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RESEARCH

Genome sizes of grasses (Poaceae), chromosomal evolution, paleogenomics and the ancestral grass karyotype (AGK)

Natalia Tkach[1] · **Grit Winterfeld**[1] · **Martin Röser**[1]

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Abstract

Grasses are one of the largest angiosperm families, widespread and economically important. Variation in genome size has functional consequences and is an essential parameter for understanding evolutionary patterns. In this study, we report the nuclear genome sizes (2C values) of 32 species and subspecies from 27 genera of Poaceae, including most of its subfamilies, examined by flow cytometry. Obtained genome sizes were analyzed together with the chromosome numbers to give information on the size of monoploid chromosome sets with the chromosome base number x and the mean chromosome size and then supplemented with the previously published data to obtain a deeper insight into the genome size evolution in grasses. Monoploid genomes of < 0.6 pg/1Cx and chromosomes of < 0.1 pg are presumably characteristic of the subfamilies Arundinoideae, Chloridoideae, Micraioideae and the Oryzoideae. The larger 1Cx values (1.2–1.8 pg) of the evolutionarily 'early diverging' subfamilies Anomochlooideae and Pharoideae are discussed in context with the origin of grasses and the pan-grass whole-genome duplication. The data indicate that the ancestral grass had a monoploid genome of this size, which is less than half the size previously assumed. Genome size data and available chromosome numbers support the concept of the ancestral grass karyotype (AGK) with $x = 12$. The AGK seems to have been conserved in some grass subfamilies (Bambusoideae, Oryzoideae, Pharoideae, parts of the Pooideae), while the major genome rearrangements are lineage-specific and occurred after the separation of the BOP and the PACMAD clades, i.e. when the diversification of their subfamilies had begun.

Keywords Chromosome base number · C-value · Flow cytometry · Genome · ρ event · WGD

Introduction

Angiosperm genomes vary spectacularly in size, ranging in non-replicated gametophytic nuclei (1C) from 61 Mbp (≈ 0.0623 pg) in the dicot *Genlisea tuberosa* to 148,881 Mbp (≈ 152.23 pg) in the monocot *Paris japonica*, a ca. 2440-fold difference (Pellicer et al. 2010; Fleischmann et al. 2014). Main drivers shaping genome size variation within comparatively short evolutionary time spans are polyploidy (whole-genome duplication) and the increase or decrease of transposable element copies, which can lead to rapid, lineage-specific change of genome size as shown in significant cereal crops such as rice, wheat, maize, barley and sorghum (Slotkin et al. 2012). These changes in genome structure, content of coding and non-coding DNA and in genome evolution represent important mechanism of speciation in plants (Chen 2007; Kejnovsky et al. 2012; Slotkin et al. 2012; Leitch and Leitch 2013).

The phylogenetic aspects of genome size variation have been studied in several angiosperm families, including the grasses (Poaceae), where there was no clear overall trend in genome size evolution: the 2C values increased in some lineages while decreasing in others (Bennetzen and Kellogg 1997; Kellogg 1998; Kellogg and Bennetzen 2004; Caetano-Anollés 2005; Leitch et al. 2010), although in one of the grass subfamilies, the Pooideae, a steady increase in genome size was observed, leading to the very large genomes of wheat and its relatives (Kellogg 1998). Recent advances in the phylogenetic analyses of this family, including phylogenomic data from the nuclear and plastid genome of the Poaceae (Blaner et al. 2014; Gallaher et al. 2019, 2022; Baker et al. 2022; Huang et al. 2022), make it worthwhile to

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✉ Martin Röser
martin.roeser@botanik.uni-halle.de

[1] Institute of Biology, Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

examine the genome sizes of representatives of the different groups in this family using the reliable method of flow cytometry (FCM) and to discuss them in the light of these new findings.

Poaceae, the study group, is one of the most successful evolutionary lineages of angiosperms. Grasses can be found on all continents and comprise approximately 11,800 species in 790 genera (Soreng et al. 2022). Leaving aside bamboo forests, which are formed by woody grasses, 30–40% of the Earth's land surface is covered by natural grasslands such as steppes, savannas, prairies, pampas and wetland (Gibson 2009; Linder et al. 2018; Griffith et al. 2020), which, due to their enormous productivity, also provide the food base for many herbivores. In Earth's history, grasses became ecologically dominant during the early to middle Miocene, when an extensive transition from forest to grassland took place. This transition occurred at different times on different continents. Grasslands spread across the globe from the mid-Miocene to the early Pliocene, and C_4 grasses played an important role in this expansion (Edwards et al. 2010; Strömberg 2011; Strömberg and Staver 2022; Wang and Lu 2022).

There is no doubt that grasses are the most important plant family for mankind. In terms of global crop production, cereals are the most important group of crops. In 2020, just four individual crops will account for half of the world's production of primary crops: sugarcane (20% of the total), maize (12%), wheat and rice (8% each), all grasses (FAO 2022). Corn, wheat and rice account for about 90% of cereal production, followed by barley, sorghum, oats, rye, and various millets (Statista 2023). This makes grasses a major focus for crop research, plant breeding, genetics, physiology and developmental biology (Kellogg 2015; McSteen and Kellogg 2022). DNA sequence and genomic analyses have contributed significantly to our understanding of the diversity, taxonomy, and phylogeny of grasses, their biogeographic patterns, and their evolutionary unfolding.

As documented by fossil remains, grasses originated between the Lower and early Upper Cretaceous (Gallaher et al. 2019, 2022; Schubert et al. 2019). Using molecular dating based on nuclear and plastid DNA sequence and genomic data, most studies converge in placing the origin of the family at about 125 Ma in the Lower Cretaceous. The beginning diversification of the family occurred at 98–105 My (Ma et al. 2021; Gallaher et al. 2022; Huang et al. 2022; Elliott et al. 2024). The first lineage to diverge phylogenetically was the small subfamily Anomochlooideae, the only grass subfamily lacking the typical spikelets, followed by the also small subfamilies Pharoideae and Puelioideae. A split about 16 Ma later, around the Cretaceous-Paleogene (K-Pg) boundary, led to the origin of the two largest clades of grasses called BOP and PACMAD clade, abbreviated after the initial letters of the subfamilies they contain (GPWG 2001; GPWG II 2012; Kellogg 2015; Soreng et al. 2022). The crown age of the BOP clade, comprising the subfamilies Bambusoideae, Oryzoideae and Pooideae with a total of 374 genera and 5941 species, was at about 75 Ma in the late Upper Cretaceous, that of the PACMAD clade, comprising the subfamilies Panicoideae, Aristidoideae, Arundinoideae, Micrairoideae, Danthonioideae and Chloridoideae with altogether 408 genera and 5815 species was younger and dates to about 65 Ma in the Lower Paleocene (Hodkinson 2018; Gallaher et al. 2019, 2022; Schubert et al. 2019; Ma et al. 2021; Orton et al. 2021, Huang et al. 2022; Elliott et al. 2024; see Soreng et al. 2022 for numbers of taxa).

The nuclear genome of grasses has long been known to show considerable variation in chromosome number (1) and DNA content (2):

(1) Somatic (sporophytic) chromosome numbers range from $2n=4$ in *Colpodium biebersteinianum* (syn. *Zingeria biebersteiniana*) and *C. versicolor* to $2n=$ca. 266 in *Poa litorosa* (Hair and Beuzenberg 1961; Hair 1968; Tzvelev and Zhukova 1974; Sokolovskaya and Probatova 1977). Polyploidy, i.e. the multiplication of chromosome sets by WGDs, is common in grasses and is virtually considered a hallmark of this family compared to other angiosperm lineages (Stebbins 1956, 1985; Estep et al. 2014). The chromosome numbers of the aforementioned *Colpodium* and *Poa* species correspond to twofold (diploid) and 38-fold ploidy, since their chromosome base numbers are $x=2$ and $x=7$, respectively. This highlights another long known variable feature of grass genome organization, namely the number of chromosomes in the monoploid chromosome sets, which ranges in the family almost continuously from $x=2$ to $x=18$. It may be quite stable in some subfamilies and tribes, such as Chloridoideae and Panicoideae with mostly $x=9$ or 10, assuming that the rarely found number $x=5$ in some Panicoideae rests on reductional dysploidy (Avdulov 1931; Stebbins 1956, 1985; de Wet 1987; Hunziker and Stebbins 1987; Hilu 2004; Kellogg 2015). The diversity of chromosome base numbers in the Poaceae has led to different hypotheses about their evolutionary pathways. According to the 'reduction hypothesis', comparatively high chromosome base numbers such as $x=12$ were ancestral and lower numbers were derived from them by descending dysploidy. This hypothesis was first proposed by Avdulov (1931), later adopted by Raven (1975) and GPWG (2001), and in principle also by Hilu (2004), who however proposed $x=11$ as the ancestral number in the Poaceae. The base number $x=12$ was therefore considered to be derived from $x=11$ by ascending dysploidy, and the lower numbers $x=7$, 9, 10, etc., occurring within the subfamilies of the BOP and PACMAD clades, by descending dysploidy. Conversely, the 'secondary polyploidy

hypothesis' (Stebbins 1982, 1985) proposed $x = 12$ and 11 as secondary chromosome base numbers derived from $x = 5$ or 6 by polyploidy and thus the lower numbers as original in grasses (Sharma 1979; de Wet 1987; Hunziker and Stebbins 1987).

(2) The nuclear genome size of grasses varies from 0.42 pg/2C in *Panicum gilvum* (chromosome number not known, probably $2n = 2x = 18$) (Chen et al. 2021) to 45.26 pg/2C in decaploid *Thinopyrum ponticum* ($2n = 70$) (Vogel et al. 1999). The 2C values, which refer to the DNA content of non-replicated diplophasic (sporophytic) nuclei, thus show a 90.5-fold variation. The highest 2C value of a diploid grass is 18.9 pg in *Secale montanum* ($2n = 14$) (Eilam et al. 2007), which already implies a 37.8-fold variation among diploids.

This astonishing genome size variability corresponds to the long-known cytogenetic observation that grass chromosome sizes can be extraordinarily different when analyzed microscopically, as extensively documented almost a century ago (Avdulov 1931). Therefore, the following questions and hypotheses are addressed in this study:

(a) Varying monoploid chromosome numbers (i.e., chromosome base number x) in a group of closely related taxa are usually not caused by aneuploid doubling or loss of single chromosomes. Rather, they are caused by chromosome rearrangements, such as (Robertsonian) chromosome fissions or chromosome fusions, which are usually caused by nested fusions or end-to-end telomeric fusions (Luo et al. 2009; Schubert and Lysak 2011; Salse 2016a, b; Lusinska et al. 2018, 2019; Winterfeld et al. 2018; Mayrose and Lysak 2021; Lysak 2022). Data on genome size and chromosome number of monoploid chromosome sets, i.e. the 1Cx value which denotes the DNA content of a non-replicated monoploid genome (chromosome set) with the chromosome base number x, and the base number x can be used to test which mechanisms underlie the strong variation in chromosome base number in grasses. To accomplish this, representative taxa from most of the major phylogenetic lineages of the grasses, including a total of 11 of their 12 subfamilies (see Soreng et al. 2022), were compared using a phylogenetic framework of their interrelationships (Saarela et al. 2018; Gallaher et al. 2019, 2022; Baker et al. 2022; Huang et al. 2022).

(b) The origin of grasses was preceded by a WGD called the ρ event (Paterson et al. 2004; Tang et al. 2010; Ming et al. 2015; McKain et al. 2016; Qiao et al. 2022), a hypothesis originally derived from phylogenetic gene pair analyses in representatives of the 'core grasses', i.e., members of the BOP and PACMAD clades mentioned above. This WGD has been confirmed for two of the three earliest diverging grass subfamilies, the Anomochlooideae, in which *Streptochaeta*, one of the two genera of this subfamily, and the Pharoideae, with only the single genus *Pharus*, have been studied (McKain et al. 2016; Ma et al. 2021; Seetharam et al. 2021). Since the Anomochlooideae are the phylogenetic sister of all other grasses, the ρ-WGD is therefore placed at the origin of the grasses. It occurred before the Anomochlooideae diverged from the lineage that gave rise to the rest of the grasses, i.e., the 'spikelet clade'. A comparison of the monoploid genome sizes (1Cx) of these 'early diverging' lineages with those of most grass subfamilies (BOP and PACMAD clade) and neighboring families of the Poaceae (Winterfeld et al. 2023) could therefore potentially provide information on the genome sizes to be assumed for the emergence of grasses after the ρ event.

(c) Genomic analyses of the arrangement of genes in the chromosomes of extant grasses revealed their remarkable collinearity despite structurally very different chromosome sets and partly distant relationship. This led to hypothesize a karyotype with five or seven protochromosomes before the ρ-WGD occurred. The ρ event resulted in duplicated 10 or 14 chromosomes from which the intermediate 'ancestral grass karyotype' (AGK) of 12 chromosomes was formed (Salse et al. 2008; Bolot et al. 2009; Murat et al. 2014, 2017; Wang et al. 2015; Salse 2016a, b; Pont et al. 2019; Bellec et al. 2023). Among the 'early diverging' grass lineages, the AGK does not appear to have changed very much, whereas it did later on a massive scale with the emergence of the 'core grass' subfamilies. These chromosomal rearrangements resulted in very different and partly lineage-specific karyotypes within the individual subfamilies of the BOP and PACMAD clades (The International Brachypodium Initiative 2010; Murat et al. 2017; Ling et al. 2018; Bellec et al. 2023). Therefore, we expect that genome size data will provide an exciting opportunity for comparison with the paleogenomic background and contribute to a deeper understanding of early Poaceae evolution. Furthermore, we aim to investigate whether changes in these genomic parameters are linked to the origin of the 'spikelet clade', specifically after the phylogenetic divergence of the subfamily Anomochlooideae, and to the divergence of the lineage leading to the 'core Poaceae', with its splitting into the BOP and PACMAD clades and their further diversification into nine subfamilies.

Material and methods

Plant material

Our sample consisted of 32 species and subspecies in 27 genera. One accession per species was examined. Fresh leaves for the genome size analyses were collected in the field, from living potted plants of our greenhouse-grown grass research collection or from the plant collections of the Botanical Garden of the University Halle-Wittenberg. Leaf samples were either processed immediately or stored in plastic bags with moist tissue in the refrigerator at 4°C for up to 5 days until processing. In other cases, silica gel-dried leaves, preferably stored at -20°C or -80°C, were successfully used. Voucher specimens of most accessions are deposited in the herbarium of the University Halle-Wittenberg (HAL). Details on the collections of the analyzed taxa can be found in the Online Resource 1.

