Review

CellPress

Plasticity in ploidy: a generalized response to stress

Daniel R. Scholes and Ken N. Paige

School of Integrative Biology, University of Illinois at Urbana-Champaign, 515 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, USA

Endoreduplication, the replication of the genome without mitosis, leads to an increase in the cellular ploidy of an organism over its lifetime, a condition termed 'endopolyploidy'. Endopolyploidy is thought to play significant roles in physiology and development through cellular, metabolic, and genetic effects. While the occurrence of endopolyploidy has been observed widely across taxa, studies have only recently begun to characterize and manipulate endopolyploidy with a focus on its ecological and evolutionary importance. No compilation of these examples implicating endoreduplication as a generalized response to stress has thus far been made, despite the growing evidence supporting this notion. We review here the recent literature of stress-induced endopolyploidy and suggest that plants employ endoreduplication as an adaptive, plastic response to mitigate the effects of stress.

The prevalence and patterns of endopolyploidy in plants

Endoreduplication is the replication of the genome without mitosis such that the nuclear DNA content (i.e., the ploidy) of the cell increases with each successive replication (generating 'endopolyploidy,' i.e., heightened ploidy within an organism; see Glossary). Although similarities exist, endopolyploidy is distinct from polyploidy in many important ways (Box 1). Endopolyploidy is common among eukaryotes, and particularly among plants, where approximately 90% of herbaceous angiosperms exhibit endopolyploidy in the majority of their tissues [1,2]. It is relatively rare among non-flowering plants, however, occurring only in the gymnosperm *Ginkgo biloba* (ginkgo) and widely among mosses (Figure 1) [3]. The occurrence of endopolyploidy in these relatively disparate groups perhaps indicates multiple evolutionary origins in land plants [4,5].

A vast mosaic of ploidy levels may be observed within an endopolyploid organism, particularly among different cell types [6–9]. For example, the highest level of endopolyploidy observed in plants is 24 576C (i.e., 24 576-fold the basic genome complement) in the endosperm of *Arum maculatum* (arum lily), although a range of 4C to 64C is typical in plants [9,10]. Very high ploidy levels (often 512C or greater) are a general feature of endosperm and suspensor cells of seed across endopolyploid taxa [9]. Very low (or the complete lack of) endopolyploidy across taxa is observed in a few cell types, including phloem companion cells and stomatal guard cells, both of which serve highly specialized functions that would possibly be disrupted by increased ploidy [7,9]. Because endoreduplication is a somatic process, the embryo and meristematic cells (e.g., procambium, root and shoot apical meristems) also lack endopolyploidy [6,7,9]. Finally, mixed ploidy among adjacent cells of the same type also occurs (e.g., leaf epidermal pavement cells range from 2C to 64C) [7,9].

Although generalized patterns of endopolyploidy may be observed within and among plants, recent evidence suggests that many plants that endoreduplicate can plastically increase their endopolyploidy beyond their 'normal' level in response to environmental factors. Given the sessile nature of plants, endoreduplication appears to be a

Glossary

C-value: the amount of DNA contained within a haplophasic nucleus (e.g., a gamete), typically measured by the number of basepairs or by mass; for a diploid organism the C-value is equivalent to genome size.
Cytokinesis: cell division, the final stage of mitosis.
Endocycle: the succession of genome replications in which the replicated genome copies remain in the same cell by omission or abortion of mitosis.
Endomitosis: the process by which mitosis is initiated but aborted before completion, where sister chromatids and chromosome segregation may or may not have occurred; can result in polyteny, doubling of nuclear chromosome number, or polynucleation depending on the timing of abortion.
Endopolyploidy: the condition in which the number of cellular genome copies has been increased through endoreduplication.
Endoreduplication: the process of complete genome replication without subsequent cytokinesis.
Genome: the complete set of chromosomes in which each is present in only one copy.
Genome size: the amount of DNA that composes a single genome copy (e.g., within a monoploid nucleus), typically measured by the number of basepairs or by mass.
Mitosis: the process of chromosome segregation and cytokinesis that results in the production of two cells with half the number of genome copies versus the initial progenitor cell.
Ploidy: an expression reflecting the number of copies of the basic genome (e.g., diploidy denotes two copies of the basic genome).
Polynucleation: the process by which mitosis is terminated after chromosome segregation, but before cytokinesis, such that multiple nuclei reform within a single cell; may also occur via cell fusion without nuclear fusion.
Polyteny: the condition of a chromosome that has been replicated but in which sister chromatid cohesion is maintained.
***n*-value:** the number of chromosomes within a haplophasic nucleus (e.g., a gamete).
Nucleotype: non-genic characters of the genome that affect phenotype.
Sister chromatids: replicated chromosomal strands that separate during mitosis to become individual chromosomes.

Corresponding author: Scholes, D.R. (scholes2@illinois.edu).
Keywords: endoreduplication; endopolyploidy; stress; environment; plant; response.

1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2014.11.007

Box 1. Polyploidy versus endopolyploidy

Polyploidy is the condition of having multiple (>2) copies of the basic genome. Polyploidy is very common in plants, with evidence of ancestral polyploidy in nearly every angiosperm and fern lineage, and in approximately 60% of moss lineages [28,106,107]. Autopolyploidy results from the doubling of the basic genome by fertilization of unreduced (2*n*) gametes or from spontaneous genome doubling of a 2*n* zygote shortly after fertilization [107,108]. Allopolyploidy results from hybridization of two species, often via fusion of unreduced gametes of each species or spontaneous genome doubling of the hybrid zygote [107,108]. Regardless, the polyploid state is generally either stabilized and perpetuated, or is lost through genome downsizing and rediploidization [106–108]. Because both polyploidy and endopolyploidy entail an increase in the number of nuclear genome copies, both may be expected to impact on nucleus volume, cell volume, and other cellular properties in similar ways [108].

Polyploidy and endopolyploidy differ in many important ways, however. While polyploid and endopolyploid cells are both bound by the allelic variation they inherited, allelic diversity can be much higher in polyploid cells. For example, a cell in a tetraploid individual could contain up to four alleles at any given locus in the genome. A polyploid individual can thus harbor tremendous allelic diversity, and the new epistatic interactions between the alleles is one of the bases for the hybrid vigor often observed shortly after polyploidy is generated [106]. A tetraploid cell generated via endoreduplication from the diploid state, by contrast, would still contain at most two alleles at any locus. The effects of increased ploidy are therefore due solely to the amount of DNA present rather than to new sequence variation, although these nucleotypic effects can be plastically induced as needed by the cell, organ, or organism. Note, however, that some polyploid species also generate endopolyploidy – for example, *Beta vulgaris* (sugar beet) [109].

While polyploidy is inherited through the germline and perpetuated throughout the soma, endopolyploidy is produced by individual somatic cells. The effects of endopolyploidy on cell size, gene expression, and cell metabolism are thus not perpetuated sexually nor shared among cells. Finally, because endopolyploidy is largely generated in differentiated somatic cells, polyteny, sister chromatid separation, or polynucleation can result after genome replication. By contrast, polyploidy necessitates sister chromatid separation for mitotic and meiotic cell division. The effects, if any, of these chromosome structural differences on genome function are not well understood, however.

valuable and commonly employed, but poorly assessed, strategy that affords plants a means of cellular optimization to local conditions that can improve their survival and fitness [1,11].

To date, no reviews have described the significance of plant endoreduplication in response to environmental stress. We draw here upon studies across disciplines, including horticulture, agronomy, ecology, evolution, and molecular biology, to review evidence that endoreduplication is employed as a plastic response by numerous plant taxa to help them mitigate the effects of environmental stress or otherwise fine-tune themselves to their local conditions. Specifically, we present the following: (i) an outline of the mechanistic basis of endoreduplication, (ii) the demonstrated roles of endopolyploidy in plant growth and development, and (iii) a broad purview of the induction of endoreduplication by environmental factors in plants. Finally, we present a synthesis of the evidence that endoreduplication is integrated into the generalized stress response of plants and is induced to mitigate the effects of a wide variety of environmental stresses.

The generation of endopolyploidy

Endoreduplication occurs via the endocycle, an alternative to the mitotic cell cycle that omits cell division but continues genome replication [12]. The induction and duration of the endocycle are developmentally regulated and environmentally influenced through an orchestrated suite of genetic and hormonal regulators (Figure I in Box 2), namely a variety of cyclins and cyclin-dependent kinases (CDKs) [12,13].

There are many variations of the endocycle, depending on the extent of mitotic progression and the nature of replication. If mitosis is completely omitted, the replicated sister chromatids remain cohesed as polytene chromosomes ([14], but see [15]). Endomitosis, by contrast, refers to the initiation but subsequent abortion of mitosis before cytokinesis [16]. Depending on the point at which mitosis is aborted, polyteny, sister chromatid separation, or even polynucleation may result [16]. Over- and under-replication of particular genomic regions can also occur, and is typically limited to regions with high gene expression or heterochromatin, respectively [14,17]. Although rare, the replication of individual chromosomes has also been observed (e.g., *Pinus sylvestris*, Scots pine, in water-logged, hypoxic conditions) [18].

