# 6 Do green plants age, and if so, how?

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## Abstract

Time-dependent ageing-like processes in green plants are discussed and compared to gerontological changes in animals and other organisms. The question of plant ageing is inseparable from the issue of the developmental architecture of plants. Modular structure and fractal, recursive patterns of plant development mean that the concept of an individual, and hence of whole-organism ageing, is ambiguous. Selective disposal of cells, tissues and organs, a major determinant of perenniality and hence lifespan, is a morphogenetic and adaptive tool that superficially resembles, but is fundamentally different from, ageing. The contrast between autotrophs and heterotrophs in the relation between resource acquisition and allocation is discussed, particularly partitioning between reproductive and vegetative development. Genetic, environmental, and epigenetic factors influencing ageing-like behaviour, including senescence, stress responses, somatic mutation and phase change, are considered. Finally, mechanisms are proposed for the origin of ageing as an intrinsic property of living cells.

## 6.1 Introduction

### 6.1.1 Why are plants of interest to gerontologists?

Green plants occupy a special niche in the field of gerontology. The yellowing, withering, and falling of leaves and other plant parts have been tropes for human ageing since poets and artists first indulged in what Ruskin (1856) called the 'Pathetic Fallacy'. Kerr, Wyllie, and Currie (1972) maintained this tradition when they introduced the term apoptosis, Greek for leaf drop, to describe programmed cell death in humans and animals. A second reason for gerontological interest in plants concerns the vast range of lifespan in the botanical world, from ephemerals that survive for a few weeks to the oldest living individuals on the planet (considered to be bristlecone pines of southeast California - Schulman 1958, Johnson and Johnson 1978). Comparing such extremes of longevity may be expected to provide insights into genetic and physiological factors underlying biological ageing. The relationship between reproduction and senescence is another feature of plant life cycles that seems to connect with patterns of ageing in many animal species. On the face of it, the death of the whole plant following flowering and fruiting in annuals and monocarpic perennials is similar in principle to suicidal reproduction

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in semelparous animals such as Pacific salmon, mayflies and many cephalopods (Patnaik et al. 2004, Carey 2002, Rocha et al. 2001).

### 6.1.2 The semantics of senescence and death

There is much confusion about the precise meanings of the various terms associated with terminal events in the lives of plants and their parts. The most striking symptom of plant senescence is the yellowing of green tissues, which in turn signifies radical alterations in the plastids of green cells. Thomas et al. (2003) pointed out that the conversion of chloroplasts to gerontoplasts is reversible, a property of senescing cells which, with other characteristic features, identifies this phase of plant development as a process of transdifferentiation or metaplasia and not one of deterioration. Both in its reversibility and in its absolute dependence on the maintenance of viability for initiation and progression, senescence is fundamentally different from programmed cell death. The timing and location of senescence is determined not only by transcription of senescence-related genes (Yoshida 2003), but also by regulatory events at the post-transcriptional and post-translational levels (Thomas and Donnison 2000, Dangl et al. 2000). Senescence reversibility means these processes must be under play-stop-rewind control, in contrast to the propagating one-way destructiveness of cell death mechanisms. Senescence and ageing are terms often used interchangeably in gerontology. In plants, senescence has a specialised meaning that relates to ageing in the sense that it is a time-based process of physiological change, but this change is not intrinsically, inevitably, or irreversibly deteriorative. This is in marked contrast to plant cell death, to which plant senescence is at best only distantly related (Thomas et al. 2003).

### 6.1.3 Criteria of viability and ageing

Related to the issue of the meanings of senescence, ageing, and death in the specific context of the plant life cycle is the question of how to tell if a plant, or one of its parts, is in an ageing condition. What criteria can be applied? What biomarkers are there that can be screened to provide an index of ageing? Genomics technologies may be expected to be informative and it is certainly true that characteristic differences in transcription pattern can be identified in pre-senescent and ageing human fibroblasts (Linskens et al. 1995). But what would such a readout of cells, tissues, or organs say about ageing in plants? For example, wood formation is a tissue death process and has a distinctive transcriptional profile (Hertzberg et al. 2001); but it is doubtful whether anything useful about ageing for a whole tree could be inferred from the molecular events associated with building the major part of its body, even if most of that body is itself dead. Interestingly, lengths of tracheids in the wood of individual bristlecone pines have been shown to have carried on increasing for more than 2000 years (Baas et al. 1986).

### 6.1.4 Distinguishing symptoms from causes of ageing

It would be useful to have a term, the equivalent of "gerontology", to cover the study of the causes and symptoms of dying as distinct from ageing. Classical Greek provides a suitable etymology. From Acheron, the river that runs through the chasm of the underworld, comes "acherontic", tottering on the brink of death ("...an old acherontic dizzard, that hath one foot in the grave..." - Burton 1624) - hence "acherontology". Examples of acherontological processes in plants include many kinds of post-harvest deterioration, malting, retting, and ensiling, as well as pre-digestive autolysis following herbivory as described by Beha et al. (2002).

Acherontology and gerontology are confronted with the same dilemma. Of the phenomena they define, which are symptoms and which are causes? For example, is vacuolar lysis in post-senescent leaf cells the agent of cell death or the consequence of lost viability? Is there such a condition as "slightly dead"? Is ageing the slow accumulation of acherontological events? How much of the active research area defined as Programmed Cell Death is really concerned with acherontology, and how many of the processes and mechanisms described are really post-mortem necrochemical changes (Thomas et al. 2003)?

### 6.1.5 Issues in plant ageing

In spite of the visibility and extreme expression of senescence and longevity in the plant kingdom, insights into ageing processes in plants do not seem to have had a particularly productive influence on understanding of human or animal ageing. Partly this is an inevitable consequence of the relatively tiny research effort on plants compared with that taking place in the biomedically-driven field of gerontology. But perhaps more significantly, it is questionable whether the mechanisms of ageing in plants are related other than very distantly to those of animals, or even whether plants undergo ageing in any gerontologically-recognisable sense (Thomas 2002). This conclusion arises from consideration of unique structural, functional, and genetic characteristics that equip plants to avoid, resist or exploit the inevitability of ageing.