Measurement of genome sizes

Relative genome sizes were estimated by FCM following the protocol of Doležel et al. (2007) with minor modifications (Winterfeld et al. 2023). In brief, fresh or silica gel-dried leaf tissue of the sample of interest and of an internal standard species were chopped together with a razor blade in a plastic Petri dish. Nuclei were extracted in 2 mL staining buffer. 10 µL propidium iodide (PI) stock solution (10 mg × mL^{-1}) and 5 µL RNase A (5 mg × 1.5 mL^{-1}) were added using the ready-to-use CyStain PI OxProtect reagent kit (Sysmex Partec) according to the manufacturer's protocol. FCM analyses were performed using a CyFlow Ploidy Analyser (Sysmex Partec GmbH, Görlitz, Germany) equipped with a green laser of 532 nm as excitation light for the DNA-intercalating fluorochrome PI was used.

Fluorescence intensity was measured for 5,000 particles (nuclei). Three replicates were performed for each sample. Only histograms with a coefficient of variation (CV) < 4% for the G0/G1 peak of the sample were considered. For CVs exceeding this threshold, the measurement was discarded and the sample was reanalyzed. The silica gel-dried samples yielded high-quality histograms comparable to those of fresh tissue, as also found in some previous studies (Šmarda and Stančík 2006; Wang and Yang 2016; Čertner et al. 2022; Loureiro et al. 2023). The following internal standards, obtained as seed from the Institute of Experimental Botany, Academy of Sciences of the Czech Republic, and grown in our greenhouses, were used for the genome size estimates (Doležel et al. 2007, 2018; Temsch et al. 2022): *Glycine max* Merr. 'Polanka' (2.50 pg/2C), *Pisum sativum* L. 'Ctirad' (9.09 pg/2C), *Raphanus sativus* L. 'Saxa' (1.11 pg/2C), *Secale cereale* L. 'Daňkovské' (16.19 pg/2C), *Solanum lycopersicum* L. 'Stupické polní rané' (1.96 pg/2C), *Vicia faba* L. 'Inovec' (26.90 pg/2C), *Zea mays* L. 'CE-777' (5.43 pg/2C). The standard used for each measurement is listed in Online Resource 1 to allow for future recalculations and corrections if a more accurate genome size estimate becomes possible due to values that need to be corrected for an internal standard. For some of the standards used here, which were calibrated against human male leucocytes, other values have already been proposed based on a calibration against the sequenced genome size of rice (*Oryza sativa*), e.g. for *G. max* (2.077 pg/2C = 83%), *P. sativum* (8.018 pg/2C = 88%), *S. lycopersicum* (1.735 pg/2C = 88%) and *V. faba* (23.796 pg/2C = 88%) (Šmarda et al. 2019). Calibration against *Agave americana* 'Aureomarginata', another long-used standard, which itself had been calibrated against human male leucocytes, resulted in values for *P. sativum* cv's (8.61 pg/2C = 95%), *R. sativus* (1.15 pg/2C = 104%), *Secale cereale* 'Petkus Spring' (15.5 pg/2C = 96%), *Solanum lycopersicum* (2.03 pg/2C = 104%), *V. faba* (26.4 pg/2C = 98%) and *Z. mays* (5.61 pg/2C = 103%) (Zonneveld 2021).

The 2C values of the samples, i.e. the amount of DNA in a somatic cell with non-replicated chromosomes, were calculated by multiplying the sample/standard ratios of the 2C peaks in the fluorescence histograms with the known genome size of each standard species used. Mean 2C values and standard deviations for each sample were calculated using FCS Express version 5 software (De Novo Software, Pasadena, CA, U.S.A.).

The genome size data obtained in this study and the standard species used for each measurement are listed in Online Resource 1. They are expressed as physical mass in picograms (pg), which can be converted to DNA content in base pairs (bp) by multiplication with the conversion factor 0.978×10^9 (Doležel et al. 2003). Previously published DNA C-values were retrieved from the 'Plant DNA C-values Database' (Leitch et al. 2019; https://cvalues.science.kew.org/; release 7.1, April 2019) or were cited from the original publications if these have been published after 2019 (see Results and discussion, References). For comparison, genome sizes estimated by FCM using an internal standard as reference genome together with the sample and PI as fluorescent dye (Doležel and Bartoš 2005), hereafter abbreviated as FCM + PI, are most often considered because they are usually reliable. Data from Feulgen microdensitometry, the most commonly used method in the past, often proved to be too unreliable for several reasons (see Greilhuber 2005; Greilhuber et al. 2007). In Figs. 1 and 2, our data were complemented by the genome size measurement for *Streptochaeta angustifolia* (Anomochlooideae) (Seetharam et al. 2021) as listed in Online Resource 2.

Chromosome numbers and monoploid genome sizes (1Cx values)

Chromosome numbers were compiled from the 'Chromosome Counts Database' (CCDB 2023: see Rice et al. 2015; Rice and Mayrose 2023), the 'Index to plant chromosome numbers' (IPCN 1979 onwards) or were cited from recent original publications (see References). For some accessions, chromosomes were counted in this study (Table 1; Online Resource 1). Young growing root tips were harvested from cultivated potted plants, immersed in distilled water, cold treated at 0 °C for 24 h to accumulate metaphases, fixed in freshly prepared 1:3 glacial acetic acid:absolute ethanol for at least 3 h and stored in absolute ethanol at − 20 °C until preparation. Before preparation, the root tips were softened in 1% cellulase (w/v) and 10% pectinase (v/v) in citric acid-sodium citrate buffer pH 4.8 at 37 °C (Schwarzacher et al. 1980). Enzyme-macerated root tips were squashed and stained on slides in a drop of 45% propionic acid with 2% carmine and covered with a coverslip. Photographs of metaphase chromosomes were taken on a Zeiss Axiophot microscope using a computer-assisted cooled CCD camera (Zeiss Axiocam HRC) using Axiovision software (Winterfeld et al. 2018). They were squashed in a drop of 45% propionic acid with 2% carmine (Winterfeld et al. 2020).

Monoploid genome sizes (1Cx values) were calculated for species with known chromosome number or ploidy by dividing the 2C values by the respective ploidy level (Greilhuber et al. 2005). The mean DNA content per chromosome (MC), expressed as the average physical mass of the chromosomes in the complement of a given plant, was calculated by dividing the 2C values by the diplophasic (sporophytic) chromosome number ($2n$) or by dividing the 1C values by the haplophasic (gametophytic) chromosome number (n), i.e. 2C/$2n$ or 1C/n, respectively.

Results and discussion

Genome and chromosome sizes of the Poaceae

2C values

The compared species from 11 of the 12 subfamilies of the grasses had 2C values (holoploid diplophasic, i.e., sporophytic genome sizes of the non-replicated nuclear DNA) between 0.67 pg and 45.26 pg (Tables 1, 2; Figs. 1a, 2; Online Resource 1; Vogel et al. 1999), thus spanning the size range from "very small" to "large" (Leitch et al. 1998). No data were available for the subfamily Puelioideae. The 2C values of most subfamilies ranged from about 2.5 pg to 8.0 pg, thus falling predominantly into the "small" category defined by ≥ 2.8 pg/2C and ≤ 7.0 pg (Leitch et al. 1998). This was true for the subfamilies Anomochlooideae, Aristidoideae, Arundinoideae, Bambusoideae, Danthonioideae, Micraioideae, Oryzoideae and Pharoideae. The only estimate for the Chloridoideae was 1.55 pg/2C, placing it in the "small" category, as well as the Oryzoideae, which had 0.84–1.84 pg/2C. The greatest variation was found in the Pooideae, which alone accounted for the aforementioned range of variation in the entire Poaceae family, followed by the Panicoideae, where 0.9–8.12 pg/2C were found, all in all comparable to the previous review of monocot genome sizes (Leitch et al. 2010).

1Cx values

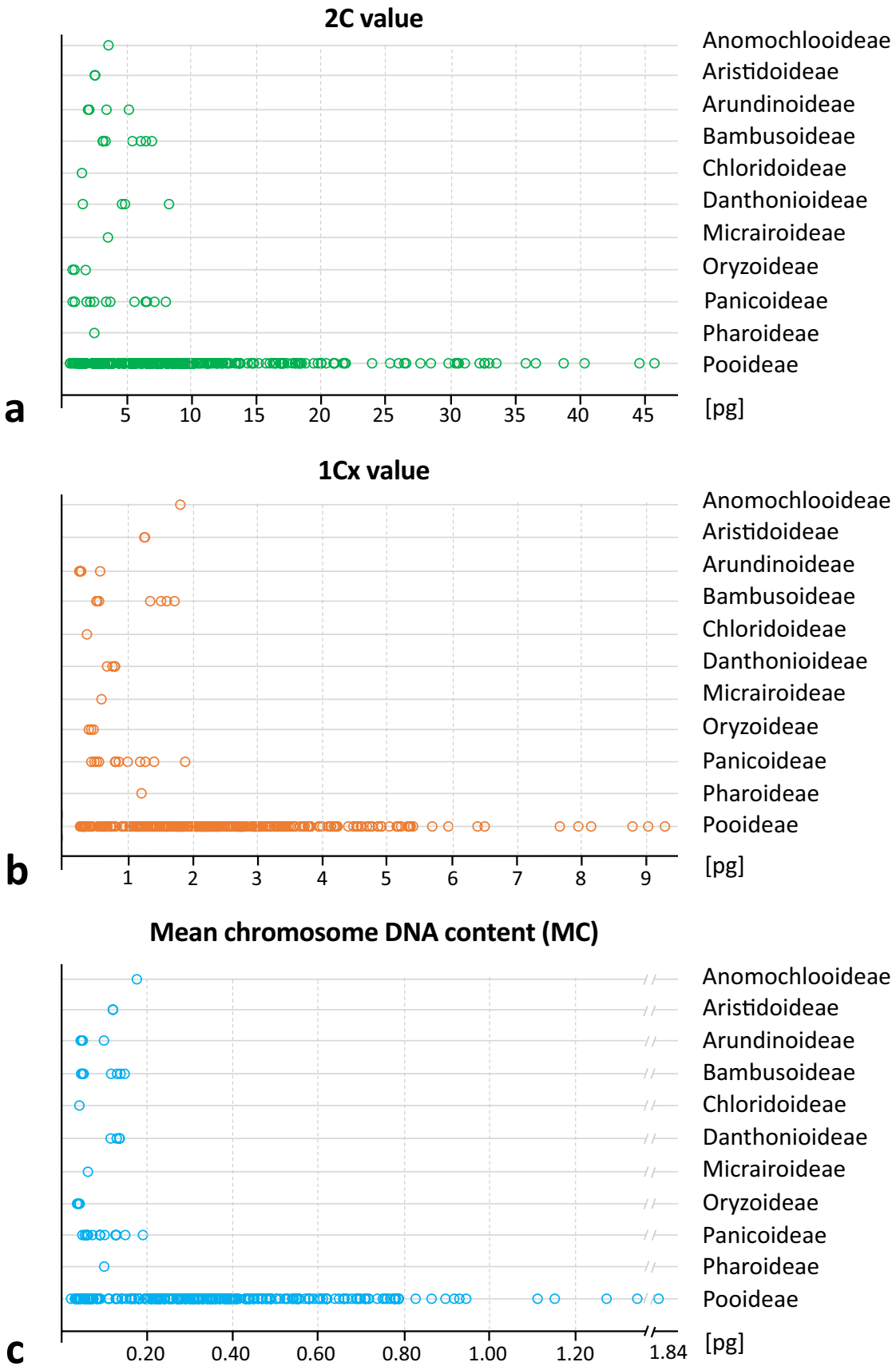
The genome size of the monoploid non-replicated chromosome sets ranged from 0.26 pg to 9.45 in the grasses, but here, too, this was mainly due to the subfamily Pooideae (Tables 1, 2; Figs. 1b, 2; Online Resource 1). This was followed by variation in the Panicoideae (0.49–1.91 pg/1Cx) and Bambusoideae (0.53–1.71 pg/1Cx). Medium-sized monoploid genomes of about 1.2–1.8 pg/1Cx occurred in the Anomochlooideae, Aristidoideae and Pharoideae, while the smallest of about 0.3–0.8 pg/1Cx were found in Arundinoideae, Chloridoideae, Danthonioideae, Micraioideae and Oryzoideae. The Oryzoideae, the subfamily of rice, thus belongs to the grasses with a small genome size, as has long been known. However, small monoploid grass genomes of < 0.4 pg/1Cx occurred also in some taxa of the Arundinoideae, Chloridoideae and Pooideae.

Mean chromosome DNA content (MC)

The chromosomes sizes varied altogether between 0.02 pg and 1.84 pg, implying a 92-fold variation, which is due to the MCs of only a single subfamily, the Pooideae. Most subfamilies had MCs of 0.04–0.19 pg (Arundinoideae, Bambusoideae, Danthonioideae, Panicoideae). The examined representatives of the Chloridoideae, Micraioideae and Oryzoideae were at the lower limit of MCs with 0.04–0.05 pg. Anomochlooideae, Pharoideae and Aristidoideae were medium-sized with MCs of 0.10–0.17 pg (Tables 1, 2; Figs. 1c, 2; Online Resource 1).

Characteristics of the subfamilies

Data on genome size (2C and 1Cx values) and chromosomal DNA content (MC) for the Poaceae subfamilies are presented in Tables 1 and 2, Online Resource 1, and illustrated in Figs. 1, 2.


2C value
Anomochlooideae
Aristidoideae
Arundinoideae
Bambusoideae
Chloridoideae
Danthonioideae
Micrairoideae
Oryzoideae
Panicoideae
Pharoideae
Pooideae
5
10
15
20
25
30
35
40
45
[pg]
a
1Cx value
Anomochlooideae
Aristidoideae
Arundinoideae
Bambusoideae
Chloridoideae
Danthonioideae
Micrairoideae
Oryzoideae
Panicoideae
Pharoideae
Pooideae
1
2
3
4
5
6
7
8
9
[pg]
b
Mean chromosome DNA content (MC)
Anomochlooideae
Aristidoideae
Arundinoideae
Bambusoideae
Chloridoideae
Danthonioideae
Micrairoideae
Oryzoideae
Panicoideae
Pharoideae
Pooideae
0.20
0.40
0.60
0.80
1.00
1.20
1.84
[pg]
c

◂**Fig. 1** Variation of genome sizes and chromosome DNA content in the subfamilies of Poaceae examined in this study. **a** Holoploid 2C genome sizes. **b** Monoploid 1Cx genome sizes. **c** Mean chromosome DNA contents (MC). For our data see Table 2 and Online Resource 1, for further data as specified in Material and methods see the individual subfamilies in Results and discussion. Data for the Anomochlooideae and the Micraroideae are based on genome size estimates of Seetharam et al. (2021) and Murray et al. (2005), respectively. Data for the Pooideae are from Tkach et al. (2024) and Winterfeld et al. (2024)

'Early diverging' grass lineages

Anomochlooideae

Genome size data for this small neotropical subfamily of 4 species in 2 genera, which were not sampled in this study, are only available for *Streptochaeta angustifolia*. Its 2C value was estimated to be 3.60–3.66 pg, also using FCM (Seetharam et al. 2021). The chromosome number is not known, but could be $2n = 22$, as has been repeatedly found in two other *Streptochaeta* species (CCDB 2023). The 1Cx value of *S. angustifolia* would be then ca. 1.82 pg and the MC ca. 0.17 pg.

