KARYOLOGY

Ploidy level versus DNA ploidy level: an appeal for consistent terminology

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There is an ever-increasing amount of cytogenetic data in plant sciences obtained by cytometric techniques, particularly flow cytometry. However, as these methods determine nuclear DNA amount irrespective of the number of chromosomes, a discrepancy between cytometric and karyological data may occur. To avoid potential bias, we appeal for consistency, distinguishing between the terms "ploidy / aneuploidy" referring to chromosome numbers and "DNA ploidy / DNA aneuploidy" to nuclear DNA content.

KEYWORDS: cytotype, cytometry, DNA ploidy, genome size, chromosome numbers, nuclear DNA content.

A variety of analytical tools have recently been employed in plant taxonomy, biosystematics, and ecology to obtain novel chromosomal and ploidy level data. Cytometric techniques such as Feulgen densitometry, image cytometry, and particularly flow cytometry (FCM) have played a major role. The latter method is based on the rapid measurement of optical properties of isolated nuclei particles moving singly in a narrow liquid stream through a powerful beam of light (Doležel, 1997). Although it was originally developed for biomedical applications (Shapiro, 2003), with the construction of user-friendly instruments, development of new fluorochromes, and a breakthrough in the methodology of sample preparation (Galbraith & al., 1983), the technique has been applied to a range of fields in plant sciences.

The great popularity of FCM in plant karyosystematics lies in its speed and simplicity as compared with conventional chromosome counting. Sample preparation is simple and convenient, allowing several dozens or even hundreds of samples to be analysed per working day. Moreover, several specimens can be analysed together when checking their genome size homogeneity, which makes the technique ideally suited for rapid and large-scale cytotype screening. Nearly all plant tissues (e.g., leaves, stems, roots, sepals, petals, seeds) can be successfully utilized in FCM assays, eliminating the need for mitotically active cells. The requirement of very small tissue amounts is another feature of paramount importance, paving the way for the use of FCM for comprehensive studies of rare and endangered species without risk to their population destruction (Sgorbati & al., 2004.)

It is hardly surprising that FCM attracts ever-increasing attention of contemporary plant researchers, and the results have had a significant impact on the taxonomy, phytogeography, population biology, and ecology of many plant alliances (Stace, 2000; Suda, 2004). Representative samplings have generated novel insights into the extent of intra- and inter-population ploidy variation, habitat requirements, and reproductive behaviour of individual cytotypes (Mandák & al., 2003; Mahelka & al., 2005). Only with the aid of FCM, has it been possible to reliably assess ploidy distribution at various spatial scales, interactions among cytotypes, and evolutionary processes in diploid-polyploid sympatric populations (Baack, 2004; Husband & Sabara, 2004). Also attractive is the potential to reshape former taxonomic concepts and propose robust classifications based on detailed knowledge of cytotype performance (Bureš & al., 2003; Rosenbaumová & al., 2004).

Although ploidy estimation by cytometric techniques is generally considered to be a trivial task, some precautions should be taken during data interpretation. In principle, the methods quantify only total nuclear DNA amount, irrespective of the exact number of chromosomes. Ploidy level (chromosome number) is inferred subsequently by comparing the sample profile with that of a reference standard (i.e., karyologically tested material of the same or related species). However, cytometric data (such as fluorescence intensity) may only be loosely correlated with chromosome counts, i.e., samples with lower chromosome number may have higher DNA content and vice versa. To avoid potential bias, the Committee on Nomenclature of the Society of Analytical Cytology (Hiddemann & al., 1984) advocated that results obtained by cytometric and karyological analyses should be treated separately. Because the terms ploidy/aneuploidy are used to describe chromosomal complement at