## 6.2 Individual or population?

### 6.2.1 Body plan

Plants and animals differ in some fundamentals of organisation and development - for example, there is no differentiation into germline and soma in plants (Walbot 1985). Also of particular significance for ageing is the body plan, which in plants, is continuously expanding by the repetitive proliferation of structural units. Variation in the spatial arrangement of modules, or in the timing of initiation and development of these units, accounts for the vast range of plant form and life cycle

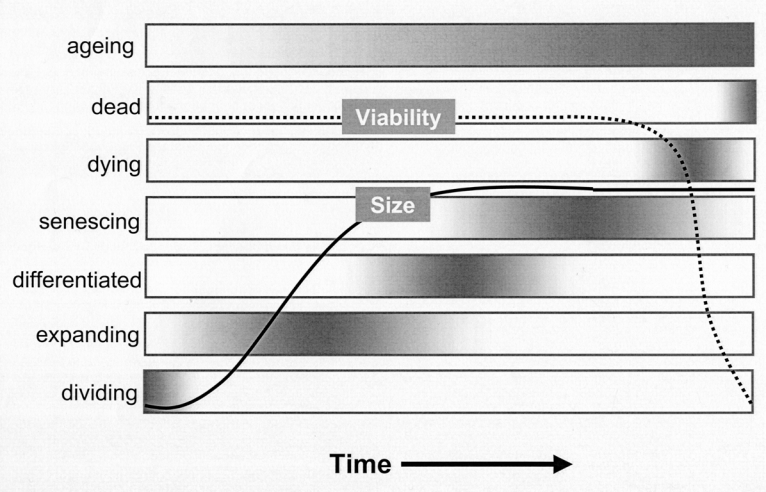


**Fig. 1.** Stages in the life-history of a plant and its parts. The sequence of events is reiterated at all levels in the hierarchy of plant organisation, from cells, tissues, and organs through to individual plants, communities, and even whole floras (Leopold 1975). Note that ageing includes, but is not limited or defined by, terminal senescence and death phases

(White 1979, Room et al. 1994). Individual plants behave as competing populations of genetically equivalent organs, interacting through hormonally-controlled vascular connections and internal competition for resources, a mode of organisation that ensures adaptation of the plant to heterogeneous environments (Sachs et al. 1993).

### 6.2.2 Fractal development

Plants are constructed from metamers, repeated units that are morphological homologues. In general, an organ or structural module follows a typical growth-curve reaching an asymptotic maximum and at some later stage, there is a period of senescence followed by death (Fig. 1). Just as organs pass through this sequence, so too do individual cells that make up its tissues. Organs, in turn, combine to impose the initiation-growth-senescence-death progression on the entire plant. As each metamer of an intact plant moves through the ageing sequence, its chemical composition, physiology, and complement of transcribed genes will change in a characteristic way and will provide measures of progress from birth to death. The interaction between different modules is the key to whole-plant longevity and the expression of mono- and poly-carpy (Thomas et al. 2000).

In this sense, plant development is fractal, a mode of organisation that has allowed the morphogenesis of real and imaginary plants to be dynamically modelled according to relatively simple heuristic principles (van Groenendael 1985, Prusinkiewicz and Lindenmayer 1990). Lindenmayer (L-) and similar systems share with fractals the property of emergence, in which complex structures are created

from simple starting data (Krieger 1996), and may therefore have some mechanistic basis as well as empirical value. For the purposes of the present discussion, it is enough to note that an important feature of the developmental hierarchy is the inclusion of a defined period of senescence at each level of organisation. The question of the relationship of this phase of cell, organ, and organism development to ageing is not straightforward and will be addressed in more detail later. For now, we propose that ageing comprises the time-based changes that occur throughout the life of the structural unit, which means that it includes, but is not identical with or bound by, the period of senescence (Fig. 1).

### 6.2.3 Plants as populations of parts

An individual plant at any given time will generally comprise a number of structural units each of which will be at a different point in its developmental or, we might say, ageing sequence. This has led some plant scientists, notably J L Harper and his colleagues, to argue that a plant is not an individual but rather a population of parts (Harper and White 1974, White 1979). Treating leaves, for example, as an age-structured population allows the application of analytical methods developed for demographic studies of individual organisms (Harper 1989a). Implicit in this approach is the idea that modules compete with each other (for resources, space, light and so on). Moreover, survival of individual structures will be determined by the intra-organismal equivalent of ecological fitness, and the higher-order organisation of the plant body (including, by extension, its ageing pattern) can be approached as a population phenomenon (Harper 1989b, Eissenstat and Yanai 1997).

### 6.2.4 What is an individual?

If a single plant is structured demographically as a population of metamers, can it be regarded as an individual for the purposes of understanding its ageing behaviour? This is a key question if we are to discover the mechanistic basis for the extremes of longevity observed in the plant kingdom. A human community that has survived in a particular place for 4000 years would be remarkable for anthropological reasons, but a 4000-year-old person would be a true gerontological wonder. The individual-as-population concept of plant organisation would argue that bristlecone pine is more like the former than the latter.

In many ways, a plant resembles a colonial animal, and not only in form. Martinez (1998) measured extremely low mortality rates in three hydra cohorts over four years and failed to find evidence for decline in reproductive rates over this period. It was concluded that, by constantly renewing the tissues of its body, this colonial metazoan probably escapes the deteriorative processes that increase the probability of death with increasing chronological age, and may therefore be potentially immortal. Interestingly, colonial cnidarians such as corals have been shown to contain lytic bodies that seem to have a role in digestion and cell senescence and these are located in the endosymbiotic algae that occupy the host cells

(Hawkridge et al. 2000). As described subsequently, expression of normally latent autolytic potential is a feature of senescence and cell death processes in terrestrial plants, so this may be another aspect of development and body organisation that plants and colonial animals share.

### 6.2.5 Scaling up and scaling down

Observation of ageing behaviour at a high level of organisation in order to identify causative time-dependent physiological processes in sub-components is a "scaling down" issue. There is an equivalent "scaling up" question concerned with the degree to which ageing is a holistic or gestalt phenomenon or else the sum of autonomous ageing events undergone by constituent structural units.

In some circumstances, events contributing to overall mortality are clearly cell- or organ-autonomous. For example if pathological cellular incidents such as hypersensitive reaction (Dangl et al. 2000) propagate extensively enough, death of the whole plant may occur. However, it is a much more subtle matter to determine whether whole-plant (or in deference to the metapopulation model, whole-module) ageing is an expression of the concerted ageing of individual cells (Kaplan and Hagemann 1991). The extreme totipotency of cultured plant cells is difficult to reconcile with the Swim-Hayflick concept that the limited lifespan of isolated animal cells in vitro is the cellular expression of the ageing process (Swim 1959, Hayflick and Moorhead 1961). Telomere attrition and apoptosis may offer plausible mechanistic explanations for cellular longevity (Bree et al. 2002); but the status and significance of both phenomena in plants are highly uncertain (see later).

