Why Some Fungi Senesce and Others Do Not

An Evolutionary Perspective on Fungal Senescence

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Short Summary

Fungi are generally considered to be modular organisms with no clear distinction of a germ line: with the expansion of the mycelium, chances for reproduction are expected to increase, and each unit under favourable circumstances may produce offspring. Fungi with such modular body plans are expected to be long-lived, as most fungi indeed seem to be. However, fungi exist that do senesce, and their growth often seems to be limited by space or time. For these fungi, we can consider the term ‘pseudo-unitary’, as life history details and ecological conditions constrain the size of the soma and the opportunities for reproduction. We may predict the life history traits and ecological conditions that favour such evolution of fungal senescence. Known proximate mechanisms of fungal senescence can be viewed in the light of this evolutionary context.

Introduction

Organisms with a ‘unitary body plan’ – such as many mobile animals – have a determinate structure composed of strict numbers of body parts, specialised organs and a separate germ line. Unitary organisms are arguably all subject to the process of senescence, resulting in death even under protected idealised conditions. Organisms with ‘modular body plans’, however – including plants, fungi and (sessile) animals such as hydroids and bryozoans – seem to escape this process and are potentially immortal. Modular organisms are composed of multiple genetically identical vegetative modules that may remain attached or become separated to form physiologically independent clones. Here all cells are in principle totipotent and capable of expansion and reproduction, so death of parts of a modular organism does not necessarily cause the death of the whole organism (Figure 17.1). In plant biology these modules are generally referred to as ‘ramets’. The ensemble of ramets that makes up a single genetic entity is generally referred to as a ‘genet’. The body plan of an organism has major implications for the way
selection can act. In modular organisms, selection rarely acts directly on the genet: the unit of selection is usually the ramet.

Fungi are considered to be modular organisms with no clear distinction of a germ line. With the expansion of the mycelium, chances for reproduction are expected to increase,

Figure 17.1  Germ-line and soma overlap to various extents in different organisms. (A) Mushroom-forming fungi are typical modular organisms with indeterminate growth. Any somatic cell (potentially with somatic mutations) can become part of the germ line (white arrows). (B) In unitary systems such as most animals (left), a small and distinct subset of cells is reserved that contributes its hereditary material to the next generation (indicated in grey; this subset of cells must be immortal). In modular systems such as plants and fungi (right) all cells can do this. We propose that an intermediary situation of these two extremes exists as well, in which germ line and soma partially overlap, for which we propose the term (pseudo-unitary) (middle). In pseudo-unitary organisms the indeterminate modular growth of the colony is restricted e.g. by the limited availability of substrate.
and each unit under favourable circumstances may produce offspring (Figure 17.1B,
right). Fungi with such modular body plans are expected to be long-lived, as most fungi
indeed seem to be. However, some fungi exist that do senesce, and their growth often
seems to be limited by space or time. For these fungi, we consider the term ‘pseudo-
unitary’ (Figure 17.1B, centre), as life history details and ecological conditions constrain
the size of the soma and the opportunities for reproduction. In this chapter we discuss
why fungi are usually long-lived and why there are exceptions to this. We predict life
history traits and ecological conditions that favour the evolution of fungal senescence.
Finally, we discuss the proximate mechanisms of fungal senescence in the light of this
evolutionary context.

Evolutionary Theory of Senescence

Evolutionary adaptations occur via natural selection of heritable traits that increase
fitness. Senescence, however, seems to be a highly maladaptive trait: it is thus not
immediately clear why it would have evolved in the first place. Charles Darwin surpris-
ingly never addressed this paradox. One of the earliest evolutionary explanations of
senescence was that made by Alfred Russel Wallace, the co-discoverer of evolution by
natural selection. In a footnote to the English translation of the 1881 essays of the
German theoretician August Weismann, he essentially suggested that immortality would
be sacrificed for the sake of reproduction, an idea that was embraced by Weismann and
would be echoed many years later in the works of Medawar (1952), Williams (1957) and
Kirkwood (1977). Paramount to the latter idea is the distinction between germ line and
soma (see Figure 17.1). Weismann stated that whereas germ-line cells are able to
transmit hereditary information to the next generation, somatic cells should not.
In other words, there should be a unidirectional flow of hereditary information from
germline to soma, but not the other way round. This concept is commonly known as the
‘Weismann barrier’, and it implies that senescence is a property of the soma and cannot
be a property of the germ line: The germ line must be evolutionarily immortal. In line
with this, modular organisms do not have a clear distinction between germ line and
soma, and indeed many appear not to senesce. Whereas individual modules such as
fruiting bodies may outlive their usefulness and die, the organism as a whole is generally
expected to be long-lived. In line with this, fungal fruiting bodies usually last at most
a single season (with the exception of, for example, polypores or bracket fungi, which
form woody perennial fruiting bodies), but mycelia can last for decades, producing new
fruiting bodies every year or whenever local conditions are permissive.

