervasive genetic control of aging by phylogenetically conserved public mechanisms indicates the action of strong selective forces. Evolution co-selected reproduction and aging/death linking them in a huge pleiotropically organized network. Data from a recent genomewide association study of human longevity with quantitative trait loci from mice shows concordance between humans and mice lifespan peaks with a very low probability that this is due to chance. Linkage disequilibrium is a tool to detect signatures of natural selection and has been observed in Drosophila and humans for genes linked to longevity. Genes that are no longer under selective pressure degenerate or are fossilized to pseudogenes. On the other hand, the phylogenetic maintenance of coding sequences and functionality of signaling pathways such as IIS and TOR is the signature of natural selection. Life history strategies including reproductive and aging trajectories are adaptive ascertaining that long-lived mutants in a variety of model systems pay high costs in terms of fitness in their natural habitat. As predicted by classical theory traits closely associated with fitness have low heritabilities. Thus the low heritability of aging is further evidence for its adaptive value. Developmental events and aging-related events are programmed by the same, phylogenetically conserved signaling pathways. Moreover, programmed cell death and organismal aging and death share the same genetic toolkits. A fundamental flaw of the ETAs is that the evolutionary rationale and mechanism for aging was searched during the period of the organism's life when it manifests, i.e. post-maturationally. However, compelling evidence indicates that aging trajectories are determined by genetic, epigenetic and environmental factors throughout life and particularly transgenerationally. Finally, the loss of totipotency and stem cell-ness of somatic cells following their genetically programmed differentiation underlies the loss of immortality of somatic cells and the soma as a whole.

It is a cornerstone of modern biology that a purposeful genetic program drives all biological processes that occur from the beginning of life to reproductive maturation. However, once reproductive maturation is reached, thought is divided with respect to whether the emerging aging process is a continuation of the

genetic program or whether it is the result of the accumulation of random, irreparable losses in molecular fidelity (Hayflick, 2007).

One feature, common to the "aging-is-not-programmed" ETAs is that aging is not likely to be determined by a genetic program, because there is a minimal selective advantage associated with longterm post-reproductive survival of the individual. The ETAs may have appeared sound when they were first put forward. At that time, aging was regarded as an evolutionarily recent phenomenon in humans and captive animals. In the natural environment, survival of many organisms is heavily dependent on extrinsic factors rather than age-associated mortality. Aging, the ETAs posited, did not evolve but developed stochastically as a result of relaxed selection. Relaxed selection, as will be demonstrated farther below, should have led to the formation of individual solutions characteristic for the ecological context in which the species lives. Thus, the ETAs expect that mechanisms associated with aging should be "private" (Martin et al., 1996)—that is, unique to each species. However, during recent decades it became evident that aging is almost universal and regulated by genes. Many studies have suggested that a variety of mechanisms associated with aging, such as insulin/IGF1 and TOR signaling, and decreased expression of the electron transport chain (Zahn et al., 2006) are "public," with similar patterns seen across diverse species (Partridge and Gems, 2002; Zahn et al., 2006; Houthoofd and Vanfleteren, 2007; Smith et al., 2008).

17.1 Aging is selected for

According to Endler (1986) natural selection can be defined as a process in which:

If a population has:

E1. variation among individuals in some attribute or trait: variation.

E2. a consistent relationship between that trait and mating ability, fertilizing ability, fertility, fecundity, and, or, survivorship: fitness differences.

E3. a consistent relationship, for that trait, between parents and their offspring, which is at least partially independent of common environmental effects: inheritance.

Then:

E4. the trait frequency distribution will differ among age classes or life-history stages, beyond that expected from ontogeny;

E5. if the population is not at equilibrium, then the trait distribution of all offspring in the population will be predictably different from that of all parents, beyond that expected from conditions E1 and E3 alone.

Conditions E1, E2, and E3 are necessary and sufficient for natural selection to occur, and these lead to deductions E4 and E5. As a result of this process, but not necessarily, the trait distribution may change in a predictable way over many generations.

Similar definitions were provided by Lewontin (1970) and Maynard Smith (1987).

Natural selection acts on an organism's phenotype, but it is the organism's genotype that is inherited. The phenotype is the result of the genotype and the environment in which the organism lives. Without any doubt, if Darwin was right, a phenotype that is universal in asymmetrically dividing unicellular and all multicellular organisms should have a strong evolutionary benefit.

17.1.1 The phenotypic/genetic signature of natural selection in aging

According to Partridge and Gems (2006) "The evolution of ageing presents a paradox for evolutionary biologists because a disadvantageous trait, namely a decline in reproductive prospects with age, has a demonstrated genetic basis and undergoes evolutionary change".

In biology, information flows from the environment to the genome by the process of natural selection (Frank, 2009). A population 'measures' the intrinsic information in the environment by differential reproduction of individuals with varying phenotypes. This fluctuation of phenotype frequencies transfers information to the population through changes in the frequencies of the hereditary particles. However, the population does not fully capture all of the intrinsic information in the frequency fluctuations caused by differential reproduction, because only a fraction of phenotypic information flows to the next generation via changes in the frequencies of the hereditary particles (Frank, 2009). In the words of Dobzhansky (1970, p. 200): "Darwinian fitness is a property...of a genotype and of the phenotypes conditioned by this genotype. Selection favors, or discriminates against, genotypes, that is, gene patterns." According to Williams (1966, p 25): "Natural selection would produce or maintain adaptation as a matter of definition. Whatever gene is favorably selected is better adapted than its unfavored alternatives. The selection of such genes of course is mediated by the phenotype, and to be favorably selected, a gene must augment phenotypic reproductive success." (It is strange that one of the founders of the ETAs, in the last half-sentence framed the conditions that determine the evolutionary rationale of aging/death while in his AP version of the ETAs he completely missed this framework. One could argue that at that time the genetic control of aging was not yet known, but even later he failed to take this

genetic control into account). Directional selection occurs when a particular allele is favored as a result of its effect on the phenotype. As molecular signature of natural selection, a favored allele can sweep towards fixation across the entire species range (Nielsen, 2005; Mitchell-Olds et al., 2007). Thus, directional selection favors genotypes dependent on their phenotype fitness and eventually carries them to fixation. To turn the argument on its head, the pervasive genetic control of a feature lets infer the action of strong selective forces. The workings of natural selection is not only visible in the maintenance or refinement of a function but also in the loss of function when it is no longer needed (see chapter 17.1.4). On the other hand, genetic drift can be precluded as the evolutionary force behind the phenomenon of aging: While there is some finite probability that some private aging mechanism, via control of e.g. IIS or TOR signaling, may be fixed by genetic drift in one species, the probability of this happening in ALL species, rendering this a public aging mechanism, is virtually zero. The random character of genetic drift is not compatible with the almost universal occurrence of aging/death and its phylogenetically conserved control by the same signaling pathways. In contrast, strong natural selection routinely can drive an allele to fixation. Thus, by inverse inference the near ubiquity of aging and conservation of its signaling pathways from yeast to mammals reveals that there must be powerful selective forces at work that render aging/death an evolutionarily stable strategy. For instance, the aging-related gene methuselah (mth) has experienced strong selection on expression level since D. melanogaster and D. simulans shared a common ancestor approximately two million years ago (Landry et al., 2005). Genetic variance for and genetic correlations among longevity, fecundity, stress resistance and other life history traits underlie predictable life history variation that reflects distinct, spatially and temporally varying, selection pressures (Schmidt et al., 2005a; b; Schmidt and Conde, 2006; Schmidt and Paaby, 2008). Together, these correlations imply that mth has experienced directional selection pressures over short and long timescales, and that mth is an important target in the selection regime driving the observed patterns of life history variation in natural populations (Paaby and Schmidt, 2008).

Natural selection, quantitative trait loci and linkage disequilibrium

Longevity and aging trajectories are selected for in the wild (Austad, 1993; Reznick et al., 2004; Korol et al., 2006). Quantitative traits refer to phenotypes with continuous characteristics, such as aging, that can be attributed to polygenic effects and their environment. Quantitative trait loci (QTLs) are stretches of DNA that contain or are linked to genes that underlie variation in a quantitative trait, which is identified by its linkage to polymorphic marker loci. Mapping regions of the genome that contain genes involved in specifying a quantitative trait is an early step in identifying and sequencing the actual genes underlying trait variation (Tanksley, 1993; Mackay, 2001; Mackay et al., 2009). A multitude of QTLs studies have identified genomic regions associated with senescence/lifespan regulation in plants (Luquez et al., 2006; Ougham et al., 2007), C. elegans (Johnson and Shook, 1997; Shook and Johnson, 1999; Ayyadevara et al., 2003), Drosophila (Johnson and Shook, 1997; Wang et al., 2004; Nuzhdin et al., 2005; Wilson et al., 2006), and mice (Yunis et al. 1984; Gelman et al. 1988; de Haan et al. 1998; Miller et al. 1998, 2002a; Klebanov et al. 2001; Jackson et al. 2002; Lang et al. 2010; Rikke et al. 2010). In Arabidopsis and winter wheat, QTL mapping found a correlation between the expression of flowering and senescence-associated genes (Wingler et al., 2010; Bogard et al., 2011).

Concordance of human and mouse QTLs has been reported previously (Sugiyama et al. 2001; Wang and Paigen 2005; Garrett et al. 2010), but for traits such as plasma lipids, hypertension, and kidney disease. Lifespan as a trait should be highly influenced by chance (according to the ETAs) and by environmental factors; so one might think that concordance for lifespan should be reduced or perhaps even nonexistent (at any rate, according to the ETAs, such a possibility is beyond any imagination). Yet the data obtained by Yuan et al. (2011), comparing data from a recent genomewide association study of human longevity (Newman et al., 2010) with QTLs from mice, clearly shows concordance between humans and mice lifespan peaks, with a very low probability that this is due to chance ($p = 0.0025$ using Fisher's exact test).

The forces of natural selection vary in space and time, resulting in genotype-environment interactions (GxE) for Darwinian fitness. QTL mapping has revealed a variety of GxE in the control of longevity (Shook and Johnson, 1999; Leips and Mackay, 2000; 2002; Vieira et al., 2000). Moreover, C. elegans QTL loci for the selected trait reproduction and longevity are coregulated (Shook and Johnson, 1999; Leips et al., 2006). In addition, aging is subject to sexual selection (Promislow and Pletcher 2002; Promislow 2003; Tower, 2006; Maklakov et al., 2007; Bonduriansky et al., 2008).

The potential for trade-offs hints at the involvement of pleiotropy in generating conditions for correlational selection surfaces. Correlational selection builds

favorable genetic correlations through the formation of linkage disequilibrium (LD) at underlying loci governing the traits (Sinervo and Svensson, 2002). Even in the absence of pleiotropy, natural selection can generate LD between alleles at separate loci thereby forming a genetic correlation (Endler, 1986; Lynch and Walsh, 1998). However, LD built up by correlational selection are expected to decay rapidly (i.e., within a few generations), unless correlational selection is strong and chronic. Biotic interactions that have 'Red Queen dynamics' (eg. host-parasite interactions, predator-prey relationships or intraspecific arms races) often fuel chronic correlational selection, which is strong enough to maintain adaptive genetic correlations (Sinervo and Svensson, 2002). LD or gametic disequilibrium is the occurrence of some combinations of alleles or genetic markers in a sexually reproducing population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies. Recombination should manifest itself as a significant decline in LD with distance (Miyashita and Langley, 1988; Schaeffer and Miller, 1993; Conway et al., 1999; Awadalla and Charlesworth, 1999). Recombination and segregation should reduce most LD within a few generations (Falconer and Mackay, 1996; Futuyma, 1998). Nevertheless, if correlational selection is strong and chronic, substantial LD can be maintained owing to a balance between recombination, segregation and selection (Hartl and Clark, 1997; Lynch and Walsh, 1998). Therefore, according to Lewontin (1964; 1974, p. 315): "The observation of significant LD that is consistent between populations is a very sensitive detector of natural selection." Hence LD has been used as tool to detect signatures of natural selection (Ennis, 2007). LD for genes linked to longevity has been observed in Drosophila (De Luca et al., 2003; Mackay, 2004, Carbone et al., 2006; Paaby et al., 2010) and humans (Bellizzi et al., 2005; Lunetta et al., 2007; Willcox et al., 2008; Flachsbart et al., 2009; Sebastiani et al., 2009; Boyden and Kunkel, 2010; Edwards et al., 2011).

Correlational selection favors trait combinations that couple underlying alleles at loci. Two major correlations underlying life history determination include the negative correlation between reproduction and survival, and the positive correlation between longevity and stress tolerance (Reznick 1985; Stearns 1991; Partridge et al., 2005; Vermeulen and Loeschcke, 2006; Harshman and Zera, 2007; Toivonen and Partridge, 2009). These relationships between traits are mediated by pleiotropic genic elements, or by genic elements that affect single traits but co-occur through LD and act as pleiotropic alleles (Paaby et al., 2010). The persistent LD throughout metazoan phylogenesis between both TOR and insulin signaling and their antagonistic control of reproduction and aging/death is a marker of correlational selection. Likewise, the coordinated regulation of stress resistance and longevity by gerontogenes (see chapter 10.1) carries the signature of correlational selection. Gerontogenes and "metabogenes" (TOR and IIS) set up another network that is shaped by correlational selection (see Illustration 1).

Taken together, the evidence provided by the QTL and LD findings demonstrates the action of correlational natural selection, presumably due to 'Red Queen type' coevolution in the modulation of aging trajectories and reproduction.

17.1.2 Aging is co-selected with reproduction

Comparative studies of life history traits in a variety of taxa identified various correlations between features of reproductive activity and longevity: low reproductive rate, slow development and long life span at one end and the opposite traits at the other end of a continuum. These strong size-independent correlations among life-history variables led to the concept of a "fast-slow continuum" of life-history variation, in which the differences between taxa evolve through adaptation to environmental factors (Stearns, 1983; Gaillard et al., 1989; Read and Harvey, 1989; Promislow and Harvey, 1990; Gaillard and Yoccoz, 2000; Ricklefs and Wikelski, 2002; Bielby et al., 2007; Jones OR et al., 2008). The absence of alternative combinations of these variables implies constraint on the diversification of life histories, but the nature of this constraint remained elusive (Ricklefs and Wikelski, 2002). The majority of these correlations have been detected in mammals (Promislow and Harvey, 1990; Sæther and Gordon, 1994; Purvis, 1995; Purvis and Harvey, 1995; Symonds, 1999; Fisher et al., 2001; Jones and MacLarnon, 2001; Oli, 2004; Isaac et al., 2005; Dobson and Oli, 2008) but other taxa (Blackburn, 1991; Owens and Bennett, 1995; Bauwens and Díaz-Uriarte, 1997; Clobert et al., 1998; Reynolds et al., 2001; Jeschke and Kokko, 2009), including plants (Poorter and Bongers, 2006; Easdale and Healey, 2009; Wright et al., 2010; Wingler, 2011), display similar covariations.

One of the predictions that most often is used to support the validity of the ETAs is the finding that increased mortality due to extrinsic causes accelerates aging trajectories (Stearns et al., 2000). Likewise, theory predicts that in safe habitats where extrinsic sources of mortality are rare, selection should favor individuals that show delayed senescence (Williams, 1957; Hamilton, 1966; Edney and Gill, 1968;

Charlesworth, 1980; Dobson, 2007; Sibly and Brown, 2007; 2009). There are numerous examples for the fact that a protected lifestyle either due to environmental conditions or the organisms' armor, palatability and means of escape delays senescence and increases longevity (Blest, 1963; Rose, 1984; Austad and Fischer, 1991; Austad, 1993; Rose, 1991; Keller and Genoud, 1997; Tatar et al., 1997, Dudycha, 2001; Sherman and Jarvis, 2002; Blanco and Sherman, 2005; Buston and García, 2007; de Magalhães et al., 2007; Dobson, 2007; Sibly and Brown, 2007; 2009; Ricklefs, 2008; Beck and Fiedler, 2009; Carroll et al., 2011). But there is also evidence to the contrary (Reznick et al., 2004). The correlated responses of reproductive behaviors illustrated that the study of mortality patterns only makes sense taking into account associated reproduction/longevity trade-offs and life history strategies (Dudycha, 2003; Lee et al., 2006). It should not come as surprise that animals that are under an increased threat of environmental death have to grow faster and reproduce earlier to avoid Damocles' sword of extinction. Thus, taxa facing high mortality risks have to live "fast" lives in order to reproduce before dying, whereas those with longer life expectancies should grow to larger size before maturing so they can invest more in reproduction (Bielby et al., 2007). The Tasmanian devil is a current example of these life history relationships (Jones et al., 2008). As discussed in chapter 17.3.4, stressors like predator pressure or resource depletion, via stress signaling pathways, shape life history traits via transgenerational genetic and epigenetic mechanisms.

