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RESEARCH

Genome size variation in the Poaceae supertribe Poodae, the major grass lineage of temperate climates (tribes Aveneae, Festuceae and Poeae)

Natalia Tkach[1] · Grit Winterfeld[1] · Matthias H. Hoffmann[1] · Martin Röser[1]

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Abstract
Nuclear DNA amounts were examined by flow cytometry for 70 genera and 214 species and subspecies taxonomically belonging to the supertribe Poodae of the grass subfamily Pooideae. This supertribe contains most of the grasses distributed in the temperate extratropical regions of the world and is important because of its many cultivated species (e.g., grains) and its prominence as a major component of grasslands. The majority of holoploid genome sizes (2C values) ranged from 5 to 10 pg, with the full range of values representing a 30-fold variation. Most monoploid genome sizes (1Cx values) were between 2 and 3 pg, with a total of 12.3-fold variation. The minimum values in Poodae are only about twice those of *Brachypodium stacei*, which has the smallest genome known for the subfamily Pooideae, and those of rice and some other species of the rice subfamily (Oryzoideae) and a few species of the subfamily Panicoideae with miniature genomes. The maximum values of our study group are among the largest found within the entire family Poaceae, only slightly exceeded by a some in the tribe Triticeae (wheat and related species). The effects of polyploidy and dysploidy, their association with genome "downsizing" as well as with cases of size increase, were analyzed in both autopolyploids and allopolyploids. The origin of the low chromosome number of only $x=2$ in some Poodae species and the transition from perennial to annual life form were addressed in the light of genome sizes, which were also discussed in a phylogenetic framework.

Keywords Chromosome base number · C-value · Dysploidy · Life form · Polyploidy · Pooideae

Introduction

Eukaryotes have highly variable genome sizes, which can be measured as the length of the genome expressed in base pairs or as physical mass in grams. The genome sizes of grasses, one of the larger angiosperm families, range from 0.42 pg in the diploid *Panicum gilvum* to 45.26 pg in the decaploid *Thinopyrum ponticum* (Vogel et al. 1999; Chen et al. 2021), where these values (2C values) represent the DNA amount of DNA from non-replicated sporophytic cell nuclei with the chromosome number 2*n*. The chromosome number of *P. gilvum* is probably $2n=2x=18$ with 9 chromosomes in a single monoploid chromosome set with the base number *x*, that of *T. ponticum* is $2n=10x=70$ with $x=7$ (Vogel et al. 1999). The approximately 108-fold difference in genome size in grasses is therefore obviously not only due to the number of chromosome sets present in the nuclei (ploidy), which is only fivefold different in the two example species (diploid vs. decaploid). The main source of variation is the different amount of DNA in each chromosome, with repetitive DNA being a highly variable component, while the actual coding DNA (genes) does not show such dramatic variation between species (Ibarra-Laclette et al. 2013; Orozco-Arias et al. 2019; Ramakrishnan et al. 2021; Mhiri et al. 2022; Moreno-Aguilar et al. 2022; Kong et al. 2023). For example, the sequenced genomes of *Brachypodium*, *Panicum*, and *Setaria* species show an overall similar proportion of coding sequences between 31 and 42 Mbp, while the total amount of repetitive DNA varies between 41 and 463 Mbp. The largest contributor to the observed variation in repetitive DNA was LTR retrotransposons, followed by DNA TEs (Lei et al. 2024). As genomic units that can move around the genome,

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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 (Salle), Germany

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transposable elements were initially considered "junk DNA", but they have since been shown to play key roles in chromosome structure, gene expression and regulation, to be associated with genome rearrangements and specific chromosome features, to act as insertional mutagens, and to play essential roles in transcriptional regulation and networks, for example, in the course of biased genome fractionation following whole-genome duplications (Ågren and Wright 2011; Bourque et al. 2018; Serrato-Capuchina and Matute 2018; Anderson et al. 2019; Orozco-Arias et al. 2019; Baduel et al. 2021; Pellicer et al. 2021; Catlin and Josephs 2022; Colonna Romano and Fanti 2022; Pulido and Casacuberta 2023; Beringer et al. 2024). The role of TEs as endogenous drivers of gene expression makes them an important factor in adaptation to environmental conditions such as heat, cold, drought and herbivory. This makes genome size an important trait that influences many characteristics of organisms, independent of the genetic information encoded, such as cytological (DNA replication, cell size), functional, phenotypic, and organ size-related traits that influence metabolic and physiological performance as well as macroevolutionary patterns (e.g. Bennett 1972; Greilhuber 1995; Leitch and Bennett 2007; Greilhuber and Leitch 2013; Souza et al. 2019; Meyerson et al. 2024; Novák et al. 2020; Plačková et al. 2021; Carta et al. 2022; Théroux-Rancourt et al. 2021; Bhadra et al. 2023; Yang et al. 2023; Bureš et al. 2024; Pinto et al. 2024; Záveská et al. 2024). It has been shown in a wide range of plant families that changes in genome size have substantial consequences at the cellular, tissue, and organismal levels, influencing phenological and ecological behavior and thus providing useful information for the understanding plant evolution and diversification (Bennett 1972; Caetano-Anollés 2005; Greilhuber and Leitch 2013; Pellicer et al. 2018; Doyle and Coate 2019; Qiu et al. 2019; Cacho et al. 2021; Faizullah et al. 2021; Pfanzelt et al. 2021; D. Wang et al. 2021a, b; Zhang and Qiu 2023).

Evolutionary history. The grass family, apart from a few 'early-diverging' lineages, is comprised of two major phylogenetic groups. One of these groups is the so-called BOP clade, which consistently has the C_3 pathway of photosynthetic carbon dioxide fixation, while the other group is the PACMAD clade with frequently C_4 grasses. The BOP clade includes the subfamilies Bambusoideae (bamboo grasses), Oryzoideae (rice subfamily), and Pooideae, which is the largest subfamily of grasses with 219 genera and 4126 species (Soreng et al. 2022a) and is typical of temperate to cool climates, thus dominating the northern hemisphere. The BOP clade originated in the Late Cretaceous about 80–90 Ma ago (Schubert et al. 2019; Gallaher et al. 2019, 2022; Orton et al. 2021; Huang et al. 2022; Zhang et al. 2022) under a warm global climate with little difference between high and low latitudes, no frost, and little or no climatic seasonality. The Late Eocene brought a gradual cooling of the climate, leading to the expansion of temperate climates and finally, at the Eocene–Oligocene transition 34 Ma ago, to global cooling with increased climate seasonality. Increased diversification of the subfamily Pooideae, showing clear adaptations to temperate climates, was associated with the Cenozoic temperature drop and expansion of temperate biomes (Schubert et al. 2019). Diversification continued through the Miocene and Pliocene, giving rise to primary grasslands in both hemispheres (Edwards et al. 2010; Pimentel et al. 2017).

The largest group of the subfamily Pooideae is characterized by $x = 7$ ('core Pooideae'), a lineage that is widespread from the Mediterranean to the Arctic, represented on mountains of the tropics and regions of the southern hemisphere with temperate climate (southern Africa, South America, the cooler regions of Australia, New Zealand, and the subantarctic islands to Antarctica).

The 'core Pooideae' is divided into two phylogenetic lineages, one of which, taxonomically treated as the supertribe Triticodae, includes wheat (*Triticum*) and its relatives such as brome grass (*Bromus*), and the other as the supertribe Poodae, which comprises many species-rich and presumably actively evolving genera typical of Holarctic and boreal vegetation, such as *Agrostis*, *Calamagrostis*, *Festuca*, *Koeleria*, *Poa* and *Puccinellia*, together containing more than 1700 species. The group originated in the Middle Eocene about 41 Ma (stem age) ago, had its early diversification at about 36 Ma and continued to diversify into numerous lineages from about 29 Ma onward (Gallaher et al. 2022). Most of these are Miocene, but some are less than 10 Ma old and are of late Miocene to Pliocene age, including the large and widespread genus *Poa* and related genera important for the Holarctic flora such as *Alopecurus*, *Phleum*, *Puccinellia*, etc. (Hoffmann et al. 2013; Soreng et al. 2022b). Much of their intense speciation, often involving polyploid speciation and apomixis, is therefore contemporaneous and likely due to the Late Pliocene and especially Pleistocene climate fluctuations and corresponding shifts of climate and vegetation zones, especially in the Northern Hemisphere (glacial-interglacial cycles), which also promoted the expansion of cold-adapted species in many other angiosperm families (Tkach et al. 2008, 2014, 2019; Hoffmann and Röser 2009; Hoffmann et al. 2010; Ebersbach et al. 2017; Kadereit and Abbott 2022).

Biosystematic background. The supertribe Poodae, with approximately 142 genera and 2562 species worldwide, is sometimes taxonomically considered to comprise only the single tribe Poeae (GPWG 2001; GPWG II 2012; Kellogg 2015; Saarela et al. 2015; Soreng et al. 2017, 2022a). Based on sequence analyses of plastid DNA, the Poeae genera were grouped into two distinct lineages, which were concordant with Sanger and whole-genome sequencing analyses (Davis and Soreng 2007; Quintanar et al. 2007; Schneider et al. 2009, 2012; Saarela et al. 2015, 2017, 2018; Orton et al. 2019, 2021; Tkach et al. 2020) and did not agree with the

separation of the traditional tribes Poeae and Aveneae. However, nuclear DNA sequence studies based on Sanger markers, single copy gene and transcriptome analyses resolved lineages that were partially distinct from the plastid DNA groupings (Table 1) (Tkach et al. 2020; Huang et al. 2022; Zhang et al. 2022). Using as complete a set of associated genera as possible and a taxonomically overlapping set of plastid and nuclear DNA data, an alternative taxonomic treatment of the supertribe Poodae was proposed by recognizing three tribes with partially revised circumscriptions, namely the tribes Aveneae, Festuceae, and Poeae (Tkach et al. 2020: p. 255). This clade structure was also reflected in nuclear transcriptome analyses, in which Aveneae and Poeae appeared as the two major lineages of 'Poeae nuclear group I' (PNG I) and Festuceae as 'Poeae nuclear group II' (PNG II), named by Zhang et al. (2022).

Each of the tribes Aveneae, Festuceae and Poeae contains several subtribes, which are color-coded in Table 1. However, seven subtribes of the Poodae (Airinae, Antinoriinae, Aristaveninae, Helictochloinae, Holcinae, Scolochloinae, Sesleriinae), cannot be unambiguously assigned to any of the three tribes because of conflicting phylogenetic signals between the plastid and the nuclear DNA-based molecular phylogenies (cytonuclear discordance), indicating a possible hybrid origin in evolutionary terms (Tkach et al. 2020: Fig. 7). These conflicting phylogenetic signals also emerge from phylogenomic plastid and nuclear low-copy gene and transcriptome data (Saarela et al. 2015, 2018; Orton et al. 2019; 2021; Huang et al. 2022; Zhang et al. 2022). In some cases, no clear phylogenetic position could be determined due to insufficient resolution of the plastid and nuclear DNA trees.

To the best of our current knowledge, the phylogenetic 'roots' of the subtribes Airinae, Antinoriinae (*Antinoria* only), Holcinae (*Holcus*, *Vahlodea*), Scolochloinae (*Dryopoa*, *Scolochloa*) and Sesleriinae (six genera) suggest an ancient hybrid origin between ancestors of the tribes Aveneae and Festuceae, listed as "Incertae sedis" subtribes in Table 1. Those of the subtribes Aristaveninae (*Deschampsia* only) and Helictochloinae (*Helictochloa*, *Molineriella*), however, are more indicative of hybridization between the tribes Festuceae and Poeae, as revised from our previous conclusion (Tkach et al. 2020: p. 255). Past hybridization between Aveneae and Poeae has not yet been detected, as previously noted (Tkach et al. 2020). The above seven subtribes have been treated as taxonomically unplaced taxa and named 'intertribe hybrids' to denote their reticulate evolutionary origin.

Cases of deep hybridization also occur in several other taxonomic groups within the supertribe Poodae (Tkach et al. 2020). The subtribe Avenulinae has a hybrid origin between ancestors both belonging to the tribe Poeae, while the subtribes Anthoxanthinae, Phalaridinae and Torreyochloinae as well as *Macrobriza maxima* (syn. *Briza maxima*) have a hybrid origin between ancestors both belonging to the tribe Aveneae, and *Arctopoa* has a hybrid origin between different lineages of the tribe Poeae (see above).

To resolve the remaining phylogenetic uncertainties, more comprehensive nuclear DNA data and a more complete taxon sampling would be very useful for a reliable, molecular phylogenetically validated taxonomic classification of the supertribe Poodae and its aforementioned tribes and subtribes. This is because the nuclear genome contains the vast majority of genes and is largely responsible for the morphological, physiological and developmental characteristics of organisms compared to plastid DNA. This means that the nuclear genome is ultimately the cause of the existing morphological diversity and complexity of species, which has always been the basis for systematics and classification of taxa.

Chromosomal and genomic research in the 'core Pooideae' has focused on Triticodae due to their enormous importance in crop plant research (e.g., Miedaner and Korzun 2018; Pont et al. 2019; Tadesse et al. 2019; Bernhardt et al. 2020; Walkowiak et al. 2020; Feldman and Levy 2023b). Cytogenetic and genomic research in Poodae has focused on the genus *Avena* (oat) and comparatively few other exemplary genera such as *Aira*, *Anthoxanthum*, *Deschampsia*, *Festuca*, *Helictochloa*, *Helictotrichon*, *Phalaris*, *Sesleria*, etc., which have been studied mainly with respect to chromosome structure and polyploid evolution and *Colpodium* (including *Zingeria*), a genus that includes species with particularly low chromosome numbers ranging from $2n = 4$ to $2n = 12$ (Kotseruba et al. 2005, 2010; Kim et al. 2009; Winterfeld and Röser 2007a, b; Winterfeld et al. 2009a, b, 2012, 2018; Chumová et al. 2015, 2021; Lazarević et al. 2015; Wölk et al. 2015; Mereďa et al. 2019; Hodálova et al. 2020; González et al. 2021). Quantitative analyses of genome sizes have revealed examples of intraspecific and interspecific variation in many genera of Poodae such as *Anthoxanthum*, *Avena*, *Festuca* and *Lolium* (Murray et al. 2005; Kopecký et al. 2010; Yan et al. 2016; Chumová et al. 2015, 2021; Rios et al. 2015; Garnatje et al. 2023). This variation has often been correlated with adaptation to different environmental, especially climatic conditions, for example in species of *Dactylis*, *Festuca* and *Koeleria* (Reeves et al. 1998; Pečinka et al. 2006; Šmarda and Bureš 2006; Šmarda et al. 2008; Martínez-Sagarra et al. 2021).

Although there are approximately 760 estimates of DNA C-values in Poodae (Leitch et al. 2019), the full range of variation is still unknown, and the consistent occurrence of large genomes is by no means certain. The present study aims to address the following questions: (1) What are the sizes and variability of nuclear DNA amounts in the supertribe Poodae? (2) Does dense sampling of the larger phylogenetic groups, as well as complete sampling of smaller units, reveal differences among evolutionary lineages? (3) How do the genome sizes of the Poodae compare with those

Table 1 Summary of taxa in the supertribe Poodae studied for genome size, their classification into tribe and subtribe (Tkach et al. 2020, 2024), our sampling density, i.e., the number of sampled genera/species compared to the total number of subtribe-affiliated genera/species, and their placement in current molecular phylogenetic studies using plastid and nuclear DNA

Tribes and subtribes	Genera / species used for genome size estimates	Total number of genera and species	Placement in contemporary molecular phylogenetic studies, and genomes used						
			Saarela et al. (2015, 2018)	Orton et al. (2019, (2021)	Gallaher et al. (2019, 2022)	Tkach et al. (2020)		Huang et al. (2022)	Zhang et al. (2022)
			plastid	plastid	plastid	plastid	nuclear	nuclear	nuclear
AVENEAE		**54/1022**							
Agrostidinae	3(5)/22	9/412	Poeae clade 1	Poeae group 1	Poeae 1	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Anthoxanthinae	1/5	1/42	Poeae clade 1	Poeae group 1	N/A	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Aveninae	15/29	21/404	Poeae clade 1	Poeae group 1	Poeae 1	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Brizinae	1/2	2/6	Poeae clade 1	N/A	N/A	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Calothecinae	1/1	10/27	N/A	N/A	N/A	Clade 1	Clade 5-1	N/A	PNG1-Clade I
Echinopogoninae	1/3	4/89	N/A	Poeae group 1	Poeae 1	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Hypseochloinae	N/A	1/2	N/A	N/A	N/A	Clade 1	Clade 5-1	N/A	N/A
Paramochloinae	N/A	2/3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Phalaridinae	1/3	1/20	Poeae clade 1	Poeae group 1	N/A	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
Torreyochloinae	2/2	2/16	Poeae clade 1	Poeae group 1	Poeae 1	Clade 1	Clade 5-1	N/A	PNG1-Clade I
Macrobriza	1/1	1/1	Poeae clade 1	Poeae group 1	Poeae 1	Clade 1	Clade 5-1	PNG1-Clade I	PNG1-Clade I
FESTUCEAE		**21/721**							
Ammochloinae	N/A	1/3	N/A	Poeae group 2	N/A	Clade 2-4	Clade 4	N/A	N/A
Cynosurinae incl. Parapholiinae	7/11	9/36	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-4	Clade 4	PNG1-Clade II	PNG1-Clade II
Dactylidinae	2/3	2/4	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-4	Clade 4	PNG1-Clade II	PNG1-Clade II
Loliinae	2(3)/23	9/678	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-4	Clade 4	PNG1-Clade II	PNG1-Clade II
POEAE		**41/878**							
Alopecurinae	2/5	2/47	Poeae clade 2	Poeae group 2	N/A	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Avenulinae	1/1	1/1	N/A	N/A	N/A	Clade 2-3	Clade 5-3	N/A	PNG-2
Beckmanniinae	2/3	4/6	N/A	Poeae group 2	Poeae 2	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Brizochloinae	N/A	1/1	N/A	N/A	Poeae 2	Clade 2-3	Clade 5-3	N/A	N/A
Cinninae	1/1	4/8	N/A	Poeae group 2	Poeae 2	Clade 2-3	Clade 5-3	N/A	PNG-2
Coleanthinae	5/10	10/175	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Dupontiinae	N/A	4/8	N/A	N/A	Poeae 2	Clade 2-3	Clade 5-3	N/A	N/A
Hookerochloinae	1/2	5/13	N/A	Poeae group 2	Poeae 2	Clade 2-3	Clade 5-3	N/A	N/A
Miliinae	1/1	1/6	N/A	Poeae group 2	N/A	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Phleinae	1/8	1/16	Poeae clade 2	N/A	N/A	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Poinae	1/21–24	1/570	Poeae clade 2	Poeae group 2	N/A	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Ventenatinae	3/5	6/20	N/A	Poeae group 2	Poeae 2	Clade 2-3	Clade 5-3	PNG-2	PNG-2
Arctopoa	1/1	1/7	N/A	N/A	N/A	Clade 2-3	Clade 5-3	N/A	N/A
INCERTAE SEDIS		**18/159**							
Airinae (Av × Fe)	3/5	4/17	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-3 or 2-4	Clade 5-1	PNG1-Clade II	PNG1-Clade II*
Antinoriinae (Av × Fe)	N/A	1/2	N/A	N/A	N/A	Clade 2-4	Clade 4 or 5-3	N/A	N/A
Aristaveninae (Fe × Po)	1/9	1/51	Poeae clade 2	Poeae group 2	N/A	Clade 2-4	Clade 4 or 5-3	PNG-2	PNG-2
Helictochloinae (Fe × Po)	1/6	2/33	N/A	Poeae group 2	Poeae 2	Clade 2-4	Clade 4 or 5-3	N/A	N/A
Holcinae (Av × Fe)	2/3	2/14	Poeae clade 2	Poeae group 2	Poeae 2	Clade 2-4	Clade 5-1	N/A	PNG1-Clade I
Scolochloinae (Av × Fe)	2/3	2/3	N/A	Poeae group 2	N/A	Clade 2-4	Clade 5-1	N/A	N/A
Sesleriinae (Av × Fe)	6/12	6/39	N/A	Poeae group 2	Poeae 2	Clade 2-4	Clade 5-1	N/A	PNG1-Clade I

Av tribe Aveneae; *Fe* tribe Festuceae; *Po* tribe Poeae; *N/A* not available or not sampled in the respective study; the asterisk indicates weakly supported placement. The color coding of clades as in Tkach et al. (2020): Figs. 11, 12, S2, S3

of the better studied Triticodae, which also have $x=7$, and with those of the other tribes of the subfamily Pooideae and the other subfamilies of grasses? Can large genomes be confirmed for the Poodae? (4) How does the variation relate to polyploidy and the often observed reduction in genome size in polyploids ('genome downsizing'), and how does it relate to dysploidy, a stepwise pattern of numerical change in chromosome number occasionally found in the Poodae? (5) Finally, how does genome size relate to changes in life form, especially the transition from perennial to annual life cycles.

Material and methods

Plant material

Our sample examined for genome size included 399 accessions from 214 species and subspecies in 70 genera (Table 2; Online Resource 1). One to 10 accessions were examined for each species studied. Data were recorded separately for each accession in Online Resource 1, but were averaged in Table 2 when multiple accessions of a species were sampled, provided they belonged to the same cytotype/ploidy level (mean value). In terms of taxonomic completeness, the sampling included, representatives of 50% of the genera of the supertribe Poodae, belonging to all three of its tribes included here and 27 of the 33 subtribes (Table 1).

Fresh leaves for the genome size measurements were collected in the field, from living potted plants of our greenhouse research grass collection, or from the plant collections of the Botanical Garden of the University of Halle-Wittenberg. Leaf samples were either processed immediately or stored in plastic bags with moist tissue in a refrigerator at 4°C for up to five days until processing. Silica gel-dried leaves, preferably stored at -20°C or -80°C, were also successfully used. Voucher specimens of most accessions have been deposited in the herbarium of the University of Halle-Wittenberg (HAL). Details of the provenances studied and the type of the plant tissue used for the experiments, whether fresh or silica gel-dried, are listed in the Online Resource 1.

Measurement of genome sizes

Genome sizes were estimated by flow cytometry (FCM) following the protocols of Doležel et al. (2007), Sliwinska et al. (2022) and Loureiro et al. (2023) with minor modifications (Tkach et al. 2025). Briefly, after grinding the plant material of the test plant without (run 1 on the instrument) or together with the internal DNA standard plant (subsequent runs), cell nuclei were extracted and stained with propidium iodide (PI) using the CyStain PI OxProtect reagent kit (Sysmex Partec GmbH, Görlitz, Germany) according to the manufacturer's protocol. A CyFlow Ploidy Analyser (Sysmex Partec) with a 532 nm green laser was used for excitation and signal detection. The first run on the cytophotometer was performed to determine the G1 peak position of the test plant and the dependent selection of suitable species from our available standard plants. Measurements together with the standard were stopped after a minimum of 3,500 nuclei and replicated three times. Only histograms with coefficients 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. Usually, fresh samples were used for the measurements, but sometimes silica-gel dried samples were used (Online Resource 1). 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). Analysis of the run data and calculation of mean 2C values and standard deviations for each sample were performed using FCS Express version 5 software (De Novo Software, Pasadena, CA, U.S.A.) as described previously (Tkach et al. 2025; Winterfeld et al. 2025a, b). The following standards, with their 2C values in brackets, were used for all individual measurements as indicated in Online Resource 1: *Glycine max* Merr. 'Polanka' (2.50 pg), *Pisum sativum* L. 'Ctirad' (9.09 pg), *Raphanus sativus* L. 'Saxa' (1.11 pg), *Secale cereale* L. 'Daňkovské' (16.19 pg), *Solanum lycopersicum* L. 'Stupické polní rané' (1.96 pg), *Vicia faba* L. 'Inovec' (26.90 pg) and *Zea mays* L. 'CE-777' (5.43 pg) (Doležel et al. 2007, 2018; Temsch et al. 2022). The 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.).