A further specification can be made for the conditions that allow for the evolution of
senescence. Theoretical work has shown that an important prerequisite for senescence to
evolve is that parents and offspring can be individually identified (Partridge & Barton
1993). This, for instance, can explain senescence in bacteria with asymmetrical division
(Ackermann et al. 2003) and even in bacteria with binary fission (Stewart et al. 2005).
In the former, parents and offspring differ in size and morphology, and in the latter,
parents and offspring can be identified by the age of the pole region of the cells.
Senescence evolves in the shadow of natural selection (Medawar 1952). This is the central tenet of the evolutionary biology of senescence. Organisms experience a certain (statistical) risk of death by predation, disease or other accidental causes of mortality. Any trait that would manifest only at an age at which the organism is long expected to have died from extrinsic causes would not be under selection. This means that regardless of the nature of this trait, which may be beneficial but also deleterious or even lethal, it would be able to drift through the population. This idea is generally known as the ‘mutation accumulation theory of ageing’ (Medawar 1952). Late-acting deleterious traits with pleiotropic, beneficial effects early in life may even be favoured by natural selection. The latter is commonly known as the ‘antagonistic pleiotropy theory of ageing’ (Williams 1957). These two ideas are not mutually exclusive. A more refined version of the antagonistic pleiotropy theory of ageing is the ‘disposable soma theory’ (Kirkwood 1977): this is based on the premise that somatic maintenance and repair are costly and states that resource allocation between maintenance and repair will be optimised so that the total reproductive output of an organism is maximised. This implies that given a certain regimen of extrinsic mortality, just enough metabolic resources will be invested to maintain an organism in a proper state only for the duration of its expected lifetime. The latter two theories use optimisation arguments balancing life span with reproduction (Partridge & Barton 1993), while the disposable soma theory translates the genetic argument of the antagonistic pleiotropy theory into candidate physiological mechanisms (Zwaan 1999).

Also, for the theory on ‘negative senescence’ (Vaupel et al. 2004), reproduction is of importance: ‘negative senescence’ is defined as a decline in mortality and is generally accompanied by an increase in fecundity. Especially, indeterminate-growth species for which size and fertility increase with age are expected to experience negative senescence.

Are the preceding ideas, which were developed largely with unitary organisms in mind, also applicable to fungi? The main issue may be the unit of selection: in unitary organisms, the unit of selection, by definition, is the genet. In a fungus, however, this can be both the genet and the ramet depending on its particular life history and ecological setting. The modular nature of fungi normally protects them from suffering systemic extrinsic mortality, thus preventing selection at the level of the genet. Some life histories and ecological settings may make fungi much more prone to systemic extrinsic mortality. These are the lack of a vegetative dispersal phase and the occupation of a spatiotemporally restricted (e.g. ephemeral) ecological niche. These conditions favour selection at the level of the genet and may result in a ‘pseudo-unitary’ growth pattern (Figure 17.1B).

**Fungi Can Be Extremely Long-Lived**

Intrinsic to their way of growing, fungi experience a strong reproductive gain with age. Fungal colonies typically expand radially, and their surface is directly proportional to the amount of fruiting bodies they can form. Therefore, the reproductive value of a colony may strongly increase with age, leading to negative senescence (Vaupel et al. 2004) when mortality rates decrease. One can think, for instance, of the increasing
circumference of the colony in fairy ring–forming basidiomycetes or as a function of the surface of the colony. In other words, fungi have much to gain by growing old and are unlikely to evolve senescence. This type of argument also applies, for example, to organisms such as size-indeterminate fish (Reznick et al. 2002) and mole-rats (Buffenstein 2005). These animals do not stop growing after reaching the age of maturity and strongly benefit from an ever-increasing reproductive output.

One of the most famous and most-quoted examples to illustrate fungal longevity is the Honey mushroom *Armillaria bulbosa*, nowadays known as *Armillaria gallica*. Smith et al. (1992) reported a single mycelial clone of this fungus that was approximately fifteen hectares in size. These authors (very conservatively) estimated it to be 1,500 years old and about ten tons heavy, which is about the mass of an adult blue whale. Since then, many more cases of ‘humongous fungus’ have been found. Similarly striking examples of longevity are also commonly found in clonal plants. Quaking aspen (*Populus tremuloides*), for example, can spread clonally via root suckers, and a single (male) stand of Aspen in Utah (USA) was estimated to be 80,000 years old and 6,000 tons (Barnes 1975; Grant et al. 1992).

