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Biological relevance of polyploidy: ecology to genomics
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Perspectives on polyploidy in plants – ancient and neo

MICHAEL D. BENNETT*

Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

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It is timely to re-examine the phenomenon of polyploidy in plants. Indeed, the power of modern molecular technology to provide new insights, and the impetus of genomics, make polyploidy a fit, fashionable and futuristic topic for review. Some historical perspective is essential to understand the meaning of the terms, to recognize what is already known and what is dogma, and to frame incisive questions for future research. Polyploidy is important because life on earth is predominantly a polyploid phenomenon. Moreover, civilization is mainly powered by polyploid food – notably cereal endosperm. Ongoing uncertainty about the origin of triploid endosperm epitomizes our ignorance about somatic polyploidy. New molecular information makes it timely to reconsider how to identity polyploids and what is a polyploid state. A functional definition in terms of a minimal genome may be helpful. Genes are known that can raise or lower ploidy level. Molecular studies can test if, contrary to dogma, the relationship between diploids and polyploids is a dynamic two-way system. We still need to understand the mechanisms and roles of key genes controlling ploidy level and disomic inheritance. New evidence for genome duplications should be compared with old ideas about cryptopolyploidy, and new views of meiosis should not ignore premeiotic genome separation. In practice, new knowledge about polyploidy will be most useful only when it reliably predicts which crops can be usefully improved as stable autopolyploids and which genomes combined to create successful new allopolyloids. *Biological Journal of the Linnean Society*, 2004, **82**, 411–423.

ADDITIONAL KEYWORDS: cryptopolyploidy – genome duplication – levels of polyploidy – limits on polyploidy – palaeopolyploidy – ploidy level genes – somatic polyploidy – spatial genome separation – terminology of polyploidy.

INTRODUCTION

April 2003 marked the 50th anniversary of Watson and Crick's landmark paper on the double helix structure of DNA, so public attention is focused on the form and significance of the genetic material at its most basic level, that of nucleotide sequence. However, this paper addresses diversity in the form of genetic material at its highest level – the multiplication of nuclear genomes in cells and taxa – known as polyploidy.

How much do we know about polyploidy? The discovery and definition of polyploidy by Winkler (1916) and others was over 80 years ago, so most questions to be discussed are not new. It is still necessary to ask: 'How is polyploidy defined?' and 'How can we recognize polyploids?'. 'How often do polyploids arise?', 'How does diploidization occur?' and 'What is its evolutionary, developmental and ecological significance?' 'How important is polyploidy for seeing the big picture and knowing the origin and nature of diversity?'. It is increasingly important to consider: 'How will understanding polyploidy help address key concerns – food security, health and environmental issues, and conserving diversity – which determine our quality of life?'.

2003 celebrates no anniversary for polyploidy, yet it is timely to re-examine the process and phenomenon of polyploidy, for several reasons. First, modern molecular cytogenetics provides striking new sights of developmental and evolutionary events at the chromosomal level, including macro views of pairing behav-

*E-mail: m.bennett@rbgkew.org.uk

iour, and of genome origins and reorganization. For example (Fig. 1A), allotetraploid *Nicotiana tabaccum* ($2n = 4x = 48$) probed with genomic DNA from *N. sylvestris* clearly shows both its allotetraploid origin and reveals numerous rearrangements between S and T genome chromosomes (Kenton *et al*., 1993; Lim *et al*., 2004 – this issue). Interestingly, probing *Sorghum bicolor* ($2n = 20$) with a BAC probe from *Sorghum* shows two subsets of five chromosomes, labelling centromeric regions on one but not the other (Fig. 1B), consistent with an allotetraploid nature (Gómez *et al*., 1998).

Second, comparative DNA analyses of complete genome sequences or synteny reveal massive duplications in many species traditionally viewed as classical diploids showing that they are actually cryptic or palaeopolyploids (Lukens *et al*., 2004 – this issue). Figure two in the landmark paper on the first complete plant genome sequence (Arabidopsis Genome Initiative, 2000) showed the surprising finding that most of the *Arabidopsis thaliana* genome comprises duplicated segments. With its tiny DNA C-value, and only five linkage groups, *A. thaliana* ($2n = 10$) was regarded as a classical diploid. Yet analysis of its DNA sequences shows its nuclear genome may be the product of three (Bowers *et al*., 2003; Kellogg, 2003) or four (Vision, Brown & Tanksley, 2000) ancient rounds of genome duplication, whilst yet another recent round of polyploidization produced its neo-tetraploid relatives such as *Arabidopsis suecica* ($2n = 26$) (Tutin *et al*., 1993).

This new ability to detect duplications is not confined to *Arabidopsis*, but extends to most plants and animals. The ancestral condition of almost any eukaryote is now seen as affected by earlier rounds of duplication upon which one or more further polyploidization events are superimposed in successive waves of doubling and subsequent diploidization (Ohno, 1999; Murray, 2002; Wendel *et al*., 2002; Durand, 2003). Thus, the total nuclear DNA contains

