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Annuality, perenniality and cell death

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Abstract

This essay considers annuality and perenniality as quantitative traits and discusses the application of established and new genetic tools to the analysis of plant life histories. Annual/perennial status is a function of meristem determinacy in combination with the processes of cell death and disposal employed by plants to generate well-adapted anatomies and morphologies. Creeping perennials, like clover or bracken, seem to move around in the environment. They do this by extending into unoccupied space while the oldest tissues behind the growing and mature regions senesce, die and decompose. Trees do essentially the same thing, except that they develop vertically and the old dead tissue does not disappear but instead persists as wood. A root system is a kind of upended vertical perennial. The balance between exploratory growth and the wave of tissue death that succeeds it is a major determinant of perenniality. So although perenniality and annuality may appear to be dramatically different traits, extremes of behaviour can arise by a relatively minor change in the relationship between growth and death. This conclusion is supported by evidence from genome dosage studies, from the practical experiences of breeding perennial-type traits into annual backgrounds and from molecular cladistics. Applications of methods for the genetic analysis of quantitative characters are described, including the exploitation of introgression mapping in *Lolium–Festuca* and quantitative trait locus mapping in cereals and other species.

Key words: Cladistics, genome dosage, introgression, longevity, meristem, mapping, quantitative trait, senescence.

Introduction

For a plant to be perennial (in this discussion, ‘perennial’ generally means ‘non-annual’) the apical meristem of at least one of its shoot axes must remain indeterminate beyond the first growth season. Determinacy is usually associated with development of the floral meristem; conversely, the shoot apical meristem is normally indeterminate for as long as it remains vegetative. But there are many exceptions to these generalizations (Battey and Lyndon, 1990), as Fig. 1 illustrates for the case of clover. Axes bearing inflorescences that are usually terminal can resume vegetative growth, as shown by the unusual genotype in Fig. 1a. The viral disease clover phyllody (Jones and Stoddart, 1971) has the effect of stimulating reproductive apices to produce leaves instead of floral organs, but these meristems remain determinate (Fig. 1b). Whilst meristem determinacy is important for annual/perennial status, it is by no means the whole story. Progressive programmed senescence and death make critical contributions too. Here the relationship between the life-history of the whole plant and that of its constituent parts is discussed. Then annuality/perenniality as the expression of competition between apical survival and progressive tissue death is considered. This leads to two broad conclusions: (1) annuality/perenniality are probably quantitative traits, and (2) there are useful new genetic tools for analysing such traits.

Development by planned obsolescence

The developmental architecture of plants is based on a modular plan, and each and every module is ultimately disposable. In recent years this view of development has become integrated with the general principles of selective death as a creative force in animal differentiation and