There is strong evidence that individual organs of animals age at a rate determined by intrinsic factors rather than by whole-organism properties. For example, heterochronic transplantation in mammals shows that the transferred organ retains the age characteristics of the donor rather than the recipient (Krohn 1966, Hollander 1970). A contemporary perspective on this issue is given by the debate following the discovery (Shiels et al. 1999) that Dolly, the cloned sheep, showed signs of premature ageing. There are countless examples of the cloning of whole plants from single cells, excised tissues, or entire organs; but clear evidence is absent that reduction in vigour or lifespan in regenerants can be related directly to the age of the plant from which the original cell or tissue was taken. Although, there are plenty of observations of somaclonal variation contributing to ageing-like changes in vitro (Kaeppler et al. 2000). Organ transplantation studies have been carried out on tobacco plants. Leaves of flue-cured tobacco varieties senesce much slower than those of burley varieties, heritable behaviour which is related to differences in the efficiency with which nitrogen fertilizer is utilized by the two types (Crafts-Brandner et al. 1987). Nitrogen use efficiency is under simple two-locus genetic control (Henika 1932, Stines and Mann 1960). Crafts-Brandner et al. (1988) carried out reciprocal leaf grafting experiments with flue-cured and burley cultivars. They found that the grafted leaf retained the compositional characteristics of the donor variety but senescence rate was determined by the genotype of

the stock. These experiments indicate that intrinsic genetic control of organ senescence exerted indirectly through resource accumulation and use can be overridden by factors that coordinate senescence at the integrated, whole-plant level. The contrast in behaviour of transplanted organs exemplifies fundamental differences between animals and plants in their relationships to resources.

## 6.3 Ageing and plant life-form

### 6.3.1 Meristems

Plant anatomy and morphology are generated by differential cell division and expansion initiated in meristems (Meyerowitz 1997). Longevity depends on the activities of terminal shoot and root meristems and the lateral meristems at each node on the plant axis. A meristem may be indeterminate (capable of continued initiation of organs while retaining a population of uncommitted proliferative cells) or determinate (differentiating a terminal set of organs and simultaneously losing generative capacity). Death of a determinate meristem is frequently preceded by a (sometimes reversible) period of proliferative arrest (Bleecker and Patterson 1997). The apical meristems of perennials remain indeterminate for more than one growth season. With notable exceptions (Battey and Lyndon 1990, Thomas et al. 2000) the apical meristem of a vegetative shoot is commonly indeterminate; when it becomes reproductive and switches from generating vegetative structures such as leaves to reproductive structures (flower parts), it becomes determinate. The interaction between meristem determinacy and the sequential or progressive programmed senescence of lateral organs determines the longevity of the axis.

### 6.3.2 Annuality and perenniality

Raunkiaer (1934) introduced a classification of plant life-forms based on the survival of apical meristems in their active or dormant forms (Table 1). The various life-forms are characterised by the extent to which shoot axes persist (phanerophytes, chamaephytes), retrench (hemicryptophytes, cryptophytes), or die outright (therophytes) at the end of the growing season. Annuals and many biennials are therophytes. Meristem determinacy is an important, but not the only, factor in determining a plant's position in the Raunkiaer classification. The formation of resting structures and the progressive programmed senescence and death of organs are critical also.

Active chamaephytes and certain hemicryptophytes, for example creeping species like clover, are horizontal perennials (Thomas 1994). They forage for resources in their environment (Stephens and Krebs 1987, Grime and Hodgson 1987, Van Kleunen and Fischer 2001) by apical proliferation, elongation growth and subsequent tissue senescence, death and decay (Gallagher et al. 1997, Turner

**Table 1.** The classification of plant life-forms (based on Raunkiaer 1934)

| Life-form | Definition | Types included |
|---|---|---|
| Phanerophytes | Generally tall plants visible throughout the year, carrying surviving buds or apices at least 25 cm up from the ground. Examples are trees, large shrubs and lianas | a) Evergreens without bud covering<br>b) Evergreens with bud covering<br>c) Deciduous with bud covering<br>d) Less than 2 m high |
| Chamaephytes | Low growing plants visible all year round, bearing perennial buds between ground-level and 25 cm up. Examples include shrubby tundra species. | a) Suffruticose (woody at the base, herbaceous above) chamaephytes that bear erect shoots which die back to the portion that bears the surviving buds<br>b) Passive chamaephytes with persistent weak shoots that trail on or near the ground<br>c) Active chamaephytes that trail on or near the ground because they are persistent and have horizontally directly growth<br>d) Cushion plants |
| Hemicryptophytes | The surviving buds or shoot apices are situated at or just below the soil surface. Includes perennial grasses, many forbs, and ferns. | a) Protohemicryptophytes with aerial shoots that bear normal foliage leaves, but of which the lower ones are less perfectly developed<br>b) Partial rosette plants bearing most of their leaves (and the largest) on short internodes near ground level<br>c) Rosette plants bearing all their foliage leaves in a basal rosette |
| Cryptophytes | At the end of the growing season, die back to bulbs, corms, rhizomes, or similar underground (in some species, underwater) structures. For example lilies, onions, garlic, potatoes, and similar forbs. | a) Geocryptophytes or geophytes which include forms with: (i) rhizomes; (ii) bulbs; (iii) stem tubers; and (iv) root tubers<br>b) Marsh plants (helophytes)<br>c) Aquatic plants (hydrophytes) |
| Therophytes | Plants that complete their life cycle from seed to seed and die within a season, or that germinate in fall, and reproduce and die in the spring of the following year. | |

and Pollock 1998). The plant survives for as long as young proliferating tissues can keep ahead of the wave of senescence and tissue death behind them. The shoots of phanerophytes (shrubs and trees) also move out into the environment but do so in the vertical plane. Older tissues become senescent and die, but do not undergo post-mortem decay, persisting instead in the form of wood. Root systems forage through the soil and pass through the apical proliferation-growth-senescence-death sequence rather like inverted shoots of vertical perennials (Spaeth and Cortes 1995; Eissenstat and Yanai 1997). We may conclude that Raunkiaer's life-forms, as they relate to degree of annuality or perenniality, are a direct expression of the extent to which proliferation at apical meristems outpaces a pursuing wave of (programmed) tissue senescence and death.

Annual plants, which grow, reproduce, and die in a single season, seem to obey the "live fast, die young" rule (Kaufmann 1996). Biennials, which generally devote the first year to vegetative growth and the second year to reproduction and death, have life cycles that are qualitatively no different from those of annuals. There are also species such as *Agave*, which may survive for many years in the vegetative condition but then produce flowers and fruits and die. In all these cases of monocarpy (semelparity), there is clearly a relationship between reproduction and whole-organism death.