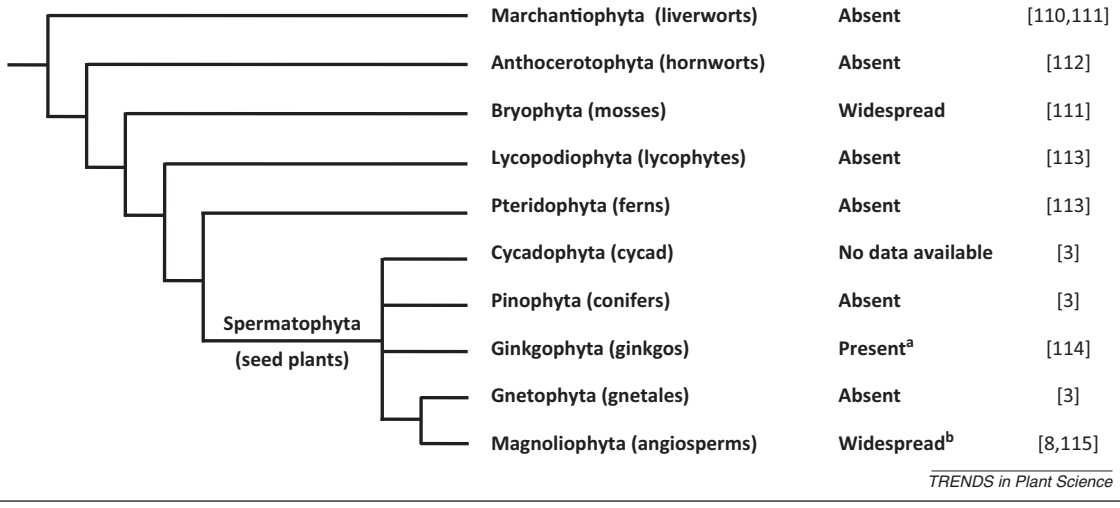

Figure 1. Occurrence of endopolyploidy in plants. Shown are extant divisions within the subkingdom Embryophyta (land plants), modified from the Tree of Life Web Project (http://www.tolweb.org). Branch lengths are arbitrary and are not correlated with divergence times [3,8,110–115]. [a]Endopolyploidy is present in the only extant ginkgo, *Ginkgo biloba*. [b]Note that many families within Magnoliophyta do not have endopolyploidy.

Box 2. Cell cycle regulation in plants

The mitotic cell cycle comprises four phases: the DNA synthesis (S) phase where the genome is replicated, mitosis (M phase) where the replicated genome is divided by half, and two 'gap' phases (G1 and G2) alternating between S and M where cell growth, metabolism, and other cellular activities primarily occur. Progression through the cell cycle occurs via the highly orchestrated interactions of numerous classes of cell cycle regulators.

Key among the cell cycle regulators are cyclins and cyclin-dependent kinases (CDKs). Cyclins are a diverse group of regulatory proteins that bind directly to CDKs. There are approximately 11 types of plant cyclins and eight types of CDKs [99,100], each containing subclasses with numerous members therein (e.g., *CYCA2;3* is the third member of the second subclass of A-type cyclins), although not all plant species have all types of cyclins and CDKs. The combinatorial nature by which cyclins interact with and activate CDKs results in a wide variety of target proteins inactivated by phosphorylation, which provides the basis for modulation of cell cycle phases. For example, the abundance of A-type cyclins rises steadily from late G1 phase through S phase, inducing genome replication by binding and activating A-type CDKs (Figure IA) [13]. The abundance of A-type cyclins drops precipitously at the G2–M transition, although the abundance of B-type cyclins peaks at this time, inducing mitosis through their interaction with A-type CDKs [13]. Throughout M phase, however, B-type cyclins bind to B-type CDKs as part of the 'mitosis-inducing factor' protein complex that maintains the balance between chromosome replication and segregation [116]. Throughout the cell cycle, cyclin abundance is typically modulated either by post-translational ubiquitination or transcriptionally by the environment and hormones – *CYCD3;1* expression, for example, is upregulated by sugar, cytokinin, auxin, gibberelins, and brassinosteroids, and down-regulated by the stress hormones jasmonic acid and abscisic acid (Figure IB) [117]. CDK inhibition seems to be particularly common under abiotic environmental stress [118].

Because the endocycle consists of alternating G and S phases without mitosis, the abundances of cyclins and CDKs are modified during endoreduplication to inhibit mitosis while continuing genome replication. Mitotic inhibition is largely achieved through the down-regulation of A and B-type cyclins, as well as B-type CDKs [10]. D-type cyclins and one subclass of A-type cyclins, CYCA3, may concurrently promote S phase by binding to A-type CDKs (Figure IA) [10,13,99].

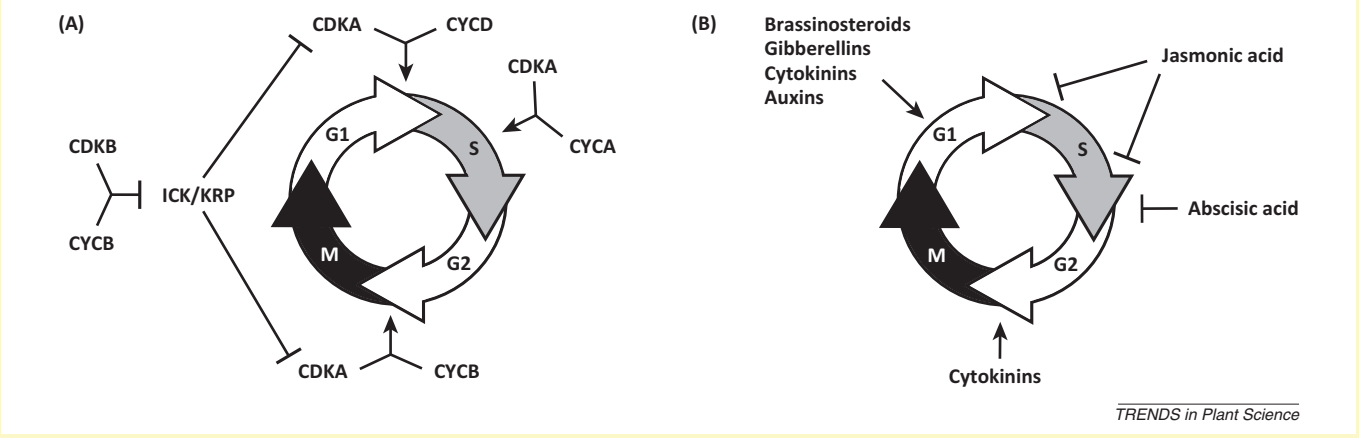

Figure I. Regulation of the cell cycle. **(A)** Genetic and **(B)** hormonal regulation of the plant mitotic cell cycle. Lines with bars (⊥) and arrows (↓) between factors indicate inhibition and promotion, respectively, in the direction of the symbol. Genetic and hormonal regulators are presented separately for simplicity but certainly interact. Several important regulators (e.g., CDK-activating kinases, CDK inhibitors, retinoblastoma protein, and E2F, among others) are omitted for simplicity, however detailed models of cell cycle regulation are presented elsewhere (cell cycle summary [13]; cyclins [99]; CDKs [100]; hormonal regulation [117,119]; in response to environmental stress [105]; endocycle summary [10]). Abbreviations: CDKA/B, cyclin-dependent kinases A,B; CYCA/B/D, cyclins A,B,D; G1, gap-phase 1; G2, gap-phase 2; ICK/KRP, cyclin-dependent kinase inhibitor; M, mitosis; S, DNA synthesis phase.

Endopolyploidy in growth and development

Owing to the breadth of taxa and cell types exhibiting endopolyploidy, endoreduplication is suspected to play important generalized roles in development and physiology [1,4,11,19–22]. These roles may include 'nucleotypic effects' – changes in the basic properties of the cell owing to changes in bulk nuclear DNA content regardless of the genome sequence [23–28]. One such nucleotypic effect is cell size – because cell size is positively correlated with nuclear DNA content [29,30], it is hypothesized that the fastest growth may be achieved when cell proliferation is followed by rapid cell expansion via endoreduplication [31–34], although the regulation and generality of this relationship remain unclear [35–37]. Endoreduplication is also important in cell differentiation [4,21]. Owing to the increase in the number of nuclear genome copies, the metabolic demands of specialized cells may be supported by an increase in transcriptional output of the genome, whether by increases in whole-genome expression or by differential expression of genes within metabolic pathways [21]. The increase in cell size with greater nuclear DNA content is also proportional to the number of particular cellular organelles, and this may further support increased metabolism and cellular specialization (Table 1 summarizes nucleotypic effects demonstrated in plants) [38,39].