Previously published DNA C-values were obtained from the 'Plant DNA C-values Database' (Leitch et al. 2019; Henniges et al. 2023; https://cvalues.science.kew.org/) and Bureš et al. (2024: Supplementary Dataset S1), with the original publications also consulted in all cases. The additional literature data used are listed in Online Resource 4.

Chromosome numbers, monoploid genome and mean chromosome sizes (1Cx values and MCs)

For many accessions used for genome size estimation, chromosomes were also counted in this study or in previous studies by our laboratory, as listed in Online Resource 1. Root tips were harvested from cultivated potted plants grown at the Botanical Garden in Halle, Germany. Pretreatment to accumulate metaphases was performed overnight in iced water, and root tips were subsequently fixed in 3:1 absolute ethanol:glacial acetic acid for at least 3 h and stored in absolute ethanol at -20°C until preparation. Enzymatically digested root tips were crushed and stained in 45% propionic

Table 2 Summary of the investigated supertribe Poodae taxa with 2C values, chromosome numbers, ploidy level, 1Cx values and mean chromosome DNA content (MC)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
AVENEAE					
Agrostidinae					
Agrostis alopecuroides Lam.	7.49	28*	4*x*	1.87	0.27
Agrostis capillaris L.	7.00	28*	4*x*	1.75	0.25
Agrostis castellana Boiss. & Reut.	10.23	42*	6*x*	1.71	0.24
Agrostis gigantea Roth	9.40	28*	4*x*	2.35	0.34
Agrostis hugoniana Rendle	13.84	N/A	N/A	N/A	N/A
Agrostis infirma Buse	8.68	N/A	N/A	N/A	N/A
Agrostis mediterranea Röser & Tkach	3.83	14*	2*x*	1.92	0.27
Agrostis nervosa Nees ex Trin.	8.51	14?	N/A	N/A	N/A
Agrostis rupestris All.	7.08	28	4*x*	1.77	0.25
Agrostis schraderiana Bech.	6.70	28	4*x*	1.68	0.24
Agrostis subspicata (Willd.) Raspail	4.76	14*	2*x*	2.38	0.34
Calamagrostis arenaria (L.) Roth	8.78	28*	4*x*	2.20	0.31
Calamagrostis arundinacea (L.) Roth	7.71	28	4*x*	1.93	0.28
Calamagrostis canescens (Weber) Roth	7.39	28	4*x*	1.85	0.26
Calamagrostis epigejos (L.) Roth	7.60	28	4*x*	1.90	0.27
Calamagrostis pseudophragmites (Haller f.) Koeler	9.08	28	4*x*	2.27	0.32
Calamagrostis purpurea (Trin.) Trin.	16.52	N/A	N/A	N/A	N/A
Calamagrostis rivalis Torges ex H.Scholz	11.45	56?	N/A	N/A	N/A
Calamagrostis scabrescens Griseb.	24.06	28?	N/A	N/A	N/A
Calamagrostis villosa (Chaix) J.F.Gmel.	7.46	28	4*x*	1.87	0.27
Gastridium phleoides (Nees & Meyen) C.E.Hubb.	11.27	28*	4*x*	2.82	0.40
Gastridium ventricosum (Gouan) Schinz & Thell.	5.43	14*	2*x*	2.72	0.39
Gastridium ventricosum (Gouan) Schinz & Thell.	11.31	28*	4*x*	2.83	0.40
Anthoxanthinae					
Anthoxanthum aristatum Boiss.	7.84	10	2*x*	3.92	0.78
Anthoxanthum australe (Schrad.) Veldkamp	9.45	14*	2*x*	4.73	0.68
Anthoxanthum monticola (Bigelow) Veldkamp	25.44	[42]	[6*x*]	4.24	0.61
Anthoxanthum nitens (Weber) Y.Schouten & Veldkamp	19.53	28	4*x*	4.88	0.70
Anthoxanthum odoratum L.	13.06	20*	4*x*	3.27	0.65
Aveninae					
Acrospelion distichophyllum (Vill.) Barberá	17.12	56*	8*x*	2.14	0.31
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl	16.55	28*	4*x*	4.14	0.59
Avena hispanica Ard.	9.95	14	2*x*	4.98	0.71
Avena macrostachya Balansa ex Coss. & Durieu	21.93	28*	4*x*	5.48	0.78
Gaudinia fragilis (L.) P.Beauv.	3.66	14*	2*x*	1.83	0.26
Graphephorum canescens (Buckley) Röser & Tkach	13.76	42*	6*x*	2.29	0.33
Helictotrichon decorum (Janka) Henrard	5.71	14*	2*x*	2.86	0.41
Helictotrichon filifolium (Lag.) Henrard subsp. *filifolium*	33.69	84*	12*x*	2.81	0.40
Helictotrichon sedenense (Clarion ex DC.) Holub subsp. *sedenense*	5.64	14*	2*x*	2.82	0.40
Helictotrichon sempervirens (Vill.) Pilg.	21.13	42*	6*x*	3.52	0.50
Helictotrichon aff. *tianschanicum* (Roshev.) Henrard	10.12	N/A	N/A	N/A	N/A
Helictotrichon tibeticum (Roshev.) Keng f.	18.48	N/A	N/A	N/A	N/A
Koeleria glauca (Spreng.) DC.	5.34	14	2*x*	2.67	0.38
Koeleria litvinowii Domin	8.59	[28]	[4*x*]	2.15	0.31
Koeleria macrantha (Ledeb.) Schult.	9.68	28*	4*x*	2.42	0.35
Koeleria pyramidata (Lam.) P.Beauv.	22.03	70*	10*x*	2.20	0.31

Table 2 (continued)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Koeleria pyramidata (Lam.) P.Beauv.	9.38	[28]	[4*x*]	2.35	0.34
Koeleria spicata (L.) Barberá, Quintanar, Soreng & P.M.Peterson	10.08	[28]	[4*x*]	2.52	0.36
Koeleria vallesiana (Honck.) Gaudin	7.75	[21]	[3*x*]	2.58	0.37
Lagurus ovatus L.	6.81	14*	2*x*	3.41	0.49
Limnodea arkansana (Nutt.) L.H.Dewey	5.51	14*	2*x*	2.76	0.39
Rostraria cristata (L.) Tzvelev	7.43	26*	4*x*	1.86	0.29
Rostraria hispida (Savi) Doğan	5.81	14*	2*x*	2.91	0.42
Sibirotrisetum sibiricum (Rupr.) Barberá	9.28	14	2*x*	4.64	0.66
Sphenopholis intermedia (Rydb.) Rydb.	5.35	14*	2*x*	2.68	0.38
Sphenopholis obtusata (Michx.) Scribn.	5.58	14*	2*x*	2.79	0.40
Tricholemma jahandiezii (Litard. ex Jahand. & Maire) Röser	32.38	28*	4*x*	8.10	1.16
Trisetopsis elongata (Hochst. ex A.Rich.) Röser & A.Wölk	17.23	28*	4*x*	4.31	0.62
Trisetum flavescens (L.) P.Beauv.	5.79	24*	4*x*	1.45	0.24
Trisetum cf. *yunnanense* Chrtek	9.36	N/A	N/A	N/A	N/A
Brizinae					
Briza media L.	6.59	14*	2*x*	3.29	0.47
Briza media L.	13.55	28	4*x*	3.39	0.48
Briza minor L.	5.73	10*	2*x*	2.87	0.57
Calothecinae					
Chascolytrum subaristatum (Lam.) Desv.	8.94	28*	4*x*	2.24	0.32
Echinopogoninae					
Pentapogon micranthus (Cav.) P.M. Peterson, Romasch. & Soreng	15.83	70*	10*x*	1.58	0.23
Pentapogon quadrifidus (Labill.) Baill. var. *quadrifidus*	14.91	56*	8*x*	1.86	0.27
Pentopogon crinitus (L.f.) P.M. Peterson, Romasch. & Soreng	16.14	70*	10*x*	1.61	0.23
Phalaridinae					
Phalaris aquatica L.	10.50	28*	4*x*	2.63	0.38
Phalaris arundinacea L.	9.82	28*	4*x*	2.46	0.35
Phalaris canariensis L.	9.43	12*	2*x*	4.72	0.79
Torreyochloinae					
Amphibromus nervosus (Hook.f.) Baill.	7.30	42*	6*x*	1.22	0.17
Torreyochloa pallida (Torr.) Church	2.48	14	2*x*	1.24	0.18
Unplaced as to subtribe					
Macrobriza maxima (L.)Tzvelev	9.32	14*	2*x*	4.66	0.67
FESTUCEAE					
Cynosurinae incl. Parapholiinae					
Catapodium marinum (L.) C.E.Hubb.	12.88	28*	4*x*	3.22	0.46
Catapodium rigidum (L.) C.E.Hubb.	6.76	14*	2*x*	3.38	0.48
Catapodium rigidum subsp. *majus* (C.Presl) F.H.Perring & P.D.Sell	6.74	14	2*x*	3.37	0.48
Ciliochloa effusa (Link) Röser, Tkach & Rasti	7.79	14*	2*x*	3.90	0.56
Cutandia maritima (L.) Barbey	7.03	14*	2*x*	3.52	0.50
Cynosurus cristatus L.	6.21	14*	2*x*	3.11	0.44
Desmazeria sicula (Jacq.) Dumort.	5.74	14*	2*x*	2.87	0.41
Falona echinata (L.) Dumort.	7.77	14*	2*x*	3.89	0.56
Parapholis cylindrica (Willd.) Romero Zarco	14.44	26*	4*x*	3.61	0.56
Parapholis filiformis (Roth) C.E.Hubb.	6.59	14*	2*x*	3.30	0.47
Parapholis incurva (L.) C.E.Hubb.	17.50	38*	6*x*	2.92	0.46
Parapholis strigosa (Dumort.) C.E.Hubb.	14.73	28*	4*x*	3.68	0.53
Dactylidinae					
Dactylis glomerata L. subsp. *glomerata*	8.52	28	4*x*	2.13	0.30

Table 2 (continued)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Dactylis glomerata subsp. *himalayensis* Domin	4.31	14	2*x*	2.16	0.31
Dactylis polygama Horv.	4.64	14	2*x*	2.32	0.33
Lamarckia aurea (L.) Moench	3.01	14*	2*x*	1.51	0.22
Loliinae					
Festuca alopecuros Schousb.	6.27	14*	2*x*	3.14	0.45
Festuca alpina Suter	4.76	14	2*x*	2.38	0.34
Festuca altissima All.	8.68	14	2*x*	4.34	0.62
Festuca drymeja Mert. & W.D.J.Koch	9.69	14	2*x*	4.85	0.69
Festuca fasciculata Forssk.	13.05	28*	4*x*	3.26	0.47
Festuca heteromalla Pourr.	16.95	42	6*x*	2.83	0.40
Festuca heterophylla Lam.	11.38	28	4*x*	2.85	0.41
Festuca heterophylla Lam.	18.13	[42]	[6x]	3.02	0.43
Festuca incurva (Gouan) Gutermann	8.27	28*	4*x*	2.07	0.30
Festuca kansuensis Markgr.-Dann.	13.74	N/A	N/A	N/A	N/A
Festuca lachenalii (C.C.Gmel.) Spenn.	10.88	14*	2*x*	5.44	0.78
Festuca leptopogon Stapf	12.04	N/A	N/A	N/A	N/A
Festuca nitidula Stapf	11.52	N/A	N/A	N/A	N/A
Festuca octoflora Walter	3.32	14*	2*x*	1.66	0.24
Festuca rubra L.	17.04	42*	6*x*	2.84	0.41
Festuca trachyphylla (Hack.) Hack.	13.87	42	6*x*	2.31	0.33
Festuca vaginata Waldst. & Kit. ex Willd.	4.87	14	2*x*	2.44	0.35
Festuca valesiaca Schleich. ex Gaudin	4.45	14	2*x*	2.23	0.32
Lolium arundinaceum (Schreb.) Darbysh. subsp. *arundinaceum*	16.63	42	6*x*	2.77	0.40
Lolium giganteum (L.) Darbysh.	20.11	42*	6*x*	3.35	0.48
Lolium perenne L.	5.67	14	2*x*	2.84	0.41
Lolium persicum Boiss. & Hohen.	7.51	14*	2*x*	3.76	0.54
Lolium subulatum Vis.	10.29	14*	2*x*	5.15	0.74
Lolium temulentum L.	8.12	14*	2*x*	4.06	0.58
POEAE					
Alopecurinae					
Alopecurus aequalis Sobol.	7.61	14	2*x*	3.81	0.54
Alopecurus cucullatus (L.) Raspail	8.64	14*	2*x*	4.32	0.62
Alopecurus myosuroides Huds.	7.16	14	2*x*	3.58	0.51
Alopecurus pratensis L.	12.73	28	4*x*	3.18	0.45
Avenulinae					
Avenula pubescens (Huds.) Dumort.	10.67	14*	2*x*	5.34	0.76
Beckmanniinae					
Beckmannia eruciformis (L.) Host	6.21	14*	2*x*	3.11	0.44
Beckmannia syzigachne (Steud.) Fernald	6.59	14*	2*x*	3.30	0.47
Pholiurus pannonicus (Host) Trin.	8.14	14*	2*x*	4.07	0.58
Cinninae					
Cinna arundinacea L.	12.40	28*	4*x*	3.10	0.44
Coleanthinae					
Catabrosa aquatica (L.) P.Beauv.	5.51	[20]	[4*x*]	1.38	0.28
Catabrosa aquatica (L.) P.Beauv.	7.65	[30]	[6*x*]	1.28	0.26
Coleanthus subtilis (Tratt.) Seidel ex Roem. & Schult.	2.71	14	2*x*	1.36	0.19
Colpodium trichopodum (Boiss.) Röser & Tkach	4.98	8*	4*x*	–	–
Colpodium versicolor Woronow ex Grossh.	2.85	4*	2*x*	1.43	0.71
Puccinellia distans (Jacq.) Parl.	8.80	42	6*x*	1.47	0.21

Table 2 (continued)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Puccinellia festuciformis (Host) Parl. subsp. *festuciformis*	8.24	42*	6*x*	1.37	0.20
Puccinellia leiolepis L.Liu	9.19	[42]	[6*x*]	1.53	0.22
Puccinellia limosa (Schur) Holmb.	2.76	14*	2*x*	1.38	0.20
Puccinellia stricta (Hook.f.) C.H.Blom	2.96	14*	2*x*	1.48	0.21
Sclerochloa dura P. Beauv.	3.26	14*	2*x*	1.63	0.23
Hookerochloinae					
Arctagrostis latifolia (R.Br.) Griseb.	24.51	56*	8*x*	3.06	0.44
Arctagrostis latifolia (R.Br.) Griseb.	36.73	84*	12*x*	3.06	0.44
Miliinae					
Milium effusum L.	9.23	28*	4*x*	2.31	0.33
Phleinae					
Phleum alpinum L.	6.55	28*	4*x*	1.64	0.23
Phleum cf. *alpinum* L.	2.77	14*	2*x*	1.39	0.20
Phleum arenarium L.	3.98	14*	2*x*	1.99	0.28
Phleum bertolonii DC.	3.56	14	2*x*	1.78	0.25
Phleum paniculatum Huds.	6.08	28*	4*x*	1.52	0.22
Phleum phleoides (L.) H.Karst.	4.01	14*	2*x*	2.01	0.29
Phleum pratense L.	8.74	42*	6*x*	1.46	0.21
Phleum rhaeticum (Humphries) Rauschert	3.13	14	2*x*	1.57	0.22
Phleum subulatum (Savi) Asch. & Graebn.	3.87	14*	2*x*	1.94	0.28
Poinae					
Poa alpina var. *vivipara* L.	6.58	N/A	N/A	N/A	N/A
Poa angustifolia L.	5.13	N/A	N/A	N/A	N/A
Poa annua L.	4.40	28	4*x*	——	–
Poa badensis Haenke ex Willd.	6.01	28*	4*x*	1.50	0.21
Poa bulbosa L.	7.08	N/A	N/A	N/A	N/A
Poa chaixii Vill.	3.15	14	2*x*	1.58	0.23
Poa cita Edgar	15.29	N/A	N/A	N/A	N/A
Poa compressa L.	5.84	N/A	N/A	N/A	N/A
Poa diaphora Trin. var. *diaphora*	9.07	N/A	N/A	N/A	N/A
Poa humilis Ehrh. ex Hoffm.	12.02	N/A	N/A	N/A	N/A
Poa laxa Haenke	8.12	[56]	[8*x*]	1.02	0.15
Poa nemoralis L.	6.25	N/A	N/A	N/A	N/A
Poa palustris L.	4.49	N/A	N/A	N/A	N/A
Poa persica Trin.	2.41	[14]	[2*x*]	1.21	0.17
Poa polycolea Stapf	10.31	N/A	N/A	N/A	N/A
Poa pratensis L.	4.93	N/A	N/A	N/A	N/A
Poa pratensis L.	6.82	N/A	N/A	N/A	N/A
Poa pratensis L.	7.61	N/A	N/A	N/A	N/A
Poa scabriculmis N.R.Cui	5.60	N/A	N/A	N/A	N/A
Poa szechuensis Rendle	5.75	N/A	N/A	N/A	N/A
Poa triodiodes (Trin.) Zotov	7.74	28*	4*x*	1.94	0.28
Poa trivialis L.	3.36	14	2*x*	1.68	0.24
Poa aff. *versicolor* subsp. *orinosa* (Keng) Olonova & G.H.Zhu	6.74	N/A	N/A	N/A	N/A
Poa sp.	5.72	N/A	N/A	N/A	N/A
Poa sp.	9.27	N/A	N/A	N/A	N/A
Poa sp.	9.50	N/A	N/A	N/A	N/A
Ventenatinae					
Apera interrupta (L.) P.Beauv.	8.68	14*	2*x*	4.34	0.62

Table 2 (continued)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Apera spica-venti (L.) P.Beauv.	9.60	14*	2*x*	4.80	0.69
Bellardiochloa variegata (Lam.) Kerguélen	7.77	14*	2*x*	3.89	0.56
Ventenata dubia (Leers) Coss. & Durieu	10.65	14*	2*x*	5.33	0.76
Ventenata macra (Steven ex M.Bieb.) Balansa ex Boiss.	12.14	14*	2*x*	6.07	0.87
Unplaced as to subtribe					
Arctopoa eminens (J.Presl) Prob.	11.75	42	6*x*	1.96	0.28
SUBTRIBUS INCERTAE SEDIS					
Airinae					
Aira caryophyllea L.	12.45	28*	4*x*	–	–
Aira elegans Willd. ex Roem. & Schult.	5.72	14	2*x*	2.86	0.41
Aira praecox L.	6.92	14	2*x*	3.46	0.49
Avenella flexuosa (L.) Drejer	11.95	28*	4*x*	2.99	0.43
Corynephorus canescens (L.) P.Beauv.	2.86	14*	2*x*	1.43	0.20
Aristaveninae					
Deschampsia argentea (Lowe) Lowe	9.62	26	4*x*	2.41	0.37
Deschampsia cespitosa (L.) P.Beauv.	9.57	26*	4*x*	2.39	0.37
Deschampsia danthonioides Munro	7.58	26*	4*x*	1.90	0.29
Deschampsia koelerioides Regel	11.24	26	4*x*	2.81	0.43
Deschampsia littoralis (Gaudin) Reut.	18.61	52	8*x*	2.33	0.36
Deschampsia media (Gouan) Roem. & Schult.	8.63	26	4*x*	2.16	0.33
Deschampsia rhenana Gremli	18.30	52	8*x*	2.29	0.35
Deschampsia setacea (Huds.) Hack.	5.25	14	2*x*	2.63	0.38
Deschampsia wibeliana (Sond. ex W.D.J.Koch) Parl.	9.96	26	4*x*	2.49	0.38
Helictochloinae					
Helictochloa agropyroides (Boiss.) Romero Zarco	30.65	70*	10*x*	3.07	0.44
Helictochloa bromoides (Gouan) Romero Zarco subsp. *bromoides*	8.40	14*	2*x*	4.20	0.60
Helictochloa hookeri (Scribn.) Romero Zarco	8.57	14*	2*x*	4.29	0.61
Helictochloa praeusta (Rchb.) Romero Zarco	35.96	112*	16*x*	2.25	0.32
Helictochloa pratensis (L.) Romero Zarco subsp. *pratensis*	38.89	126*	18*x*	2.16	0.31
Helictochloa versicolor (Vill.) Romero Zarco	7.31	14*	2*x*	3.66	0.52
Holcinae					
Holcus lanatus L.	3.36	14*	2*x*	1.68	0.24
Holcus mollis L.	4.53	28*	4*x*	1.13	0.16
Holcus mollis L.	7.50	N/A	N/A	N/A	N/A
Vahlodea atropurpurea (Wahlenb.) Hartman	6.32	N/A	N/A	N/A	N/A
Scolochloinae					
Dryopoa dives (F.Muell.) Vickery	30.76	70*	10*x*	3.08	0.44
Scolochloa festucacea (Willd.) Link	9.75	28*	4*x*	2.44	0.35
Scolochloa marchica M.Duvel, Ristow, H.Scholz	14.80	42*	6x	2.47	0.35
Sesleriinae					
Echinaria capitata (L.) Desf.	16.59	18*	2*x*	8.30	0.92
Mibora minima (L.) Desv.	10.05	14*	2*x*	5.03	0.72
Oreochloa disticha (Wulfen) Link	9.02	14*	2*x*	4.51	0.64
Psilathera ovata (Hoppe) Deyl	6.81	14	2*x*	3.41	0.49
Sesleria alba Sm.	9.04	28*	4*x*	2.26	0.32
Sesleria albicans Kit. ex Schult.	10.14	28	4*x*	2.54	0.36
Sesleria argentea (Savi) Savi	8.62	28*	4*x*	2.16	0.31
Sesleria autumnalis (Scop.) F.W.Schultz	8.83	28*	4*x*	2.21	0.32
Sesleria comosa Velen.	19.87	56*	8*x*	2.48	0.35

Table 2 (continued)

Taxon	2C value [pg]	2*n* chromosome number	Ploidy level	1Cx value [pg]	MC [pg]
Sesleria sadleriana Janka	18.49	56*	8*x*	2.31	0.33
Sesleria tenuifolia Schrad.	16.37	56	8*x*	2.05	0.29
Sesleriella sphaerocephala (Ard.) Deyl	8.10	14	2*x*	4.05	0.58

In the case of multiple accessions of the same taxon and cytotype, the mean values are given. Chromosome numbers were counted in our laboratory (asterisk) or were taken from the CCDB (2023) and original literature. Square brackets indicate inferred chromosome numbers and ploidy levels based on 2C values and available congeneric species data. The dash indicates values that were not calculated due to allopolyploidy. Online Resource 1 provides full details of samples analyzed and measurements made. *N/A* not available

acid with 2% carmine (Winterfeld et al. 2018). Chromosome preparations on the microscopic slides were examined on a Zeiss Axioskop 2 microscope, and images with well-spread metaphases were captured with a computerized CCD camera (Zeiss Axiocam HRC) using Zeiss Axiovision software. Details of the cytogenetic study, including karyotypes, images, and discussions, are in preparation for publication (G. Winterfeld et al. unpublished data). For the other taxa studied for genome size, the 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 cited from original publications (see Results and discussion and References).

