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Flow cytometry protocols, relative genome size and ploidy levels for 1,103 species of non-apomictic angiosperms from the Eastern Alps: a community resource based on the screening of 45,000 samples

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Abstract: Flow cytometry provides a reliable and fast method for estimating genome size and ploidy levels in plants. Until recently, most studies employed fresh tissues that usually provide high-quality results. However, the need for fresh tissues limits the use of the method with samples from remote areas or when an extremely high number of samples needs to be processed in a short time. Although there is growing evidence that fixed silica-dried material can be used for ploidy estimation in some taxa, no flora-wide study has been available so far. Here, we provide methodological aspects of an unprecedented study exploring ploidy variation of non-apomictic angiosperms in the Eastern Alps. We have analysed ~45,000 silica-dried samples of 1,134 species using flow cytometry with DAPI as stain. Surprisingly, we were able to obtain ploidy level information from as much as 1,103 (97%) of species. The unsuccessful species included succulent plants of the family *Crassulaceae* (genera *Jovibarba*, *Rhodiola*, *Sedum*, *Sempervivum*), the achlorophyllous parasitic or mycoheterotrophic genera *Orobanche* and *Hypopitys*, and a handful of others. About 80% of samples were successfully analysed using a single 'universal' protocol and leaf tissue, while in the remaining species the use of alternative tissues (such as petioles or flowers) and/or protocol modifications were needed (targeting composition of buffers, duration of incubation or staining time or use of alternative buffers). A total of 384 species (35%) included polyploid cytotypes. Among them, 151 (14%) included diploid and polyploid cytotypes, 25 species (2%) being ploidy variable with multiple polyploid cytotypes, and 208 (19%) being polyploids with just one cytotype. The remaining 719 (65%) species were diploid only. As a community resource, we provide relative genome sizes and ploidy assignments of 1,328 cytotypes retrieved from 1,103 species along with methodological details (e.g. buffers, standards, analysed plant organs, histogram quality, and other remarks). We believe that this dataset will facilitate future research in particular species as well as in flora-wide investigations of ploidy level variation of the central-European flora in general. We are confident that novel cytotypes of many species will be discovered in other geographic areas, and we would be delighted if the present dataset could serve the botanical community for comparison.

Keywords: flow cytometry, silica-dried samples, Eastern Alps, central Europe, angiosperms, ploidy level, polyploidy

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Introduction

Polyploidy, i.e. the presence of more than two chromosome sets in zygotes and more than one set in gametes, is a common feature in plants. In angiosperms, which is by far the most diverse extant plant group, about 35% of species are polyploid (Wood et al. 2009). Polyploidization is one of the most prominent speciation mechanisms in plants, as it creates an instantaneous reproductive barrier (Husband & Sabara 2004, but see Bartolić et al. 2024). Wood et al. (2009) estimate that ~15% of speciation events in angiosperms (and even more in other groups, such as ferns) are directly connected to ploidy level increase. Polyploidization offers evolutionary advantages by enhancing gene redundancy, which buffers against deleterious mutations and provides new genetic material for the evolution of novel functions through subfunctionalization or neofunctionalization (Birchler & Yang 2022). It can also increase heterosis leading to improved vigour and potentially higher fitness (Comai 2005, Otto 2007).

Increased genetic diversity and adaptability in polyploids can facilitate successful colonization of new environments and may be linked to an increase of the rate of asexual reproduction (te Beest et al. 2012). Polyploidy can also confer robustness to environmental changes, enhancing survival under stress and offering resistance to pathogens (Van de Peer et al. 2021). Genomic studies have shown that most plants underwent at least one, but usually several, rounds of polyploidization in their evolution; polyploidization events can be traced also in the genomes of animals and fungi (Jiao et al. 2011, Van de Peer et al. 2017). Despite that, most extant plant species are functionally diploid, and often have low chromosome numbers (an example being 2n = 10 in the model species *Arabidopsis thaliana*; Rice et al. 2015). Polyploid genomes undergo progressive reduction of many duplicated regions, a process called diploidization (Wendel 2015, Van de Peer et al. 2017). Therefore, in the long-term perspective, polyploidy may be only a temporary phenomenon in the evolutionary history of a plant species. This also implies that the definition of a taxon's ploidy level is not absolute but rather depends on the phylogenetic context in which it is considered.

Since the early studies (e.g. Löve & Löve 1943), it is well established that the distribution of polyploids is non-random. The available studies suggest an increasing frequency of polyploidy towards higher latitudes, although the drivers are not clear (Rice et al. 2019). However, due to the scarcity of karyological data, each species in such large-scale studies as well as in the available flora-wide studies (e.g. Šmarda et al. 2019, Chytrý et al. 2021) is typically assigned a single (dominant) ploidy level even though it is widely acknowledged (Rice et al. 2015) that multiple cytotypes are known in about a quarter of species of land plants. Such intraspecific ploidy variation may then confer differences in genetic variability, genes underlying environmental adaptations, traits or even the ecological niche within a species (Celestini et al. 2025, Meirmans & Kolář 2025, Vlček et al. 2025). Our limited understanding of intraspecific variation in ploidy level is mostly due to the laborious methodology of chromosome counting. Progress in the application of flow cytometry on plant samples over the last two decades (Kron et al. 2007, Loureiro et al. 2010, Galbraith et al. 2021) has enabled researchers to generate flora-wide datasets with a sufficient number of replicates for each species. The presented research is part of a project focussing on the distribution of polyploids in the Eastern Alps and the underlying factors. The main questions relate to the altitudinal distribution of polyploids and to

their occurrence related to distance from major Pleistocene refugia, since better tolerance of polyploids to harsh environments such as low temperatures in high elevations and better colonization abilities of polyploids are often hypothesized (Van de Peer et al. 2021). To answer these questions while taking possible infraspecific ploidy variation into account, we aimed at generating a flora-wide dataset from multiple sampling sites over the Eastern Alps.

Fresh leaf tissue is the preferred material for flow cytometry (Sliwinska et al. 2022). However, the need for fresh material limits the use of the method when a large number of samples need to be collected and processed in a short time and/or when samples from remote places are to be analysed. Therefore, attempts were made to use fixed tissues (see Čertner et al. 2022 for a detailed review). Some studies tested the use of deep-frozen tissues (e.g. Cires et al. 2009, Sabara et al. 2013). Various methods of chemical fixation have been tested, among them preservation of isolated nuclei in glycerol (e.g. Kolář et al. 2012, Kobrlová et al. 2020), which gives good results in non-model plants. However, these methods require basic laboratory equipment and a freezer/refrigerator available and some of them are rather laborious and thus not suitable for processing a lot of samples in field conditions. For field studies, the use of desiccated material is the most effective approach. Some authors report the use of the herbarium material (e.g. Šmarda & Stančík 2006). Suda & Trávníček (2006) tested herbarium specimens of multiple species dried at 40 °C and were able to get scorable histograms from 52 out of 60 tested species (86%). However, rapid drying of plant tissue in silica gel is the most convenient method for field studies due to simplicity and no need for special equipment. It is increasingly being used and there is growing evidence that silica-dried material can be used for ploidy estimation in various plant species (e.g. Sonnleitner et al. 2010, Frajman et al. 2015, Kravanja et al. 2025). Compared to fresh material, dried samples usually provide histograms with higher peak coefficients of variation (CV) and higher background noise (Bainard et al. 2011). A mild decrease of fluorescence intensity of a few % up to ~10% is often reported (Čertner et al. 2022), although studies employing propidium iodide as a stain surprisingly report fluorescence intensity increase in some species (Cires et al. 2009, Bainard et al. 2011, Vejvodová et al. 2024). The changes of the histogram quality and fluorescence seem to be species-specific. The use of dried tissues is therefore not recommended for absolute genome size estimation, while it is generally well suitable for ploidy level analysis (Sliwinska et al. 2022).

