Developmentally Regulated Systemic Endopolyploidy in Succulents with Small Genomes

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Nuclei from *Mesembryanthemum crystallinum* (ice plant) exhibit multiple levels of ploidy in every tissue as revealed by flow microfluorometric analysis of isolated nuclei stained with mithramycin. Multiples of the haploid nuclear genome complement (1C) corresponding to 2C, 4C, 8C, 16C, 32C, and 64C were observed. The distribution of nuclei among the different ploidy levels is tissue-specific and in leaves is characteristic of the stage of development. This type of genome organization has been identified in eight other succulent CAM (crassulacean acid metabolism) plant species with small genomes. Multiploidy may be a common property of this type of plant.

PARTIAL ELIMINATION, SELECTIVE AMplification, and multiplication of entire genomes are some of the alterations to a constant 2C DNA content (where 1C is the haploid genome complement) seen in a variety of organisms (*1*). For plants, the most widely known form of genome variation is generative or germline polyploidy, in which haploid germline cells contain more than a single complement of the genome as, for example, in tetraploid, hexaploid, and octaploid varieties of wheat (*2*). Less widely known are the various forms of somatic polyploidy, which are observed in plants and which can arise by polyteny, endomitosis, and other mechanisms (*3*). Most examples of somatic polyploidy have been restricted to highly specialized cell types such as vascular elements, embryo-associated cells, or storage cells of cotyledons (*4*). About 90% of angiosperms are thought to be polyploid in at least some of their somatic cells (*5*). However, previous analytical studies of the DNA content of nuclei have been limited to studies of a single tissue for a given plant, the simple presence or absence of less or more than two genomic copies of DNA, or the quantitation of relatively small numbers of nuclei. Flow cytometry allows accurate determination of DNA contents of large numbers of nuclei from numerous tissues so that a study can be made of the proportion of cells in the different phases of the cell cycle (*6*) or the association of polyploidy with tissue type, developmental stage, or environmental factors. We now describe a systemic pattern of multiple ploidy in *Mesembryanthemum crystallinum* (ice plant). We refer to the simultaneous presence of three or more integer multiples of the diploid DNA complement in all the tissues of a plant as multiploidy.

Nuclei were isolated from *M. crystallinum*, stained with the fluorescent dye mithramycin, and analyzed for DNA content by flow cytometry (*6*). Nuclei from somatic tissue would be expected to fall into two distinct peaks of fluorescence corresponding to a 2C complement of DNA in G_0 and G_1 phase cells, and a 4C complement for G_2 and M (mitosis) phase cells. However, as many as six distinct peaks of fluorescence occurred in *M. crystallinum* nuclei isolated from single plants (Fig. 1A).

The average DNA content for each of the classes of nuclei was calculated from fluorescence measurements made from six separate isolations from leaves, each with ~2000 stained nuclei. Chicken red blood cells, with a DNA content (with standard error) of 2.33 ± 0.22 pg (*6*), were included in the samples as an internal standard. The positions of peaks relative to the control were used to calculate the average DNA content of the nuclei in each peak. Each successive class of nuclei has twice the DNA of the preceding class (*7*). This pattern is not the result of aggregation of nuclei, which would result in classes that are related to the smallest class by multiples of two rather than by exponentials of two.

Nuclei were isolated from mature anthers to determine whether the nuclei with the least DNA were 2C. For anthers rich in haploid pollen cells, a peak was observed corresponding to a DNA content of 0.38