Some models for the "fast-slow continuum" of life-history variation view life-history rates and timings as adaptations to extrinsic rates of mortality (Promislow and Harvey, 1990; 1991; Charnov 1991; 1993; Kozlowski and Weiner, 1997; Reznick et al., 2001). In other models, reproductive timing and output represented by interbirth interval, age at sexual maturity, or weaning age feature prominently as surrogate measure of a species' position on the fast–slow continuum axis (Bielby et al., 2007; Dobson and Oli, 2007). Morever, a semelparity-iteroparity axis has been described (Gaillard et al., 1989). Removing statistically the influences of body mass from the fast–slow continuum revealed that a general trade-off of reproduction and survival shapes the residual pattern of the fast–slow continuum (Harvey and Zammuto, 1985; Gaillard et al., 1989; Dobson and Oli, 2008; Dobson and Jouventin, 2010). Importantly, life-history correlations are characterized both by phenotypic plasticity and genetic/physiological constraints and modulated by neuroendocrine, particularly stress response, axes (Ricklefs and Wikelski, 2002) and resource availability (Sibly and Brown, 2007).

Thousands of papers demonstrated the trade-offs between reproduction and longevity in iteroparous organisms (see chapters 8 and 20) and the relationship between reproduction and death in semelparous organisms (see chapters 6.3 and 7). Co-selection is the mechanism of Red Queen coevolutionary dynamics. Trade-offs are evolutionary footprints of conflict (see chapter 12) and opposing, respectively correlational, selective forces (see chapters 13 and 17.1.1). The importance of trade-offs as dynamic rather than static relationships was emphasized (Roff et al., 2002). Theory predicts that correlational selection on two traits will cause the major axis of the bivariate G matrix to orient itself in the same direction as the correlational selection gradient. A meta-analysis utilizing empirical estimates of correlational selection gradients and measures of the correlation between the two focal traits supported the underlying hypothesis (Roff and Fairbairn, 2012). A multitude of papers have shown that longevity and reproduction are co-selected (e.g. Sokal, 1970; Enesco et al., 1989; Heininger, 2002a; de Magalhães and Church, 2005; Carey and Molleman, 2010). In Drosophila, laboratory selection for reproduction trajectories co-selects for lifespan (Rose and Charlesworth, 1980; Clare and Luckinbill, 1985; Luckinbill and Clare, 1985; Rose, 1991; Partridge et al., 1999; Sgró and Partridge, 1999; Linnen et al., 2001; Phelan et al., 2003; Valenzuela et al., 2004), mimicking phenomena that are pervasive in the wild throughout phylogeny (Craig, 1985; Pyle et al. 1997; Miller et al., 2002a; Charmantier et al., 2006; Ricklefs, 2010a). In an elegant study, Sgró and Partridge (1999) demonstrated that reduced early fecundity was causally involved in the retarded aging of their long-lived Drosophila lines. Abolishing reproduction through either irradiation or genetic manipulation removed the differences in aging rate between their controls and long-lived selected lines (Sgró and Partridge, 1999).

Hormones are intimately related to fitness, and they often mechanistically underlie life-history trade-offs such as survival and reproduction (Ketterson and Nolan, 1992; Roff, 1992; 2002; Stearns, 1992; Sinervo and Svensson, 1998; Zera and Harshman, 2001; Ricklefs and Wikelski, 2002; Adkins-Regan, 2005; Breuner et al., 2008; Lessells, 2008; Bonier et al., 2009; Mills et al., 2009; Hau et al., 2010; Flatt and Heyland, 2011). However, individual variation in quality, condition, or resource acquisition may mask the functional trade-off (van Noordwijk and de Jong

1986; McGlothlin et al., 2010).

A variety of mutations extend the lifespan in various model organisms. Consistently, however, these long-lived strains are outcompeted by wild-type animals, particularly under resource-restricted conditions (see chapter 12.1). The proponents of the ETAs are so focused on aging as fitness-eroding maladaption that they ignore that the essence of aging can only be grasped in the context of reproduction and the heavy toll evolution put on reproducing organisms to constrain the emergence of a Darwinian demon.

In an analysis of 26,530 single nucleotide polymorphisms (SNPs) with allele frequencies that were determined in three human populations, statistically significant evidence was obtained supporting the hypothesis that selection has influenced extant patterns of human genetic variation. Importantly, one candidate gene for death and one for reproduction were identified among 174 candidate genes with distribution of genetic variation that indicates that they have been targets of selection (Akey et al., 2002).

It is intriguing (remember Orgel's second rule) that evolution linked reproduction, the ultimate cause of resource limitation (see chapter 5.1), and aging/death in an intricately tuned and plastic network effecting transgenerational resource management. The selection of longevity together with reproductive activity makes perfect sense for the evolutionary optimization of fitness.

17.1.3 Natural selection and network design

The design of molecular networks is regarded as a molecular phenotype which is shaped by natural selection and reflects a successful adaptation towards certain functional demands (Gerhart and Kirschner, 1997; Wagner, 2003; 2008; Barab'asi and Oltvai, 2004; Babu et al., 2006; Balaji et al., 2006; Davidson, 2006). Biological networks have evolved to allow for a robust performance of their functions (Stelling et al., 2004; Kitano, 2004; Hammerstein et al., 2006) to support the maintenance of functionality as reflected by a stable ground state. This frees up the kinetic parameters of the system to meet other demands. Therefore, both a higher kinetic and structural off-state robustness of network design could confer a better evolvability (Kirschner and Gerhart, 1998; Wagner and Wright, 2004; Wagner, 2005; Lenski et al., 2006; Shinar et al., 2007; Kartal and Ebenhöh, 2009). The Red Queen dynamics of the germ-soma conflict over the management of future resources orchestrated a huge molecular network that linked development (see chapter 17.3.2), reproduction and aging/death in antagonistic pleiotropic signals integrating resource allocation and stress response pathways. The phylogenetic conservation of these canonical signaling pathways, the public aging mechanisms (Partridge and Gems, 2002; Zahn et al., 2006; Managbanag et al., 2008; Houthoofd and Vanfleteren, 2007; Smith et al., 2008), are molecular evidence for the strength of the underlying selective force.

17.1.4 "Relaxed selection" and genetic decay

The ETAs posit that the underlying evolutionary principle of aging is the postreproductive decline of natural selection. The examination of the phenotypic and genetic sequelae of relaxed selective pressure draws a different picture.

Environmental change often eliminates or weakens a source of selection that was formerly important for the maintenance of a particular trait. Such circumstances are often termed 'relaxed selection'. Evidently this is a misnomer, since selection is not relaxed but is directed against the maintenance of a costly function that is no longer needed. Selection against a trait can be considered in terms of fitness costs incurred in the maintenance or expression of the trait. Some costs are automatically incurred in the act of maintaining or expressing the trait ('constitutive' costs). By contrast, other costs are 'contingent,' or arise owing to particular features of the environment where the traits are expressed (Lahti et al., 2009). The loss or vestigialization of traits that no longer contribute to fitness is a widespread phenomenon that highlights the importance of natural selection for the maintenance of adaptive traits (Fong et al. 1995; Wiens 2001; Porter and Crandall 2003). Adaptive plasticity is lost during long periods of environmental stasis (Masel et al., 2007). In evolving populations of E. coli adapting to a single nutrient in the medium, unused catabolic functions decayed and their diet breadth became narrower and more specialized (Cooper and Lenski, 2000). In constant, nutrient-rich environments where benefits associated with spores are absent and no longer balance the cost of constructing spores, sporulation ability of B. subtilis was lost over 6,000 generations (Maughan et al., 2009). Propagation of B. subtilis for less than 2,000 generations in a nutrient-rich environment where sporulation is suppressed led to rapid initiation of genomic erosion including biosynthetic pathways, sporulation, competence, and DNA repair (Brown et al., 2011). The social prokaryote M. xanthus loses its social behavior when propagated in a nutrient-rich habitats in which their social behaviors for starvation-induced spore production or predatory efficiency were not under positive selection (Velicer et al., 1998; Velicer and Stredwick, 2002). The majority

of 24 parasitoid species of wasps were found to be incapable of synthesizing lipids, and phylogenetic analyses showed that the evolution of lack of lipogenesis is concurrent with that of parasitism in insects (Visser et al., 2010). In Drosophila melanogaster, the frequency of genotypes that express diapause increase over time when flies are exposed to environmental stress, whereas the frequency of non-diapause genotypes increased when flies were cultured under benign control conditions (Schmidt and Conde, 2006). In natural populations, the propensity to express reproductive diapause is subject to strong selection in temperate habitats. In isofemale lines of differing diapause genotype, diapause genotype explained the majority of the variance for lifespan, fecundity, cold shock tolerance, and mortality rates in the sampled populations (Schmidt and Paaby, 2008), arguing for the strong adaptive value of these traits. Under relaxed selection, a function, organ or property degenerates and becomes dysfunctional. Eye involution and albinism evolved multiple times and independently in a variety of organisms ranging from arthropods, teleosts, amphibians, to reptiles and mammals (Howe, 1919; Shlaifer, 1951; Durand, 1976; Springer et al., 1997; Kos et al., 2001; Protas et al., 2006; 2011; Jeffery, 2009).

Genes that are no longer under selective pressure degenerate or are fossilized to pseudogenes (Carroll, 2006; Hall and Colegrave, 2008). In the absence of selection, genes will be lost from a bacterial genome, owing to a mutational bias that favors deletions (Mira et al., 2001; Kuo and Ochman, 2009). In the marsupial mole, the interphotoreceptor retinoid binding protein gene is a single-copy gene that functions in the visual cycle sequence and contains three frameshift indels and numerous stop codons in all three reading frames. Given that the marsupial mole is blind with degenerate eyes, this finding suggests that phenotypic degeneration of the eyes is accompanied by parallel changes at the molecular level as a result of relaxed selective constraints (Springer et al., 1997). In blind cave fishes, opsin genes undergo rapid degeneration even when there is still some residual visual function (Yokoyama et al., 1995). A nocturnal lifestyle that is thought to mark the early evolution of mammals around 150–200 Ma may be the cause of the reduction in the number of cone pigment genes to only two classes (Hunt et al., 2009). In coelacanths, cetaceans, blind mole rats, owl monkeys, and bush babies the degeneration of the short-wavelength-sensitive opsin gene is associated with the taxa's habitat and lifestyle, demonstrating that relaxed selection on a gene leads to its decay (Jacobs et al., 1996; Fasick et al., 1998; Yokohama et al., 1999; David-Gray et al., 2002; Levenson and Dizon, 2003; Kawamura and Kubotera, 2004; Carroll, 2006). Olfactory receptors (ORs) form the largest multigene family in vertebrates. The numbers of functional OR genes vary enormously, ranging from 1,200 in rats and 400 in humans to 150 in zebrafishes and 15 in pufferfishes. Most species have a considerable fraction of pseudogenes, e.g. 400 in humans (Niimura, 2009). Apes, Old World monkeys and one New World monkey, the howler monkey that possess full trichromatic vision, have a significantly higher proportion of olfactory receptor pseudogenes than do other New World monkeys or the lemur (a prosimian), suggesting that the deterioration of the olfactory repertoire occurred concomitant with the acquisition of full trichromatic color vision in primates (Gilad et al., 2004). There are many more examples of pseudogene evolution and trait degeneration demonstrating that functional genes, sensory systems and other traits are costly (Niven and Laughlin, 2008) and degenerate under relaxed selection (Hall and Colegrave, 2008; Lahti et al., 2009). In mammals and Drosophila, studies of pseudogene evolution that are assumed to accumulate various types of spontaneous mutations unbiased by natural selection suggest that any aging-specific public mechanisms should have readily undergone degeneration by random genetic drift (Petrov and Hartl, 2000). Even pleiotropic proteins may suffer from a loss of one of their functions. The rudimentary eyes of the blind mole rat Spalax ehrenbergi have lost their visual function, but are still required for the control of circadian rhythms. α A-crystallin, a major eye lens protein in other mammals, evolved much faster in the mole rat than in rodents with normal vision (Hendriks et al., 1987). Yet, although mole rat ?A-crystallin degenerated as a lens protein, its rate of change is still much slower than that of pseudogenes, suggesting some remaining function. In fact, the pleiotropic, chaperone-like activity of mole rat αA-crystallin is considerably lower than that of its rat orthologue (Smulders et al., 2002).

Following the mathematical insight and the analysis of sequence data that became available in the late 1960s that most mutagenic changes are selectively neutral or near-neutral, Kimura (1968; 1983; 1991) put forward the neutral theory of molecular evolution. "…the neutral theory claims that the overwhelming majority of evolutionary changes at the molecular level are not caused by Darwinian natural selection acting on advantageous mutants, but by random fixation of selectively neutral or very nearly neutral mutants through the cumulative effect of sampling drift (due to finite population number) under continued input of new

mutations" (Kimura, 1991). At face value (but see Heininger, in preparation), confirmation of various predictions of the theory provides evidence for its phenotypic correctness:

in protein sequences, conservative changes—substitutions of amino acids that have similar biochemical properties and are therefore less likely to affect the function of a protein—occur much more frequently than radical changes.

synonymous base substitutions of the third nucleotide in a triplet (i.e., those that do not cause amino acid changes) occur almost always at a much higher rate than nonsynonymous substitutions.

noncoding sequences, such as introns, evolve at a high rate similar to that of synonymous sites.

entirely untranslated pseudogenes, or dead genes, evolve at a high rate, and this rate is about the same in three-codon positions.

However, focusing on the rate of selectively neutral nucleotide substitutions, the neutral theory totally ignored that the maintenance of coding sequences (whose products have been functioning well over millions of years) is the signature of natural selection (otherwise, as in pseudogenes the genes would degenerate). Thus, from the phylogenetic conservation of "public" genetic programming of aging the action of strong selective forces can be inferred. Accordingly, the rate of amino acid replacements, similar to the pattern observed in several other signal transduction pathways, correlates negatively with the position of a protein in the insulin/TOR-signaling pathway in Caenorhabditis, Drosophila and vertebrates (Alvarez-Ponce et al. 2009, 2010; Cui et al. 2009; Jovelin and Phillips, 2011) arguing that more downstream components may be under stronger purifying selection because they are the common final pathways required to transduce an increasing amount of signals in the cell (Cui et al., 2009; Guirao-Rico and Aguade 2009, 2011; Jovelin and Phillips, 2011).

17.1.5 Extrinsic mortality and selection pressure

Limitation of resources being a pervasive phenomenon, natural selection within its constraints exerted by e.g. sexual selection (think of the peacock's tail), tends to favor the most economic organisms. Hence, obsolete, energy-consuming, functions such as visus in caves (Wilkens, 2010) or metabolic capacities that are no longer used, are lost. Thus, relaxed selection only means that selection for visus is relaxed in dark environments but there should be a strong selection pressure to eliminate redundant, energy-consuming functions and organs. Likewise, there should be a strong selection pressure to eliminate redundant, post-reproductive organisms.