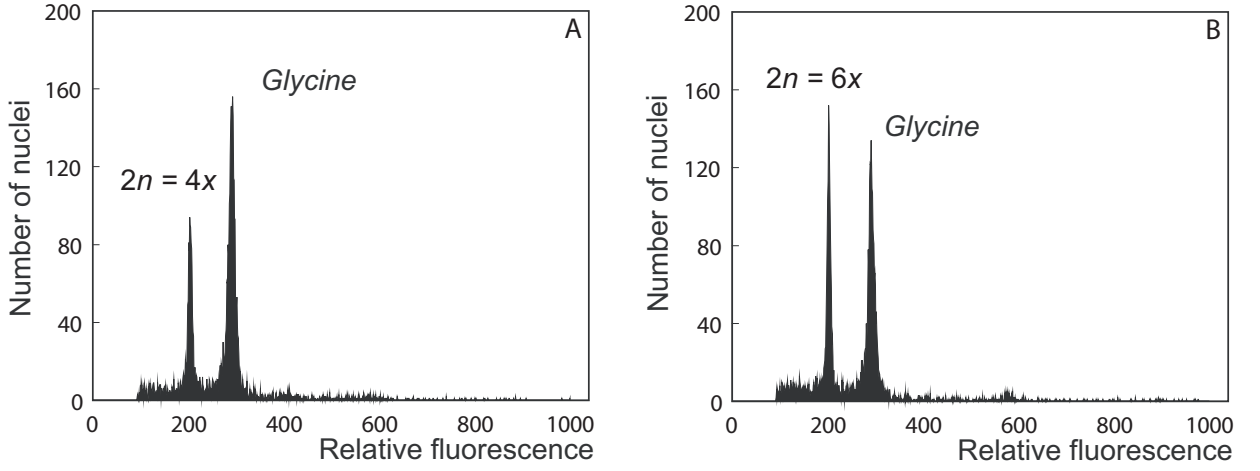

Fig. 1. Flow cytometric histograms documenting apparent discrepancy between ploidy level and DNA ploidy level. DAPI-stained nuclei isolated from karyologically-checked tetraploid Potamogeton perfoliatus (2*n* = 4*x* = 52, peak ratio 1.428; A) and hexaploid *P. filiformis* (2n = 6x = 78, peak ratio 1.441; B) were analysed together with *Glycine max* cv. Polanka (2C = 2.5 pg) as internal standard. Despite different numbers of chromosomes (i.e., different ploidy level), both species have a nearly identical nuclear DNA content and the same DNA ploidy level could erroneously be inferred.

cellular, individual or population levels (Rieger & al., 1976), the prefix "DNA" (i.e., DNA ploidy, DNA aneuploidy) should denote cytometric data, referring to nuclear genome in particular tissues, individuals or populations. DNA diploid then describes a sample with nuclear DNA content corresponding to that from a sample with a diploid number of chromosomes determined using a conventional chromosome counting. Similarly, the term DNA aneuploidy describes a sample with DNA content deviating from karyologically checked euploid material. This proposed terminology is consistently used already, for instance, in clinical studies (see the Web of Science).

In contrast to clinical studies, distinguishing between DNA ploidy level and (chromosomal) ploidy level is still only rarely done in plant sciences (but see the recommendation in FCM reviews of Doležel, 1991, 1997). We were able to retrieve less than three dozen botanical articles from the Web of Science that correctly adopted the term DNA ploidy, while the total number of similar cytometric papers was well over one order higher. Yet the lack of precise terminology may make it difficult to compare data from different sources, and can lead to misunderstandings, and even to the introduction of big errors. The plant kingdom is well known for (i) high incidence of polyploidy, (ii) large divergence in genome size, even among closely related taxa with the same number of chromosomes, and (iii) often non-proportional changes of nuclear DNA amount with respect to ploidy level. Consequently, several problematic cases may be encountered:

1. Homoploid species within the same genus with markedly different nuclear DNA contents. — Such plants will mimic different ploidy levels during cytometric analyses. A survey of data from the Plant DNA C-values Database (Bennett & Leitch, 2005) revealed that this phenomenon is quite common. The median of 1C-value range for 381 homoploid species (at diploid to dodecaploid levels) was 1.59 (range 1.00–9.03, with one outlying value of 40.24 in the genus *Oxalis*). In 131 (34.4%) and 33 (8.7%) cases, the infrageneric DNA content variation at the same ploidy level exceeded the thresholds 2.00 and 4.00, respectively (i.e., diploid plant could erroneously be considered to be at least tetraploid and octoploid, respectively). For example, species with identical numbers of chromosomes but dramatically different genome sizes are *Cypripedium molle* (2*n* = 20, 1C = 4.14 pg) and *C. macranthos* (2*n* = 20, 1C = 37.4 pg), *Bulnesia sarmientoi* (2*n* = 26, 1C = 0.35 pg) and *B. retama* (2*n* = 26, 1C = 2.28 pg), *Lonicera nigra* (2*n* = 18, 1C = 0.65 pg) and *L. vesicaria* (2*n* = 18, 1C = 3.88 pg), and *Vicia lunata* (2*n* = 14, 1C = 1.83 pg) and *V. kalakhensis* (2*n* = 14, 1C = 9.18 pg).

2. Heteroploid species from the same genus with (nearly) identical nuclear DNA contents. — Despite different number of chromosomes, cytometric analyses will yield (nearly) matching flow cytometry profiles and the same ploidy may be erroneously inferred. Representative examples are tetraploid *Potamogeton perfoliatus* (2*n* = 4*x* = 52) and hexaploid *P. filiformis* (2*n* = 6*x* = 78), both with 1C = ca. 0.87 pg (Fig. 1), and pentaploid *Hieracium bauhinii* (2*n* = 5*x* = 45) and hexaploid *H. pilosella* (2*n* = 6*x* = 54), both with 1C = ca. 5.2 pg (our unpublished results).

3. Reverse relationship between the number of chromosomes and nuclear DNA content. —

Selected species pairs from genera *Tradescantia* (*T. gigantea*, 2*n* = 2*x* = 12, 1C = 30.03 pg and *T. fluminensis*, 2*n* = 12*x* = 72, 1C = 7.18 pg), *Oxalis* (*O. dispar*, 2*n* = 2*x* = 12, 1C = 16.5 pg and *O. sellowii*, 2*n* = 12*x* = 72, 1C = 7.5 pg), and *Acacia* (*A. simsii*, 2*n* = 2*x* = 26, 1C = 2.1 pg and *A. seyal*, 2*n* = 8*x* = 104, 1C = 1.08 pg) may serve as the most blatant examples.