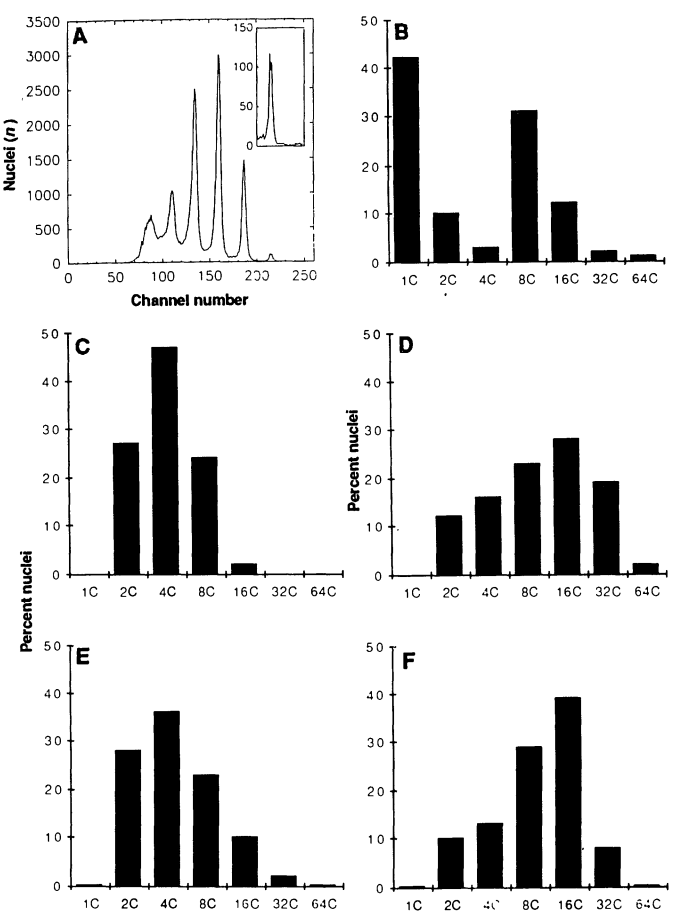

Fig. 1. Representative ploidy distributions for nuclei isolated from tissues of *M. crystallinum*. (**A**) Plot of numbers of nuclei as a function of fluorescence intensity (log scale) resulting from the flow cytometric analysis of nuclei isolated from a mature leaf. Fluorescence intensity increases with increasing channel number. Inset: vertical expansion of sixth peak. (B to F) Percentages of nuclei in each ploidy class for nuclei isolated from (**B**) anthers and petals, (**C**) young leaf, (**D**) mature leaf, (**E**) root tip, and (**F**) cotyledons. Nuclei were isolated and analyzed as described (*6*) from plants grown under greenhouse conditions in soil and hydrophonics. Data were collected for at least 5000 nuclei for each distribution. The relative abundance of each ploidy class was calculated by means of the integration routine provided by the Coulter Electronic Analysis System.

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pg. On the basis of the DNA content for the 2C peak, 0.86 ± 0.034 pg (7), we calculate a genome size of 391,400 ± 13,300 kb. This falls at the low end of the range of known plant genome sizes. It is 0.3% of the *Fritillaria assyriaca* genome (115,974,000 kb) (2) and is approximately five times as large as the genome of *Arabidopsis thaliana* (70,000 kb) (8), the smallest known plant genome. Because the 0.86-pg peak corresponds to 2C nuclei and each successive class is greater than the previous one by a factor of 2, the nuclei classes are referred to as 2C, 4C, 8C, 16C, 32C, and 64C. *Mesembryanthemum crystallinum* appears to become endopolyploid by repeated rounds of replication of its entire genome in the absence of mitosis.

Table 1. Extent of endopolyploidy determined for 11 succulent species. Nuclei were isolated from leaves and analyzed by flow cytometry. The species are grouped according to phylogenetic classification (*14*). The DNA content given is for the smallest nuclei class for each species. The *M. crystallinum* value is 2C. Pollen was not available for the other species and, therefore, the values given have not been proven to be 2C. Since the genome size of *Aloe juveana* was too large to be on scale with the internal standard and thus could not be measured accurately, it is reported as being ~42.00 pg.

Order	Family	Species	Ploidy classes (no.)	DNA (pg)
Caryophyllidae				
	Aizoaceae	*Mesembryanthemum crystallinum*	6	0.86
	Cactaceae	*Pereskia grandifolia*	3	2.05
	Cucurbitaceae	*Xerosicyos danguyi*	4	1.24
	Passifloraceae	*Adenia sp.*	5	1.71
Rosidae				
	Crassulaceae	*Crassula phyteris*	4	1.50
	Crassulaceae	*Echeveria ramiletta*	3	2.68
	Crassulaceae	*Kalanchoe blosfeldiana*	4	3.40
	Crassulaceae	*Kalanchoe fedtshenkoi*	3	1.75
	Crassulaceae	*Sedum adolphi nova*	4	2.02
Liliidae				
	Liliaceae	*Aloe hyb.*	2	32.10
	Liliaceae	*Aloe juveana*	2	~42.00

Table 2. Summary of 11 succulent and 39 nonsucculent (*11*) plants for which the genome size and number of ploidy classes have been determined by flow cytometry. Numbers given under the three genome size ranges represent the numbers of species in each range for each of the taxonomic families (*13*) studied.