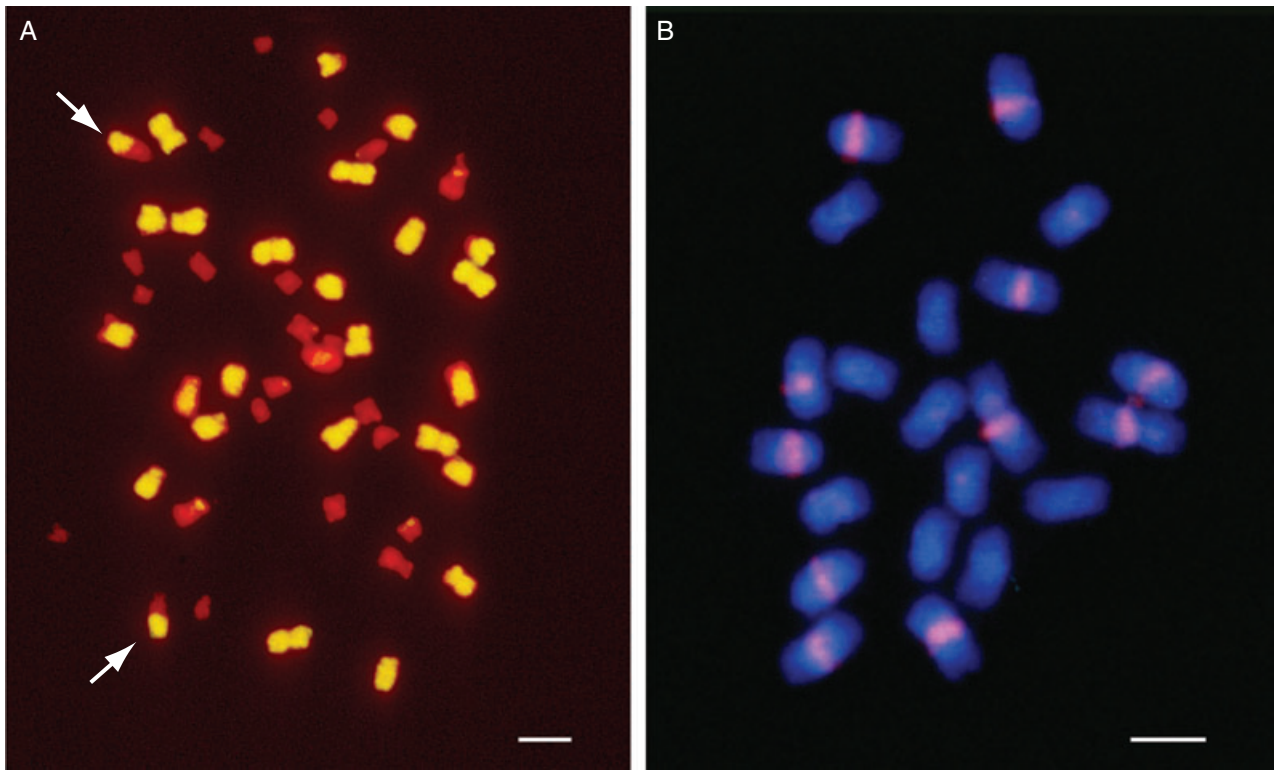

Figure 1. Novel views of chromosomes in polyploid taxa using molecular techniques. A, GISH on a root tip metaphase of *Nicotiana tabaccum* ($2n = 4x = 48$) probed with total genomic DNA from *N. sylvestris* ($2n = 2x = 24$) confirms its allopolyploid nature, and shows intergenomic recombination between S and T genome chromosomes (arrowed). Reproduced with permission from Kenton *et al*. (1993) *Molecular and General Genetics* **240:** 159–169. Scale bar = 5 μm. B, Fluorescent *in situ* hybridization (FISH) of *Sorghum bicolor* bacterial artificial chromosome (BAC 22B2) to *S. bicolor* root tip metaphase chromosomes in a cell trisomic ($2n = 20 + 1$) for chromosome E with strong FISH signals on centromeres of 11 chromosomes (labelled pink) but weak or no signal on ten chromosomes provides strong evidence that *S. bicolor* is at least of allotetraploid origin ($2n = 4x = 20$) with five chromosomes in each genome (from Gómez *et al*., 1998, *Journal of Heredity* **89:** 188–190, with permission). Scale bar = 2 μm.

smaller nested representations of itself revealed by disassembling its higher order structure.

Third, pressing global problems due to human population pressure and environmental pollution demand new information to inform key endeavours such as plant breeding and conservation action plans to slow or prevent species loss and underpin the management and sustainable use of genetic resources. It is impossible to develop meaningful strategies to address these key concerns while ignoring polyploidy, given its distribution and significance in diversity and development.

Together, these reasons combine to make polyploidy a fit, fashionable and futuristic topic, worthy and overdue for new in-depth reassessment. Some historical and terminological perspective is essential to understand the origin and meaning of the terms, to recognize what is already known and what is dogma, and to frame incisive questions for future research.

SOME BASIC TERMINOLOGY OF POLYPLOIDY

It would be easy just to cite key references where the terminology of polyploidy originates or is explained. However, it is probably more helpful for modern readers, who may find access to pre-electronic publications difficult, to give some basic information here, so it is accessible with this compendium of papers on the topic.

Chromosome number is characteristic of, but varies widely between, taxa, ranging in plants from four to over 600 in angiosperms, and to 1440 in the fern *Ophioglossum reticulatum* (Stace, 1993). The whole group of chromosomes derived from a gametic or zygotic nucleus is known as its chromosome complement, but this may contain from one to many basic chromosome sets or genomes. Nuclei with one basic chromosome set are monoploid, those with two are diploid, but nuclei with three or more basic chromosome sets are defined as polyploid – and termed triploid (three sets), tetraploid (four sets) and so on (Darlington, 1932).

At a trivial level, too many do not know the correct term for eight chromosome sets, and so the literature abounds with both the incorrect 'octaploid' and the correct 'octoploid'. However, how many who can spell 'octoploid' do not know its origin? How many see diploid and polyploid as 'di - ploid' and 'poly - ploid', not realizing their origin as 'diplo - id' and 'polyplo - id', from Weissman's (1892) 'id' theory of heredity, referring to a number of 'ids' or 'units of germ plasm'.

To complicate matters, 'diploid' has two commonly used modern uses in plants. It can refer either to 'diplophase' of the life cycle (including in polyploid species with three or more genomes), or to describe taxa with two basic chromosome sets (in contrast to polyploid species). Combining both can produce confusing statements, e.g. 'Bread wheat is a hexaploid containing 42 chromosomes in its diploid cells.'

Organisms with three or more chromosome sets in their zygotic and meiotic nuclei are polyploid taxa. However, nuclei with three or more chromosome sets can occur in cells that do not assure the genetic continuity of an individual in taxa at all ploidy levels (monoploids, diploids and polyploids). Such nuclei are said to exhibit 'somatic polyploidy', which can be of several forms.

Once formed, by whatever mechanism, polyploid taxa may undergo processes of diploidization that affect chromosome behaviour, chromosome number, gene copy number and DNA amount. Thus, diploidization may involve changes that constitute decay of the original wholesale duplications of genomic characters such as DNA amount, gene copy number and chromosome number. Such decay, if protracted, may eventually obscure a polyploid origin and state, and restore a near diploid condition.