These nucleotypic effects at the cellular level may then impact aspects of organismal phenotype. For example, genome size is positively correlated with the duration of the mitotic cell cycle, which in turn affects rates of cellular growth and development, that in combination can modulate the phenotype of the whole organism [23–28]. Depending on its ecological environment, an organism with a small basic genome size may therefore benefit from rapid genome replication and cell division – and organismal growth and development – during times and in cell types where advantageous. It may then endoreduplicate during times and in cells that would benefit from the effects associated with greater cellular DNA content (e.g., increased gene expression and metabolic capacity in specialized cells involved in reproduction) [19,20]. Indeed, endopolyploidy is perhaps

Table 1. Nucleotypic effects demonstrated in plants[a]

Parameter		Taxa	Refs
Generation time	Genome size	31 herbaceous angiosperms, including 19 annual and 12 perennial species	[24]
	Ploidy		N/A
	Endopolyploidy	54, including 14 angiosperm and 2 gymnosperm families	[8]
Mitotic cycle duration	Genome size	110 angiosperms, including 58 monocots and 52 dicots	[120]
	Ploidy	15 angiosperms, including 8 monocots and 7 dicots	[121]
	Endopolyploidy		N/A[c]
Meiotic duration	Genome size	24 herbaceous angiosperms, including 9 annual and 13 perennial	[24]
	Ploidy[b]	16 angiosperms, including 3 polyploid species	[23]
	Endopolyploidy		N/A[c]
Nuclear volume	Genome size	14 herbaceous angiosperms	[122]
	Ploidy	widespread	[123]
	Endopolyploidy	5 herbaceous angiosperms	[29]
Cell volume	Genome size	101 angiosperms, including 32 monocots, 62 dicots, and 7 magnoliids	[30]
	Ploidy	widespread	[123]
	Endopolyploidy	5 herbaceous angiosperms	[29]
Gamete size	Genome size	464, including 437 angiosperms and 27 gymnosperms	[124][d]
	Ploidy	7 species of *Avena*, including multiple accessions of each	[125]
	Endopolyploidy		N/A[c]
Seed size	Genome size	1222, including 139 families and 48 orders	[126]
	Ploidy	18 diploid and 27 tetraploid cultivars of *Lolium perenne*	[127]
	Endopolyploidy	Hybrid crosses between 5 varieties of *Pisum sativum*	[128]
Gene expression	Genome size		N/A
	Ploidy	Ploidy series of *Zea mays*	[129]
	Endopolyploidy	*Solanum lycopersicum*	[39]
Mitochondria number	Genome size		N/A
	Ploidy		N/A
	Endopolyploidy	*Solanum lycopersicum*	[39]
Chloroplast number	Genome size	111 species across 28 families of Magnoliiadae	[38]
	Ploidy	38 species of Solanaceae	[130]
	Endopolyploidy	*Beta vulgaris*	[131]

[a]Plant taxa and references listed for each parameter are examples and are not considered definitive nor exhaustive. All parameters have a positive relationship with nuclear DNA content unless noted. Parameters are not necessarily independent (e.g., cell volume is likely correlated with nuclear volume). N/A, data not available.

[b]There is a negative relationship between ploidy and meiosis duration.

[c]An effect is not expected because of the limitation of endoreduplication within differentiated somatic cells.

[d]A positive relationship exists but with phylogenetic influence.

most common among large, specialized cells with high metabolic output, and in organisms with small basic genome sizes with rapid development and short generation times (e.g., herbaceous angiosperms) [1,4,8,9]. The scaling of cellular nucleotypic effects to the organism is often unclear, however, because a balance in cell size and cell number may prevent increases in overall organ or organism size [31,40,41].

Environmentally induced endopolyploidy in plants

The induction of endoreduplication has been observed in response to numerous environmental stressors across a wide variety of plants. We present here examples by stressor and discuss the mechanisms by which the authors assert increased ploidy helps to mitigate the effects of stress.

Abiotic factors

Light. One environmental variable that seems to directly influence endopolyploidy in plants is light availability. Etiolated hypocotyls of *Lupinus albus* (white lupin), *Raphanus sativus* (radish), *Glycine max* (soybean), *Pisum sativum* (garden pea), *Arabidopsis thaliana* (thale cress), *Brassica oleracea* (wild cabbage), and others all show greater numbers of endoreduplication cycles than do hypocotyls of light-grown seedlings [9,42]. The rapid elongation of dark-grown hypocotyls appears to be an adaptive light-searching strategy, likely developed to combat deep seed burying or crowding in nature. Similar examples of low-light induction of endopolyploidy include *Kalanchoe blossfeldiana* (flaming katy) and *Triticum durum* (durum wheat), which both display enlarged leaf epidermal cells by endoreduplication under low light, seemingly to increase photosynthetic cell surface area for improved light capture [9].

Endoreduplication is also induced in continuous high-light environments, where prolonged exposure to UV-B irradiation stimulates endoreduplication, cell expansion, peroxidase activity, and the production of polyphenolic compounds in *Cucumis sativus* (cucumber) cotyledons [43,44]. Endopolyploidy in this context may be protective in several ways, given that more DNA templates could help to sustain genome integrity under damaging UV-B and promote the genetic pathways that produce peroxidase and polyphenols, which are active in the stress response and UV-B absorption, respectively [43,44]. Specifically, gene manipulation experiments in *A. thaliana* revealed that an E2F endocycle regulator also regulates *PHR1*, a photolyase

DNA repair gene such that upon UV-B treatment, plants employed a coordinated induction of DNA repair and endoreduplication to better tolerate UV-B stress [45]. Similar results have been reported for the endocycle regulator *UV-B INSENSITIVE 1* [46]. In *A. thaliana*, endopolyploidy also appears to serve in protecting the genome through increased copy number – UV-B tolerant genotypes generally either had high endopolyploidy and low quantities of UV-absorbing pigments, or vice versa, such that endopolyploidy may be an alternative adaptive strategy to pigment production under UV stress [47].

Endopolyploidy in high-light environments is also stimulated in *A. thaliana* and *Phaseolus vulgaris* (common bean) [47,48]. Specifically, endopolyploidy supported cell expansion in *P. vulgaris* under high-light, where epidermal cells of the adaxial (upward-facing) leaf surface maintained higher levels of endopolyploidy, and subsequently cell size, than did either cells of the shaded abaxial (downward-facing) surface or adaxial cells in low light [48]. Epidermal endoreduplication and cell expansion are thus induced directly by the light treatment, and this could aid in the protection against irradiation damage [47,48].

Temperature. Changes in temperature can also have profoundly negative effects on cellular and physiological processes as enzyme-mediated reactions depart from their temperature optima. In natural systems, high temperature often coincides with water and/or light stress such that it is difficult to ascertain the response of endoreduplication to temperature relative to its induction by other factors. Generally, endoreduplication is repressed by both very high and very low temperatures [9,49]. Endoreduplication in response to moderate, short-term cold, however, can support organ growth via ploidy-mediated cell expansion rather than by an increase in cell number when mitosis is repressed [8]. In addition, increased endopolyploidy has been observed in hypocotyls of cold-grown seedlings of one genotype of *Medicago truncatula* (barrel medic) relative to other genotypes, speculated to be an adaptation of this genotype to the high-altitude regions to which it is native [50].

Water. Drought is another environmental stressor that can be particularly damaging to plants, and in many species water stress can repress both the mitotic cell cycle and the endocycle through decreases in CDK activity [51–53]. Endoreduplication can increase under slight water stress, however, and this is evident in leaf mesophyll cells of *A. thaliana* that maintain leaf area in drought via ploidy-mediated cell expansion [54]. This induction of endoreduplication may be through jasmonate signaling, a plant hormone commonly produced in response to drought and other stresses. In *A. thaliana*, jasmonate activates cyclins and CDKs that stimulate the switch from mitotic cycles to endocycles via the downregulation of the mitotic cyclin *CYCB1;1*; this downregulation ultimately promotes endoreduplication under water stress (Box 2) [55,56]. Drought also stimulates the expression of *ETHYLENE RESPONSE FACTOR5* (*ERF5*) and *ERF6* in *A. thaliana*, two regulators that affect leaf growth by inducing a cascade of osmotic stress-response genes that ultimately promote leaf cell expansion via endoreduplication under drought [57]. Water use efficiency may be further enhanced by endopolyploidy via the action of GTL1, a trihelix transcription factor downregulated under drought in *A. thaliana* [58]. Downregulation of *GTL1* leads to increased endopolyploidy and a decrease in stomatal number, which collectively serve to maintain leaf area for photosynthesis while reducing transpirational water loss through fewer stomata [58].

Perhaps as evidence of endoreduplication as a long-term adaptive strategy, many drought-adapted succulents (e.g., *Mesembryanthemum crystallinum*, ice plant) exhibit constitutive endopolyploidy widely throughout their tissues [59]. The employment of systemic endoreduplication in drought-adapted species suggests the value of endopolyploidy for some fundamental processes in severely water-stressed environments. Among the various effects presumed to accompany endopolyploidy, cell expansion may be particularly advantageous for growth in drought conditions where less-adapted species presumably suffer because of their reliance on cell expansion via water importation [33].