**But Some Grow Old and Die within a Matter of Weeks**

Despite their reputation as long-lived organisms, some fungi do grow old and die within a matter of weeks (for an overview of fungi in which senescence/degeneration has been observed, see Table 17.1). One of the most intensively studied examples of senescing fungi is the ascomycete *Podospora anserina*. All natural isolates of this species that have been collected show mitotic (a.k.a. replicative or proliferative) senescence; their growth reduces over time and eventually stops (Rizet 1953; van der Gaag et al. 1998; van Diepeningen et al. 2008a). Post-mitotic senescence – loss of viability of formed mycelium – also occurs but has not been studied in great detail. The life span of a *P. anserina* strain is usually tested in long glass tubes referred to as ‘race tubes’ and expressed as distance (e.g. centimetres) or time (e.g. days) of growth. As cultures ‘go down the tube’, they progressively decline in vigour, usually commencing with a decline in female fertility, followed by a decline in mycelial growth rate and ending in arrest and death of the mycelial growth front. At the dying mycelial front, hyphae show various types of morphological aberrations and accumulate large amounts of lipofuscin, a yellow-brown pigment that is a rest product of fatty acid oxidation, also seen as the ‘ageing pigment’ in, for example, human tissue (Munkres & Rana 1978b). The formation of lipofuscin may be symptomatic of mitochondrial degeneration (see later).

The transition of the culture to a senescent state (i.e. the onset of senescence) correlates with the appearance of an infectious element originally referred to as the ‘determinant’: when senescent and non-senescent (young) sub-cultures of the same isolate are inoculated together, the mixture typically adopts the shorter life span of the senescent culture. To date, there is no consensus on the exact nature of this infectious determinant element, but it is possible (if not likely) that multiple elements are involved,
including both suppressive mitochondrial DNA (mtDNA) lesions and protein-based epigenetic factors. Examples of the multiple candidates for determinant elements in fungi are, for instance, mitochondrial plasmids, mobile mitochondrial introns and retrotransposon-like elements (e.g. Jamet-Vierny et al. 1980; Kudryavtseva et al. 2012; Osiewacz & Esser 1980; Tudzynski & Esser 1979; Tudzynski et al. 1980; Wright et al. 1982).

Similar phenomena have been described in other fungal genera, including *Neurospora*. However, fungal populations are typically polymorphic for the senescence trait (i.e. it is found in only a fraction of the strains). Senescence has, for example, been found in about a third of all the Hawaiian *N. intermedia* wild types and in about a fifth of *N. tetrasperma* isolates (Debets et al. 1995; Griffiths & Bertrand 1984, Ulrich & Bertrand 1985; Maas et al. 2003; Rieck et al. 1982; see also Maheshwari & Navaraj 2008).

In *Neurospora*, senescence can also be demonstrated in race tubes, as is routinely done in *Podospora*, but it is more commonly demonstrated by serial sub-culturing the isolates

### Table 17.1 Fungi with Observed Senescence or ‘Degeneration’

<table>
<thead>
<tr>
<th>Genus</th>
<th>Senescing/degenerating species</th>
<th>Life style</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascobolus</em></td>
<td><em>A. immersus</em></td>
<td>Coprophilic</td>
<td>Francou 1981; Marcou 1960</td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
<td><em>A. amstelodami</em></td>
<td>Stored products</td>
<td>Caten 1972; Lazarus et al. 1980; Lazarus &amp; Kuntzel 1981; van Diepeningen et al. 2006, 2008b</td>
</tr>
<tr>
<td></td>
<td><em>A. niger</em></td>
<td>saprobic</td>
<td></td>
</tr>
<tr>
<td><em>Cercophora</em></td>
<td><em>C. gradiuascela, C. samala</em></td>
<td>Coprophilic</td>
<td>Geydan et al. 2012</td>
</tr>
<tr>
<td><em>Chaetomium</em></td>
<td><em>C. nigricolor, C. pachypodioides</em></td>
<td>Coprophilic, saprobic</td>
<td></td>
</tr>
<tr>
<td><em>Cordyceps</em></td>
<td><em>C. militaris</em></td>
<td>Entomopathogenic</td>
<td>Xiong et al. 2013</td>
</tr>
<tr>
<td><em>Cryphonectria</em></td>
<td><em>C. parasitica</em></td>
<td>Plant pathogenic</td>
<td>Baidyaroy et al. 2011</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td><em>F. tricinctum, F. fujikuroi</em></td>
<td>Plant pathogenic</td>
<td>Leslie &amp; Summerell 2006; Geydan et al. 2012</td>
</tr>
<tr>
<td><em>Heterobasidion</em></td>
<td><em>H. parviporum</em></td>
<td>Forest pathogen</td>
<td>Vainio et al. 2014</td>
</tr>
<tr>
<td><em>Humicola</em></td>
<td><em>H. variabilis</em></td>
<td>Coprophilic</td>
<td>Geydan et al. 2012</td>
</tr>
<tr>
<td><em>Menthazizium</em></td>
<td><em>M. anisoplae</em></td>
<td>Entomopathogenic</td>
<td>Wang et al. 2005</td>
</tr>
<tr>
<td><em>Neurospora</em></td>
<td><em>N. crassa, N. intermedia</em></td>
<td>Burned substrate</td>
<td>Court et al. 1991; Griffiths &amp; Bertrand 1984, Marcinko-Kuehn et al. 1994; Rieck et al. 1982</td>
</tr>
<tr>
<td><em>Phomopsis</em></td>
<td><em>P. subordinaria</em></td>
<td>Plant pathogenic</td>
<td>Geydan et al. 2012</td>
</tr>
<tr>
<td><em>Podospora</em></td>
<td><em>P. anserina, P. curvicolta</em></td>
<td>Coprophilic</td>
<td>Rizet 1953; Gagny et al. 1997; Geydan et al. 2012</td>
</tr>
<tr>
<td></td>
<td><em>P. flatula, P. setosa, P. tetraspera</em></td>
<td>Coprophilic</td>
<td></td>
</tr>
<tr>
<td><em>Sordaria</em></td>
<td><em>S. macrospora</em></td>
<td>Coprophilic</td>
<td>Gagny et al. 1997; Marcou 1961</td>
</tr>
<tr>
<td><em>Zopfella</em></td>
<td><em>Z. longicaudata</em></td>
<td>Coprophilic</td>
<td>Geydan et al. 2012</td>
</tr>
<tr>
<td><em>Unknown</em></td>
<td><em>Pezizales sp.</em></td>
<td>Coprophilic</td>
<td>Geydan et al. 2012</td>
</tr>
</tbody>
</table>
using vegetative spores (macroconidia). After a strain-specific number of transfers, the fertility of the cultures, their macroconidial production and growth rates decline. The last cultures typically still show conidiation, though none of the spores produced are able to germinate. Similar to *Podospora*, death often (but not always) coincides with morphologically aberrant hyphae and lipofuscin accumulation (Munkres & Rana 1978a).