Similarly to fresh material, and even more importantly due to generally lower quality of the analyses, the results may also be influenced by the choice of a buffer and DNA stain. Various nuclei isolation buffers have been published and cross-tested with several species (e.g. Loureiro et al. 2006, 2007); further modifications of existing buffers were recently published by Šmarda et al. (2019) and buffer composition was reviewed by Loureiro et al. (2021) in detail. In general, there is no universal buffer and for problematic species (for example containing staining inhibitors or mucilage) one needs to seek a suitable specific buffer. Among the two most common DNA stains, propidium iodide and 4',6-diamidino-2-phenylindole (DAPI), the latter is preferred with dried material due to higher resolution of the histograms and lower influence of the staining inhibitors, despite that it is AT-selective and the data can only be used for ploidy level analysis, but not for absolute genome size estimation (Sliwinska et al. 2022).

Here, our principal aim is to provide a community resource by reporting on methodological aspects of an unprecedented flora-wide study exploring ploidy variation of non-apomictic angiosperms in the Eastern Alps spanning parts of Austria, Italy, Slovenia, Germany and Switzerland. While several studies focused on individual species/species complexes are available from the study area (e.g. Sonnleitner et al. 2010, Kirchheimer et al. 2016, Regele et al. 2017, Zozomová-Lihová et al. 2026), for most species information on ploidy variation is limited to a few published chromosome counts, or lacking. Using flow cytometry of silica-dried material, we have analysed ~45,000 samples, collected along 101 elevational transects. While the distribution of polyploids in the Eastern Alps and the underlying factors will be analysed in follow-up studies, the aim of this study is to present (i) the relative genome sizes and ploidy assignments of 1,328 cytotypes retrieved from 1,103 species. Further, we show that (ii) the vast majority (97%) of species can be analysed from silica-dried samples, and discuss for which taxa this was not possible. Finally, (iii) we provide various methodological details (e.g. buffers, standards, analysed plant organs, histogram quality), with the aim of facilitating future research in particular species as well as in flora-wide investigations of nuclear genome size variation.

Materials and methods

Field sampling

We sampled 101 transects positioned on south-exposed mountain slopes in the Eastern Alps (Fig. 1) during the summers 2021 and 2022. A transect typically consisted of 5 sampling belts, spanning 100 m of elevation each, separated by 150 m. Each complete transect therefore spanned 1,100 m of elevation, with the median belt centred at the timberline. We chose to position all transects on southern slopes in order to keep the position of the timberline, which we used as a reference altitude, comparable. In each of the belts, the pool of native (i.e. non-neophytic) angiosperms was sampled as completely as possible from all types of natural or semi-natural vegetation except for aquatic macrophytes. The avoidance of neophytes or strongly anthropogenically altered habitats reflects the overall project aims outlined above. Species from Annex II of the Habitats Directive (in our case *Campanula zoysii* and *Cypripedium calceolus*) and the taxonomically highly intricate, predominantly apomictic genera *Alchemilla*, *Hieracium*, *Pilosella* and *Taraxacum* as well as *Rubus* sect. *Rubus* and the *Rosa canina* species group, which could not be exhaustively sampled without the permanent presence of a specialist for each group, were not sampled. For each species, material of one individual per belt was collected. We always collected leaves; other organs such as flowers, parts of the inflorescence, fruits, or seeds were collected to aid determination or when we anticipated their use for flow cytometry. Healthy tissues in good condition (i.e. not withering, not infested by insect herbivores or fungi) were collected and kept in humid conditions. At the end of a sampling day, samples were put into labelled 8 × 19 cm cellulose bags (tea bags) and dried and stored intermingled with layers of silica gel in air-tight ~36 × 56 × 27 cm plastic boxes. Even if much of the silica gel was translocated towards the bottom of the boxes when driving on unpaved roads, this approach proved very efficient for quickly drying large numbers of samples. When the samples were desiccated, silica gel was reused after drying at ~100 °C for several days. As an exception, samples of *Crassulaceae* (*Jovibarba*,

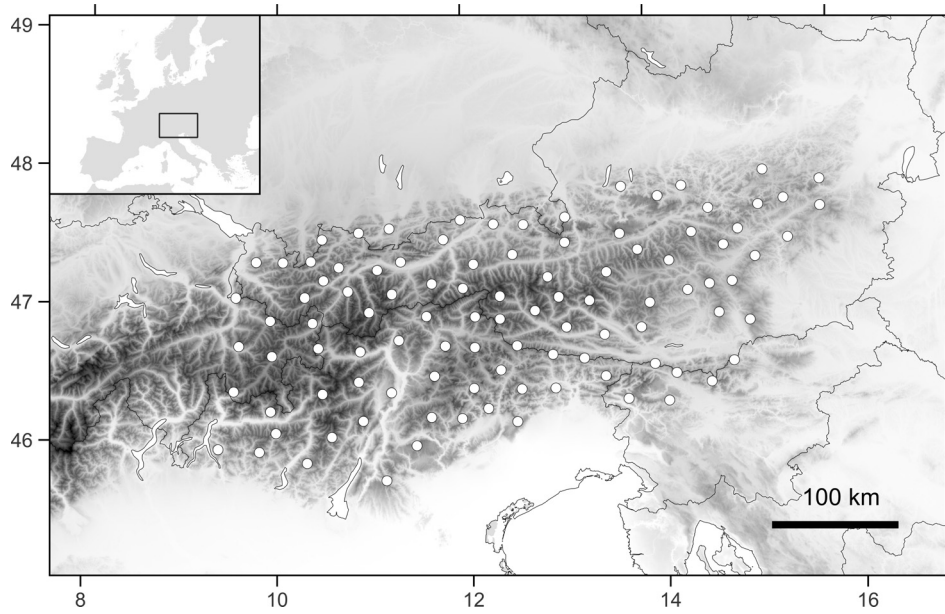

Fig. 1. Study region and position of the transects in the Eastern Alps.

Sedum, *Sempervivum*) were slightly squashed and dried with paper tissue before putting them into tea bags.

The nomenclature in the manuscript and Supplementary Table S1 follows Schratt-Ehrendorfer et al. (2022) and Bartolucci et al. (2018) for the species not included in the former source. However, we also retained selected older synonyms following Fischer et al. (2008) in the Supplementary Table S1 to facilitate the search for taxa.

Flow cytometry

The relative genome size was measured using flow cytometry. Samples from one species were mostly analysed in one laboratory. We used Sysmex (formerly Partec) flow cytometers (Sysmex CyFlow Space or Sysmex CyFlow Space equipped with a CyFlow Space Autoloading Station), using 365 nm LED as light source. The samples were stained with DAPI (4',6-diamidino-2-phenylindole). Since an AT-selective dye was used, results are expressed as relative genome size (RGS), that is the ratio of the mean fluorescence intensity of the sample and the standard G_1/G_0 peaks (i.e. the peaks containing nuclei with 2C DNA content).