Amongst polycarpic (iteroparous) perennial species, where flowering and whole-plant senescence are not obligately linked, the range of lifespans is striking, ranging from less than 10 years in some herbaceous species to more than 2000 years in woody conifers (Table 2). Asexual reproduction propagates clones, which often remain attached to the parent plant and can proliferate to establish community-sized "individuals" of extraordinary longevity, maybe in excess of 10000 years (Table 2). In this respect, clonal plants resemble the huge underground hyphal networks of certain fungi, some of which may be even older (Smith et al. 1992). Clonal behaviour like this really does stretch the concept of organismal individuality beyond breaking-point and may not be especially illuminating when it comes to fathoming the functional basis of ageing.

### 6.3.3 Body piercing and body sculpture

The structural complexity of plants arises from repetition and variation in time and space between metamers (White 1979, Room et al. 1994). Structural modules turn over, that is, there is a flux of metamers through the plant body. In the case of shoots, this usually takes the form of recruitment by propagation of new metamers at terminal meristems, progression partly or entirely through the age-structured strata of the modular plant body and ultimate loss through programmed senescence and death (Thomas 1992). Turnover is, of course, a central factor in biology at all levels of organisation, from the subcellular (metabolism determines pool sizes and fluxes of intermediates through synthesis, interconversion, and breakdown) to the demographic (population structures defined by births, deaths and migrations). Indeed, Leopold (1975) visualised a continuum of biological turnover running from molecules to entire floras, with turnover at each level in this

**Table 2.** Maximal lifespans of individual and clonal plants (Nooden 1988)

| Species | Age (years) |
|---|---|
| **Single plants** | |
| Bristlecone pine (*Pinus longaeva*) | 4,600 |
| Giant sequoia (*Sequoia gigantea*) | 3,200 |
| Huon pine (*Dacrydium franklinii*) | 2,200+ |
| Common juniper (*Juniperus communis*) | 2,000 |
| Stone pine (*Pinus cembra*) | 1,200 |
| Queensland kauri (*Agathis microstachya*) | 1,060 |
| European beech (*Fagus sylvatica*) | 600-930 |
| Olive (*Olea europaea*) | 700 |
| Scots pine (*Pinus silvestris*) | 500 |
| Pear (*Pyrus communis*) | 300 |
| Black walnut (*Juglans nigra*) | 250 |
| European ash (*Fraxinus excelsior*) | 250 |
| Apple (*Pyrus malus*) | 200 |
| English ivy (*Hedera helix*) | 200 |
| Arctic willow (*Salix arctica*) | 130 |
| Flowering dogwood (*Cornus florida*) | 125 |
| European white birch (*Betula verrucosa*) | 120 |
| Quaking aspen (*Populus tremuloides*) (ramet) | 100 |
| European grape (*Vitis vinifera*) | 80-100 |
| European cyclamen (*Cyclamen europaeum*) (tuber) | 60 |
| Scots heather (*Calluna vulgaris*) | 42 |
| Myrtle whortleberry (*Vaccinium myrtillus*) | 28 |
| Spring heath (*Erica carnea*) | 21 |
| European elder (*Sambucus racemosus*) | 20 |
| Eurasian solomon seal (*Polygonatum multiflorum*) (root stock) | 16-17 |
| Scandinavian thyme (*Thymus chamaedrys*) | 14 |
| Crossleaf heather (*Erica tetralix*) | 10 |
| Broadleaf solomon seal (*Polygonatum latifolium*) (root stock) | 8 |
| Yellow wood anemone (*Anemone ranunculoides*) (root stock) | 7 |
| **Clonal plants** | |
| Huckleberry (*Gaylussacia brachycerium*) | 13,000+ |
| Creosote (*Larrea tridentata*) | 11,000+ |
| Quaking aspen (*Populus tremuloides*) | 10,000+ |
| Bracken (*Pteridium aquilinum*) | 1,400 |
| Velvet grass (*Holcus mollis*) | 1,000+ |
| Sheep fescue (*Festuca ovina*) | 1,000+ |
| Red fescue (*Festuca rubra*) | 1,000+ |
| Ground pine (*Lycopodium complanatum*) | 850 |
| Lily of the valley (*Convallaria majalis*) | 670+ |
| Reed grass (*Calamagrostis epigeios*) | 400+ |
| Black spruce (*Picea mariana*) | 330+ |

hierarchy conceived as being the integral of the turnover processes occurring below it, with an overlay of emergent properties. This view is essentially fractal and is of recurring value in attempting to define the relationship between plants, their parts, and the ageing process.

A unique feature of the modular developmental architecture of plants is the ultimate disposability of each and every structural unit. Disposability is programmed into plant development, which in turn implies the timely operation of programmes for the senescence and death of component cells, tissues and organs. Selective senescence and death is a creative force throughout the life of the plant and its parts (Bleecker and Patterson 1997). For example, pervasion by holes and tubes ensures the surface area:volume ratio of a tissue or organ is sufficient to sustain vital transport and exchange processes. The rigid wall immobilizes plant cells, which means that hole and tube formation by cell migration, such as occurs during gastrulation in animals, is not possible. Instead, tissue perforation in plants, like disposal of individual metamers, comes about through controlled, localised cell death. Thus, selective cell and tissue death are critical for plant architecture, adaptation, and life cycle. For example, hypersensitive response cell death is fundamental for plant reactions to biotic and other stressful challenges (Heath 2000). Localised cell death in apices and primordia is also decisive for the generation of organ form (e.g .Calderon-Urrea and Dellaporta 1999). Plant morphogenesis is not just origami - it employs scissors too.

### 6.3.4 Origins of lysigeny and schizogeny

The capacity for controlled autolysis is present even in single-celled and filamentous plants (Park et al. 1999, Moriyasu 1995) so it is reasonable to conclude that it probably arose very early in plant evolution. Certainly, the first terrestrial plants that colonised the land were already actively exploiting lysigeny (intracellular dissolution of protoplasm) and schizogeny (cell separation) to differentiate conducting tissues and shed reproductive structures and other parts (Raven 1986, Edwards 1993). The secretory pathway within plant cells (Hadlington and Denecke 2000) is critical for lysogeny and schizogeny. The vacuole represents "inner space", into which lytic enzymes and other components are secreted. The vacuole's role in terminal processes of cell development is more than simply to act as a leaky bag of aggressive catabolic enzymes (Thomas et al. 2003). Vacuolar accumulation of phenylpropanoid pigments accounts for the colour changes that occur in ripening fruit such as strawberry and in highly pigmented senescent leaves such as those of maples. The final products of chlorophyll catabolism are directed to the vacuole (Matile et al. 1999; Thomas et al. 2001). Sequestration of pigments and catabolites provides direct or indirect protection from photodamage (Matile et al. 1999, Feild et al. 2001). The vacuole also defends against pathogens and pests and is the destination for some of the pathogenesis-related proteins encoded by certain senescence up-regulated genes (Hanfrey et al. 1996; Thomas and Donnison 2000). At one time, it was thought that senescence in green plant cells resulted from vacuoles flooding the cytosol with hydrolases or even engulfing whole organelles such as chloroplasts. More plausibly, vacuoles perform a non-lytic protective role during senescence and only at the extreme stage of cell death (see Fig. 1) does release of vacuolar hydrolases occur. Autolysis of cell contents in the death phase of *Zinnia* tracheid transdifferentiation exemplifies the sequential control of vacuolar

function (Fukuda 1996). In schizogeny, the secretory system directs lytic enzymes to the "outer space" of the apoplast, where cleavage of bonds in the extracellular matrix weakens cell-cell adhesion, leading to cell separation and ultimate shedding of the tissue or organ (Roberts et al. 2002)