Reminiscent of the ‘senescence determinant’ from *Podospora*, the onset of senescence correlates with the appearance of an infectious element. Similar to *Podospora*, there is no consensus on the nature of this element: although the senescence process in *Neurospora* involves horizontally transmitted mitochondrial plasmids (see later), plasmids can be transmitted independently from senescence (Debets et al. 1994).

Both in *Podospora* and in *Neurospora*, the onset of senescence is a strain-specific trait, and life span is inherited largely (but not strictly) maternally (Marcou 1961; Rizet 1953, 1957). This is because mitochondria play a major role in the senescence phenomenon. Senescence is always accompanied by a ‘mutational meltdown’ of the mitochondrial genome, whereby certain regions are amplified and/or mtDNA molecules with large rearrangements (e.g. insertions and deletions) accumulate. The energetic decline that results from this mitochondrial meltdown is likely the major proximate agent of senescence.

Also within the genus *Aspergillus*, senescence or senescence-like phenomena have been reported. In ‘ragged’ mutants of *A. amstellodami*, for example, a retro-transposon-like element inside the mitochondrial genome seems to be the cause of senescence (Lazarus et al. 1980). In *A. niger* colonies that are heavily infected with double-stranded RNA (dsRNA) mycoviruses, a senescence-like phenotype can sometimes be observed in sectors of the mycelium. The majority of mycovirus infections (occurring in approximately 10 per cent of *A. niger* wild-type isolates), however, do not seem to cause such effects (van Diepeningen et al. 2006, 2008b).

In a virus-free strain of *Cryphonectria parasitica*, an altered form of mtDNA has been associated with hypo-virulence and senescence (Baidyaroy et al. 2011). Recently, Vainio et al. (2014) described how the forest pathogen *Heterobasidion parviporum* – a typical candidate for immortality/postponed or negative senescence – senescence and dies through its dsRNA mycovirus infection. Also, the colony sectorisation of *Metarhizium anisopliae*, accompanied by oxidative stress and loss of fertility, can be seen as a sign of senescence, though there the cause of the phenomenon has not been linked to any (extra) genomic element (Wang et al. 2005).

**Ecological Conditions that Favour the Evolution of Senescence**

Many fungi occupy niches in which they are limited neither spatially nor temporally. The earlier-mentioned species of *Armillaria*, for example, is usually found as an innocuous saprophyte living on organic matter in the soil. It can spread over large distances via specialised root-like bundles of hyphae called ‘rhizomorphs’. Spatially as well as temporally it is therefore potentially unlimited in its growth and expected to be extremely long-lived. Fungi that grow on more ephemeral and/or spatially restricted
substrates, however, may be expected to be short-lived: after depletion of the short-lived substrate and spore production, the remaining mycelium has lost its usefulness and has become disposable. These ‘pseudo-unitary’ fungi include parasites of short-lived hosts, endophytes, saprophytic species specialising on dung, bones, feathers and similar substrates, etc. Several examples are illustrated in Figure 17.2.

Most of the fungi that have been observed to date to senesce prove to have a coprophillic life style and belong to ascomycetous genera, including *Podospora*, *Ascobolus*, *Sordaria*, *Chaetomium* and *Cercophora* spp. (Böckelmann & Esser 1986; Gagny et al. 1997; Geydan et al. 2012; Marcou 1961). A broad phylogenetic approach that included also non-coprophillic members of these genera showed that fungal senescence evolved independently in several clades within the sordariomycetes as a function of the ephemerality of the substrate (Geydan et al. 2012). In the study by Geydan et al. (2012), approximately 70 per cent of the coprophilic species proved to senesce (seven of ten of the strains isolated from dung in that study and nine of thirteen of all the coprophilic fungi tested).