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. Mean DNA content per chromosome (MC) was calculated by dividing the 2C values by the diplophasic (sporophytic) chromosome number (2*n*) or by dividing the 1C values by the haplophasic (gametophytic) chromosome number (*n*), i.e. 2C/2*n* or 1C/*n*, respectively (Tkach et al. 2025). 1Cx values were calculated for 350 out of 399 samples (88%). Despite of known chromosome number, values were not calculated for 10 samples (2.5%) due to allopolyploidy. For 39 samples (9.7%), the chromosome number (ploidy level) was unknown (Online Resource 1).

Statistical analyses

Two-way ANOVAs were calculated for the 2C, 1Cx and MC data using the stats library of the R statistical package (R Core Team 2024). Independent factors were tribe (4 levels) and life form (2 levels, annual vs. perennial) and a possible interaction term between the factors. Contrasts among all marginal means were calculated with the package emmeans (Lenth 2024), using the Tukey method to adjust *p*-values for multiple comparisons. The results of the analyses are presented in Online Resource 3.

Results and discussion

Genome and chromosome size variation in the study group Poodae

2C values. Representatives of the supertribe Poodae, including the tribes Aveneae, Festuceae, Poeae and the intertribe hybrids, had 2C values, i.e. holoploid diplophasic or sporophytic genome sizes of the non-replicated nuclei, ranging from 1.49 pg in diploid *Poa supina* to 44.75 pg in 16–18*x Anthoxanthum amarum*, respectively. All of the 214 species and 399 accessions sampled in our study fell between these minimum and maximum values, which were also found in previous studies using FCM + PI (Mao and Huff 2012; Chumová et al. 2015). According to the genome size categories of Leitch et al. (1998), 2.3% of our studied Poodae species had "very small" genomes of ≤ 2.8 pg/2C, 31.3% had "small genomes" (≤ 7 pg/2C), while most, 49%, had "medium-sized" (> 7 and < 14 pg/2C), 16.4% "large" (≥ 14 and < 35 pg/2C) and 1.4% "very large genomes" of ≥ 35 pg/2C (Tables 2, 3; Figs. 1, 2a; Online Resource 1). Compared to the rest of the subfamily Pooideae, the 2C values of the Poodae were mostly similar to those of their sister supertribe Triticodae (mostly about 6–35 pg/2C) but higher than those of the phylogenetically 'early-diverging' lineages of the subfamily Pooideae (mostly about 1–14 pg/2C) (Winterfeld et al. 2025b).

1Cx values. The genome size of the monoploid non-replicated Poodae chromosome sets found in this study ranged from 1.13 pg/1Cx in one of the *Holcus mollis* accessions to 8.30 pg/1Cx in *Echinaria capitata* (Tables 2, 3; Figs. 1, 2b, 3; Online Resource 1). However, these values were exceeded by a minimum value of 0.75 pg/1Cx found in *Poa supina* (Mao and Huff 2012) and the maximum value of 9.19 pg/1Cx found in *Anthoxanthum gracile* (Chumová et al. 2015). Thus, compared to the supertribe Triticodae, the sister lineage of the Poodae, the values were therefore not much different from those of the tribe Bromeae (mostly about 2–6 pg/1Cx), but mostly smaller

than those of the Triticeae (mostly about 5–9 pg/1Cx) (Winterfeld et al. 2025b).

Mean chromosome DNA content (MC). The calculated mean chromosome sizes of the studied Poodae ranged from 0.16 pg in the same *Holcus mollis* accession to 1.16 pg in *Tricholemma jahandiezii* (Tables 2, 3; Figs. 1, 2c; Online Resource 1), but the values were exceeded by the minimum MC of 0.11 pg previously recorded for *Poa supina* and the maximum value of 1.84 pg for *Anthoxanthum gracile* (Mao and Huff 2012; Chumová et al. 2015).

Table 3 Genome sizes (holoploid 2C and monoploid 1Cx values) and mean chromosome DNA content (MC) in Poodae tribes and subtribes

Tribes, subtribes and chromosome base numbers	2C value [pg]	1Cx value [pg]	MC [pg]
AVENEAE ($x=4, 5, 6, \mathbf{7}$)	2.48–44.75	1.22–9.19	0.18–1.84
Agrostidinae ($x=7$)	3.83–24.06	1.68–2.83	0.24–0.40
Anthoxanthinae ($x=5$)	5.52–44.75	2.46–9.19	0.53–1.78
Anthoxanthinae ($x=7$)	7.83–29.97	2.32–4.88	0.33–0.78
Aveninae ($x=7$, including hypoploids)	3.66–33.69	1.45–8.10	0.24–1.16
Brizinae ($x=5$)	5.73	2.87	0.57
Brizinae ($x=7$)	6.59–13.56	3.29–3.39	0.47–0.49
Calothecinae ($x=7$)	8.94	2.24	0.32
Echinopogoninae ($x=7$)	14.91–16.14	1.58–1.86	0.23–0.27
Phalaridinae ($x=6$)	9.43	4.72	0.79
Phalaridinae ($x=7$)	9.82–10.50	2.46–2.63	0.35–0.38
Torreyochloinae ($x=7$)	2.48–7.30	1.22–1.24	0.17–0.18
Macrobriza ($x=7$)	9.32	4.66	0.67
FESTUCEAE ($x=7$)	3.01–30.48	1.51–5.44	0.22–0.78
Cynosurinae incl. Parapholiinae ($x=7$, including hypoploids)	5.74–17.50	2.87–3.90	0.41–0.56
Dactylidinae ($x=7$)	3.01–8.52	1.51–2.24	0.22–0.35
Loliinae ($x=7$)	3.32–30.48	1.66–5.44	0.24–0.78
POEAE ($x=2, 4, 5, \mathbf{7}$)	1.49–36.73	0.75–6.07	0.11–0.90
Alopecurinae ($x=7$)	7.16–12.73	3.18–4.32	0.45–0.62
Avenulinae ($x=7$)	10.67	5.34	0.76
Beckmanniinae ($x=7$)	6.21–8.12	3.11–4.07	0.44–0.58
Cinninae($x=7$)	10.24–12.40	2.56–3.10	0.37–0.44
Coleanthinae ($x=2$)	2.85–6.96	1.16–1.80	0.58–0.90
Coleanthinae ($x=5$)	5.51–7.65	1.28–1.38	0.26–0.28
Coleanthinae ($x=7$)	2.71–10.70	1.34–1.63	0.19–0.23
Hookerochloinae ($x=7$)	20.51–36.73	3.06	0.44
Miliinae ($x=4$)	6.28	3.14	0.79
Miliinae ($x=7$)	9.23	2.31	0.33
Phleinae ($x=7$)	2.77–8.74	1.39–2.01	0.20–0.29
Poinae ($x=7$)	1.49–15.29	0.75–1.94	0.11–0.28
Ventenatinae ($x=7$)	7.77–12.14	3.89–6.07	0.56–0.87
Arctopoa ($x=7$)	11.75	1.96	0.28
SUBTRIBUS INCERTAE SEDIS ($x=4, \mathbf{7}, 9$)	2.86–38.89	1.13–8.30	0.16–0.92
Airinae ($x=7$)	2.86–12.45	1.43–3.46	0.20–0.49
Aristaveninae ($x=7$, including hypoploids)	5.25–18.61	1.89–2.81	0.29–0.43
Helictochloinae ($x=7$)	7.31–38.89	2.16–4.29	0.31–0.61
Holcinae ($x=7$)	3.36–7.50	1.13–1.68	0.16–0.24
Scolochloinae ($x=7$)	9.75–30.76	2.44–3.08	0.35–0.44
Sesleriinae ($x=7$)	6.81–19.87	2.05–5.03	0.29–0.72
Sesleriinae ($x=9$)	16.59	8.30	0.92

The most common chromosome base numbers, if there are several in a tribe, are printed in bold. For details of our data, see Table 2 and Online Resource 1. For additional data as specified in Materials and methods, see individual tribes in Results and discussion

N/A not available

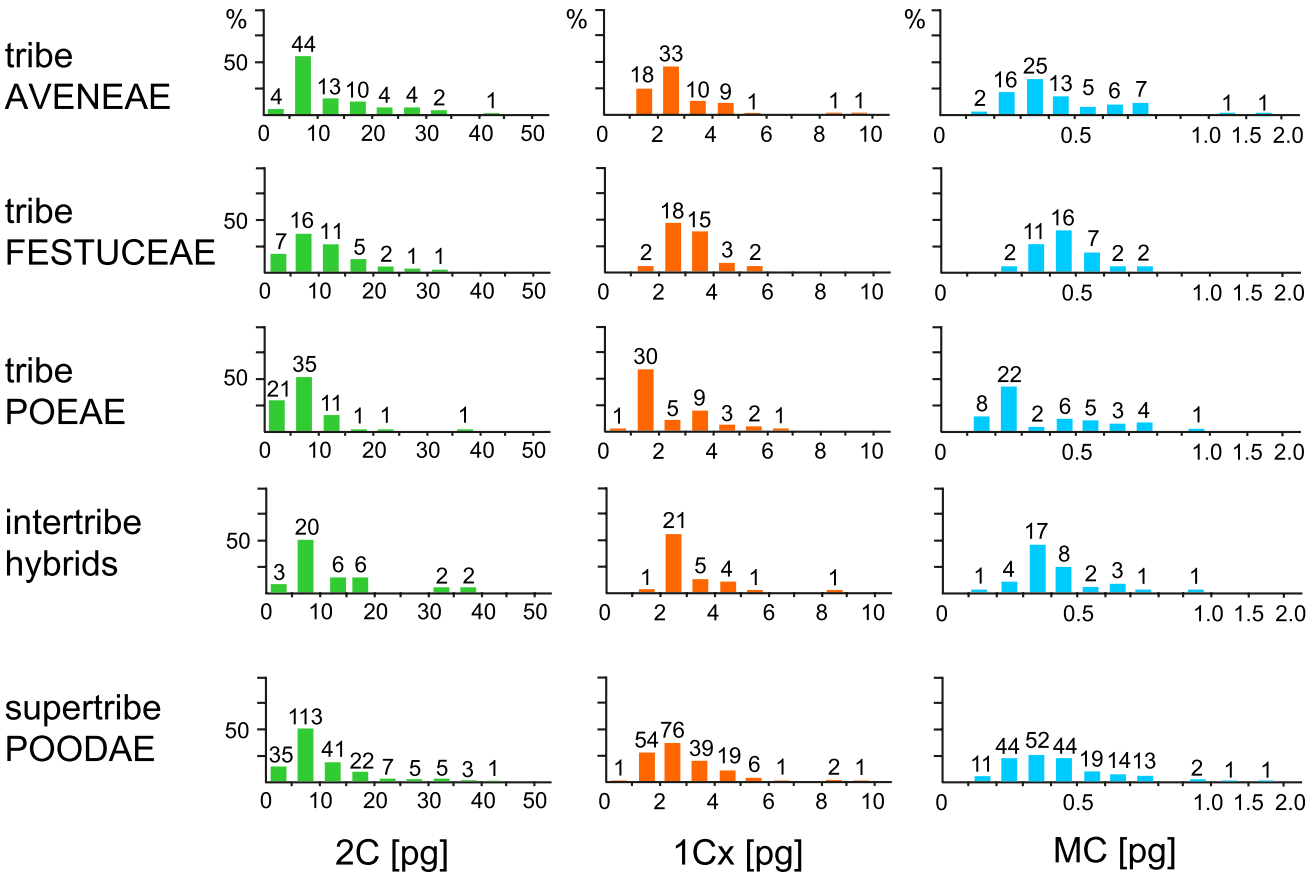


Fig. 1 Variation in genome size and chromosome DNA content in the tribes and the intertribe hybrid taxa of the supertribe Poodae analyzed in this study. The bar graphs show holoploid (2C) and monoploid (1Cx) genome sizes and mean chromosome DNA content (MC) arranged in intervals on the x-axis and the corresponding percentage estimates, which sum to 100%, on the y-axis. The number of estimates falling within each interval is shown above the corresponding bar. Data for the tribe Aveneae include supplementary genome size estimates from Murray et al. (2005) and Chumová et al. (2015) in the subtribe Anthoxanthinae, for the tribe Festuceae from Martínez-Sagarra et al. (2021) in the subtribe Loliinae, for the tribe Poeae from Murray et al. (2005) in the subtribe Cinninae, from Houben et al. (2003), Kotseruba et al. (2003, 2010) and Zonneveld (2019) in the subtribe Coleanthinae, from Zonneveld (2019) in the subtribe Miliinae and from Mao and Huff (2012) in the subtribe Poinae

The majority of the values were between 0.2 and 0.5 pg. As in the case of the 1Cx values, the MCs of the Poodae were similar to those of the tribe Bromeae (mostly ca. 0.3–0.8 pg) in the sister supertribe Triticodae, but significantly smaller than those of the Triticeae with mostly larger chromosome sizes (mosty about 0.6–1.4 pg) (Winterfeld et al. 2025b).

Tribe Aveneae and its subtribes

The 2C values in the tribe Aveneae varied from 2.48 pg (mean value) in diploid *Torreya pallida* to 33.69 pg in 14*x Helictotrichon filifolium* subsp. *filifolium* (Tables 2, 3; Figs. 1, 2a; Online Resource 1) with a maximum value of this tribe of 44.75 pg mentioned above for 16*x*–18*x Anthoxanthum amarum* (Online Resource 4), implying an approximately 18-fold variation. Most holoploid genome sizes was between 5 and 10 pg.

The monoploid genome sizes were mostly between 1.22 pg/1Cx found for *Amphibromus nervosus* and 8.1 pg/1Cx in *Tricholemma jahandiezii*, the latter value being exceeded only by 9.19 pg in *Anthoxanthum gracile* (see above). Most monoploid genome sizes were 2–3 pg/1Cx (Tables 2, 3; Figs. 1, 2b; Online Resources 1, 3).

With respect to chromosome sizes, most MCs were 0.2–0.5 pg, with the outliers of exceptionally large chromosome sizes of > 1.1 pg again in *T. jahandiezii* and *A. gracile* (Tables 2, 3; Figs. 1, 2c; Online Resources 1, 3).

Considering only the diploid Aveneae taxa, the maximum 1Cx value of species with $x = 7$ was 4.98 pg/1Cx in *Avena hispanica* ($2n = 14$) (Table 2; Online Resource 1), while the diploids with $x = 5$ had up to 9.19 pg in *Anthoxanthum gracile* ($2n = 10$) (Chumová et al. 2015).

The tribe Aveneae comprises about 54 genera and 1022 species and has a cosmopolitan distribution with a focus outside of the tropics and subtropics, namely in the temperate

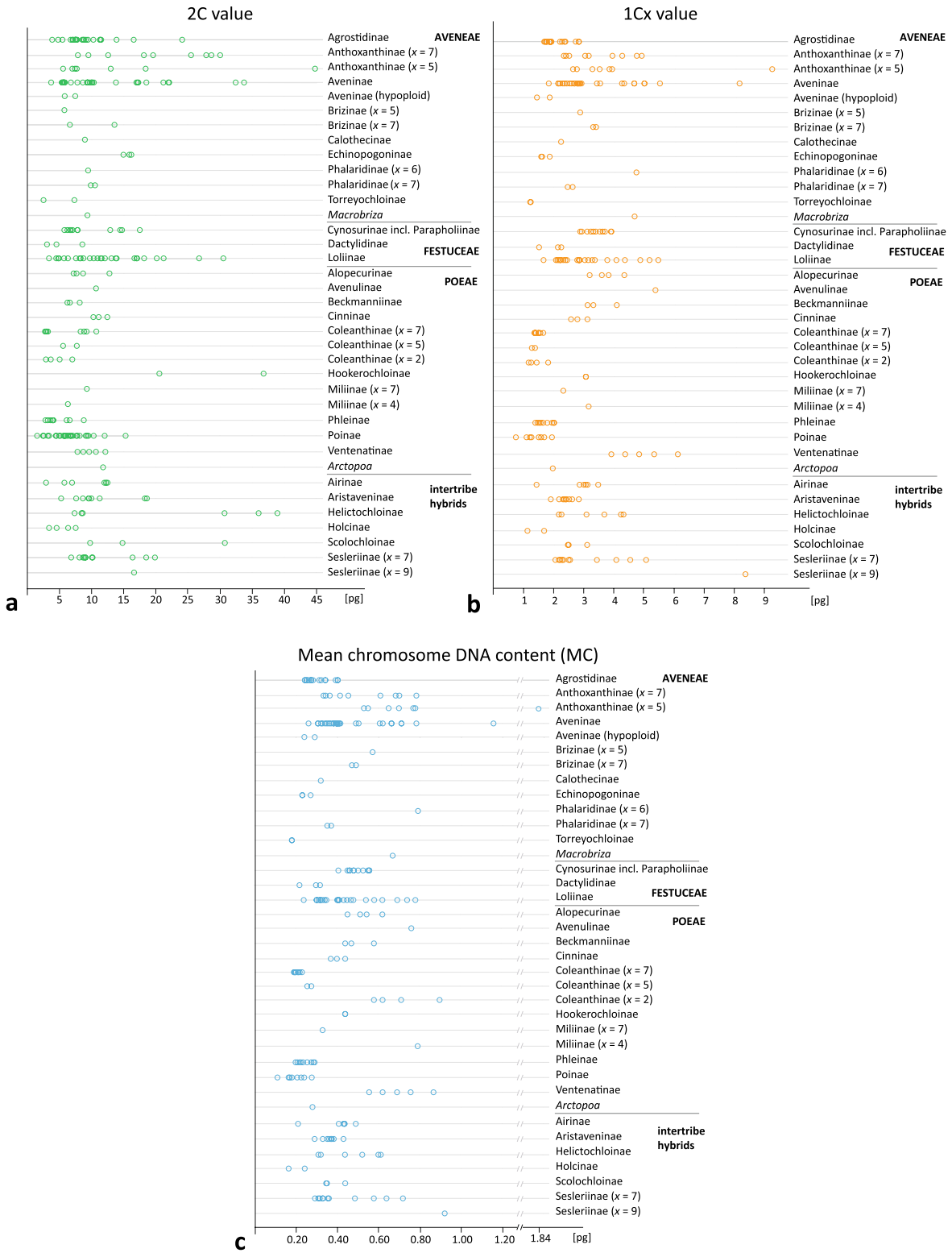


Fig. 2 Variation in genome size and chromosome DNA content in the subtribes of the supertribe Poodae. **a** Holoploid 2C genome sizes. **b** Monoploid 1Cx genome sizes. **c** Mean chromosome DNA content (MC). For our data see Table 2 and Online Resource 1, for further data as specified in Material and methods see the individual subtribes in Results and discussion. Data for the subtribe Anthoxanthinae include supplementary genome size estimates from Murray et al. (2005) and Chumová et al. (2015), for the subtribe Loliinae from Martínez-Sagarra et al. (2021), for the subtribe Cinninae from Murray et al. (2005), for the subtribe Coleanthinae from Houben et al. (2003), Kotseruba et al. (2003, 2010) and Zonneveld (2019), for the subtribe Miliinae from Zonneveld (2019), and for the subtribe Poinae from Mao and Huff (2012). These additional data are listed in Online Resource 4

climatic zones of the Earth. Representatives of eight subtribes of the Aveneae were examined, arranged in alphabetical order (Tables 1, 2, 3; Figs. 2, 4; Online Resource 1).

Agrostidinae. The genera *Agrostis* s.l. (including *Chaetopogon*, *Polypogon*), *Calamagrostis* (including *Ammophila*, *Deyeuxia*) and *Gastridium* were studied for this predominantly northern hemisphere subtribe, comprising a total of 22 examined species and 31 accessions. The 2C values ranged from 3.83 pg for the diploid *Agrostis mediterranea* (syn. *Polypogon maritimus*) to 24.06 pg for the polyploid *Calamagrostis scabrescens*. For this species, only a tetraploid chromosome number was reported (CCDB 2023), whereas judging by the 2C value, our specimen must have a higher ploidy of probably 10*x*–12*x*. The 1Cx values of Agrostidinae ranged from 1.68 pg in *Agrostis schraderiana* to 2.83 pg (mean value) in *Gastridium ventricosum*, and the MC from 0.24 pg to 0.40 pg in the same species. The highest 1Cx values and MCs of the whole subtribe were found in *G. phleoides* and *G. ventricosum* with 2.72–2.82 pg/1Cx and an MC of about 0.40 pg.

Among the *Agrostis* species studied, *A. gigantea* and *A. subspicata* (syn. *Chaetopogon fasciculatus*) had the highest 1Cx values of 2.35 pg and 2.38 pg, respectively (mean values). The two studied species of the former *Polypogon*, a genus included in *Agrostis* (Röser and Tkach 2024), the diploid *A. mediterranea* and the tetraploid *A. alopecuroides* largely agreed with the majority of *Agrostis* species with respect to their 1Cx values (1.87–1.92 pg) and MCs (both 0.27 pg). *Agrostis* species have often been placed in different sections (reviewed by Saarela et al. 2017; Peterson et al. 2020), mainly based on the presence/absence and length of the palea. However, the genome size data of representatives of both groups do not differ significantly, e.g. *A. rupestris* (short palea) with a 1Cx value of 1.77 pg and a MC of 0.25 pg compared to *A. capillaris* and *A. castellana* (long palea) with 1.71–1.75 pg/1Cx and MCs of 0.24–0.25 pg.

Most *Calamagrostis* species had 1Cx values of 1.85–2.27 pg/1Cx and MCs of 0.26–0.32 pg. Thus, the overall variation of 1Cx values and chromosome sizes (MC) was moderate and the representatives of the different lineages identified in *Calamagrostis* by molecular phylogenetics (Peterson et al. 2022), such as *C. arundinacea* (Deyeuxia group, clade B1), *C. epigejos* and *C. pseudophragmites* (Epigejos group, clade B2), *C. scabrescens* (Orientalis group, clade C1), *C. purpurea* (Purpurea group, clade C2), *C. canescens* and *C. villosa* (Calamagrostis group, clade C3), did not show clear differences, considering that chromosome numbers are not known or not reliably known for all taxa.