The sample preparation protocols are described below in detail and indicated in Supplementary Table S1 along with other technical information, such as standards, pooling of samples, and use of the autoloading station. In general, we followed best-practice recommendations outlined in Sliwinska et al. (2022), Temsch et al. (2022), and Koutecký et al. (2023). All samples from the first collection season were analysed individually. We used pooled samples of two individuals from the second season in selected species under the following conditions: (a) high-quality histograms, (b) no missing data (unsuccessful analyses) in the first season, (c) no substantial RGS variation within a ploidy level in the

first season, (d) samples from the same transect. If RGS variation in a pooled sample was suspected based on the fluorescence histogram, the samples were reanalysed individually. We always used internal standardization with one of the following standards (fresh tissue): *Carex acutiformis*, *Solanum pseudocapsicum*, *Bellis perennis*, *Pisum sativum* 'Ctirad', *Pisum sativum* 'Kleine Rheinländerin', *Chlorophytum comosum*, *Vicia faba* 'Inovec' (see Temsch et al. 2022 for details on the individual standards). We aimed at using one standard for all samples of one genus. However, in large genera with diverse genome sizes (e.g. *Campanula*, *Cerastium*, *Festuca*, *Salix*, *Saxifraga*), several standards had to be used to avoid peak overlap or more than 4-fold difference between the sample and the standard mean fluorescence. To allow comparison across species, all RGS values were recalculated to the same standard (i.e. the sample RGS was multiplied by the RGS ratio of the original to the new standard). We measured the RGS ratio (mean of three or more repeats on different days) for all pairs of standards that do not differ more than 4-fold; for more distant pairs, we computed the expected ratio based on standards with intermediate RGS in a cascade-like manner. The recalculation matrix is presented in Supplementary Table S2. We also recalculated the RGS of all species to *Bellis perennis* to allow comparison over the entire dataset.

Initially, we tested the 'universal' sample preparation protocol with Otto buffers, which are frequently used in plant flow cytometry (i.e. protocol 1C in Doležel et al. 2007, simplified two-step protocol). About 4–25 mm^2 (depending on the species) of the dried sample and the fresh internal standard was chopped with a sharp razor blade in ice-cold Otto I buffer (0.1 M citric acid monohydrate, 0.5% Tween 20), and filtered through a 42 µm nylon mesh (Uhelon 130T, Silk&Progress, Brněnec, Czech Republic). After ~5 min the filtrate was stained with Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$, 0.2% 2-mercaptoethanol, DAPI 4 µg/ml) and analysed after ~5 min of staining. If this approach did not yield satisfactory results (i.e. histograms with coefficients of variation (CV) below 5%, low background), different optimizations of the protocol were tested. Frequent modifications included: (a) use of alternative plant tissue, if available (e.g. leaf petioles instead of leaf blades, flowers, seeds; in case of seeds, the embryo peak was scored), (b) addition of 0.1 M HCl to the Otto I buffer (1:1), supplemented with Tween 20 to keep its concentration at 0.5% (this corresponds to the 'lh3' buffer from Šmarda et al. 2019), (c) addition of 20 mg/ml polyvinylpyrrolidone (PVP), (d) changing incubation time (in Otto I buffer) to short (< 3 min) or prolonged (> 10 min). In some cases, different buffers were tested, especially LB01 buffer (Doležel et al. 2007), with or without PVP.

Depending on the species (amount of material, histogram quality), fluorescence of 3,000–5,000 particles was recorded. The gain/voltage parameter was adjusted in a way that no peak was located below channel 100 (on the 1,024-channel linear scale), leaving at least 10 'empty' channels before the first peak. During the first measurements of each species, the lower limit of the scale was set such that half of the fluorescence of the putative left-most (lowest-fluorescence) peak was visible to not overlook any peaks; this practice is especially important in plants with endopolyploidy.

A subset of samples, comprising unproblematic species represented by multiple individuals, was analysed using a flow cytometer with autoloading station, which required modified sample preparation. Approximately 3–4 mm^2 of plant tissue of both the fresh internal standard and the silica-dried sample were inserted into microtubes arranged in a 96-well rack (DNeasy 96 Plant Kit, Qiagen, Hilden, Germany). A tungsten carbide bead

with 3 mm diameter (Qiagen) was added to each microtube, followed by the addition of 400 µl ice-cold Otto I buffer. The plant material was ground in microtubes at 30 Hz for ~2 min using a TissueLyser II (Qiagen); grinding time was adjusted for each species. Orientation of the rack was reversed after half of the grinding time had elapsed to ensure homogenous grinding. Separation from the debris was achieved by filtering 100 µl of the nuclei suspension through a 42 µm nylon mesh into a 96-well microplate. The suspension was incubated for ~15 minutes before staining with 200 µl of Otto II buffer. CyPAD 1.3 software (Sysmex Partec) was used for sample analysis, with the sample volume set to 100 µl. Adjustments of speed and gain/voltage parameters were done similarly as described above, but generally not changed during the run of a plate. Total running time per plate – and consequently the maximum incubation time in Otto II for the final sample – varied between 2 and 3 hours.

Histograms were evaluated using FloMax 2.9 software (Sysmex Partec) or the package flowPloidy (Smith et al. 2018) for R version 4.4.2 (R Core Team 2024). Mean fluorescence intensities of the G_1/G_0 peaks of the sample and the standard – and their CVs – were recorded and the relative genome size (RGS) calculated.

In *Orchidaceae*, a special approach was used to estimate the RGS, due to the peculiar pattern of endoreduplication specific for this family. Some orchid species are known for partial endoreduplication (Trávníček et al. 2015). During the endoreduplication cycles, only a part of the genome is replicated in those species; the genome size ratio between the successive peaks is then less than two. Instead of 2C, 4C, 8C, etc., the DNA content of the individual peak is marked as 2C, 2C+P, 2C+3P etc., P being the replicated part of the genome ($P < 2C$ under partial endoreduplication, while under conventional endopolyploidy $P = 2C$). The ratio between successive peaks is stable and specific for the individual peak pairs (Fig. 2), i.e. it is different between the first (2C) and the second peak (2C+P), the second (2C+P) and the third peak (2C+3P), etc. The difference of the successive peak ratios is greater, the smaller the value of P is. The height of the peaks (i.e. the number of cells having a particular DNA content) is variable and partly tissue-specific (Fig. 2). In leaf tissue of species showing partial endoreduplication (and especially those in which only a small part of the genome is replicated), the 2C peak is sometimes missing, while it is always present in immature ovaries (Trávníček et al. 2015). Absence of the 2C peak may lead to wrong estimation of the sample RGS. On the other hand, when P is known and at least two peaks are recorded, their specific RGS ratio allows identifying the peak identity and calculating the true RGS even if the 2C peak is missing. We used this approach for the analysis of *Orchidaceae* data within our dataset. First, RGS values of all peaks (at least two, but often three peaks), were recorded. From samples where ovaries were available (all species, 1–52 samples per species, one measurement per sample), we estimated the species-specific value of P. The estimated P-values and the successive peak ratios are listed in Supplementary Table S3 to make them available to other researchers. We analysed leaf material from the same individuals (up to 20 samples per species, if available) to verify the calculations; the recorded peak ratios always corresponded to the respective values obtained from ovaries. Last, for the samples where only leaves were available, we identified the individual peaks as 2C, 2C+P, 2C+3P etc. based on their RGS ratios, and calculated the 2C RGS based on the species-specific P-value, even if the 2C peak was absent.