The genome size of monospecific *Anomochloa*, the second genus of this subfamily, is yet unknown. Its chromosome number is $n = 18$, well documented by a microphotograph showing 18 bivalents at diakinesis (Hunziker et al. 1989; Judziewicz and Soderstrom 1989).

Pharoideae

This tropical African subfamily with 3 genera and 12 species was represented in this study only by *Pharus latifolius* with a 2C value of 2.48 pg, which is in good agreement with previous studies using FCM + PI as fluorescent dye that found 2,467 Mbp (≈ 2.52 pg/2C) and 2,270 Mbp (≈ 2.32 pg/2C), respectively (Šmarda et al. 2014; Ma et al. 2021). The *P. latifolius* genome assembled from sequencing data had a length of 1,002.88 Mbp/1C (≈ 2.05 pg/2C), with an estimated genome completeness of approximately 92.6% (Ma et al. 2021). Therefore, the interpolated 2C value would be about 2.21 pg/2C. *Pharus latifolius* has $2n = 24$ (CCDB 2023) probably based on $x = 12$, resulting in a 1Cx value of 1.24 pg and an MC of 0.10 pg.

Puelioideae

For this tropical African subfamily of 11 species in 2 genera, $2n = 24$ has been found repeatedly in two *Puelia* species (CCDB 2023), but their genome size is apparently still unknown.

BOP clade

The BOP clade comprises three subfamilies with a total of more than 5,900 species in 374 genera (Soreng et al. 2022), all of which are characterized by C_3 photosynthetic pathway. The clade is distributed worldwide. In this study, the clade was represented by ten example taxa in nine genera.

Bambusoideae

The mainly tropical to subtropical, partly warm temperate distributed subfamily Bambusoideae (bamboos) are the third largest subfamily of grasses and comprise about 1700 species in 120–140 genera (Clark et al. 2015; Soreng et al. 2022; Clark 2023). The studied members of this subfamily had 2C values between 3.19 pg and 7.01 pg.

The three studied species of the genera *Bambusa* and *Gigantochloa*, which belong to the paleotropical members of the consistently woody and polyploid bamboos of the tribe Bambuseae, had rather uniform 2C genome sizes of 3.19–3.42 pg. The values for *B. multiplex*, *B. vulgaris* and *G. verticillata* were in the same order of magnitude as those previously found for the same or other species of both genera (Zhou et al. 2017 and Chalopin et al. 2021, both using FCM + PI). The chromosome numbers of the species we studied are $2n = 68–72$ (CCDB 2023), suggesting a sixfold ploidy probably based on $x = 12$. The resulting 1Cx values were 0.53–0.57 pg and the MCs 0.04–0.05 pg. For neotropical Bambuseae species of the genera *Guadua* and *Chusquea,* genome sizes of 3.63 pg/2C and 3.99 pg/2C (both *G. angustifolia*), 3.98 pg/2C (*G. chacoensis*) and 4.77 pg/2C (*C. tenella*), all tetraploid, were found in estimates with FCM + PI (Guo et al. 2019; Zappellini et al. 2020). Their 1Cx values would be 0.91–1.19 pg and MCs 0.08– 0.11 pg. Genome sequencing of *G. angustifolia* found a genome size of 1,580 Mbp (≈ 3.23 pg/2C) (Guo et al. 2019).

The sampled species of *Arundinaria*, *Fargesia* and *Pseudodasa*, which belong to the tribe Arundinarieae, the temperate woody and also consistently polyploid bamboos, had 5.49–7.01 pg/2C. The genome sizes of the Arundinarieae are thus significantly larger than those of the paleotropical Bambuseae, as previously noted (Zhou et al. 2017; Chalopin et al. 2021). Chromosome numbers are not available for our sampled species, but for many congeners, all of which consistently had $2n = 48$ (CCDB 2023), presumably also based on $x = 12$. Thus, we can assume that our Arundinarieae taxa have 1Cx genome sizes of 1.37–1.75 pg, about three times than those of the paleotropical woody Bambuseae studied. The MCs were 0.11–0.15 pg, which is also considerably larger (about 2–3 times). A difference to the neotropical woody bamboo species is less pronounced, but also recognizable.

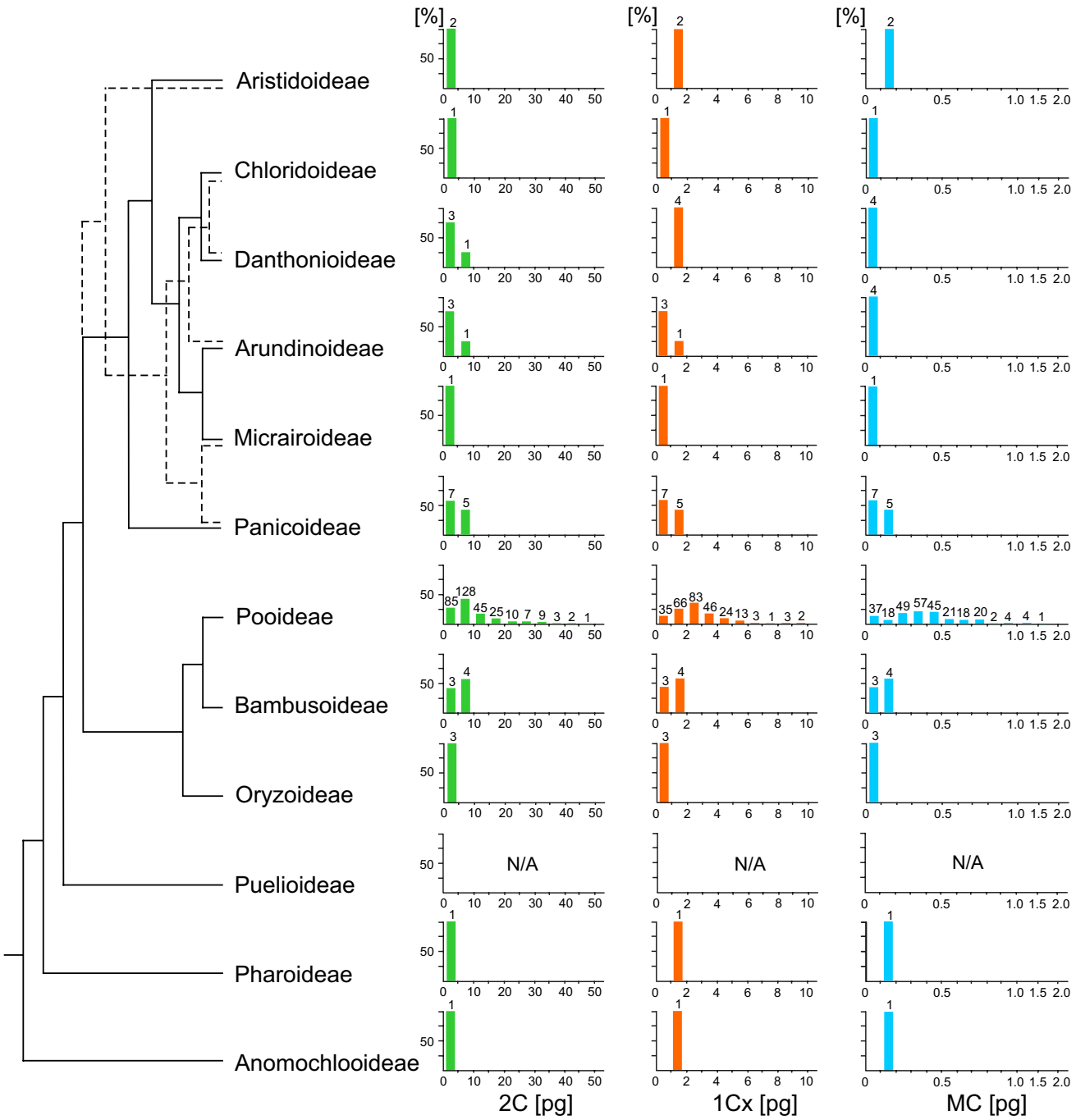


Fig. 2 Holoploid (2C) and monoploid (1Cx) genome sizes and mean chromosome DNA content (MC) arranged according to a Poaceae phylogenetic tree. DNA content intervals are shown on the x-axis of the bar graphs, while the y-axis represents the corresponding percentage estimates, which sum to 100% for each subfamily. The number of estimates falling within each interval is displayed above the corresponding bar. The simplified phylogenetic tree is adapted from plastome-based phylogenetic analyses (Gallaher et al. 2019, 2022). Dashed lines indicate alternative sister relationships within the PACMAD clade based on nuclear phylogenomic analyses (Baker et al. 2022; Huang et al. 2022). For our data see Table 2 and Online Resource 1, for further data as specified in "Material and methods" section see the individual subfamilies in Results and discussion. Data for the Anomochlooideae and the Micrairoideae are based on genome size estimates of Seetharam et al. (2021) and Murray et al. (2005), respectively. Data for the Pooideae are from Tkach et al. (2024) and Winterfeld et al. (2024)

Table 1 Summary of the Poaceae taxa studied, providing their 2C values, chromosome numbers, ploidy levels, 1Cx values and mean chromosome DNA content (MC)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Arundinoideae					
Arundo donax L.	5.25	108	18*x*	0.29	0.05
Molinia caerulea (L.) Moench	3.52	36	6*x*	0.59	0.10
Phragmites australis (Cav.) Trin. ex Steud. subsp. *australis*	2.14	48	8*x*	0.27	0.04
Phragmites australis subsp. *humilis* (de Not) Kerguélen	2.09	48	8*x*	0.26	0.04
Bambusoideae					
Arundinaria pygmaea (Miq.) Makino	6.14	[48]	[4*x*]	1.54	0.13
Bambusa multiplex (Lour.) Raeusch. ex Schult. & Schult.f.	3.19	72	[6*x*]	0.53	0.04
Bambusa vulgaris Schrad. ex J.C.Wendl.	3.42	68	[6*x*]	0.57	0.05
Fargesia nitida (Mitford ex Bean) Keng f. ex T.P.Yi	5.49	[48]	[4*x*]	1.37	0.11
Gigantochloa verticillata (Willd.) Munro	3.23	68	[6*x*]	0.54	0.05
Pleioblastus amarus (Keng) Keng f.	7.01	[48]	[4*x*]	1.75	0.15
Pseudosasa japonica (Siebold & Zucc. ex Steud.) Makino ex Nakai	6.53	[48]	[4*x*]	1.63	0.14
Chloridoideae					
Cleistogenes mucronata Keng f.	1.55	[40]	[4*x*]	0.39	0.04
Danthonioideae					
Cortaderia selloana (Schult. &Schult.f.) Asch. & Graebn.	8.30	72	12*x*	0.69	0.12
Danthonia alpina Vest	4.89	36* **	6*x*	0.82	0.14
Danthonia decumbens DC.	4.66	36**	6*x*	0.78	0.13
Schismus arabicus Nees	1.63	12*	2*x*	0.82	0.14
Oryzoideae					
Hygroryza aristata (Retz.) Nees ex Wight & Arn.	0.84	24	2*x*	0.42	0.04
Leersia oryzoides (L.) Sw.	1.84	48	4*x*	0.46	0.04
Oryza sativa L.	0.99	24	2*x*	0.50	0.04
Panicoideae					
Cenchrus flaccidus (Griseb.) Morrone	1.99	36	4*x*	0.50	0.06
Chasmanthium latifolium (Michx.) H.O.Yates	3.50	48	4*x*	0.88	0.07
Coix lacryma-jobi L.	3.82	20	2*x*	1.91	0.19
Digitaria sanguinalis (L.) Scop.	2.27	36	4*x*	0.57	0.06
Miscanthus sinensis Andersson cv. *giganteus*	7.24	57	6*x*	1.21	0.13
Miscanthus sinensis Andersson cv. *gracillimus*	5.70	38	4*x*	1.43	0.15
Oplismenus hirtellus (L.) P.Beauv.	6.55	72	8*x*	0.82	0.09
Oplismenus undulatifolius (Ard.) P.Beauv.	6.65	[72]	[8*x*]	0.83	0.09
Panicum capillare L.	0.90	18	2*x*	0.45	0.05
Saccharum officinarum L.	8.12	80	8*x*	1.02	0.10
Setaria viridis (L.) P.Beauv.	1.07	18	2*x*	0.54	0.06
Tripidium ravennae (L.) H.Scholz	2.58	20	2*x*	1.29	0.13
Pharoideae					
Pharus latifolius L.	2.48	24	2*x*	1.24	0.10

Chromosome numbers were taken from the CCDB (2023) and original literature or were counted in our laboratory (asterisks). Square brackets indicate inferred chromosome numbers and ploidy levels based on 2C values and available congeneric species data. Online Resource 1 provides complete details of the analyzed samples and measurements

*This study

**Winterfeld (2006)

Table 2 Genome sizes (holoploid 2C and monoploid 1Cx values) and mean chromosome DNA content (MC) of the examined representatives of the grass subfamilies

Subfamilies and chromosome base numbers	2C value [pg]	1Cx value [pg]	MC [pg]
Anomochlooideae ($x=9?, 11$)	3.63	1.82	0.17
Pharoideae ($x=12$)	2.48	1.24	0.10
Puelioideae ($x=12$)	N/A	N/A	N/A
BOP clade			
Bambusoideae ($x=7, 9, 10, 11, \mathbf{12}$)	3.19–7.01	0.53–1.75	0.04–0.15
Oryzoideae ($x=\mathbf{12}, 15, 17$)	0.84–1.84	0.42–0.50	0.04
Pooideae ($x=2, 4, 5, 6, \mathbf{7}, 8, 9, 10, 11, \mathbf{12}, 14$)	0.67–45.26	0.33–9.45	0.02–1.84
PACMAD clade			
Aristidoideae ($x=11, 12$)	2.6–2.62	1.30–1.31	0.12
Arundinoideae ($x=6, 9$)	2.09–5.25	0.26–0.59	0.04–0.10
Chloridoideae ($x=6?, 7, 8, \mathbf{9}, \mathbf{10}, 12$)	1.55	0.39	0.04
Danthonioideae ($x=\mathbf{6}, 7, 9$)	1.63–8.30	0.69–0.82	0.12–0.14
Micraioideae ($x=10$)	3.64	0.61	0.06
Panicoideae ($x=3, 4, 5, 7, 8, \mathbf{9}, \mathbf{10}, 11, 12$)	0.9–8.12	0.45–1.91	0.05–0.19

The most frequent chromosome base numbers in a subfamily are in bold. For details on our data see Table 1 and Online Resource 1. For further data as specified in "Material and methods" section see the individual subfamilies in Results and discussion. Data for the Pooideae are from Tkach et al. (2024) and Winterfeld et al. (2024). *N/A* not available

The predominantly tropical New World herbaceous bamboos of the tribe Olyreae, which were not sampled in this study, are mostly diploid, typically with $2n=20$ or 22, although lower numbers such as $2n=14$ or 18 have rarely been found (Kellogg 2015; CCDB 2023). Genome sizes of 1,265 Mbp/2C (≈ 1.29 pg/2C) and 1,384 Mbp/2C (≈ 1.42 pg/2C) for *Olyra latifolia* and 1,370 Mbp/2C (≈1.40 pg/2C) for *Raddia guianensis* have been reported (Šmarda et al. 2014; Guo et al. 2019). Their 1Cx values would be 0.65–0.70 pg and MCs uniformly 0.06 pg, so Olyreae seem to have the smallest monoploid genomes and the smallest chromosomes of the whole Bambusoideae. Genome sizes of 681 Mbp (≈ 1.39 pg/2C) and 629 Mbp (≈ 1.28 pg/2C) were estimated for *O. latifolia* and *R. guianensis*, respectively, by genome sequencing (also Guo et al. 2019).