Calamagrostis rivalis, endemic to Saxony, Germany, has been reported to be octoploid (Heine 1970, 1972; Schiebold et al. 2009 as *C. pseudopurpurea*), but the accession we studied (11.45 pg/2C) probably had a lower ploidy level and therefore 1Cx and MC values that are probably consistent

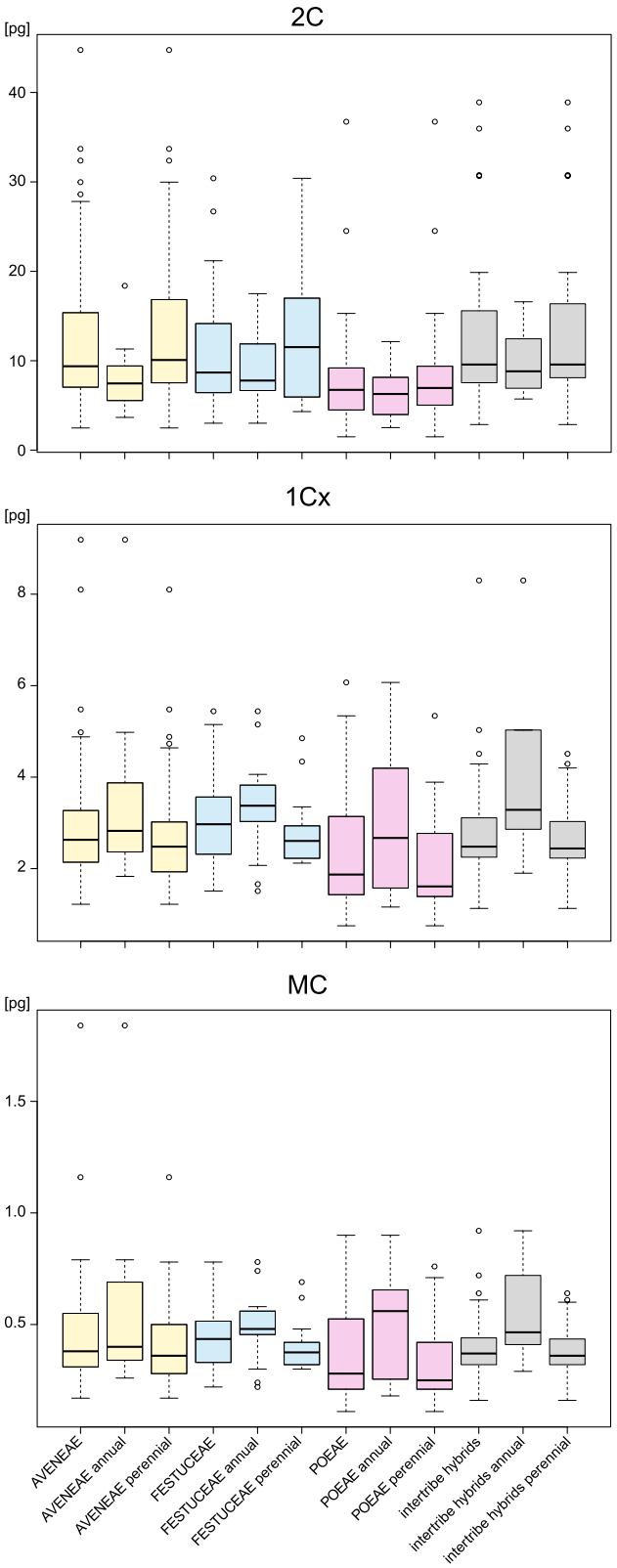
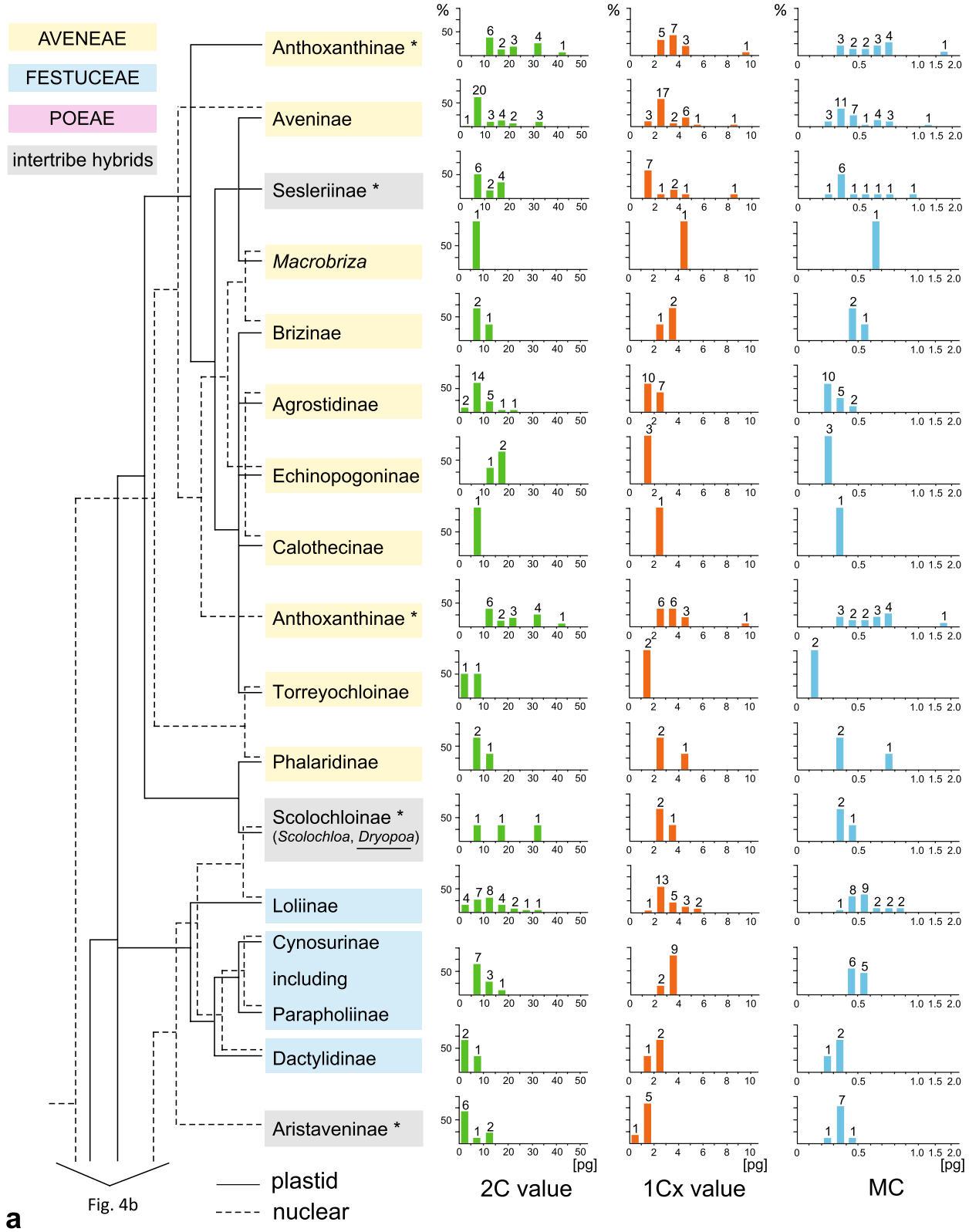


Fig. 3 Comparison of genome (2C and 1Cx values) and chromosome sizes (MC) and life forms among the studied tribes and intertribe hybrid taxa


AVENEAE
FESTUCEAE
POEAE
intertribe hybrids
Anthoxanthinae *
Aveninae
Sesleriinae *
Macrobriza
Brizinae
Agrostidinae
Echinopogoninae
Calothecinae
Anthoxanthinae *
Torreyochloinae
Phalaridinae
Scolochloinae *
(Scolochloa, Dryopoa)
Loliinae
Cynosurinae
including
Parapholiinae
Dactylidinae
Aristaveninae *
Fig. 4b
plastid
nuclear
%
[pg]
2C value
1Cx value
MC

a

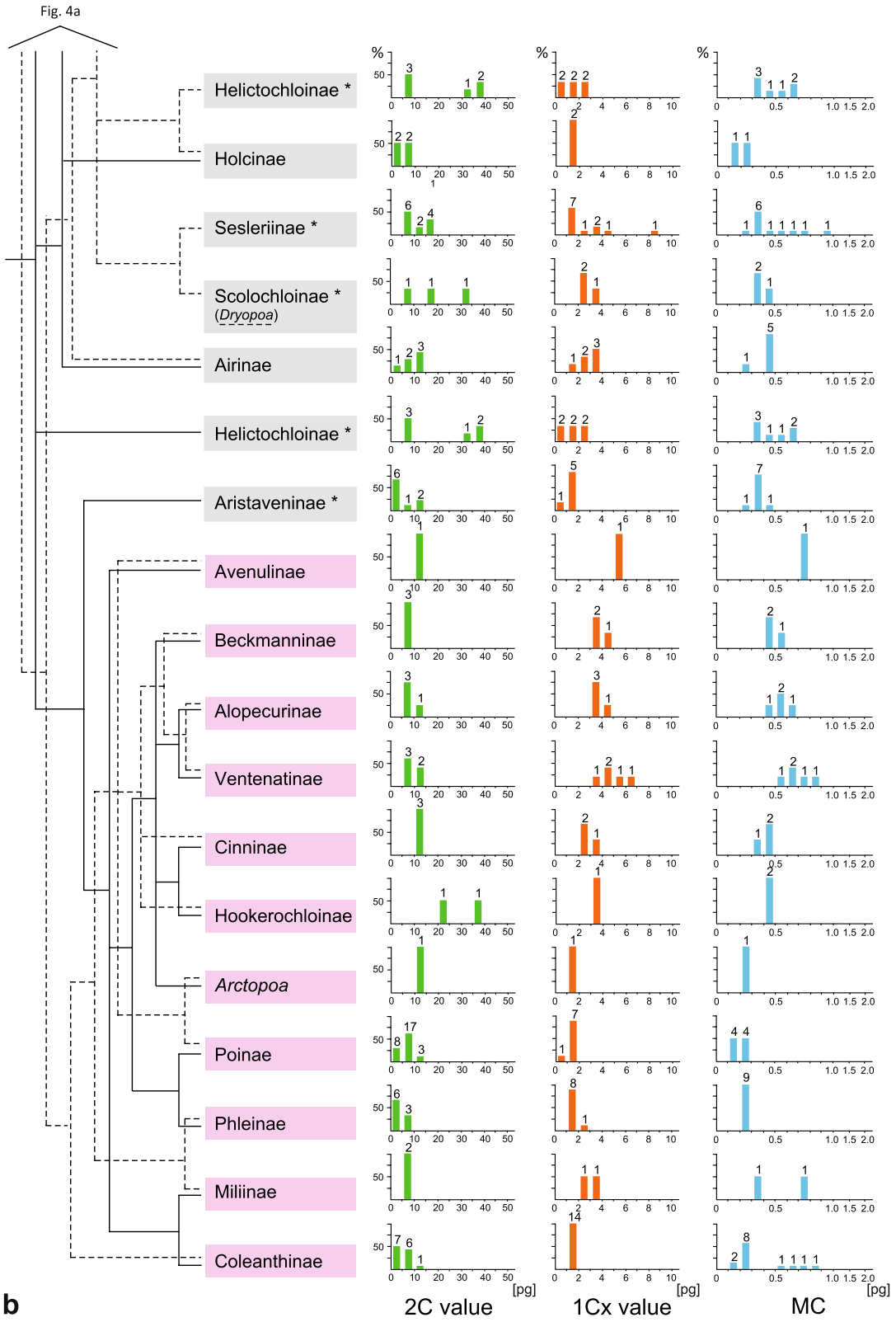


Fig. 4 (continued)

Fig. 4 Holoploid (2C) and monoploid (1Cx) genome sizes and mean chromosome DNA content (MC) arranged according to a phylogenetic tree of the subtribes belonging to the supertribe Poodae. 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 tribe. The number of estimates falling within each interval is shown above the corresponding bar. The simplified phylogenetic tree is adapted from plastome-based phylogenetic analyses (Gallaher et al. 2019, 2022; Saarela et al. 2018; Schubert et al. 2019; Orton et al. 2021). Dashed lines indicate relationships based on nuclear Sanger sequence and phylogenomic analyses (Tkach et al. 2020; Baker et al. 2022; Huang et al. 2022; Zhang et al. 2022). For our data see Table 2 and Online Resource 1. For further data as specified in Material and methods, see individual subtribes in Results and discussion. Asterisks mark subtribes that appear twice in the scheme, to show the connecting lines in each of the two phylograms without overlap. *Dryopoa* and *Scolochloa* (subtribe Scolochloinae) are sister in the nuclear DNA tree, but have different positions in the plastid DNA tree, where *Dryopoa* is sister to the Sesleriinae, whereas *Scolochloa* is sister to the Loliinae. The color coding of the subtribes indicates their tribe affiliation and is consistent with Table 1 and Tkach et al. (2020): Figs. 11, 12, S2, S3. Data for the subtribe Anthoxanthinae include supplementary genome size estimates from Murray et al. (2005) and Chumová et al. (2015), for the subtribe Loliinae from Martínez-Sagarra et al. (2021), for the subtribe Cinninae from Murray et al. (2005), for the subtribe Coleanthinae from Houben et al. (2003), Kotseruba et al. (2003, 2010) and Zonneveld (2019), for the subtribe Miliinae from Zonneveld (2019), and for the subtribe Poinae from Mao and Huff (2012). These additional data are listed in Online Resource 4

with those of the other *Calamagrostis* species. The same is true for the *C. scabrescens* accession studied (24.06 pg/2C). This species has been reported as tetraploid (CCDB 2023), but probably has a higher ploidy.

Previous studies using FCM + PI in *Agrostis capillaris*, *A. gigantea*, *A. rupestris*, *Calamagrostis arundinacea*, *C. canescens* and *C. epigeios* in accessions with probably the same ploidy level as our samples (Šmarda et al. 2013, 2019) found genome sizes comparable in magnitude to this study, although these values were consistently lower (7–16%), except for *A. rupestris*, which differed by as much as 27%, a case that needs to be re-examined. The genome size of 5.42 pg/2C for *C. arundinacea*, also determined by FCM + PI (Pustahija et al. 2013), was about 20% lower than our estimate, although both accessions were presumably tetraploid. However, the values recorded for *A. capillaris* and *C. canescens* (Zonneveld 2019) differ from our estimates by only 0.1–0.35%, while *A. gigantea* is reported to have 13.2 pg/2C instead of 9.40 pg (mean value) in this study, which is 1.4 times higher, indicating that it was obtained in an accession belonging to the 6*x* cytotype with $2n = 42$, whereas in this study the 4*x* cytotype with $2n = 28$ was used, as verified by chromosome counting for one of our three accessions studied.

However, the 2C values of 1.1 pg and 1.5 pg reported by Bai et al. (2012) for *A. gigantea*, which were also determined by FCM + PI, appear to be incorrect and are likely based on misidentification of the plants studied.

Anthoxanthinae. This subtribe consists only of the genus *Anthoxanthum* s.l. if *Hierochloe* is included, and its species have either $x = 7$ (traditional *Hierochloe*) and $x = 5$ (*Anthoxanthum* s.s.) as base numbers. The 2C values of the studied species ranged from 7.83 pg/2C (mean value) in diploids to 25.44 pg/2C in polyploids. However, the whole range in *Anthoxanthum* s.l. is even higher, ranging from 5.52 pg/2C in diploid *A. alpinum* to 44.75 pg/2C in 16*x*–18*x A. amarum* (Chumová et al. 2015), both with $x = 5$.

For the $x = 7$ taxa, the 1Cx values found were 4.73 pg in the diploid Central European *A. australe* (mean value) and 4.88 pg in the presumably tetraploid *A. nitens*, a widespread Holarctic species. The 2C value of 25.44 pg for *A. monticola* from Alaska suggests that this accession was hexaploid (6*x*) rather than octoploid (8*x*) as usually found in this species (CCDB 2023). The 1Cx value of *A. monticola* would then be 4.24 pg, which is consistent with the other $x = 7$ species. The MC of all these $x = 7$ species was 0.61–0.70 pg.

Genome sizes of *Anthoxanthum* species from the southern hemisphere were estimated by FCM + PI in five polyploid species (4*x*–12*x*, $2n = 28$–84) from New Zealand (as *Hierochloe*) (Murray et al. 2005). Their 2C values ranged from 18.10 pg to 29.97 pg, the (here calculated) 1Cx values from 2.32 pg to 3.14 pg and the MCs from 0.33 pg to 0.45 pg. Monoploid genome sizes (1Cx values) and chromosomes (MC) are thus significantly smaller than in the northern hemisphere $x = 7$ taxa. This is most likely real and is not an artifact based on methodological issues, since the DNA C-values reported by Murray et al. (2005) always agree very well with ours when the same species have been studied in both, e.g. in *Deschampsia*, *Koeleria* (*Trisetum*), *Pentapogon* (*Dichelachne*) and *Poa* (see below), and also in the grasses of the tribe Stipeae (Winterfeld et al. 2025b).

The perennial tetraploid *Anthoxanthum odoratum*, belonging to the $x = 5$ taxa, had a 1Cx value of 3.27 pg (mean value). The MC was 0.65 pg (mean value) and therefore not significantly different from that of the $x = 7$ taxa. For *A. odoratum*, 1Cx values of 3.22 pg and 3.27 pg were previously found, which were also determined by FCM (Chumová et al. 2015; Zonneveld 2019). These values agree with our results, whereas the values of 2.83 pg and 2.89 pg found by Šmarda et al. (2013, 2019) are somewhat lower. The highly polyploid perennial *A. amarum* (16*x*–18*x*) with a 2C value of 44.75 pg (Chumová et al. 2015) had a 1Cx value of 2.48–2.80 pg and an MC of 0.50–56 pg, which is lower than in tetraploids and diploids.

The 1Cx values of diploids based on $x = 5$ in the perennials *A. alpinum* and *A. maderense* averaged 2.76 pg and 3.48 pg, respectively (Chumová et al. 2015). *Anthoxanthum alpinum* thus has the smallest monoploid genome found in

this genus. The diploid annual *A. aristatum* had 3.92 pg/1Cx (mean value) (Table 2), which corresponds to the previously reported 3.83 pg (Chumová et al. 2015), while the diploid annual *A. gracile* had 9.19 pg/1Cx (Chumová et al. 2015), the highest 1Cx value found so far in the genus.

Aveninae. A total of 15 genera, 29 species and 57 accessions of the mainly northern hemisphere subtribe Aveninae were studied. The 2C values ranged from 3.66 pg in the diploid *Gaudinia fragilis* (mean value) to 33.69 pg in the dodecaploid (12*x*) *Helictotrichon filifolium* subsp. *filifolium* when both diploids and polyploids are included. The 1Cx values of the diploids ranged from 1.83 pg (mean value) in *G. fragilis* to 4.98 pg in *Avena hispanica*, both annuals. Their MCs ranged from 0.26 pg to 0.71 pg, respectively. In marked contrast, the 1Cx values of the polyploid species, considering those with $x=7$, ranged from 2.14 pg in the verified octoploid *Acrospelion distichophyllum* (syn. *Trisetum distichophyllum*) to 8.10 pg in the tetraploid *Tricholemma jahandiezii*, a narrowly distributed endemic of the Moroccan Middle Atlas with an exceptionally large monoploid genome. Both are perennials.

Comparatively high 1Cx values (4.14–5.48 pg) were found in *Arrhenatherum elatius* (mean value), *Trisetopsis elongata*, *Sibirotrisetum sibiricum* (syn. *Trisetum sibiricum*) and the aforementioned *Avena hispanica* and *A. macrostachya*, all perennials except for *A. hispanica*. Their MCs were 0.59–0.78 pg.

In the majority of the Aveninae studied (genera *Graphephorum*, *Helictotrichon*, *Koeleria*, *Lagurus*, *Limnodea*, *Rostraria*, *Sphenopholis*) the 1Cx values ranged from 2.15 pg/1Cx in *Koeleria litvinowii* to 3.52 pg/1Cx in *Helictotrichon sempervirens*.

Within the genera *Helictotrichon* and *Koeleria*, several species were examined. In *Helictotrichon*, six species and seven accessions covering the diploid to dodecaploid level, had 2C values ranging from 5.64 pg (mean value) in *H. sedenense* subsp. *sedenense* to 33.69 pg in *H. filifolium* subsp. *filifolium*. The 1Cx values ranged from 2.82 pg/1Cx (mean value) in *H. sedenense* to 3.52 pg/1Cx in *H. sempervirens*. The MCs were 0.40–0.50 pg. The chromosome numbers of *H.* aff. *tianschanicum* and *H. tibeticum* are not known, but judging from the 2C values, these species are probably tetra- and hexaploid, respectively.

A total of six species and ten accessions of the genus *Koeleria* were sampled, covering the ploidy levels of 2*x*–10*x* and including also *K. spicata* (syn. *Trisetum spicatum*). Chromosome numbers were counted for two accessions, in other cases taken from CCDB (2023), and in the case of 3*x K. vallesiana* inferred from the 2C value. The 2C values ranged from 5.34 pg/2C in diploid *K. glauca* to 22.03 pg/2C (mean value) in decaploid *K. pyramidata*. The 1Cx values were 2.67 pg/1Cx in *K. glauca* and slightly lower, 2.15–2.58 pg/1Cx, in the polyploids, and the MCs were relatively uniform in all species (0.31–0.38 pg).

Our sample of the Aveninae included two species with a dysploid chromosome number, *Trisetum flavescens* ($2n=24$) and *Rostraria cristata* ($2n=26$) with 5.79 pg/2C and 7.43 pg/2C, respectively. Assuming that they are hypotetraploids, the 1Cx values would be about 1.45 pg and 1.86 pg, respectively, which is significantly lower than in all other Aveninae polyploids. The MCs were 0.24 pg and 0.29 pg, respectively.

The previously reported 2C values of 5.20–22.89 pg for 2*x*–10*x* taxa of *Koeleria*, also estimated using FCM + PI, agree with our results, as do the 1Cx values of (recalculated) 2.29–2.60 pg/1Cx (Pečinka et al. 2006). The 2C values of 5.01–5.42 pg and 8.72 pg (Zonneveld 2019) most likely refer to diploid and tetraploid *Koeleria* accessions, respectively. 4.06 pg/2C was recorded for diploid *K. glauca*, 4.43 pg/2C for a diploid *K. macrantha* accession, and 19.94 pg/2C for 10*x K. pyramidata* (Šmarda et al. 2019), which are values slightly lower than ours, as mentioned above. The 2C value of 9.92 pg estimated for tetraploid *K. spicata* (as *Trisetum spicatum*) from New Zealand (Murray et al. 2005) agrees with our result of 10.08 pg for an accession from the Tibetan Plateau.

Comparatively low 2C values determined by FCM for other Aveninae taxa are 14.74 pg and 15.15 pg in *Arrhenatherum elatius* (Šmarda et al. 2013, 2019), 8.80 pg in *Avena hispanica* and 21.78 pg in *A. macrostachya* (both Yan et al. 2016), 3.44 pg in *Gaudinia fragilis*, 6.68 pg in *Lagurus ovatus*, 7.49 pg in *R. cristata* and 5.53 pg in *Trisetum flavescens* (Zonneveld 2019), which is in good agreement with our estimates, while the 4.71 pg/2C recorded for *T. flavescens* (Šmarda et al. 2013) is slightly lower.

Brizinae. The studied representatives of the widespread Eurasian subtribe Brizinae, excluding *Macrobriza maxima* (see below), had 2C values of 5.73 pg (mean value) in the annual Mediterranean *Briza minor* ($2n=2x=10$) and 6.59 pg/2C and 13.55 pg/2C (mean value), respectively, in the widespread Eurasian perennial *B. media* with $x=7$. The former value refers to a diploid accession of *B. media* with $2n=14$ verified by chromosome counting from the Swiss Alps, the latter to tetraploids ($2n=4x=28$) of unknown origin. The 1Cx values were 2.87 pg (mean value) in *B. minor*, 3.29 pg and 3.39 pg (mean value) in the two cytotypes of *B. media*. MCs were 0.57 pg (mean value) in *B. minor* and 0.47–0.48 pg (mean value) in *B. media*. Thus, *B. minor* with $x=5$ has an approximately 0.9-fold smaller monoploid genome than *B. media* with $x=7$, but approximately 1.2-fold more chromosomes. Previously reported 2C values were 6.68 pg, 11.92 pg and 12.38 pg for *B. media* (Šmarda et al. 2013, 2019; Zonneveld 2019), which obviously also refer to diploids and tetraploids, respectively. The value of 0.58 pg for *B. minor* (Siljak-Yakovlev et al. 2019) is most likely

a calculation or typing error, as 5.8 pg would correspond to our result.