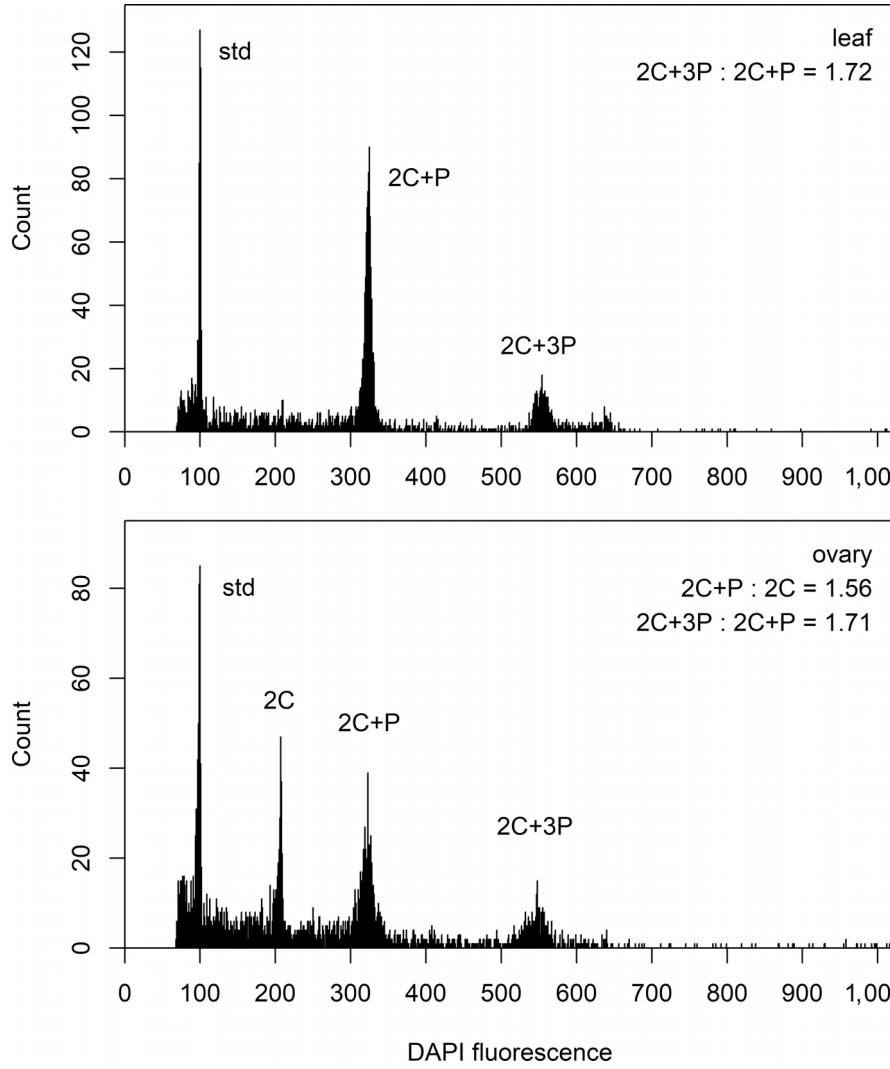

Fig. 2. Flow cytometric analysis of a leaf (top) and an ovary (bottom) of the same individual of *Gymnadenia conopsea*. Note the missing 2C peak in the leaf sample and the specific RGS ratios of the successive peaks. *Bellis perennis* was used as the internal standard (std).

Histogram quality was evaluated based on the CV of the sample peak and the amount of background noise; it was ranked in one of three categories (high/medium/low). Additional features were recorded if present, for example the regular presence of multiple peaks due to pronounced endopolyploidy or fluorescence shifts due to secondary metabolites (i.e. stable RGS, but both the sample and the standard peaks located at different positions of the fluorescence axis despite the same settings of the cytometer). As a quality check, histograms of the RGS and basic descriptive statistics (mean, standard deviation, coefficient of variation, minimum, maximum) were computed for each species. Outlying RGS values were checked for the analysis quality and possible peak scoring errors; they

were reanalysed, if needed. If the outlying RGS was confirmed, we consulted the literature whether karyological/genome size variability is known for a species (e.g. due to aneuploidy or B chromosomes). Further, we checked the plant material for possible determination errors and redetermined the specimen, if necessary. After this procedure, final descriptive statistics was calculated. These statistics are presented in Supplementary Table S1.

Ploidy assignment

For each species, we extracted the available chromosome counts from the literature and online databases. We preferably used the Austrian chromosome number checklist (Dobeš & Vitek 2000) and online databases and floras of the surrounding countries: Italy, i.e. Bedini & Peruzzi (2021+), available at https://bot.biologia.unipi.it/chrobase/index.php; Germany: Paule et al. (2017), available at https://chromosomes.senckenberg.de/item/1; Switzerland: InfoFlora (2025), available at https://www.infoflora.ch; Czechia: Flora of the Czech Republic, mainly based on Měsíček & Jarolímová (1992); Slovakia: Marhold et al. (2007), available at https://www.chromosomes.sav.sk. We also consulted global chromosome databases such as IPCN (IPCN 2025) and CCDB (Rice et al. 2015), the Pladias database of the Czech flora (Chytrý et al. 2021; available at https://pladias.cz) and specialized literature on individual genera or species; all sources are indicated in Supplementary Table S1.

For each genus, the lowest available chromosome count was considered diploid and we derived the genus' base chromosome number from it. We preferred records from Europe in general; doubtful unique counts (i.e. those from older literature not documented by photographs or drawings of the chromosome preparations) were disregarded, while reliable extra-European counts were used for alternative ploidy assignment (see below). We assigned ploidy levels to individual species based on their published chromosome counts as well as the measured RGS values. In large and karyologically diverse genera such as *Androsace*, *Saxifraga* and *Viola*, we applied the same procedure to more homogeneous infra-generic units (subgenera, sections or subsections) instead of the entire genus. In species, for which several chromosome counts are reported in the literature, we prioritized data (chromosome counts and RGS) published for the Eastern Alps. In addition, we compared the measured RGS values with those of closely related species (same section, same genus) in our dataset and with other FCM studies (Šmarda et al. 2019, taxonomic studies available for some genera or species). If multiple cytotypes within a species were revealed in our sampling, we also compared their RGS. If the RGS ratio deviated from the assigned ploidy level by more than 10% and one of the cytotypes was not supported by published chromosome counts, we marked its ploidy as uncertain (with an asterisk in the column 'Ploidy' in Supplementary Table S1, in total 22 cytotypes of 17 species). Although we assigned such cytotypes the nearest euploid levels for simplicity, we are aware that they may also include aneuploids, individuals bearing B chromosomes or hybrids.

Because the link between a measured RGS and a chromosome count/ploidy level is indirect, all inferred ploidies should be understood as 'DNA ploidy' as defined by Suda et al. (2006). However, for simplicity we omit the prefix 'DNA' in the text.

In rare cases, our approach may lead to odd-ploidy levels (e.g. 10x scored as 5x). If no chromosome count was available for a species, we assigned it the same ploidy as the other species of the same genus (subgenus/section) with similar RGS. If the RGS did not match the expected ploidy, we assigned the ploidy based on available taxonomic and karyological literature; all such cases are commented on in Supplementary Table S1. In a few cases (63 species, 6%), where two different ploidy assignments (e.g. 2x and 4x versus 4x and 8x) are equally likely or when only the extra-European chromosome counts suggested a different base chromosome number, we used the lower ploidy assignments but marked the alternative higher assignments in Supplementary Table S1.

Our approach based on the lowest chromosome count within a genus or a large subgeneric unit yields a more conservative estimate of the frequency of polyploidy compared to the analyses by Rice et al. (2019), who used models of chromosome evolution within an entire genus and scored also diploidized species as polyploids. However, if a species is paleo-polyploid but is (partly) diploidized and thus behaves as a functional diploid (concerning chromosome inheritance, number of copies of most genes etc.), we preferred to score it as diploid, reserving the polyploid state for evolutionarily young taxa where multiple copies of all chromosomes are still present and likely to segregate.

Results and discussion

We analysed 1,137 species. Three species of *Poa* (*P. alpina*, *P. pratensis* s.l., *P. nemoralis*) were excluded from the dataset after the first collection season due to the impossibility to link the obtained continuous RGS variation with ploidy levels; apomixis and frequent aneuploidy are known in *Poa* (Åkerberg 1942, Müntzing 1966, Speckmann & Van Dijk 1972). Among the remaining 1,134 species, RGS and ploidy information was obtained for 1,103 species, i.e. 97% (Supplementary Table S1). This is a higher success rate than 87% reported by Suda & Trávníček (2006) based on an analysis of air-dried tissues of 60 species. The difference may be at least partly caused by testing various buffers and other adjustments of the methodology in our study (see below). Slightly different methods of drying may also contribute to the better performance of our flow cytometry analyses: Suda & Trávníček (2006) used air-drying at 40 °C, while we used silica gel at room temperature, which is expected to desiccate the tissues more quickly (Bainard et al. 2011).