## 6.4 Ageing in relation to resource allocation

### 6.4.1 Ageing in autotrophs

Plant body-plan and life-form follow radically distinct design specifications from those of animals. Fundamentally different expressions of ageing in plants and animals are a direct consequence of contrasting structural and developmental principles. Another definitive difference between plants and animals concerns the acquisition and internal allocation of energy and raw materials. Heterotrophs have constantly to trade off investment in repair and maintenance against growth and reproduction, and the major theories of ageing have their mechanistic basis in this relationship (Kirkwood 2002). Clearly, no plant can survive if denied light, water, or nutrients for long enough (though tolerance of such deprivation can be astonishingly high in some cases - see Thomas and Sadras 2001). Furthermore, the productivity of many natural plant communities is commonly limited by one or more of these environmental inputs. Nevertheless, the appropriation and utilisation of resources by green plants follow sufficiently different rules from those of heterotrophs that they call into question the generality of "trade-off" theories of biological ageing. Obeso (2002) reported a number of case studies where the cost-of-reproduction model did not fit observations. For example, dioecious woody perennials seemed to fit the hypothesis, but dioecious herbs did not. Obeso concluded that plants were able to compensate for reproductive costs through the plasticity of assimilation and growth responses, somatic architecture and physiological integration. Thomas and Sadras (2001) described examples of apparently deliberate "inefficiency" in some characteristic developmental and metabolic processes in plants and argued that the evolutionary legacy of promiscuous resource capture has driven the adoption of apparently wasteful developmental and physiological adaptations.

### 6.4.2 Ageing as a starvation or neglect process

Individuals in a plant community interact with each other in a number of ways. They compete for nutrients, water, light, and space (Grime 2001). They sometimes conduct chemical warfare by exuding allelopathic compounds (Harborne 1993). There is evidence that they can communicate pathological danger to each other by emitting volatile signals (Tscharntke et al. 2001). There are parallels in the behaviour of metamers within an individual plant. For example, leaves are both assimilatory and storage organs. During senescence, the photosynthetic function declines

and redistribution of reserves becomes the dominant activity. Nutrient status is amongst the most important internal factors triggering the transition from assimilatory to mobilisation function. Young developing plant parts have a high requirement for N. When the appetites of growing tissues cannot be satisfied by import from the rhizosphere alone, N will be sourced from older tissues and distinctive senescence patterns will arise (Thomas et al. 2002). Transfer of nutrients from leaves to seeds and fruits is a feature of reproductive senescence. During seasonal senescence of deciduous trees, N is relocated to storage structures such as bark. N export to the growing apex is related to progressive or sequential senescence in the vegetative phase of development.

Although there is a clear relationship between senescence pattern and internal distribution of nutrients in the whole plant, it is doubtful that the latter is the cause of the former. This is discussed further in the context of reproduction-triggered senescence. On the other hand, there are instances when physical factors might influence viability and ageing by limiting supply of raw materials. For example, as a tree grows, increasing distances between the roots and the extremities of the crown impose increasing stress on the hydraulic functions of the vascular system. Ryan and Yoder (1997) considered this to be more likely than nutrient allocaton, respiratory patterns or increasing mutational load as a determinant of tree growth and form (and, by implication, ageing). Hubbard et al. (1999) showed that declining photosynthesis in older ponderosa pine trees is associated with decreases in hydraulic conductance and whole-tree sap flow. Significant as this mechanism might be for certain phanerophytes (Table 1), it clearly cannot be a general cause of plant ageing. Lanner and Connor (2001) could find no evidence for age-related deterioration in the function of xylem and phloem in bristlecone pines over the age range 23 to 4713 years.

### 6.4.3 Reproductive development and ageing

Senescence, as a consequence of exhaustion or starvation, was one of the original hypotheses proposed for the mechanism of monocarpy (Molisch 1938). It is certainly true that seed development depends on a supply of current fixed carbon, which in turn requires maintenance of the photosynthetic apparatus in source leaves. But for the growth of the same seeds, reduced nitrogen compounds must be provided to support synthesis of enzymes and storage proteins. The leaves that supply photosynthate are also the major potential sources of mobilised reduced N. In moncarpic species like soybean or sunflower (which have high-protein seeds), there is a clear functional conflict between the photosynthetic and protein storage functions of the foliage (Sadras et al. 1993).

Nevertheless, the evidence against nutrient diversion as a cause of whole-plant death in monocarpic species is strong and has been often reviewed (see, for example Thomas 1992, 2002, Nooden et al. 1997). An alternative hypothesis states that young sinks seek to satisfy their requirement for recycled nutrients by exporting a "death hormone" (Wilson 1997), which promotes senescence and remobilisation in source tissues. A related hypothesis proposes that older leaves are out-competed