Calothecinae. A member of this mainly South American subtribe, *Chascolytrum subaristatum* with verified $2n = 4x = 28$, had a 2C value of 8.94 pg, a 1Cx value of 2.24 pg and an MC of 0.32 pg.

Echinopogoninae. This Australasian and South American subtribe was represented by three *Pentapogon* species. *Pentapogon crinitus* and *P. micranthus*, both previously mostly assigned to their own genus *Dichelachne*, were decaploid with $2n = 10x =$ about 70, verified by chromosome counting, and had similar 2C values of 16.14 pg and 15.83 pg and 1Cx values of 1.61 pg and 1.58 pg, respectively, and an MC of 0.23 pg each. The octoploid *Pentapogon quadrifidus* var. *quadrifidus* ($2n = 8x =$ about 56) had a 2C value of 14.91 pg, a 1Cx value of 1.86 pg and an MC of 0.27 pg. These values are consistent with previous genome size reports of 16.36 pg/2C in the decaploid *P. crinitus* (as *D. crinita*) and 16.85 pg/2C in the also decaploid *P. micranthus* (as *D. micrantha*) (Murray et al. 2005).

Phalaridinae. Within the monogeneric, almost cosmopolitan subtribe Phalaridinae, the three species studied had 2C values ranging from 9.43 pg in the diploid annual *P. canariensis*, and 10.50 pg in the tetraploid perennial *P. aquatica* (mean values). *Phalaris canariensis* is characterized by the dysploid monoploid chromosome number $\boldsymbol{x = 6}$, its 1Cx was 4.72 pg and the MC was 0.79 pg (mean values). *Phalaris aquatica* and *P. arundinacea*, both with $\boldsymbol{x = 7}$, had significantly lower 1Cx values of 2.63 pg (mean value) and 2.46 pg and MCs of 0.38 pg (mean value) and 0.35 pg, respectively. Our results are consistent with previous estimates obtained using FCM + PI, i.e. 9.61 pg/2C for *P. canariensis* (Zonneveld 2019), and 9.80–10.50 pg, 9.08 pg and 10.50 pg/2C for *P. arundinacea* (Bai et al. 2012; Šmarda et al. 2019; Zonneveld 2019).

Torreyochloinae. Both genera of this subtribe were studied, the disjunct Australasian–South American *Amphibromus* and the North American–East Asian *Torreyochloa*. The hexaploid Australian *A. nervosus* had a 2C value of 7.30 pg, a 1Cx value of 1.22 pg and an MC of 0.17 pg (mean values), while the diploid American *T. pallida* had 2.48 pg/2C, 1.24 pg/1Cx and an MC of 0.18 pg (mean values). The monoploid genome sizes and mean genome sizes of both taxa are thus quite similar.

Macrobriza. This genus probably originated as a hybrid taxon between a subtribe Brizinae (maternal line) and an ancestor of the subtribe Aveninae (Tkach et al. 2020). The only species is the annual Mediterranean *M. maxima* (syn. *Briza maxima*). The mean 2C value of diploid *M. maxima* ($2n = 14$) was 9.32 pg, in good agreement with a previous genome size estimate of 9.0 pg (Zonneveld 2019). The 1Cx value of *M. maxima* was 4.66 pg and the MC was 0.67 pg. Thus, the monoploid genome of *M. maxima* is approximately 1.4–1.6 times larger and the chromosomes approximately 1.2–1.4 times larger than in *B. minor* and *B. media*.

Tribe Festuceae and its subtribes

The holoploid 2C genome sizes in the studied Festuceae taxa ranged from 3.01 pg/2C in the diploid *Lamarckia aurea* to 20.11 pg/2C in the hexaploid *Lolium giganteum* (syn. *Festuca gigantea*). The apparently highest 2C value of 30.48 pg in Festuceae was found in 14*x Festuca yvesii* (Martínez-Sagarra et al. 2021). The monoploid 1Cx values varied between 1.51 pg/1Cx in *Lamarckia aurea* and 5.44 pg/1Cx in *Festuca lachenalii* (syn. *Micropyrum tenellum*), and the chromosome sizes (MCs) varied between 0.22 pg and 0.78 pg in the same species, respectively (Tables 2, 3; Online Resource 1). The variation of all of these quantitative genome parameters was therefore very similar to that of the tribe Aveneae, as shown in the corresponding plots (Figs. 1, 2, 3; Online Resource 3).

The tribe Festuceae, comprising about 22 genera and 721 species (Table 1), is almost cosmopolitan, with the center of diversity in the temperate regions of the northern hemisphere, including the Mediterranean region. The tribe also extends into the tropics, the southern hemisphere along the Andes in South America and the high mountains of Africa.

Representatives of all subtribes of the Festuceae, except of the Ammochloinae, have been studied and are listed below in alphabetical order (Tables 1, 2, 3; Figs. 2, 4; Online Resource 1).

Cynosurinae. This subtribe, which has been merged with Parapholiinae into a single subtribe based on molecular phylogenetic analyses (Tkach et al. 2024), comprises 10–11 genera, seven of which have been studied, with a total of eleven species and 41 accessions. The 2C values of this Eurasian group, which evolved mainly in drier regions from the Mediterranean to the Middle East, ranged from 5.74 pg in the diploid *Desmazeria sicula* ($2n = 14$) to 17.50 pg (mean values) in the polyploid *Parapholis incurva* ($2n = 38$).

The 2C values of all diploids ($2n = 14$) were only moderately variable (i.e., *Catapodium rigidum*, *Ciliochloa effusa* (syn. *Cynosurus effusus*), *Cutandia maritima*, *Cynosurus cristatus*, *Desmazeria sicula*, *Falona echinata* (syn. *Cynosurus echinatus*) and *Parapholis filiformis*. The minimum values were again found in *D. sicula* (2.87 pg/1Cx, MC of 0.41 pg), the maximum values in *F. echinata* (7.77 pg/2C, 3.88 pg/1Cx, MC of 0.56 pg (mean values).

The tetraploid *Catapodium marinum* ($2n = 28$) had a 2C value of 12.88 pg, which was about twice that of the diploids. It had a similar 1Cx value of 3.22 pg and an MC of 0.46 pg (mean values).

Within the genus *Parapholis* (including *Hainardia*), the two euploid (orthoploid) species examined were two

accessions of the diploid *P. filiformis* ($2n = 14$), and one of the tetraploid *P. strigosa* ($2n = 28$) with 2C values of 6.59 pg (mean value) and 14.73 pg, 1Cx values of 3.30 pg (mean value) and 3.68 pg, and MCs of 0.47 pg (mean value) and 0.53 pg, respectively. In the dysploid and presumably hypotetraploid *P. cylindrica* (syn. *H. cylindrica*) with $2n = 4x = 26$ and the presumably hypohexaploid *P. incurva* ($2n = 6x = 38$) the 2C values were 14.44 pg and 17.50 pg, and the MCs were 0.56 pg and 0.46 pg (mean values), respectively. Assuming that these two dysploid species have essentially tetraploid and hexaploid chromosome complements, respectively, the approximate 1Cx values would be 3.61 pg for *P. cylindrica* and 2.92 pg for *P. incurva*.

The only taxa in Cynosurinae that have so far been studied with FCM appear to be *Cynosurus cristatus* with 2C values of 5.38 pg, 5.43 pg, 5.94 pg and 6.10 pg (Šmarda et al. 2008, 2013, 2019; Zonneveld 2019) and *P. strigosa* with 13.0 pg (Zonneveld 2019). Some of these values are broadly consistent with our results, while the relatively low value of 5.38 pg represents a 1.3-fold difference from our estimate.

Dactylidinae. This small subtribe is widespread in the Old World and includes the consistently perennial genus *Dactylis*, which is widespread in Eurasia and the Mediterranean, and *Lamarckia* with the single species *L. aurea*, a characteristic annual of the Saharo-Sindian and Mediterranean regions. The 2C values of the Dactylidinae ranged from 3.01 pg/2C (mean value) in the diploid *L. aurea* to 8.52 pg/2C in the tetraploid cytotype of *D. glomerata* subsp. *glomerata*.

Dactylis was represented by accessions of diploid *D. glomerata* subsp. *himalayensis* and *D. polygama* and the tetraploid *D. glomerata* subsp. *glomerata* mentioned above. The diploids had holoploid genome sizes of 4.31–4.64 pg/2C (mean values), the latter slightly less than twice as much (8.52 pg/2C). The 1Cx values and MCs of both cytotypes were almost identical, i.e. 2.24 pg/1Cx versus 2.13 pg/1Cx, while the MCs were 0.32 pg versus 0.30 pg, respectively. Several previous genome size studies in *Dactylis* using FCM + PI, and partly recognizing narrowly defined separate species or using taxonomic synonyms of *D. glomerata*, are in broad agreement with our results and recorded 2C values of 4.04–4.53pg/2C in diploids (Šmarda et al. 2008, 2019) and 7.81–9.04 pg/2C in tetraploids (Greilhuber and Baranyi 1999; Zonneveld et al. 2005; Šmarda et al. 2008, 2019; Pustahija et al. 2013; Vallès et al. 2014; Zonneveld 2019). However, slightly different and lower values have been reported for the 4*x* cytotype of *D. glomerata* without specifying the fluorescent dye (Marie and Brown 1993) and for the 2*x*, 4*x* and 6*x* cytotypes using DAPI (Horjales et al. 1995). In the latter case, the low estimates were most likely caused by the preferential binding of DAPI to AT base pairs, as opposed to the intercalating and not base pair-specific propidium iodide (Sumner 1990).

The second genus of the Dactylidinae, the monospecific *Lamarckia* with $2n = 2x = 14$ verified in *L. aurea*, had a 1Cx value of 1.51 pg (see above) and a MC of 0.22 pg (mean values), thus a significantly smaller genome and chromosome size than *D. glomerata*.

Loliinae. This large subtribe, which mainly contributes to the almost worldwide distribution of the tribe Festuceae, was sampled by 17 exemplary species of the genus *Festuca* (including *Micropyrum*, *Psilurus*, *Vulpia*) and six of *Lolium*, comprising a total of 32 accessions.

The 2C values ranged from 3.32 pg in the diploid annual *F. octoflora* (syn. *V. octoflora*) ($2n = 14$) to 20.11 pg in the hexaploid perennial *L. giganteum* (syn. *F. gigantea*) ($2n = 42$). The 1Cx values varied between 1.66 pg in *F. octoflora* and 5.44 pg (mean value) in *F. lachenalii* (syn. *M. tenellum*), also a diploid annual.

Otherwise, the 1Cx values showed a more or less continuous range of variation between 2.07 pg (mean value) in the tetraploid *F. incurva* (syn. *P. incurvus*) ($2n = 28$) and 5.15 pg in the diploid *L. subulatum* ($2n = 14$), of which two accessions each were examined. These species are both annuals like *F. octoflora* and *F. lachenalii*. In contrast, the perennials have intermediate 1Cx values with also continuous variation from 2.23 pg in *F. valesiaca* to 4.34 pg in *F. altissima* and 4.85 pg in *F. drymeja*, all of which are diploids with $2n = 14$.

Accordingly, the chromosomes (MC) were comparatively small in the annual *F. octoflora* (0.24 pg) and *F. incurva* (mean value of 0.30 pg), followed by the perennial *F. alpina, F. trachyphylla*, *F. vaginata* and *F. valesiaca*, which had MCs of 0.32–0.35 pg. The perennials *F. altissima* and *F. drymeja* had comparatively large chromosomes (MCs of 0.62–0.69 pg), surpassed only by those of the annuals *L. subulatum* (mean value 0.74 pg) and *F. lachenalii* (mean value 0.78 pg).

Chromosome numbers were verified in this study for eleven Loliinae accessions including what appears to be the first chromosome count for *L. subulatum* ($2n = 14$). One of the two *F. heterophylla* accessions studied appears to be hexaploid rather than tetraploid, as is common in this species. The new ploidy level of this accession is indicated by its 2C value, which is about 1.5 times higher than that of the other (18.13 pg/2C vs. 11.38 pg/2C).

The 2C values of *Festuca* species extensively studied by Šmarda et al. (2008), also using FCM + PI, are mostly in good agreement with our results, e.g. 4.25–4.59 pg for *F. alpina* (4.76 pg in this study), 8.04 pg for *F. altissima* (8.68 pg in this study), 9.78 pg for *F. drymeja* (9.69 pg in this study), 16.39 pg for *F. heteromalla* (16.95 pg in this study), 11.32 pg for tetraploid *F. heterophylla* (11.38 pg in this study), 4.90 pg for *F. vaginata* (4.87 pg in this study), 16.98–17.22 pg for *Lolium arundinaceum* (as *F. arundinacea*) (16.63 pg in this study), 20.75 pg for *L. giganteum* (as *F. gigantea*) (20.11 pg in this study) and 5.51 pg for

the diploid cytotype of *L. perenne* (5.67 pg in this study). However, the 2C values of 5.72 pg and 5.04 pg recorded for *L. temulentum* (Šmarda et al. 2008, 2019), possibly from the same accession, do not agree with our estimates of 7.98 pg/2C and 8.25 pg/2C for two different accessions, which also confirm a previous estimate of 8.27 pg/2C (Zonneveld 2019).

Furthermore, the 2C values of 9.15 pg for *F. heterophylla* (Šmarda et al. 2019), 4.02 pg for *F. valesiaca* (4.45 pg in this study) (Šmarda et al. 2019), 18.3 pg and 17.3 pg for *L. giganteum* (as *F. gigantea*) (Zonneveld 2019; Šmarda et al. 2019) (20.11 pg in this study), 15.59 pg, 15.94 pg, 17.45 pg and 17.3 pg for *L. arundinaceum* (as *F. arundinacea*) (Arumuganathan et al. 1999; Loureiro et al. 2007; Kopecký et al. 2010; Zonneveld 2019), 5.36 pg, 4.93 pg, 5.26 pg, 5.56 pg and 5.51 pg for diploid *L. perenne* (Kopecký et al. 2010; Šmarda et al. 2019; Zonneveld 2019; Frei et al. 2021 using a double-haploid plant; Moreno-Aguilar et al. 2022) and 6.4 pg for *L. persicum* (Moreno-Aguilar et al. 2022) (7.51 pg in this study) are broadly consistent with our estimates.

Genome sequencing studies have reported lengths of approximately 2.26 Gbp (≈ 4.6 pg/2C), 2.467 Gbp (≈ 5.04 pg/2C) and 2.55 Gbp (≈ 5.21 pg/2C) for the *Lolium perenne* genome (Byrne et al. 2015; Frei et al. 2021; Nagy et al. 2022), with the more recent data closer to the value of 5.67 pg/2C in this study (see above) than the older ones, and moreover, Frei et al. (2021) also considered the genome size of 5.44 pg/2C they determined by FCM to be more reliable than their estimate by sequencing (see above).

The above values for *L. arundinaceum* all refer to the hexaploid cytotype ($2n = 6x = 42$), which was also examined in this study. However, *L. arundinaceum* includes additional $4x$, $8x$ and $10x$ cytotypes (Bulińska-Radomska and Lester 1986). The octo- and decaploids are often referred to as taxonomically distinct varieties or subspecies. Their 2C values were also determined by FCM + PI as 16.22 pg in the octoploid subsp. *atlantigenum* and 19.70 pg in the decaploid var. *letourneuxianum*, while the hexaploid subsp. *corsicum* had 16.62 pg (Ezquerro-López et al. 2017 sub *Festuca*). The latter is in good agreement well with the values for the two hexaploid accessions we studied (16.54–16.72 pg/2C), although they belonged to the type subspecies/variety.

Considering the whole range of variation in holoploid genome sizes within the subtribe Loliinae, the highest known 2C values occur in dodeca- and tetradecaploid accessions of *F. yvesii* subspecies from the Iberian Peninsula, namely 26.69 pg and 30.40 pg (mean values calculated here), respectively (Martínez-Sagarra et al. 2021), while the lowest value seems to be that of the aforementioned diploid *F. octoflora*, giving a total range of 3.25–29.7 Gbp and making the total variation in the Loliinae somewhat larger than previously thought (Moreno-Aguilar et al. 2022).

Regarding the classification of *Festuca* and its segregate genera, it can be noted that some of the different infraspecific taxa (sections or subsections) seem to have relatively characteristic and different monoploid genome sizes: The species of *F.* subsect. *Festuca* had 1Cx values of 2.23–2.44 pg (2*x F. alpina*, 6*x F. trachyphylla*, 2*x F. vaginata*, 2*x F. valesiaca*), those of sect. *Phaeochloa* 4.34–4.85 pg (2*x F. altissima*, 2*x F. drymeja*) and those of sect. *Aulaxyper* 2.83–3.02 pg (6*x F. heteromalla*, 4*x* and 6*x F. heterophylla*, 6*x F. rubra*).

Considering the taxa often ascribed to *Vulpia*, the low 2C value of 3.32 pg of the diploid *F. octoflora* is interesting from a biogeographical and phylogenetic point of view, as this species is one of only four native American species (Soreng et al. 2000 onwards, Stace 2022) of this segregated genus. *Vulpia* has a center of diversity mainly in the Mediterranean, a region, where 17 of the 21 species occur (Euro+Med PlantBase 2006 onwards; Stace 2022). Compared to *F. octoflora*, the only American *Vulpia* species whose genome size has been studied so far, the diploid Old World Vulpias have 1.5–1.9 times larger genomes of about 5.11–6.27 pg/2C (see also Šmarda et al. 2008, 2019; Zonneveld 2019). *Festuca octoflora* (1.16 pg/1Cx) is taxonomically placed in the same section, i.e. the sect. *Vulpia* (Stace 2022), as 2*x V. bromoides* (5.12–5.86 pg/2C and 2.66–2.93 pg/1Cx), 4*x V. ciliata* (8.28–8.99 pg/2C and 2.06–2.25 pg/1Cx) and 6*x V. myuros* (12.1–13.86 pg/2C and 2.21–2.31 pg/1Cx). However, molecular phylogenetic data did not support this relationship, but showed that *F. octoflora* (North America and Cono Sur) and the other American Vulpias studied to date are closer to American fescues than to Old World *Vulpia*, revealing that traditional *Vulpia* is polyphyletic and most likely polytopic in origin (Inda et al. 2008; Díaz-Pérez et al. 2014; Minaya et al. 2017). This phylogenetic background is consistent with the large genome size difference between *F. octoflora* and the Old World Vulpias, so that at least the phylogenetically close South American 2*x F. australis* (syn. *V. australis*) may also have a small genome, possibly also the North American 6*x F. microstachys* (syn. *V. microstachys*) and other related American taxa (clade I_{FL6} of Díaz-Pérez et al. 2014). This suggests that the origin of the American *F. octoflora* (and its relatives?) was accompanied by a genome size reduction similar to that found in the Old World *Vulpia* (Šmarda et al. 2008), but even larger.

In the Old World *Vulpia*, it has been suggested that the origin of the hexaploids is based on allopolyploidy (Stace 2005). In particular, in the case of 6*x V. myuros*, an origin from a diploid species such as *V. muralis* and a tetraploid member of the *V. ciliata-Psilurus incurvus* clade has been suggested. Although the genome size of *V. muralis* is still unknown, that of the presumably closely related 2*x V. bromoides* is known to be 5.48 pg/2C, that of 4*x V. ciliata* 8.70 pg/2C (mean value), that of 6*x V. myuros* 13.60 pg/2C (mean value) (Šmarda et al. 2008; Zonneveld 2019) and that of

4x *F. incurva* (syn. *Psilurus incurvus*) 8.27 pg/2C. The additivity of the found genome sizes of the diploids and tetraploids (Online Resource 2a) would be compatible with the proposed origin of the hexaploid *V. myuros*, with the caveat that the genome sizes of other potential parental species are not known.

Tribe Poeae and its subtribes

The 2C values ranged from 2.41 pg in the diploid *Poa persica* to 36.73 pg in the 12x cytotype of *Arctagrostis latifolia* (Tables 1, 2, 3; Figs. 2, 4; Online Resource 1). The apparently smallest 2C value recorded for Poeae to date was 1.49 pg/2C in *P. supina* (Mao and Huff 2012).

The 1Cx values ranged from 0.75 pg/1Cx in *Poa supina*, 1.21 pg/1Cx in *Poa persica* to 6.07/1Cx in *Ventenata macra*, all of which are diploids with $2n = 2x = 14$. Their MCs were therefore 0.11–0.87 pg.

The largest chromosomes known so far for the Poeae occurred in the dysploid *Colpodium biebersteinianum* ($2n = 2x = 4$) with an MC of 0.90 pg (mean value), corresponding to the recorded genome sizes of 3.5–3.7 pg/2C (as *Zingeria biebersteiniana*), also estimated by using FCM + PI (Houben et al. 2003; Kotseruba et al. 2003, 2010).

The variation in all of these quantitative genome parameters therefore was therefore very similar to that of the tribe Aveneae, as shown in the corresponding plots (Figs. 1, 2).

The tribe Poeae comprises about 41 genera and 878 species (Table 1) and is distributed in temperate to often rather cool climates of both the northern and southern hemispheres, reaching the Arctic and Antarctic zones. Ecologically, this group appears to have a wide amplitude. It often grows in wetlands, but also occurs in forests, at high altitudes, and in relatively arid habitats.

Alopecurinae. The almost cosmopolitan Alopecurinae were represented in this study by four species of the genus *Alopecurus*, which comprises altogether about 44 species worldwide in temperate climates, while the small Asian, mainly Siberian to Arctic genus *Limnas* was not sampled. The 2C values ranged from 7.16 pg and 7.61 pg in the diploid annuals *A. myosuroides* and *A. aequalis* (both $2n = 14$), respectively, to 12.73 pg (mean value) in a tetraploid accession of the perennial *A. pratensis* ($2n = 28$). *Alopecurus myosuroides* had a 1Cx value of 3.58 pg and an MC of 0.51 pg, which were similar to those of *A. pratensis* with 3.18 pg/1Cx and an MC of 0.45 pg and those of *A. aequalis* with 3.81 pg/1Cx and an MC of 0.54 pg.

Previous FCM + PI data for *Alopecurus* were 7.2 pg/2C and 7.06 pg/2C for *A. aequalis*, 7.74 pg/2C and 7.52 pg/2C for *A. myosuroides*, 13.25 pg/2C and 12.6 pg/2C for *A. pratensis* (Wentworth et al. 2004; Zonneveld 2019), which are in agreement with our results, while those of 5.88 pg in *A. aequalis* and 6.58 pg in *A. myosuroides* (Šmarda et al. 2019) and 11.19 pg in *A. pratensis* (Šmarda et al. 2013) are again lower. Taxonomically, all of our studied species represent at the same time different sections of *Alopecurus*:

The annual *A. cucullatus* (syn. *Cornucopiae cucullatum*) had a higher 1Cx value of 4.32 pg and a higher MC of 0.62 pg (mean values) than the other studied *Alopecurus* species.

Although our sample of *Alopecurus* species is limited to four species, three different sections of this genus (Tzvelev 1976; Doğan 1999; Cabi et al. 2017; Tzvelev and Probatova 2019; Gnutikov et al. 2024) are represented: (1) sect. *Pseudophalaris* with diploid *A. myosuroides* (3.58 pg/1Cx), (2) sect. *Alopecurium* with diploid *A. aequalis* (3.81 pg/1Cx) and (3) sect. *Alopecurus* with tetraploid *A. pratensis* (3.18 pg/1Cx) and diploid *A. cucullatus* (4.32 pg/1Cx), a species that presumably also belongs to this section based on its placement in the trees of molecular phylogenetic analyses (Boudko 2014). The tetraploid *A. pratensis* has a smaller calculated monoploid genome size than all diploids of *Alopecurus* studied, especially with respect to the presumably closely related *A. cucullatus*. This may indeed reflect "genome downsizing" following polyploidization, as previously suggested for the tetraploid *A. geniculatus* (Wentworth et al. 2004), but molecular phylogenetic data support a possible hybrid origin of the sect. *Alopecurus* and the presence of different maternal lines in *A. pratensis* (Cabi et al. 2017; Gnutikov et al. 2024). This implies that allopolyploidy involving the donor of a small genome, rather than genome downsizing, may indeed be the case in *A. pratensis* (Table 5 in Online Supplement 2), which is supported by meiotic studies suggesting segmental allopolyploidy for this species (Sieber and Murray 1979; Wentworth et al. 2004).