In line with the expectations of a link between ephemeral substrate and senescence, cases of senescence or senescence-like phenomena have also been observed in both

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**Figure 17.2** Examples of spatiotemporally restricted ecological niches in which fungal senescence is expected to evolve. (A) Mosquito (*Anopheles* sp.) that was killed by the fungal parasite *Beauveria bassiana*. (B) Spruce cone with fruiting bodies of the Sprucelon cap (*Strobilurus esculentus*). (C) Birch bolete (*Leccinum scabrum*) with a parasitic fungus. (D and E) Cow bones with an unidentified basidiomycete.

All wild-type isolates of *P. anserina* senesce, but they do so in a strain-dependent manner: some strains senesce faster than others, and life spans on nutrient-rich media vary from less than one week to over three weeks (van der Gaag et al. 1998; van Diepeningen et al. 2008a). In the case of the different senescent *Neurospora* species, growing on burned vegetation, the fraction of senescent strains varies between species and even populations, and 70 per cent or more of a population may have a non-senescent phenotype (Debets et al. 1995; Griffiths & Bertrand 1984; Maas et al. 2005; Rieck et al. 1982; see also Maheshwari and Navaraj 2008). Thus, fungal strains can show (genetic) variation in whether or not they senesce and in their rate of senescence. Natural variation in susceptibility to senescence plasmids may exist, and restrictions in somatic fusion between mycelia can limit the spread of fungal senescence diseases (Bastiaans et al. 2014).

Fungi show an extreme diversity in ecological strategies, and these are notoriously difficult to classify in a single scheme. In *r/K* selection theory, for example (after the growth rate parameter *r* and carrying-capacity parameter *K* from the Verhulst equation of population dynamics), a distinction is made between growth-rate-selected species (*r*-strategists) that exploit less crowded niches (e.g. primary colonisers) and species that exist at densities close to the carrying capacity of their ecological setting (*K*-strategists). According to *r/K* selection theory, the former are species with a high reproductive output and low survival, and vice versa. There are relatively few species that can be classified as pure *r*- or *K*-strategists, however: most fungi simultaneously exhibit features of both. It is certainly not true that fungal species with *r*-selected traits are always short-lived and those with *K*-selected traits are always long-lived. For example, in the ecological succession on dung, primary colonisers (including mainly zygomycetes) specialise on easily degradable carbon sources. They are all fast-growing species and poor competitors that invest mainly in short-lived vegetative spores. The mycelia of these primary colonisers, however, tend to be long-lived. Late species (including mainly ascomycetes and basidiomycetes) specialise on complex carbon sources such as cellulose. They are all relatively slow-growing species and good competitors that invest in robust sexual spores. These late species, however, tend to be short-lived. As discussed later, there are important differences in the life histories of these early and late species that may explain why.

Also, when we consider an alternative scheme such as Grime’s triangle theory (Grime & Pierce 2012), which was developed for plant ecology, it is not easy to classify typical senescent or long-lived fungal species. Grime’s three extreme ecological strategies are competitor (*C*), stress tolerator (*S*) and ruderal (*R*). Looking again at the ecological succession on dung, the primary colonisers could be considered typical ruderals that are fast-growing and rapidly producing large amounts of spores. Tested in the laboratory, these species will keep growing as long as there is available substrate, but in nature they are rapidly outcompeted. The late species on dung with their specialised carbon source utilisation are these better competitors, but also they are limited in their life span by the ephemeral dung and
need to reproduce fast. To do so, the obligate sexual species *P. anserina* has adapted its reproductive mode: instead of being heterothallic and needing a chance partner of opposite mating type, it has become pseudo-homothallic and solved the mate problem by containing two different nuclei of opposite mating type.

**Reproductive Strategies that Favour the Evolution of Senescence**

Life history traits like reproductive mode or place in the succession of a new environment can also influence the evolution of senescence: *P. anserina* is an obligate sexually reproducing fungus and is an example of a relatively late species in the ecological succession on dung. The most relevant difference with early species (primary colonisers) is that late species such as *Podospora* usually do not spread via vegetative spores (Figure 17.3): all vegetative mycelium modules of an individual colony consequently share the same fate. This implies that the unit of selection will always coincide with the genetic individual (genet) as a whole. In this sense, it is similar to unitary organisms. Many coprophilic ascomycetes and basidiomycetes share these properties and are similarly expected to be short-lived.

In contrast, in fungi that have an important vegetative dispersal stage, the unit of selection will usually coincide with the ramet. Species of *Neurospora*, for example, are primary colonisers of burnt vegetation (Figure 17.4). Although the true spatiotemporal restrictions of this niche are unknown (a burning site may remain open for many years, but it is unknown to what degree it will be suitable for *Neurospora*), *Neurospora* species can spread very efficiently across post-fire sites via wind and/or insect dispersal of vegetative spores (macroconidia). In *Neurospora*, the unit of selection will therefore usually coincide with the individual clone (ramet) rather than directly with the genetic individual (genet) as a whole. In line with this, *Neurospora* cultures are much longer-lived than those of *Podospora*.