Thus, polyploids divide into several types (depending on their progress through this process), which should not be seen as rigid classes but as forming a continuum. According to Ehrendorfer (1980) those very similar and closely related to extant diploids (or lower polyploids), so that they can be placed in the same species (comparium, or species aggregate), are neopolyploids. Those clearly diverged from extant diploids (or lower polyploids), but which are still close enough to them or related polyploids that they can be placed in the same section, or small genus are mesopolyploids. Mature stages of polyploid complexes isolated because all of their diploid and lower polyploid ancestors are now extinct or have diverged beyond recognition are palaeopolyploids.

If there has been a gross reduction in chromosome number that masks their polyploid nature, species are known as cryptic polyploids (Murray, 2002). Polyploids are also described as recent polyploids or ancient polyploids. These terms are not rigidly defined in any absolute temporal or geological basis, but are only relative to each comparison.

Polyploids have also been classified depending on the genetic and taxonomic similarity of the genomes involved, and reflected in their meiotic chromosome pairing behaviour. Thus polyploids are traditionally divided into autopolyploids and allopolyploids, on the basis of their assumed or known origin, using concepts dating back to the classic paper of Kihara & Ono (1926). The terms autopolyploidy and allopolyploidy parallel intraspecific and interspecific polyploidy, respectively. It is possible to distinguish two extreme cases in which three or more chromosome sets are present in a complement. First, those in which the sev-

eral sets are all homologous and which arise within a species by a process of genome multiplication. These types are called autopolyploids. Second, those in which some sets at least are dissimilar, hybridization and genome multiplication having occurred. These are called allopolyploids (Lewis & John, 1963). Between the extreme auto- and allopolyploids, however, there is a complete range of intermediate types, reflecting the range of genetic variation found in different genotypes and taxa. Indeed, considerable variation exists within these classes. Autopolyploidy embraces 'cases ranging from the homozygous individual…at one extreme, to the polyploid derivatives of a hybrid between subspecies…of a species at the other' (Lewis, 1980). The classification by Stebbins (1947, 1950, 1971) is still the one generally accepted and used in the literature.

As with most classification systems, that for types of polyploid is imperfect. Thus, the categories are not always sharply refined, and the distinctions are sometimes difficult and arbitrary. Yet the terms are usefully applied to the majority of situations in which polyploidy is understood within or between species, although species complexes beyond the tetraploid level may involve both phenomena in autoallopolyploids (Lewis, 1980).

HOW MANY ANGIOSPERM SPECIES ARE POLYPLOIDS?

There are about 250 000 angiosperm species, but there has long been no agreement on what proportion are polyploids. Estimates ranged from a liberal 70–80% to a conservative 30%. The difference of 40–50% represents over 100 000 species, which is an unacceptably high error for this key element of plant evolution. The basis for such estimates was shaky, resting on an assumption by an expert that all species with more than a particular number of chromosomes are polyploids. However, experts differ, some setting a threshold as low as $2n = 20$ (Goldblatt, 1980), others as high as $2n = 28$ (Grant, 1981). The lower the threshold is set, the higher the estimated proportion of polyploidy (Stace, 1993). So 87 years after polyploidy was discovered, we do not know with any precision what proportion of angiosperms is polyploid, or allopolyploid.

Recent molecular research has cast serious doubt on the value of such expert opinion as a substitute for experimental evidence, and of the ability of experts to recognize polyploids and ploidy levels. Until recently, most experts agreed that *Zea mays* is a diploid, yet work on synteny proved conclusively that it is a tetraploid (Moore *et al*., 1995; Gaut & Doebley, 1997). Clearly, a radical reassessment of the proportion of polyploids in angiosperms, and of our ability to recognize individual polyploids, was already overdue. Moreover, if all flowering plants are palaeopolyploids, this can render estimates of the frequency and level of polyploids in extant groups (e.g. angiosperms) academic, unless 'polyploid' is carefully defined in each case.

TOWARDS A FUNCTIONAL DEFINITION OF POLYPLOIDY

'What is recognized as a diploid at the generic level may represent an ancient polyploid at higher levels of taxonomic categories.' Clearly, defining polyploidy is often a relative truth or subjective concept, based either on the opinion of an expert, or on a starting point in a phylogeny, arbitrarily assumed to be diploid for the sake of comparison. Is any more absolute definition of polyploidy possible?

A functional definition in terms of a minimal genome may be helpful. There is a minimum compendium of nuclear genes essential for the life and reproduction of any organism. Taxa with two copies of a minimal genome are functionally diploid for a life form. Those with wholesale duplication in their ancestry, which possess, or retain substantial parts of, three or more complete copies of the minimal genome are functionally polyploid.

This concept is behind Craig Venter's recently declared intention to synthesize from scratch a minimal bacterial genome (Check, 2002). A project for a minimal eukaryote genome may follow. If so, a successful organism with two copies of a minimal genome would be diploid in absolute terms. Moreover, its duplication would produce an exquisite new model for a unique study of diploidization in a minimal autotetraploid genome, uncluttered by extraneous DNA sequences.

The ancestral genome for each life form was probably less streamlined than Venter's minimal genome concept, and included some redundant DNA. However, it would contain all the genes coding its essential characters at divergence. As such, it would define a meaningful diploid baseline against which any later genome duplication affecting the level and occurrence of polyploidy can be measured and expressed.

GENOME DUPLICATION THEORIES

Today the term polyploidy is used interchangeably with complete genome duplication (e.g. Kellogg, 2003). Interestingly, there has long been interest in the possibility of repeated whole genome duplications, with or without polyploidy. Wallace & Morowitz (1973) proposed that total genome doubling may have had an evolutionary role as a means of independent development of early prokaryotes (by genome size increase) and of eukaryotes (by genome number increase). Sparrow's group reported a series of doublings of a mini-

mum genome size in many widely separated taxonomic groups, a phenomenon they called cryptopolyploidy. Unlike conventional polyploidy, which denotes a multiplication of a basic chromosome number, cryptopolyploidy 'results in larger chromosomes'. Comparisons suggested at least eight doublings of a basic ancestral genome common to many groups (Sparrow & Nauman, 1976). Some have claimed the existence of multistranded chromosomes, e.g. in *Vicia* (Martin & Shanks, 1966), which possibly explained the phenomenon, but this idea was soon discounted. Others claimed that DNA C-values for diploid species show an approximate doubling series within a genus, e.g. *Anemone* (Rothfels *et al*., 1966), or large incremental increases of a basal DNA C-value, e.g. in *Lathyrus* and *Allium* (Narayan, 1998). These ideas were also discounted (Nandini *et al*., 1997), but some of the data on which they were based merit re-examination in the light of new understanding of complete genome duplications (Wendel *et al*., 2002; Durand, 2003) and subsequent genome downsizing (Leitch & Bennett, 2004 – this issue).