Avenulinae. *Avenula pubescens* ($2n = 2x = 14$), the only species of this monospecific subtribe, had a comparatively large holoploid genome (10.67 pg/2C), a 1Cx value of 5.34 pg and an MC of 0.76 pg. A quite similar value of 10.1 pg/2C was found previously (Zonneveld 2019 as *Helictotrichon pubescens*), while 8.38 pg and 9.40 pg (Šmarda et al. 2013, 2019) again were lower.

Beckmanniinae. This small subtribe with only four genera was studied using the genera Beckmannia, with two species in the Holarctic, and the monospecific *Pholiurus* from western Eurasia, both characterized by $2n = 2x = 14$. The two *Beckmannia* species examined, with two accessions each of the perennial *B. eruciformis* and the perennial to annual *B. syzigachne*, had fairly uniform 2C values of 6.21–6.59 pg, 1Cx values of 3.11–3.30 and MCs of 0.44–0.47 pg (mean values). For *B. eruciformis*, 5.37 pg/2C was previously reported (Šmarda et al. 2019), a value lower than ours. *Pholiurus pannonicus* had a larger holoploid genome than *Beckmannia*, with 8.14 pg/2C, a 1Cx value of 4.07 pg and an MC of 0.58 pg (mean values).

Cinninae. The subtribe Cinninae, which includes the widespread Holarctic *Cinna* and four other genera with disjunct distributions (Gillespie et al. 2022), was represented in this study only by *C. arundinacea* with a verified $2n = 4x = 28$, the 2C value of 12.40 pg, the 1Cx value of 3.10 pg and an MC of 0.44 pg. Three previously examined *C. arundinacea* accessions were found to have 10.6–10.8 pg/2C (Bai et al. 2012).

For the endemic New Zealand genus *Simplicia*, a southern hemisphere outlier of the Cinninae, 2C values of 11.07 pg and 10.24 pg were found for two of its three species, *S.* buchananii and *S. laxa*, respectively (Murray et al. 2005). Both are tetraploid, therefore their 1Cx values are 2.77 pg and 2.56 pg and the MCs are 0.40 pg and 0.37 pg, all in remarkable similarity to the values of the northern hemisphere *Cinna*.

Coleanthinae. This subtribe has an almost worldwide distribution and represents a lineage with about ten genera, of which five were studied. The 2C values ranged from 2.71 pg in the annual diploid *Coleanthus subtilis* to 8.80 pg (mean values) in the hexaploid Puccinellia *distans* and 9.19 pg in the, judging by the 2C value, also hexaploid *P. leiolepis*. An octoploid accession of *P. maritima* had a 2C value of 10.70 pg (Zonneveld 2019), the highest value recorded so far for the subtribe Coleanthinae. However, available chromosome counts indicate that up to 11-fold polyploidy occurs in the Coleanthinae (CCDB 2023), suggesting that the maximum 2C value in this subtribe may actually be somewhat higher.

The subtribe Coleanthinae is characterized by the occurrence of three different base numbers, $x = 2$, 5 and 7. The presumably phylogenetically original $\underline{x = 7}$ is found in the genera *Puccinellia* with five sampled species and *Sclerochloa* with only one species (monospecific genus). The short-lived small *Coleanthus subtilis* had the lowest 1Cx value of 1.36 pg and an MC of 0.19 pg, followed by diploid to hexaploid, perennial *Puccinellia* species with 1Cx values of 1.37–1.53 pg and an MC of 0.20–0.22 pg. The six examined accessions of the diploid annual *S. dura* had the highest 1Cx values of 1.63 pg and an MC of 0.23 pg (mean values).

Catabrosa aquatica, characterized by a monoploid chromosome set of $\underline{x = 5}$, was represented by one tetraploid and one hexaploid accession, as judged by the 2C values. The 1Cx values were 1.38 and 1.28 pg, the MCs were 0.28 pg and 0.26 pg, respectively.

The two *Colpodium* (syn. *Zingeria*) species studied, the diploid *C. versicolor* ($2n = 4$) and the tetraploid *C. trichopodum* ($2n = 8$) represent the $\underline{x = 2}$ lineage. Their 2C values were 2.85 pg and 4.98 pg. The 1Cx value of *C. versicolor* was 1.43 pg and its MC was 0.71 pg, while these values were not calculated for $4x$ *C. trichopodum*, an allopolyploid containing differently sized monoploid genomes (Kotseruba et al. 2003), as detailed below (see chapter "Dysploidy…"). These data are broadly consistent with a previous genome size estimate also using FCM + PI of 2.40 pg/2C in *C. versicolor* (Kotseruba et al. 2005). However, an estimate of 5.30 pg/2C recorded for *C. trichopodum* (as *Z. trichopoda*) (Kotseruba et al. 2003) actually refers to *C. pisidica* (Kotseruba et al. 2010), a close relative of *C. trichopodum* that was not previously considered a separate species. In *C. biebersteinianum* ($2n = 4$), genome sizes of 3.5 pg/2C and (recalculated) 3.7 pg/2C, also determined by FCM + PI, were recorded (Kotseruba et al. 2003; Houben et al. 2003), while the hexaploid *C. kochii* (as *Z. kochii*) ($2n = 12$) with 6.96 pg/2C had the largest holoploid genome found so far in the $x = 2$ lineage (Kotseruba et al. 2010).

The previously reported DNA C-values using FCM + PI for *Sclerochloa dura* (3.12 pg/2C) (Šmarda et al. 2008), *Catabrosa aquatica* (6.29 pg/2C) and *Puccinellia distans* (8.70 pg/2C) (Zonneveld 2019) as well as *P. limosa* (2.59 pg/2C for the diploids of Central Germany that are also taxonomically treated under *P. distans* agg.) (Kúr et al. 2023) mostly agree well with our data, while the values recorded for *Coleanthus subtilis* (2.30 pg/2C), *Catabrosa aquatica* (5.12 pg/2C) and *Sclerochloa dura* (2.61 pg/2C) (Šmarda et al. 2019) are again up to 20% lower.

Hookerochloinae. Hookerochloinae comprises five genera, four of which are scattered throughout the southern hemisphere (Gillespie et al. 2022), while the genus *Arctagrostis* is widespread in the Holarctic and comprises only two species, one of which was sampled. Different specimens examined from a collection of *A. latifolia* from Alaska had 2C values of 24.51 pg and 36.73 pg. Their chromosome numbers $2n = 56$ and $2n = 84$, representing the octo- and dodecaploid levels, respectively, were determined by counting. The 1Cx value of 3.06 pg and the MC of 0.44 pg were identical in both.

Miliinae. The monogeneric subtribe Miliinae has about six species in the Holarctic region, of which the perennial forest species *Milium effusum*, the most widespread species, was sampled. This tetraploid species ($2n = 28$), as verified by chromosome counting, had a 2C value of 9.23 pg, a 1Cx of 2.31 pg and an MC of 0.33 pg. The 2C value is comparable to one of the previous FCM + PI estimates, namely 8.30 pg/2C (Šmarda et al. 2019), while 5.17 pg/2C could refer to a diploid accession of *M. effusum* (Zonneveld 2019) or another species, as this accession is also listed as '*M.* cf. *effusum*' (Zonneveld 2019: Electr. Suppl. Table 5). However, the four other estimates of 8.86–9.22 pg/2C for *M. effusum* (Zonneveld 2019: Electr. Suppl. Table 5) fit our result much better and should therefore actually belong to the tetraploid *M. effusum*.

For *M. vernale*, an annual diploid with the diverging dysploid chromosome number $2n = 8$, a 2C value of 6.28 pg was previously obtained (Zonneveld 2019), which largely agrees

with the value of 5.73 pg previously estimated by Feulgen densitometry (Bennett and Thomas 1991). The 1Cx value of *M. vernale* would therefore be 3.14 pg and the MC would be 0.79 pg (Table 3).

Phleinae. The Holarctic and Andean subtribe Phleinae comprises only the genus *Phleum*, when its segregate genera are included. Of the approximately 15 species, nine were examined in this study, including diploid ($2n = 14$) to hexaploid ($2n = 42$) species and cytotypes, with 2C values ranging from 2.77 pg in the diploid *P.* cf. *alpinum* to 8.74 pg (mean value) in the hexaploid *P. pratense*. The other diploids studied (*P. arenarium*, *P. bertolonii*, *P. phleoides*, *P. rhaeticum*, *P. subulatum*) had 3.13–4.01 pg/2C, the tetraploids *P. alpinum* and *P.* paniculatum had 6.08–6.55 pg/2C. Previous FCM + PI genome size data mostly agree well with these values, e.g. 8.1 pg/2C in presumably also 6*x P. pratense* (Bai et al. 2012), 2.44 pg/2C in 2*x P. alpinum*, 3.49 pg/2C in 4*x P. phleoides* and 7.63 pg–7.99 pg/2C in 6*x P. pratense* (Šmarda et al. 2013, 2019), 3.13 pg/2C for 2*x P. arenarium* and 8.47–8.78 pg/2C in 6*x P. pratense* (Zonneveld 2019). Several diploid accessions of *P. alpinum* (as *P. commutatum*) and *P. rhaeticum* had mean 2C values of 2.68 pg and 2.61 pg, respectively, while tetraploid *P. alpinum* accessions (as *P. commutatum*) from America and Europe uniformly had about 6.14–6.20 pg (Kula et al. 2006), which is also consistent with our results. The 1Cx values of all *Phleum* accessions examined in our study varied between 1.39 pg and 2.01 pg, the MCs between 0.20 and 0.29 pg.

Poinae. This subtribe consists only of the genus *Poa*, into which several segregate genera have been included. *Poa* is the largest of all grass genera with about 570 species (Soreng et al. 2022a, b). It is distributed almost worldwide, even reaching the Arctic region and Antarctica.

The total of 24 *Poa* species and 35 accessions analyzed had 2C values ranging from 2.41 pg in a presumably diploid accession of *P. persica* (syn. *Eremepoa persica*) to 15.29 pg in 12*x*–14*x P. cita*. Two other diploids examined, *P. chaixii* and *P. trivialis*, had 3.15 and 3.36 pg/2C, respectively.

The tetraploids examined were the annual *P. annua* with 4.40 pg/2C (mean value) and the perennials *P. badensis* with 6.01 pg/2C (mean value) and *P. triodioides* (syn. *Austrofestuca littoralis*) with 7.74 pg/2C. Chromosome counts were not performed for the other species and accessions, but they all were probably polyploid. Also *P. palustris* with only 4.49 pg/2C (mean value) was probably not diploid but tetraploid (CCDB 2023).

The 1Cx values of the diploids ranged from 1.21 to 1.68 pg, those of the polyploids from 1.07 to 1.94, the latter in the Australasian *P. triodioides*. The MCs showed similar variation, ranging from 0.17 to 0.24 pg in diploids, and from 0.15 to 0.28 pg in polyploids, the latter value again in *P. triodioides*, indicating that this species has the largest monoploid genome and the largest chromosomes found so far in *Poa*.

For most of the species we studied, the 1Cx values and MCs cannot be given because the chromosome numbers of these accessions are unknown. All these species are known to have more than one cytotype (ploidy level), some show in additional aneuploidy and facultative apomixis (e.g. *P. bulbosa* and *P. pratensis*), and it is not clear to which cytotype our accessions used for genome size estimates belong.

Our genome size data in *Poa* are largely consistent with the results of several previous studies also using FCM + PI, such as 4.21 pg/2C and 4.19 pg/2C (mean values) recorded for *P. annua* (Zonneveld 2019; Siljak-Yakovlev et al. 2020), 14.71 pg/2C (mean value) for *P. cita* and 7.42 pg/2C for *P. triodioides* (as *Austrofestuca littoralis*) (both Murray et al. 2005), 4.2–5.8 pg/2C for *P. palustris* (Bai et al. 2012), and 3.32 pg/2C and 3.48 pg/2C for *P. trivialis* (Šmarda et al. 2008; Zonneveld 2019). For *P. annua*, 3.88 pg/2C (Mao and Huff 2012) and 3.87 pg/2C (Šmarda et al. 2019) have also been found, which is closer to the genome size of 1,778 Mbp (≈ 3.64 pg/2C) suggested by sequencing (Robbins et al. 2023; Benson et al. 2023) than our FCM estimate. The same discrepancy between FCM- and sequencing-based genome size estimates as in *P. annua* is evident in the case of *P. trivialis*, whose sequenced genome length of 1,350 Mbp (≈ 2.76 pg/2C) (Brunharo et al. 2024) is about 17–21% lower than the available FCM genome size estimates of 3.32–3.48 pg/2C (see above).

However, it is difficult to say which are the more accurate estimates, since sequencing approaches, although depending on the method used, typically underestimate the true genome size due to the mostly incomplete representation of the amount of repetitive DNA as has often been noted (e.g. Bennett et al. 2003; Doležel et al. 2018; Kapustová et al. 2019; Blommaert 2020; Becher et al. 2022; Tkach et al. 2025; Winterfeld et al. 2025a, b), due to the difficulty of assembling repeats with monomers longer than the sequencing read length and the representation of two or more copies, which may be of different lengths, in only one sequence of the assembly.

Some other previously reported C-values of *Poa* species were also about 6–18% lower than ours, such as 2.74 pg/2C and 2.97 pg/2C instead of 3.36 pg/2C for *P. trivialis* (Wieners et al. 2006: p. 1537; Šmarda et al. 2013), 4.38 pg/2C instead of 5.13 pg/2C for *P. angustifolia*, 2. 83 pg/2C instead of 3.15 pg/2C for *P. chaixii*, 5.28 pg/2C instead of 6.25 pg/2C for *P. nemoralis* and 3.73 pg/2C instead of 4.49 pg/2C for *P. palustris* (Šmarda et al. 2019), while these samples, either confirmed by chromosome counting or most likely, have the same ploidy level as the accessions we used.

In other cases, different ploidy levels were studied, resulting in 2.48 pg/2C for diploid (Šmarda et al. 2019) and 6.01 pg/2C for tetraploid *P. badensis,* and 4.08 pg/2C for tetraploid *P. laxa* (Šmarda et al. 2019), suggesting that our

accessions of *P. laxa* were octoploid, judging by its genome size of 8.12 pg/2C.

The three accessions of *P. pratensis* studied, with a genome size of 4.93–7.61 pg/2C probably had different chromosome numbers. The numerous cytotypes of this facultatively apomictic species (Bonos and Huff 2013), comprising 4*x*–22*x* ploidy levels, cause considerable variation in the size of the holoploid genomes (Eaton et al. 2004; Wieners et al. 2006; Dennhardt et al. 2016; Zonneveld 2019; Ghanbari et al. 2023; Phillips et al. 2023). Using FCM + PI, an octoploid accession of *P. pratensis* subsp. *angustifolia* was found to have 3,525.59 Mbp (≈ 7.21 pg/2C), while other accessions of unknown ploidy from subsp. *angustifolia* had 3,248.04 Mbp (≈ 6.64 pg/2C) and from subsp. *pratense* had 4,030.61 Mbp (≈ 8.24 pg/2C) and 4,856.38 Mbp (≈ 9.93 pg/2C), respectively (Phillips et al. 2023).

The highest value of 18.66 pg/2C recorded so far in the genus *Poa* also belongs to *P. pratensis* (Raggi et al. 2015, using DAPI as the fluorescent dye), while for most other accessions of *P. pratensis* have 2C values below 11 pg/2C have been recorded.

The lowest 2C values recorded in this genus refer to the perennial *P. supina* and the annual *P. infirma*, which are the diploid parental species of the aforementioned weedy annual to perennial, cosmopolitan allotetraploid *P. annua* (Nannfeld 1937 using the synonym *P. exilis* for *P. infirma*; Tutin 1952, 1957; Soreng et al. 2010; Mao and Huff 2012; Chen et al. 2016; Nosov et al. 2019; Benson et al. 2023).

The genome size of *P. infirma* was 2.52 pg/2C and 2.84 pg/2C (mean values) according to FCM data (Mao and Huff 2012; Zonneveld 2019) and was 1,125.5 Mbp (≈ 2.30 pg/2C) according to genome sequencing (Benson et al. 2023; Robbins et al. 2023). *Poa supina* had an even smaller genome size estimated at 1.48 pg/2C and 1.49 pg/2C by FCM (Mao and Huff 2012; Šmarda et al. 2019) and 636 Mbp (≈ 1.30 pg/2C) by DNA sequencing (Benson et al. 2023).

Unclear are some other genome size estimates for *Poa* species (Joshi et al. 2016), which were also performed using FCM + PI. For all eight species considered diploid (*P. asiae-minoris*, *P. badensis*, *P. bucharica*, *P. chaixii*, *P. hybrida*, *P. pumila*, *P. sibirica*, *P. trivialis* with two accessions), the identical 2C value of 2.5 pg was found, which is hardly credible.

Ventenatinae. This subtribe comprises six genera and is distributed in western Eurasia, the Mediterranean and the Middle East. Representatives of three genera were examined, all them diploid (2*n* = 14). The perennial *Bellardiochloa violacea* had the smallest holoploid genome size of 7.77 pg/2C, a 1Cx value of 3.89 pg and an MC of 0.56 pg.

Two species and three accessions of the annual genus *Apera* had 8.68–9.60 pg/2C, 1Cx values of 4.34–4.80 pg and MCs of 0.62–0.69 pg (*A. interrupta* and *A. spica-venti*). Previously recorded genome sizes were 8.66 pg/2C (Šmarda et al. 2019) and 10.3 pg/2C for *A. spica*-venti and 8.73 pg/2C for *A. interrupta* (both Zonneveld 2019), in agreement with our results.

The also annual *Ventenata*, in which two species and four accessions were studied, showed some variation, as *V. dubia* had 10.65 pg/2C, a 1Cx of 5.33 pg and an MC of 0.76 pg (mean values), while *V. macra* had a larger holoploid genome of 12.14 pg/2C, a 1Cx of 6.07 pg and an MC of 0.87 pg.

Arctopoa. This hybrid taxon of the tribe Poeae, has ancestors from the subtribes Poinae and Cinninae each (Gillespie et al. 2010, 2022; Tkach et al. 2020). The studied hexaploid *Arctopoa eminens* (2*n* = 42) had a genome size of 11.75 pg/2C and an MC of 0.28 pg. The 1Cx value of 1.96 pg and the MC of 0.28 pg are average values calculated for this allopolyploid, but the combination of very different sized monoploid genomes is also possible in this species.

Intertribe hybrid groups

The subtribes that are taxonomically unplaced due to presumed hybrid origin or not yet assigned to a tribe, had 2C values ranging from 2.86 pg (mean value) in the diploid *Corynephorus canescens* to 38.89 pg in 18*x Helictochloa pratensis* (Tables 2, 3; Figs. 1, 2a; Online Resource 1.

The largest holoploid genome size among the diploids with *x* = 7 was found in *Mibora minima* with 10.05 pg/2C (mean value). In the diploid *Echinaria capitata*, characterized by the dysploid number *x* = 9, the 2C value was even higher and amounted to 16.59 pg/1Cx (mean value) (Table 2, 3; Figs. 1, 2b).

Monoploid genome sizes ranged from 1.13 pg/1Cx in a tetraploid of *Holcus mollis* accession to 5.03 pg/1Cx in *Mibora minima*, both with *x* = 7, exceeded by 8.30 pg/1Cx (mean values) in *Echinaria capitata* with *x* = 9. Similarly, the MCs varied between a minimum of 0.16 pg in *H. mollis* and a maximum of 0.72 pg in *M. minima* and 0.92 in *E. capitata*, respectively (Figs. 1, 2c).

The size distribution of 2C values, 1Cx values, and MCs of the intertribe hybrid groups as a whole closely resembles that of the tribe Aveneae (Fig. 1), which is consistent with the involvement of Aveneae as one of the parental taxa in their origin, as shown in Table 1, which updates the parental tribe designations for two subtribes compared to Tkach et al. (2020: p. 255).

The intertribe hybrid groups comprise 18 genera and approximately 159 species, representing seven subtribes, six of which were sampled in this study (Table 1). Most of their genera are distributed in the temperate regions of Eurasia and the Mediterranean, only a few are Holarctic or nearly so (*Helictochloa*, *Scolochloa*, *Vahlodea*), while *Dryopoa* is SE Australian, and only *Deschampsia* is nearly cosmopolitan. The subtribes of the intertribe hybrid groups are listed

below in alphabetical order (Tables 1, 2, 3; Figs. 2, 4; Online Resource 1).

Airinae. This subtribe with four genera and nearly worldwide distribution had 2C values ranging from 2.86 pg in the diploid perennial *Corynephorus canescens* to 11.95 pg in the tetraploid perennial *Avenella flexuosa* (mean values). The consistently annual *Aira* species studied had 2C values of 5.72 pg (mean value) in *A. elegans* and 6.92 pg in *A. praecox* (mean value), both of which were diploid. Tetraploid *A. caryophyllea* had 12.45 pg/2C (mean value). The 1Cx values and MC of the Airinae species varied accordingly, with the lowest values occurring in *C. canescens* (1Cx value of 1.43 pg, MC of 0.20 pg) and the highest in the diploid *A. praecox* (1Cx value of 3.46 pg, MC of 0.49 pg). Due to its allopolyploid (amphidiploid) status (Albers 1973, 1978, 1980a, b), the 1Cx and MC were not calculated for A. *caryophyllea* (Online Resource 2a).

Genome size data from most previous FCM studies were largely in agreement with our estimates and amounted to values of 6.12 pg/2C and 6.78 pg/2C for *Aira praecox*, 2.24 pg/2C and 3.07 pg/2C for *C. canescens*, 10.87 pg/2C and 12.20 pg/2C for *Avenella flexuosa* (as *Deschampsia flexuosa*) (Šmarda et al. 2019; Zonneveld 2019). *Aira caryophyllea* has been reported with 12.70 pg/2C (Zonneveld 2019), which agrees with our estimate, as well as the previous Feulgen-densitometric estimates of 5.87 pg for *A. praecox* and 12.05 pg for *A. caryophyllea* (Albers 1980a).

Using the non-intercalating DAPI as a fluorescent dye for FCM, the ratio between the genome sizes of *A. praecox* and *A. caryophyllea* was 0.56–0.59 pg, calculated from the data of Gregor et al. (2023), which is consistent with the 0.56 pg (mean value) found in our estimates.

Aristaveninae. The genus *Deschampsia*, with about 62 species, is the only representative of the subtribe, which is distributed almost worldwide, including Antarctica. *Deschampsia* is widespread in the Holarctic, but also has a center of species diversity in South America. The 2C values varied from 7.58 pg in *D. danthonioides* to 18.61 pg in *D. littoralis* (mean values). All but one of the chromosomally studied species have $2n = 26$ or 52 (CCDB 2023) or slightly different chromosome numbers due to aneuploidy or B chromosomes (Albers 1972), which at first glance implies $x = 13$.