One of the central tenets of the ETAs is that extrinsic mortality is high enough to eliminate the postreproductive organisms so that this potential selection pressure does not occur in the wild. Until recently, it was assumed that individuals in the wild were highly unlikely to show signs of senescence (Holmes and Austad 1995; Kirkwood and Austad 2000). However, this assumption has been refuted. Definitely, senescence has evolved outside of a "selection shadow" (Turbill and Ruf, 2010). Postreproductive survival, somatic and reproductive senescence in natural populations have now been documented in a wide range of taxa (Calder 1984; Nesse, 1988; Promislow 1991; Carey and Gruenfelder, 1997; McDonald et al., 1997; Ricklefs, 1998; Loison et al., 1999; Catchpole et al., 2000; Holmes et al., 2001; Ericsson and Wallin, 2001; Bennett and Owens, 2002; Reznick et al., 2002b; 2006; Cichon et al., 2003; Lozano and Lank, 2003; Saino et al., 2003; Bryant and Reznick 2004; Cameron and Siniff, 2004; Bronikowski and Promislow, 2005; Haussmann et al., 2005a; Mysterud et al., 2005; Beauplet et al., 2006; Bowen et al. 2006; Brunet-Rossinni and Austad, 2006; Catry et al., 2006; Ujvari and Madsen, 2006; Palacios et al., 2007; Bronikowski, 2008; Jones et al., 2008; Nussey et al., 2008; Ricklefs, 2008; Wilson et al., 2008; Bowhuis et al., 2009; 2010; 2012; Holmes and Martin, 2009; Turbill and Ruf, 2010; Péron et al., 2010; Dugdale et al., 2011), including short-lived organisms (e.g. insects, small birds) where it was long deemed not to occur in the wild (Adamo et al., 2001; Bennet and Owens 2002; Bonduriansky and Brassil 2002; 2005; Kurtz, 2002; Moya-Laraño, 2002; Dukas, 2008; Kawasaki et al., 2008; Zajitschek et al., 2009a; b; Sherratt et al., 2010; 2011). In fact, senescence is widespread in the wild and equally likely to occur with regard to survival and reproduction (Jones et al., 2008; Bowhuis et al., 2012). Moreover, for many groups of organisms the extrinsic mortality risk is dependent on an organism's intrinsic condition and susceptibility to such hazards. Intrinsic aging renders organisms more vulnerable to predation (Fuller and Keith 1980; Bjorge and Gunson 1989; Boyd et al. 1994; Mech et al. 1995; Ricklefs and Scheuerlein, 2001; Smith et al., 2004) and immunosenescence-related infection (Ashman et al., 1999; Møller and de Lope 1999; Doums et al., 2002). Beyond the direct mortality effect of greater susceptibility to pathogens, higher parasite burdens might also impose additional costs through, for example, diminished foraging ability and/or increased risk of predation (Lafferty and Morris 1996; Bakker et al. 1997). Hence, extrinsic mortality is not a cause of aging (frustrating the efforts for somatic maintenance) (Edney and Gill 1968; Promislow 1991; Rose 1991;

Stearns 1992; Abrams, 1993), but to a significant extent already a sequel of aging. In a series of papers, Ricklefs and Scheuerlein showed that aging-related mortality almost certainly is intrinsic to the organism and likely represents various failures of organismal function with the accumulation of damage to molecules, cells, and tissues. To the extent that predators and disease tend to remove older individuals in natural populations, these causes of death might come to individuals that are terminally weakened by intrinsic processes. According to the statistical computations derived from animal demographics they concluded that between 2% and 78% of deaths were due to senescent decline, with the higher percentages in long-lived species. Thus, even when brought into predator-free conditions, such individuals might die at similar ages due to 'intrinsic' causes (Ricklefs 1998; 2000; 2008; 2010a; Ricklefs and Scheuerlein, 2001; 2002).

17.2 Aging is adaptive

Darwin's theory of evolution by natural selection explains both the process and the purpose of adaptation (Fisher, 1930, Hamilton, 1970). The process of adaptation occurs through the action of natural selection, which is mediated by differential reproductive success of individual organisms, and resulting changes in gene frequency (Fisher, 1930). According to Rose and Lauder (1996), adaptation refers both to a process and its product, the process of modifying one thing to another and the condition of being adapted. Adaptation looks to the past and to a trait's adaptive history while fitness points to future reproductive success. In a general sense, adaptation can be defined as the process of change in an organism to conform better with (new) environmental conditions, whereby the organism (or group of organisms) acquires characteristics, involving changes in morphology, physiology or behaviour that improve their survival and reproductive success in the particular environment. Such changes can occur phenotypically, within a set genotype, and then phenotypic adaptation is the result of what is called 'phenotypic plasticity', the capability of a genotype to change its phenotype according to prevailing environmental conditions. Adaptation can also occur through changes in allele frequencies as a result of the selection pressure exerted by the environment (e.g. David et al., 2005; Lindgren and Laurila, 2005; Sørensen et al., 2005). This process is known as genotypic adaptation or evolutionary adaptation (Bijlsma and Loeschke, 2005). The process of adaptation leads individual organisms to appear designed as if for the purpose of maximizing their inclusive fitness, which is defined as the effect of one individual's actions on its genetic contribution to future generations through its direct descendants and those of its relatives (Hamilton, 1964; Grafen, 2006). The inclusive fitness approach to adaptation has been extremely successful, especially in the fields of behavioral and evolutionary ecology, providing explanations for a wide range of traits (Stearns, 1992; Krebs and Davies, 1993). Adaptation is characterized by the movement of a population towards a phenotype that best fits the present environment (Fisher, 1930). Without any doubt, adaptation has a genetic basis (Rose and Lauder, 1996; Brock, 2000; Elena and Lenski, 2003; Mauricio 2005; Orr, 2005) and is subject to a phenotype/genotype-by-environment interaction (Clare and Luckinbill, 1985; Brock, 2000; Carnes and Olshansky, 2001; Nevo, 2001; 2009; Svensson et al., 2001; Cooper and Lenski, 2003; Mangel et al., 2007; Suryan et al., 2009; Bell, 2010).

17.2.1 Life history strategies are adaptive

Adaptation of life-history traits to a variable environment during the lifetime of the organism (in contrast to a pre-determined lifespan) should increase its reproductive fitness and have a strong selective advantage (Ergon et al., 2001). Hence, the genetic control for reproduction-aging trajectories is plastic (Finch, 1990; 1997, Scheiner, 1993; Arking, 1998; Kenyon, 2005), subject to environmental modulation (Clare and Luckinbill, 1985) and mediated to a substantial extent through resource utilization (see chapter 9) and stress resistance (Buck et al., 1993; Krebs and Loeschcke, 1999) (see chapter 10). Longevity, is the phenotype of multiple divergent mechanisms, e.g. at the redox balance or DNA repair level (Arking et al., 1996; 2000a; b). Accordingly, identical longevity phenotypes may be achieved by different genotypic and effector mechanisms (Service et al., 1988; Rose, 1991; Arking et al., 1996; 2000b; Johnson et al., 2000) according to the 'multiple solutions to a given problem' strategy of evolutionary mechanisms (Travisano and Lenski, 1996; Heininger, 2001; MacLean and Bell, 2003; Novak et al., 2006; Barrick and Lenski, 2009). In a continued legacy from their unicellular ancestors and mediated by oxidative stress, these evolutionary processes unfold with a programmed randomness at the cellular level (Heininger, 2001).

There is no doubt that life history strategies are adaptive (Williams, 1966; Wilbur et al., 1974; Roff, 1992; 2002; Stearns, 1992; Gotthard and Nylin, 1995; Daan and Tinbergen, 1997; Nylin and Gotthard, 1998; Ricklefs and Wikelski, 2002). In a strange squirm of logic, the ETAs made us believe that aging and death,

that are undoubtedly life history traits, are maladaptive. As discussed earlier, semelparity and iteroparity, coupling reproductive and aging trajectories, are threshold traits and rather than representing a dichotomy are opposite ends of a continuum of life history variation (Woolhouse, 1983; Pontier et al., 1993; Silvertown, 1996; Benton and Grant, 1999; Thomas et al., 2000; Boonstra et al. 2001; 2007; Hautekèete et al., 2001; Iguchi and Tsukamoto, 2001; Woods and Hellgren, 2003; Boonstra, 2005). Obviously, aging trajectories are subject to adaptation to environmental conditions such as nutrient limitation (mimicked by DR) and other environmentally harsh conditions (Allen, 2008). That the aging trajectories of animals in their natural habitat is adaptive is highlighted by their competitive advantage compared to longevity mutants. It is maintained that “In all species examined to date, endocrine manipulations can slow aging without concurrent costs in reproduction” (Tatar et al., 2003). This may be true in the laboratory where organisms with abundant resource availability may be exempted to pay the costs of reproduction (see chapter 8). If the above dictum would also be true in the wild, it would question Darwinian evolution. However, long-lived mutants pay a high cost in terms of fitness in their natural habitat. Long-lived yeast variants show a significant defect in fitness in a direct competition assay with wild type cells (Fabrizio et al., 2004a; b; Herker et al., 2004; Delaney et al., 2011). In Dictyostelium, cheating entails fitness costs in terms of reduced dispersal due to shorter stalks and migration of slugs (Hilson et al., 1994; Pál and Papp, 2000; Castillo et al., 2005; Strassmann and Queller, 2011a). By selecting Drosophila strains for breeding at late ages, or directly for increased lifespan, several experiments have found that the evolution of increased longevity is correlated with decreases in fitness traits such as early fecundity or egg-to-adult survival (viability), suggesting the existence of a negative genetic correlation, or trade-off, between adult survival and other fitness components (Luckinbill et al., 1984; Luckinbill and Clare, 1985; Zwaan et al., 1995; Partridge et al., 1999; Buck et al., 2000). The longevity mutation in the Drosophila Indy gene is not associated with any cost when flies are well fed. However, mutants on a decreased-calorie diet have a reduced fecundity (Marden et al., 2003). The lifespan-extending effect of the methuselah mutation in D. melanogaster is offset by an effect on reproductive output (Mockett and Sohal, 2006). Long-lived C. elegans mutants have a lower fecundity than a wild strain (Gems et al., 1998; Hekimi et al., 1998; Chen et al., 2001) and are outcompeted by wild C. elegans both under laboratory conditions (Jenkins et al., 2004;Van Voorhies et al., 2006; Chen et al., 2007) and even more under simulated conditions of the wild (Walker et al. 2000; Jenkins et al., 2004; Van Voorhies et al., 2005). Under limited resources, the long-lived mutants competing with their wild conspecifics became extinct within four generations (Jenkins et al., 2004), a situation reminiscent of the rapid extinction of cheater phenotypes in unicellular, facultative multicellular, microbes (Ennis et al., 2003; Fiegna and Velicer, 2003; Kuzdzal-Fick et al., 2011). Overall, the longevity mutants can be regarded as cheater phenotypes. The ETAs provide no reason why cheater phenotypes should not have evolved. In fact, under the basic assumptions of the ETAs (“An animal that grows to maturity and thereafter reproduces indefinitely has, other things being equal, a greater Darwinian fitness than one that grows to maturity and then survives and reproduces for only a fixed period of time.” [Kirkwood and Melov, 2011]), cheaters should have evolved repeatedly, given the ease of one-step mutations e.g. in the insulin signaling pathway (see chapter 9.3) and their spontaneous evolution (Buss, 1982; Ennis et al., 2003; Fiegna and Velicer, 2003; Fortunato et al., 2003), but evolution appears to select against these social parasites (Grosberg and Strathmann, 1998). These experimental findings clearly identify the programmed reproduction-longevity network as fitness optimum in the wild.

17.3 Aging is programmed

The Darwinian evolutionary process can be summarized into three components: struggle for existence, variation in characters that influence success in that struggle, and transmission of that variation from parents to offspring. Understanding the causes of variation among individuals in their contribution to future generations—variation in fitness—and the way in which that variation is inherited—its genetic basis—thus lies at the heart of our understanding of evolution (Ellegren and Sheldon, 2008). Any theory that sets out to explain the genetic programming of aging has to take into account: i) why aging is phylogenetically conserved and ii) why aging is regulated by reproductive activity, resource utilization and stress resilience.

Although there is a general consensus that aging is regulated by genes (tenthousands of papers should suffice to witness this genetic control) (e.g. reviewed by Finch and Rose, 1994; Finch and Tanzi, 1997; Johnson et al., 1999; Guarente and Kenyon, 2000; Heininger, 2002a, Hekimi and Guarente, 2003; Helfand and Rogina, 2003; Kenyon, 2005; 2010; Warner, 2005; Antebi, 2007; Braeckman and Vanfleteren, 2007; Houthoofd and Vanfleteren, 2007;

Vijg, 2007; Kennedy, 2008; Kuningas et al., 2008; Flatt and Schmidt, 2009) there is still substantial controversy whether aging is evolutionarily programmed (e.g. Austad, 2004a; 2004b; Bredesen 2004a; 2004b; Kirkwood and Melov, 2011; Skulachev, 2011; Goldsmith, 2012).

Goldsmith (2010) described the situation as follows: "For example, noted biologists Olshansky, Hayflick, and Carnes say in a Scientific American article in 2002: 'The way evolution works makes it impossible for us to possess genes that are specifically designed to cause physiological decline with age or to control how long we live.' This statement was made after the discovery of aging genes in multiple organisms…. Note again the use of the word 'impossible' (used previously by Williams in similar context), which is relatively seldom used in scientific papers. This paper is said to have been endorsed by 51 prominent scientists.". And in a further article: ''. . . longevity determination is under genetic control only indirectly'' and ''. . . aging is a product of evolutionary neglect, not evolutionary intent'' (Olshansky et al., 2002a; b).

Intriguingly, aging is the only major biological phenomenon where a sizable number of scientists maintains that although its genetic control is undeniable that this phenomenon is not programmed. In fact, the majority of those that agree that there is some regulatory role of genes in aging think that aging is not programmed (e.g. Lithgow, 2006; de Grey, 2007; Kirkwood, 2008; Kirkwood and Melov; 2011). The semantic rollercoaster that is required by this denial occasionally brings forth strange blossoms (Kirkwood and Melov; 2011): "What is regulated, or indeed programmed, is the setting of the levels of survival functions (DNA repair, antioxidant defences, etc.)."

I doubt whether those of the ETA aficionados that agree that longevity is "genetically regulated" are aware that they advocate some Lamarckian type of inheritance: The ETAs infer that some "declining selective forces" (whatever that is, see chapter 3) act during the parent's postmaturational lifetime. If these forces, or their absence leave their heritable footprints in the genome of the progeny (notabene phylogenetically conserved from yeast to humans) some Lamarckian-type inheritance (in its original sense of genetic heritability of somatic change) must be involved.

The arguments in the genetic-regulation/programmed-aging debate, in their admittedly simplified version, go like this: We acknowledge that there is some genetic regulation of aging, but according to the ETAs aging cannot be programmed. These arguments value theories higher than data and are highly circular. Of course, this denial has method. To admit that aging is programmed would mean, given the principles of the Modern Synthesis, a first-class funeral for the ETAs. Yet, even the most staunch proponents of the ETAs agree that semelparity is programmed and subject to neuroendocrinological control (Hayflick, 1995; Holliday, 1995; Austad, 2004; de Grey, 2007). On the one hand according to the ETAs, semelparity is considered an extreme version of the decline in the force of natural selection with age (Kirkwood and Austad, 2000). On the other hand, the semelparity-programmed aging discussions reveal a general strategy of the proponents of the ETAs. If there is a feature related to aging that they may be unable to dismiss as non-programmed they either resort to some semantics about the meaning of the word "program" (e.g. Kirkwood and Cremer, 1982; Austad, 2004; Kirkwood and Melov, 2011). A sample with a particularly nice haut-goût of circular reasoning (Kirkwood and Cremer, 1982; Kirkwood and Melov, 2011): "If ageing is assumed to have evolved in a non-adaptive way, there seems no reason to suppose that such a well-defined programme would exist. This is not to say, however, that non-adaptive ageing would not be genetically determined. In fact, it is obvious from interspecies comparisons and from studies on inbred strains of laboratory animals that duration of life is dependent on genotype. Thus, the issue that distinguishes programmed from non-programmed ageing is not whether the factors that determine longevity are specified within the genome, but rather, how this is arranged." Or (Kirkwood and Melov, 2011): "it is not the deterioration and death of the post-reproductive adult that is programmed, but the events associated with the act of reproduction.".

It should be now beyond doubt that aging is regulated (to avoid for now the term programmed) by various signal transduction pathways involved in reproduction, resource utilization and stress response. Further it is uncontested that these pathways program a multitude of developmental processes. Moreover, these ontogenetic processes are linked to aging in a variety of antagonistic pleiotropic trade-offs. That the same pathways program development but only "regulate" aging leave the ETAs in an irresolvable contradiction.

17.3.1 The heritability of fitness traits

The modest heritability of aging (McGue et al., 1993; Curtsinger et al. 1995, Herskind et al., 1996; Finch and Tanzi, 1997; Finch and Kirkwood, 2000; De Benedictis et al., 2001; Mitchell et al., 2001) has been taken as an argument for its randomness and against a genetic regulation of aging (Finch and Kirkwood, 2000). According to Finch and Tanzi (1997) heritability of

lifespan accounts for ≤ 35% of its variance in short-lived invertebrates [the nematode and fruit fly] and in mammals [the mouse and human]. Studies of human twins attribute most (> 65%) of the variance to nonshared (individually unique) environmental factors. Twins reared apart share even less heritability of lifespan. Other human twin studies, however, suggest a heritability of about 50% (Yashin and Iachine, 1995; Yashin et al., 1999). A genome-wide association study of exceptional longevity in 801 centenarians (median age at death 104 years) and 914 genetically matched healthy controls discriminated between cases and controls with 89% sensitivity and specificity, and with 58% specificity and 60% sensitivity in an independent cohort of 341 controls and 253 genetically matched nonagenarians and centenarians (median age 100 years). Consistent with the hypothesis that the genetic contribution is largest with the oldest ages, the sensitivity of the model increased in the independent cohort with older and older ages (71% to classify subjects with an age at death >102 and 85% to classify subjects with an age at death >105) (Sebastiani et al., 2012).