Contrary to expectation, however, as also discussed earlier, senescence can be found in some *Neurospora* strains and, depending on the geographical location, even at a relatively high frequency (e.g. in about a third of the *N. intermedia* isolates from Hawaii; see earlier). One possible explanation for this is that the agricultural practice of regular sugar cane burning in these locations has created conditions that would limit the fungus to a ‘pseudo-unitary’ system: the regular pre-harvest fires kill the somatic tissue (mycelium and spores) but not the sexual spores (ascospores). Instead, these fires induce germination of sexual spores, thus introducing discrete generations (separating parent from offspring) and effectively increasing the shadow of selection.

**Proximate Mechanisms of Fungal Senescence: Evidence for Trade-Offs between Life Span and Reproduction**

The core premise of the antagonistic pleiotropy theory is that genetic trade-offs shape the evolution of senescence. In fungi, there is substantial evidence for such
trade-offs. First of all, fungi respond to calorie restriction by postponing reproduction for the sake of longevity: ‘calorie restriction’ refers to a dietary regimen that is low in calories but without malnutrition. Its life-span-extending effect was first noted in rodents (McCay et al. 1935), and since its initial discovery, it has been documented in many different organisms ranging from yeast (Lin et al. 2002) to primates (Ingram et al. 2004; Lane et al. 2001). Calorie restriction is associated with increased resistance to various kinds of stress, including, for example, heat and oxidative stress, and appears to forestall many late-onset diseases, including cancer (Berrigan et al. 2002; Hursting et al. 2003; Sohal & Weindruch 1996; Weindruch & Walford 1988). This kind of response allows organisms to postpone reproduction and survive unfavourable conditions. When food intake is restored, calorie-restricted individuals are typically still able to reproduce, whereas controls that are fed ad

Figure 17.3 Life cycle of Podospora anserina. P. anserina is a secondary homothallic ascomycete that grows on dung. It produces sexual spores that are shot onto the surrounding vegetation and eaten by herbivores. Passage through the gut subsequently induces germination and the cycle starts over. Because it does not spread via vegetative spores (unlike Neurospora, see Figure 17.4), there is a discrete vegetative phase.
libitum are post-reproductive or no longer alive. The plasticity of these (life history) traits induced by calorie restriction is therefore of clear selective value (Holliday 1989). This also applies to fungi: in *P. anserina*, calorie restriction increases life span by forestalling both the onset and the progression of mtDNA instability (van Diepeningen et al. 2009). Since mtDNA integrity is required for producing quality offspring, this response allows a postponement of reproduction. *Podospora* cultures that are grown on highly reduced glucose levels (e.g. <0.02 per cent (w/v)) do not produce any fruiting bodies, and they are at least five-fold longer-lived than controls grown on high glucose levels (e.g. 2.0 per cent (w/v)). When the medium is changed to a high-glucose one, these cultures are able to produce fruiting bodies again.

*Figure 17.4* Life cycle of *Neurospora crassa*. *N. crassa* is a heterothallic ascomycete that grows on burnt vegetation. It produces both sexual spores (ascospores) and vegetative spores (macroconidia). The sexual spores remain in the soil until their germination is triggered by fire. The vegetative spores are spread by wind and/or insects. Due to the vegetative dispersal phase, a single genet can spread across burning sites and potentially survive indefinitely.
The exact molecular mechanisms by which calorie restriction works have still to be elucidated, but it probably acts through a conserved regulatory programme that induces a wide array of physiological changes rather than by simply delaying the overall metabolic rate (reviewed by Guarente and Picard 2005). There are clear indications that mitochondria play a vital role in this regulatory programme: from the bakers’ yeast *Saccharomyces cerevisiae* it is known that calorie restriction requires an up-regulation of mitochondrial respiration. This is because calorie restriction requires energy conversion to become more efficient, and the most efficient means of energy conversion is respiration. Under high-glucose conditions, it is affordable or even favourable to ferment, since fermentation is much faster than respiration. Under low-glucose conditions, however, it is favourable to respire in order to conserve energy. Similar rules apply to *P. anserina*, in which a shift can be predicted from a situation (under high-glucose conditions) where respiration and (e.g. lactic acid) fermentation occurs simultaneously to a situation (under low-glucose conditions) where respiration is exclusively favoured. In line with this, factors that interfere with mitochondrial respiration effectively block the life-span-extending response to calorie restriction in *Podospora* (Maas et al. 2004).