Order	Family	Ploidy classes (no.)	Number of species with 2C nuclear DNA content (pg)		
			0.7–3.5	3.5–25.0	>25.0
		Succulents			
Caryophyllidae					
	Aizoaceae	6	1		
	Cactaceae	3	1		
	Cucurbitaceae	4	1		
	Passifloraceae	5	1		
Rosidae					
	Crassulaceae	3	2		
		4	3		
Liliidae					
	Liliaceae	2			2
		Nonsucculents			
Asteridae					
	Apocynaceae	2		1	
	Compositae	2	1	2	
	Convulvulaceae	2	1		
	Gesneriaceae	2	1		
	Labiatae	2	1		
	Scrophulariaceae	2	1		
	Solanaceae	2	2	10	
Caryophyllidae					
	Caryophyllaceae	2		1	
Commelinidae					
	Gramineae	2	3	6	1
Dillenidae					
	Cruciferae	2	1		
Rosidae					
	Euphorbiaceae	2	1		
	Geraniaceae	2		1	
	Leguminosae	2	1	1	
	Lythraceae	2	2		
	Rosaceae	2	1		

Nuclei isolated from specific tissues of *M. crystallinum* were analyzed for multiple ploidy (Fig. 1, B to F), which was subsequently observed in all tissue types examined. The most abundant nuclei found belonged to the classes greater than 2C. The distributions of nuclei among the ploidy classes varied, but were characteristic of each tissue. For root tips and young leaves, the distribution was skewed toward 2C, 4C, and 8C nuclei. In mature leaves, cotyledons, and flowers (excluding the haploid pollen cells) 8C and 16C nuclei were prevalent. These general patterns were observed in at least three independent isolations for each tissue type.

To test the possibility that multiploidy was associated with the overall age of the plant, we isolated nuclei from the stems and cotyledons of 3-day-old seedlings, the cotyledons of 1.5-week-old plants, and the leaves of plants that were 1.5, 4, 5, 6, 7, 8, and 10 weeks old, covering a large part of the 14- to 16-week life-span of this species. Multiploidy was observed in all cases, although the exact distribution varied. Ploidy classes of 2C, 4C, and 8C were always present. The stems and cotyledons of the 3-day-old seedlings and the cotyledons of the 1.5-week-old plants had 2C, 4C, 8C, 16C, and 32C nuclei. In leaves, the presence of 16C, 32C, and 64C nuclei depended on leaf position. While the young, upper leaves had little or no 16C and 32C nuclei, the mature, lower leaves were always found to have 16C and 32C nuclei in addition to 2C, 4C, and 8C nuclei.

A developmental gradient is formed by the series of leaves from the top to the bottom of the plant. To test whether changes in the relative abundance of each ploidy class correlated with leaf development, we determined distributions for nuclei from three developmental series of leaves (Fig. 2). The percentage of nuclei that is 8C is uniform over the course of leaf development. However, the ploidy distribution shifts gradually from a bias toward low ploidy in the young, upper leaf to a bias toward higher ploidy in the mature lower leaf. In the youngest leaves, 57 ± 3.6% of the nuclei were 2C or 4C. This percentage declined to 33 ± 6.7% of the nuclei from the mature leaves. In dicotyledonous plants, most cell divisions are completed early in leaf development, and enlargement to the

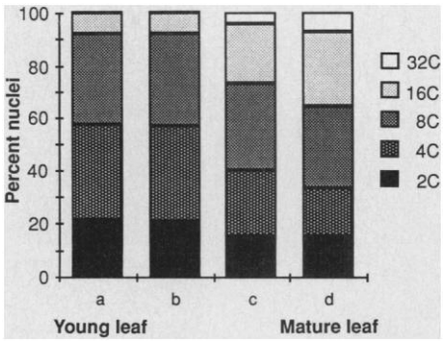

Fig. 2. The change in ploidy distribution with leaf development. Average ploidy distributions for four sets of leaves from three plants are shown starting with (a) the topmost leaf pair through the (b) second, (c) third, and (d) fourth leaf pair down the central axis of the plant. Standard errors range from 0.6 to 6.0.

fully expanded mature leaf is primarily the result of cell expansion. The higher proportion of low ploidy nuclei in young leaves may result from the developmental program. The high rate of cell division in early leaf development may delay the accumulation of cells with elevated genome copy numbers.