Aging has both programmed and stochastic features (see chapter 18). Nonheritable variations in lifespan are found in laboratory populations of inbred lines of nematodes, fruit flies, and mice. Within each inbred line, individuals show wide variations in lifespan that, expressed as the coefficient of variation, approximate those of outbred populations (Finch and Tanzi, 1997). However, the maintenance of substantial levels of genetic variation in fitness-related traits has been widely appreciated (Price and Schluter, 1991; Pomiankowski and Møller, 1995; Houle et al., 1996; Rowe and Houle, 1996). Classical theory (Fisher, 1930; Falconer and Mackay, 1996) predicts that traits closely associated with fitness will have low heritabilities while more distantly related traits should have higher heritabilities. Reviews of the literature have confirmed the fact that heritabilities of fitness components are lower than those of other traits (Falconer, 1981; Gustafsson, 1986; Roff and Mousseau, 1987; Mousseau and Roff, 1987; Price and Schluter, 1991; Houle, 1992; Messina, 1993; Falconer and Mackay, 1996; Houle et al., 1996; Kruuk et al., 2000; 2001; Merilä and Sheldon, 1999; 2000; McCleery et al., 2004; Pelletier et al., 2007; Teplitsky et al., 2009). The high additive genetic and residual variability of fitness traits might be explained by the great number of genetic and environmental events they are affected by. Thus, a substantial stochasticity and variability is displayed by genetically programmed processes like development (Smith-Gill, 1983; Vom Saal et al., 1990; Molenaar et al., 1993; Harvell, 1994; Debat and David, 2001; Kurakin, 2005; Uller, 2008; Vogt et al., 2008; Huang, 2009; Tomkins and Moczek, 2009; Hochberg et al., 2011) and reproductive activity (Gustafsson, 1986; Tuljapurkar, 1990; Finch and Tanzi 1997; Finch and Kirkwood, 2000 p. 19; te Velde and Pearson, 2002; Oliveira et al., 2008; Carey and Molleman, 2010). Stochastic gene expression in fluctuating environments appears to be, at least in part, the underlying feature (McAdams and Arkin, 1997; Kepler and Elston 2001; Elowitz et al., 2002; Swain et al., 2002; Raser and O'Shea, 2004; Thattai and van Oudenaarden, 2004; Kurakin, 2005; Cai et al., 2006; Mettetal et al., 2006; Raj et al., 2006; Kaufmann et al., 2007; Acar et al., 2008; Losick and Desplan, 2008; Raj and van Oudenaarden, 2008; Raj et al., 2010). This has confirmed the empirical generalization from laboratory studies that additive genetic variance varies with respect to trait type: characters under strong selection have low heritabilities, high additive genetic variance, and even higher environmental variance (Houle, 1992; Kruuk et al., 2000; Coltman et al., 2005; Foerster et al., 2007; Ellegren and Sheldon, 2008; Clements et al., 2011). The highly differing lifespans of e.g. leukocytes and neurons in the same organism, or queens and workers in eusocial insect colonies that are programmed by the same genome but regulated by epigenetic mechanisms argue against the validity of the low heritability argument against the genetic programming of longevity. On the other hand, the plasticity of aging is of great relevance for the fitness of organisms that can adapt their life history trajectories including reproduction and aging to fluctuating environmental conditions (Kenyon, 2005).

17.3.2 Phylogenetic continuity of genetic programming of ontogeny and aging/death.

The proponents of the ETAs are constantly walking on eggshells to find more or less plausible explanations for the many inconsistencies of the theories. After they have denied the genetic programming of aging: “Specific genes selected to promote ageing are unlikely to exist.” (Kirkwood and Austad, 2000), they argue in a strange twist of logic that “there may be adverse gene actions at older ages arising either from purely deleterious genes that escape the force of natural selection or from pleiotropic genes that trade benefit at an early age against harm at older ages” (Kirkwood and Austad, 2000). As usual, they do not dwell upon the underlying selective forces that may turn benefit into harm and obviously, genetic decay under “relaxed” selection as outlined in chapter 17.1.4 does not occur in the world of the ETAs. These arguments have not been revised in the meantime but were maintained until very recently (Kirkwod and

Melov, 2011). A simple literature search with the terms differentiation, development or ontogeny retrieves hundreds of thousands of publications where we meet old acquaintances. All the transcription factors, signaling pathways and agents that are involved in aging, e.g. p53, NF-κB, PTEN, Wnt, FOXO, TNF, JNK, GATA factors, HSPs, AMPK, sirtuins, or GSK3 have a role in ontogenetic events. Aging, co-selected with reproduction, is a process that is integrated into a huge network of pleiotropic cellular and organismal functions.

None of these manifold connections can be regarded as the result of a chance event, all are canonical signaling pathways and public aging mechanisms. There is a continuity of genetic programming and the "fossil record" of the genome (Buss, 1988) from primordial reproductive events associated with postreproductive death to multicellular ontogeny and aging/death. (Heininger, 2001; de Magalhães and Church, 2005; Budovskaya et al., 2008; Pincus and Slack, 2008). As discussed in chapter 9, conserved from yeast to humans, the nutrient-sensing IIS and TOR signaling pathways play a central role in the control of somatic longevity. TOR is a conserved Ser/Thr kinase that controls cell growth by activating an array of anabolic processes including protein synthesis, transcription and ribosome biogenesis, and by inhibiting catabolic processes such as mRNA degradation and autophagy. Phylogenetically conserved, TOR also regulates reproductivity, gamete maturation and function by coupling growth factor signaling to nutrient availability (Sananes et al., 1998; Walensky et al., 1998; Schwab et al., 1999; Weisman et al., 2001; Menand et al., 2002; 2004; Hansen et al., 2004; Deprost et al., 2005; 2007; Zhang et al., 2006; Arsic and Guerin, 2008; Chen et al., 2009; Maestro et al., 2009; Roos et al., 2009; Guiboileau et al., 2010; LaFever et al., 2010; Roa and Tena-Sempere, 2010; Ren et al., 2011; Roy and Raikhel, 2011). Importantly, downregulation of TOR signaling promotes somatic survival and inhibits reproductive activity. In D. discoideum, through the regulation of chemotaxis and signal relay, TOR plays an essential role in controlling aggregation by coordinating the two essential arms of the developmental pathway that leads to multicellularity (Lee et al., 2005; Kamimura et al., 2008; Cai et al., 2010; Liao et al., 2010). Thus, through nutrient sensing TOR controls Dictyostelium multicellular development linking nutrient stress to spore formation and stalk cell death.

17.3.3 Deep homology of cellular and organismal aging/death

Mayr (1960) argued that the emergence of new structures reflects intensification of existing selection rather than a new selective regime. Such a concept is consistent with the view characterized by Jacob (1977) as 'evolution by tinkering' and by Duboule and Wilkins (1998) as 'the evolution of bricolage,' and with the insight that has been called the deep homology of shared genetic, biochemical, cellular and developmental mechanisms (Shubin et al., 1997; 2009; Gerhart, 2000; Gilbert and Bolker, 2001; Hall, 2003).

We take for granted that both microbial programmed cell death (PCD) (Lewis, 2000; Engelberg-Kulka et al., 2006; Rice and Bayles, 2008) and developmental cell death in multicellular organisms (Potten and Wilson, 2004; Green 2010) are genetically programmed. Likewise it is beyond dispute that cell death mechanisms have been conserved over large evolutionary distances from bacteria to metazoa (Aravind et al., 1999; Heininger, 2001; Debrabant et al., 2003; Nedelcu, 2009). Further, apoptosis and the programmed cellular apoptotic mechanisms such as those related to autophagy (Lockshin and Zakeri, 2004; Xiao, 2007; Tavernarakis et al., 2008; He and Klionsky, 2009; Salminen and Kaarniranta, 2009; Seo et al., 2010; Markaki and Tavernarakis, 2011), and mitochondria (Kujoth et al., 2005; Skulachev and Longo, 2005; Lee and Wei, 2007; Bratic and Trifunovic, 2010; Seo et al., 2010) have been acknowledged as central to the proximate causes of aging. Moreover, cellular senescence is a programmed process in which telomere shortening and both pro-apoptotic and anticancerogenic processes play a role (see chapters 17.3.3 and 18). The links between cellular senescence and apoptotic cell death are evolutionarily conserved (Campisi, 2003; Fabrizio and Longo, 2008; Vicencio et al., 2008). Skulachev (1999; 2002) presented cogent evidence that programmed apoptosis and organismal death (which he termed phenoptosis) are causally linked and thus, phenoptosis is programmed. Bredesen (2004a) argued on a similar vein of thought. This, however, was deemed to imply group selection and found few supporters in the prejudiced climate of the ETAs. Phylogenetically conserved signaling pathways and effectors including p53 (Bauer et al., 2005; Rutkowski et al., 2010; Vigneron and Vousden, 2010), sirtuins (Kyrylenko and Baniahmad, 2010; Yi and Luo, 2010), DAF-16/FOXO (Birkenkamp and Coffer, 2003; Giannakou and Partridge, 2004; Salih and Brunet, 2008), mitochondrial MnSOD (Curtis et al., 2007), β-catenin (Hoogeboom and Burgering, 2009), and NF-κB (Xiao, 2007; Salminen and Kaarniranta, 2010a; b) are involved in the regulation of both development, cellular apoptosis and organismal aging. There is also extensive cross-talk and networking between these signaling pathways

(Giannakou and Partridge, 2004; You and Mak, 2005; Salminen et al., 2008b; Kawahara et al., 2009; van Leeuwen and Lain, 2009).

It would be devoid of any evolutionary logic to assume that the same effectors that program cellular senescence and death would regulate but not program senescence and death in an organismal context. These pleiotropic effectors constitute the nodes of a highly integrated cellular and organismal network that orchestrate stress, nutrition sensing, and metabolism, cellular and organismal survival, death decisions and reproductive activity (Coussens et al., 2008; Klysik et al., 2008; Hu, 2009, Holness et al., 2010; Kriete et al., 2010).

Autophagy is a catabolic membrane-trafficking process whereby cells recycle cytosolic proteins and organelles under stress conditions or during development. Induction of autophagy in response to starvation is a highly conserved ability of eukaryotic cells, indicating a critical and ancient role of this process in adapting to nutrient conditions. TOR inhibits autophagy. Under TOR-inactivating conditions, i.e., nutrient limitation, cells undergo autophagy (Díaz-Troya et al., 2008; Chang et al., 2009; Jung et al., 2010; Neufeld, 2010). Autophagy is a prerequisite for sporulation in D. discoideum (Otto et al., 2003; 2004; Tekinay et al., 2006; Rigden et al., 2009) and other unicellular eukaryotes (Kiel, 2010; Bartoszewska and Kiel, 2011). Inhibition of TOR signaling and stress activate autophagy in Chlamydomonas reinhardtii (Crespo et al., 2005; Pérez-Pérez et al., 2010). Overall, autophagy is a survival enhancing process in unicellular eukaryotes and regulator of survival/death pathways. In metazoans, longevity-promoting regimens, including DR and inhibition of TOR with rapamycin, resveratrol or the natural polyamine spermidine, have been associated with autophagy and in some cases were reported to require autophagy for their effects (Salminen and Kaarniranta, 2009; Tavernarakis et al., 2008; Vellai, 2009; Madeo et al., 2010; Jia and Levine, 2010). In a ongoing legacy, TOR signaling and the cellular autophagy machinery link the primordial sporulation response and its survival/death dichotomy to the aging phenotype of multicellular plants and animals. Mitochondria and a variety of their functions in energy metabolism, apoptosis, cellular stress responses, and oxidative stress are determinants of organismal longevity (Ku et al., 1993; Barja, 1999; De Benedictis et al., 1999; Salvioli et al., 2001; Lee et al., 2003; Curtis et al., 2007; Caldeira da Silva et al., 2008; He and Klionsky, 2009; Raffaello and Rizzuto, 2011). Mammal and bird species maximal longevities correlate with mitochondrial mutagenesis and evolutionary patterns of mitochondrial DNA diversity (Nabholz et al., 2008; 2009), arguing for the fundamental role of a mitochondrial molecular clock in both the germline and soma and its role for phylogenetic and organismal dynamics and trajectories (Heininger, in preparation). Oxidative stress as phylogenetically conserved pleiotropic mediator of trade-offs between life-history traits has been discussed in chapter 9.2. Thus, a multitude of programmed cellular processes are causally linked to organismal lifespan. It would make little sense from an evolutionary point of view why (and how?) these programmed processes should lead to a non-programmed process of organismal decay.

Several genes that can affect apoptosis (and senescence) have been found to affect Drosophila lifespan, including DPOSH, MnSOD and p53 (Bauer et al., 2005; Curtis et al., 2007; Aigaki et al., 2002; Tower, 2006). In mammals hyperactive p53 can produce an accelerated-aging-like phenotype (Ungewitter and Scrable, 2009) and in Drosophila a dominant-mutant p53 transgene can inhibit insulin-like signaling and cause increased lifespan (Bauer et al., 2007). Over-expression of both wild-type and mutant p53 transgenes indicated that, in adult flies, p53 limits lifespan in females but extends lifespan in males. In contrast, during larval development, moderate over-expression of p53 produced both male and female adults with increased lifespan (Waskar et al., 2009). Over–expression of the caspase inhibitor baculovirus p35 during larval development reduced the mean lifespan of male and female adults, and also produced a subset of females with increased lifespan (Shen et al., 2009).

It has been shown that the evolutionarily conserved links of insulin/insulin-like and TOR signaling with aging are public aging processes with some private, lineage-specific, variations at the level of individual, orthologous genes or paralogous genes under IIS regulation (Partridge and Gems, 2002; McElwee et al., 2007). Insulin signaling evolved with the appearance of multicellularity, allowing primordial metazoans to respond to a greater diversity of environmental signals. The insulin signaling pathway is split into two complementary and interacting subsystems. The functional separation of IGF and insulin signaling that is seen in mammals dates to approximately 600 million years ago, as the two distinct types of molecules are already present in the lower metazoan tunicate phylum (McRory and Sherwood, 1997). Insects have a single insulin/IGF system that may correspond to the ancestor of the dual insulin/IGF system. The TOR signaling pathway is a serine/threonine kinase of the phosphatidylinositol kinase-related kinase family and is phylogentically even older, being conserved over

large evolutionary distances in eukaryotes from unicellular algae to plants and from yeast to man (Bögre et al., 2003; Crespo et al., 2005; Jacinto and Lorberg, 2008). From the pattern of evolutionary conservation of TOR in senescence it appears to be feasible to date the evolutionary programming of aging between the advent of eukaryotes and the origin of photosynthetic eukaryotes before 1,558 Myr (Yoon et al., 2004) when eukaryotes 'enslaved' cyanobacteria to become chloroplasts (Mereschkowsky, 1905). According to these considerations the programming of aging can be dated at least to the period between these two evolutionary cornerstones before eukaryotic splitting into the animal and plant trajectories. Circumstantial evidence of aging in prokaryotes (Ackermann et al., 2003; 2007; Stewart et al., 2005; Nyström, 2007; Veening et al., 2008b; Turke, 2008; Gómez, 2010; Lele et al., 2011; Rang et al., 2011; 2012) may even reflect a more ancient origin of aging. A timing of this evolutionary novelty is however, difficult due to the phenomenon of bacterial horizontal gene transfer. At any rate, the repeated evolution of aging in asymmetrically reproducing organisms emphasizes the strong evolutionary rationale for aging in age-structured populations. Importantly, some of these microorganisms, although reproducing morphologically symmetric, were found to divide functionally asymmetric (Desnues et al., 2003; Stewart et al., 2005) corroborating the notion that aging is a feature of asymmetrically reproducing organisms (Partridge and Barton, 1993; Stearns 2000; Heininger, 2002a).

17.3.4 Life-long and transgenerational modulation of aging

A fundamental flaw of the ETAs is that the evolutionary rationale for aging was searched during the period of the organism's life when it manifests, i.e. post-maturationally. However, cumulative evidence indicates that aging trajectories are determined by genetic, epigenetic and environmental factors throughout life and even transgenerationally. Development and aging trajectories are linked genetically throughout phylogenesis (Buck et al., 1993; Birkhead et al., 1999; Jennings et al., 1999; Zwaan, 2003; Antebi, 2005; Boehm and Slack, 2005; Brakefield et al., 2005; de Magalhães and Church, 2005; Barker, 2007; Chen et al., 2007; Schippers et al., 2007; Schmidt et al., 2008; Walker, 2011). From the multifaceted evidence of a continuity of genetic factors programming ontogeny and aging, only the developmental role of TOR and IIS in both growth, maturation and aging are mentioned (see chapter 9). Intriguingly, a microRNA and its target transcription factor that control the timing of larval development in C. elegans also regulate lifespan in the adult. Lifespan extension was dependent on DAF-16 and HSF-1 transcription factors, suggesting that lifespan control is effected through the TOR and IIS pathways (Antebi, 2005; Boehm and Slack, 2005; Ibanez-Ventoso et al., 2006; Curran and Ruvkun, 2007).