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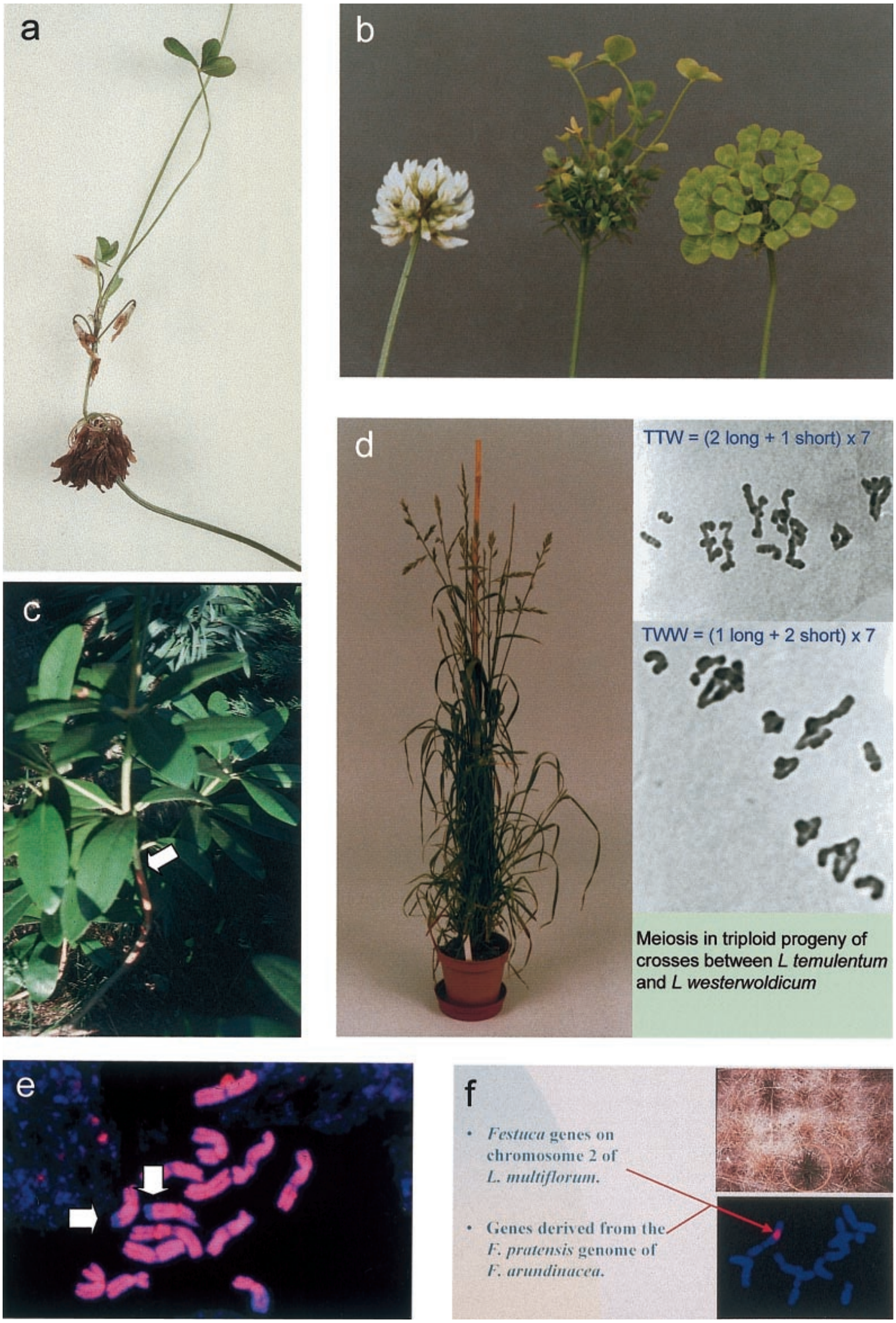