Aside from calorie restriction, fungal life span can be extended by pharmacological interventions and genetic modifications in the respiratory chain. All these interventions and modifications have a profound effect on sexual development. This is probably best explained by the dual effect of mitochondria: mitochondria produce the energy required for sexual development, but they also form free radicals that can damage the cell. They convert the energy that is stored in foodstuffs via a series of redox reactions into a proton gradient across their inner membrane. This proton gradient is subsequently used by the ATP synthase (complex V) to phosphorylate ADP. The force of this proton gradient can act as a counter-force of the redox reactions themselves: excessive gradients therefore potentially inhibit (or even invert) electron transport, which causes free-radical formation. This constitutes a direct mechanistic link between cellular energy production and senescence (Merry 2002; Miwa & Brand 2003).

In *Podospora*, as in most other organisms, there are three complexes that are together responsible for the bulk of the inner-membrane gradient: complexes I, III and IV. Targeted knockouts and spontaneous mutations of these complexes all cause an extreme life span extension (immortalisation) at the cost of female fertility: complex I deletion strains have a strongly reduced male and female fertility (Kudryavtseva et al. 2012; Maas et al. 2010); complex III/IV deletion strains are female sterile (Dufour et al. 2000; Maas et al. 2009; Sellem et al. 2007). These mutations decrease the formation of free radicals and stabilise the mitochondrial genome; mutations in mtDNA-encoded subunits or chaperones and co-factors of these complexes have a similar effect (Borghouts et al. 2002; Osiewacz & Nuber 1996).

Individual knockouts of the main respiratory complexes are viable in fungi due to the existence of alternative electron transport chain components. An alternative NADH dehydrogenase (ND1p), for example, bypasses complex I (Maas et al. 2010), and an alternative oxidase (AOXp) bypasses complex III and IV (Lorin et al. 2001). Therefore, complex I and the ensemble of complexes III and IV can operate semi-independently. Interestingly, the over-expression of these alternative pathways in the respiratory
mutants simultaneously restores both senescence and fertility: over-expression of NDI1 restores all physiological defaults associated with complex I malfunction (Maas et al. 2010); over-expression of AOXp restores those associated with complex III/IV malfunction (Lorin et al. 2001; Maas et al. 2009; Sellem et al. 2007). It is unknown how this effect occurs, but it could be through an effect on the activity of the remnant complexes or the up-regulation of an upstream pathway (Sellem et al. 2009).

Recent work by Plohnke et al. (2014) analysing the mitochondrial proteome in *P. anserina* suggests that reactive oxygen species (ROS) produced by the respiratory complexes may not cause a gross general and non-selective accumulation of damaged proteins during senescence, as suggested in Harman’s mitochondrial free-radical theory of ageing (Harman 1972). Instead, ROS may be effective by controlling specific regulators of mitochondrial function, and in that way they may control the age-dependent expression of specific proteins (Plohnke et al. 2014).

In short, the calorie-restriction response and the response to respiratory-chain modifications provide a clear mechanistic basis for antagonistic pleiotropy. Aside from antagonistic pleiotropy, however, there is also substantial evidence in fungi for an accumulation of detrimental factors. There is a plethora of plasmids, viruses and other molecular parasites that exert their influence only in the shadow of natural selection. These are not mutations in the strict sense of the mutation accumulation theory, but they do restrain the life span of fungi when the selection shadow is removed.

**Proximate Mechanisms of Fungal Senescence: Evidence for Mutation Accumulation**

In *P. anserina*, a variety of mitochondrial DNA elements has been described that could classify as molecular parasites. In their molecular behaviour, many of these elements bear striking resemblances to bacteriophages, including a ‘lytic’ and a ‘lysogenic’ phase, as well as possible conjugation behaviour. They do not have an effect on reproduction early in life, at least none that we know of, and their ubiquitous presence is therefore likely the result of mutation accumulation mechanisms rather than antagonistic pleiotropy.

In *P. anserina*, the most prominent of these elements is ‘senDNAα’, in earlier scientific literature also referred to as ‘pl-DNA’ for ‘plasmid-like DNA’ because of its resemblance to bacterial plasmids. SenDNAα is a 2.5-kb circular element that derives from the self-splicing and reverse transcription of the first intron of the mitochondrial *COX1* gene. The latter intron is present in all isolates that have been collected thus far (Kück et al. 1985; van Diepeningen et al. 2010a). Intron α is a retro-transposon (Sellem et al. 1993) and can integrate elsewhere in the mitochondrial genome, leading to major structural rearrangements (e.g. via recombination events between the native intron and an ectopically integrated copy). These rearrangements are usually large deletions partially or entirely covering intron α and including a variably sized mtDNA region bordering the intron. The fact that virtually all long-lived derivatives that were found contained such rearrangements and lacked senDNAα (or otherwise did not accumulate it) initially suggested a causal link between the element and the senescence process.
(Belcour & Vierny 1986; Koll et al. 1985; Kück et al. 1985; Osiewacz et al. 1989; Schulte et al. 1988; Vierny et al. 1982). This led to a widespread belief that senDNAα itself was the proximate cause of senescence. Ultimately, however, it proved to be just one of many factors: a more precise deletion of the intron from the mitochondrial genome (in strain mid26, for mitochondrial intron deletion), for example, did not abolish senescence (Begel et al. 1999), and it is now clear that the life-span-extending effect of most retro-transposition mutations involving intron α are in fact due to the respiratory chain defects associated with these mutations (see preceding section). It is possible, if not likely, that the molecular behaviour of intron α is both a consequence of the senescence process and an integral part of the problem, reinforcing it via positive feedback: the degree of senDNAα accumulation may reflect transcriptional activity at the COX1 locus and, hence, respiratory activity. Its destabilising retro-transposition behaviour may itself be triggered by mtDNA damage resulting from respiratory activity (e.g. free-radical- or replication-error-mediated damage). The molecular behaviour of intron α thus may be a proxy for respiratory chain activity and its damaging effects while at the same time reinforcing them.