Oryzoideae

The worldwide distributed rice subfamily of 117 species in 19 genera (Soreng et al. 2022) was sampled using three taxa.

The diploid *Hygroryza aristata* ($2n=24$) had a 2C DNA value of 0.84 pg, which is comparable to an unpublished previous estimate of 1.00 pg/2C for this species using Feulgen microdensitometry (Leitch et al. 2019).

A studied accession (subspecies and cultivar not known) of rice, *Oryza sativa* ($2n=24$), had 0.99 pg/2C. For *O. sativa*, 0.87–1.20 pg/2C have been estimated in previous studies using FCM or Feulgen microdensitometry (e.g., Martinez et al. 1993; Kurata and Fukui 2003; Loureiro et al. 2007; Yamamoto et al. 2018; Panibe et al. 2021; Dai et al. 2022). The reference genome of *O. sativa* subsp. *japonica* cv. Nipponbare, which is often used also as standard in FCM studies, has been reported to be 384.2–386.5 Mbp and 375.1 ± 20.9 Mbp in genome sequencing projects (Kawahara et al. 2013; Wang et al. 2018), corresponding to 0.79 pg/2C and 0.77 ± 0.04 pg/2C, respectively. Gap-free reference genomes of two subsp. *indica* varieties were 392 Mbp (≈ 80.0 pg/2C) and 396 Mbp (≈ 80.1 pg/2C), respectively (Song et al. 2021).

The tetraploid *Leersia oryzoides* ($2n=48$) had 1.84 pg/2C, which is consistent with previously found values of 1.84 pg/2C and 1.83 pg/2C also using FCM + PI (Bai et al. 2012; Zonneveld 2019). All three Oryzoideae taxa examined in this study had 1Cx genome sizes of 0.42–0.50 pg and their MCs were uniformly around 0.04 pg.

The genus *Zizania* with four species, used as wild rice for grain harvest in North America and as a vegetable in China due to its *Ustilago*-infected, enlarged stems, is characterized by a WGD that occurred after the *Zizania-Oryza* phylogenetic split. For East Asian *Z. latifolia*, genome sizes of 586 Mbp (≈ 1.20 pg/2C) and of 604.1 Mbp (≈ 1.24 pg/2C) were found by FCM + PI and by genome sequencing, respectively (Guo et al. 2015). Recent sequencing studies found 547.38 Mbp and 545.36 Mbp (both ≈ 1.12 pg/2C) (Yan et al. 2022; Xie et al. 2023). The holoploid genome size of *Z. latifolia* is therefore about 1.1–1.2 times larger than that of *O. sativa*. Conflicting chromosome numbers have been reported for this species (see CCDB 2023). However, assuming that $2n=34$ is correct, although $2n=30$ has also been reported (Probatova and Sokolovskaya 1982; Tzvelev and Probatova 2019), the MCs would be 0.03–0.04 pg. The genome size of another *Zizania* species, North American *Z. palustris* ($2n=30$), was estimated to be 3.68–3.87 pg/2C by FCM + PI and 1,289 Mbp (≈ 2.63 pg/2C) by genome sequencing (Haas

et al. 2021). Its MC is therefore 0.12–0.13 pg, about 3 times larger than that of *O. sativa*. The *Z. palustris* genome has strongly restructured chromosomes compared to rice and is characterized by a massive amplification of repetitive elements, comprising about 74% of the total genome, compared to about 50% in rice and 53% in *Z. latifolia* (Haas et al. 2021; Yan et al. 2022).

Pooideae

This is the largest subfamily of grasses, comprising nearly 220 genera with 4130 species (Soreng et al. 2022), slightly more than one-third of all grass species. The Pooideae are most abundant in the temperate to cool regions of both hemispheres. The subfamily is taxonomically further subdivided into 10 to 16 tribes, depending on the width of the respective delineations (GPWG 2001; Schneider et al. 2009, 2011, 2012; GPWG II 2012; Kellogg 2015; Tkach et al. 2020; Soreng et al. 2022). The holoploid genome sizes found for the subfamily Pooideae ranged from the low estimates of 0.56 pg/2C and 0.67 pg/2C in *Brachypodium stacei* (Catalán et al. 2012; Winterfeld et al. 2024) to 45.26 pg/2C in *Thinopyrum ponticum* (Vogel et al. 1999). The variation was therefore greater than in any other grass subfamily (Tables 1, 2; Figs. 1, 2), as already noted (Bennetzen and Kellogg 1997; Kellogg and Bennetzen 2004; Caetano-Anollés 2005; Leitch et al. 2010; Kellogg 2015). The 1Cx values varied widely from 0.33 pg in hexaploid *Austrostipa scabra* to 9.45 pg in diploid *Secale montanum* (Eilam et al. 2007) and the MCs from 0.02 pg to 1.84 pg in the same species (Table 2; Figs. 1, 2). The genome size data of the Pooideae also showed strong differences between the phylogenetic lineages and tribes of this subfamily and will be discussed in more detail elsewhere (Tkach et al. 2024; Winterfeld et al. 2024).

PACMAD clade

This clade comprises six subfamilies with over 5800 species (Soreng et al. 2022) and is characterized by the frequent occurrence of the highly efficient C_4 photosynthetic pathway. In this study, the clade was represented by 21 example taxa in 18 genera.

Panicoideae

This subfamily is the second-largest subfamily of grasses, with over 3300 species in 242 genera (Soreng et al. 2022), and distributed from tropical to warm temperate regions. The DNA 2C values of the sampled species ranged from 0.9 to 8.12 pg, and their ploidy levels ranged from 2× to 8*x* (CCDB 2023). The chromosome base numbers in the Panicoideae vary depending on the tribes to which the genera studied belonged: $x=9$ in *Cenchrus*, *Digitaria*, *Oplismenus*, *Panicum* and *Setaria* (tribe Paniceae), which had 1Cx values of 0.45–0.83 pg and MCs of 0.05–0.09 pg; $x=10$ in the tribe Andropogoneae genera *Coix*, *Saccharum* and *Tripidium*, which were characterized by distinctively larger 1Cx values of 1.02–1.91 pg and MCs of 0.10–0.19 pg. This also applies to *Miscanthus* from this tribe, which has $2n=38$ or 57 based on $x=19$. This chromosome number is derived from ancestors with $x=9$ and $x=10$ through allopolyploidy/amphidiploidy (Adati and Shiotani 1962; Chramiec-Głąbik et al. 2012); and $x=12$ in *Chasmanthium* (tribe Chasmanthieae) with intermediate values of 1Cx and MC, specifically 0.88 pg and 0.07 pg, respectively.

There were no distinct differences between the perennial (1Cx of 0.50–1.81 pg in *Cenchrus*, *Chasmanthium*, *Miscanthus*, *Oplismenus*, *Saccharum*, *Tripidium*) and annual taxa (1Cx of 0.45–1.91 pg in *Coix*, *Digitaria*, *Panicum*, *Setaria*), nor between the taxa with C_4 (0.45–1.91 pg in *Cenchrus*, *Coix*, *Digitaria*, *Miscanthus*, *Panicum*, *Saccharum*, *Tripidium*) and C_3 photosynthesis (0.54–1.11 pg in *Chasmanthium*, *Oplismenus*, *Setaria*). Previous genome size estimates using FCM + PI in *Coix*, *Digitaria*, *Miscanthus* (both cytotypes), *Panicum* and *Setaria*, including the same species as used in this study, agree with our data (Rayburn et al. 2009; Nishiwaki et al. 2011; Chramiec-Głąbik et al. 2012; Zhang et al. 2013; Chae et al. 2014; Zonneveld 2019: Table 5 electron. supplement). This also largely applies to the genome sizes estimated by sequencing, i.e. 1,560 Mbp (≈ 3.19 pg/2C) in *Coix lacryma-jobi*, and 395.1 Mbp and 397 Mbp (both ≈ 0.81 pg/2C) in *Setaria viridis* (Kang et al. 2020; Mamidi et al. 2018; Thielen et al. 2020).

Arundinoideae

The subfamily Arundinoideae is distributed worldwide and has a consistently C_3 photosynthetic pathway. According to its current narrow taxonomic definition, it includes only about 14 genera and 36 species (Hardion et al. 2017; Soreng et al. 2022). The most likely chromosome base number is $x=6$ (see Hardion et al. 2011, 2013 with a review of previous literature data). The 2C value of 5.25 pg found in *Arundo donax*, most likely the 18× cytotype with $2n=108$, agrees with the previously recorded amounts of 5.6 pg and 4.5–4.8 pg, respectively, also estimated by using FCM + PI (Zonneveld et al. 2005; Hardion et al. 2011). The studied *A. donax* accession had a 1Cx value of 0.29 pg and an MC of 0.05 pg. The two subspecies of *Phragmites australis* investigated, both likely $2n=48$, had proportionally lower 2C values of 2.09–2.18 pg compared to *Arundo*, but similar 1Cx values of 0.26–0.27 pg and MCs of 0.04–0.05 pg. This estimate agrees with the genome sequence length of 1,140 Mbp (≈ 2.33 pg/2C) for a presumably tetraploid accession of subsp. *australis*, invasive in North America (Oh et al. 2022).

The holoploid genome size of *Molinia caerulea* (3.52 pg/2C) was found to be intermediate between those of the *Arundo* and *Phragmites* accessions. Assuming a chromosome number $2n = 36$, which is the most common in *M. caerulea* (CCDB 2023), the 1Cx value of our accession would be 0.59 pg and the MC would be 0.10 pg, which is larger than in the other Arundinoideae taxa studied. The 2C value of 3.52 pg, which was recalculated from previously reported data for the tetraploid cytotype of *M. caerulea* (Dančák et al. 2012), agrees with our findings and suggests that our accession is also tetraploid, although the chromosome number has not been determined. The estimated 2C values for Arundinoideae species are largely consistent also with the results of previous estimates using FCM + PI. *Phragmites australis* was recorded as having 1.89 pg and 2.26 pg, while *M. caerulea* had 3.04–3.13 pg and 3.51–3.54 pg (Šmarda et al. 2019; Zonneveld 2019).

Chloridoideae

The subfamily Chloridoideae, distributed mainly in the tropics to subtropics, rarely in temperate zones, with an almost uniform C_4 photosynthetic pathway, has about 120 genera and 1,600 species. It is represented in this study only by the C_4 species *Cleistogenes mucronata*. Its 2C value was 1.55 pg, but its chromosome number is unknown. According to CCDB (2023), other *Cleistogenes* species usually have $2n = 40$, which is probably based on $x = 10$. So, if we assume a fourfold ploidy for *C. mucronata*, the 1Cx value would be 0.39 pg and the MC would be 0.04 pg. However, it has often been argued that $x = 10$ is already a polyploid number in Chloridoideae, which was originally based on $x = 5$, but reports of $2n = 10$ are still extremely rare, as noted by Roodt and Spies (2003), and would need to be confirmed.

Danthonioideae

The mainly African to Australasian subfamily Danthonioideae (C_3 throughout) with about 19 genera and 290 species had 2C values ranging from 1.63 pg in *Schismus arabicus*, 4.66 pg in *Danthonia decumbens*, 4.89 pg in *D. alpina* to 8.30 pg in *Cortaderia selloana*. Considering $x = 6$ as the established chromosome base number in this subfamily, the 1Cx values are quite uniform, namely 0.82 pg in *S. arabicus* ($2x$), 0.78 pg and 0.82 pg in the two sampled *Danthonia* species (both $6x$) and 0.69 pg in the highly polyploid *C. selloana* ($12x$). The MCs of 0.12–0.14 pg were also quite uniform. Comparable 2C values were obtained for *D. alpina* (3.90 pg) and *D. decumbens* (3.873 Mbp ≈ 3.96 pg and 4.19 pg) in previous studies also using FCM + PI (Šmarda et al. 2014, 2019; Zonneveld 2019).

Aristidoideae and Micraroideae

These tropical to subtropical subfamilies, not sampled in this study, each include both C_3 and C_4 grasses and have 3 and 9 genera, respectively, with a nearly cosmopolitan distribution. Genome size data using FCM + PI have been previously obtained for some species. *Aristida purpurea* (diploid with $2n = 22$) and *A. tuberculosa* had 2.60 pg/2C and 2.62 pg/2C (Bai et al. 2012; Šmarda et al. 2014), suggesting that the latter species is also diploid and implying 1Cx values of 1.30 pg and 1.31 pg, respectively, and an MC of 0.12 pg each. The Micraroideae species *Isachne globosa* with $2n = 6x = 60$ had 3.64 pg/2C, a 1Cx of 0.61 pg and an MC of 0.06 pg (Murray et al. 2005). It should be noted that Murray et al. (2005: p. 1300) explicitly corrected previous estimates (Murray et al. 2003), stating that they were about 30% too low.