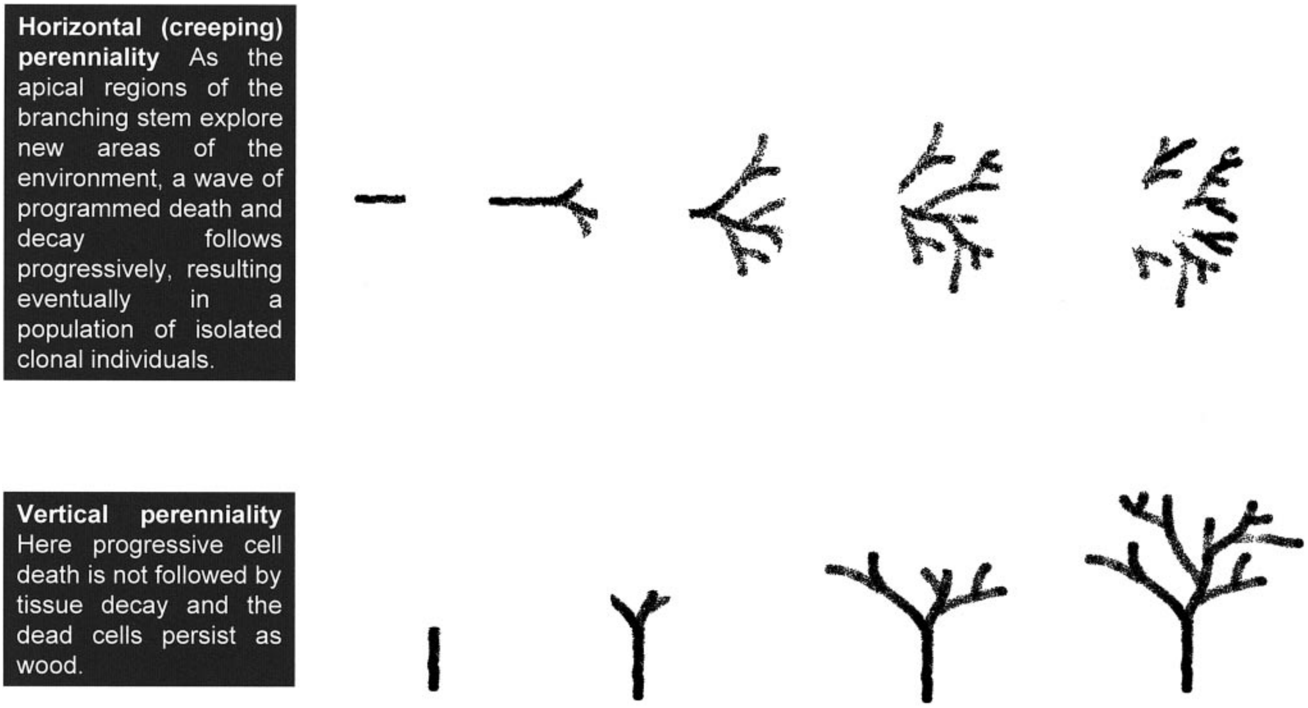
Fig. 1. (a) An unusual genotype of white clover, *Trifolium repens*, in which further vegetative shoot growth is reinitiated at normally terminal inflorescences. (b) A white clover inflorescence (left) and two examples showing severe symptoms of clover phyllody virus infection, which causes substitution of leaflets for flower parts. (c) Shoot of *Rhododendron*. Arrowed is the junction between current (green) and previous (suberized) year's growth. (d) *Lolium westerwoldicum* and metaphase spreads of meiotic chromosomes from hybrids with *L. temulentum*. (e) Chromosomes of *Lolium temulentum* visualized by genomic *in situ* hybridization (GISH) to show a matching pair of alien segments (arrowed) from *Festuca pratensis*. (f) Introgression of extreme persistence under drought into *Lolium multiflorum* from *Festuca arundinacea* is associated with the presence in the *L. multiflorum* genome of a specific alien segment identifiable by GISH.

adaptation (Hengartner and Bryant, 2000). Instances of localized cell elimination in animals are abundant. To take one example, the default model for development of the limb of a bird is not the chicken's foot, but that of a duck. The embryonic chick limb has clear interdigital webs that disappear during development as a consequence of the death of particular groups of cells (Hinchliffe, 1982). Essentially, the same thing happens to make the finger-like leaflets of a palm leaf (Kaplan *et al.*, 1982). Morphogenesis in apical meristems is currently studied almost exclusively in terms of the pattern of expression of genes that regulate cell proliferation. But localized cell death in apices and primordia is also decisive for the generation of organ form and is becoming an increasingly fruitful area for research (Calderon-Urrea and Dellaporta, 1999).