HOW DO ANGIOSPERMS COMPARE WITH OTHER PLANTS?

Despite the difficulties just mentioned, polyploidy *s.l.* among angiosperm species is certainly high compared with some, though not all, other plant groups. With 80-ploid reported in the stonecrop *Sedum suaveolans* (Uhl, 1978), maximum ploidy level is high in angiosperms compared with some other groups.

Polyploidy is much rarer among gymnosperms (<5% of species). Khoshoo (1959) listed only 11 real polyploids out of 240 taxa (4.6%). Moreover, the maximum ploidy level was also low, restricted to tetraploidy except in one hexaploid species, *Sequoia sempervirens* with $2n = 66$.

By contrast, both the proportion and the maximum level of polyploidy are even higher among pteridophytes than among angiosperms. Thus, at least 90% of pteridophyte species are thought to be polyploids, and a race of *Ophioglossum reticultum* with 1440 chromosomes is thought to be 96-ploid (Khandelwal, 1990).

Many accept a high proportion (*c*. 80%) of bryophytes are polyploids, assuming low basic chromosome numbers of 5–7 in mosses (Newton, 1984; Kuta & Pryzwara, 1997), but others challenge this, suggesting it is much lower (e.g. Voglmayr, 2000). According to Newton (1988) polyploidy in liverworts is rare (<13% – assuming $n = 8$, 9 or 10), and ~2% of hornworts are believed to be polyploids (Wyatt *et al*., 1988). Regardless, maximum ploidy level is lower in bryophytes than in angiosperms, reaching only about 32× or 16×.

Polyploidy is also common among algae, but estimates of its incidence are rare. Nevertheless, algae seem similar to angiosperms. Polyploidy must reach very high levels in algae, as chromosome numbers from eight to over 500 are known, and allopolyploidy is regarded as a major factor in the evolution of some groups (such as Rhodophyta) (Nichols, 1980).

Depending on what is true in bryophytes, the proportion and maximum level of polyploidy in different plants may be positively correlated, but rigorous tests await better estimates for these characters in the various groups. Clearly, most plant species are polyploid *s.l.*, and pteridophytes and angiosperms have more polyploids and a higher maximum ploidy level than other higher plant groups. As Stebbins (1971) correctly noted: 'The most wide-spread and distinctive cytogenetic process which has affected the evolution of higher plants has been polyploidy, the multiplication of entire chromosome complements.'

SOMATIC POLYPLOIDY WITHIN SPECIES

Not only are most angiosperm species polyploids, but so are many cells, even in diploid species. Whereas the ploidy level found in the zygote is maintained in embryonic, meristem and other cell lines that assure the genetic continuity of an individual, higher ploidy, as the result of endopolyploidy or endoreduplication, is common in other living cells (De Rocher *et al*., 1991). The proportions of such polyploid cells in the diploid species *Beta vulgaris* and *Scilla decidua* were estimated at 70–80% (Frisch & Nagl, 1979). Similarly, DNA contents in nuclei from *Arabidopsis thaliana* as seen by flow cytometry led Galbraith, Harkins & Knapp (1991) to note that somatic polyploidy includes 'a majority of the somatic cells comprising the body of the plant'. So in this and other 'diploid' species, most of its living cells are not diploid but polyploid systems. Similarly, most cells of polyploid taxa have higher ploidy levels than are listed for them in a Chromosome Atlas.

Some form of somatic polyploidy is found in nearly every angiosperm taxon examined (Nagl, 1982), but not all; Evans & Van't Hof (1975) found no polyploid cells in any tissue of *Helianthus annuus* (roots, cotyledons, stems, leaves, sepals, petals, pistils and stamens). However, somatic polyploidy occurs in many species in a wide variety of root, stem, leaf and flower cell types, including: root cap, xylem vessel, stem and leaf epidermis, trichome, nectary trichoblast, elaiosome, tapetum, antipodals, endosperm, cotyledon, suspensor and endosperm haustorium.

Not only is somatic polyploidy widespread, but it can reach high levels in both diploid and polyploid species [as excellent reviews by Barlow (1978), Nagl (1982) and D'Amato (1984) show]. For example, based on cytophotometry, somatic polyploidy is known to reach 64C in cotyledon cells of *Pisum sativum*, 90C in

endosperm of *Zea mays* (Kowles & Phillips, 1985), 256C in trichomes of *Urtica* spp. and hairs of *Bryonia dioica*, 1024C in antipodal cells of *Scilla bifolia* and 8192C in suspensor cells of *Phaseolus coccineus*. Based on its relationship with nuclear volume, it is estimated to reach 4096*n* in elaiosomes of *Scilla bifolia*, and 24 576*n* in endosperm haustorium of *Arum maculatum*. Thus, it seems that most living cells in most angiosperm taxa show somatic polyploidy.

RATIONALE FOR POLYPLOIDY RESEARCH

There are several strong reasons why a better understanding of polyploidy in plants is needed, and why new polyploidy research is timely.

First, it is estimated that plants form 90% of the world's biomass. Moreover, most plant species and most of their cells are polyploid. So most of the world's biota (our life support system) is polyploid, and life on earth is predominantly a polyploid plant phenomenon. Not understanding this represents a failure to know how most cells in most organisms are, and work.

Second, we face a mass extinction of biodiversity, losing species at 10 000 times the background rate (May, Lawton & Stork, 1995). A third of angiosperms (80 000 species) may be lost by 2050 (World Conservation Monitoring Centre, 1992). Most of the world's threatened flora (our global gene bank) are polyploid. We need to know if diploids and polyploids are equally at risk. If so, because of allopolyploidy, the percentage loss of genomes may be more than the percentage loss of species. However, if polyploids are more adaptable and likely to survive, the proportion of polyploids among surviving species will rise.