Aside from intron α, almost 40 per cent of all P. anserina strains contain invertron-type mitochondrial plasmids of the pAL2-1 homology group. These plasmids are autonomously replicating linear DNA molecules that accumulate in the mitochondrial matrix (analogous to the accumulation of senDNAα) and insert into the mitochondrial genome, leading to structural rearrangements (analogous to the retro-transposition of intron α). Under nutrient-rich culturing conditions, the pAL2-1 homologues are effectively neutral (Maas et al. 2004; van der Gaag et al. 1998). This is because the accumulation and insertion of the plasmids only occur during the senescent phase of the culture, in the shadow of other physiological problems. However, under life-span-extending conditions, their effect becomes clear: under calorie-restricted conditions, pAL2-1 homologue-carrying cultures die within a matter of weeks, whereas plasmid-free controls are several-fold longer-lived (Maas et al. 2004; van Diepeningen et al. 2008; 2009; 2010b). In other words, their effect is drawn out of the shadow of other mortality factors.

Despite the analogy with intron α, interesting asymmetries exist between intron α and pAL2-1 homologues, in that intron α is transmitted vertically in a harmless ‘prophage-like’ mode, whereas the pAL2-1 homologues seem to rely also on horizontal transmission (van der Gaag et al. 1998). Also, calorie restriction seems to ‘tame’ intron α (van Diepeningen et al. 2008a), but not the pAL2-1 homologues (Maas et al. 2004). A point could thus be made that pAL2-1 constitutes an infectious disease, whereas intron α is an inherent senescence factor.

Similar elements exist in species of Neurospora: for example, the invertron-type plasmids KALILO (pKAL) and MARANHAR pMAR) and the retro-plasmids VARKUD (pVAR) and MAURICEVILLE (pMAU). These show a behaviour that is similar to the pAL2-1 homologues and senDNAα, respectively, except that the retro-plasmids from Neurospora are not an integral part of the main mitochondrial genome. All these elements show accumulation and/or (re)insertion into the mitochondrial
genome, leading to demise of the culture (Chan et al. 1992; Chiang & Lambowitz 1997; Chiang et al. 1994; Court & Bertrand 1992; Griffiths 1995; Maas et al. 2005).

Double-stranded RNA mycoviruses occur commonly in many fungi and in some phyto-pathogens and are known to be able to cause ‘hypovirulence’ (Ghabrial & Suzuki 2009; Nuss 2005). In some cases, mycoviruses have been linked to senescence phenomena such as reduced growth rate and reduced fertility (e.g. van Diepeningen et al. 2006, 2008b), while they are both targets and suppressors of the RNA silencing in the fungal cell (Hammond et al. 2008). Recently, the forest pathogen *H. parviporum* was shown to senesce and die because of mycovirus infection (Vainio et al. 2014).

**Conclusion**

The evolutionary theories of senescence have focused mainly on unitary organisms with a clear distinction between germ line and soma and predicted that modular organisms lacking such distinction would not senesce. However, crossing the Weismann barrier does not guarantee immortality: some fungi with a modular body plan do senesce and behave ‘pseudo-unitary’ in a way that the total parental mycelium can be sacrificed in favour of offspring production, in agreement with the disposable soma theory. These fungi also behave in accordance to Medawar’s mutation accumulation theory, as the fungus’s ecological niche seems to determine whether senescence evolved and in accordance to William’s antagonistic pleiotropy theory of ageing with clear trade-offs between life span and reproduction. The processes observed in senescent fungi are similar to those observed in senescing species of other kingdoms: reduced growth and reduced fertility signifying a reduced energy level, (mitochondrial) DNA instability, the accumulation of lipofuscin pigments and even the slowing down of these processes by calorie restriction. Several (extra)genomic elements such as mitochondrial plasmids and introns with retro-transposon activity seem to act in fungi as the most important senescing diseases. Thus, even exceptional modular organisms such as senescent fungi senesce in a normal way with respect to the senescing phenomena as well as with respect to senescence theory.

**References**


Munkres, K. & Rana, R. S. (1978b). Antioxidants prolong life span and inhibit the
senescence-dependent accumulation of fluorescent pigment (lipofuscin) in clones of
a mobile intron of a mitochondrial gene. *Current Genetics, 8*, 299–305.
Reviews Microbiology, 3*, 632–42.