Comparison of the grass subfamilies

Holoploid genomes

The 2C values do not show an overall trend of increase or decrease across the sampled grass subfamilies (Table 2, Figs. 1a, 2). Comparatively small 2C values occur in the Oryzoideae, Panicoideae and Pooideae. While the Oryzoideae have consistently small 2C values, the Panicoideae and Pooideae are highly variable and also have the largest values found in our sample, followed by the Bambusoideae. The phylogenetically 'early diverging' grass subfamilies Anomochlooideae and Pharoideae have small but not strikingly small genome sizes. They are therefore not characterized by their own conspicuous 2C values compared to the 'core grasses', but correspond to the average of the grass subfamilies of the BOP and PACMAD clades.

Monoploid genomes

Regarding the monoploid chromosome sets, the lowest values of less than 0.4 pg are found in some species of Arundinoideae and Pooideae, the highest in Chloridoideae, Panicoideae and also Pooideae (Table 2, Figs. 1b, 2). With values of about 1.2–1.8 pg/1Cx, the Anomochlooideae and Pharoideae do not even belong to the small genome species. Comparatively large monoploid genomes occur in Bambusoideae, Panicoideae and the (more extensively sampled) Pooideae. The monoploid genomes of the Oryzoideae, including that of cultivated rice, are therefore among the smaller, but not the smallest, of the grasses.

Chromosome sizes

The mean chromosome DNA content (MC values) is below 0.1 pg in most subfamilies, i.e. the chromosomes are relatively small (Table 2, Figs. 1c, 2). In the 'early diverging' lineages such as the subfamilies Anomochlooideae and Pharoideae, it is even at or above 0.1 pg. The small chromosomes and monoploid genomes found in most subfamilies, but particularly noticeable in the Oryzoideae, may be the result of a secondary reduction in genome size compared to the Anomochlooideae and Pharoideae. However, the Puelioideae, which is the third 'early diverging' lineage of grasses (Fig. 2), has not been studied in this regard. The Aristidoideae and Panicoideae from the PACMAD clade as well as the Bambusoideae and particularly the Pooideae from the BOP clade, also exhibit similarly high values as those found in the Anomochlooideae and Pharoideae. This could represent an ancestral character state in a phylogenetic sense. The Pooideae, however, have larger chromosomes in many cases due to an increase in chromosome sizes in its lineages with reductional dysploidy, resulting in the chromosome base number $x = 7$ (Winterfeld et al. 2024).

Genome sizes and the origin of the grasses

The Anomochlooideae and Pharoideae, which have also been shown to be characterized by the ρ-WGD typical of all other grasses (McKain et al. 2016; Ma et al. 2021; Seetharam et al. 2021), have neither particularly small nor particularly large genomes (1Cx) or chromosome sizes (MC) compared to the other Poaceae, but are somehow intermediate (Table 2; Figs. 1b,c, 2). Both are also characterized by a more or less unspectacular content of repetitive sequences in the genome of 51% and 78.9%, respectively (Ma et al. 2021; Seetharam et al. 0.2021), which is in the order of magnitude of grasses with medium-sized genomes such as *Sorghum bicolor* (62.8%) of the subfamily Panicoideae, but higher than in small-genome grasses such as *Oryza sativa* (32.3%) or *Brachypodium distachyon* (28.0%) of the subfamilies Oryzoideae and Pooideae, respectively.

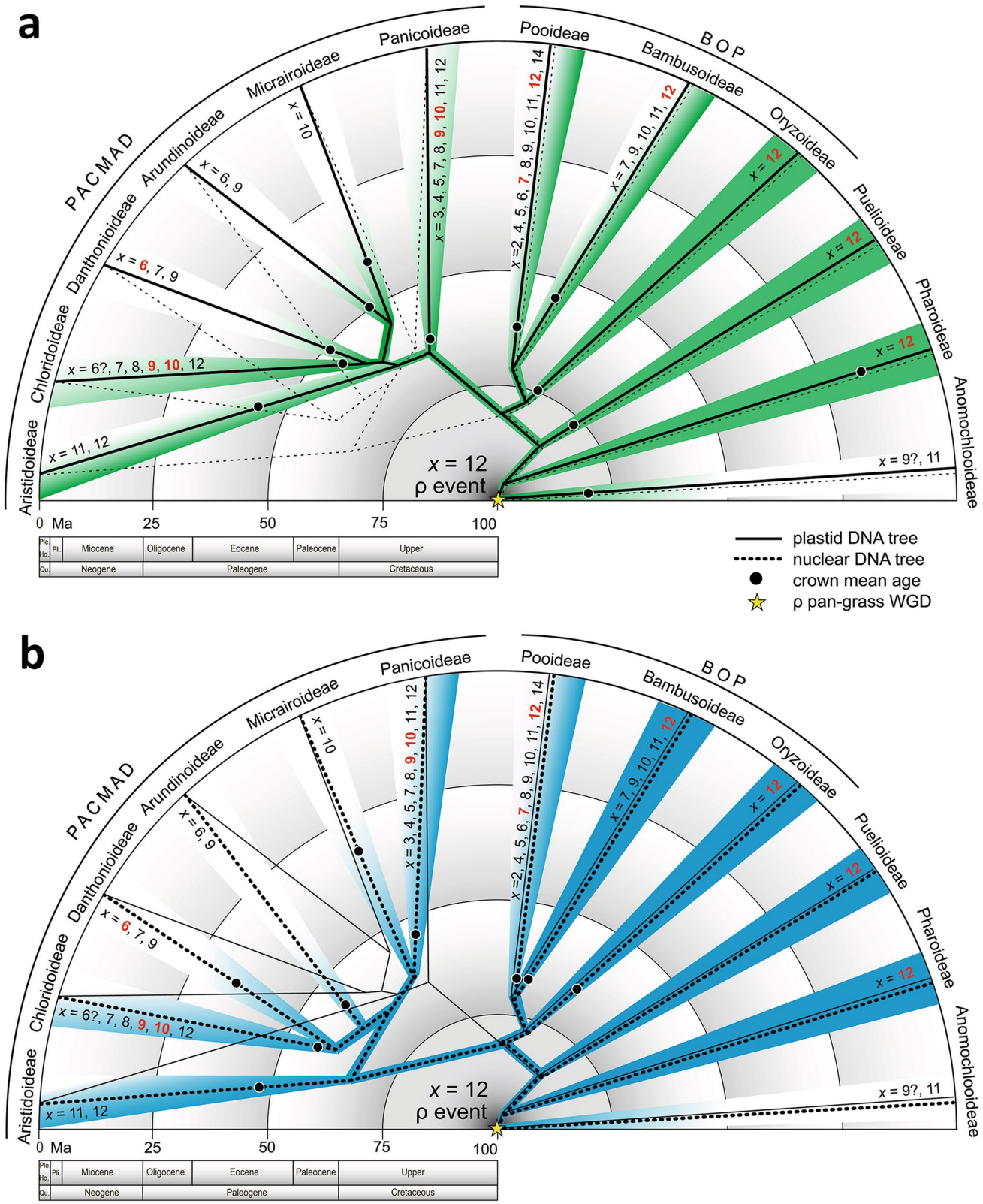
The sister families of the Poaceae are Ecdeiocoleaceae and Joinvilleaceae, all of which form the 'graminid clade' within Poales, but Ecdeiocoleaceae and Joinvilleaceae lack the ρ-WGD of the Poaceae (McKain et al. 2016). Their genome sizes of 1.98–2.72 pg/2C (Winterfeld et al. 2023) are comparable to, but not half as large as that of the Anomochlooideae and Pharoideae (2.48–3.63 pg/2C), as might be expected in principle from the WGD event. However, assuming that their chromosome numbers $2n = 36$, ca. 38 and ca. 48 reflect a fourfold ploidy based on $x = 9$ and 12, their 1Cx values are 0.50–0.68 pg (Winterfeld et al. 2023), about half that of Anomochlooideae and Pharoideae (1.24–1.82 pg). In addition, their MCs are 0.05–0.08 pg (Winterfeld et al. 2023), about half that of Anomochlooideae and Pharoideae (0.10–0.17 pg). This all implies that the ρ event would indeed still be reflected in the genome and chromosome size data of the 'basal' grass subfamilies compared to the closest sister families of Poaceae, suggesting a pre-ρ karyotype of 9–12 chromosomes as an intermediate stage between 7 protochromosomes (Murat et al. 2014; Pont et al. 2019) and the formation of the AGK.

Genome sizes and the origins of the `spikelet clade' and the 'core grasses'

The 'spikelet clade', i.e. all grass subfamilies except for the Anomochlooideae, and the 'core grasses', which include the BOP and PACMAD clades as sister lineages, do not appear to be characterized by a consistent clear difference in 1Cx genome size or chromosome sizes (MC) compared to the Anomochlooideae or the Anomochlooideae and Pharoideae (Table 2; Figs. 1B,C, 2).

It is therefore conceivable that genome sizes of about 1.2–1.8 pg/1Cx, such as those of the studied representatives of the Anomochlooideae (*Streptochaeta angustifolia*) and Pharoideae (*Pharus latifolius*), can be considered as ancestral for the grasses. This value is much lower than the previous suggestion of 3.0 pg to 5.2 pg DNA per 2C nucleus for the genome size of the ancestor of the grass family (Caetano-Anollés 2005). The very small genomes found in the Oryzoideae and parts of the Bambusoideae could therefore be the result of genome shrinkage and thus of secondary origin. Within the BOP clade, this probably applies to some Pooideae as well (Winterfeld et al. 2024). Furthermore, within the PACMAD clade, secondarily reduced 1Cx genome sizes (Table 2) are also plausible for the Arundinoideae, parts of the Chloridoideae and the Panicoideae compared to the Aristidoideae.

In the opposite case, small genomes such as that of rice (Oryzoideae) would have been ancestral within the grasses, with a corresponding (apomorphic) genome enlargement already occurring within the 'early diverging' lineages Anomochlooideae and Pharoideae, as well as within the lineages of the BOP clade except for the Oryzoideae, and additionally within the PACMAD clade. This hypothesis cannot be excluded in principle, but seems less plausible. Genome size data on the second genus of the Anomochlooideae, the monospecific genus *Anomochloa*, the other two genera of the Pharoideae (*Leptaspis* and *Scrotochloa*), and especially the third subfamily of the 'early diverging' lineages, namely the subfamily Puelioideae (*Guaduella*, *Puelia*), which has not yet been investigated in this respect and which together with the 'core grasses' (BOP and PACMAD clades) forms the monophyletic 'bistigmatic clade', would be needed for a final clarification of this question.


a
PACMAD
Aristidoideae
Chloridoideae
Danthonioideae
Arundinoideae
Micrairoideae
Panicoideae
Pooideae
BOP
Bambusoideae
Oryzoideae
Puelioideae
Pharoideae
Anomochlooideae
x = 11, 12
x = 6?, 7, 8, 9, 10, 12
x = 6, 7, 9
x = 6, 9
x = 10
x = 3, 4, 5, 7, 8, 9, 10, 11, 12
x =2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
x = 7, 9, 10, 11, 12
x = 12
x = 12
x = 12
x = 9?, 11
x = 12
ρ event
0 Ma
25
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Plio.
Plei.
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Miocene
Oligocene
Eocene
Paleocene
Upper
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Paleogene
Cretaceous
plastid DNA tree
nuclear DNA tree
crown mean age
ρ pan-grass WGD
b
PACMAD
Aristidoideae
Chloridoideae
Danthonioideae
Arundinoideae
Micrairoideae
Panicoideae
Pooideae
BOP
Bambusoideae
Oryzoideae
Puelioideae
Pharoideae
Anomochlooideae
x = 11, 12
x = 6?, 7, 8, 9, 10, 12
x = 6, 7, 9
x = 6, 9
x = 10
x = 3, 4, 5, 7, 8, 9, 10, 11, 12
x =2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
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x = 12
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Neogene
Paleogene
Cretaceous

◂**Fig. 3** Ancestral grass karyotype (AGK) and evolution of chromosome base numbers in Poaceae. The monoploid numbers (x) are given for each grass subfamily, and the most frequent numbers are printed in red. Blue and green background colors indicate lineages where the AGK is largely conserved, as shown by nuclear genome sequencing for Bambusoideae, Oryzoideae and Pharoideae, or is expected based on the conserved chromosome base number $x=12$, such as in some lineages within the subfamily Pooideae of the BOP clade, but also within the PACMAD clade, for example in the subfamilies Aristidoideae, Chloridoideae and Panicoideae. Dated phylogenetic trees based on plastome (**a**) and nuclear phylogenomic (**b**) analyses adapted from Gallaher et al. (2019, 2022) and Huang et al. (2022). *Ho.* Holocene, *Ple.* Pleistocene, *Pli.* Pliocene, *Qu.* Quaternary

Ancestral grass karyotype (AGK)

The paleogenomic reconstruction of the AKG with 12 chromosomes, which arose after the ρ event, a genome duplication that resulted in a chromosome set of most likely 14 chromosomes that was restructured to 12, is supported by the well preserved synteny at the chromosomal level the in studied species of the different grass subfamilies. Comparatively few chromosomal rearrangements occurred between *Pharus latifolius* and rice, with some more changes with respect to *Phyllostachys edulis* (Bambusoideae), for example, suggesting that the AGK remained evolutionarily rather static for a long time after the origin of grasses (Ma et al. 2021). Differences were larger for *Sorghum bicolor* and *Cenchrus americanus* (both Panicoideae) and particularly dramatic for representative taxa from other lineages of the 'core Poaceae', i.e. *Oropetium thomaeum* (Chloridoideae), *Brachypodium distachyon* and *Aegilops tauschii* (both Pooideae). Most rearrangements therefore were lineage-specific and occurred within subfamilies, with the AGK remaining largely unchanged in the lineage leading to the 'core Pooideae', after the split of Pharoideae (Ma et al. 2021) and, by implication, Puelioideae.

The chromosome base numbers of grasses show a prevalence of $x=12$ in most subfamilies (Table 2; Fig. 3). This is true for the Pharoideae and Puelioideae within the 'early diverging lineages', while $x=11$ was recorded for two *Streptochaeta* species of the Anomochlooideae. *Anomochloa marantoidea*, on the other hand, has $2n=36$, suggesting $x=18$, which is supported by the occurrence of 18 bivalents in the meiotic prophase of this species (Hunziker et al. 1989). However, since nearly half of the sexually reproducing polyploid plants show bivalent chromosome pairing and are functionally diploid (Li et al. 2021a, b), *Anomochloa* could also represent a diploidized polyploid species, making $x=9$ a possible monoploid number for *Anomochloa*. It could have arisen, like $x=11$ in *Streptochaeta*, by descending dysploidy from $x=12$.