Soil quality. Owing to their sessile nature, plants are presumably under selection to tolerate or otherwise contend with unsuitable soil pH, high salt, or adulteration by heavy metals. Numerous studies have suggested the role of endoreduplication for this purpose – roots of tolerant varieties of *Sorghum bicolor* (sorghum), for example, endoreduplicate following exposure to NaCl while non-salt tolerant genotypes do not [60]. The authors suggest that the increased root cell volume following endoreduplication may serve in the sequestration or cellular processing of excess soil salt [60]. Endoreduplication in response to salt stress could occur via proline signaling, because lines of *Saccharomyces cerevisiae* (yeast) that overproduce this amino acid from a *Solanum lycopersicum* (tomato) transgene also have increased endoreduplication and growth under mild salt-stress relative to controls [61]. Cellular acclimation of *M. truncatula* to salt stress has been linked to the expression of the important endoreduplication regulators *CCS52* and *WEE1*, as well as of the proline synthesis gene *P5CS*, further supporting the roles of endoreduplication and proline in salt tolerance [62].

Increasing ploidy in response to cadmium has been observed in roots of *Pisum sativum* [63]. Endoreduplication of roots in contaminated soil is presumed to improve the absorptive capacity for sequestration of heavy metals, as well as improve metabolic activity and transport efficiency with fewer, larger cells [18,63].

Biotic factors

While some evidence of plant endoreduplication in response to biotic interactions has accumulated, to our knowledge its induction for the mitigation of the effects of biotic stress is largely limited to specific scenarios of herbivory. The role of endopolyploidy in mitigating other biotic stressors such as competition is less clear, but may be inferred by its response to factors such as allelochemicals (below) and shading [9,42]. We note a growing body of literature detailing the induction of plant endoreduplication by pathogenic (e.g., fungi, nematodes) and symbiotic

(e.g., rhizobia, mycorrhizae) biotrophs, although in these circumstances plant endoreduplication is largely considered to be a symptom of the stress (by pathogens) or to facilitate a positive interaction (for symbionts). These examples are thus omitted (but see [64–67]).

Herbivory. Endopolyploidy may help to mitigate damage from shoot herbivory. Particular genotypes of *A. thaliana*, a species which endoreduplicates systemically [6], endoreduplicate more following shoot damage than when undamaged [68,69]. Genotypes with the greatest increases in endopolyploidy during regrowth also often increase their seed production relative to undamaged plants in a phenomenon termed 'overcompensation' [68,69]. Further, the experimental overexpression of *INCREASED LEVEL OF POLYPLOIDY1*, an endoreduplication enhancer, in a genotype of *A. thaliana* that typically suffers reduced fitness when damaged allowed it to completely mitigate the otherwise detrimental effect of damage on its biomass, fruit, and seed production [70]. In this system, endoreduplication may be integrated into the damage-induced oxidative stress-response pathway, because overcompensation and increased endopolyploidy correlate with increased expression of *GLUCOSE-6-PHOSPHATE DEHYDROGENASE 1*, an important regulator of the oxidative phase of the pentose phosphate pathway (PPP) [71]. The PPP plays a central role in plant metabolism and produces the nucleotides necessary for endoreduplication – endopolyploidy may thus not only enhance growth by cell expansion, but may also enhance metabolism by increases in the expression of PPP genes [69,71]. Endoreduplication in this system may thus promote compensation to damage in two ways: (i) via rapid stem elongation, tissue expansion, and general regrowth of plant tissue by endopolyploidy-associated increases in cell volume throughout the plant, and (ii) via the size, gene expression, and metabolic effects of endopolyploidy on specialized cell types involved in reproduction [68–70].

While plant regrowth following large-scale herbivory may be aided by endoreduplication, the process may also serve in the prevention of small-scale herbivory. Trichomes, which protect the plant surface from feeding by small insects, as well as from evaporative water loss, UV radiation, frost damage, and other stressors [21,72], typically endoreduplicate to 32C to achieve the large volume and specialized shape needed to fulfill these roles [21,73].

Competition. Allelochemicals are often transferred in plant–plant, plant–microbe, and plant–herbivore interactions through the soil [74]. These compounds, such as benzoic and cinnamic acids, are often used in competition within or among species by causing oxidative stress in nearby plants [74]. For example, exposure of *Cucumis sativus* seedlings to the *C. sativus* root autotoxin cinnamic acid induces the activity of NADPH oxidase, superoxide dismutase, guaiacol peroxidase, and catalase, all enzymes involved in the oxidative stress response [75]. Other hallmarks of oxidative stress, including hydrogen peroxide and reactive oxygen species (ROS), were also produced upon exposure [75]. This response corresponded with earlier onset of endoreduplication in the developing root radicles, and was also stimulated by exposure to *C. sativus*-derived benzoic acid [76]. These allelochemicals inhibit mitosis, and thus cell proliferation, such that radicle growth can continue by cell expansion via endoreduplication [76]. Endoreduplication in this context may also be a protective and adaptive mechanism for the sequestration or detoxification of allelochemicals [76]. Increased endopolyploidy in *Zea mays* (maize) root tips has been similarly observed when exposed to cyanamide, an allelochemical produced by four Fabaceae species [77]. Following an initial arrest of the mitotic cell cycle, and a decrease in root tip growth rate, the induction of endoreduplication upon prolonged exposure in this system may support continued growth by cell enlargement when cell proliferation is inhibited [77].

Endoreduplication as a generalized stress response

Because of its commonality across taxa and cell types, endoreduplication is assumed to play important roles in the normal development and function of differentiated cells [1,4,11,21,22]. The differentiation of a cell generally entails the permanent change in its basic properties to support a gain in specialized function – endopolyploidy may thus stimulate the nucleotypic, metabolic, and gene expression changes required for a cell to achieve its fully differentiated state [4,21]. Considering the above examples of induced endoreduplication in response to a variety of environmental stimuli, endoreduplication may also play a role in the generalized stress response by providing a mechanism for plastically modifying basic cellular properties. For example, the increase in cell volume upon increased DNA content may improve the stress tolerance of a plant by providing the necessary cell volume for toxin sequestration or water storage, or for enhancing growth rates, as examples [8,18,59,60]. These cellular effects of endopolyploidy may thus affect development and physiology in an environmentally-influenced, plant-mediated manner. In fact, one might even speculate about the evolutionary significance of endopolyploidy in handling environmental stress, given that plants that endoreduplicate generally maintain some basal level of endopolyploidy and plastically enhance this level to mitigate the effects of stress outside the norm.

A proposed model for the integration of endoreduplication and stress-responsive pathways

If endopolyploidy does in fact promote the mitigation of environmental stress by enhancing gene expression and cell metabolism, among other possible mechanisms, evidence of the integration of endocycle regulation with stress-responsive metabolic pathways would be supportive of this role. One important metabolic pathway linked to endoreduplication is the PPP, which produces the reductant NADPH and the intermediate compounds necessary for generalized biosynthesis (Figure 2) [78]. This pathway is key in normal development, but is also important in the oxidative stress response [78]. Specifically, the PPP supplies compounds to the shikimate pathway for the production of secondary defense metabolites [79]. The PPP and shikimate pathway are stimulated by numerous environmental factors, including high light/UV, low temperature,