The species that did not yield any scorable histograms included succulent plants of the family *Crassulaceae* (all species of the genera *Jovibarba*, *Rhodiola*, *Sedum*, *Sempervivum*), the achlorophyllous parasitic or mycoheterotrophic genera *Orobanche* and *Hypopitys* (syn. *Monotropa*), and a handful of others. We hypothesize that in succulent *Crassulaceae* desiccation was not fast enough to preserve the nuclei. Moreover, plants of this family are fairly recalcitrant even when analysed from fresh material (specific buffers and procedures must be used; Šmarda et al. 2019). The genera *Orobanche* and *Hypopitys* (here, all three analyses of *H. monotropa* were unsuccessful while one out of three analyses of *H. hypophegea* was successful) are somewhat similar to the *Crassulaceae* succulents in having fleshy tissues, in which desiccation might have failed. Among the other unsuccessful species, *Potentilla micrantha* and *Rubus idaeus* belong to the family *Rosaceae* that is known to contain staining inhibitors (Čertner et al. 2022), while we were not able to isolate enough nuclei from most of the *Helianthemum alpestre* samples due to the

extremely high amount of mucilage. We have no simple explanation why samples of *Pseudofumaria lutea* did not yield any results. The other species, for which the analyses failed (*Dianthus seguieri*, *Rumex nivalis*, *Saxifraga adscendens*, *Tozzia alpina*) were represented by a single sample each, and the failure might rather be a random event. A low success rate (50% or less) was observed in five additional species, of which only *Galium odoratum* (*Rubiaceae*) and *Pinguicula leptoceras* (*Lentibulariaceae*) were represented by multiple samples, while the other species were represented by two samples each (*Epilobium dodonaei*, *Saxifraga aspera*, *Xerolekia speciosissima*)

Almost half of the successfully analysed species (527 out of 1,103, i.e. 48%) were measured using the standard protocol with Otto buffers, without any specific modifications (such as adjusting buffer composition, incubation time or staining time). Additional 349 species (32%) were measured using a flow cytometer with autoloading station and with Otto buffers used for sample preparation; these samples would also most likely be successfully measured with the standard protocol. This shows that about 80% of the samples were successfully analysed using the standard methodology; this number roughly corresponds to the results of Suda & Trávníček (2006) who obtained acceptable histograms in 52 out of 60 (86.7%) species using air-dried samples and the same flow cytometry protocol. In 20% of species in our study, modifications of the protocol were needed to obtain scorable results or were advantageous due to improved quality of the analyses. In 7% of species, we adjusted the sample preparation procedure, especially incubation time in Otto I buffer before staining; both very short (< 3 min) and prolonged times (> 10 min) were tested. In the last 13% of species, buffer composition was adjusted (addition of HCl and/or PVP to the Otto I buffer; see Methods for details) or the alternative buffer (LB01) was used, sometimes combined with an adjusted incubation time. All modifications are detailed in Supplementary Table S1. The top five families that required modifications most often (compared to their frequency in the dataset) include *Poaceae*, *Rosaceae*, *Salicaceae*, *Boraginaceae* and *Onagraceae*.

The analysis quality was scored as high or medium in 1,033 species (93.7% of the successful species and 91.1% of all species). In the remaining 70 species, the quality was scored as low or low to medium. Among the species with mostly low quality histograms, there are members of several families listed by Čertner et al. (2022) as problematic for FCM analysis, such as *Boraginaceae* (*Myosotis*, *Pulmonaria*, *Symphytum*), *Rosaceae* (*Aruncus dioicus*, *Crataegus monogyna*, *Geum urbanum*, *Rubus saxatilis*, *Sanguisorba dodecandra*) and *Violaceae* (*Viola*). We observed low quality of histograms also in some *Apiaceae* (*Anthriscus nitidus*, *Chaerophyllum hirsutum*, *C. villarsii*, *Coristospermum seguieri*), *Lentibulariaceae* (*Pinguicula*), *Onagraceae* (*Circaea alpina*, *Epilobium*), *Santalaceae* (*Thesium*) and some other species from different families. The genus *Helianthemum* (*Cistaceae*) appeared extremely challenging due to the high content of mucilage that hampered isolation of a sufficient number of nuclei.

In total, we discovered 129 new cytotypes (with no corresponding chromosome counts in the literature) in 118 species. In 105 of these species cytotype variation has not been known before. 384 species (35%) included polyploid cytotypes (i.e. they were exclusively polyploid or mixed diploid-polyploid), while 719 (65%) were exclusively diploid. In addition, 45 species were classified as polyploid under the alternative ploidy assignment (see above), raising the estimated frequency of species including polyploids to 39%. However, the above numbers also include species in which a dominant cytotype

is diploid, while polyploids are present only as minority cytotypes. Excluding them, the numbers of species comprising polyploid cytotypes decrease to 323 (29%) and to 369 (33%) under the alternative ploidy assignment. These numbers are lower than other estimates. Rice et al. (2019) report 42.9% of polyploids in the ecoregion 'Alps conifer and mixed forests' (the spatially relatively limited alpine and subalpine zone being included in this ecoregion, with no separate estimate available), and 43.4% in lower elevations in central Europe ('Central European mixed forests'). The latter number is nearly identical to 42.7% found in a detailed study of the Czech flora targeting the same ecoregion (Šmarda et al. 2019). The lower estimated polyploid frequency in our sampling probably reflects mainly our more conservative strategy of ploidy assignment. To some degree it can also be caused by omission of apomictic complexes and ferns (that are often polyploid) and the exclusion of neophytes (although their number is rather limited in (semi)natural montane and subalpine vegetation, where we mostly worked).

In total, we recorded 176 (16%) ploidy-variable species. Among them, 151 (14%) comprised diploid and polyploid cytotypes, while 25 (2%) comprised only multiple polyploid cytotypes. However, based on our sampling, we consider 70 cytotypes to be recent neo-autopolyploids that occasionally form as single individuals in plant populations due to the rare involvement of viable unreduced gametes and are very unlikely to establish as a separate cytotype (e.g. one triploid found among 164 diploids of *Dryas octopetala*). Such individuals are sometimes reported in extensive flow cytometry screenings (e.g. Slovák et al. 2009, Dušková et al. 2010, Kolář et al. 2016). The number of ploidy-variable species is well-comparable to estimates based on global variation of plant ploidy in the CCDB database reporting that 16.2% of plant species harbour intraspecific variation in their ploidy levels (Rice et al. 2015). Given the high sampling effort for many species in our dataset (for 482 and 268 species, we sampled at least 20 and 50 accessions, respectively), the number of ploidy variable species in our dataset can still be considered relatively low. As for many species we sampled only a part of their ranges, some species are uniform in the study area and elevations covered by our dataset, but include other cytotypes elsewhere (e.g. *Arabidopsis arenosa*, *Astragalus australis*, *Potentilla caulescens*, *Teucrium montanum*, *Urtica dioica*; see Supplementary Table S1 for references). Finally, within five species (*Juncus jacquinii*, *Luzula spicata*, *Pseudorchis albida*, *Pulsatilla alpina*, *Thesium alpinum*), we discovered cytotypes that significantly differ in RGS (by 20–38%) but are probably of the same ploidy level as the difference is far less than full multiple. Based on the RGS and occurrence on multiple sites, these case probably do not represent karyological variation such as aneuploidy or presence of B-chromosomes. They are also most likely not hybrids, since they do not morphologically differ from the other cytotypes of the respective species nor there are other species on the sites with the RGS large enough to serve as the second parent of a hybrid.