A mass of cells only becomes large and structurally complex if it has been penetrated by holes or tubes. In this way surface area keeps pace with volume and vital transport and exchange processes are sustained. Development of the biological mechanisms for creating perforations was thus an essential prerequisite for the evolution of higher plants. A senescence-like autolytic programme is present in unicellular and filamentous green algae (Park *et al.*, 1999; Moriyasu, 1995). Fossil evidence shows that the first land plants were already actively exploiting lysigeny (intracellular dissolution of protoplasm) and schizogeny (cell separation) to differentiate conducting tissues and to shed reproductive structures and other parts (Raven, 1986). Controlled cell death, part of the developmental and adaptive armoury of plants since early in evolution, is deployed on an enormous scale in the modern flora (Dangl *et al.*, 2000). For example, most of the biomass of a tree is dead. To generalize: cell death and disposal are universally employed by plants to generate their anatomies (inter- and intracellular apertures), and to build well-adapted morphologies.

Death in the life-cycle

The way in which programmed death can define life-form is illustrated by what may be called the horizontal and vertical variations of the perennial habit (Thomas, 1994). A creeping perennial such as white clover pushes out into new areas of the environment by proliferation at its apices, occasional branching, and subsequent elongation growth. Behind this zone of environmental invasion is a wave of cell senescence, death and necrotrophic disappearance (Gallagher *et al.*, 1997; Turner and Pollock, 1998). Loss of apical viability may be preceded by a more or less prolonged and reversible period of proliferative arrest (Wang and Woolhouse, 1982; Bleecker and Patterson, 1997). As long as the rate of exploration and proliferation does not fall below the pace of pursuing tissue death and disappearance, the plant will persist. As the zone of death passes branch points, fragmentation occurs and a population of isolated clones develops (Fig. 2). In this way creeping perennials appear to move



Horizontal (creeping) perenniality As the apical regions of the branching stem explore new areas of the environment, a wave of programmed death and decay follows progressively, resulting eventually in a population of isolated clonal individuals.

Vertical perenniality Here progressive cell death is not followed by tissue decay and the dead cells persist as wood.

Fig. 2. Horizontal and vertical perenniality.

around their environment, very like a herd of foraging animals (and models of foraging behaviour apply effectively to clonal perennials; Stephens and Krebs, 1987; Grime and Hodgson, 1987). There are examples of very long-lived clones, for example, bracken communities that date back more than 1500 years, and there are prairie grass clones maybe twice as old as this (Molisch, 1938; Stebbins, 1958; Oinonen, 1967).

Turning to shrubs and trees, the same principles are at work, but with two characteristic differences. First, penetration of the environment is essentially in the vertical plane rather than horizontal. Second, the tissues that have been through programmed senescence and death, instead of disappearing through post-mortem decay, persist as mummified corpses—namely as wood. The wave of cell death moving along the shoots of woody plants is seen clearly in species such as *Rhododendron* (Fig. 1c). Here the current year's growth is green and the previous year's growth suberizes and lignifies in a zone that moves up, killing axillary buds and triggering senescence and abscission of leaves as it passes.

Root systems are populations of foraging units that behave, in a sense, like inverted vertical perennials. A range of lifespans for cohorts of roots, from 2–3 weeks (apple, strawberry) to over 35 weeks (sugar maple) has been compiled (Eissenstat and Yanai, 1997). Roots that have sloughed off entire epidermal and cortical regions often retain transport functions in the stele and may even initiate new laterals from the viable pericycle (Spaeth and Cortes, 1995). This further reinforces the analogy with the shoots of woody perennials and the dynamic role of cell death in development and function.

Without labouring the point further, it can be concluded that the habits of ephemerals, annuals or perennials can be understood in terms of the extent to which apical meristems can stay at least one step ahead of the succeeding wave of cell death. This view of life-history is reminiscent of the concept of the 'blastozone' and 'necrozone' as archetypal features of morphogenesis in the earliest land plants (Hagemann, 1999).