Accumulating evidence indicates that both genetic and non-genetic inheritance, and the interactions between them, have important effects on evolutionary outcomes. Environmental exposures early in development have a role in susceptibility to disease in later life and mortality (Gluckman et al., 2007). In addition, some of these environmental effects seem to be passed on through subsequent generations (Jirtle and Skinner, 2007). There is increasing awareness that non-genetic information can also be inherited across generations (Jirtle and Skinner, 2007; Burton et al., 2011; Danchin et al., 2011). Non-genetic inherited information can arise through several interacting mechanisms, including epigenetics, parental effects and ecological and cultural inheritance (Hercus and Hoffmann, 2000; West-Eberhard, 2003; Jablonka and Lamb, 2005; Bonduriansky and Day, 2009; Helanterä and Uller, 2010). An extrinsic transgenerational phenotype requires a continued multigenerational exposure to the factor (often at only a specific period of development) triggering an epigenetic change. For example, good maternal behavior towards offspring (e.g., early postnatal pup licking in rodents) can program the same good maternal behavior in the female adults that then pass this on to their offspring in a similar manner (Champagne, 2008; Mcgowan et al., 2008; Szyf et al., 2008). Without the continued generational maternal behavior and epigenetic programming of the brain and behavior in the female offspring, the transgenerational phenotype would be lost (Mcgowan et al., 2008; Szyf et al., 2008). In the event a germline epigenetic alteration is involved, the exposure has the potential to promote intrinsic transgenerational phenomena, which will promote a transgenerational phenotype independent of continued environmental exposures (Anway et al., 2005; Skinner and Guerrero-Bosagna, 2009). Exposures of mother rats to particular endocrine disruptors can induce epigenetic changes in the male germline that are associated with changes in male fertility and reproductive behaviour up to four generations later (Anway and Skinner, 2006; 2008). Several studies in mammals support the hypothesis that transgenerationally inherited epigenetic alterations affect the health and longevity of future generations (Morgan et al., 1999; Lane et al., 2003; Rakyan et al., 2003; Pembrey et al., 2006; Youngson and Whitelaw,

2008; Bonduriansky et al., 2012). Transgenerational epigenetic inheritance has also been demonstrated in other eukaryotic organisms, for example, plants, yeast, nematodes, rotifers, insects and fishes (Grewal and Klar, 1996; Hercus and Hoffmann, 2000; Chandler and Stam, 2004; Bashey, 2006; Galloway and Etterson, 2007; Youngson and Whitelaw, 2008; Burton et al., 2011; Kaneko et al., 2011; Bonduriansky et al., 2012). In plants and aquatic invertebrates, intra- and interspecific competitive interactions leave their transgenerational signature on life history traits (Galloway and Etterson, 2007; Allen et al., 2008). Intriguingly, the longevity-extending effect of DR is transgenerationally inherited by rotifer offspring (Kaneko et al., 2011). Even in B.subtilis, the physiological state of the cell's ancestor (more than two generations removed) does affect the outcome of cellular differentiation and bacterial aging by epigenetic inheritance (Veening et al., 2008b). In placental animals, maternal conditions such as nutritional stress affect growth rate, immune capacity, survival and breeding performance of offspring (Festa-Bianchet and Jorgenson, 1998; Lummaa and Clutton-Brock, 2002; Lummaa, 2003; Jones et al., 2005). Germline and fetal programming of obesity, cardiovascular disease and insulin resistance has been investigated in a wide range of epidemiological and animal studies; these investigations elucidated transgenerational adaptations that effect epigenetic modification of genes involved in a number of key regulatory pathways with long-term sequelae for morbidity and mortality (Symonds et al., 2009; Carone et al., 2010; Alfaradhi and Ozanne, 2011; Ferguson-Smith and Patti, 2011; Nätt, 2011; Ozanne et al., 2011; Rakyan et al., 2011; Skilton et al., 2011; Guilmatre and Sharp, 2012; Herring et al., 2012). In humans, a link between grandparental and parental periods of low or high food availability and offspring mortality and morbidity was found (Bygren et al., 2001; Kaati et al., 2002; 2007; Pembrey et al., 2006). Similarly, F1 sons of female mice that are 50% dietary restricted during late gestation but fed ad libitum throughout their own life develop metabolic syndrome and indeed their own F2 offspring also exhibit impaired glucose tolerance (Jimenez-Chillaron et al., 2009). In another study, when the fertilized eggs of adult females born to dietary restricted dams were embryo transferred to control dams, the inheritance of metabolic syndrome was still observed suggesting that this transmission may occur via alterations in the germline of both parents (Thamotharan et al., 2007). Conditions of stress seem to be particularly important as inducers of heritable epigenetic variation, and lead to changes in epigenetic and genetic organization that are targeted to germline specific genomic sequences (Jablonka and Lamb, 2005; Jablonka and Raz, 2009; Curley and Mashoodh, 2010; Nätt, 2011; Seong et al., 2011). Intriguingly, stress exposure in intrauterine life is associated with shorter leukocyte telomere lengths in young adulthood (Entringer et al., 2010; 2011) which is a predictor for earlier onset of age-related disease and mortality (Blackburn, 2000; Epel et al., 2004; Serrano and Andrés, 2004; Epel, 2009; Lin et al., 2009; Mather et al., 2011) (see chapter 18). The telomere-shortening action also extends to early childhood (Drury et al., 2011). Embryonal, maternal-derived, stress hormones appear to be the mediators, arguing for a common neuroendocrinological mediation via stress-sensing pathways (Dufty et al., 2002; Drake et al., 2005; Robert and Bronikowski, 2010). In fishes and birds, egg yolk and albumen content of stress hormones has adverse effects on offspring development and longevity (Eriksen et al., 2003; 2007; Hayward and Wingfield, 2004; Love et al., 2005; Rubolini et al., 2005; Saino et al., 2005; Love and Williams, 2008; Gagliano and McCormick, 2009; McCormick and Gagliano, 2009). Importantly, these effects are adaptive under environmental adversity (Meylan and Clobert, 2005; Love and Williams, 2008; Love et al., 2009). Sirtuins may be one of the molecular links between food sensing and availability and both lifetime and transgenerational epigenetic modulation of aging trajectories via histone acetylation/deacetylation (Olaharski et al., 2005; Kawahara et al., 2009; Gravina and Vijg, 2010). The inducibility and transmissibility of epigenetic variants depend on developmental conditions. There are a multitude of mechanisms that may convert reversible epigenetic changes into stable epigenetic and genetic transgenerational effects (Pembrey, 1996, Young, 2001; Beaudet and Jiang, 2002; Jablonka, 2004; Cullis, 2005; Gallou-Kabani and Junien, 2005; Mittelman and Wilson, 2010). Thus, demographic responses can, over time, evolve into new, genetically mediated life-history parameters (Reznick et al., 1990; Kokko and López-Sepulcre, 2007; Jones et al., 2008; Mittelman and Wilson, 2010). Aging is still amenable to modulation in late-reproductive and post-reproductive organisms. Although not consistently (see Masoro, 2006), adult and late-life onset DR is able to modulate aging-related changes, morbidity and longevity in worms, flies and mammals (Weindruch and Walford, 1982; Yu et al., 1985; Pugh et al., 1999; Lane et al., 2000; Cao et al., 2001; Berrigan et al., 2002; Goto et al., 2002; Mair et al., 2003; Dhahbi et al., 2004; Magwere et al., 2004; Rae, 2004; Goto, 2006; Lenaerts et al., 2007; Mattison et al., 2007; Smith et

al., 2008; Sharma et al., 2010; Wang et al., 2010; Cameron et al., 2011). Likewise, late-life exercise allows to decrease morbidity and mortality (Paffenbarger et al., 1993; Sandvick et al., 1993; Bean et al., 2004; Manini et al., 2006; Middleton et al., 2008; Byberg et al., 2009; Yates et al., 2008; Safdar et al., 2011; Wen et al., 2011). This is additional evidence that the DST is flawed. According to its predictions, deviating energy away from bodily maintenance and repair should shorten, and not extend, longevity. That aging is still plastic even at advanced age and can be delayed by TOR inhibition was demonstrated in mice (Harrison et al., 2009; Miller et al., 2011).

17.3.5 A tale of immortality and mortality

It is commonplace that germline cells are virtually immortal while the soma is mortal. In fact life has been transmitted by an unbroken line of germlike unicellular organisms and later germline cells in multicellular organisms since the dawn of life approx. 4 billion years ago. Stem cells that can produce the entire spectrum of cell types found in an organism are 'totipotent' (from the Latin totus, entire). In most animals, the only cell that is truly totipotent is the fertilized egg — the zygote and its immediate descendants. Stem cells in an adult animal, such as those that continually generate blood cells, give rise to a restricted spectrum of mature cell types and are considered to be merely pluripotent (from the Latin pluris, many or several).

Many cells in plant and basal metazoa, in contrast, continue to be totipotent throughout the organism's life — a whole organism can be propagated from small pieces of tissue or even from single cells (Weigel and Jürgens, 2002). The self-renewing pool of totipotent stem cells is the cellular basis of the reproductive strategy, including sexual and asexual reproduction; totipotent cells share morphofunctional features of embryonic stem and germline cells of invertebrate metazoa (Isaeva et al., 2008; Watanabe et al., 2009). Some mortal medusae, the mobile jellyfishes, which represent the sexual form of many Hydrozoans and Scyphozoans, retain the potential for ontogeny reversal (Schmid 1972; 1992; Bode et al., 1986; Alder and Schmid 1987; Bavestrello et al. 1992; Piraino et al. 1996; 2004, Seipel et al., 2004; Boero et al., 2005; De Vito et al. 2006; Schmich et al., 2007). Retro/transdifferentiation could enable the medusae to evade death and attain potential immortality. Planarian somatic cells, called neoblasts, have retained a strong regenerative potential and express a vasa-like gene (Agata and Watanabe, 1999; Shibata et al., 1999). However, immortality does not come cost-free. Regeneration and transdifferentiation are fuelled by apoptosis of stem cells in both Hydra and Planarians (Carlà et al. 2003; Valentini, 2006; Galliot and Chera, 2010; Pellettieri et al., 2010; Chera et al., 2011). Likewise, asexually reproducing lower metazoa may achieve continuous rejuvenation and immortalization by vegetative differentiation/dedifferentiation cycles (Balser, 1998; Kawamura and Fujiwara, 2000) which appear to be related to apoptotic events (Balser, 1998). Thus the immortalization of cells is traded against the death of other somatic cells.

The telomere/telomerase system plays a role in the distinction between toti/pluripotent germline/stem cells and the soma (Monaghan, 2010). The importance of the telomere/telomerase complex as a marker of cellular and organismal senescence is discussed in chapter 18.

Mitochondria play a key role in life, death and immortalization decisions. Mitochondria are of paramount importance in germ cell specification and rejuvenation (Iida and Kobayashi, 1998; Machesky et al., 1998; Kashikawa et al., 1999; Isaeva and Reunov, 2001; van Werven and Amon, 2011; Ünal and Amon, 2011). Mitochondria are also the drivers of elevated ROS production during malignant transformation and "immortalization" of cancer cells (Heininger, 2001; Galluzzi et al., 2010; Ralph et al., 2010).

The function of vasa and other transcription repressors, e.g. nanos, are important for preserving totipotency. Upon differentiation, the planarian neoblasts lose their vasa expression and totipotency (Shibata et al., 1999). One mechanism for preserving totipotency is to inhibit expression of genes that would lead to somatic differentiation (Seydoux and Strome, 1999; Raz, 2000). If transcription is activated in primordial germ cell (PGC) precursors, PGCs are not formed. If transcription is turned on after PGC formation, PGCs often exhibit migration defects and die, or they reach the somatic gonad but their descendents degenerate (Blackwell, 2004; Schaner and Kelly, 2006). Lineage analyses have shown that loss of PGCs in C. elegans mutants is due to transformation of the germ cell precursors to a somatic cell fate (Schaner and Kelly, 2006). Similarly, inhibition of the cell death program in Drosophila Nanos mutants revealed that the dying pole cells express somatic markers, consistent with a germline-to-soma transformation (Hayashi et al., 2004). Collectively, these observations suggest that repression of mRNA transcription is essential to inhibit somatic differentiation and promote germ cell fate and viability (Seydoux and Braun, 2006). The loss of toti/pluripotency corresponds to the loss of immortality. Differentiation makes the difference between cellular and organismal immortality and mortality. Vasa and other RNA helicases are the guardians of totipotency and germline cell immortality (Navarro et al., 2001).

Several lines of evidence suggest that the potential for totipotency resides primarily in oocytes. In the male, totipotency is retained through the adult germline stem cells and then likely is lost during sperm differentiation (Seydoux and Braun, 2006). Remarkably, eggs of many species can also develop entirely normally when provided with the nucleus of a somatic cell in place of egg chromosomes or a sperm nucleus. Somatic cells do not have the ability to generate a complete organism and the nucleus of a somatic cell must be reprogrammed if it is to participate in normal development with an enucleated egg (Gurdon and Wilmut, 2011).

Taken together, the immortality of germline cells is programmed by their totipotency and lost by somatic differentiation during devlopment.

18. Program and stochasticity in cellular and organismal aging: telomeres and telomerases

I agree that theorizing is to be approved, provided that it is based on facts, and systematically makes deductions from what is observed. -Hippocrates, Precepts

Summary

Telomeres protect the chromosome extreme ends and program the number of mitotic divisions of somatic cells. Telomere length is related to cellular proliferative capacity and replicative senescence. Lifestyle, environmental and risk factors, all of them proven or suspected to be linked to increased morbidity and mortality, have been associated with shortening of white blood cell telomere length. The telomere-telomerase complex links cellular and organismal aging and epitomizes the intricate relationship of program, programmed variability and chance variation in biological systems optimizing fitness in responding to often unpredictable and stochastic environments.

For a long time, scientists thought that cells once removed from an organism would be able to replicate indefinitely, mainly as a consequence of a long-held claim by Alexei Carrel that chicken embryonic fibroblast cultures could be kept in culture indefinitely (Witkowski, 1980). Hence, there was a general perception that the finiteness of somatic life had to be unrelated to the virtual immortality of somatic cells. However, in 1961 Hayflick and Moorhead found that embryo-derived fibroblasts can divide 50 ± 10 times before arresting irreversibly (Hayflick and Moorhead, 1961). Olovnikov (1971; 1973) suggested that the cause of cellular senescence is the gradual loss of telomeres due to the end-replication problem-i.e., the inability of DNA polymerase to completely replicate the 3' end of linear duplex DNA (Watson, 1972; Harley et al., 1990; Blackburn, 1991). Specialized ribonucleoprotein complexes, the telomeres, consist of a short repeat sequence (5'-TTAGGG-3' in vertebrate chromosomes) (Moyzis et al., 1988; Meyne et al, 1989) that cap the chromosome extreme ends and protect the end of linear chromosomes from recognition as DNA double-stranded breaks and activation of a DNA damage response (Blackburn, 2000; 2005; de Lange, 2009). The number of mitotic divisions of somatic cells is programmed and telomeres appear to be a mitotic clock (Harley et al., 1990; 1992; Allsopp et al., 1992; Vaziri et al., 1994; de Lange, 1998; Wright and Shay, 2001). The mean telomere length was found to decrease progressively during serial passages of human fibroblasts (Harley et al, 1990), to be related to remaining proliferative capacity (Allsopp et al., 1992; Martens et al., 2000) and to be shorter in samples from older donors (Chang and Harley, 1995; Lansdorp, 1995). In vitro studies have shown that once a critical telomere length and state is reached, cells stop dividing and enter a state of replicative senescence (Blackburn 1991; Counter et al., 1992; Shay and Wright, 2000; Karlseder et al., 2002; von Zglinicki and Martin-Ruiz, 2005; Blasco 2007). Telomere-based replicative senescence is thought to function as a potent mechanism of tumor protection in humans (Lansdorp, 2009).