The use of dried plant tissue may pose problems due to shifts in fluorescence intensity as compared to fresh material. While fluorescence intensity decrease of several percent is often reported after desiccation (Suda & Trávníček 2006, Čertner et al. 2022), some studies surprisingly observed mild fluorescence increase in some species using propidium iodide dye (e.g. Bainard et al. 2011). Suda & Trávníček (2006) also observed a negative effect of the age of the material, but concluded that the lifetime of dried material stored at room temperature is between 1 and 4 years. In some species even older material can be used: Šmarda & Stančík (2006) were able to analyse ~40% of four to five and a half years

old herbarium specimens of *Festuca*. We compared our data with the extensive dataset of Šmarda et al. (2019), who analysed fresh material and reported sample/standard ratios (i.e. RGS) for DAPI dye. After recalculating the RGS values to the same standard (see Supplementary Table S2), we identified 500 cytotypes that are most likely common to both studies (similar RGS and ploidy invariable-species or the same chromosome counts/ploidy levels reported from both study areas, the Eastern Alps and Czechia). There is no systematic shift in the RGS values between these two datasets. When considering values for fresh material as 100%, the difference between mean RGS values from dried material (our study) and fresh material (Šmarda et al. 2019) is within ± 10% for 488 cytotypes (97.6%), with the mean difference being close to zero, – 0.10%. Differences up to 10% were observed when different laboratories/instruments analysing identical material are compared (Doležel et al. 1998, Sliwinska et al. 2022). Since we analysed most of the samples within one year after the collection (exceptionally up to three years, in case samples needed to be reanalysed), we did not observe any pronounced effect of aging on the success rate or quality of the analyses. We conclude that for most of the studied species, the use of dried material is suitable for assigning the samples to different ploidy levels.

Our results demonstrate that flow cytometry can be effectively used for ploidy level screening from dried material across a broad diversity of plant species, often using a single universal protocol. This is relevant as ploidy level is increasingly recognized as an evolutionarily important trait, which is regularly scored in phylogenetic, evolutionary or ecological studies. By making a flora-wide database of RGS values, ploidy levels and methodological details available, we aim to advance the knowledge of the Eastern Alpine flora in particular and of the central European flora in general. Indeed, the advanced knowledge of ploidy and genome size variation across the Eastern Alpine flora has pinpointed several groups, where taxonomic changes appear necessary. For instance, the detection of a hitherto unknown diploid relative of widespread tetraploid *Homogyne alpina* as well as of a hexaploid Southern Alpine entity within the *Festuca halleri* complex likely require the descriptions of new species; according efforts are underway. Further, our RGS data are currently aiding an improved, integrative characterization of doubtful taxa based on intra-ploidy differences in RGS (*Pseudorchis albida* subsp. *tricuspis*) or ploidy level (*Cerastium carinthiacum*, *Lotus corniculatus*, *Molinia caerulea* agg., *Oreojuncus trifidus*, *Trisetum alpestre*). Further cases such as the obvious RGS variation in *Juncus jacquinii* and *Thesium alpinum*, are still awaiting scrutiny. Overall, we are confident that novel cytotypes of many species will be discovered in other geographic areas, and we would be delighted if the present dataset could serve the botanical community for comparison.

Supplementary data

Table S1. Relative genome sizes, flow cytometry methodology and ploidy assignment.
Table S2. Recalculation matrix between genome size standards (DAPI staining).
Table S3. Relative genome sizes (RGS) of *Orchidaceae* (DAPI staining).

Supplementary materials are available at https://www.preslia.cz

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References

Åkerberg E. (1942) Cytogenetic studies in *Poa pratensis* and its hybrid with *Poa alpina*. – Hereditas 28: 1–126.

Bainard J. D., Husband B. C., Baldwin S. J., Fazekas A. J., Gregory T. R., Newmaster S. G. & Kron P. (2011) The effects of rapid desiccation on estimates of plant genome size. – Chromosome Research 19: 825–842.

Bartolić P., Morgan E. J., Padilla-García N. & Kolář F. (2024) Ploidy as a leaky reproductive barrier: mechanisms, rates and evolutionary significance of interploidy gene flow. – Annals of Botany 134: 537–550.

Bartolucci F., Peruzzi L., Galasso G., Albano A., Alessandrini A., Ardenghi N. M. G., Astuti G., Bacchetta G., Ballelli S., Banfi E., Barberis G., Bernardo L., Bouvet D., Bovio M., Cecchi L., Di Pietro R., Domina G., Fascetti S., Fenu G., Festi F., Foggi B., Gallo L., Gottschlich G., Gubellini L., Iamonico D., Iberite M., Jiménez-Mejías P., Lattanzi E., Marchetti D., Martinetto E., Masin R. R., Medagli P., Passalacqua N. G., Peccenini S., Pennesi R., Pierini B., Poldini L., Prosser F., Raimondo F. M., Roma-Marzio F., Rosati L., Santangelo A., Scoppola A., Scortegagna S., Selvaggi A., Selvi F., Soldano A., Stinca A., Wagensommer R. P., Wilhalm T. & Conti F. (2018) An updated checklist of the vascular flora native to Italy. – Plant Biosystems 152: 179–303.

Bedini G. & Peruzzi L. (eds) (2021–) Chrobase.it – Chromosome numbers for the Italian flora v. 2.0. – URL: https://bot.biologia.unipi.it/chrobase.

Birchler J. A. & Yang H. (2022) The multiple fates of gene duplications: deletion, hypofunctionalization, subfunctionalization, neofunctionalization, dosage balance constraints, and neutral variation. – The Plant Cell 34: 2466–2474.

Celestini S., Lipánová V., Vlček J. & Kolář F. (2025) Whole-genome duplication reshapes adaptation: autotetraploid *Arabidopsis arenosa* leverages its high genetic variation to compensate for selection constraints. – Molecular Biology and Evolution 42: msaf298.

Čertner M., Lučanová M., Sliwinska E., Kolář F. & Loureiro J. (2022) Plant material selection, collection, preservation, and storage for nuclear DNA content estimation. – Cytometry Part A 101: 737–748.

Chytrý M., Danihelka J., Kaplan Z., Wild J., Holubová D., Novotný P., Řezníčková M., Rohn M., Dřevojan P., Grulich V., Klimešová J., Lepš J., Lososová Z., Pergl J., Sádlo J., Šmarda P., Štepánková P., Tichý L., Axmanová I., Bartušková A., Blažek P., Chrtek J. Jr, Fischer F. M., Guo W.-Y., Herben T., Janovský Z., Konečná M., Kühn I., Moravcová L., Petřík P., Pierce S., Prach K., Prokešová H., Štech M., Těšitel J., Těšitelová T., Večeřa M., Zelený D. & Pyšek P. (2021) Pladias database of the Czech flora and vegetation. – Preslia 93: 1–87.

Cires E., Cuesta C., Peredo E. L., Revilla M. Á. & Prieto J. A. F. (2009) Genome size variation and morphological differentiation within *Ranunculus parnassifolius* group (*Ranunculaceae*) from calcareous screes in the northwest of Spain. – Plant Systematics and Evolution 281: 193–208.

Comai L. (2005) The advantages and disadvantages of being polyploid. – Nature Reviews Genetics 6: 836–846.

Dobeš C. & Vitek E. (2000) Documented chromosome number checklist of Austrian vascular plants. – Naturhistorisches Museum, Wien.

Doležel J., Greilhuber J., Lucretti S., Meister A., Lysák M. A., Nardi L. & Obermayer R. (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. – Annals of Botany 82 (Suppl. A): 17–26.