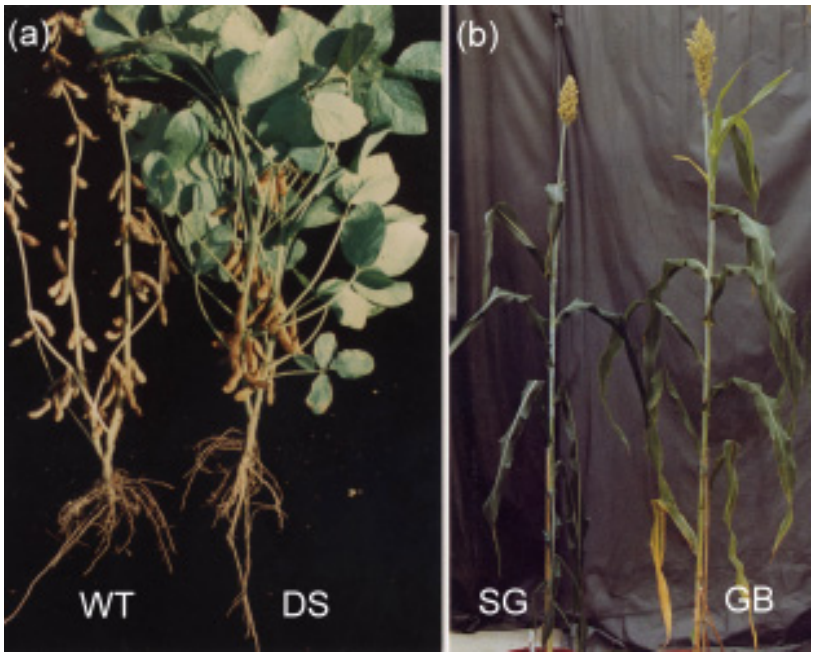


**Fig. 2.** Genetic disturbance of the relationship between reproduction and total senescence of vegetative parts. (a) Wild type (WT) soybean and an individual (DS) with alleles for early flowering and determinate growth habit, in which fruit set occurs without leaf senescence and abscission (Abu-Shakra et al. 1978, Thomas and Smart 1993). (b) Go-brown (GB) and stay-green (SG) lines of sorghum, a species in which retention of green leaf area during grain fill has been achieved by introgression of genes from polycarpic land race germplasm (Thomas and Howarth 2000).

by young tissues for "anti-death" hormones in the transport system (Nooden and Letham 1993). In many species, ethylene has some of the characteristics of the senescence-promoting hormone (Jing et al. 2002), while cytokinins exhibit many of the senescence-inhibiting properties of an anti-death hormone (Hwang and Sheen 2001). Surgical, physiological, and transgenic experiments have provided reasonable evidence in support of roles in senescence for either or both regulators (Dangl et al. 2000).

The nutrient-diversion and (anti-) death hormone hypotheses for the integrative control of senescence in the whole plant may not be fundamentally different, because many translocated raw materials (sucrose, nitrate for example) have both a nutritional and a morphogenetic role in plants. It is significant in this connection that a number of senescence-enhanced genes have been shown to be hormone and carbohydrate-regulated (Ono et al. 2001, Yoshida 2003). Thus, internal competition for resources is a factor in plant ageing by virtue of its direct influence on interacting developmental programmes, one of which specifies a senescence syndrome that is intrinsic, with variations, to every element in the modular plant body.

### 6.4.4 Evolution of reproductive habit in relation to ageing

Even in a species like soybean, which physiological models suggest may be obligately self-destructive at seed-set (Sinclair and deWit 1975), reproduction and overall senescence are readily unlinked by genetic means (Guiamét et al.1991, Thomas and Smart 1993, Nooden et al. 1997, Fig. 2). It is clear that the link has been repeatedly forged and broken during evolution, as annuality/moncarpy/semelparity has given way to perenniality/polycarpy/iteroparity within taxonomic groupings. In Charnov and Schaffer's (1973) view, the annual or ephemeral habit is favoured in hostile environments. Perennials invest in long-lived vegetative biomass rather than big-bang monocarpism and so tend to outcompete annuals in more benign habitats by closing the canopy and shading them out. The development of molecular systematics has allowed evolutionary trends in life-history within taxa to be reconstructed. Bena et al. (1998) have developed such a scheme for the genus Medicago and presented evidence that the ancestral form was a selfing annual, from which there has been recurrent evolution towards perenniality and outcrossing. Self-fertilization, which is much more common in annual than in perennial plants, may itself be an important factor in evolution of the annual habit (Zhang 2000). Silvertown et al. (2001) mined data on lifespan and fecundity for 65 species of polycarpic perennials to test hypotheses about the evolution of senescence and life-history traits. A conclusion from this work is that plant senescence rate is independent of initial mortality rate (in contrast to the relationship in animals) but positively related to reproductive lifespan (though fecundity generally did not decline with age). The implication that the risk of death increases with each additional cycle of reproduction is consistent with the quantitative genetic regulation of monocarpy/polycarpy as described by Thomas et al. (2000). Interestingly, these analyses suggested strongly that the clonal habit, particularly where clones fragment rather than remain physiologically integrated, is an effective means by which plants have escaped the evolution of senescence.

## 6.5 Genetics and epigenetics of plant ageing

### 6.5.1 Time and entropy

In engineering, the term stress describes an environmental factor which, when applied to an object or system, invokes a corresponding strain. Biologists have requisitioned the concept of stress and used it, not always very fastidiously, to describe the experience of non-optimal environments by living organisms. In a sense, time is a stress, though it differs from all other stresses in that, except at absolute zero, it is always present. Even when all controllable environmental influences are fixed or excluded, time accumulates as thermal time. Ageing is the biological response to time-stress. The physiology of individuals and their substructures is envisaged as reacting to non-optimal environments by invoking specific stress genes, stress

proteins, and stress metabolites. By the same token, it could be argued that ageing expresses the activities of time-stress genes and their products (Thomas 1994).

Viable organisms and their components can adopt any of three different strategies to deal with environmental stress: avoidance, resistance, or exploitation. Therophytes (Table 1) are stress-avoiders, whereas phanerophytes survive by resisting stress. All plants exploit stress to some degree or other - for example low seasonal temperature is used for time-measurement by winter-dormant structures such as seeds and buds. Indeed, many or even most plants are absolutely dependent on environmental deviations from optimality to cue normal progress through their developmental cycles. Moreover, as Thomas (1992) has pointed out, a common plant adaptation to stress is to mimic the state that lack of adaptation would have imposed. Thus, a winter dormant deciduous tree looks like a dead tree.

By analogy, for an organism not to succumb to time-stress (and hence ageing), it has the choice of avoidance, resistance, or exploitation. Time-stress can be avoided by outrunning it: that is, by growing, developing, and differentiating (avoid growing old by staying young). It can be resisted, by building-in structural and functional durability and by repairing wear and tear. Or time-stress can be pre-empted, through the adoption of programmed senescence as a developmental and adaptive resource so that ageing and death take place on the organism's own terms. Accordingly, genes with functions in ageing are of three kinds. Ageing avoidance genes include all the programmes for embryological development, structural and functional specialisation, and maturation. Ageing resistance genes regulate metabolic homeostasis, balanced turnover, macromolecular repair and maintenance, and resilience towards pathological influences such as diseases or free radicals. Pre-emptive or suicide genes function in the purposeful destruction of cells, tissues, and organs in defiance of entropy (Thomas 1994).