Third, any consideration of the importance of polyploidy must mention endosperm, which forms the greater part of many seeds. Early endosperm is triploid, but later higher polytriploid levels are common (Kowles & Phillips, 1985). This polyploid tissue provides most of our food and hence powers our civilization. Better knowledge about endosperm seems vital, but we still do not know the origin or significance of triploid endosperm (Donoghue & Scheiner, 1992).

We know little about the significance of polyploidy in endosperm, but even less about its role in the antipodal cells that abut endosperm, and become highly endopolyploid in many species, including breadwheat. Figure 2A compares a 256C antipodal cell nucleus of breadwheat with a somatic 4C interphase nucleus. The antipodal nuclei can have polytene chromosomes in some taxa (Nagl, 1981). For example, Figure 2B shows four 256-stranded chromosomes of rye compared with 2–4C ovular nuclei. It might be expected that these highly polyploid cells are well researched, but not so. Rather they epitomize our deep ignorance of the role and control of somatic endopolyploidy in plants.

Their many templates and active nucleoli have led to proposals that polyploid antipodal cells play a secretory function in seed development, but if so, its nature remains uncertain. Perhaps the main role of high polyploidy in antipodals is phenological or anatomical. By facilitating rapid nuclear doubling in coenocytic endosperm they may cut generation time by about a week. Highly polyploid antipodal cells have a large volume before resorption, so their main role may be to create a large hole, which endosperm can fill later. Their main role may not be in their life, but only after they are dead and gone. If so, antipodal cells would not be unique, as polyploidy in developing xylem helps to create the large empty vessels familiar in plant plumbing after the lysis of their cell contents.

Fourth, most agricultural production of food, fodder and fibre comes from polyploids. Table 1 lists the world's 21 most important crops measured by area under cultivation using 2002 FAO data: 71% are polyploids occupying 83.7% of the area. Polyploids predominate irrespective of whether cereal, pulse or fodder crops are listed separately, and no matter whether importance is measured by area, production or its cash value. Polyploidy is important because we depend primarily on polyploids for most natural products. Consequently, and fifthly, most plant breeding involves the genetic modification of polyploid species.

Excitement after the discovery of how to make polyploids using colchicine was great, but the practical impact of the new knowledge fell far short of what many expected. Apart from just a few examples, such as triticale, the number of artificial polyploid crops successfully introduced into agriculture in the 70 years since this discovery is disappointingly few compared with early predictions (Dewey, 1980). Consequently, we must ask whether recent new advances in understanding the role of polyploidy in genome evolution will also prove to be one more small theoretical step, rather than a great practical leap forward for mankind, which delivers exciting benefits to improve significantly our quality of life.

New knowledge about polyploidy will be most useful when it can reliably predict which crops can be usefully improved as stable autopolyploids, and which genomes can be successfully combined to create new allopolyploids at higher ploidy levels (Bennett, 1981). However, this still awaits discoveries of how nuclear stability and fertility are achieved. We still need understanding of diploidization mechanisms, which puts in our hands generic control of the molecular levers controlling cell development, including those able to order and repattern the spatial and temporal arrangements and behaviour of genomes, chromosomes and DNA sequences.