The only exception to $x = 13$ is found in *D. setacea*, a rare species from the comparatively humid regions of western Europe, which is diploid and has $2n = 14$. It was split off at some point as the genus *Aristavena*. All other species with $x = 7$ that have occasionally been listed under *Deschampsia* (e.g., CCDB 2023), such as *D. atropurpurea*, *D. flexuosa* and *D. minor* actually belong to other genera, namely *Avenella*, *Holcus* and *Vahlodea*. However, based on molecular phylogenetic data (N. Tkach and M. Röser unpublished data), *D. setacea* forms a maximally supported monophyletic group with all other *Deschampsia* species, making it plausible to accept its inclusion within *Deschampsia*. The 2C value of *D. setacea* was 5.25 pg (mean value), the 1Cx value was 2.63 pg and the MC was 0.38 pg.

The *Deschampsia* species with $2n = 26$ are either hypotetraploids, i.e. tetraploids with $2n = 28$, in which two chromosomes have been lost in some way, e.g. by centric fusion of two acrocentric chromosomes, or they are allopolyploids derived from a diploid ancestor with $x = 7$, such as *D. setacea* and an unknown diploid with $x = 6$ (Albers 1980a, García Suarez et al. 1997; González et al. 2021). *Deschampsia argentea* from Madeira (three accessions studied), the *D. cespitosa* (five accessions), *D. media* (one accession) and *D. wibeliana* (one accession specifically from the Elbe estuarine zone) from central Europe had 8.63–9.96 pg/2C, 1Cx values of about 2.16–2.49 pg when considered tetraploid and an MCs of 0.33–0.38 pg. *Deschampsia koelerioides* from China had a larger genome of 11.24 pg/2C, a 1Cx of 2.81 pg and an MC of 0.43 pg (mean values).

Deschampsia littoralis from northwestern Switzerland and *D. rhenana* from the Lake Constance are both octoploids with $2n = 52$ (CCDB 2023). They had 18.61 and 18.30 pg/2C, 2.33 and 2.29 pg/1Cx and MCs of 0.36 and 0.35 pg, respectively (mean values for *D. littoralis*). The 1Cx and MC values were similar to those of the $2n = 26$ species mentioned above.

The annual North American *D. danthonioides* has a smaller holoploid genome, 7.58 pg/2C, than the other $2n = 26$ *Deschampsia* species. Its 1Cx value was 1.90 pg and the MC was 0.29 pg (mean value each).

Previous estimates of 2C genome size using FCM in *Deschampsia* were 4.94 pg for *D. setacea* with $2n = 14$ (Zonneveld 2019). Considering the taxa with $2n = 26$, the 2C values were 9.51 pg for *D. argentea* (Greimler et al. 2022), 7.51–10.88 pg for *D. cespitosa* (Murray et al. 2005; Šmarda et al. 2019; Zonneveld 2019; Greimler et al. 2022 including many accessions) and 9.73–10.47 pg for *D. koelerioides* (Greimler et al. 2022). In the taxa with $2n = 52$, the 2C values were 17.95–18.08 pg for *D. littoralis* and 16.96–17.72 pg for *D. rhenana* (Greimler et al. 2022). All these estimates are in good agreement with our data. Some variation in genome size in *D. cespitosa* could also be due to B chromosomes, which occur in variable numbers and sizes in this species (Albers 1972). In addition, occasional accessions of *D. cespitosa* with $2n = 52$ have also been found, with holoploid genome sizes of 15.89–17.95 pg/2C (Greimler et al. 2022).

Helictochloinae. The genus *Helictochloa*, all species of which are perennial and characterized by $x = 7$, forms the subtribe Helictochloinae together with the dysploid Mediterranean annual *Molineriella* ($x = 4$; not studied). *Helictochloa* is widespread in the northern hemisphere and is characterized by a wide range of ploidy levels from $2x$ to $22x$ (e.g., Gervais 1973 as *Avenochloa*; Röser 1996, 1998

as *Helictotrichon*). The 2C values of the six studied species ranged from 7.31 pg in the diploid *Helictochloa versicolor* ($2n = 14$) to 38.89 pg in the highly polyploid *H. pratensis* ($2n = 18x = 126$). The 1Cx values varied between 3.66 and 4.29 pg in diploids and 2.16–3.07 in polyploids, which had correspondingly smaller chromosome sizes (an MC of 0.52–0.61 vs. 0.31–0.44 pg). For $18x$ *H. pratensis* accessions, previous estimates using FCM were 30.29–33.22 pg/2C and 35.8 pg/2C (Šmarda et al. 2019 and Zonneveld 2019 as *Helictotrichon pratense*), which are in the same order of magnitude as our data.

Holcinae. Both genera of this small subtribe were studied, the mainly European genus *Holcus* and the mainly amphi-Beringian and arctic *Vahlodea*. The 2C values were 3.36–7.5 pg in the two *Holcus* species studied and 6.32 pg (only one measurement made) in *V. atropurpurea*. Three accessions of the diploid *H. lanatus* examined had 3.36 pg/2C, 1.68 pg/1Cx, and MC of 0.24 pg (mean value each). These values are consistent with 3.41 pg/2C (Zonneveld 2019), while 2.97–2.99 pg/2C are slightly lower values (Šmarda et al. 2013, 2019). A verified tetraploid *H. mollis* accession had 4.53 pg/2C, a 1Cx value of 1.13 pg and an MC of 0.16 pg. Another accession had 7.50 pg/2C, indicating higher polyploidy. *Holcus mollis* is known to be $3x$–$7x$ (CCDB 2023), which explains why values of 5.42 pg/2C and 5.99 pg/2C were also previously found for this species (Šmarda et al. 2019; Zonneveld 2019). *Vahlodea atropurpurea* has been reported as diploid, but there seems to be only one chromosome available for this species (CCDB 2023), while our accession is most likely polyploid, considering the high 2C value.

Scolochloinae. This subtribe is characterized by a remarkably disjunct distribution of its only two genera, the northern hemisphere genus *Scolochloa* with two species and the southeastern Australian monospecific genus *Dryopoa*. The tetraploid *S. festucacea* ($2n = 28$) had 9.75 pg/2C, 2.44 pg/1Cx and an MC of 0.35 pg (mean values). The hexaploid *S. marchica*, a rare local endemic of eastern Germany ($2n = 42$), had 14.80 pg/2C, 2.47 pg/1Cx and an MC also of 0.35 pg (mean values), which agrees with the recently reported values of 9.96 pg/2C and 14.88 pg/2C for these species (Kruk et al. 2024, also using FCM + PI). The genome size data suggest that *S. marchica* is autopolyploid and arose from *S. festucacea* by fusion of reduced and unreduced gametes, without the involvement of another parental taxon (Kruk et al. 2024; M. Röser et al. unpublished data).

The Australian *Dryopoa dives* has a much larger holoploid genome of 30.76 pg/2C. The sampled accession was verified to be decaploid ($2n = 70$), resulting in a comparatively high 1Cx value of 3.08 pg and an MC of 0.44 pg, consistent with the large chromosome size under the microscope (not shown).

Sesleriinae. This European to Mediterranean subtribe was represented in this study by all of its six genera. Five of them have a monoploid chromosome set of $x = 7$, namely the annual, species-poor genus *Mibora* and the perennial, also species-poor genera *Oreochloa*, *Psilathera* and *Sesleriella* as well as the perennial but species-rich genus *Sesleria* with about 36 species. The 2C values ranged from 6.81 pg in the diploid *P. ovata* to 19.87 pg in the hexaploid *Sesleria comosa.*

$\underline{x = 7}$. *Mibora minima*, a diploid short-lived, ephemeral and winter annual species ($2n = 14$) had a 2C value of 10.05 pg, a 1Cx of 5.03 pg and an MC of 0.72 pg (mean values), confirming a previous estimate of 9.92 pg/2C (Zonneveld 2019). The perennial diploids ($2n = 2x = 14$) *Oreochloa*, *Psilathera* and *Sesleriella* had 9.02 pg/2C (mean value), 6.81 pg/2C and 8.10 pg/2C, respectively, with 1Cx values of 4.51 pg (mean value), 3.41 pg and 4.05 pg, and MCs of 0.64 pg (mean value), 0.49 and 0.58 pg, respectively. The genome size of *P. ovata* (as *Sesleria ovata*) was previously reported to be 5.95 pg/2C, also determined by FCM + PI (Lazarević et al. 2015).

Interestingly, *M. minima* is a diminutive, short-lived annual from the winter-mild regions of Western Europe that germinates already in autumn and survives the winter as a plantlet. Its large genome size may be related to such a life form and ecological traits, as discussed for the 'neotenic' centrolepids of the family Restionaceae and other examples mentioned by Winterfeld et al. (2025a).

The consistently polyploid genus *Sesleria* was represented by seven species and nine accessions. The four tetraploid species examined had 8.62–10.14 pg/2C, the three octoploid species 16.37–19.78 pg/2C. The values of 9.26 pg/2C, 9.78 pg/2C (mean value), and 9.55 pg/2C for tetraploid *S. albicans* (Lysák and Doležel 1998 and Lysák et al. 2000, also using FCM + PI; Zonneveld 2019) are largely consistent with our estimate of 10.14 pg/2C (mean value). For the tetraploids *S. alba* and *S. argentea* we obtained 9.04 pg/2C (mean value) and 8.62 pg/2C, which agrees with previous estimates of 8.58 pg/2C and 8.97 pg/2C (mean value), respectively (Lazarević et al. 2015). Similarly, our estimate of 18.49 pg/2C in the octoploid *S. sadleriana* is broadly consistent with that of 18.00 pg/2C (mean value) (Lysák and Doležel 1998).

The 1Cx values of all examined *Sesleria* species ranged from 2.05 pg to 2.54 pg (mean values), the MC from 0.29 pg to 0.36 pg (mean values).

$\underline{x = 9}$. The remaining genus of the subtribe Sesleriinae, the annual monospecific *Echinaria* with $2n = 18$ has the divergent monoploid number $x = 9$. *Echinaria capitata* had a relatively high 2C value of 16.59 pg, a 1Cx of 8.30 pg and an MC of 0.92 pg (mean values).

Are there any trends in the evolution of genome sizes in the Poodae?

Compared to the tribes Aveneae and Festuceae, the Poeae are characterized by significantly lower 2C values, especially due to a comparatively high proportion of taxa with low 2C values < 5 pg/2C. The other genome size parameters were not fundamentally different from the other tribes, however, their 1Cx values with monoploid genomes < 2.0 pg were mostly smaller than in the intertribe hybrids, and small chromosomes with MCs < 0.3 pg predominated (Figs. 1, 2, 3, 4; Online Resource 3). This is mainly caused by the monogeneric subtribes Phleinae (*Phleum*) and Poinae (*Poa*), and most of the Coleanthinae, while one of the late-diverging lineages of the tribe Poeae has mostly large 2C values, but also large monoploid genomes (1Cx values) and large chromosomes (MCs). This lineage consists of the subtribes Alopecurinae, Beckmanniinae, Cinninae, Hookerochloinae and Ventenatinae (Fig. 4), forming the 'ABCV clade' (Tkach et al. 2020), which was subsequently renamed the 'Alopecurinae superclade' (Gillespie et al. 2022). However, this genome expansion is also seen in the monogeneric subtribe Avenulinae (*Avenula*), a relative of both, Phleinae/Poeae and the ABCV clade (Tkach et al. 2020: Fig. 7).

Regarding the tribe Festuceae (Figs. 1, 2, 3, 4), comparatively large 2C values and monoploid genome sizes and MCs are found in the subtribe Cynosurinae/Parapholiinae compared to the Dactylidinae and the Loliinae, a subtribe that apparently diverged earlier in the phylogeny. This could also imply a 'secondary' genome and chromosome enlargement, but the variation within the Loliinae is strong and there is no clear trend (Fig. 4).

The variation in genome and chromosome size in the tribe Aveneae is also very striking and there seems to be no clear direction of 'evolutionary progress'. However, the enormous variability of these parameters within the monogeneric subtribe Anthoxanthinae (*Anthoxanthum*) (Tables 2, 3; Figs. 2, 4) is particularly remarkable (Chumová et al. 2015, 2017, 2021). This is also true for the occurrence of the relatively small genome and chromosome sizes in the subtribe Torreyochloinae (Tables 2, 3; Figs. 2, 4), which show a surprisingly uniform monoploid genome size (1Cx value) and MC in the studied species of the diploid *Torreya pallida* from Canada and the hexaploid *Amphibromus nervosus* from Australia, which are almost maximally geographically separated.

The variability of genomic parameters appears to be associated less with long-term phylogenetic and evolutionary events and possibly more with evolutionary events within smaller groups of related taxa or individual genera. Features such as polyploidization, which per se is associated with a sudden change in genome size, but also chromosomal mutations associated with dysploid changes in chromosome number, or the evolution of different life forms (annual vs. perennial) have been discussed previously as possible factors for changes in genome size and associated traits and biological properties of organisms (Greilhuber and Leich 2013; Šmarda et al. 2019; Liddell et al. 2021; Zhan et al. 2021; Heslop-Harrison et al. 2023). The available data on numerous representatives of a relatively narrowly defined group of plants, such as the investigated subtribes grouped in the supertribe Poodae, provide good opportunities to address these questions.

Polyploidy (whole-genome duplication, WGD) and genome size

Polyploidy, the multiplication of chromosome sets by whole genome duplication (WGD) as it is commonly called today, represents the most significant and drastic change in holoploid genome size (2C values) because it takes effect immediately. In contrast, changes caused by a gradual increase or decrease in the proportion of certain, typically repetitive elements in the DNA are much slower. Since all angiosperms have undergone one or more cycles of genome duplication in their evolutionary past, holoploid genome sizes should in principle have become larger and larger (Leitch and Bennett 2004; Bennett and Leitch 2005; Wendel 2015; Wendel et al. 2018; Wang et al. 2021a, b). However, since many angiosperms living today have very small genomes, there must inevitably be mechanisms that counteract this genome expansion through polyploidy, which is very well documented by numerous examples. Within the investigated genome sizes of grasses, there are polyploids in which the holoploid genome is the sum of the individual monoploid genomes ('additivity'), as well as those in which a reduction of the holoploid genome has already occurred ('downsizing') (Online Resource 2a). Both processes can occur in both autopolyploids and allopolyploids, providing new insight into the long-standing debate as to whether alloploidy promotes downsizing, whereas autoploidy is associated with genome size additivity (Ozkan et al. 2001; 2006; Soltis et al. 2003; Leitch and Bennett 2004; Bennett and Leitch 2005; Johnston et al. 2005; Garnatje et al. 2006; Suda et al. 2007; Vaio et al. 2007; Eilam et al. 2008, 2009, 2010; Leitch and Leitch 2008; Pellicer et al. 2010; Husband et al. 2013; Zenil-Ferguson et al. 2016; Becher et al. 2021; X. Wang et al. 2021a, b; Feldman and Levy 2023b).

Additivity of monoploid genome sizes in polyploids. Polyploids whose holoploid genome sizes reflect the sum of the genome sizes of their diploid or lower polyploid relatives document a lack of or ineffective, genome size reduction after polyploidization. They may be autopolyploids or allopolyploids.

Examples found of the most likely autopolyploid taxa with little or no downsizing include (Online Resource 2b):

(A) Triploid (3*x*) *Koeleria vallesiana*,
(B) Tetraploid (4*x*) *Anthoxanthum nitens* (syn. *Hierochloe nitens*), *Briza media* (as also noted by Murray 1975, 1976), *Catapodium marinum*, *Dactylis glomerata* subsp. *glomerata*, *Festuca trachyphylla*, *F. fasciculata* (syn. *Vulpia fasciculata*), *Gastridium ventricosum*, *G. phleoides*, *Koeleria macrantha*, *K. spicata* (syn. *Trisetum spicatum*), *Poa badensis* and *P. laxa*,
(C) Hexaploid (6*x*) *Amphibromus nervosus*, *Festuca heteromalla*, *F. heterophylla*, *F. rubra* and *Scolochloa marchica*,
(D) Octoploid (8*x*) *Sesleria sadleriana*,
(E) Hypo-tetraploid (4*x*-2) *Deschampsia argentea*, *D. cespitosa*, *D. koelerioides*, *D. media* and *D. wibeliana* and
(F) Hypo-octoploid (8*x*-4) *D. littoralis*, *D. rhenana* compared to (E).

Examples of allopolyploids with little or no downsizing, characterized by an almost invariant additivity of the different parental genomes include (Online Resource 2c):
(G) Tetraploid (4*x*) *Aira caryophyllea*, *Anthoxanthum odoratum*, *Poa annua* and
(H) Hexaploid (6*x*) *Festuca muyros* (syn. *Vulpia myuros*).

Auto- or allopolyploids (unresolved) with little or no downsizing are (Online Resource 2d):
(I) Tetraploid (4*x*) *Phleum alpinum*,
(II) Hexaploid (6*x*) *Puccinellia distans* and *P. leiolepis*,
(III) Octoploid (8*x*) *Sesleria comosa*.

Polyploids with significant genome downsizing and decrease in the mean chromosome DNA content. Genome downsizing (2C and 1Cx values) and decrease of chromosome size (MC) (MC) > 10% were found in some of the most likely autopolyploids (Online Resource 2e):
(A) Tetraploid (4*x*) *Koeleria litvinowii* and *K. pyramidata*,
(B) Hypo-octoploid (8*x*-4) *Deschampsia littoralis* and *D. rhenana* compared to D. setacea (2*x*),
(C) Decaploid (10*x*) *Helictochloa agropyroides*, *Koeleria pyramidata*, *Pentapogon crinitus* and *P. micranthus*.

Examples of allopolyploids with significant genome downsizing and decrease in MC include (Online Resource 2f) are:
(D) Tetraploid (4*x*) *Alopecurus pratensis*, *Colpodium pisidicum*/*C. trichopodum* (syn. *Zingeria pisidica*/*Z. trichopoda*), *Holcus mollis, Phleum paniculatum*,
(E) Hexaploid (6*x*) *Phleum pratense*,
(F) Hypohexaploid (6*x*-4) *Parapholis incurva*,
(G) Octodecaploid (18*x*) *Helictochloa praeusta*, *H. pratensis* subsp. *pratensis*.

Taxa with unresolved status of polyploidy, whether autopolyploid or allopolyploid, that show significant downsizing include (Online Resource 2g):
(H) Tetraploid (4*x*) *Anthoxanthum odoratum*, *Koeleria litvinowii*,
(I) Hexaploid (6*x*) *A. monticola* (syn. *Hierochloe alpina*),
(J) Octoploid (8*x*) *Sesleria tenuifolia*.

Polyploids with uncertain diploid ancestors. In some cases, the genome size data help to exclude certain diploid taxa as ancestors or genome donors of some diploids (Online Resource 2a, h):

The genome size of 1.66 pg/1Cx and the MC of 0.24 pg of *Festuca octoflora* (syn. *Vulpia octoflora*) suggest that this New World diploid species was not involved in the origin of the sampled Old World 4*x F. fasciculata* (syn. *V. fasciculata*), which had 3.26/1Cx and an MC of 0.47 pg, i.e. approximately twice the chromosome size of *F. octoflora*. Comparable size data, however, were found in the Mediterranean diploid *F. alopecuros* (syn. *V. alopecuros*), i.e. 3.14 pg/2C and an MC of 0.45 pg, thus belonging to the putative diploid ancestors of the Old World polyploids.

The highly polyploid western Mediterranean *Helictotrichon filifolium* subsp. *filifolium* (12*x*) had 2C, 1Cx and MC values that almost exactly matched the parameters of the diploid *Helictotrichon* species studied, *H. decorum* and *H. sedenense* subsp. *sedenense*, and could therefore be considered a good example of allopolyploidy without significant genome downsizing. However, the actual genome donors have been shown to be different species, namely *H. parlatorei* and *H. sarracenorum* or related taxa (Wölk et al. 2015; Winterfeld et al. 2016), for which genome size data are not yet available.

A similar case is the allohexaploid *H. sempervirens* from the Western Alps, which even showed an increase in 2C and 1Cx values and MC compared to the above diploid species, but again, the relevant diploid species that acted as actual genome donors, *H. parlatorei* and *H. setaceum* or related taxa (Wölk et al. 2015; Winterfeld et al. 2016), have not been studied.

In the genus *Parapholis*, the sampled diploid *P. filiformis* apparently did not represent a genome donor for the tetraploid *P. strigosa* and the hypotetraploid (4*x*-2) *P. cylindrica* due to its genome size parameters with too low a 1Cx value and too low a MC.

Dysploidy and changes in genome and chromosome size

Dysploidy is a less dramatic change in chromosome number than polyploidy, and it usually refers to one or a few chromosomes of a monoploid set of chromosomes. The result is a gradual change in chromosome number, typically as a decrease, which is referred to as descending or

Table 4 Comparison of monoploid genome sizes (1Cx values) and mean chromosome DNA content (MC) of closely related euploid and dysploid taxa/cytotypes of the studied Poodae

Dysploid vs. related euploid taxon	Difference of		Reference for genome sizes
	1Cx value	MC	
Briza minor ($x=5$)—*B. media* ($x=7$)	↘ 13%	↗ 21%	This study
Catabrosa aquatica ($x=5$) — Ø other Coleanthinae ($x=7$)	↘ 22%	↗ 23%	This study
Colpodium biebersteinianum ($x=2$) — Ø other Coleanthinae ($x=7$)	↘ 23%	↗ 429%	This study
Colpodium versicolor ($x=2$) — Ø other Coleanthinae ($x=7$)	↘ 10%	↗ 314%	This study
Echinaria capitata ($x=9$) — Ø other Sesleriinae ($x=7$)	↗ 95%	↗ 35%	This study
Phalaris canariensis ($x=6$) — Ø *Phalaris* species ($x=7$)	↗ 363%	↗ 214%	This study
Rostraria cristata ($x=6$) — *R. hispida* ($x=7$)	↘ 36%	↘ 31%	This study
Trisetum flavescens ($x=6$) — Ø 2*x* perennial Aveninae ($x=7$)	↘ 54%	↘ 47%	This study
Trisetum flavescens ($x=6$) — Ø 4*x* perennial Aveninae ($x=7$)	↘ 59%	↘ 53%	This study

To exclude the potential impact of polyploidy on genome size, only diploid taxa are considered, except for *Phalaris* taxa with $x=7$, for which 1Cx and MC values were calculated from tetraploids. For *Trisetum flavescens*, both diploids and tetraploids with $x=7$ were used for comparison. See Table 2 and Online Resource 1 for full details of the samples analyzed and the measurements made, and Online Resource 2h for the calculations. Arrows ↗ and ↘ indicate increase and decrease, respectively

reductional dysploidy (Rieger et al. 1991; Schubert and Lysák 2011; Mandáková and Lysák 2018; Lysák 2022). It is associated with structural rearrangements leading to fusion of two chromosomes, usually as nested fusions, less often as end-to-end fusions, whereas gain is caused by chromosome fissions and appears to be a rarer event than descending dysploidy (Stebbins 1950; Lysák et al. 2009; Escudero et al. 2014; Lysák 2014; Carta et al. 2020; Winterfeld et al. 2020a; Mayrose and Lysák 2021; Senderowicz et al. 2021; Chase et al. 2023; Xavier et al. 2024).

Dysploidy is less common than polyploidy in the Poodae taxa studied. Of the 98 grass taxa with known chromosome number, 39 (about 40%) are polyploid and 59 (about 60%) are diploid. Dysploidy occurs in only 20 cases (about 20%), sometimes combined with polyploidy (*Anthoxanthum*, *Catabrosa*, *Colpodium*, *Deschampsia*, *Parapholis*) (Table 2; Online Resource 1). In addition, many dysploid grass taxa are annuals, supporting the hypothesis "that a low chromosome number has a selective advantage in a cross-fertilized annual species, since it increases the amount of linkage and therefore the degree of constancy of a population" (Stebbins 1950: p. 458).