Evolution of life cycles

Here just one of many views of the evolution of annuality/perenniality is briefly discussed as a prelude to considering the genetic basis of life-history. Age at first reproduction was emphasized as a critical determinant of the fitness of individuals (Cole, 1954). This argument was further developed (Charnov and Schaffer, 1973) by taking into account age-related differences in mortality and relative growth rate, with the conclusion that the annual or ephemeral habit would be favoured in hostile environments. Moreover, in a benign habitat, with nutrient-rich soils for example, perennials are likely to be more competitive than annuals because canopy closure and light limitation favour a life-history in which there has been some investment in long-lived vegetative biomass rather than total commitment to big-bang monocarpism. This is related to an important constraining principle, the self-thinning rule (Westoby, 1984), which identifies shorter plants as more likely to die than taller. Early-season extension growth of annuals on rich soils is generally less than that of perennials, and their likelihood to be culled correspondingly greater. Consistent with these generalizations are observations of broad correlations at the level of whole floras between, among other features, seed size (in turn a correlate of the extent of nutrient remobilization from vegetative tissues), plant height and perenniality (Leishman *et al.*, 1995). The different relationships of perennials and annuals to the resource and stress status of the habitat and the fitness consequences of competition between the different life-forms have profound and complex implications for the dynamics of heterogeneous populations (Venable and Brown, 1993). These factors are also significant in the evolution of self (in)compatibility (Morgan *et al.*, 1997; Pannell and Barrett, 1998).

The availability of detailed phylogenies based on cladistics and molecular systematics (Doyle, 1998) allows traits such as life-history to be put in an evolutionary context within broad or narrow taxonomic groupings. In a recent study, for example, a putative history of the genus *Medicago* was developed using molecular data (Bena *et al.*, 1998). Based on parsimony rules they inferred that the ancestral form was a selfing annual and that there has been recurrent evolution in the direction of perenniality and outcrossing. Building in other assumptions gave other possible reconstructions. However, a general conclusion that can be drawn from this and other studies of life-history is that annuality and perenniality are traits that recur time and again across the taxonomic range and that, with the right selection pressure, the propensity to generate either form of phenotype can be realized without the need for large-scale genetic innovation.

Perenniality as a quantitative trait

To develop the last point further: although many genes have been described that individually participate in the progression through cell division, growth, maturity, senescence, and death, life-history looks more like a matter of the quantitative relationships between expression of these genes than of simple on–off switching. Evidence for this comes from studies of crosses between annuals and perennials. Temperate forage grasses of the genera *Lolium* and *Festuca* are good subjects for seeking the genetic basis of perenniality. There is an excellent range of longevity within the complex—from virtual ephemerals such as *Lolium temulentum* to extremely persistent perennials such as *Festuca arundinacea*.

L. temulentum is strictly annual. *L. westerwoldicum*, though usually treated as an annual, is a short-lived perennial which, although lacking persistency, will survive to flower again in the second and sometimes succeeding years (Fig. 1d). Inverse triploid hybrids have been created between the two species by crossing tetraploid *L. temulentum* (T) with diploid *L. westerwoldicum* (W) and diploid *L. temulentum* with tetraploid *L. westerwoldicum* (Thomas, 1995). The resulting triploid hybrids had the genomic constitutions TTW and TWW. What was not reported in the original paper is that the hybrid plantlets generated by embryo culture flowered within a very few weeks. None of the plants was allowed to set seed, but inflorescences were fixed for meiotic analysis before the plants were cut back. The TTW triploids were strictly monocarpic and all the plants died. TWW plants, on the other hand, survived the winter and flowered again the following summer. The influence of *L. westerwoldicum* on perenniality seems to be dose-dependent. Similarly, it was found that the degree of perenniality in progeny was related to genome dosage in a cross between annual wheat and the perennial × *Agrotriticum intermediodurum* (a durum wheat × *Thinopyrum* hybrid) (Jones *et al*., 1999).