4. Non-proportional change of nuclear DNA content in relation to ploidy level within the same species. — Whereas the above-mentioned examples concerned infrageneric genome size variation and a selection of conspecific reference standards might have reduced the risk of inaccurate ploidy inference, the examples described below provide particularly good illustrations of the errors which may arise when inferring ploidy levels from flow histograms. Both decreases and increases of DNA amount per monoploid genome (i.e., Cx value = 2C DNA content/ploidy level) with increasing ploidy level have been reported. For example, tetraploid cytotypes of *Plantago ovata* (2*n* = 4*x* = 16, 1C = 0.8 pg) have only 1.5-fold more DNA (imitating thus 3*x*) than diploids (2*n* = 2*x* = 8, 1C = 0.53 pg), and hexaploid cytotypes of *Larrea tridentata* (2*n* = 6*x* = 78, 1C = 1.6 pg) have only 1.25-fold more DNA (imitating thus 5*x*) than the tetraploids (2*n* = 4*x* = 52, 1C = 1.28 pg). These examples illustrate the former case where ploidy level may be underestimated (see also Fig. 2). On the other hand, pentaploid cytotypes of *Holcus mollis* (2*n* = 5*x* = 35, 1C = 4.1 pg) may be erroneously regarded as hexaploids when using a tetraploid standard (2*n* = 4*x* = 28, 1C = 2.78 pg), and pentaploidy may be erroneously estimated for tetraploid cytotypes of *Saxifraga granulata* (2*n* = 4*x* = 44, 1C = 1.78 pg) using a diploid standard (2*n* = 2*x* = 22, 1C = 0.68 pg).

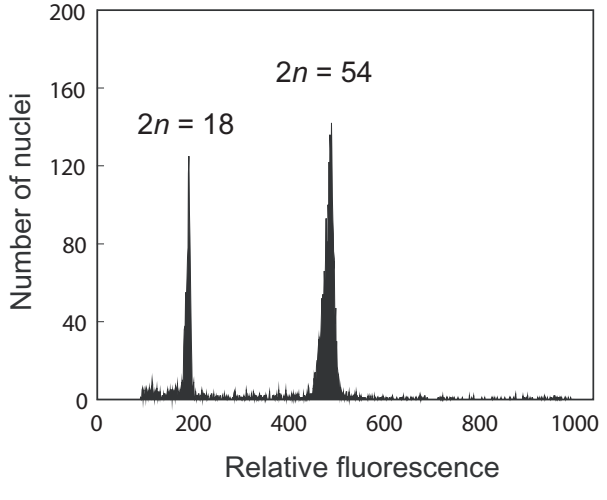

Fig. 2. Simultaneous FCM analysis of DAPI-stained nuclei isolated from karyologically-checked diploid (2*n* = 2*x* = 18) and hexaploid (2*n* = 6*x* = 54) cytotypes of *Aster amellus*. Both plants differ threefold in the number of chromosomes but only 2.54-fold in the nuclear DNA content. Pentaploidy may thus be erroneously estimated for the latter cytotype.

5. A lack of correlation between the degree of intraspecific aneuploidy (chromosome(s) gain/loss) and corresponding change in nuclear DNA amount. — Under certain conditions, a high-chromosome aneuploid individual may have a lower nuclear DNA amount than its conspecific low-chromosome counterpart (and vice versa). Examples include species with bimodal karyotypes or allopolyploids combining parental genomes with markedly different chromosomal sizes. Nuclear DNA content will then depend on the actual chromosome constitution. Analogously, some aneuploid products of recent hybridization between species with markedly different genome sizes may also fall into this category. Examples include a hybrid between hexaploid and heptaploid plants within a population of *Elytrigia intermedia*, which was hybridised in the past with an unknown species with higher DNA content. A specimen with 47 somatic chromosomes had about 6% more DNA than its counterpart with 49 somatic chromosomes (Mahelka & al., unpubl.).

All of the above examples clearly illustrate that serious discrepancies may occur between karyological and cytometric data. Therefore, the exact number of chromosomes is needed for every sample with a distinct cytometric profile. If chromosome counts are lacking, which is still quite common, the results should always be designated as "DNA ploidy" or "DNA aneuploidy".

Stabilization of the terminology concerning ploidy/aneuploidy is nowadays particularly important (similar problems surrounding the term "genome size" have been settled recently, see Greilhuber & al., 2005). With the routine use of cytometric equipment (particularly flow cytometers) in plant taxonomy and related disciplines, a wealth of cytometric data are being published additional to conventional karyological counts (e.g., Mráz, 2005; Flora of the Czech Republic, vol. 8., in prep.). A boom of such kind of data is undoubtedly forthcoming. We therefore appeal for the meticulous distinction between "DNA ploidy level/DNA aneuploidy" and "ploidy level/aneuploidy" in research articles and in the Indexes to Plant Chromosome Numbers (e.g., Goldblatt & Johnson, 2003). Adherence to this policy will allow reliable data juxtaposition and facilitate better understanding of results obtained by different methods and in different laboratories.

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