### 6.5.2 Programmes for cell death and senescence

We have seen, there are many ways by which plant cells and tissues can die. They may be programmed to die during normal development as part of processes that create complex organ shapes and specialized cell types. Alternatively, there may be necrotic death resulting from exposure to environmental deviations beyond the tissue's adaptive limits (Pennell and Lamb 1997). Another route to inviability is pathological programmed hypersensitivity (Heath 2000). A common fate for plant biomass is to be ingested by vertebrates or invertebrates, during which a distinctive type cell death process is triggered with far-reaching ecological implications (Kingston-Smith and Theodorou 2000). Then there is senescence in its special plant sense, a component of normal development intimately associated with, but significantly different from, autolytic, and/or pathological cell death. Figure 3 summarises the interrelationships and semi-independence of the physiological (senescence, cell specialisation) and acherontological (biotic and abiotic stress) routes to plant cell death (Thomas and Donnison 2000).

The genetic programs underlying these modes of impending mortality are becoming better understood. Some recent reviews covering regulation of the

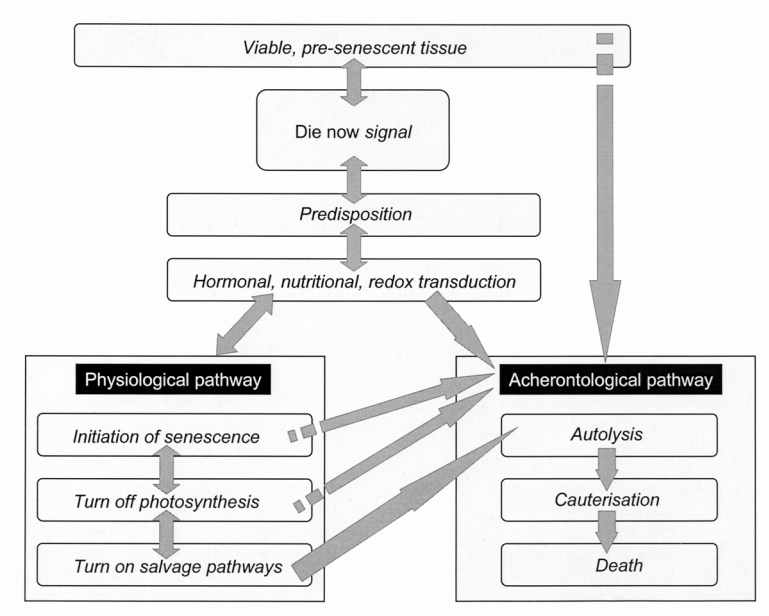


**Fig. 3.** Terminal processes in the life of plant tissues. The physiological (senescence) route and the acherontological (pathological) course can work in series or in parallel, making it possible for death to occur without senescence, or (through reversal of transdifferentiation) for senescence to happen without death. It is not known how much, if any, of the regulatory and metabolic machinery of senescence and cell death are common to the two pathways (modified from Thomas and Donnison 2000).

initiation and progress of programmed death in plants are: Jones and Dangl (1996), Pennell and Lamb (1997), Dangl et al. (2000), Ono et al. (2001), Yoshida (2003). Programs for cell death and senescence will not be discussed in detail here, but the relationship of such mechanisms to the wider issue of ageing may be noted. As discussed previously, in some cases, components of the syndrome of declining viability are likely to be at least partially acherontological. Others contribute directly or indirectly to the generation of form and complex function. The senescence of leaves and other green tissues is a (trans)differentiation rather than a deteriorative process. Take away these elements of programmed cell senescence and death in plants and very little remains that could be identified with a gerontological role.

### 6.5.3 Non-optimal environments and ageing

Seasonal plant senescence symbolises ageing, even though it is not itself a true gerontological process. Senescence happens to viable cells, tissues, and organs and, as a physiological activity, is responsive to non-optimal environmental condi-

tions. Seasonal senescence is strategic and anticipates future stress, exemplified by deciduous trees that sense declining day lengths after midsummer and initiate mass leaf senescence in preparation for winter. Senescence may also be tactical, deployed when an unpredictable environmental challenge is experienced, such as pathogen attack. A given stress may have one effect on the initiation of tactical or strategic senescence and a completely different influence on execution of the syndrome. For example, drought often invokes premature foliar senescence but reduces the rate of yellowing. If the stress develops quickly or severely enough it can overwhelm the tactical or strategic deployment of programmed senescence and divert the tissue directly into the acherontological pathway leading to death (Fig. 3).

Plants and their parts employ this kind of pre-emptive proxy ageing response to deal with many of the environmental challenges that are thought to contribute to gerontological changes in animals. For example, ultraviolet radiation induces deteriorative changes in plant tissues, including enhanced expression of genes associated with senescence (John et al. 2001). Reactive oxygen has moved centre stage in the drama of ageing mechanisms. On the animal side, there is a substantial body of evidence for pathological oxidation as a major player at the cellular level (Martin et al. 1996, Lithgow 2000, Finkel and Holbrook 2000). There is also a rapidly expanding literature on a range of effects of reactive oxygen in plant physiological processes, including claims that oxidative damage has a leading role in senescence and ageing. It is necessary for the present author to declare a prejudice: he is deeply sceptical about most of these claims and sees fundamental difficulties in most models of plant ageing based on reactive oxygen. Within plant cells, there are many sources of reactive oxygen species and in some cases; these become more active with age (e.g. Munné-Bosch and Alegre 2002). For example, superoxide anion or its disproportionation product are normal products of metabolism in peroxisomes, chloroplasts, and other cell compartments. Oxidative metabolism is active in and necessary for plant senescence to proceed normally. Senescence, which is an energy-demanding process, can be immediately suspended by treatment with respiratory poisons. In a more subtle way, senescence is also sensitive to cellular redox conditions (Chen et al. 1998). During senescence, peroxisomes redifferentiate into glyoxysomes (Nishimura et al. 1993) a transition that changes the profile of superoxide-producing and antioxidant enzymes (del Rio et al.1998). In these examples, reactive oxygen species act as components of regulated, integrated signalling and metabolic mechanisms in coherent, viable cells. When they break free of control and promote rapid destruction of cells through the propagation of free radical cascades, they become agents of acherontological change, which, as argued repeatedly, is distinct from ageing. Finally, it is an ironic fact that plants are generally so replete with defences against harmful build-up of reactive oxygen species that curative or anti-ageing claims are often made for cosmetics, herbal remedies, and functional foods containing plant products. It might therefore be argued (perhaps not wholly seriously) that it is difficult to see how reactive oxygen species could be effective in promoting the ageing of such well-defended organisms.