Increase in 1Cx and MC. Several dysploid taxa of the studied grasses show an increase in their monoploid genome size (1Cx value) and an increase in the mean chromosome DNA content (MC) compared to their euploid relatives (Online Resource 2i). The most striking example is *Phalaris canariensis* ($x=6$), whose 1Cx and MC are increased by 363% and 214%, respectively, compared to its euploid congeners ($x=7$) (Table 4; Online Resource 2i).

Within the genus *Colpodium*, the diploids *C. biebersteinianum* and *C. versicolor* have $2n=2x=4$ chromosomes. This is one of the few cases of such low chromosome number in angiosperms. Their chromosomes are 310–430% larger than those of related euploid species with $x=7$ from the same subtribe Coleanthinae. The size of the monoploid genomes (1Cx values) shows less striking differences. *Colpodium biebersteinianum* has an increase of 23%, while *C. versicolor* has a decrease of 10%. This shows that chromosome fusions were the main mechanism of chromosome enlargement in these $x=2$ taxa.

Echinaria capitata ($2n=2x=18$) is characterized by a 95% increase in the 1Cx value and a 35% increase in MC compared to the other diploid but euploid species within the subtribe Sesleriinae, which have $x=7$. This is a notable increase, although less pronounced than that observed in *Phalaris canariensis*. It appears to be associated with a change in base number from $x=7$ to $x=9$, which may involve chromosome fissions, but this has not been investigated. It is the only example of ascending dysploidy found among the Poodae species studied.

Decrease in 1Cx and increase in MC. A moderate decrease in monoploid genome size (1Cx value) and a moderate increase in MC of about 13–23% each were found in *Briza minor* ($x=5$) compared to its congener *B. media* ($x=7$) and *Catabrosa aquatica* ($x=5$) compared to the its related taxa with x = 7 in the subtribe Coleanthinae.

Decrease in 1Cx and MC and ambiguous cases. The hypotetraploid *Rostraria cristata* ($2n=4x\text{-}2=26$) shows a 31–36% decrease in 1Cx and MC compared to its diploid euploid congener *R. hispida* ($2n=2x=14$). However, it is not certain that this is actually caused by dysploidy, as this species is allopolyploid (N. Tkach unpublished data) and may possibly also contain a smaller chromosome set than *R. hispida* in its tetraploid chromosome complement, causing its lower (mean) 1Cx value and MC.

Trisetum flavescens ($2n=4x=26$) stands out for its comparatively small 1Cx and MC values, which are the smallest within the comparatively extensively sampled subtribe Aveninae (Table 2). Both values are 43–59% lower than the

mean values of the diploids and tetraploids of Aveninae used for comparison (Table 4). The values would be even lower, if *Tricholemma jahandiezii*, with its exceptionally high and rather atypical 1Cx value and MC (chromosome size), had not been excluded from the calculation of the mean values of the tetraploid Aveninae (Appendix to Online Resource 2i). It would be interesting to include other cytotypes of the cytogenetically rather heterogeneous *Trisetum flavescens*, which also comprises hexaploids ($2n=36$) (Winterfeld 2006; Winterfeld and Röser 2007b) and euploid tetraploid populations with $2n=28$ based on $x=7$ (CCDB 2023). This would also provide a more reliable means of comparing whether genome size reduction in *T. flavescens* actually occurred in association with dysploidy than the average genome size values from other Aveninae genera used here, or whether it occurred independently of dysploidy.

The genus *Anthoxanthum*, including euploid taxa with $x=7$ (former *Hierochloe*) such as *A. nitens* and dysploid taxa with $x=5$ such as the *A. aristatum*, both of which are diploids (Tables 2, 3). This appears to show a decrease in 1Cx from 4.73 pg to 3.92 pg, a 17% reduction in genome size, and an increase in chromosome size from 0.68 pg to 0.78 pg associated with this dysploid chromosome change. The values recorded for other diploid $x=5$ taxa (*A. alpinum*, *A. maderense*) seem to show the same or even stronger decrease of the monoploid genome size (1Cx value) and in- or decrease of MCs (Chumová et al. 2015). However, the situation in *Anthoxanthum* is rather ambiguous, as one of the $x=5$ diploids, *A. gracile*, has a very large genome of 9.19 pg/1Cx and an MC of 1.84 pg (Chumová et al. 2015), implying a dramatic increase. Due to the contradictory data on genome up- and down-scaling, it must be assumed that very different processes of genome evolution with very different outcomes overlap in *Anthoxanthum*, a conclusion supported by cytogenetic and molecular analyses (Chumová et al. 2017, 2021) (see also the following chapter).

In the genus *Deschampsia*, there is no clear difference between *D. setacea*, the only euploid and diploid species (2.63 pg/1Cx and MC of 0.38 pg), and the dysploid and at the same time polyploid taxa ($2n=4x$-2 or $8x$-4 and 2.16–2.81 pg/1Cx, MC of 0.33–0.43 pg). All of them are perennials, whereas *D. danthonioides*, the only annual species of this genus studied, has a much lower 1Cx and MC (1.90 pg/1Cx; MC 0.29 pg). As *D. danthonioides* also has $2n=4x$-2, its small genome size values rather represent a change related to the life form (see below).

In summary, the enlargement of genomes (1Cx values) and chromosomes (MC) associated with a dysploid change in chromosome number, as found in about half of the cases of dysploidy among the grasses studied, seems to be the most common pattern observed in angiosperms in general (Lysák et al. 2006; Cheng et al. 2013; Vaio et al. 2013; Winterfeld et al. 2018, 2020b). Such an increase in genome size has been shown to be caused by massive transposon amplification due to loss of repression and elimination of transposable elements as a result of chromosome rearrangements (Theuri et al. 2005; Lou et al. 2012; Pellicer et al. 2014; Yang et al. 2014; Rockinger et al. 2016; Ferraz et al. 2023).

Genome size reduction in the course of dysploid change, as apparently observed in some of the studied taxa (*Rostraria*, *Trisetum*), is a pattern that has rarely been observed in other angiosperms (Chase et al. 2023).

Nevertheless, the current results seem to indicate that there is no simple causal relationship between changes in chromosome number and changes in genome size in the case of dysploidy.

Different life forms and genome sizes

Plants that are able to complete their life cycle in one year or less, i.e. annuals (therophytes), are typically adapted to a highly seasonal climate where there is usually a period of drought, requiring the production of seeds that can survive this hostile season. Therefore, the Mediterranean region and the Middle East, with its winter rainfall regime, is one of the most important centers for the development of annual plants, including grasses. In summary, annuals should be favored when adult mortality is high and seed persistence and seedling survival are relatively high (Hjertaas et al. 2023; Poppenwimer et al. 2023).

Ploidy and genome size of perennials and annuals. A characteristic of the annual grasses studied is that they are mostly diploid and only more rarely polyploid, with ploidy levels of mostly $4x$ and only very rarely $6x$, the latter occurring among the taxa studied only in the presumably hypohexaploid *Parapholis incurva* ($2n=6x$-4=38). Hexaploid annuals also are otherwise quite rare in the Poodae, but are known, for example, from a few wild annual species of *Avena* (oat) and of *Aegilops* from the Triticodae (wheat and relatives), the sister supertribe of the Poodae, while hexaploids are quite common among the cultivated annuals (crop species) of the tribes Aveneae and Triticeae (Rajhathy and Thomas 1974; Baum 1977; Yan et al. 2016; Feldman and Levy 2023a).

The perennial species, on the other hand, do not seem to have such restrictive constraints regarding the ploidy level, being found up to $18x$ in the species studied (*Helictochloa pratensis*) (Table 2).

2C values. The likely explanation for this striking difference between annuals and perennials seems to be that annuals have an upper limit on the 2C value, i.e. the holoploid genome size, which slows down the cell cycle by requiring the replication of a larger amount of DNA during the interphase. Although larger genome size correlates with larger cell size (nucleus/plasma ratio), which could in principle be advantageous for faster growth through fewer cell divisions,

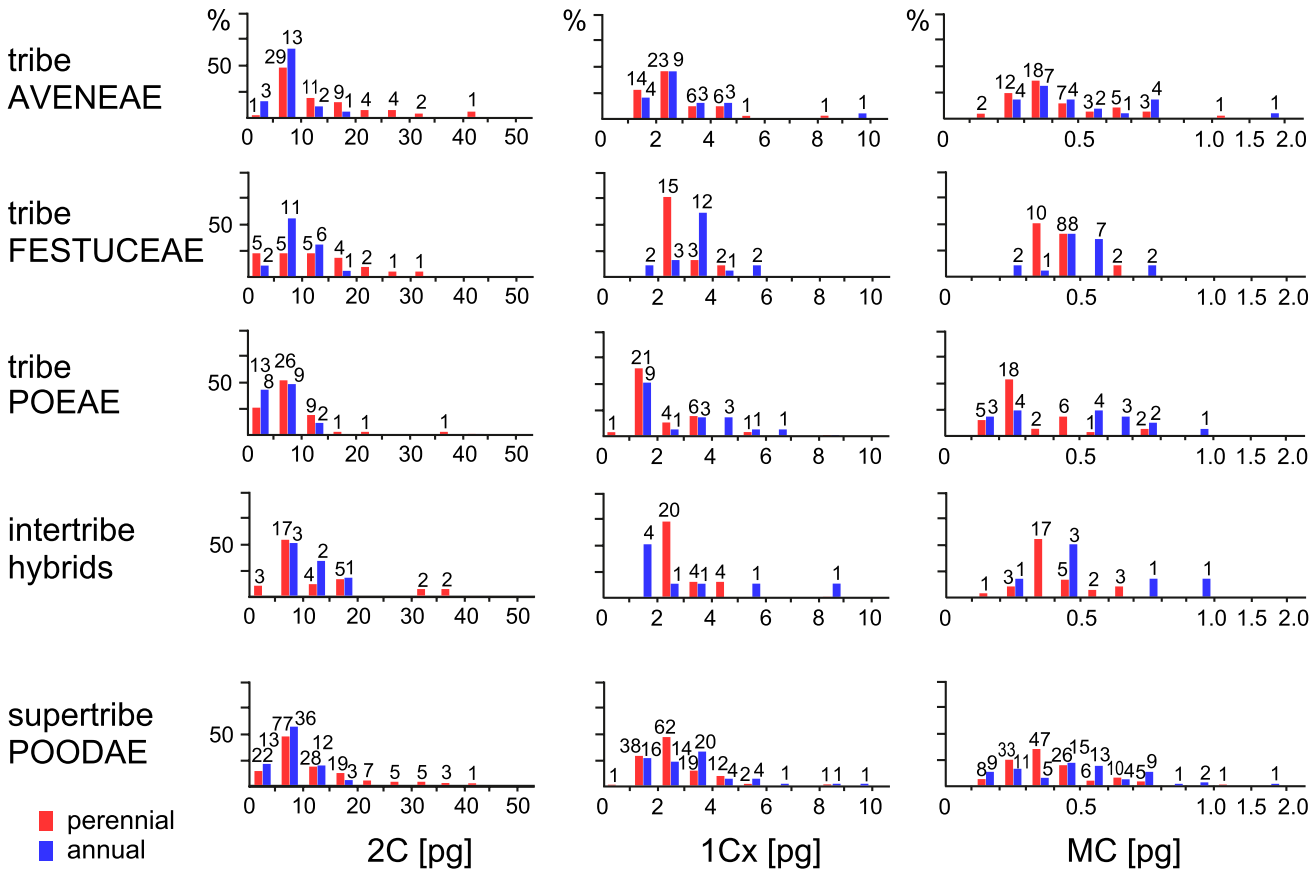


Fig. 5 Life form (perennial/annual) in relation to genome size (holoploid 2C and monoploid 1Cx values) in the tribes and the intertribe hybrid taxa of the supertribe Poodae analyzed in this study. The values are arranged in intervals on the x-axis, and the number of taxa falling within each interval is indicated above the corresponding bar. The height of the bars corresponds to the percentage, with the total number of all perennial (red) and all annual (blue) taxa taken as 100% each. The facultative perennial/annual species *Beckmannia syzigachne* and *Sphenopholis obtusata* (Finot et al. 2004; Clayton et al. 2006 onwards) are not included. See Table 2 and Online Resource 1 for the taxa used. Supplementary data for ten species of the tribe Aveneae are from Murray et al. (2005) (*Hierochloe brunonis*, *H. equiseta*, *H. fusca*, *H. novae-zelandiae*, *H. redolens*) and Chumová et al. (2015) (*Anthoxanthum alpinum*, *A. amarum*, *A. aristatum*, *A. gracile*, *A. maderense*), for two taxa with three cytotypes of the tribe Festuceae from Martínez-Sagarra et al. (2021) (*Festuca yvesii* subsp. *summilusitana* and subsp. *lagascae*) and seven species of the tribe Poeae from Houben et al. (2003) (*Zingeria biebersteiniana*), Kotseruba et al. (2003, 2010) (*Z. biebersteiniana*, *Z. kochii*), Murray et al. (2005) (*Simplicia buchananii*, *S. laxa*), Mao and Huff (2012) (*Poa infirma, P. supina*) and Zonneveld (2019) (*Milium vernale*). These additional data are listed in Online Resource 4

the cell cycle retardation appears to be more negative for annuals, while it may be less significant for perennials (Bennett 1972, 1987; Grant 1987; Bennett and Leitch 1995, 1997; Greilhuber and Leitch 2013).

2C values of > 20 pg were found only in perennials among the species studied (Fig. 5), namely in 21 (≈ 9.0%) of all 234 species (perennials and annuals) and 31.3% of all 167 perennial species studied (excluding facultative perennials). For the lower 2C values, which make up the majority, there is little difference between perennials and annuals, and the lowest values of < 5 pg/2C are found in both. On average, however, the 2C values of the annuals were higher than those of the perennials (Online Resource 3).

1Cx values. The vast majority of monoploid chromosome sets had 1–5 pg/1Cx, again with apparently little difference between perennials and annuals, although perennials had higher 1Cx values than annuals (Fig. 5; Online Resource 3). The comparatively few large values of > 5 pg/1Cx also occur in both, as does the largest of 8–10 pg/1Cx in the tetraploid perennial *Tricholemma jahandiezii*, and the diploid annuals *Echinaria capitata* (Table 2) and *Anthoxanthum gracile* (Chumová et al. 2015).

All three species have special features: *Tricholemma jahandiezii* is a taxonomically isolated endemic of the Middle Atlas of Morocco with a comparatively small range in the highlands; *A. gracile* is a phylogenetically early-diverging species of its genus (Chumová et al. 2021) with a huge genome compared to the other species. For both taxa, an ancient relict status and a small past or present population

size could be assumed, which could be responsible for the spread of genome-enlarging repetitive elements in the DNA (genetic bottleneck). Although *E. capitata* is instead a very widespread Mediterranean species, it is characterized by a dysploid chromosome set $x=9$ as opposed to $x=7$, which prevails in its close relatives of the subtribe Sesleriinae. As in some other cases in the species studied, the dysploidy and the associated chromosome rearrangements probably contributed significantly to genome enlargement in the monospecific genus *Echinaria* (see *Phalaris* above).

Mean DNA content of the chromosomes. The MCs showed a similar distribution of comparable sizes of perennials and annuals as noted for the 1Cx values (Online Resource 3). Most values were between 0.1 and 0.8 pg. Only five species had chromosomes larger than 0.8 pg. These were, in addition to the three species just mentioned, the dysploid *Colpodium biebersteinianum* ($2n=2x=4$) and the euploid *Ventenata macra* with $2n=2x=14$, which belongs to a phylogenetic lineage that also includes some other species with comparatively large genomes (Poeae subtribes Ventenatinae and Beckmanniinae) (Tables 2, 3; Online Resource 1).

Genera with perennial and annual species. Few of the genera studied contain both perennial and annual species:

Briza and *Macrobriza*. The annual *B. minor* (1Cx of 2.87 pg and MC of 0.57) has a smaller monoploid genome but slightly larger chromosomes than *B. media* (1Cx of 3.34 pg and MC of 0.47), the only perennial of this genus studied. This opposite change in both parameters is apparently related to the dysploidy in *B. minor* ($x=5$) compared to the euploid *B. media* ($x=7$) and the associated chromosome restructuring.

Macrobriza maxima, a diploid euploid annual ($2n=2x=14$), differs markedly from both *Briza* species due to its large genome of 9.32 pg/1Cx and large chromosomes (MC of 0.67 pg). However, due to its evolutionary hybrid origin (Tkach et al. 2020), it must be taxonomically excluded from *Briza* and therefore cannot serve as an example of genomic changes in the course of the emergence of the therophyte life form within *Briza*.

Deschampsia. The only annual *Deschampsia* species examined, *D.* danthonioides ($2n=4x\text{-}2=26$), has a 1Cx genome size and MC about 23% lower than its also dysploid but perennial congeners with ($2n=4x\text{-}2=26$ or $8x\text{-}4=52$) (Table 2; Online Resource 1).

Anthoxanthum. The opposite case occurs among the diploid *Anthoxanthum* species, where the perennial *A. alpinum* has the lowest 1Cx value of 2.70 pg/1Cx and the smallest chromosomes (MC of 0.55 pg), whereas the annual *A. gracile* has the highest 1Cx value of 9.19 pg/1Cx and the largest chromosomes (MC of 1.8 pg) (Chumová et al. 2015). This may be related to the above-mentioned different processes of genome evolution that seem to occur in this genus.

Festuca and *Lolium*. In the genera *Festuca* and *Lolium*, as taxonomically understood in this study, there is no clear distinction between annuals and perennials based on genome and chromosome sizes. The smallest 1Cx values and chromosome sizes in *Festuca* actually occur in the annuals, namely the diploid *F. octoflora* (syn. *Vulpia octoflora*) and the tetraploid *F. incurva* (syn. *Psilurus incurvus*) (1.66 and 2.07 pg/1Cx; MC of 0.24 and 0.30 pg), but also the highest one, in the diploid *F. lachenalii* (syn. *Micropyrum tenellum*) (5.44 pg/1Cx; MC of 0.78 pg), while the numerous perennials studied are intermediate (Table 2; Online Resource 1).

The annuals of *Lolium*, which are all diploid (*L. persicum*, *L. subulatum*, *L. temulentum*), also have larger monoploid genomes and larger chromosomes (3.76–5.15 pg/1Cx; MC 0.54–0.74 pg) than the studied perennials (*L. arundinaceum*, *L. giganteum*, *L. perenne*), which include diploid and hexaploid species (2.77–3.35 pg/1Cx; MC 0.40–0.48 pg).

These results for these genera with both annual and perennial species support the conclusion drawn above from the overall distribution of monoploid genome sizes (1Cx values) across all species studied, that the two life forms do not differ per se in this genomic trait and the associated mean chromosome sizes (MCs). Thus, the transition from a perennial to an annual life form is not necessarily associated with either genome downsizing or genome inflation, as has also been observed in the genus *Hordeum* from the related grass tribe Triticeae (Jakob et al. 2004).

Two species in our sample were facultative annuals/perennials (winter annuals), *Beckmannia syzigachne* (6.59 pg/2C) and *Sphenopholis obtusata* (5.58 pg/2C) (Finot et al. 2004; Clayton et al. 2006 onwards). Their genome sizes were similar to those of their strictly perennial congeneric taxa *B. eruciformis* (6.21 pg/2C) and *S. intermedia* (5.35 pg/2C), respectively. All species were diploid. Thus, a significantly higher genome size in obligate compared to facultative perennial monocotyledons, as suggested by Bennett (1972), is not evident from these grass examples.

Systematic affiliation and life form

Two-way ANOVAs were calculated to examine the effects of the taxonomic group (Aveneae, Festuceae, Poeae and intertribe hybrids) and life form (annual, perennial) and their interactions on three different types of DNA content (2C value, 1Cx value and MC) of the Poodeae (Online Resource 3; Fig. 3). All ANOVAs and contrasts showed a significant difference between annual and perennial life form of the pooled data. However, the 2C values of the annuals were higher than those of the perennials, a pattern that was reversed for the 1Cx and MC. There was always a non-significant interaction

between taxonomic group and life form. The group was significant for the 2C and 1Cx values but not for the MC.

The estimated marginal contrasts showed an inconsistent pattern in the sense that some factors had significant effects of one but not all dependent variables. The 2C values of the Poeae were significantly lower than those of the Aveneae and Festuceae, their 1Cx values were lower than those of the intertribe hybrids. The MC was not significantly different between the four groups.

Contrasts of these groups within a life form category showed no significant difference for the annuals. This means that the annuals of the four groups had no significantly different 2C and 1Cx values and MCs, whereas the perennial species of Aveneae, Festuceae and intertribe hybrids had higher 2C values than the perennial Poeae. The perennial Aveneae had marginally significantly higher 1Cx values compared to the perennial Poeae. The MC showed no significant differences between the perennials of all groups.

Contrasts of the life forms within groups showed that the annual Aveneae had higher 2C values than the perennials. Annual Poeae and annual intertribe hybrids had higher 1Cx values than the corresponding perennials. Annual Aveneae and annual intertribe hybrids had significantly higher MCs than the corresponding perennials.

Conclusions

The large amount of available genome size data and the comparatively dense taxonomic sampling allow us to study also the effects of polyploidy and dysploidy in several different lineages of our study group. Polyploidy, as a major source of genomic variation, appears to be consistently associated with genome downsizing, i.e., a reduction in genome size, only in highly polyploids, whereas this is not necessarily the case in polyploids with lower valences. Furthermore, the presence or absence of downsizing can be found in both autopolyploids and allopolyploids. The same is true for dysploidy, a rarer type of genomic change in the study group, which can lead to an increase in genome size, but often also to a decrease. The origin of the strikingly low chromosome number taxa of the Poodae with only $x = 2$ resulted from chromosome translocations from $x = 7$ taxa, as suggested by the almost uniform monoploid genome sizes. The transition from a perennial to an annual life form was not associated with a consistent reduction of the genome, as indicated by the partly large monoploid genome sizes of annuals. However, there may be an upper limit to holoploid genome size (and ploidy level) in annuals that is not strongly operative in perennials, allowing the latter to show greater variability in holoploid genome size. Overall, there are apparently divergent trends in genome evolution that often overlap in Poodae taxa, making the study of genome evolution a rather surprising field of research. The widely held view that Poodae (and their sister group Triticodae) are characterized by consistently large genomes and chromosomes cannot be confirmed in any way. They are characterized by an immense variability that makes them appear to be a group that is actually undergoing an active evolutionary unfolding.

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, collection details and sample/standard ratio.
Online Resource 2. Comparison of genome sizes of diploid and polyploid (**a**), of auto- and allopolyploid (**b**, **c**, **d**, **e**, **f**, **g**, **h**) and of eu- and dysploid (**i**) taxa/cytotypes of the studied Poodae.
Online Resource 3. Statistical analyses of the studied genome (2C and 1Cx values) and chromosome sizes (MC) and life forms among the studied taxa (tribes Aveneae, Festuceae, Poeae and intertribe hybrids).
Online Resource 4. Genome sizes (2C values) of taxa taken from the literature and used in this study. Chromosome numbers (2n) as stated in the original publication; if in curly brackets, added in this study (see text for references). Ploidy levels, 1Cx values and mean chromosome DNA content (MC) partly revised or calculated in this study.

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Declarations

Conflict of interest The authors declare no competing interests.

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