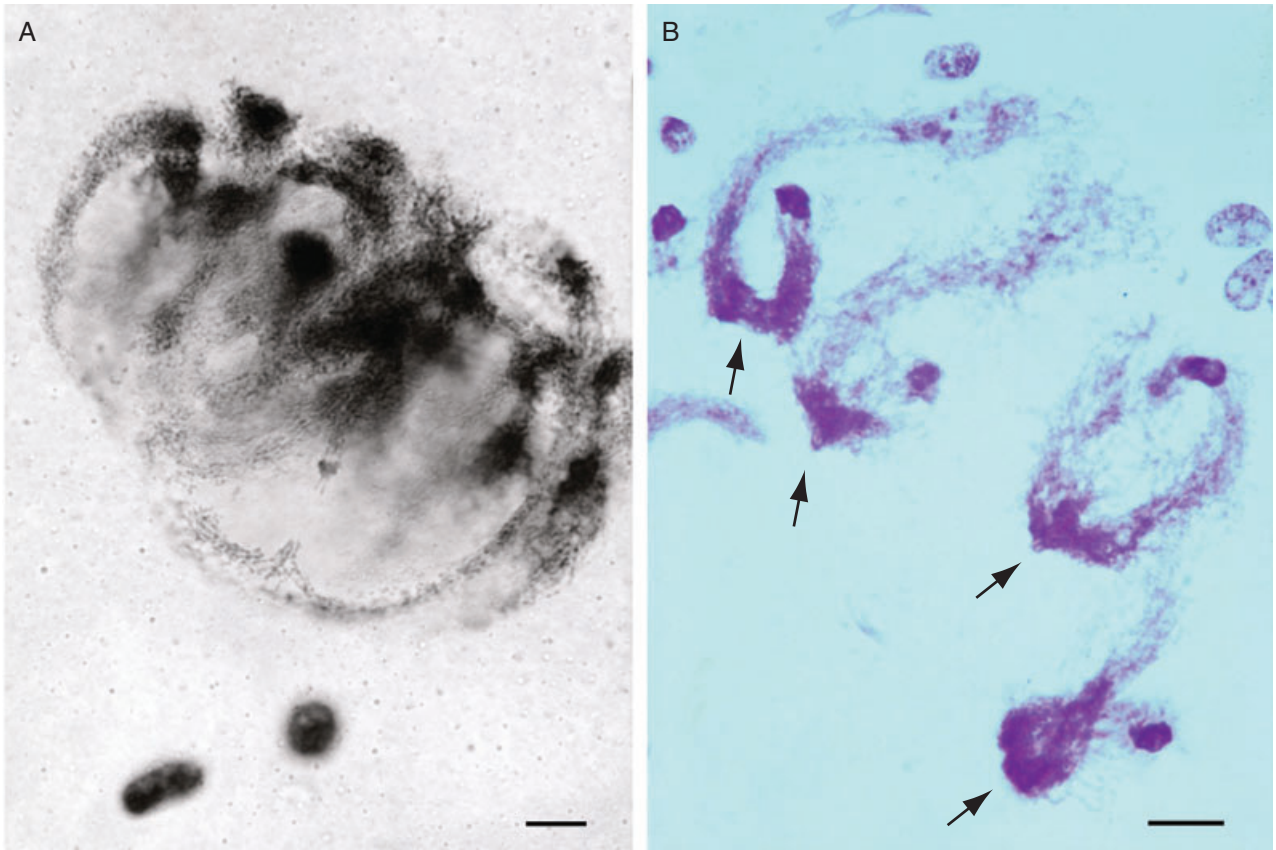

Figure 2. Somatic polyploidy in Feulgen-stained antipodal cell nuclei of grasses. A, Nuclei of hexaploid *Triticum aestivum* ($2n = 6x = 42$) comparing an endopolyploid antipodal cell nucleus with a 256C DNA content and two somatic nuclei with 4C DNA contents. B, Four 256-stranded chromosomes (arrowed) from an antipodal cell of *Secale cereale* compared with ovular nuclei with 2C or 4C DNA contents. Scale bar = 10 μm.

CHROMOSOME PAIRING IN POLYPLOID PLANTS

The key problem still to be resolved concerns how bivalent pairing seen at first metaphase of meiosis is determined in many polyploids despite the presence of multiple homologues or homoeologues. As Stebbins (1971) noted: 'In many plants having high numbers of chromosomes such as the adder's tongue fern *Ophioglossum* the regular formation of hundreds of bivalents in every meiotic cell, each of which represents the association of a chromosome with only one specific mate of the hundreds which surround it, is nothing short of miraculous.' Genomic and other DNA probes have been used to study the meiotic process in breadwheat and other grasses to good effect (Martinez-Perez, Shaw & Moore, 2001; Golubovskaya *et al*., 2002), but the molecular mechanisms behind the colourful new cell biology seem as inscrutable as for the monochromic views of past decades (Thomas & Kaltsikes, 1976; Bennett, 1979).

Other data from electron microscopy reconstructions should not be forgotten as they also concern genome organization in polyploids. Such work asked: How are basic genomes spatially organized in the nucleus? Are they random, or do they occupy separate domains? In diploid hybrids between *Hordeum vulgare* and *Secale africanum* parental sets were distinguishable by size; chromosomes from *S. africanum* were all larger than any from *H. vulgare*. Studies on somatic cells from root tip meristems showed that that these two parental genomes always tended to occupy separate spatial domains throughout development, and in different plants, years and tissues, as in the root-tip metaphases shown in Figure 3A (Finch, Smith & Bennett, 1981; Schwarzacher-Robinson *et al*., 1987). The separation of monoploid parental sets was mostly 'concentric' but sometimes it was 'side-by-side' (Bennett, 1988; Leitch *et al*., 1991). In a remarkable technical feat, J. B. Smith cut tens of thousands of serial thin sections of several entire anther locules to look at premeiotic mitosis and showed that this highly signifi-

Table 1. Diploids and polyploids among all the world's top crops grown on over 10 million hectares, ranked by area harvested (Ha) – FAO www.2002*

Rank	Crop	Polyploid(s)	Diploid(s)
1	Wheat	210 785 147	
2	Rice, Paddy[1]	146 029 456	
3	Maize	138 896 695	
4	Soybeans	79 167 520	
5	Barley		54 012 738
6	Sorghum	42 103 351	
7	Millet		36 885 951
8	Seed cotton	32 281 621	
9	Groundnuts in shell	25 863 695	
10	Beans, dry		24 698 382
11	Rapeseed	22 855 090	
12	Sugar cane	19 733 548	
13	Sunflower seed		19 568 213
14	Potato	19 256 031	
15	Cassava	16 907 529	
16	Alfalfa for forage + silage	15 870 041	
17	Oat	13 493 832	
18	Coconut		10 792 364
19	Oil palm fruit	10 782 450	
20	Chickpea		10 660 511
21	Coffee, green	10 644 040	
	Total	**804 670 046** (83.7%)	**156 618 159** (16.3%)

*Site for FAO statistics: http://faostat.fao.org/faostat/collections?subset=agriculture (accessed Feb. 2002).

[1]Rice (*Oryza sativa*) is listed as a polyploid because studying its draft DNA sequence showed that 59% of markers were present as two or more copies, indicating an apparent whole genome duplication (Goff *et al*., 2002).

cant separation of monoploid sets (Fig. 3B) persisted until just before meiosis (Bennett, 1988), as seen previously in *Hordeum vulgare* (Bennett, 1984) and noted later in an F_1 *H. vulgare* × *H. bulbosum* interspecific hybrid (Schwarzacher *et al*., 1992). Part of one male archeporium had spontaneously become allotetraploid, with about 28 rather than 14 chromosomes (Bennett, 1988), and a strong tendency towards 'concentric' genome separation of *Secale* and *Hordeum* was clearly displayed in all three reconstructed allopolyploid premeiotic nuclei (Fig. 3C).

Another unpublished result also showed that monoploid sets are spatially separated in some plant reproductive tissues. Work at RBG Kew on fertile amphidiploids between naturally occurring autotetraploids of *Gibasis consobrina* and *G. karwinskyana* (both $2n = 20$) confirmed that their identically sized monoploid sets are easily distinguished by genomic *in situ* hybridization (GISH) (Fig. 4A) (Parokonny *et al*., 1992). These species in the Commelinaceae have a generative cell mitosis to produce two sperm nuclei in the developing pollen tube. The narrow bore of the pollen tube causes the large chromosomes to form a linear array at metaphase as seen in an unreduced generative cell of *Gibasis karwinskyana* with $2n = 10$ (Fig. 4B). This behaviour is ideal for testing for genome and chromosome order. We successfully performed GISH on chromosome arrays and interphase generative nuclei in intact pollen tubes of fertile amphidiploids between autotetraploids of *Gibasis consobrina* and *G. karwinskyana* (both $2n = 20$). Figure 4C shows typical generative nuclei probed with genomic DNA from *G. karwinskyana* fluorescing yellow, quite separate from *G. consobrina* DNA, which is red. These results show unequivocally that these identically sized monoploid sets from either parent are spatially separate soon after meiosis, whereas other results in allotetraploid nuclei of barley × rye showed