### 6.5.4 Chimeras and somatic mutations

The indeterminate meristems of a long-lived perennial plant would be expected to have accumulated somatic mutations and it is reasonable to conclude that these will contribute to the ageing and ultimate death of the whole individual. The age-related increase in the frequency with which chimeras and sports arise is evidence that such mutational events do occur; but the case for somatic mutation as a mechanism of whole-plant ageing is weak. There is good evidence that mutations of this sort can be important sources of adaptive fitness (Gill et al. 1995; Salomonson 1996; Pineda-Krch and Fagerstrom 1999). New genotypes better adapted to variable environments can arise, for example by a surge of transposon activation in meristems (Chaparro et al 1995; Walbot et al 1998). Klekowski (1988) modelled the genetic load characteristics of two fern species differing in the longevity of clonal genotypes based on the propagation of mutations in a microbial cell culture. Such models of genetic mosaicism in plants and other organisms with a modular, clonal architecture (Fischer and Van Kleunen 2001) show that intraorganismal selection is effective in purging most deleterious somatic mutations (eg Pineda-Krch and Fagerstrom 1999, Byers and Waller 1999, Orive 2001). The mutational load hypothesis of plant ageing has been put to the severest test by Lanner and Connor (2001) who assessed the frequency of mutations in the pollen, seed, and seedlings of bristlecone pines up to more than 4700 years old. They found no statistically significant relationship between mutation frequency and age of individual.

### 6.5.5 Telomeres and telomerases

Shortening of telomeres occurs during human differentiation and ageing (Harley et al.1990). The reverse transcriptase-type enzyme telomerase is responsible for maintenance of telomeres (Lundblad 1998). Loss of telomerase activity results in progressive reduction in telomere length until, at a critical point; chromosomal fusions and rearrangements become frequent enough to cause replicative senescence (Counter et al. 1992). All the elements of the telomere-telomerase system are present in plants, and there have been a few observations of apparent age-related shortening of telomeres in some species (Kilian et al. 1995). Nevertheless, mutagenic manipulation of plant telomerase activity has failed to show any consistent consequence for ageing or related processes. Riha et al. (2001) generated *Arabidopsis* mutants lacking telomerase that were able to survive for up to 10 generations. Beyond the 5th generation, there was progressive accumulation of severe cytogenetic defects, including end-to-end chromosome fusions and anaphase bridges. These late-generation plants exhibited malformations of organs and meristems and ultimately arrested in a vegetative and partially de-differentiated condition. Interestingly, the lifespans of mutants at this terminal stage were longer than comparable wild type individuals. The authors conclude that fundamental differences between animals and plants in their response to telomere disruption reflect differences in developmental and genomic architecture.

### 6.5.6 Phase-change

By all observable criteria, bristlecone pine shows no sign of undergoing an intrinsic ageing process (Lanner and Connor 2001). The present discussion concludes that this is true of green plants in general. Nevertheless, there is one aspect of plant development that arguably has some kind of relationship to ageing, namely phase-change, also referred to as maturation or heteroblasty (Grenwood 1995). Poethig (1990) described four phases or maturation stages in the life cycle: embryonic; post-embryonic juvenile; adult vegetative; adult reproductive. Each phase is associated with a characteristic package of morphological and physiological traits. If juvenile tissue of, for example, ivy (Bauer and Bauer 1980) is cultured and plants are regenerated from it, these plants have a stable juvenile phenotype; similarly, mature tissue yields regenerants with mature characteristics. Although juvenile and mature types are genetically identical, it is often so difficult to bring about reversion by external treatment that the phenotype appears to be fixed by some kind of epigenetic mechanism resembling genomic imprinting (Martienssen 1998, Kierszenbaum 2002). Studies of gene expression have identified a few phase-specific transcripts, a number of phase-change mutants have been described, and a vegetative-phase regulator has recently been described (Berardini et al. 2001). The latter was identified as the product of the *Arabidopsis* gene *SQUINT*. It is a homologue of cyclophilin 40, a component of the Hsp90 chaperone complex found in animals and yeast as well as plants. There is a long way to go before it will be possible even to frame a hypothesis about the mechanisms underlying phase change; but this area seems in some respects closer to the field of ageing research than many of the superficially related terminal developmental and acherontological plant processes that have been covered in this discussion.

## 6.6 Valediction

### 6.6.1 Poise

Within cells, aggressive lytic enzymes and metabolites are often physically separated from cytosol, for example by sequestration in lysosomes, vacuoles, zymogen bodies or the apoplast (Bursch 2001, Matile 1997, Donepudi and Grutter 2002; Lazure 2002, Hoson 2002). Of course, this does not mean that cytosol is therefore a benign environment in which macromolecules and their ligands enjoy a life free from the threat of destruction. On the contrary, the fidelity and fitness of molecules, complexes, and cell structures are continuously being tested by cytosolic systems that prowl the cell and pick off damaged, badly folded, mis-assembled, idle, inappropriate, or superfluous components (Thomas 1997). This means that cytosol is in reality a severe and stringent milieu and its survival critically depends on metabolic poising, which we recognise as cell viability. This poising is like that of a tightrope walker, inching along a potentially endless high-wire. For cells, the wire is time, and in the end, poise will not be enough, and the ropewalker will fall

one way or the other. Is this, writ large, what we call ageing? If so, and the author considers it could well be, then ageing is a non-negotiable property of protoplasm and inseparable from viability. Life proceeds and proliferates because living organisms have the means to re-establish poise. Sex is one way. For example, reinstatement of cellular poise can be clearly seen in pollen development, where cells undergo what Dickinson and Heslop-Harrison (1977) strikingly referred to as "cytoplasmic restandardization". Through a different route, involving intraorganismal cell selection at the somatic level, cytoplasmic stability is sustained in terminal meristems.

### 6.6.2 It's not what you do...

One of the great biological principles is that the development, adaptation, and survival of living organisms are the results of closing down options. The genome represents the impractical unedited totality of what the organism is capable of. Successful organisms do more than possess, express, and pass on the right genes - they refrain from expressing inappropriate potential. Music provides an analogy. Western music uses the 12 notes of the chromatic scale. Imagine sitting at the keyboard of an organ. Simultaneously hold down the 12 keys corresponding to the chromatic scale. Cease playing 3 minutes later. Within that cacophonous 3 minute block of sound are all possible 3-minute musical works. But "Tea for two" (Tatum 1933) is 3 minutes of musical genius, and why? Overwhelmingly, because of the notes that were not played. So it is with living organisms - the genome is the chromatic scale, the surviving organism is the harmonised musical line. The selectivity that orchestrates expression of genomic potential comprises cellular processes that repress and destroy. Might it not be that ageing is the long-term revelation of these negative, but nonetheless essential, forces that animate the machinery of living matter?

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