In mammals and amphibians, telomere length is reset during embryogenesis by the action of telomerase or through a recombination-based mechanism (Schaetzlein et al., 2004; Liu et al., 2007; Vizlin-Hodzic et al., 2009). Telomerase contains two components, an RNA component and a catalytic subunit referred to as the telomerase reverse transcriptase (TERT). Telomerase elongates the telomeres and thus reverses the normal telomere attrition (Greider and Blackburn, 1985). Expression of telomerase is usually required for cell immortalization and long-term tumor growth. In humans, telomerase activity is tightly regulated during development and oncogenesis (Cong et al., 2002). Overexpression of TERT, the catalytic subunit of telomerase, counteracts telomere shortening, extends the replicative potential and prevents/delays replicative senescence of cells (Bodnar et al., 1998; Vaziri and Benchimol, 1998; Yang et al., 1999; Rufer et al., 2001). In addition, there is evidence from other model animals for telomere length-independent functions of telomerase, which

appear to promote cell growth, DNA repair, stress resistance, and protection from apoptosis (Ludwig et al., 2001; Oh et al., 2001; Zhang et al., 2003; Kang et al., 2004; del Bufalo et al., 2005; Bollmann, 2008). For instance, overexpressing a protein that lengthens telomeres extends also the lifespan of C. elegans dependent on daf-16 (Joeng et al., 2004). This is intriguing because the somatic cells of C. elegans are postmitotic and are not susceptible to replicative telomere shortening (Kenyon, 2005).

Although highly variable, white blood cell (WBC) telomere length is heritable (Slagboom et al., 1994; Jeanclos et al., 2000; Nawrot et al., 2004; Bischoff et al., 2005; Vasa-Nicotera et al., 2005; Andrew et al., 2006; Hunt et al, 2008), longer in women than men (Jeanclos et al., 2000; Benetos et al., 2001; Nawrot et al., 2004; Bischoff et al., 2005; Vasa-Nicotera et al., 2005; Mayer et al., 2006; Bekaert et al., 2007; Fitzpatrick et al., 2007; O'Donnell et al., 2008) and modified by paternal age at conception (Unryn et al., 2005; De Meyer et al., 2007; Njajou et al., 2007; Kimura et al., 2008a). In humans, rare mutations in genes that are involved in telomere length regulation have been identified in monogenic diseases such as dyskeratosis congenita (Vulliamy et al., 2001; 2008; Armanios et al., 2005; Mason et al., 2005; Savage et al., 2008; Walne et al., 2008), idiopathic pulmonary fibrosis (Tsakiri et al., 2007; Armanios et al., 2007) and Werner syndrome (Chang et al., 2004; Crabbe et al., 2004; 2007; Du et al., 2004; Opresko et al., 2004) which are associated with shortened leukocyte telomere length and/or a progeroid syndrome.

Cross-sectional analyses have demonstrated that telomere length of WBCs is inversely correlated with age (Slagboom et al., 1994; Iwama et al., 1998; Benetos et al., 2001; Cherif et al., 2003; Nawrot et al., 2004; Mayer et al., 2006). Telomeric repeats are lost rapidly (at a rate of >1 kilobase per year) from the WBCs of young children, followed by an apparent plateau between age 4 and young adulthood, and by gradual attrition later in life (Frenck et al., 1998). Longitudinal studies support the finding that telomere attrition with age is the rule (Aviv et al., 2009; Ehrlenbach et al., 2009; Nordfjäll et al., 2009; Farzaneh-Far et al., 2010; Chen et al., 2011; Houben et al., 2011; Weischer et al., 2012). Telomere lengthening may occur within shorter but not longer intervals and appears to reflect measurement errors of WBC telomere lengths in relation to the duration of follow-up periods (Chen et al., 2011). The estimated mean rate of WBC telomere length shortening was 31 bp/y with a range from 23 to 47 bp/y with none of the individuals showing WBC telomere lengthening over the average 12.4 years of follow-up (Chen et al., 2011). In addition to chronological aging, WBC telomere attrition can serve as a marker of the cumulative oxidative stress and inflammation and, consequently, show the pace of biological aging. That it is a marker of biological rather than chronological aging independent of genetic influences (Bakaysa et al., 2007) was suggested by a study of Bischoff et al. (2006) among elderly and oldest old. Longer telomeres were associated with better survival (hazard ratios = 0.89 [95% confidence interval = 0.76-1.04] per 1 kb in males and 0.79 [0.72-0.88] per 1 kb in females, respectively). On the other hand, including age in the analyses changed the estimates to 0.97 (0.83-1.14) and 0.93 (0.85-1.03), respectively (Bischoff et al., 2006). Another study found WBC telomere length positively associated with more years of healthy life, but not survival (Njajou et al., 2009). The value as marker of biological aging was confirmed by recent studies in same-sex elderly twins that have found that the twin with shorter telomere length was more likely to die before the twin with longer telomere length and that longer telomere length was associated with better physical and cognitive functioning (Bakaysa et al., 2007; Kimura et al., 2008b; Christensen et al., 2009). However, the failure to establish telomere length as predictor of survival in very advanced age (Martin-Ruiz et al., 2005; Harris et al., 2006; Njajou et al., 2009; Houben et al., 2011) may be related to the high degree of telomere instability in populations of advanced age (Martin-Ruiz et al., 2005).

Shorter leukocyte telomere length is a predictor for earlier onset of age-related disease and mortality (Blackburn, 2000; Aviv, 2004; Epel et al., 2004; Serrano and Andrés, 2004; Epel, 2009; Epel et al., 2009; Lin et al., 2009; Mather et al., 2011), has been found associated with the incidence of major age-related diseases, i.e. cardiovascular disease (Benetos et al., 2001; 2004; Samani et al., 2001; Brouilette et al., 2003; 2007; Cawthon et al., 2003; Demissie et al., 2006; Fitzpatrick et al., 2007; Weischer et al., 2012), diabetes mellitus (Adaikalakoteswari et al., 2005; Sampson et al., 2006; Uziel et al., 2007; Salpea et al., 2010), cancer incidence and mortality (Willeit et al., 2010), dementia (von Zglinicki et al., 2000; Panossian et al., 2003; Honig et al., 2006), and was associated with age-related disease burden across multiple physiologic systems (Sanders et al., 2012). Certain lifestyle and environmental and risk factors, all of them proven or suspected to be linked to increased morbidity and mortality, have been associated with shortening of telomere length (Monaghan and Haussmann, 2006), including obesity (Valdes et al., 2005; Cherkas et al., 2006; Nordfjäll et al., 2008;

Zannolli et al., 2008; Kim et al., 2009; O'Callaghan et al., 2009; Al-Attas et al., 2010; Farzaneh-Far et al, 2010; Buxton et al., 211; Strandberg et al., 2011), low HDL cholesterol levels (Chen et al., 2009; Dei Cas et al., 2011; Maeda et al., 2011), carotid artery intimal medial thickness (O'Donnell et al., 2008), insulin resistance (Gardner et al., 2005a; b; Aviv et al., 2006; Demissie et al., 2006; Fitzpatrick et al., 2007; Al-Attas et al., 2010), glycemic control in diabetes mellitus (Uziel et al., 2007), low physical activity (Cherkas et al., 2008; LaRocca et al., 2010), cigarette smoking (Nawrot et al., 2004; Valdes et al., 2005; Morla et al., 2006; O'Donnell et al., 2008; Aviv et al., 2009; Strandberg et al., 2011), lower socio-economic status/educational attainment (Cherkas et al., 2006; Steptoe et al., 2011; Surtees et al., 2012), radiation (Derradiji et al. 2008), and a history of psychosocial stress such as early maltreatment, mood disorders, self-reported psychological stress and stress exposure related to being the caretaker of a chronically ill individual (Epel et al. 2004; Sapolsky, 2004; Simon et al. 2006; Damjanovic et al., 2007; Lung et al., 2007; Parks et al., 2009; Tyrka et al., 2009; Kananen et al. 2010; Surtees et al., 2011). Weight loss, induced by calorie-restricted diets, increased telomere length in the rectal mucosa of obese men (O'Callaghan et al., 2009). Thus, in addition to the stress response axis of aging (von Zglinicki et al., 2003), the metabolism axis of aging leaves its footprints on telomere attrition that is accelerated by increased resource utilization (Ahima, 2009; Kark et al., 2012).

Cells of the immune system are under enormous proliferative demand. The immune system is highly sensitive to shortening of telomeres as its competence depends strictly on cell renewal and clonal expansion of T- and B-cell populations. Cells of the immune system are unique among normal somatic cells as they can up-regulate telomerase and limit telomere attrition in the process of cell proliferation undergoing in activated cells (Kaszubowska, 2008; Andrews et al., 2010). The telomerase knockout mouse shows severe telomere dysfunction, a reduced proliferative capacity of B- and T-cells, as well as a reduction of germinal center reactivity upon immunization. Both immune system defects are landmarks of immunosenescence (Blasco, 2002). The causal role of telomerase downregulation in CD8+ T cell immunosenescence has been demonstrated (Valenzuela and Effros, 2002; Dagarag et al., 2004; Fauce et al., 2008). Short telomeres in WBCs might be indicative of immunosenescence (Effros and Pawelec, 1997), while failure of immunosurveillance may contribute to the development of age-related diseases (Franceschi et al., 2000; Pawelec et al., 2002). The important costimulatory receptor, CD28, is perhaps the closest to a biomarker of aging for human lymphocytes. Both in vivo and in vitro, the proportion of CD28+ cells decreases with age (Effros et al., 1994; Boucher et al., 1998). Moreover, telomere lengths in $CD28^{-}$ cells are shorter than in CD28+ cells from the same donors, implying that the former has undergone more rounds of cell division than the latter (Effros, 1998). The link between telomere attrition in WBCs and human immunosenescence on one hand and between immunosenescence and aging-related morbidity and mortality on the other hand (see chapter 11.3.2) may provide a plausible mechanistic explanation for the association of WBC telomere attrition with aging-related morbidity and mortality.

Which signals may provide the link between cellular and organismal aging and vice versa? There is evidence of a bidirectional feedback loop. Telomere-initiated cellular senescence depends strongly on p53 (d'Adda di Fagagna et al., 2003; Herbig et al., 2004; Cosme-Blanco et al., 2007) that is also involved in organismal aging (see chapter 17.3.3). Cellular telomerase insufficiency leads to aging-related organismal dysfunctions (Epel et al., 2006), to bone marrow failure (Marrone et al., 2005), impaired glucose tolerance and insulin secretion (Kuhlow et al., 2010) and in vitro, telomerase insufficiency has been linked to cardiovascular pathobiology (Yang et al., 1999; Oh, 2003).

Age-dependent defects in niche cells are systemically regulated and can be reversed by exposure to a young circulation (Conboy et al., 2005; Mayack et al., 2010). Metabolic cues integrate organismal and cellular aging trajectories. An increase in IIS contributes to aging of bone marrow stem cells (Mayack et al., 2010; Ratajczak et al., 2010; Kucia et al., 2012). A rise in insulin resistance was associated with an increased WBC telomere erosion (Gardner et al., 2005). Hyperglycemia induced premature replicative senescence of human skin fibroblasts after 44.42 ± 1.5 population doublings compared to 57.9 ± 3.83 population doublings under normoglycemia ($p < 0.0001$). The "point of no return," beyond which hyperglycemia resulted in irreversible progression to premature replicative senescence, occurred after exposure to hyperglycemia for as few as 20 population doublings (Blazer et al., 2002). The accelerated senescence response in cells exposed to high glucose is strongly related to oxidative stress (Ksiazek et al., 2007; Yi et al., 2009). Oxidative stress, the common final pathway of a variety of stress responses, including metabolic and psychosocial stress (Lindquist, 1986; Sanchez et al., 1992; Finkel and Holbrook, 2000; Heininger, 2001; Mittler, 2002; Mikkelsen and

Wardman, 2003; Sørensen et al., 2003; Apel and Hirt, 2004; Epel et al., 2004), has been consistently suggested to be the agent mediating telomere attrition (Ren et al., 2001; von Zglinicki, 2002; Forsyth et al., 2003; Tchirkov and Lansdorp, 2003; Kurz et al., 2004; Demissie et al., 2006; Richter and von Zglinicki, 2007; Bull and Fenech, 2008; Cattan et al. 2008; Houben et al. 2008; Ilmonen et al. 2008). Free radicals preferentially induce lesions at triplet G (Oikawa and Kawanishi, 1999), making telomeres with many kilobases of the triplet G containing repetitive sequence TTAGGG an ideal target for oxidative damage.

Public programs of aging, like the IIS, TOR and a variety of stress response-specific signaling pathways such as FOXO are phylogenetically conserved. An almost genome-wide RNAi screen in C. elegans, searching for genes that extend the lifespan of wild-type worms, identified 89 distinct candidate genes, of which 32 have a clear orthologous partner in Drosophila, mouse, and/or human (Hamilton et al., 2005). In contrast, there are private programs that are species-specific (Partridge and Gems, 2002). As a rule of thumb, public pathways appear to give clues about the ultimate, evolutionary causes of aging/death while the private programs characterize the proximate mechanisms that may differ from species to species. The telomere/telomerase complex represents a private aging program.

Telomerase-based chromosome end maintenance is a very ancient mechanism in unicellular and multicellular organisms. In Saccharomyces cerevisiae, although telomere shortening in EST1 mutants causes rapid loss of cell viability (Lundblad and Szostak, 1989), telomere length holds constant over the lifespan of normal cells, implying that telomere shortening is not a cause of aging in yeast (D'Mello and Jazwinski, 1991; Austriaco and Guarente, 1997; Sinclair et al., 1998). Both asexual and sexual planarian Schmidtea mediterranea display age-related decline in telomere length (Tan et al., 2012). However, immortal asexual planarians are able to maintain telomere lengths somatically during fission and regeneration, whereas mortal sexual animals restore telomeres by passage through a germline and embryogenesis stage (Tan et al., 2012). In invertebrates, fishes, amphibians, and reptiles persistent telomerase activity in somatic tissues might allow the maintenance of extensive regenerative potentials of these species (Gomes et al., 2010). Telomerase repression among birds and many mammals suggests that they may use replicative aging as an organismal aging and tumor protection mechanism (Gomes et al., 2010). No telomere shortening occurs throughout life of sea urchins (Francis et al., 2006; Ebert et al., 2008) and only in young, but not old, oysters (Godwin et al., 2012). Likewise, in some long-lived snake, fish and bird telomere length does not appear to decrease with increasing age (Vleck et al., 2003; Hall et al., 2004; Horn et al., 2008; 2011; Ujvari and Madsen, 2009). On the other hand, in some bird species longitudinal telomere dynamics rather than age predicted life expectancy in the wild (Haussmann et al., 2005b; Pauliny et al., 2006; Bize et al., 2009; Salomons et al., 2009; Foote et al., 2011) while cross-sectional studies often (Delany et al., 2000; Hausmann et al., 2003; Juola et al., 2006) but not always (Vleck et al., 2003; Foote et al., 2011) suggested an aging-related shortening. Similar results have been obtained in studies of telomere length in fishes (Hatakeyama et al. 2008; Hsu et al., 2008; Hartmann et al., 2009) and reptiles (Scott et al., 2006; Bronikowski, 2008; Hatase et al., 2008). Shortening of telomeres and both organismal and cellular senescence appear not to be closely related in the wild-type mouse (Wright and Shay 2000; Wang et al., 2010). On the other hand, although in the telomerase null mouse loss of telomere function did not elicit a full spectrum of classical pathophysiological symptoms of aging, age-dependent telomere shortening and accompanying genetic instability were associated with shortened lifespan as well as a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation (Rudolph et al., 1999).

On the basis that individual cells from clonally derived populations show heterogeneous division potential (Smith and Whitney, 1980) and a large heterogeneity in telomere length both between chromosome ends within individual cells and between cells (Lansdorp et al., 1996; Baird et al., 2003; Martin-Ruiz et al., 2004; Zou et al., 2004) it has been maintained that telomere attrition is no cellular aging program (Passos et al., 2007a). It was argued that the 'Hayflick limit' can only be applied to mass populations of cells, and that the lifespan of an individual cell lineage is not controlled by a defined genetic programme, but governed by stochastic factors upstream of telomere shortening, probably related to oxidative stress (Passos et al., 2007a). Along a similar line of thought it was argued that due to the large and unpredictable variability of organismal aging (Finch and Kirkwood, 2000; Kirkwood and Finch, 2002; Kirkwood et al., 2005; Sánchez-Blanco and Kim, 2011) a program that regulates aging can be excluded.