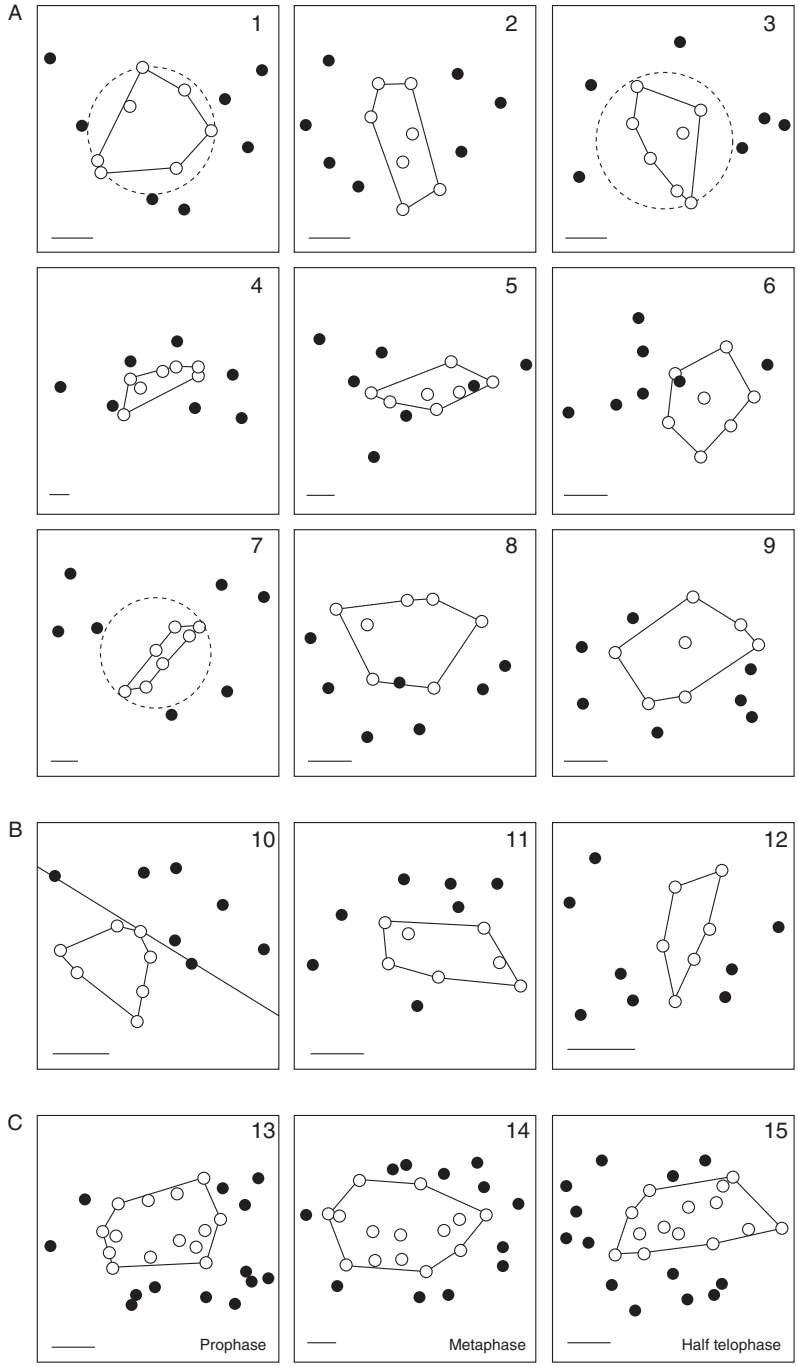

Figure 3. Spatial separation either side-by-side or radially of parental genomes in 15 out of 16 serially thin-sectioned reconstructed cells of F_1 *H. vulgare* × *S. africanum* ($2n = 14$). A, Polar views of positions of *Hordeum* (○) and *Secale* (●) centromeres on metaphase plates, and polygons of least perimeter including all *Hordeum* centromeres, in nine root-tip cells. Reproduced from Schwarzacher-Robinson *et al*. (1987) *J. Cell Sci* 87: 291–304 with the permission of the Company of Biologists Ltd. B, Polar views of positions at the polfeld of *Hordeum* (○) and *Secale* (●) centromeres at prophase, and polygons of least perimeter including all *Hordeum* centromeres, in three diploid ($2n = 14$) male archesporial cells at premeiotic mitosis. Centromeres from parental genomes separate either side of a line in cell 175. C, Polar views of the positions of *Hordeum* (○) and *Secale* (●) centromeres, and polygons of least perimeter including all *Hordeum* centromeres, in all three spontaneously doubled ($2n = 26$ or 27) near tetraploid male archesporial cells at different stages of premeiotic mitosis. B & C are reproduced with permission from Bennett (1988) Proceedings of the Third Kew Chromosome Conference, 195–208. Scale bars = 2 μm.