The number $x=12$ prevails in the subfamilies of the BOP clade, only in Bambusoideae lower numbers $x=7, 9, 10, 11$ are well documented and were most likely also derived by reductional dysploidy. This is also true for the Pooideae, where $x=7$, the chromosome base number often considered to be characteristic of this subfamily, actually prevails only in its phylogenetically late diverging lineages, summarized as the 'core Pooideae', while its early diverging lineages mostly have $x=12$ (Fig. 3) (Winterfeld et al. 2024).

The same might apply to the PACMAD clade, where the higher monoploid numbers $x=11, 12$ are represented in the Aristidoideae, which represents the earliest diverging lineage of this clade according to the nuclear DNA phylogenetic analyses (Fig. 3b). Comparatively high numbers of $x=9, 10$ are also found in the Panicoideae and Chloridoideae, from which much lower chromosome base numbers are derived, similar to Pooideae (Table 2; Fig. 3).

As mentioned above, rice has largely preserved the AGK (Wang et al. 2015), but so have bamboos with few changes in genome structure after their split from other clades. The studied genomes of bamboos, including diploid, tetraploid and hexaploid species, show genome-wide collinearity with the rice genome (Guo et al. 2019; Ma et al. 2021), while major genomic repatterning processes such as chromosome fusions and subsequent chromosome base number reduction are widespread in grasses but concentrated in the phylogenetically late diverging lineages. The increasing number of genomic analyses in different grass clades shows that the AGK with 12 chromosomes is unexpectedly well conserved in grasses and has remained evolutionarily almost unchanged for almost 100 million years in some grass lineages (Fig. 3). The majority of major genome rearrangements, as seen in both the BOP and the PACMAD clades, are lineage-specific and occurred after the diversification of their subfamilies had begun.

Conclusion and outlook

Data on genome sizes, which can be obtained relatively easily by FCM and for which field fixations of leaf samples using silica gel can also be used, in conjunction with knowledge of chromosome numbers, i.e. classical cytogenetic data and the increasing number of sequenced genomes of wild grasses, allow a completely new perspective on the genome and chromosome evolution of this fascinating and successful group of plants. Unfortunately, for many of the mainly tropical-subtropical groups of grasses, even the baseline data of chromosome numbers or C-values are completely missing or only a few are available.

It would be particularly interesting for future studies to sequence the genomes of representatives of the subfamily Puelioideae ($x=12$) of the basal grass lineages, as well as those of the subfamilies Pooideae (BOP clade) and Aristidoideae, Chloridoideae, Panicoideae (PACMAD clade), in which the chromosome base number $x=12$ is found. This

would make it possible to clarify the possible occurrence of the evolutionarily unexpectedly static and in some lineages almost unchanged AGK also for other grass subfamilies.

Information on Electronic Supplementary Material

Online Resource 1. Examined taxa with 2C values and standard deviation, 1C values, chromosome numbers, ploidy levels, 1Cx values, mean chromosome DNA content (MC), FCM standard species and collection details.
Online Resource 2. Genome sizes (2C values) of taxa taken from the literature and used in this study.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Adani S, Shiotani I (1962) The cytotaxonomy of the genus *Miscanthus* and its phylogenic status. Bull Fat Agric Mie Univ 25:1–14

Avdulov N (1931) Kario-sistematicheskoe issledovanie semeistva zlakov. Trudy Prikl Bot Selekts Suppl 44:1–428. https://doi.org/10.1017/cbo9780511525445.002

Baker WJ, Bailey P, Barber V, Barker A, Bellot S, Bishop D, Botigué LR, Brewer G, Carruthers T, Clarkson JJ, Cook J, Cowan RS, Dodsworth S, Epitawalage N, Françoso E, Gallego B, Johnson MG, Kim JT, Leempoel K, Maurin O, Mcginnie C, Pokorny L, Roy S, Stone M, Toledo E, Wickett NJ, Zuntini AR, Eiserhardt WL, Kersey PJ, Leitch IJ, Forest F (2022) A comprehensive phylogenomic platform for exploring the angiosperm tree of life. Syst Biol 71:301–319. https://doi.org/10.1093/sysbio/syab035

Bai C, Alverson WS, Follansbee A, Waller DM (2012) New reports of nuclear DNA content for 407 vascular plant taxa from the United States. Ann Bot (Oxford) 110:1623–1629. https://doi.org/10.1093/aob/mcs222

Bellec A, Sow MD, Pont C, Civan P, Mardoc E, Duchemin W, Armisen D, Huneau C, Thévenin J, Vernoud V, Depège-Fargeix N, Maunas L, Escale B, Dubreucq B, Rogowsky P, Bergès H, Salse J (2023) Tracing 100 million years of grass genome evolutionary plasticity. Pl J 114:1243–1266. https://doi.org/10.1111/tpj.16185

Bennetzen JL, Kellogg EA (1997) Do plants have a one-way ticket to genomic obesity? Pl Cell 9:1509–1514. https://doi.org/10.1105/tpc.9.9.1509

Blaner A, Schneider J, Röser M (2014) Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single copy locus topoisomerase 6 compared with chloroplast DNA. Syst Biodivers 12:111–124. https://doi.org/10.1080/14772000.2014.890137

Bolot S, Abrouk M, Masood-Quraishi U, Stein N, Messing J, Feuillet C, Salse J (2009) The 'inner circle' of the cereal genomes. Curr Opin Pl Biol 12:119–125. https://doi.org/10.1016/j.pbi.2008.10.011

Caetano-Anollés G (2005) Evolution of genome size in the grasses. Crop Sci (Madison) 45:1809–1816. https://doi.org/10.2135/cropsci2004.0604

Catalán P, Müller J, Hasterok R, Jenkins G, Mur LAJ, Langdon T, Betekhtin A, Siwinska D, Pimentel M, López-Alvarez D (2012) Evolution and taxonomic split of the model grass *Brachypodium distachyon*. Ann Bot (Oxford) 109:385–405. https://doi.org/10.1093/aob/mcr294

CCDB (2023) The chromosome counts database. Version 1.58. Available at: http://ccdb.tau.ac.il/home/. Accessed 9 August 2023

Čertner M, Lučanová M, Sliwinska E, Kolář F, Loureiro J (2022) Plant material selection, collection, preservation, and storage for nuclear DNA content estimation. Cytometry 101:737–748. https://doi.org/10.1002/cyto.a.24482

Chae WB, Hong SJ, Gifford JM, Rayburn AL, Sacks EJ, Juvik JA (2014) Plant morphology, genome size, and SSR markers differentiate five distinct taxonomic groups among accessions in the genus *Miscanthus*. GCB Bioenergy 6:646–660. https://doi.org/10.1111/gcbb.12101

Chalopin D, Clark LG, Wysocki WP, Park M, Duvall MR, Bennetzen JL (2021) Integrated genomic analyses from low-depth sequencing help resolve phylogenetic incongruence in the bamboos (Poaceae: Bambusoideae). Frontiers Pl Sci 12:725728. https://doi.org/10.3389/fpls.2021.725728

Chen Y, Zhu X, Loukopoulos P, Weston LA, Albrecht DE, Quinn JC (2021) Genotypic identification of *Panicum* spp. in New South Wales, Australia using DNA barcoding. Sci Rep 11:16055. https://doi.org/10.1038/s41598-021-95610-6

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annual Rev

Pl Biol 58:377–406. https://doi.org/10.1146/annurev.arplant.58.032806.103835
Chramiec-Głąbik A, Grabowska-Joachimiak A, Śliwińska E, Legutko J, Kula A (2012) Cytogenetic analysis of *Miscanthus ×giganteus* and its parent forms. Caryologia 65:234–242. https://doi.org/10.1080/00087114.2012.740192
Clark LG (2023) Integrating genomic and morphological data into bamboo taxonomic and evolutionary studies. Pl Diversity 45:123–124. https://doi.org/10.1016/j.pld.2023.02.002
Clark LG, Londoño X, Ruiz-Sanchez E (2015) Bamboo taxonomy and habitat. In: Liese W, Köhl M (eds) Bamboo. Tropical forestry, Springer, Cham, pp 1–30. https://doi.org/10.1007/978-3-319-14133-6_1
Dai SF, Zhu XG, Hutang GR, Li JY, Tian JQ, Jiang XH, Zhang D, Gao LZ (2022) Genome size variation and evolution driven by transposable elements in the genus *Oryza*. Frontiers Pl Sci 13:921937. https://doi.org/10.3389/fpls.2022.921937
Dančák M, Duchoslav M, Trávníček B (2012) Taxonomy and cytogeography of the *Molinia caerulea* complex in central Europe. Preslia 84:351–374
de Wet JMJ (1987) Hybridization and polyploidy in the Poaceae. In: Soderstrom TR, Hilu KW, Campbell CS, Barkworth ME (eds) Grass systematics and evolution. Smithsonian Institution Press, Washington, DC, pp 188–194
Doležel J, Bartoš J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot (Oxford) 95:99–110. https://doi.org/10.1093/aob/mci005
Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51:127–128. https://doi.org/10.1002/cyto.a.10013
Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:2233–2244. https://doi.org/10.1038/nprot.2007.310
Doležel J, Čížková J, Šimková H, Bartoš J (2018) One major challenge of sequencing large plant genomes is to know how big they really are. Int J Molec Sci 19:3554. https://doi.org/10.3390/ijms19113554
Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, C4 Grasses Consortium, Bond WJ, Christin P, Cousins AP, Duvall MR, Fox DL, Freckleton RP, Ghannoum O, Hartwell J, Huang Y, Janis CM, Keeley JE, Kellogg EA, Knapp AK, Leakey ADB, Nelson DM, Saarela JM, Sage RF, Sala OE, Salamin N, Still CJ, Tipple B (2010) The origins of C_4 grasslands: integrating evolutionary and ecosystem science. Science 328:587–591. https://doi.org/10.1126/science.1177216
Eilam Y, Anikster Y, Millet E, Manisterski J, Sagi-Assif O, Feldman M (2007) Genome size and genome evolution in diploid Triticeae species. Genome 50:1029–1037. https://doi.org/10.1139/G07-083
Elliott TL, Spalink D, Larridon I, Zuntini AR, Escudero M, Hackel J, Barrett RL, Martín-Bravo S, Márquez-Corro JI, Granados Mendoza C, Mashau AC, Romero-Soler KJ, Zhigila DA, Gehrke B, Andrino CO, Crayn DM, Vorontsova MS, Forest F, Baker WJ, Wilson KL, Simpson DA, Muasya AM (2024) Global analysis of Poales diversification—parallel evolution in space and time into open and closed habitats. New Phytol 242:727–743. https://doi.org/10.1111/nph.19421
Estep MC, McKain MR, Vela Diaz D, Zhong J, Hodge JG, Hodkinson TR, Layton DJ, Malcomber ST, Pasquet R, Kellogg EA (2014) Allopolyploidy, diversification, and the Miocene grassland expansion. Proc Natl Acad Sci USA 111:15149–15154. https://doi.org/10.1073/pnas.1404177111
FAO (2022) World food and agriculture—statistical yearbook 2022. FAO, Rome. https://doi.org/10.4060/cc2211en
Fleischmann A, Michael TP, Rivadavia F, Sousa A, Wang W, Temsch EM, Greilhuber J, Müller KF, Heubl G (2014) Evolution of genome size and chromosome number in the carnivorous plant genus *Genlisea* (Lentibulariaceae), with a new estimate of the minimum genome size in angiosperms. Ann Bot (Oxford) 114:1651–1663. https://doi.org/10.1093/aob/mcu189
Gallaher TJ, Adams DC, Attigala L, Burke SV, Craine JM, Duvall MR, Klahs PC, Sherratt E, Wysocki WP, Clark LG (2019) Leaf shape and size track habitat transitions across forest—grassland boundaries in the grass family (Poaceae). Evolution (Lancaster) 73:927–946. https://doi.org/10.1111/evo.13722
Gallaher TJ, Peterson PM, Soreng RJ, Zuloaga FO, Li D-Z, Clark LG, Tyrrell CD, Welker CA, Kellogg EA, Teisher JK (2022) Grasses through space and time: an overview of the biogeographical and macroevolutionary history of Poaceae. J Syst Evol 60:522–569. https://doi.org/10.1111/jse.12857
Gibson DJ (2009) Grasses and grassland ecology. Oxford University Press, New York
GPWG (Grass Phylogeny Working Group) (2001) Phylogeny and subfamilial classification of the grasses (Poaceae). Ann Missouri Bot Gard 88:373–457
GPWG (Grass Phylogeny Working Group) II (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C_4 origins. New Phytol 193:304–312. https://doi.org/10.1111/j.1469-8137.2011.03972.x
Greilhuber J (2005) Intraspecific variation in genome size in angiosperms: identifying its existence. Ann Bot (Oxford) 95:91–98. https://doi.org/10.1093/aob/mci004
Greilhuber J, Doležel J, Lysak MA, Bennett MD (2005) The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. Ann Bot (Oxford) 95:255–260. https://doi.org/10.1093/aob/mci019
Greilhuber J, Temsch EM, Loureiro JM (2007) Nuclear DNA content measurement. In: Doležel J, Greilhuber J, Suda J (eds) Flow cytometry with plant cells. Wiley-Verlag, Weinheim, pp 67–101
Griffith DM, Osborne CP, Edwards EJ, Bachle S, Beerling DJ, Bond WJ, Gallaher TJ, Helliker BR, Lehmann CER, Leatherman L, Nippert JB, Pau S, Qiu F, Riley WJ, Smith MD, Strömberg CAE, Taylor L, Ungerer M, Still CJ (2020) Lineage-based functional types: characterising functional diversity to enhance the representation of ecological behaviour in land surface models. New Phytol 228:15–23. https://doi.org/10.1111/nph.16773
Guo L, Qiu J, Han Z, Ye Z, Chen C, Liu C, Xin X, Ye CY, Wang YY, Xie H, Wang Y, Bao J, Tang S, Xu J, Gui Y, Fu F, Wang W, Zhang X, Zhu Q, Guang X, Wang C, Cui H, Cai D, Ge S, Tuskan GA, Yang X, Qian Q, He SY, Wang J, Zhou XP, Fan L (2015) A host plant genome (*Zizania latifolia*) after a century-long endophyte infection. Pl J 83:600–609. https://doi.org/10.1111/tpj.12912
Guo ZH, Ma PF, Yang GQ, Hu JY, Liu YL, Xia EH, Zhong MC, Zhao L, Sun GL, Xu YX, Zhao YJ, Zhang YC, Zhang YX, Zhang XM, Zhou MY, Guo Y, Guo C, Liu JX, Ye XY, Chen YM, Yang Y, Han B, Lin CS, Lu Y, Li DZ (2019) Genome sequences provide insights into the reticulate origin and unique traits of woody bamboos. Molec Pl 12:1353–1365. https://doi.org/10.1016/j.molp.2019.05.009
Haas M, Kono T, Macchietto M, Millas R, McGilp L, Shao M, Duquette J, Qiu Y, Hirsch CN, Kimball J (2021) Whole-genome assembly and annotation of northern wild rice, *Zizania palustris* L., supports a whole-genome duplication in the *Zizania* genus. Pl J 107:1802–1818. https://doi.org/10.1111/tpj.15419
Hair JB (1968) Contributions to a chromosome atlas of the New Zealand flora—12. New Zealand J Bot 6:267–276. https://doi.org/10.1080/0028825X.1968.10428812
Hair JB, Beuzenberg E (1961) High polyploidy in a New Zealand *Poa*. Nature 189:160. https://doi.org/10.1038/189160a0