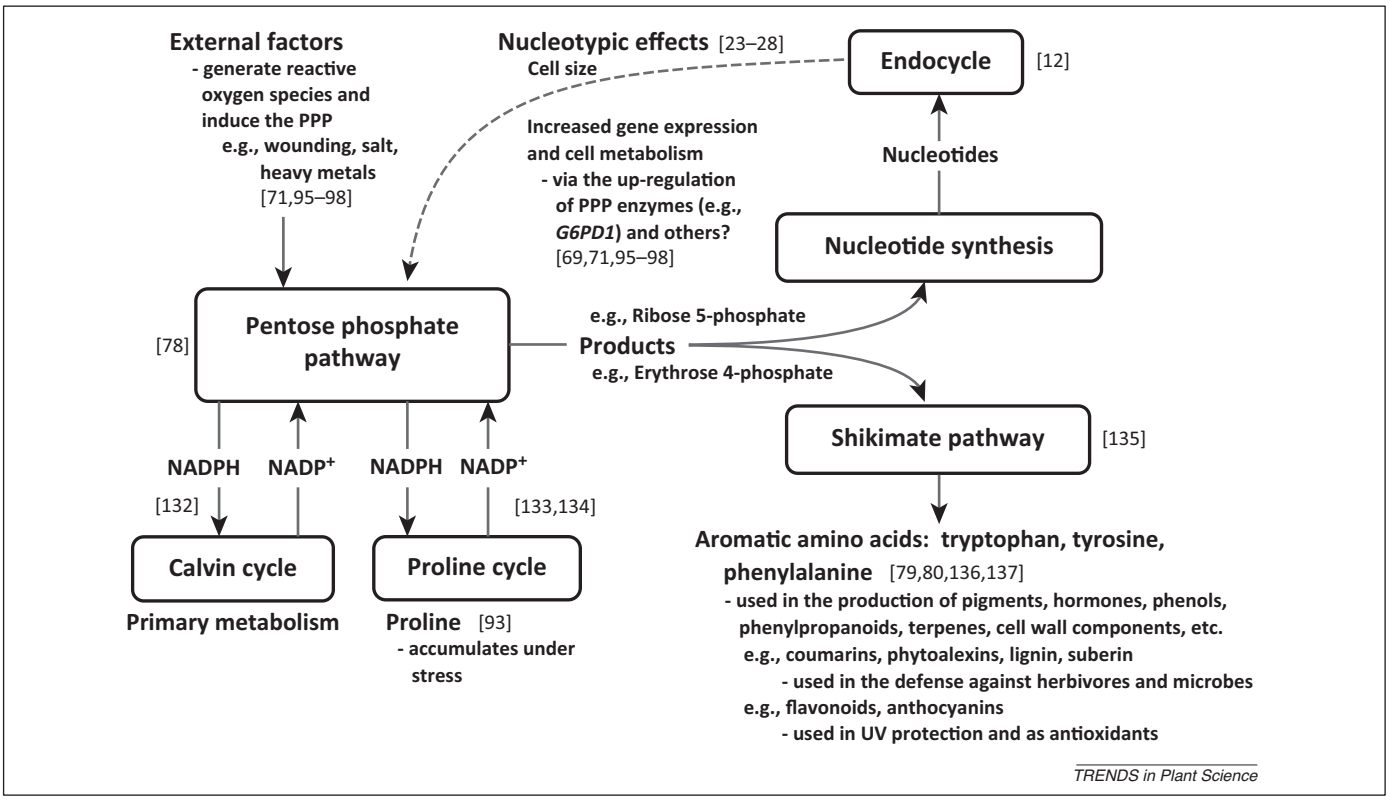

Figure 2. Integration of the endocycle with stress-responsive pathways. Shown are the relationships between the pentose phosphate pathway (PPP), the proline cycle, the shikimate pathway, and the endocycle. Arrows (↓) indicate the production or the movement of compounds from one pathway to another in the direction of the arrow. The broken line between the endocycle and the PPP depicts our model for the positive feedback of endopolyploidy on the expression of PPP genes (namely *G6PD1*) to increase cell metabolism. Detailed models of each pathway are presented elsewhere [12,23–28,69,71,78–80,93,95–98,132–137].

wounding, water stress, and soil adulteration by heavy metals, among others ([80] for review) [71,81–84]. For example, UV-A, UV-B, and UV-C all stimulate shikimate pathway gene expression and the production of phenolic anthocyanins in *Vitis vinifera* (grape), which absorb UV, protect DNA from radiation damage, and serve as antioxidants of stress-induced ROS [83]. In addition to the PPP, ROS generated by exposure to environmental stressors are also known to signal proline accumulation in plants [85]. For example, the production of phenylpropanoid defensive compounds is concurrent with an increase in proline content in numerous plants (e.g., *Thymus vulgaris*, thyme [86]; *Origanum vulgare*, oregano [87]; *Vigna radiata*, mungbean [88]; and *Vicia faba*, fava bean [89,90]) and in response to numerous stressors (e.g., salt [61]; drought [91]; and cold [92]) ([93] for review), suggesting that the proline-linked PPP can directly stimulate the shikimate production of phenolic compounds (Figure 2) [94].

One enzyme important in the PPP, and thus likely in the production of shikimate defense compounds, is the glucose-6-phosphate dehydrogenase G6PD1 which catalyzes the rate-limiting first reaction of the PPP [78]. Upregulation of *G6PD1* has been implicated in the remediation of oxidative stress caused by salt and heavy metals in *Triticum aestivum* (wheat) and *Phaseolus vulgaris*, among others [95–98]. Further, in addition to the positive relationship between endopolyploidy and tolerance of damage in *A. thaliana* [68–70], recent evidence shows that *G6PD1* expression is also positively correlated with tolerance where genotypes that overcompensate (i.e., damaged plants produce greater seed yield than do undamaged plants) upregulate *G6PD1* expression when damaged, whereas those that incur reduced fitness do not [71]. The timing of *G6PD1* expression in these plants also corresponds with *ILP1* expression, an endocycle regulator known to impact compensatory performance ([70], D.R.S. *et al.*, unpublished). The integration of these genetic pathways is presumably driven by *G6PD1* and the PPP, which lead to the production of DNA and RNA nucleotides for genome replication and gene transcription, respectively (Figure 2) [78]. Although proline content was not measured in these *A. thaliana* plants, recall that proline content is important for salt tolerance in *Solanum lycopersicum* and *Medicago truncatula*, and is accompanied in both systems by increased endoreduplication [61,62]. Taken together, we thus speculate that the PPP, the shikimate pathway, the proline cycle, and the endocycle are all integrated in the generalized stress response in the following ways: stress generates ROS which induce the PPP; the PPP produces the raw materials for the shikimate production of a variety of defense compounds; the PPP produces the reducing power in NADPH for the production of proline and other metabolites that help to mitigate the effects of stress; the PPP produces RNA nucleotides to support the increased expression of stress-related genes; and the PPP produces DNA nucleotides to support endoreduplication, which produces more gene templates per cell to increase the expression of enzymes in the PPP, the shikimate pathway, the production of defensive compounds, and

endoreduplication in a positive feedback loop (Figure 2) [69,71]. The integration of the endocycle in the stress-response pathway may therefore provide the increases in transcriptional output, metabolism, stress-mitigating compounds, and nucleotypic effects necessary to mitigate the effects of a variety of environmental stresses.

Ecological and evolutionary constraints on endopolyploidy

Given the plasticity that endopolyploidy affords organisms to respond to their environments, why do many plants not endoreduplicate? The most direct limitation may be the underlying genetics. There is tremendous diversity in cell cycle regulators, not only in their sheer number but also in the allelic diversity that exists for each, the ways in which they interact, and even their presence or absence among taxa (Box 2) [99,100]. Because the induction and maintenance of endoreduplication require precise orchestration in the interaction and timing of these regulators, the prevalence of endopolyploidy is likely limited in part by the ability of the organism to actually undergo this process, and this might explain in part the strong phylogenetic signal of endopolyploidy in plants [1,4,101] (but note that phylogeny also correlates with phenotype and life history [8,102]). Even if an organism can endoreduplicate, the results of doing so are likely not unconditionally positive. Endopolyploidy may be beneficial to the function of one cell type but may be detrimental for another – the high endopolyploidy characteristic of plant trichomes, for example, is presumed to aid in their large size and specialized structure. These traits would likely interfere with the size flux-dependent function of stomatal guard cells, which predictably lack endopolyploidy [7]. Other effects of increased cell size, such as a lower surface area-to-volume ratio, could negatively impact intracellular transport efficiency and reduce the relative area available for membrane-based reactions, as examples [28]. The severity of these nucleotypic effects may be particularly disadvantageous if the basic genome size of an organism is already relatively large.

While various environmental factors seem to induce endopolyploidy, the environment of an organism can also constrain it. Endoreduplication is presumably an expensive process, particularly in phosphorus and nitrogen which are often limited for plant uptake in natural soils [103]. This phosphorus constraint is evident in the positive association between the distribution of polyploid species (or those with large basic genome sizes) and soil phosphorus availability across vascular plants [104]. Regardless, the benefit of endopolyploidy must at least balance its costs. For example, although efficient cell expansion may typically be achieved by water importation, the expense of endoreduplication for this purpose is apparently justified for the many succulents adapted to extremely water-limited environments [33,59]. If environmental factors become too severe, however, both the mitotic cell cycle and the endocycle may be repressed [105]. A final constraint on endopolyploidy may be the limitation of its plasticity – mitotic division of endopolyploid cells is rare, such that changes in cellular ploidy are almost exclusively limited to successive doubling [20,21]. If there is an environmental change during the lifetime of a plant, or if the plant experiences a different environment than its parent (e.g., if dispersed as seed), endoreduplication provides the plant some means of acclimation. In an unstable environment, however, the 'ratcheting up' of ploidy largely cannot be undone for those cells committed to the endocycle, such that the endopolyploidy generated in response to one environmental factor may later become disadvantageous if the environment changes again. Perpetually embryonic meristems allow plants to maintain their ability to respond to current conditions, however, by constantly generating new cells of their basic genomic state.

Concluding remarks

Given that endopolyploidy is known to play important roles in cell growth, development, and function, plasticity in cellular ploidy may be important in the response to environmental stress by providing a mechanism for organisms to fine-tune themselves to their local environment via control at the level of individual cells. The increase in ploidy may then provide the increased cell volume, metabolic output, and/or gene expression to combat the stress

Box 3. Outstanding questions

Since the first observation of endopolyploidy well over a century ago [138], and theorization of its ecological and evolutionary importance in the 1970s [1,4,139], fundamental questions have remained unresolved.