In microorganisms and mammalian cells, stochasticity in gene expression gives rise to cell-to-cell variability in protein concentrations (McAdams and Arkin, 1997; Kepler and Elston 2001; Elowitz et al., 2002; Ozbudak

et al., 2002; Swain et al., 2002; Blake et al., 2003; Raser and O'Shea, 2004; Thattai and van Oudenaarden, 2004; Colman-Lerner et al., 2005; Golding et al., 2005; Rosenfeld et al., 2005; Cai et al., 2006; Mettetal et al., 2006; Raj et al., 2006; Sigal et al., 2006; Kaufmann et al., 2007; Acar et al., 2008; Chang et al., 2008; Feinerman et al., 2008; Losick and Desplan, 2008; Raj and van Oudenaarden, 2008; Raj et al., 2010) and individual cells differ widely in responsiveness to uniform physiological stimuli (Goldstein et al., 2000; Lahav et al., 2004; Colman-Lerner et al., 2005; Geva-Zatorsky et al., 2006; Albeck et al., 2008a; b; Spencer et al., 2009). Importantly, cellular oxidative stress-dependent responses, although undoubtedly programmed, such as FOXO nuclear translocation (Essers et al., 2004), NF-KB signaling (Hayot and Jayaprakash, 2006), pro- or antiapoptotic states (Nair et al., 2004; Bagci et al., 2006; Rangamani and Sirovich, 2007; Albeck et al., 2008a; Spencer et al., 2009), and immune responses (Lipniacki et al., 2006; Feinerman et al., 2008) are also highly variable. A variety of these features may be based on the stochasticity of mitochondrial bioenergetic/oxidative events (Hüser et al., 1998; Genova et al., 2003; Passos et al., 2007b; Wang et al., 2008). Stochasticity is an integral component of cellular life-death decisions, e.g. when Dictyostelium amoebae that only differ in their cell cycle phase that it happens to be in at the time that it starves (Jang and Gomer, 2011) undergo completely different cell fates. Likewise, the differentiation-apoptosis cell fate decision during development is largely based on stochastic elements in which minute differences in both the cellular redox and energy state decide the cell fate (Heininger, 2001). Oxidative stress signaling is the prime example of a stochastic agent that can give rise to random mutations, e.g. in mtDNA, with diametrically opposed consequences for cellular outcome (Heininger, 2001). On the other hand, evidence from microorganisms and metazoan single cells indicates that variability at the cellular level is not stochastic but largely predetermined and part of the microorganisms/cells' bet-hedging strategy that increases fitness at the population level (Caporale, 1999; Avery, 2006; Veening et al., 2008a; b; Snijder and Pelkmans, 2011). Likewise, phenotypic bet-hedging was demonstrated at the organismal level when mothers, faced with unpredictable environments, succeeded to increase offspring trait variation (Crean and Marshall, 2009). It was suggested that variability of aging trajectories, like other random variabilities in quantitative and developmental traits that occur in addition to genetic and environmental influences (Gärtner, 1990; Molenaar et al., 1993; Wong et al., 2005; Kan et al., 2010), results from epigenetic "gambling" and may serve a bet-hedging purpose (Martin, 2009; 2012). A stable strategy within the ecological constraints of a species must 'cover all bases'. Chance favors the prepared genome and organisms have evolved manifold systems that generate programmed stochasticity (Caporale, 1999 and Vol. 870 of Ann NY Acad Sci). Stress response pathways with their final stochastic messengers reactive oxygen and nitrogen species and their role in stochastic mutagenesis are of utmost importance to the manifestation of aging (Parsons, 1995; 2002; 2005). The eminent importance of stress response pathways as an early predictor of longevity was demonstrated in C. elegans. Induction of a heat shock protein in response to a sub-lethal heat stress showed significant, not heritable, variation among isogenic individuals and was an excellent predictor of life expectancy on the second day of adult life (Rea et al., 2005). Stochastic as well as genetic factors are operative in C. elegans aging, with extensive variability both among same-age animals and between cells of the same type within individuals (Herndon et al., 2002). As elaborated by Frank (2011), developmental variability and learning enhance nonheritable phenotypic variation, which in turn can accelerate evolutionary response. Like developmental variation, variation of aging trajectories may smooth the fitness landscape. A smoothed fitness landscape profoundly alters evolutionary dynamics in a way that greatly accelerates adaptation to novel or extreme environmental challenges (Frank, 2011).

Taken together, telomere attrition is not only a biomarker of cellular senescence but also of biological organismal aging (Bekaert et al., 2005; von Zglinicki and Martin-Ruiz, 2005). The telomere/telomerase complex epitomizes the intricate relationship of program, programmed variability and chance variation in biological systems optimizing fitness in responding to often unpredictable and stochastic environments.

19. Germ-soma conflict theory and its implications for evolutionary theory

Summary

The germ-soma conflict theory of aging/death has implications for concepts of evolutionary theory, specifically fitness and units of selection. Apart from reproductive fitness, a multitude of definitions of fitness emphasize survival as a component of fitness. However, survival is only

evolutionarily relevant in terms of "survive to reproduce" which gives the inclusion of survival into the definition of fitness a tautological connotation. Like the vectorial, unidirectional design of genes, fitness of genotypes is a vectorial, directed entity that is relayed like a baton from generation to generation.

The different lifestyles of sessile and mobile organisms were causal to their different germline segregation strategies. The reproductive division of labor and the ensuing germ-soma conflict with its Red Queen coevolutionary motor underlie the evolutionary dynamics of functional differentiation within and between organisms and their speciation. Evolution shaped the bauplan of organisms as result of hierarchical or multi-level selection.

19.1 Fitness

According to Ariew and Lewontin (2004), no concept in evolutionary biology has been more confusing and has produced such a rich philosophical literature as that of fitness. Krimbas (2004) stressed that fitness is a conceptual device, a useful tool, only for descriptive purposes of selective processes, changing from case to case, and thus devoid of any substantial physical counterpart. Natural selection is an implicit or explicit constituent of any definition of fitness. And like natural selection, fitness is a forward-looking concept, its most defining element being the change in representation of the organism's genes in the population (Ariew and Lewontin, 2004). Apart from reproductive fitness, a multitude of definitions of fitness, particularly in the literature on aging and longevity, emphasize survival as a component of fitness (e.g. Williams, 1957; Brandon, 1990, p. 15; Schluter et al., 1991; Barton and Partridge, 2000; Michod and Nedelcu, 2003; Michod, 2005; Drenos et al., 2006; Jones et al., 2008; Kirkwood and Melov, 2011; Bouwhuis et al., 2012). However, as stressed by Krimbas (2004): "Fitness is the mean number of progeny left; therefore viability components (survival, longevity) are important as far as they affect the net reproductive effect. Longevity may be important in those cases where it may affect the net reproductive effect. Selection is blind to longevity at a post-reproductive age". Thus, survival is only evolutionarily relevant in terms of "survive to reproduce", respectively "survive to increase offspring fitness". Thus, the inclusion of "survival" into the definition of fitness gives it a tautological connotation. The ETAs claim that in terms of fitness, aging and death are maladaptive and according to this thinking increased longevity, i.e. higher fidelity of somatic maintenance is deemed to have a fitness value in itself. Directional selection is roughly twice as strong on mating success (in sexually reproducing populations) and fecundity as compared with survivorship (Endler, 1986; Hoekstra et al, 2001; Kingsolver et al., 2001; Siepielski et al., 2011). The cumulative evidence allows to redefine fitness in a world of limited resources. Somatic survival has no end in itself but has only an evolutionary value if it is in the interest of reproductive success. Thus, it makes sense that female salmon arriving early at their spawning grounds have a longer post-spawning survival (to guard their nests so that the site cannot be reused, which would drastically reduce the survival of her eggs) than late-arriving females (Morbey and Ydenberg, 2003; Hendry et al., 2004; Morbey et al., 2005). Or that female Octopus cyanea survive for a month after spawning, during which time they brood their eggs (which are otherwise unprotected) and generally die a few days after the eggs hatch (Wodinsky, 1977; Van Heukelem, 1983). Another behavior enhancing the fitness of the progeny (and selecting for postreproductive longevity) appears to be the ability of postreproductive aphids to defend the colony by immobilizing predators with abdominal waxy secretions that accumulate during the course of adult aging (Uematsu et al., 2010). This insight entails another understanding: like the vectorial, unidirectional design of genes, fitness of genotypes is a vectorial, directed entity that is passed on like a baton. Iteroparous organisms can pass on batons at several occasions but after their last reproductive event their only evolutionary raison d'être is to increase the fitness of their progeny.

Natural selection shapes genomes to optimize their reproductive success (de Magalhães and Church, 2005). The evolutionary emphasis is on optimization, not maximization. Thus it makes sense that gonadal hormones limit the reproductive potential by effecting reproductive senescence (chapter 11.2.4). Most species have "learned" the evolutionary lesson that survival at the carrying capacity of their habitat is preferable to oscillations between feast and famine (see chapter 5), each population bottleneck carrying the risk of extinction. In conjunction with the limited resources paradigm and the tragedy of the commons (see chapter 5.4), evolution provided the framework to constrain the evolution of a Darwinian demon. That these constraints may prove insufficient if a species, like the humans, is able to excel at resource acquisition is currently shown in the man-made ecological crisis.

19.2 Units of selection

There is a long-standing controversy about the units of selection. In the 1960ies and '70ies there was a strong opposition to any group-selective concepts (Williams

1966; Maynard Smith, 1964; 1976). In the last decades there has been a renaissance of group-selective concepts often formulated as hierarchical or multi-level selection theory (Frank, 1998; Sober and Wilson, 1998; Keller, 1999; Michod, 1999a; Gould, 2002; Hammerstein, 2003; Rice, 2004; Okasha, 2006; Godfrey-Smith, 2009). I think the controversy is predominantly fuelled by its focus on mobile animals. A close look at the selective pressures in a community of sessile organisms should have ended the controversy in an instant (Stevens et al., 1995; Goodnight and Stevens, 1997). Strangely enough, neither the books of Sober and Wilson (1998), Gould (2002), nor Okasha (2006), to name only a few, base their arguments on plants or sessile animals (but see Wilson and Sober, 1989; Clarke, 2011). In fact, the different germline segregation strategies are already the outcome of the different selection pressures acting on sessile and mobile organisms and, compared to mobile animals, the stronger impact of community level selection pressures in modular organisms (Goldberg et al., 2001). It has been shown (Simpson, 2012) that the total extent of functional differentiation is larger if there is a reproductive division of labor. Thus, the early segregation of the germline during ontogeny in mobile animals was causal to the evolution of their different bauplans and life histories. Sessile and mobile organisms and their lifestyles prove that there is a hierarchy of levels of selection as elaborated by Gould (2002). In fact, plant ecosystems can be regarded as superorganisms (Wilson, 1987; Wilson and Sober, 1989). It is the degree of integration of genes, cells, organisms, species, and communities, the intensity of their interactions that determines the strength of natural selection at the different levels.

20. The ecological shaping of lifespan

From the elephant to butyric acid bacterium—it is all the same! -Albert Jan Kluyver, 1926
Anything found to be true of E. coli must also be true of elephants. -Jacques Monod, 1954

Summary
Aging trajectories are shaped by environment by genotype interactions. A multitude of genetic and ecological factors determined life history traits during deep evolutionary time. Laboratory experiments on reproduction-longevity trade-offs rarely take ecological conditions into account. The ecological conditions underlying negligible senescence and differential lifespans of breeding and non-breeding individuals in socially breeding species are further case studies demonstrating the wide validity of germ-soma conflict theory-related ecological reasoning.

Among the basal assumptions of the Modern Synthesis are: (i) organisms are largely genetically homogeneous; (ii) the parts work for the good of the whole; (iii) within-organism selection is of no evolutionary consequence (Okasha, 2006). At least the latter two of the three assumptions are questioned by the present work. Within an organism there is a bitter fight over the utilization of resources, a fight that ends with the death of the soma. A multitude of genetic and ecological factors determined life history traits during deep evolutionary time (Illustration 2). And the coevolutionary dynamics of the conflict of interests between germline cells and soma determine the major evolutionary events and phenomena, the Cambrian explosion, the evolutionary success of sexual reproduction and the mortality of the soma.

Studies with mutated model animals provided invaluable insights into the signaling pathways controlling aging. However, they do not reflect the natural environments in which the life history traits of the organism including the aging phenotype evolved. Many animal models are so highly inbred, and are maintained under such unnaturally favorable conditions that fitness decline is to be expected when these animals are released into the wild (Bryant and Reed, 1999; Lynch and O'Hely, 2001; Araki et al., 2007; 2009). Likewise, genetic effects become difficult to interpret in the context of "normal" aging (e.g., Hoffmann and Harshman, 2000; Van Voorhies et al., 2005; Kawasaki et al., 2008; Burke and Rose, 2009). For instance, there is experimental evidence that lifespan and reproductive investment can be decoupled in benign circumstances (Stearns, 1992; Reznick et al., 2000; Tatar et al., 2003; Partridge et al., 2005; Ricklefs and Cadena, 2007; Lee et al., 2008; Grandison et al., 2009b; Khazaeli and Curtsinger, 2010; Anderson et al., 2011; Flatt, 2011), including conditions that mitigate oxidative stress or its sequelae (Golden et al., 2002; Ruan et al., 2002). Long-term laboratory evolution in benign conditions may even result in a break down of correlations between longevity and stress resistance (Archer et al., 2003; Phelan et al., 2003), one of the most robust associations. Multiple stressors, however, may unmask the trade-offs and fitness costs of non-adaptive, non-ecologically determined longevity (Marden et al., 2003; Jenkins et al., 2004). At any rate, whether and how these ecological factors affect the reproduction/longevity trade-off depends on the

genetic background and thus the evolutionary history of a species (Arking et al., 1993; Leips and Mackay, 2000; Vieira et al., 2000; Spencer et al., 2003; Kaeberlein et al., 2005).

Stearns (1992, pp. 214–218) elaborated a table listing experiments that were designed to look for evidence of trade-offs between fertility and longevity in diverse animal species. About half the studies found some relationship and half found none. Mitteldorf (2004) argued that the "observation of an artificially evolved fly that is fitter in every aspect of its life history than the wild type calls into question a postulate of the evolutionary theory of life histories (Stearns, 1992; Charlesworth, 1994). A great deal of this mathematical science derives from the premise that natural selection optimizes life histories for reproductive potential, which is a weighted integral of fertility over an individual lifetime. If this quantity is under strong directional selection in nature, it should be very difficult to increase it further by laboratory selection. Rose's O strain, evolved in just a few hundred generations, appears to have both a longer lifespan and a higher fertility than natural selection has been able to evolve over a vastly longer period in the wild." The mostly abundant resource availability in the laboratory makes these studies hardly transferable to conditions in the wild habitat. Resource availability that underlies the evolutionary rationale of life history trade-offs clearly is one of its most essential moderators.

Ecology of "negligible" senescence

Species with negligible senescence can have a shorter or a longer lifespan (Finch 1990). A long lifespan says nothing about whether a species shows senescence or not (Roach 1993). Thus, species with extreme lifespans should not rashly be cited as species that have managed to escape senescence – they only live at a very slow pace. Whether they escape senescence is a different question. Theoretical results from evolutionary demographic models (Vaupel et al. 2004; Baudisch 2008) suggest that the pace (the time-scale on which mortality progresses) and shape (time-standardized pattern of mortality) of aging are two distinct dimensions that can vary independently (Baudisch, 2011). In the rockfishes, longevity increases with maximum depth (Cailliet et al. 2001) and competitive environment (Bonsall and Mangel, 2004; Mangel et al., 2007). Rockfishes (Sebastes spp.) are relatively sedentary, deeper depths are relatively colder and have low O2 concentrations, and food resources are scarce: all of which suggest a decline in rockfish metabolic activity (rate of living) with depth (Bonsall, 2006). Moreover, the habitats of rockfishes appear to be structured with regard to age and water depth: older individuals living in greater depths (Cailliet et al. 2001; Ingram, 2011) thereby minimizing resource competition with younger individuals. Generally, arctic populations, due to their metabolic rate depression and resistance to oxidative stress, live much longer than their temperate or tropical counterparts (Haldorson and Craig, 1984; Munch and Salinas, 2009; Buttemer et al., 2010; Philipp and Abele, 2010; Ungvari et al., 2011).

Among both mammals and birds, long lifespans are associated with low fecundity (Holmes et al., 2001; Read and Harvey, 1989). Holliday (1995, p. 114) has shown that longevity of mammals has an inverse correlation with their fecundity. Hamilton (2011) showed that fecundity is a function of body mass in mammals and that female placentals, marsupials and monotremes of the same body size all allocate energy to reproduction at essentially the same rate. "Extrapolating" these relationships to other classes of organisms and taking into account the constraints imposed on the evolution of a Darwinian demon it can be inferred that organisms with negligible senescence have either poor reproductive success or otherwise they would become the dominant species in their habitat. In turtles, for example, a long lifespan and high reproductive investment (Congdon et al., 2003; Wallace et al., 2007) is counterbalanced by a very low egg-to-adulthood survival (Frazer, 1986).