Hardion L, Verlaque R, Fridlender A, Vila B (2011) [Records] p 1221, E6–E8. In: Marhold K (ed) IAPT/IOPB chromosome data 11. Taxon 60:1220-E14. https://doi.org/10.1002/tax.604032

Hardion L, Verlaque R, Fridlender A, Zehzad B, Vila B (2013) [Records] p 1075, E9–E11. In: Marhold K (ed) IAPT/IOPB chromosome data 15. Taxon 62(5):1073-E14. https://doi.org/10.12705/625.16

Hardion L, Verlaque R, Haan-Archipoff G, Cahen D, Hoff M, Vila B (2017) Cleaning up the grasses dustbin: systematics of the Arundinoideae subfamily (Poaceae). Pl Syst Evol 303:1331–1339. https://doi.org/10.1007/s00606-017-1451-6

Hilu KW (2004) Phylogenetics and chromosomal evolution in the Poaceae (grasses). Austral J Bot 52:13–22. https://doi.org/10.1071/BT03103

Hodkinson TR (2018) Evolution and taxonomy of the grasses (Poaceae): a model family for the study of species-rich groups. Annual Pl Rev 1:1–39. https://doi.org/10.1002/9781119312994.apr0622

Huang W, Zhang L, Columbus JT, Hu Y, Zhao Y, Tang L, Guo Z, Chen W, McKain M, Bartlett M, Huang CH, Li DZ, Ge S, Ma H (2022) A well-supported nuclear phylogeny of Poaceae and implications for the evolution of C_4 photosynthesis. Molec Pl 15:755–777. https://doi.org/10.1016/j.molp.2022.01.015

Hunziker JH, Stebbins GL (1987) Chromosomal evolution in the Gramineae. In: Soderstrom TR, Hilu KW, Campbell CS, Barkworth MA (eds) Grass systematics and evolution. Smithsonian Institution Press, Washington, DC, pp 179–187

Hunziker JH, Wulf AF, Soderstrom TR (1989) Chromosomes studies on *Anomochloa* and other Bambusoideae (Gramineae). Darwiniana 29:41–45

IPCN (1979 onwards) Index to plant chromosome numbers; Goldblatt P, Johnson DE (eds), Missouri Botanical Garden, St. Louis. Available at: http://legacy.tropicos.org/Project/IPCN. Accessed 19 August 2023

Judziewicz EJ, Soderstrom TR (1989) Morphological, anatomical, and taxonomic studies in *Anomochloa* and *Streptochaeta* (Poaceae: Bambusoideae). Smithsonian Contrib Bot 68:1–52. https://doi.org/10.5479/si.0081024X.68

Kang SH, Kim B, Choi BS, Lee HO, Kim NH, Lee SJ, Kim HS, Shin MJ, Kim HW, Nam K, Kang KD, Kwon SJ, Oh TJ, Lee SC, Kim CK (2020) Genome assembly and annotation of soft-shelled adlay (*Coix lacryma-jobi* variety *ma-yuen*), a cereal and medicinal crop in the Poaceae family. Frontiers Pl Sci 11:630. https://doi.org/10.3389/fpls.2020.00630

Kawahara Y, de la Bastide M, Hamilton JP, Kanamori H, McCombie WR (2013) Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice 6:4. https://doi.org/10.1186/1939-8433-6-4

Kejnovsky E, Hawkins JS, Feschotte C (2012) Plant transposable elements: biology and evolution. In: Wendel J, Greilhuber J, Doležel J, Leitch IJ (eds) Plant genome diversity, Springer, Vienna, pp 17–34. https://doi.org/10.1007/978-3-7091-1130-7

Kellogg EA (1998) Relationships of cereal crops and other grasses. Proc Natl Acad Sci USA 95:2005–2010. https://doi.org/10.1073/pnas.95.5.2005

Kellogg EA (2015) The families and genera of vascular plants. Flowering plants: monocots; Poaceae. Springer, Cham

Kellogg EA, Bennetzen JL (2004) The evolution of nuclear genome structure in seed plants. Amer J Bot 91:1709–1725. https://doi.org/10.3732/ajb.91.10.1709

Kurata N, Fukui K (2003) Chromosome research in genus *Oryza*. In: Nanda JS, Sharma SD (eds) Monograph on genus *Oryza*. Science, Enfield, pp 213–261

Leitch IJ, Leitch AR (2013) Genome size diversity and evolution in land plants. In: Leitch IJ, Greilhuber J, Doležel J, Wendel JF (eds) Plant genome diversity, Physical structure, behavior and evolution of plant genomes. Springer, Wien, pp 307–322. https://doi.org/10.1007/978-3-7091-1160-4

Leitch IJ, Chase MW, Bennett MD (1998) Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Ann Bot (Oxford) 82(suppl 1):85–94. https://doi.org/10.1006/anbo.1998.0783

Leitch IJ, Beaulieu JM, Chase MW, Leitch AR, Fay MF (2010) Genome size dynamics and evolution in monocots. J Bot 2010:1–18. https://doi.org/10.1155/2010/862516

Leitch IJ, Johnston E, Pellicer J, Hidalgo O, Bennett MD (2019) Plant DNA C-values Database (Release 7.1, April 2019). Available at: https://cvalues.science.kew.org/. Accessed 19 August 2023

Li K, Jiang W, Hui Y, Kong M, Feng LY, Gao LZ, Li P, Lu S (2021a) Gapless indica rice genome reveals synergistic contributions of active transposable elements and segmental duplications to rice genome evolution. Molec Pl 14:1745–1756. https://doi.org/10.1016/j.molp.2021.06.017

Li Z, McKibben MTW, Finch GS, Blischak PD, Sutherland BL, Barker MS (2021b) Patterns and processes of diploidization in land plants. Annual Rev Pl Biol 72:387–410. https://doi.org/10.1146/annurev-arplant-050718-100344

Linder HP, Lehmann CER, Archibald S, Osborne CP, Richardson DM (2018) Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. Biol Rev 93:1125–1144. https://doi.org/10.1111/brv.12388

Ling HQ, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zheng S, Li Y (2018) Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. Nature 557:424–428. https://doi.org/10.1038/s41586-018-0108-0

Loureiro J, Čertner M, Lučanová M, Sliwinska E, Kolář F, Doležel J, Garcia S, Castro S, Galbraith DW (2023) The use of flow cytometry for estimating genome sizes and DNA ploidy levels in plants. In: Heitkam T, Garcia S (eds) Plant cytogenetics and cytogenomics, Methods in molecular biology. Humana, New York, pp 25–64. https://doi.org/10.1007/978-1-0716-3226-0_2

Loureiro J, Suda J, Doležel J, Santos C (2007) FLOWER: a plant DNA flow cytometry database. In: Doležel J, Greilhuber J, Suda J (eds) Flow cytometry with plant cells. Wiley, Weinheim, pp 423–438. https://doi.org/10.1002/9783527610921.ch18

Luo MC, Deal KR, Akhunov ED, Akhunova AR, Anderson OD, Anderson JA, Blake N, Clegg MT, Coleman-Derr D, Conley EJ, Crossman CC, Dubcovsky J, Gill BS, Gu YG, Hadam J, Heo HY, Huo N, Lazo G, Ma Y, Matthews DE, McGuire PE, Morrell PL, Qualset CO, Renfro J, Tabanao D, Talbert LE, Tian C, Toleno DM, Warburton ML, You FM, Zhang W, Dvorak J (2009) Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. Proc Natl Acad Sci USA 106:15780–15785. https://doi.org/10.1073/pnas.0908195106

Lusinska J, Majka J, Betekhtin A, Susek K, Wolny E, Hasterok R (2018) Chromosome identification and reconstruction of evolutionary rearrangements in *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. Ann Bot (Oxford) 122:445–459. https://doi.org/10.1093/aob/mcy086

Lusinska J, Betekhtin A, Lopez-Alvarez D, Catalan P, Jenkins G, Wolny E, Hasterok R (2019) Comparatively barcoded chromosomes of *Brachypodium* perennials tell the story of their karyotype structure and evolution. Int J Molec Sci 20:5557. https://doi.org/10.3390/ijms20225557

Lysak MA (2022) Celebrating Mendel, McClintock, and Darlington: on end-to-end chromosome fusions and nested chromosome fusions. Pl Cell 34:2475–2491. https://doi.org/10.1093/plcell/koac116

Ma PF, Liu YL, Jin GH, Liu JX, Wu H, He J, Guo ZH, Li DZ (2021) The *Pharus latifolius* genome bridges the gap of early grass evolution. Pl Cell 33:846–864. https://doi.org/10.1093/plcell/koab015

Mamidi S, Healey A, Huang P, Grimwood J, Jenkins J, Barry K, Sreedasyam A, Shu A, Lovell JT, Feldman M, Wu J, Yu Y, Chen C, Johnson J, Sakakibara H, Kiba T, Sakurai T, Tavares R, Nusinow DA, Baxter I, Schmutz J, Brutnell TP, Kellogg EA (2018) A genome resource for green millet *Setaria viridis* enables discovery of agronomically valuable loci. Nat Biotechnol 38:1203–1210. https://doi.org/10.1038/s41587-020-0681-2

Martinez CP, Arumuganathan K, Kikuchi H, Earle ED (1993) Estimation of nuclear DNA content in *Oryza* by flow cytometry. Rice Genet News 10:116–119

Mayrose I, Lysak MA (2021) The evolution of chromosome numbers: mechanistic models and experimental approaches. Genome Biol Evol 13:evaa220. https://doi.org/10.1093/gbe/evaa220

McKain MR, Tang H, McNeal JR, Ayyampalayam S, Davis JI, de Pamphilis CW, Givnish TJ, Pires JC, Stevenson DW, Leebens-Mack JH (2016) A phylogenomic assessment of ancient polyploidy and genome evolution across the Poales. Genome Biol Evol 8:1150–1164. https://doi.org/10.1093/gbe/evw060

McSteen P, Kellogg EA (2022) Molecular, cellular, and developmental foundations of grass diversity. Science 377:599–602. https://doi.org/10.1126/science.abo5035

Ming R, VanBuren R, Wai CM, Tang H, Schatz MC, Bowers JE, Lyons E, Wang ML, Chen J, Biggers E, Zhang J, Huang L, Zhang L, Miao W, Zhang J, Ye Z, Miao C, Lin Z, Wang H, Zhou H, Yim WC, Priest HD, Zheng C, Woodhouse M, Edger PP, Guyot R, Guo HB, Guo H, Zheng G, Singh R, Sharma A, Min X, Zheng Y, Lee H, Gurtowski J, Sedlazeck FJ, Harkess A, McKain MR, Liao Z, Fang J, Liu J, Zhang X, Zhang Q, Hu W, Qin Y, Wang K, Chen LY, Shirley N, Lin YR, Liu LY, Hernandez AG, Wright CL, Bulone V, Tuskan GA, Heath K, Zee F, Moore PH, Sunkar R, Leebens-Mack JH, Mockler T, Bennetzen JL, Freeling M, Sankoff D, Paterson AH, Zhu X, Yang X, Smith JAC, Cushman JC, Paull RE, Yu Q (2015) The pineapple genome and the evolution of CAM photosynthesis. Nat Genet 47:1435–1442. https://doi.org/10.1038/ng.3435

Murat F, Zhang R, Guizard S, Flores R, Armero A, Pont C, Steinbach D, Quesneville H, Cooke R, Salse J (2014) Shared subgenome dominance following polyploidization explains grass genome evolutionary plasticity from a seven protochromosome ancestor with 16K protogenes. Genome Biol Evol 6:12–33. https://doi.org/10.1093/gbe/evt200

Murat F, Armero A, Pont C, Klopp C, Salse J (2017) Reconstructing the genome of the most recent common ancestor of flowering plants. Nat Genet 49:490–496. https://doi.org/10.1038/ng.3813

Murray BG, de Lange PJ, Ferguson AR (2005) Nuclear DNA variation, chromosome numbers and polyploidy in the endemic and indigenous grass flora of New Zealand. Ann Bot (Oxford) 96:1293–1305. https://doi.org/10.1093/aob/mci281

Murray BG, Weir IE, Ferguson AR, de Lange PJ (2003) Variation in DNA C-value and haploid genome size in New Zealand native grasses. New Zealand J Bot 41:63–69. https://doi.org/10.1080/0028825X.2003.9512832

Nishiwaki A, Mizuguti A, Kuwabara S, Toma Y, Ishigaki G, Miyashita T, Yamada T, Matuura H, Yamaguchi S, Rayburn AL, Akashi R, Stewart JR (2011) Discovery of natural *Miscanthus* (Poaceae) triploid plants in sympatric populations of *Miscanthus sacchariflorus* and *Miscanthus sinensis* in southern Japan. Amer J Bot 98:154–159. https://doi.org/10.3732/ajb.1000258

Oh DH, Kowalski KP, Quach QN, Wijesinghege C, Tanford P, Dassanayake M, Clay K (2022) Novel genome characteristics contribute to the invasiveness of *Phragmites australis* (common reed). Molec Ecol 31:1142–1159. https://doi.org/10.1111/mec.16293

Orton LM, Barberá P, Nissenbaum MP, Peterson PM, Quintanar A, Soreng RJ, Duvall MR (2021) A 313 plastome phylogenomic analysis of Pooideae: exploring relationships among the largest subfamily of grasses. Molec Phylogen Evol 159:107110. https://doi.org/10.1016/j.ympev.2021.107110