- Ecologically, what generalities exist across the breadth of taxa and environments where endopolyploidy occurs? What role does endopolyploidy have in the response to the environment, and to what degree can it be plastically modified?
- Evolutionarily, what are the origins of endoreduplication, and under what conditions was the process initially under selection? How does this context differ from the wide range of taxa now known to endoreduplicate, their varied life histories, and the environments in which they occur?
- Further, truly understanding the ecology and evolution of endopolyploidy requires knowledge of its effects from the cellular to the organismal level. There is correlational evidence for the nucleotypic effects of ploidy, but what are the true contributions of cell size, gene expression, cell metabolism, and other effects to the organismal phenotype and fitness? How are these effects modulated by the environment?

These are merely examples of the many fundamental questions remaining from ecological, evolutionary, and mechanistic perspectives. To begin addressing these issues, broad surveys analyzing examples of endopolyploidy across taxa, cell types, cellular characteristics, and environments will help to identify generalities and exceptions to our current notions regarding endopolyploidy and the associated nucleotypic effects. Targeted assessments manipulating environmental factors and/or the endocycle itself will undoubtedly strengthen our understanding of the nature and impact of endopolyploidy beyond the correlational and observational data that currently pervade the literature. We point to our own work in the response to damage of *A. thaliana*, which began with an initial correlation between endopolyploidy and fitness that was later followed by genetic manipulation of the endocycle, genetic manipulation of a key metabolic pathway, quantitative trait loci mapping, microarray analyses, and integration with current literature to more explicitly link endopolyploidy with damage tolerance and develop our proposed working model (see Figure 2 in main text) [68–71]. We believe that integrative research employing methods across levels of biological organization (i.e., molecular, cellular, organismal, etc.) will be paramount in making strides in understanding this process in ecological and evolutionary contexts and addressing these outstanding questions.

through the production of secondary defense compounds, compound detoxification and sequestration, rapid organismal growth, increased metabolism, or even reproductive output [21]. Plasticity in ploidy may be particularly important for plants, which are sessile in nature and thus are under strong selection for means to mitigate the effects of stress. Although endopolyploidy research has so far been largely limited to agricultural systems and molecular description, many examples of the induction of endoreduplication by environmental factors have been accumulating. Recently, new insights have brought questions of the ecological importance and evolutionary origin of endopolyploidy to the forefront (Box 3). With continued research on a variety of taxa and environmental factors, we predict that endoreduplication will be recognized as a generalized, adaptive mechanism by which plants plastically control cell development and function, ultimately helping them to mitigate the negative impacts of environmental stress.

Acknowledgments

We thank the numerous research groups whose work (both cited and uncited) inspired great thought on this topic and made this review possible. This manuscript was greatly improved with comments by A.D.B. Leakey, A.V. Suarez, A.L. Rayburn, and two anonymous reviewers. Our research on this topic was supported by awards from the National Science Foundation (K.N.P. DEB1146085, K.N.P and D.R.S DEB1308941) and the University of Illinois Campus Research Board (K.N.P.).

References

1 Nagl, W. (1976) DNA endoreduplication and polyteny understood as evolutionary strategies. *Nature* 261, 614–615
2 D'Amato, F. (1984) Role of polyploidy in reproductive organs and tissues. In *Embryology of angiosperms* (Jori, B.B., ed.), pp. 519–566, Springer-Verlag
3 Leitch, A.R. and Leitch, I.J. (2012) Ecological and genetic factors linked to contrasting genome dynamics in seed plants. *New Phytol.* 194, 629–646
4 Nagl, W. (ed.) (1978) *Endopolyploidy and Polyteny in Differentiation and Evolution: Towards an Understanding of Quantitative and Qualitative Variation of Nuclear DNA in Ontogeny and Phylogeny*, North-Holland
5 Edgar, B.A. *et al.* (2014) Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. *Nat. Rev. Mol. Cell Biol.* 15, 197–210
6 Galbraith, D.W. *et al.* (1991) Systemic endopolyploidy in *Arabidopsis thaliana*. *Plant Physiol.* 96, 985–989
7 Melaragno, J.E. *et al.* (1993) Relationship between endopolyploidy and cell size in epidermal tissue of *Arabidopsis*. *Plant Cell* 5, 1661–1668
8 Barow, M. and Meister, A. (2003) Endopolyploidy in seed plants is differently correlated systematics, organ, life strategy, and size. *Plant Cell Environ.* 26, 571–584
9 Barow, M. (2006) Endopolyploidy in seed plants. *Bioessays* 28, 271–281
10 Maluszynska, J. *et al.* (2013) Endopolyploidy in plants. In *Plant Genome Diversity. Vol. 2: Physical Structure, Behaviour and Evolution of Plant Genomes* (Leitch, I.J. *et al.*, eds), pp. 99–119, Springer-Verlag
11 Brodsky, V.Y. and Uryvaeva, I.V. (1985) The biological significance of polyploidy and polyteny. In *Genome Multiplication in Growth and Development* (Brodsky, V.Y. and Uryvaeva, I.V., eds), pp. 175–233, Cambridge University Press
12 Vlieghe, K. *et al.* (2007) Physiological relevance and molecular control of the endocycle in plants. In *Cell Cycle Control and Plant Development* (Inzé, D., ed.), pp. 227–248, Blackwell
13 Magyar, Z. *et al.* (2013) Cell cycle modules in plants for entry into proliferation and for mitosis. In *Plant Genome Diversity. Vol. 2: Physical Structure, Behaviour and Evolution of Plant Genomes* (Leitch, I.J. *et al.*, eds), pp. 77–97, Springer-Verlag
14 Nagl, W. (1981) Polytene chromosomes of plants. *Int. Rev. Cytol.* 73, 21–53
15 Schubert, V. *et al.* (2006) Sister chromatids are often incompletely aligned in meristematic and endopolyploid interphase nuclei of *Arabidopsis thaliana*. *Genetics* 172, 467–475
16 Nagl, W. (1972) Molecular and structural aspects of the endomitotic chromosome cycle in angiosperms. *Chromosomes Today* 3, 17–23
17 Nagl, W. (1990) Gene amplification and related events. In *Biotechnology in Agriculture and Forestry 11, Somaclonal Variation in Crop Improvement I* (Bajaj, Y.P.S., ed.), pp. 153–201, Springer-Verlag
18 Biskup, A. and Izmaiłow, R. (2004) Endosperm development in seeds of *Echium vulgare* L. (Boraginaceae) from polluted sites. *Acta Biol. Cracov. Bot.* 46, 39–44
19 Traas, J. *et al.* (1998) Endoreduplication and development: rule without dividing? *Curr. Opin. Plant Biol.* 1, 498–503
20 Larkins, B.A. *et al.* (2001) Investigating the hows and whys of DNA endoreduplication. *J. Exp. Bot.* 52, 183–192
21 Lee, H.O. *et al.* (2009) Endoreplication: ploidy with purpose. *Gene Dev.* 23, 2461–2477
22 Breuer, C. *et al.* (2014) Endocycling in the path of plant development. *Curr. Opin. Plant Biol.* 17, 78–85
23 Bennett, M.D. (1971) The duration of meiosis. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 178, 259–275
24 Bennett, M.D. (1972) Nuclear DNA content and minimum generation time in herbaceous plants. *Proc. R. Soc. Lond. B: Biol. Sci.* 181, 109–135
25 Bennett, M.D. (1987) Variation in genomic form in plants and its ecological implications. *New Phytol.* 106, 177–200
26 Gregory, T.R. and Hebert, P.D.N. (1999) The modulation of DNA content: proximate causes and ultimate consequences. *Genome Res.* 9, 317–324
27 Gregory, T.R. (2001) Coincidence, coevolution, or causation? DNA content, cell size, and C- value enigma. *Biol. Rev. Camb. Philos. Soc.* 76, 65–101
28 Gregory, T.R. (2005) The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Ann. Bot. (Lond.)* 95, 133–146
29 Jovtchev, G. *et al.* (2006) Nuclear DNA content and nuclear and cell volume are positively correlated in angiosperms. *Cytogenet. Genome Res.* 114, 77–82
30 Beaulieu, J.M. *et al.* (2008) Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol.* 179, 975–986
31 Sugimoto-Shirasu, K. and Roberts, K. (2003) 'Big it up': endoreduplication and cell-size control in plants. *Curr. Opin. Plant Biol.* 6, 544–553
32 Kondorosi, E. *et al.* (2003) Plant cell-size control: growing by ploidy? *Curr. Opin. Plant Biol.* 3, 488–492
33 Skirycz, A. and Inzé, D. (2010) More from less: plant growth under limited water. *Curr. Opin. Biotechnol.* 21, 197–203
34 Edgar, B.A. and Orr-Weaver, T.L. (2001) Endoreplication cell cycle: more for less. *Cell* 105, 297–306
35 Beemster, G.T.S. *et al.* (2002) Variation in growth rate between *Arabidopsis* ecotypes is correlated with cell division and A-type cyclin-dependent kinase activity. *Plant Physiol.* 129, 854–864
36 Breuer, C. *et al.* (2010) Developmental control of endocycles and cell growth in plants. *Curr. Opin. Plant Biol.* 13, 654–660
37 Tsukaya, H. (2013) Does ploidy level directly control cell size? Counterevidence from *Arabidopsis* genetics. *PLoS ONE* 8, e83729
38 Butterfass, T.H. (1983) A nucleotypic control of chloroplast reproduction. *Protoplasma* 118, 71–74
39 Bourdon, M. *et al.* (2012) Evidence for karyoplasmic homeostasis during endoreduplication and a ploidy-dependent increase in gene transcription during tomato fruit growth. *Development* 139, 3817–3826
40 John, P.C.L. and Qi, R. (2008) Cell division and endoreduplication: doubtful engines of vegetative growth. *Trends Plant Sci.* 13, 121–127
41 Harashima, H. and Schnittger, A. (2010) The integration of cell division, growth and differentiation. *Curr. Opin. Plant Biol.* 13, 66–74
42 Kudo, N. and Mii, M. (2004) Endoreduplication cycles during hypocotyl growth of cabbage (*Brassica oleracea* L.) under light and dark conditions. *Plant Biotechnol.* 21, 295–298
43 Yamasaki, S. *et al.* (2007) Continuous UV-B irradiation induces morphological changes and the accumulation of polyphenolic