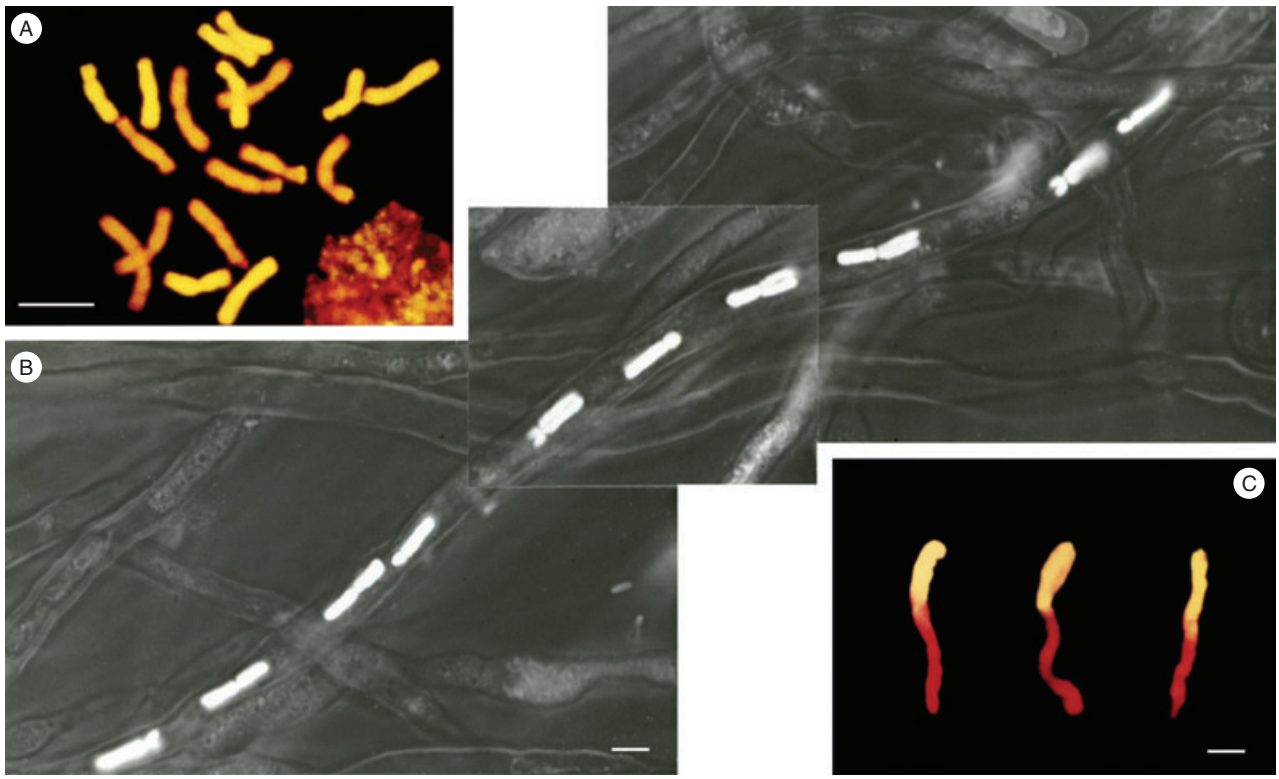

Figure 4. A, Genomic *in situ* hybridization distinguishes parental genomes from *Gibasis karwinskyana* (yellow) and *G. consobrina* (orange/red) in their F_1 hybrid ($2n = 10$) probed with total genomic DNA from *G. karwinskyana* (NB, two chromosomes are missing). B, Chromosomes form a linear array in the narrow bore of a pollen tube at mitotic metaphase in an unreduced generative nucleus of tetraploid *Gibasis karwinskyana* ($2n = 20$). C, GISH of interphase generative nuclei in intact pollen tubes of fertile amphidiploids between autotetraploids of *Gibasis consobrina* and *G. karwinskyana* (both $2n = 20$) reveals post-meiotic parental genome separation. Typical generative nuclei probed with genomic probe DNA from *G. karwinskyana* (fluorescing yellow) show that *G. karwinskyana* DNA is separate from *G. consobrina* DNA, which is red. Scale bar = 5 μm.

they remain separate until premeiotic mitosis. Thus, as previously noted (Bennett, 1984), the premeiotic switch leading to meiotic pairing must involve two steps, first switching off whatever controls genome separation, and second switching on some controls of homologous pairing. Diploids and polyploids may differ in the timing of such steps, which may be concurrent in one but not in the other.

If all angiosperms are indeed palaeopolyploids, then no new genes are needed to explain bivalent pairing in new allopolyploids, as these can reuse existing mechanisms that controlled this early step in diploidization after previous waves of polyploidization.

UPPER LIMIT TO POLYPLOIDY IN PLANT TAXA

There is a large literature on the effects of higher genome dosage, which may explain the advantages of polyploidy and the success of polyploids in nature. However, optimum ploidy levels seem to vary between different groups (Brandham, Fraser & West, 1995), and there are clearly limits to multiplying copies of the nuclear genome before this becomes a liability. The different effects of autohexaploidy in barley (which is sterile) and allohexaploidy in wheat (which is the world's number one crop – Table 1) epitomizes our ignorance on these matters.

Rommel (1960) reported six rare autohexaploid barley plants, noting that none progressed beyond grass dwarfs. Finch & Bennett (1982) made large populations of plants with one, two, three, four, five or six copies of a genetically identical *H. vulgare* genome, and easily obtained over 90 seedlings counted as autohexaploid ($2n = 42$) among progeny of autotetraploid *tri*. From monoploid to tetraploid all made healthy mature green plants that produced seed. However, the autohexaploid *tri* mutant plants of the different cultivar (Parvo) all ceased mitosis before meiosis (only three progressing beyond grass dwarfs) after appear-

ing normal during early vegetative growth. So the maximum ploidy level that a species can tolerate seems limited. In *H. vulgare* the maximum viable genome dosage is four, as autopentaploidy and autohexaploidy induced sterility and premature death. This maximum clearly varies between species, as breadwheat and some other *Hordeum* taxa are fertile hexaploids, but the mechanism(s) that determines the limit is totally unknown.

GENES FOR TWO-WAY PLOIDY LEVEL CHANGE

Triploid inducer *tri* (Ahokas, 1977) is a gene for polyploidization that may map to 5HL (Finch & Bennett, 1982). Other genes that control the elimination of complete parental sets are also known and mapped to two other linkage groups in barley (Kasha, 1974). Their potentially opposite roles in nature are also unknown. Genes such as *tri* (in barley) and *elongate* (in maize) may be important in both the evolution and the breeding of plants. Polyploidy in plants is almost invariably seen as a one-way process, involving only a progressive increase in the number and types of genomes (Stebbins, 1971). Apart from reversible tetraploidy (in which fertile diploids are recovered from autotetraploid taxa), which is commonly regarded as playing at most a minor role in population evolution in higher plants (Stebbins, 1971), reductions in ploidy level are regarded as rare and inconsequential in angiosperm evolution, although de Wet (1980) questioned this dogma. Moreover, the genes controlling uniparental genome loss may determine a decrease in the number of genome types and dosage, and the recovery in nature as autonomous entities of diploid species whose nuclear genomes had once coexisted with those of other species in the nuclear environment of allopolyploids (Bennett, 1981). Thus the relationship between diploids and polyploids may be a dynamic system with strong two-way traffic both increasing and decreasing genome dosage in nature. This possibility has not received serious experimental attention, probably because it is contrary to dogma, but its consideration would now be both timely and practical using molecular techniques that could provide evidence of a previous allopolyploid association in a now diploid taxon.

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