Doležel J., Greilhuber J. & Suda J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. – Nature Protocols 2: 2233–2244.

Dušková E., Kolář F., Sklenář P., Rauchová J., Kubešová M., Fér T., Suda J. & Marhold K. (2010) Genome size correlates with growth form, habitat and phylogeny in the Andean genus *Lasiocephalus* (*Asteraceae*). – Preslia 82: 127–148.

Fischer M. A., Adler W. & Oswald K. (2008) Exkursionsflora für Österreich, Liechtenstein und Südtirol. Ed. 3. – Land Oberösterreich und Biologiezentrum der OÖ Landesmuseen, Linz.

Frajman B., Rešetnik I., Weiss-Schneeweiss H., Ehrendorfer F. & Schönswetter P. (2015) Cytotype diversity and genome size variation in *Knautia* (*Caprifoliaceae*, *Dipsacoideae*). – BMC Evolutionary Biology 15: 140.

Galbraith D., Loureiro J., Antoniadi I., Bainard J., Bureš P., Cápal P., Castro M., Castro S., Čertner M., Čertnerová D., Chumová Z., Doležel J., Giorgi D., Husband B. C., Kolář F., Kouteckỳ P., Kron P., Leitch I. J., Ljung K., Lopes S., Lučanová M., Lucretti S., Ma W., Melzer S., Molnár I., Novák O., Poulton N., Skalický V., Sliwinska E., Šmarda P., Smith T. W., Sun G., Talhinhas P., Tárnok A., Temsch E. M., Trávníček P. & Urfus T. (2021) Best practices in plant cytometry. – Cytometry Part A 99: 311–317.

Husband B. C. & Sabara H. A. (2004) Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (*Onagraceae*). – New Phytologist 161: 703–713.

InfoFlora (2025) InfoFlora. The national data and information center on the Swiss flora. – URL: https://www.infoflora.ch.

IPCN (2025) Index to plant chromosome numbers (IPCN). – URL: https://legacy.tropicos.org/Project/IPCN.

Jiao Y., Wickett N. J., Ayyampalayam S., Chanderbali A. S., Landherr L., Ralph P. E., Tomsho L. P., Hu Y., Liang H., Soltis P. S., Soltis D. E., Clifton S. W., Schlarbaum S. E., Schuster S. C., Ma H., Leebens-Mack J. & dePamphilis C. W. (2011) Ancestral polyploidy in seed plants and angiosperms. – Nature 473: 97–100.

Kirchheimer B., Schinkel C. C. F., Dellinger A. S., Klatt S., Moser D., Winkler M., Lenoir J., Caccianiga M., Guisan A., Nieto-Lugilde D., Svenning J.-C., Thuiller W., Vittoz P., Willner W., Zimmermann N. E., Hörandl E. & Dullinger S. (2016) A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. – Journal of Biogeography 43: 716–726.

Kobrlová L., Dančák M., Sukmaria Sukri R., Metali F. & Hroneš M. (2020) Application of glycerol-preserved nuclei protocol for genome size estimation in the field conditions of a tropical rainforest. – Plant Systematics and Evolution 306: 73.

Kolář F., Lučanová M., Těšitel J., Loureiro J. & Suda J. (2012) Glycerol-treated nuclear suspensions – an efficient preservation method for flow cytometric analysis of plant samples. – Chromosome Research 20: 303–315.

Kolář F., Lučanová M., Záveská E., Fuxová G., Mandáková T., Španiel S., Senko D., Svitok M., Kolník M., Gudžinskas Z. & Marhold K. (2016) Ecological segregation does not drive the intricate parapatric distribution of diploid and tetraploid cytotypes of the *Arabidopsis arenosa* group (*Brassicaceae*). – Biological Journal of the Linnean Society 119: 673–688.

Kouteckỳ P., Smith T., Loureiro J. & Kron P. (2023) Best practices for instrument settings and raw data analysis in plant flow cytometry. – Cytometry Part A 103: 953–966.

Kravanja M., Koce J. D. & Frajman B. (2025) Out of Liguria: how polyploidy affected diversification of the sweet spurge (*Euphorbia dulcis*, *Euphorbiaceae*), European widespread forest species. – Perspectives in Plant Ecology, Evolution and Systematics 67: 125861.

Kron P., Suda J. & Husband B. C. (2007) Applications of flow cytometry to evolutionary and population biology. – Annual Review of Ecology, Evolution and Systematics 38: 847–876.

Loureiro J., Kron P., Temsch E. M., Kouteckỳ P., Lopes S., Castro M. & Castro S. (2021) Isolation of plant nuclei for estimation of nuclear DNA content: overview and best practices. – Cytometry Part A 99: 318–327.

Loureiro J., Rodriguez E., Doležel J. & Santos C. (2006) Comparison of four nuclear isolation buffers for plant DNA flow cytometry. – Annals of Botany 98: 679–689.

Loureiro J., Rodriguez E., Doležel J. & Santos C. (2007) Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. – Annals of Botany 100: 875–888.

Loureiro J., Trávníček P., Rauchová J., Urfus T., Vít P., Štech M., Castro S. & Suda J. (2010) The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. – Preslia 82: 3–21.

Löve A. & Löve D. (1943) The significance of differences in the distribution of diploids and polyploids. – Hereditas 29: 145–163.

Marhold K., Mártonfi P., Mereďa P. & Mráz P. (eds) (2007) Chromosome number survey of the ferns and flowering plants of Slovakia. – Veda, Bratislava.

Meirmans P. G. & Kolář F. (2025) Whole-genome duplication leads to significant but inconsistent changes in climatic niche. – Proceedings of the National Academy of Sciences of the United States of America 122: e2424785122.

Měsíček J. & Jarolímová V. (1992) List of chromosome numbers of the Czech vascular plants. – Academia, Praha.

Müntzing A. (1966) Apomixis and sexuality in new material of *Poa alpina* from middle Sweden. – Hereditas 54: 314–337.

Otto S. P. (2007) The evolutionary consequences of polyploidy. – Cell 131: 452–462.

Paule J., Gregor T., Schmidt M., Gerstner E.-M., Dersch G., Dressler S., Wesche K. & Zizka G. (2017) Chromosome numbers of the flora of Germany: a new online database of georeferenced chromosome counts and flow cytometric ploidy estimates. – Plant Systematics and Evolution 303: 1123–1129.

R Core Team (2024) R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna. URL: https://www.R-project.org.

Regele D., Grünebach M., Erschbamer B. & Schönswetter P. (2017) Do ploidy level, morphology, habitat ecology and genetic relationships in Alpine *Vaccinium uliginosum* allow for discrimination of two entities? – Preslia 89: 291–308.

Rice A., Glick L., Abadi S., Einhorn M., Kopelman N. M., Salman-Minkov A., Mayzel J., Chay O. & Mayrose I. (2015) The Chromosome Counts Database (CCDB): a community resource of plant chromosome numbers. – New Phytologist 206: 19–26.

Rice A., Šmarda P., Novosolov M., Drori M., Glick L., Sabath N., Meiri S., Belmaker J. & Mayrose I. (2019) The global biogeography of polyploid plants. – Nature Ecology & Evolution 3: 265–273.

Sabara H. A., Kron P. & Husband B. C. (2013) Cytotype coexistence leads to triploid hybrid production in a diploid-tetraploid contact zone of *Chamerion angustifolium* (*Onagraceae*). – American Journal of Botany 100: 962–970.

Schratt-Ehrendorfer L., Niklfeld H., Schröck C. & Stöhr O. (2022) Rote Liste der Farn- und Blütenpflanzen Österreichs. Dritte, völlig neu bearbeitete Auflage. – Stapfia 114: 1–357.