When *M. crystallinum* is subjected to high NaCl or drought stress, it undergoes a major physiological and metabolic conversion from C_3 to CAM (crassulacean acid metabolism) CO_2 fixation (*9*) as a result of altered gene expression (*10*). This conversion is a water conservation strategy. Because differences in gene copy number could be one aspect of changes in gene expression in response to stress, ploidy distributions were determined for nuclei from leaves and roots of nonstressed and NaCl-stressed plants (*11*). No significant differences in ploidy distribution in response to salt stress were observed in five independent experiments. Subtle changes that might be limited to a specific cell type within these tissues are not ruled out.

Multiploidy was not observed in 39 plant species previously analyzed by slow cytometry (*12*). Since *M. crystallinum* differs from these other plants in that it is a succulent CAM plant, we analyzed nuclei from the leaves of ten other succulent CAM species to test the possibility that multiploidy is associated with succulence or CAM. We have identified eight species that exhibit multiple ploidy at least in their leaves and two species that do not (Table 1). As does *M. crystallinum*, the eight multiple ploidy species have a small genome size, and 2C nuclei make up only a minor part of the total nuclei population. Two species of aloe, although succulent CAM plants, do not exhibit multiple classes of ploidy. These aloes are distinct from the other succulents in that they have very large genomes. This agrees with the observation that the tendency for somatic polyploidization in plants is inversely related to genome size and that somatic polyploidization in small genome plants and germline polyploidization in large genome plants may be two strategies for accomplishing a similar result (*13*). Multiple ploidy is not exhibited by 16 nonsucculent species that have genome sizes similar to the multiploid succulents (Table 2). Multiploidy may be a general property of succulents that have small genomes. The function of multiploidy in these plants is unknown. In mature *M. crystallinum* leaves, the presence of a range of ploidy, including 2C, indicates that not all cells are destined to become endopolyploid. The gradual shift in the bias of ploidy distributions toward higher levels seen during leaf development stops once the leaf is fully expanded. A ploidy distribution is then maintained that is typical of mature leaves. This pattern of differential endopolyploidization may be linked to the process of cellular differentiation that yields the different types of cells present in the mature leaf. Cells that could reasonably be expected to reach high levels of ploidy are large-volume cells such as the water-storing mesophyll cells that give the leaves the property of succulence. The capacity for synthesis from a small genome may be insufficient for the large volume of these cells. For succulents, it may be that large genomes and multiploidization of small genomes are alternative strategies for adaptation to arid environments. Correlation of ploidy with particular cell types would give clues as to the possible role of multiploidy in these plants.

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Photoacoustic "Signatures" of Particulate Matter: Optical Production of Acoustic Monopole Radiation

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Absorption of pulsed laser radiation by a single particle generates a photoacoustic wave whose time profile can be measured with a wideband pressure transducer. Solution of the wave equation for pressure in one, two, and three dimensions shows that the photoacoustic wave is determined by the geometry and dimensions of the particle, and by its sound speed and density relative to the fluid that surrounds it. Photoacoustic waves, referred to here as signatures, are reported in experiments in which fluid droplets, cylinders, and layers are irradiated with 10-nanosecond laser pulses.

THE ABSORPTION OF OPTICAL RADIATION by matter causes heating and, in general, subsequent expansion of the irradiated body, thereby launching an acoustic wave. Owing to its high sensitivity and its response to evolved heat, this process, known as the photoacoustic effect, has found application in a number of fields including spectroscopy, nondestructive testing, photochemistry, microscopy, semiconductor physics, and trace detection (*1*). We report here a study of a facet of the photoacoustic effect that has heretofore received only scant attention: the temporal profile of acoustic waves generated by particulate mat-

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