Like unitary organisms, tree species appear to display trade-offs between longevity and growth/reproductive success (Poorter and Bongers, 2006; Easdale and Healey, 2009; Wright et al., 2010). Due to a heavy investment in growth, extremely long-lived tall plant populations (Finch, 1990, p. 209) have a poor reproductive success (Van Valen, 1975; Falster and Westoby, 2003; Petit and Hampe, 2006). As trees tend to live in comparatively stable habitats and generation turnover is slow, only an extremely small fraction of the seeds produced during an individual's lifetime will eventually survive to maturity. This has important consequences for trees' evolution. First, the considerable selection potential during early life stages should favor local adaptation of recruits, particularly for traits that enhance competitive ability (such as early growth and delayed maturity). By contrast, selective culling during trees' establishment appears to have little influence on population demography (Franco and Silvertown, 1997). Second, because much of the density-dependent mortality takes place before maturity in trees, their effective population size should be closer to the actual adult census size compared to herbs, contributing to preserve genetic diversity (Dodd and Silvertown, 2000). There is an apparent trade-off between growth rate to the age of 50 years and longevity, i.e. fast early growth is associated with

decreased longevity in trees (Bigler and Veblen, 2009; Schweingruber and Wirth, 2009). Conversely, individuals growing in extreme sites within the natural range of a given species exhibit a higher longevity than those thriving in mesic sites (Schulman, 1954; LaMarche, 1969; Ward, 1982; Kelly et al., 1992; Schweingruber and Wirth, 2009). Tree leaf and stem lifespans were found to be highly correlated to metabolism, stems displaying a much lower metabolism than leaves. Consequently, the extremely high longevity of trees may be explained by the lower metabolism displayed by the stems. These results clearly reflect different energy allocation and energy expenditure rate strategies between leaves and stems, which may result in different senescence rates (and lifespans) in these organs (Issartel and Coiffard, 2011).

Sociality

The ETAs are unable to explain the differential lifespans of breeding and non-breeding individuals in social species. Particularly, why should a reproductively inactive organism that can allocate all resources to somatic maintenance have a shorter lifespan than breeding organism, contrary to the predictions of the DST? In eusocial insects like hymenopterans (e.g. ants, bees, wasps) and termites, reproductive individuals live significantly longer than non-reproductive "helpers" (Hölldobler and Wilson, 1990; Keller, 1998; Heinze and Schrempf, 2008; Remolina and Hughes, 2008; Gordon, 2010). Several eusocial, cooperatively breeding species of subterranean African mole-rats, live in multigenerational families where reproduction is skewed towards a few breeding individuals (Jarvis, 1981; Bennett and Faulkes, 2000). Due to strict inbreeding avoidance (Burda, 1995), most of their offspring remain as reproductively quiescent ''helpers'' in their natal families unless removed from their colonies and paired to an unfamiliar mate. This holds true also when one or both breeding animals die. Breeding individuals live approximately twice as long as non-breeding kin irrespective of social rank or other potentially confounding factors (Dammann and Burda, 2006; Dammann et al., 2011). Studies in sterile worker honey bees may provide mechanistic clues to eusocial differential longevity. Ovarian signaling in these honey bees tie social behaviour and longevity to variation in ovary size (Amdam et al. 2006, 2007; Page and Amdam 2007; Münch et al., 2008). The germ-soma conflict theory can readily explain the phenomenon. As the conflict is shaped predominantly by the interests of the germline cells, their breeding success that is assured by the helpers, the latter's status as helpers and fallback germ cell reservoir determines their evolutionary value. When both functions become obsolete, the organisms are eliminated as redundant competitors for resources.

21. Concluding remarks

What is utterly baffling to me is why one cannot be a reductionist and a holist at the same time. -John Tyler Bonner, The Evolution of Complexity, 1988

Pauca sed matura (few, but ripe) -Favorite quotation of Carl Friedrich Gauss (1777-1855)

The presented theory is based on the cumulative and compelling evidence that was compiled for almost 20 years from some 400,000 publications and a plethora of books from evolutionary biology, ecology, microbiology, epidemiology, paleontology, molecular biology, botany, gerontology, endocrinology, immunology, and neurobiology. Its early stages date back to my occupation with Alzheimer's disease (Heininger, 1999a; b; 2000a; b), when I realized that a deeper understanding of biological and pathological processes can only be achieved by "unearthing" the "fossil record" of the genome (Buss, 1988). So far, aging theories suffered from a too narrow scope of data and gauge into evolutionary space of time. The awareness that the evolutionary roots of aging date back to unicellular organisms had to prompt a quest for the unicellular/facultative multicellular processes that were later co-opted for multicellular life history strategies. The genetic and phenotypic evidence indicates that reproduction and aging/death are inextricably linked. Thus, this theory is the result of a highly comprehensive study of aging and death, integrating all known facts about aging into a single holistic, consilient concept and realizing, in its truest sense, a systems biology and eco-evo-devo approach (Blute, 2008; West and Bergman, 2009).

As Kuhn (1970) pointed out, science is puzzle solving. When embarking on my tour d'horizon I found a couple of "island solutions" to the picture of aging in its ecological-evolutionary context which, although they appeared to be coherent within themselves, did not fit together at their margins. Thus, the ETAs, apart from their logical fallacies, were found to be incompatible with: (1) the ecological concept of a world of limited resources; (2) life history theory suggesting a continuum of semelparous/iteroparous reproduction strategies; (3) the genetic evidence of a programmed aging process; (4) the phylo-/ontogenetic findings that development and aging are regulated by the same, phylogenetically conserved signaling pathways. The island solutions had to be broken up, the puzzle had to

be rearranged completely. The resulting solution to the puzzle is highly robust in terms of invading alternative solutions due to the plausibility, size and coherence of the eco-evo-devo network. It also resolves a variety of scientific controversies that were fuelled by the island solutions.

Postreproductive death and aging are interpreted as caused by an intricately tuned, dynamic, co-evolved network of environmental and genetic factors, a network that gains evolutionary robustness by the sheer number of interacting nodes (Hintze and Adami, 2008; 2010; Yukilevich et al., 2008; Kartal and Ebenhöh, 2009; Kriete et al., 2010; Whitacre and Bender, 2010; Bryden et al., 2011; Parshany et al., 2011, Whitacre, 2011), a network that can be modulated but that for evolutionary and ecological reasons no living being can escape.

The data that have been accumulated build, in my opinion, a strong case for the germ-soma conflict theory of aging/death:

* It could be shown that the basic assumption of the ETAs that natural selection does not "see" postreproductive organisms is flawed. Likewise, the other assumptions are flawed and the ETAs are based both on circular reasoning and a fallacious post hoc, ergo propter hoc argumentation. In the light of genetic evidence these arguments even imply some sort of Lamarckian-type inheritance (in its original sense of genetic heritability of somatic change). There are dozens of inconsistencies of the ETAs with the ecological and experimental data and with evolutionary theory. Some are minor, others are, at least based on the cumulative evidence of the last 20 years, literally hair-raising.

* A simple change of perspective from a soma-centered to a germ/offspring-centered point of view renders somatic aging/death fitness-enhancing and adaptive for the offspring and hence favored by natural selection.

* Evolution cannot be indifferent to postreproductive individuals due to the limited resources paradigm and the fitness costs of resource competition. In unitary organisms, evolution "prevented" the intergenerational "tragedy of the commons" not by a group selective mechanism but by making the germline cells the ultimate censor of parental somatic longevity.

* The evolutionary roots of reproduction as stress/starvation response with its survival/death dichotomy are reflected by the resource and stress-dependent signaling pathways that control aging/death and exhibit antagonistic pleiotropic patterns throughout phylogenesis.

* Reproduction and aging/death have been co-selected. Reproduction is not only a circumstantial factor causing its cost on somatic survival by drainig resources due to a source-sink relationship but by actively advancing aging through its energetic costs necessitating increased resource utilization and by actively eroding the somatic immune competence and stress resilience.

* In the oligotrophic environment, postreproductive survival of the ancestors beyond the brood care period has a negative fitness impact for the offspring. Life history strategies, including postreproductive survival are adaptive and are shaped by the co-evolutionary mediation of conflict.

* The aging soma is under both metabolic and oxidative stress, witnessing that the aging process does not happen to the soma due to wear and tear or waning selective forces but is imposed upon the soma by extrasomal forces. And there is no evolutionary rationale in the concept that the soma, due to resource limitation, should voluntarily let happen the decay in the first place and later invest resources into fighting it.

* Germ cell signaling promotes aging/death by a multitude of effector molecules that limit longevity, and impair immune competence and stress resilience. The mortality plateau of old age is a logical consequence of the reproductive aging-related declining intensity of germline cell signaling. The human gender gap of life expectancies is also readily explained from the gender dimorphic dynamics of gonadal hormone persistence in old age.

* As a corollary to the germ-soma conflict theory and its Red Queen coevolutionary dynamics, a conceptional framework for the Big Bang evolution of the Cambrian Explosion is put forward. The same rationale applies for the fitness and evolvability advantage of sexual reproduction (Heininger, in preparation).

With some right, during the first decades of their lifetime the ETAs carried the "evolutionary" in their name. However, the ETAs and their proponents completely ignored the ecological and developmental aspects of aging that emerged later. At the latest, when the genetic programming of aging became undeniable the ETAs lost the factual authority to carry this adjective. The ETAs hitchhiked on the Darwinian principles but perverted their central tenets, misinterpreted epiphenoma of aging and due to a logical fallacy held them for the evolutionary rationale of aging. The founders of the ETAs formulated their theories based on the facts that were known at that time. Particularly, they did not know that aging is genetically programmed (a fact that their adherents deny until today). The scientific establishment followed their arguments for too long although sufficient evidence emerged that invalidated the basic

assumptions.

Common descent with modification was not a discovery of Charles Darwin (e.g. Gould, 2002; Ruse, 2009). When he published The Origin of Species (1859), evolutionary thoughts were around for quite a while and Lamarck (1809) was the first to present a full-blown evolutionary theory. Ruse (2009) wrote: “Unlike earlier thinkers, what was of great concern to Darwin was the cause or causes of evolution. Without causes, he was no more than one among many evolutionists. Without causes, he could never be the Newton of biology.” In fact, what Darwin accomplished and because of which he is rightly celebrated as the founding father of evolutionary theory was that he devised a plausible mechanism, natural selection, by which evolution brings about macroevolutionary change. When I presented the abstract of my paper to science journalists for review, asking for support in a media campaign (in the hope to draw the attention of the scientific community to take note of my theory), one of them commented that the idea of a resource competition between parents and offspring is not new! To reiterate the role of limited resources in the causation of aging was never the primary topic of this work. The idea has been around for at least 120 years (and dismissed by the ETAs). Another version of this concept hardly would have justified a major effort of 20 years’ duration. Rather, I present compelling arguments for the first plausible mechanism, the germ-soma conflict theory, extending an earlier publication (Heininger, 2002) and elaborating how evolution co-selected reproduction and aging/death to coerce organisms into a resource management program.

So far, the germ-soma conflict theory (Heininger, 2002) was totally ignored by the scientific community. (Was it because of its length of 55 pages so that it could not be published in one of the mainstream journals; because in the era of information overload such a format requiring an intense occupation with the topic is selected against [but see above Carl Gauss's favorite quotation]; because so far I did not “sell” the concept at a scientific conference; or a mixture of all three?). In his biography, Max Planck (1949, p. 33) remarked: A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it. In “The Structure of Scientific Revolutions” TS Kuhn argued that Planck overstated the case. With Hull et al. (1978) (showing that, ten years after the publication of the Origin of Species, less than 10 percent of the variation in acceptance of evolutionary theory is explained by the age of the scientist) I am optimistic (or naïve?) enough to hope that Max Planck was wrong. During his whole life, Francis Crick emphasized the importance of theories and models in guiding experimental work and helping to eliminate lines of research leading to dead ends (Morange, 2008). My evidence-based theory will be instrumental in revising the unrealistic hopes, fostered by the ETAs, for a “cure of aging” (which does not mean that aging cannot be modulated along its proximate mechanisms). It can be expected that the implementation of this change of paradigm will have a huge impact on the life sciences altogether, and evolutionary biology, life history theory, ecology, and gerontology in particular.

22. Abbreviations

AMPK: AMP-activated protein kinase
AP: antagonistic pleiotropy
CE: Cambrian explosion
DIF: differentiation-inducing factors
DR: dietary restriction
DST: disposable soma theory
EDCs: endocrine-disrupting chemicals
ETAs: “evolutionary theories of aging”
FGF: fibroblast growth factor
FGFR: FGF receptor
GC: glucocorticoid
GnRH: gonadotropin
GSCs: germ stem cells
GSK3: glycogen synthase kinase-3
GSR: general stress response
HPA: hypothalamic-pituitary-adrenal
HPG: hypothalamic-pituitary-gonadal
IIS: insulin, insulin-like growth factor (IGF) and insulin/IGF-like signaling
JH: juvenile hormone
LD: linkage disequilibrium
MA: mutation accumulation
MnSOD: manganese superoxide dismutase
PCD: programmed cell death
QTLs: quantitative trait loci
ROS: reactive oxygen species
TERT: telomerase reverse transcriptase
TNF: tumor necrosis factor
TOR: target of rapamycin
WBC: white blood cell
Wnt: derived from the Drosophila Wingless (Wg) and the mouse Int-1 genes
20E: 20-hydroxy-ecdysone

23. References

Note: References can be found in the additional files section.

Illustrations

Illustration 1

Figure 1

Antagonistic pleiotropy of signaling pathways in the adult organism. ↑ denotes activation/protection; ⊤ denotes inhibition. Consistently, pathways that act to increase germline fitness, decrease adult somatic fitness and vice versa.

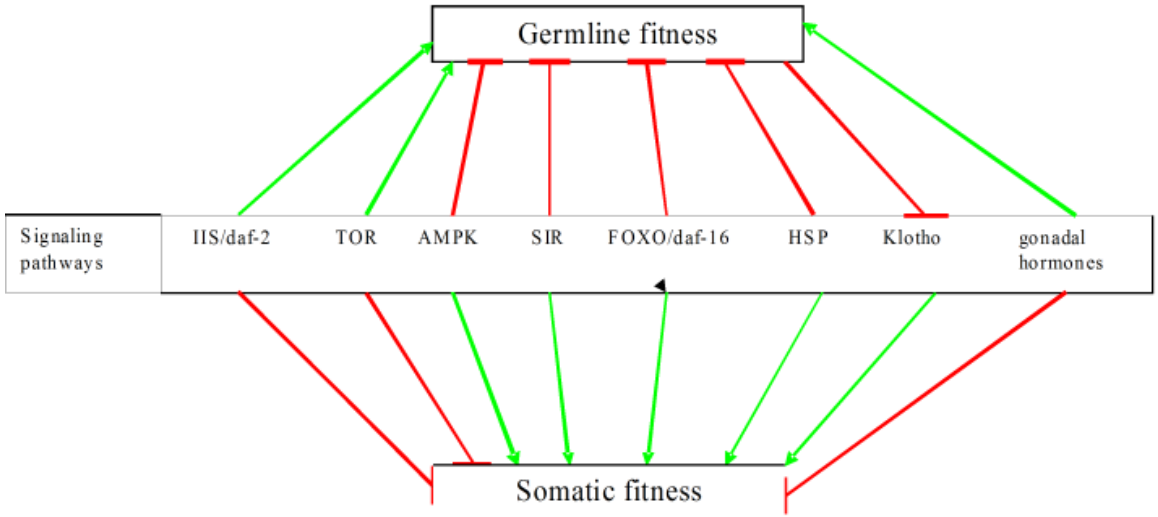

Illustration 2

Table 1

	Environment			
Genes/Development	Resources	Stressors	Behavior	Miscellaneous
genotype	availability	competitors	dominance	timing
epigenetics	acquisition	density	coping	stochasticity
constraints	allocation	infectious agents	sociality	threshold trait
genetic	utilization	predators	dispersal	isolation
developmental	economy	abiotic stressors	migration	body mass
physiological	nutrient composition	temperature		
gender	variability	saisonality		
unitary/modular	herbivore/carnivore	water/drought		
maturation		irradiation		
		unpredictability		
		toxins		

Covariants shaping the reproduction/longevity network. The table should be understood as network, each variable being an interactive node (with different weight) linked with a variety of other variables in a large regulatory network. The list does not claim to be complete.

Disclaimer