compounds on the surface of cucumber cotyledons. *J. Radiat. Res. (Tokyo)* 48, 443–454
44 Yamasaki, S. *et al.* (2010) Continuous UV-B irradiation induces endoreduplication and peroxidase activity in epidermal cells surrounding trichomes on cucumber cotyledons. *J. Radiat. Res. (Tokyo)* 51, 187–196
45 Radziejwoski, A. *et al.* (2011) Atypical E2F activity coordinates PHR1 photolyase gene transcription with endoreduplication onset. *EMBO J.* 30, 355–363
46 Hase, Y. *et al.* (2006) A mutation in the *uvi4* gene promotes progression of endo-reduplication and confers increased tolerance towards ultraviolet B light. *Plant J.* 46, 317–326
47 Gegas, V.C. *et al.* (2014) Endopolyploidy as a potential alternative adaptive strategy for *Arabidopsis* leaf size variation in response to UV-B. *J. Exp. Bot.* 65, 2757–2766
48 Kinoshita, I. *et al.* (2008) Difference in light-induced increase in ploidy level and cell size between adaxial and abaxial epidermal pavement cells of *Phaseolus vulgaris* primary leaves. *J. Exp. Bot.* 59, 1419–1430
49 Engelen-Eigles, G. *et al.* (2001) DNA endoreduplication in maize endosperm cells is reduced by high temperature during the mitotic phase. *Crop Sci.* 41, 1114–1121
50 Pierre, J. *et al.* (2014) Cellular changes during *Medicago truncatula* hypocotyl growth depend on temperature and genotype. *Plant Sci.* 217–218, 18–26
51 Schuppler, U. *et al.* (1998) Effect of water stress on cell division and cdc2-like cell cycle kinase activity in wheat leaves. *Plant Physiol.* 117, 667–678
52 Granier, C. *et al.* (2000) Spatial distribution of cell division rate can be deduced from that of $p34^{cdc2}$ kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. *Plant Physiol.* 124, 1393–1402
53 Setter, T.L. and Flannigan, B.A. (2001) Water deficit inhibits cell division and expression of transcripts in cell proliferation and endoreduplication in maize endosperm. *J. Exp. Bot.* 52, 1401–1408
54 Cookson, S.J. *et al.* (2006) Cell and leaf size plasticity in *Arabidopsis*: what is the role of endoreduplication? *Plant Cell Environ.* 29, 1273–1283
55 Harb, A. *et al.* (2010) Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiol.* 154, 1254–1271
56 Noir, S. *et al.* (2013) Jasmonate controls leaf growth by repressing cell proliferation and the onset of endoreduplication while maintaining a potential stand-by mode. *Plant Physiol.* 161, 1930–1951
57 Dubois, M. *et al.* (2013) ETHYLENE RESPONSE FACTOR6 acts as a central regulator of leaf growth under water-limiting conditions in *Arabidopsis*. *Plant Physiol.* 162, 319–332
58 Kaplan-Levy, R.N. *et al.* (2012) The trihelix family of transcription factors – light, stress and development. *Trends Plant Sci.* 17, 163–171
59 de Rocher, J.E. *et al.* (1990) Developmentally regulated systemic endopolyploidy in succulents with small genomes. *Science* 250, 99–101
60 Ceccarelli, M. *et al.* (2006) Chromosome endoreduplication as a factor of salt adaptation in *Sorghum bicolor*. *Protoplasma* 227, 113–118
61 Maggio, A. *et al.* (2002) Does proline accumulation play an active role in stress-induced growth reduction? *Plant J.* 31, 699–712
62 Elmaghrabi, A. *et al.* (2013) Enhanced tolerance to salinity following cellular acclimation to increasing NaCl levels in *Medicago truncatula*. *Plant Cell Tiss. Org. Cult.* 114, 61–70
63 Fusconi, A. *et al.* (2006) Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. Frisson seedlings. *Environ. Exp. Bot.* 58, 253–260
64 Vinardell, J.M. *et al.* (2003) Endoreduplication mediated by the anaphase-promoting complex activator CCS52A is required for symbiotic cell differentiation in *Medicago truncatula* nodules. *Plant Cell* 15, 2093–2105
65 Wildermuth, M.C. (2010) Modulation of host nuclear ploidy: a common plant biotroph mechanism. *Curr. Opin. Plant Biol.* 13, 449–458
66 Bainard, L.D. *et al.* (2011) Mycorrhizal symbiosis stimulates endoreduplication in angiosperms. *Plant Cell Environ.* 34, 1577–1585
67 de Almeida Engler, J. and Gheysen, G. (2013) Nematode-induced endoreduplication in plant host cells: why and how? *Mol. Plant Microbe Interact.* 26, 17–24
68 Scholes, D.R. and Paige, K.N. (2011) Chromosomal plasticity: mitigating the impacts of herbivory. *Ecology* 92, 1691–1698
69 Scholes, D.R. *et al.* (2013) The genetic basis of overcompensation in plants: a synthesis. *Int. J. Mod. Bot.* 3, 34–42
70 Scholes, D.R. and Paige, K.N. (2014) Plasticity in ploidy underlies plant fitness compensation to herbivore damage. *Mol. Ecol.* 23, 4862–4870
71 Siddappaji, M.H. *et al.* (2013) Overcompensation in response to herbivory in *Arabidopsis thaliana*: the role of glucose-6-phosphate dehydrogenase and the oxidative pentose-phosphate pathway. *Genetics* 195, 589–598
72 Johnson, H.B. (1975) Plant pubescence: an ecological perspective. *Bot. Rev.* 41, 233–258
73 Larkin, J.C. *et al.* (2007) Insights into the endocycle from trichome development. In *Cell Cycle Control and Plant Development* (Inzé, D., ed.), pp. 249–268, Blackwell
74 Li, Z.H. *et al.* (2010) Phenolics and plant allelopathy. *Molecules* 15, 8933–8952
75 Ding, J. *et al.* (2007) Physiological basis of different allelopathic reactions of cucumber and figleaf gourd plants to cinnamic acid. *J. Exp. Bot.* 58, 3765–3773
76 Zhang, Y. *et al.* (2009) Effects of phenylcarboxylic acids on mitosis, endoreduplication and expression of cell cycle-related genes in roots of cucumber (*Cucumis sativus* L.). *J. Chem. Ecol.* 35, 679–688
77 Soltys, D. *et al.* (2014) Phytotoxic cyanamide affects maize (*Zea mays*) root growth and root tip function: from structure to gene expression. *J. Plant Physiol.* 171, 565–575
78 Kruger, N.J. and von Schaewen, A. (2003) The oxidative pentose phosphate pathway: structure and organisation. *Curr. Opin. Plant Biol.* 6, 236–246
79 Maeda, H. and Dudareva, N. (2012) The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annu. Rev. Plant Biol.* 63, 73–105
80 Dixon, R.A. and Paiva, N.L. (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell* 7, 1085–1097
81 Crifò, T. *et al.* (2011) Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. *Gene* 476, 1–9
82 Sánchez-Rodríguez, E. *et al.* (2011) Differential responses of five cherry tomato varieties to water stress: changes on phenolic metabolites and related enzymes. *Phytochemistry* 72, 723–729
83 Zhang, Z.Z. *et al.* (2012) Three types of ultraviolet irradiation differentially promote expression of shikimate pathway genes and production of anthocyanins in grape berries. *Plant Physiol. Biochem.* 57, 74–83
84 Gajewska, E. *et al.* (2013) Differential response of wheat roots to Cu, Ni and Cd treatment: oxidative stress and defense reactions. *Plant Growth Regul.* 71, 13–20
85 Rejeb, K.B. *et al.* (2014) How reactive oxygen species and proline face stress together. *Plant Physiol. Biochem.* 80, 278–284
86 Kwok, D. and Shetty, K. (1998) Effect of proline and proline analogs on total phenolic and rosmarinic acid levels in shoot clones of thyme (*Thymus vulgaris* L.). *J. Food Biochem.* 22, 37–51
87 Yang, R. and Shetty, K. (1998) Stimulation of rosmarinic acid in shoot cultures of oregano (*Origanum vulgare*) clonal line in response to proline, proline analog and proline precursors. *J. Agric. Food Chem.* 46, 2888–2893
88 McCue, P. and Shetty, K. (2002) Clonal herbal extracts as elicitors of phenolic synthesis in dark-germinated mungbeans for improving nutritional value with implications for food safety. *J. Food Biochem.* 26, 209–232
89 Shetty, P. *et al.* (2002) Effects of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-DOPA synthesis in dark germinated *Vicia faba*. *Process Biochem.* 37, 1285–1295
90 Shetty, P. *et al.* (2003) Stimulation of total phenolics, L-DOPA and antioxidant activity through proline-linked pentose phosphate pathway in response to proline and its analogue in germinating fava beans (*Vicia faba*). *Process Biochem.* 38, 1707–1717
91 Seki, M. *et al.* (2007) Regulatory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol.* 10, 296–302
92 Sarkar, D. and Bhowmik, P.C. (2009) Cold acclimation responses of three cool-season turfgrasses and the role of proline-associated pentose phosphate pathway. *J. Am. Soc. Hortic. Sci.* 134, 210–220