Barley, *H. vulgare*, is annual but most species in the genus *Hordeum* are perennial. A large number of interspecific hybrids was examined and it was discovered that perenniality is dominant over annuality in all cases (von Bothmer *et al*., 1983). *Thinopyrum turcicum* ($2n=10x=70$) was crossed with hexaploid bread wheat and the hybrid chromosome-doubled to produce a $16x$ amphiploid (I King and J Harper, unpublished results). The amphiploid, which is perennial, has been backcrossed to wheat; the backcross has the polyhaploid chromosome set of *Th. turcicum* plus the hexaploid complement of wheat (EEEEEAABBDD). This is also perennial and continues to flower freely after three years. It has also been reported (M Leggett, personal communication) that hybrids between annual *Avena* species and the perennial *A. macrostachya* still remain alive after 14 years.

Selective transfer of agronomically useful traits between annuals and perennials has long been a mainstay of crop improvement. An example of an annual-type character exploited to rectify a deficiency in a potentially valuable perennial crop is the introduction of high seed quality from pearl millet into perennial elephantgrass hybrids used for forage and biomass (Diz and Schank, 1993). An important instance of a perennial trait into annual backgrounds is the introduction of the stay-green or non-senescence character into sorghum. Perennials tend to have low harvest indices and reproductive sink strengths. Accordingly, the monocarpic influence that would trigger and sustain wholesale foliar senescence in an annual is weak in such plants. The vegetative tissues of perennials may also be systemically less sensitive to senescence signals. Comparison of closely-related annual and perennial species often suggests that they differ in the intrinsic rate of leaf senescence. For example, detached leaves of *Lolium temulentum* (annual) turn yellow significantly faster than those of the perennial *Festuca pratensis* and have higher rates of proteolysis (A Kingston-Smith and H Thomas, unpublished results). Grain yield in cereals is principally a reflection of starch accumulation, which relies on current photosynthate and a non-senescent canopy. Seed storage protein is generally laid down towards the end of grain fill and relies principally on recycled N from senescing vegetative tissues. The canopy is, therefore, not only the source of assimilated carbon but also an intermediate storage tissue for salvageable N (Sinclair and Sheehy, 1999).

The ideotype for a high carbohydrate grain crop such as sorghum includes a canopy with extended photosynthetic duration followed by a rapid and efficient senescence and N remobilization. Late and/or slow decline in photosynthesis is a perennial-type character that could make a big contribution to grain yield in an annual background, provided there is no unbreakable genetic linkage that prevents high levels of storage and recycling of vegetative N. Sorghum and pearl millet, staples of tropical African agriculture for over 3000 years, have been grown in a pattern of shifting cultivation and are truly multifunctional crops that are used not just for grain but also for straw (Kelly *et al*., 1991). In Duncan's words, sorghum 'is a coarse, perennial grass that is treated as an annual in temperate or subtropical climes . . .' (Duncan, 1996). The practice of ratooning exploits the perennial tendencies of some land-races and cultivars of sorghum (Escalada and Plucknett, 1975). Germplasm from Sudan and Ethiopia has been an important foundation for breeding programmes aimed at increasing green leaf retention (Duncan *et al*., 1981). Stay-green genotypes have additional beneficial attributes, notably the capacity to continue normal grain-fill under drought conditions (Rosenow *et al*., 1983; Borrell and Douglas, 1996), reduced lodging and improved disease and pest resistance (Rosenow, 1984).

This dosage effect of annual and perennial genomes, and the practical experiences of breeding perennial-type green leaf retention into annual-type cereals, are entirely consistent with the view that life-form and longevity can be treated as quantitative traits for the purposes of genetic analysis.

Genetic analysis of perennials

An advantage of species from the *Lolium–Festuca* complex as subjects for analysing perenniality is the convenience of introgression as a powerful genetic tool in these species. Introgression substitutes a gene by its homeologue from an alien background, allowing the locus to be identified, analysed and, ultimately, isolated. Several barriers must be overcome to achieve useful introgression: pollination incompatibility mechanisms, intolerance of alien chromosomes, inability of homeologous chromosomes to pair and recombine, anonymity of the introgressed segment. Between species across the range of the *Lolium*s and *Festuca*s, these barriers are readily surmounted. A unique feature of alien DNA introgressed by interspecific or intergeneric hybridization in *Lolium–Festuca* is that it is detectable by genomic *in situ* hybridization (GISH; Thomas *et al*., 1994) and using species-specific molecular markers (King *et al*., 1998). A range of introgression populations has been generated, each showing segregation for many different traits. Some of these populations will be particularly useful for analysing the genetic control of perenniality.