Sliwinska E., Loureiro J., Leitch I. J., Šmarda P., Bainard J., Bureš P., Chumová Z., Horová L., Koutecký P., Lučanová M., Trávníček P. & Galbraith D. W. (2022) Application-based guidelines for best practices in plant flow cytometry. – Cytometry Part A 101: 749–781.

Slovák M., Vít P., Urfus T. & Suda J. (2009) Complex pattern of genome size variation in a polymorphic member of the *Asteraceae*. – Journal of Biogeography 36: 372–384.

Šmarda P., Knápek O., Březinová A., Horová L., Grulich V., Danihelka J., Veselý P., Šmerda J., Rotreklová O. & Bureš P. (2019) Genome sizes and genomic guanine+cytosine (GC) contents of the Czech vascular flora with new estimates for 1700 species. – Preslia 91: 117–142.

Šmarda P. & Stančík D. (2006) Ploidy level variability in South American fescues (*Festuca* L., *Poaceae*): use of flow cytometry in up to 5 1/2 -year-old caryopses and herbarium specimens. – Plant Biology 8: 73–80.

Smith T. W., Kron P. & Martin S. L. (2018) flowPloidy: an R package for genome size and ploidy assessment of flow cytometry data. – Applications in Plant Sciences 6: e1164.

Sonnleitner M., Flatscher R., García P. E., Rauchová J., Suda J., Schneeweiss G. M., Hülber K. & Schönswetter P. (2010) Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (*Asteraceae*) in the Eastern Alps. – Annals of Botany 106: 967–977.

Speckmann G. J. & Van Dijk G. E. (1972) Chromosome number and plant morphology in some ecotypes of *Poa pratensis* L. – Euphytica 21: 171–180.

Suda J., Krahulcová A., Trávníček P. & Krahulec F. (2006) Ploidy level versus DNA ploidy level: an appeal for consistent terminology. – Taxon 55: 447–450.

Suda J. & Trávníček P. (2006) Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry: new prospects for plant research. – Cytometry Part A 69: 273–280.

te Beest M., Le Roux J. J., Richardson D. M., Brysting A. K., Suda J., Kubešová M. & Pyšek P. (2012) The more the better? The role of polyploidy in facilitating plant invasions. – Annals of Botany 109: 19–45.

Temsch E. M., Koutecký P., Urfus T., Šmarda P. & Doležel J. (2022) Reference standards for flow cytometric estimation of absolute nuclear DNA content in plants. – Cytometry Part A 101: 710–724.

Trávníček P., Ponert J., Urfus T., Jersáková J., Vrána J., Hřibová E., Doležel J. & Suda J. (2015) Challenges of flow-cytometric estimation of nuclear genome size in orchids, a plant group with both whole-genome and progressively partial endoreplication. – Cytometry Part A 87: 958–966.

Van de Peer Y., Ashman T.-L., Soltis P. S. & Soltis D. E. (2021) Polyploidy: an evolutionary and ecological force in stressful times. – The Plant Cell 33: 11–26.

Van de Peer Y., Mizrachi E. & Marchal K. (2017) The evolutionary significance of polyploidy. – Nature Reviews Genetics 18: 411–424.

Vejvodová K., Krejčí J., Koutecký P., Lučanová M., Hornych O. & Ekrt L. (2024) High mountains of central Europe as a refuge of surprising cytotype diversity of *Huperzia selago* (*Lycopodiaceae*). – Alpine Botany 134: 87–100.

Vlček J., Hämälä T., Cobo C. V., Curran E., Šrámková G., Slotte T., Schmickl R., Yant L. & Kolář F. (2025) Whole-genome duplication increases genetic diversity and load in outcrossing *Arabidopsis arenosa*. – Proceedings of the National Academy of Sciences of the United States of America 122: e2501739122.

Wendel J. F. (2015) The wondrous cycles of polyploidy in plants. – American Journal of Botany 102: 1753–1756.

Wood T. E., Takebayashi N., Barker M. S., Mayrose I., Greenspoon P. B. & Rieseberg L. H. (2009) The frequency of polyploid speciation in vascular plants. – Proceedings of the National Academy of Sciences of the United States of America 106: 13875–13879.

Zozomová-Lihová J., Majerová H., Šlenker M., Kminiaková E., Šrámková G. & Marhold K. (2026) *Cardamine rivularis* Schur is not the same as *Cardamine rivularis* auct. non Schur and why does it matter in the genomic era: resolving the long-standing taxonomic confusion. – Perspectives in Plant Ecology, Evolution and Systematics 70: 125921.

Protokoly pro průtokovou cytometrii, relativní velikosti genomu a ploidní úrovně 1103 druhů neapomiktických krytosemenných rostlin z Východních Alp – databáze založená na analýze 45 000 vzorků

Průtoková cytometrie je v současnosti nejpoužívanější, rychlou a spolehlivou metodou pro stanovení velikosti genomu a ploidie rostlin. Většina dosavadních měření pochází ze vzorků živých tkání, což je standardní materiál, který obvykle poskytuje kvalitní výsledky. Použití živých tkání ale omezuje využití metody pro vzorky z odlehlých oblastí nebo pokud je potřeba analyzovat velké množství vzorků najednou, v krátkém čase. Přestože existují i práce, ve kterých byl pro stanovení ploidie vybraných taxonů využit fixovaný materiál vysušený v silikagelu, tato metodika dosud nebyla využita pro studie zahrnující mnoho druhů. V tomto článku popisujeme metodiku a výsledky dosud nejrozsáhlejší studie stanovení ploidie využívající vysušený materiál, která zahrnovala celkem 1134 druhů neapomiktických krytosemenných rostlin z Východních Alp. Pomocí průtokové cytometrie s využitím barviva DAPI jsme analyzovali přibližně 45 000 vzorků. Překvapivě se podařilo změřit ploidii u naprosté většiny analyzovaných druhů, konkrétně 1103 druhů (97 %). Měření nebylo úspěšné pouze u sukuletních rostlin z čeledi *Crassulaceae* (rody *Jovibarba*, *Rhodiola*, *Sedum*, *Sempervivum*), u nezelených parazitických nebo mykoheterotrofních rodů *Orobanche* a *Hypopitys* (*Monotropa*) a několika dalších druhů. Pro přibližně 80 % druhů bylo možné využít stejný „univerzální“ protokol a listovou tkáň, zatímco u zbývajících druhů bylo nutné použít jiné tkáně (např. řapíky nebo květy) nebo modifikovat protokol přípravy vzorků (úprava složení pufrů nebo použití zcela jiných pufrů, úprava doby inkubace nebo barvení vzorků). Celkem 384 druhů (35 %) zahrnovalo polyploidní cytotypy. Z nich 151 druhů (14 %) zahrnovalo jak diploidy, tak polyploidy. 25 druhů (2 %) bylo pouze polyploidních, přičemž zahrnovaly více cytotypů, zatímco 208 druhů (19%) bylo polyploidních s jediným cytotypem. Zbylých 719 druhů (65 %) bylo pouze diploidních. Jako zdroj dat pro botanickou komunitu uvádíme naměřené relativní velikosti genomu a odpovídající odhad ploidie pro celkem 1328 cytotypů v rámci studovaných 1103 druhů. Uvádíme také podrobnou metodiku měření (použité pufry, standardy, jaké orgány byly měřeny, kvalitu získaných histogramů a další metodické poznámky). Věříme, že tyto údaje usnadní budoucí výzkum studovaných druhů nebo podobně zaměřené studie flóry střední Evropy. Jsme si jisti, v jiných oblastech budou dosud neznámé cytotypy objeveny i pro dalšídruhy, přičemž srovnání s našimi daty může posloužit pro jejich identifikaci.

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