Panibe J, Wang L, Li J, Li MY, Lee YC, Wang CS, Ku MSB, Lu MYJ, Li WH (2021) Chromosomal-level genome assembly of the semi-dwarf rice Taichung Native 1, an initiator of Green Revolution. Genomics 113:2656–2674. https://doi.org/10.1016/j.ygeno.2021.06.006

Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci USA 101:9903–9908. https://doi.org/10.1073/pnas.0307901101

Pellicer J, Fay MF, Leitch IJ (2010) The largest eukaryotic genome of them all? Bot J Linn Soc 164:10–15. https://doi.org/10.1111/j.1095-8339.2010.01072.x

Pont C, Wagner S, Kremer A, Orlando L, Plomion C, Salse J (2019) Paleogenomics: reconstruction of plant evolutionary trajectories from modern and ancient DNA. Genome Biol 20:29. https://doi.org/10.1186/s13059-019-1627-1

Probatova NS, Sokolovskaya AP (1982) Konspekt khromosomnykh chisel Poaceae Sovetskogo Dalnego Vostoka. I. Triby Oryzeae, Brachypodieae, Triticeae. Bot Zhurn SSSR 67:62–70

Qiao X, Zhang S, Paterson AH (2022) Pervasive genome duplications across the plant tree of life and their links to major evolutionary innovations and transitions. Comput Struct Biotechnol J 20:3248–3256. https://doi.org/10.1016/j.csbj.2022.06.026

Raven PH (1975) The bases of angiosperm phylogeny: cytology. Ann Missouri Bot Gard 62:724–764. https://doi.org/10.2307/2395272

Rayburn AL, Crawford J, Rayburn CM, Juvik JA (2009) Genome size of three *Miscanthus* species. Pl Molec Biol Report 27:184–188. https://doi.org/10.1007/s11105-008-0070-3

Rice A, Mayrose I (2023) The chromosome counts database (CCDB). In: Garcia S, Nualart N (eds) Plant genomic and cytogenetic databases, Methods in molecular biology. Humana, New York, pp 123–129. https://doi.org/10.1007/978-1-0716-3389-2_10

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The chromosome counts database (CCDB)—a community resource of plant chromosome numbers. New Phytol 206:19–26. https://doi.org/10.1111/nph.13191

Roodt R, Spies JJ (2003) Chromosome studies in the grass subfamily Chloridoideae. I Basic Chromosome Numbers. Taxon 52:557–583. https://doi.org/10.2307/3647454

Saarela JM, Burke SV, Wysocki WP, Barrett MD, Clark LG, Craine JM, Peterson PM, Soreng RJ, Vorontsova MS, Duvall MR (2018) A 250 plastome phylogeny of the grass family (Poaceae): topological support under different data partitions. PeerJ 2018:e4299. https://doi.org/10.7717/peerj.4299

Salse J (2016a) Ancestors of modern plant crops. Curr Opin Pl Biol 30:134–142. https://doi.org/10.1016/j.pbi.2016.02.005

Salse J (2016b) Deciphering the evolutionary interplay between subgenomes following polyploidy: a paleogenomics approach in grasses. Amer J Bot 103:1167–1174. https://doi.org/10.3732/ajb.1500459

Salse J, Bolot S, Throude M, Jouffe V, Piegu B, Quraishi UM, Calcagno T, Cooke R, Delseny M, Feuillet C (2008) Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. Pl Cell 20:11–24. https://doi.org/10.1105/tpc.107.056309

Schneider J, Döring E, Hilu KW, Röser M (2009) Phylogenetic structure of the grass subfamily Pooideae based on comparison of plastid *matK* gene–3'*trnK* exon and nuclear ITS sequences. Taxon 58:405–424. https://doi.org/10.1002/tax.582008

Schneider J, Winterfeld G, Hoffmann MH, Röser M (2011) Duthieeae, a new tribe of grasses (Poaceae) identified among the early diverging lineages of subfamily Pooideae: molecular phylogenetics, morphological delineation, cytogenetics and biogeography.

Syst Biodivers 9:27–44. https://doi.org/10.1080/14772000.2010.544339
Schneider J, Winterfeld G, Röser M (2012) Polyphyly of the grass tribe Hainardieae (Poaceae: Pooideae): identification of its different lineages based on molecular phylogenetics, including morphological and cytogenetic characteristics. Organisms Divers Evol 12:113–132. https://doi.org/10.1007/s13127-012-0077-3
Schubert I, Lysak MA (2011) Interpretation of karyotype evolution should consider chromosome structural constraints. Trends Genet 27:207–216. https://doi.org/10.1016/j.tig.2011.03.004
Schubert M, Marcussen T, Meseguer AS, Fjellheim S (2019) The grass subfamily Pooideae: Cretaceous-Palaeocene origin and climate-driven Cenozoic diversification. Global Ecol Biogeogr 28:1168–1182. https://doi.org/10.1111/geb.12923
Schwarzacher T, Ambros P, Schweizer D (1980) Application of Giemsa banding to orchid karyotype analysis. Pl Syst Evol 134:293–297. https://doi.org/10.1007/BF00986805
Seetharam AS, Yu Y, Bélanger S, Clark LG, Meyers BC, Kellogg EA, Hufford MB (2021) The *Streptochaeta* genome and the evolution of the grasses. Frontiers Pl Sci 12:710383. https://doi.org/10.3389/fpls.2021.710383
Sharma ML (1979) Some considerations on the phylogeny and chromosomal evolution in grasses. Cytologia 44:679–685. https://doi.org/10.1508/cytologia.44.679
Slotkin RK, Nuthikattu S, Jiang N (2012) The impact of transposable elements on gene and genome evolution. In: Wendel J, Greilhuber J, Doležel J, Leitch I (eds) Plant genome diversity, Springer, Vienna, pp 35–58. https://doi.org/10.1007/978-3-7091-1130-7
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 Biol (Stuttgart) 8:73–80. https://doi.org/10.1055/s-2005-872821
Šmarda P, Bureš P, Horová L, Leitch IJ, Mucina L, Pacini E, Tichý L, Rotreklová O (2014) Ecological and evolutionary significance of genomic GC content diversity in monocots. Proc Natl Acad Sci USA 111:E4096–E4102. https://doi.org/10.1073/pnas.1321152111
Sokolovskaya AP, Probatova NS (1977) O naimenshem chisle khromosom ($2n = 4$) u *Colpodium versicolor* (Stev.) Woronow. Bot Zhurn (Moscow & Leningrad) 62:241–245
Song JM, Xie WZ, Wang S, Guo YX, Koo DH, Kudrna D, Gong C, Huang Y, Feng JW, Zhang W (2021) Two gap-free reference genomes and a global view of the centromere architecture in rice. Molec Pl 14:1757–1767. https://doi.org/10.1016/j.molp.2021.06.018
Soreng RJ, Peterson PM, Zuloaga FO, Romaschenko K, Clark LG, Teisher JK, Gillespie LJ, Barberá P, Welker CAD, Kellogg EA, Li D-Z, Davidse G (2022) A worldwide phylogenetic classification of the Poaceae (Gramineae) III: an update. J Syst Evol 60:476–521. https://doi.org/10.1111/jse.12847
Statista (2023) Statista. Available at: https://www.statista.com/statistics/263977/world-grain-production-by-type/. Accessed 28 Nov 2023
Stebbins GL (1956) Cytogenetics and evolution of the grass family. Amer J Bot 43:890–905. https://doi.org/10.1002/j.1537-2197.1956.tb11182.x
Stebbins GL (1982) Major trends of evolution in the Poaceae and their possible significance. In: Estes JR, Tyrl RJ, Brunken JN (eds) Grasses and grasslands: systematics and ecology. University of Oklahoma Press, Norman, pp 3–36
Stebbins GL (1985) Polyploidy, hybridization, and the invasion of new habitats. Ann Missouri Bot Gard 72:824–832
Strömberg CAE (2011) Evolution of grasses and grassland ecosystems. Annual Rev Earth Planet Sci 39:517–544. https://doi.org/10.1146/annurev-earth-040809-152402
Strömberg CAE, Staver AC (2022) The history and challenge of grassy biomes. Science 377:592–593. https://doi.org/10.1126/science.add1347
Tang H, Bowers JE, Wang X, Paterson AH (2010) Angiosperm genome comparisons reveal early polyploidy in the monocot lineage. Proc Natl Acad Sci USA 107:472–477. https://doi.org/10.1073/pnas.0908007107
Temsch EM, Koutecký P, Urfus T, Šmarda P, Doležel J (2022) Reference standards for flow cytometric estimation of absolute nuclear DNA content in plants. Cytometry 101:710–724. https://doi.org/10.1002/cyto.a.24495
The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768. https://doi.org/10.1038/nature08747
Thielen PM, Pendleton AL, Player RA, Bowden KV, Lawton TJ, Wisecaver JH (2020) Reference genome for the highly transformable *Setaria viridis* ME034V. G3 Genes Genomes Genet 10:3467–3478. https://doi.org/10.1534/g3.120.401345
Tkach N, Schneider J, Döring E, Wölk A, Hochbach A, Nissen J, Winterfeld G, Meyer S, Gabriel J, Hoffmann MH, Röser M (2020) Phylogeny, morphology and the role of hybridization as driving force of evolution in grass tribes Aveneae and Poeae (Poaceae). Taxon 69:234–277. https://doi.org/10.1002/tax.12204
Tkach N, Winterfeld G, Röser M (2024) Genome size variation in the Poaceae supertribe Poodae, the major grass lineage of temperate climates (tribes Aveneae, Festuceae and Poeae). Research Square (preprint). https://doi.org/10.21203/rs.3.rs-5003188/v1
Tzvelev NN, Probatova NS (2019) Zlaki Rossii. KMK Scientific Press, Moscow
Tzvelev NN, Zhukova PG (1974) O naimenshem osnovnom chisle khromosom v sem. Poaceae. Bot Zhurn (Moscow & Leningrad) 59:265–269
Vogel KP, Arumuganathan K, Jensen KB (1999) Nuclear DNA content of perennial grasses of the Triticeae. Crop Sci (Madison) 39:661–667. https://doi.org/10.2135/cropsci1999.0011183X003900020009x
Wang G, Yang Y (2016) The effects of fresh and rapid desiccated tissue on estimates of Ophiopogoneae genome size. Pl Divers 38:190–193. https://doi.org/10.1016/j.pld.2016.08.001
Wang H, Lu H (2022) Climate controls on evolution of grassland ecosystems since Late Cenozoic: a phytolith perspective. Earth Sci Rev 231:104059. https://doi.org/10.1016/j.earscirev.2022.104059
Wang X, Wang J, Jin D, Guo H, Lee TH, Liu T, Paterson AH (2015) Genome alignment spanning major Poaceae lineages reveals heterogeneous evolutionary rates and alters inferred dates for key evolutionary events. Molec Pl 8:885–898. https://doi.org/10.1016/j.molp.2015.04.004
Wang W, Mauleon R, Hu Z, Chebotarov D, Tai S, Wu Z, Li M, Zheng T, Fuentes RR, Zhang F (2018) Genomic variation in 3,010 diverse accessions of Asian cultivated rice. Nature 557:43–49. https://doi.org/10.1038/s41586-018-0063-9
Winterfeld G (2006) Molekular-cytogenetische Untersuchungen an Hafergräsern (Aveneae) und anderen Poaceae. Stapfia 86:1–170
Winterfeld G, Becher H, Voshell S, Hilu K, Röser M (2018) Karyotype evolution in *Phalaris* (Poaceae): the role of reductional dysploidy, polyploidy and chromosome alteration in a widespread and diverse genus. PLoS ONE 13:e0192869. https://doi.org/10.1371/journal.pone.0192869
Winterfeld G, Tkach N, Röser M (2023) Genome size variation and whole-genome duplications in the monocot order Poales. Research Square (preprint). https://doi.org/10.21203/rs.3.rs-3721310/v1
Winterfeld G, Tkach N, Röser M (2024) Reductional dysploidy and genome size diversity in Pooideae, the largest subfamily of

grasses (Poaceae). Research Square (preprint). https://doi.org/10.21203/rs.3.rs-4359976/v1

Xie YN, Qi QQ, Li WH, Li YL, Zhang Y, Wang HM, Zhang YF, Ye ZH, Guo DP, Qian Q, Zhang ZF, Yan N (2023) Domestication, breeding, omics research, and important genes of *Zizania latifolia* and *Zizania palustris*. Frontiers Pl Sci 14:1183739. https://doi.org/10.3389/fpls.2023.1183739

Yamamoto N, Garcia R, Suzuki T, Solis CA, Tada Y, Venuprasad R, Kohli A (2018) Comparative whole genome re-sequencing analysis in upland new rice for Africa: insights into the breeding history and respective genome compositions. Rice 11:33. https://doi.org/10.1186/s12284-018-0224-3

Yan N, Yang T, Yu XT, Shang LG, Guo DP, Zhang Y (2022) Chromosome-level genome assembly of *Zizania latifolia* provides insights into its seed shattering and phytocassane biosynthesis. Commun Biol 5:36. https://doi.org/10.1038/s42003-021-02993-3

Zappelini J, Souza LG, Guerra MP, Pescado R (2020) First cytomolecular characterization of three Neotropical woody bamboos (Bambusoideae, Poaceae) suggests ancient diploidized karyotypes. Acta Bot Brasil 34:673–679. https://doi.org/10.1590/0102-33062019abb0261

Zhang L, Cao B, Bai C (2013) New reports of nuclear DNA content for 66 traditional Chinese medicinal plant taxa in China. Caryologia 66:375–383. https://doi.org/10.1080/00087114.2013.859443

Zhou M, Xu C, Shen L, Xiang W, Tang D (2017) Evolution of genome sizes in Chinese Bambusoideae (Poaceae) in relation to karyotype. Trees 31:41–48. https://doi.org/10.1007/s00468-016-1453-y

Zonneveld BJM (2019) The DNA weights per nucleus (genome size) of more than 2350 species of the flora of The Netherlands, of which 1370 are new to science, including the pattern of their DNA peaks. Forum Geobot 8:24–78. https://doi.org/10.3264/FG.2019.1022

Zonneveld BJM (2021) Selected perennial plants do provide convenient standards for the determination of genome sizes with flow cytometry. Pl Syst Evol 307:28. https://doi.org/10.1007/s00606-021-01747-2

Zonneveld BJM, Leitch IJ, Bennett MD (2005) First nuclear DNA amounts in more than 300 angiosperms. Ann Bot (Oxford) 96:229–244. https://doi.org/10.1093/aob/mci170