For example, lines of *Lolium temulentum* were derived from a cross with *L. multiflorum* which in turn carried an introgression from *Festuca pratensis* (Thomas *et al*., 1999). The location of the alien segment in the *L. temulentum* genome was shown by GISH (Fig. 1e). Associated with this segment are *Festuca*-derived morphological characters; expressed genes carrying polymorphisms clearly originating in the *Festuca* progenitor; and *Festuca*-specific genomic repeat sequences (Thomas *et al*., 1997; I Donnison, B Hauck, H Jones, H Thomas, I King, unpublished results). These observations were made on advanced backcross lines that are substantially *L. temulentum* in morphology and annuality (Thomas *et al*., 1999). However, earlier generations in the crossing programme leading to these lines clearly segregate for longevity (G Morgan, unpublished results) and represent *Lolium temulentum* backgrounds with perennializing introgressions that will be identifiable via GISH and molecular markers. Figure 1f is a demonstration of what is possible with this approach, applied here to an issue related to perenniality, namely survival under stress. Genes from *Festuca arundinacea* (highly persistent and drought tolerant) were introgressed into *Lolium multiflorum* (low persistence and drought susceptible) (Humphreys and Pasakinskiene, 1996). Progeny surviving a severe drought treatment were shown to carry an introgression clearly carrying genes related to the drought-resistant trait introduced from *Festuca* (Fig. 1f; Humphreys *et al*., 1998). The corresponding genomic locus can now be identified by tagging with species-specific markers (Pasakinskiene *et al*., 2000).

The quantitative nature of perenniality and associated characters means that they should be analysable by genetic mapping as QTLs (quantitative trait loci). Senescence in sorghum has been approached in this way (Crasta *et al*., 1999). A set of recombinant inbred lines was derived from a cross between the extreme stay-green B35 and Tx430, a line with relatively high susceptibility to post-flowering drought stress. Seven stay-green QTLs were identified, three of which accounted for 42% of the variability in stay-green ratings. In a similar study on pearl millet (Howarth *et al*., 1994; Yadav *et al*., 1999; Thomas and Howarth, 2000) at least five QTLs for green leaf retention have been identified. Furthermore, up to six QTLs for leaf senescence have been observed in certain *Lolium* populations (Thorogood *et al*., 1999).

If the genetic basis of the annual/perennial character is related to a quantitative interaction between on the one hand, factors influencing apical meristem determinacy and, on the other, the pattern of progressive cell death, then some degree of correlation between corresponding QTLs might be expected. In the sorghum study (Crasta *et al*., 1999) one of the stay-green loci mapped on top of a maturity locus. This co-localization is consistent with the notion of life-history as an expression of programmed senescence and death in combination with apical determinacy, since meristem activity and the timing of floral induction are likely to be major components of maturity. The observation that one of the QTLs for senescence in pearl millet maps at the same position as a major QTL for flowering time (Thomas and Howarth, 2000) is similarly suggestive. A gene complex for annuality in sugar beet has been described in which bolting and its suppression was controlled by a number of genes and specific day-length requirements (Abe *et al*., 1997). It would be interesting to discover whether loci related to programmed death processes are part of this complex, as the ideas developed in this discussion would predict.

In conclusion, when the lifespan of *Drosophila*, *C. elegans* or mouse has been experimentally extended by a few days or weeks it often makes headlines. But for extreme contrasts in longevity combined with the potential to generate populations for mapping quantitative loci related to longevity, plants offer extreme possibilities.

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