93 Szabados, L. and Savouré, A. (2010) Proline: a multifunctional amino acid. *Trends Plant Sci.* 15, 89–97
94 Shetty, K. (1997) Biotechnology to harness the benefits of dietary phenolics; focus on *Lamiaceae*. *Asia Pac. J. Clin. Nutr.* 6, 162–171
95 van Assche, F. (1988) Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. *Environ. Pollut.* 52, 103–115
96 Ślaski, J.J. *et al.* (1996) Aluminum resistance in wheat (*Triticum aestivum*) is associated with rapid, Al-induced changes in activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in root apices. *Physiol. Plant.* 98, 477–484
97 Nemoto, Y. *et al.* (2000) Specific expression of glucose-6-phosphate dehydrogenase (G6PDH) gene by salt stress in wheat (*Triticum aestivum* L.). *Plant Sci.* 158, 53–60
98 Liu, Y. *et al.* (2007) Glucose-6-phosphate dehydrogenase plays a pivotal role in nitric oxide-involved defense against oxidative stress under salt stress in red kidney bean roots. *Plant Cell Physiol.* 48, 511–522
99 Nieuwland, J. *et al.* (2007) The plant cyclins. In *Cell cycle Control and Plant Development* (Inzé, D., ed.), pp. 31–61, Blackwell
100 Dudits, D. *et al.* (2007) The growing family of plant cyclin-dependent kinases with multiple functions in cellular and developmental regulation. In *Cell Cycle Control and Plant Development* (Inzé, D., ed.), pp. 1–30, Blackwell
101 Bainard, J.D. *et al.* (2013) A multivariate analysis of variation in genome size and endoreduplication in angiosperms reveals strong phylogenetic signal and association with phenotypic traits. *New Phytol.* 196, 1240–1250
102 Tschermak-Woess, E. (1956) Karyologische Pflanzenanatomie. *Protoplasma* 46, 798–834
103 Vitousek, P.M. *et al.* (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol. Appl.* 20, 5–15
104 Šmarda, P. *et al.* (2013) Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytol.* 200, 911–921
105 Granier, C. *et al.* (2007) Cell cycle and environmental stresses. In *Cell Cycle Control and Plant Development* (Inzé, D., ed.), pp. 335–355, Blackwell
106 Adams, K.L. and Wendel, J.F. (2005) Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8, 135–141
107 Tate, J.A. *et al.* (2011) Polyploidy in plants. In *The Evolution of the Genome* (Gregory, T.R., ed.), pp. 371–426, Academic Press
108 Comai, L. (2005) The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6, 836–846
109 Sliwinska, E. and Lukaszewska, E. (2005) Polysomaty in growing in vitro sugar-beet (*Beta vulgaris* L.) seedlings of different ploidy level. *Plant Sci.* 168, 1067–1074
110 Bainard, J.D. *et al.* (2013) Nuclear DNA content variation and evolution in liverworts. *Mol. Phylogenet. Evol.* 68, 619–627
111 Bainard, J.D. and Newmaster, S.G. (2010) Endopolyploidy in bryophytes: widespread in mosses and absent in liverworts. *J. Bot.* 2010, 316356
112 Bainard, J.D. and Villarreal, J.C. (2013) Genome size increases in recently diverged hornwort clades. *Genome* 56, 431–435
113 Bainard, J.D. *et al.* (2011) DNA content variation in monilophytes and lycophytes: large genomes that are not endopolyploid. *Chromosome Res.* 19, 763–775
114 Avanzi, S. and Cionini, P.G. (1971) A DNA cytophotometric investigation on the development of the female gametophyte of *Ginkgo biloba*. *Caryologia* 24, 105–116
115 Barow, M. and Jovtchev, G. (2007) Endopolyploidy in plants and its analysis by flow cytometry. In *Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes, and Genomes* (Doležel, J. *et al.*, eds), pp. 349–372, Wiley
116 Boudolf, V. *et al.* (2004) The plant-specific cyclin-dependent kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in *Arabidopsis*. *Plant Cell* 16, 2683–2692
117 John, P.C.L. (2007) Hormonal regulation of cell cycle progression and its role in development. In *Cell Cycle Control and Plant Development* (Inzé, D., ed.), pp. 311–334, Blackwell
118 Kitsios, G. and Doonan, J.H. (2011) Cyclin dependent protein kinases and stress responses in plants. *Plant Signal. Behav.* 6, 204–209
119 Tank, J.G. *et al.* (2014) Phytohormones in regulation of the cell division and endoreduplication process in the plant cell cycle. *R. Soc. Chem. Adv.* 4, 12605–12613
120 Francis, D. *et al.* (2008) A strong nucleotypic effect on the cell cycle regardless of ploidy level. *Ann. Bot. (Lond.)* 101, 747–757
121 Evans, G. *et al.* (1972) The relationship between nuclear DNA amount and the duration of the mitotic cycle. *Chromosomes Today* 3, 24–31
122 Price, H.J. *et al.* (1973) Correlations between nuclear volume, cell volume and DNA content in meristematic cells of herbaceous angiosperms. *Experientia* 29, 1028–1029
123 Stebbins, G.L. (1971) *Chromosomal Evolution in Higher plants*, Addison-Wesley
124 Knight, C.A. *et al.* (2010) On the relationship between pollen size and genome size. *J. Bot.* 2010, 612017
125 Katsiotis, A. and Forsberg, R.A. (1995) Pollen grain size in four ploidy levels of genus *Avena*. *Euphytica* 83, 103–108
126 Beaulieu, J.M. *et al.* (2007) Correlated evolution of genome size and seed mass. *New Phytol.* 173, 422–437
127 Smith, K.F. *et al.* (2003) The effects of ploidy and seed mass on the emergence and early vigour of perennial ryegrass (*Lolium perenne* L.) cultivars. *Aust. J. Exp. Agric.* 43, 481–486
128 Lemontey, C. *et al.* (2000) Maternal genotype influences pea seed size by controlling both mitotic activity during early embryogenesis and final endoreduplication level/cotyledon cell size in mature seed. *J. Exp. Bot.* 51, 167–175
129 Guo, M. *et al.* (1996) Dosage effects on gene expression in a maize ploidy series. *Genetics* 142, 1349–1355
130 Butterfass, T.H. (1973) Control of plastid division by means of nuclear DNA amount. *Protoplasma* 76, 167–195
131 Butterfass, T.H. (1963) Die Abhängigkeit der Plastidenvermehrung von der Reproduktion der Erbsubstanz im Kern. *Ber. Dtsch. Bot. Ges.* 76, 123–134
132 Berg, J.M. (2006) The Calvin cycle and the pentose phosphate pathway. In *Biochemistry* (Berg, J.M. *et al.*, eds), pp. 739–755, W.H. Freeman
133 Shetty, K. and Wahlqvist, M.L. (2004) A model for the role of proline-linked pentose phosphate pathway in phenolic phytochemical biosynthesis and mechanism of action for human health and environmental applications. *Asia Pac. J. Clin. Nutr.* 13, 1–24
134 Perassolo, M. *et al.* (2011) Role of reactive oxygen species and proline cycle in anthraquinone accumulation in *Rubia tinctorum* cell suspension cultures subjected to methyl jasmonate elicitation. *Plant Physiol. Biochem.* 49, 758–763
135 Herrmann, K.M. and Weaver, L.M. (1999) The shikimate pathway. *Annu. Rev. Plant Phys.* 50, 473–503
136 Tzin, V. and Galili, G. (2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Mol. Plant* 3ssss, 956–972
137 Shetty, K. (2004) Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochem.* 39, 789–804
138 Balbiani, E.G. (1881) Sur la structure du noyau des cellules salivaires chez les larves de *Chironomus*. *Zool. Anz.* 4, 662–667
139 Barlow, P.W. (1978) Endopolyploidy: towards an understanding of its biological significance. *Acta Biotheor.* 27, 1–18