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ORIGINAL ARTICLE

Genome size variation in *Deschampsia cespitosa* sensu lato (Poaceae) in Eurasia

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Abstract

The grass *Deschampsia cespitosa* is a variable taxon out of which many varieties, subspecies and endemic species have been separated. In this paper, the variation in genome size (GS) and ploidy of this grass including several of its subspecies and two related species in Eurasia was investigated by flow cytometric (FCM) measurements. GS and ploidy data were also related to specific environments and reproduction mode. Ploidy levels found by FCM were confirmed by chromosome counts of diploid ($2n = 28$) and tetraploid ($2n = 52$) samples. Seminiferous (seed bearing) *D. cespitosa* was mainly diploid (GS between 3.754 and 5.438 pg/1C). GS variation in diploids showed a geographic pattern with a significant difference ($H = 41{,}441$, $P < 0.001$) between European (median = 4.377 pg) and Asian (median = 4.881 pg) accessions. Genome size (1C) in tetraploids ranged from 7.9426 to 9.0399 pg. Tetraploid seminiferous *D. cespitosa* was found mostly in disturbed habitats in western and southern Europe, while tetraploids in Asia were registered in wet Arctic habitats. Genome size (1C between 8.3278 and 8.8603 pg) of the pseudoviviparous plants (spikelets produce plantlets asexually) of wet habitats in central and northern Europe indicated tetraploidy. A putative triploid (GS 6.6817 pg) was detected in Iceland. Summing up, we found a high variation in GS on the geographic scale with significant regional differences in diploid *D. cespitosa*. Among the tetraploids, the asexually reproducing plants were bound to specific habitats, while the seminiferous plants showed a habitat preference similar to the diploids.

Keywords *Deschampsia* · Flow cytometry · Genome size · Geographical variation · Polyploidy · Pseudovivipary

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Introduction

There is increasing evidence that all plant lineages have undergone several whole-genome doubling (WGD) events, i.e., cycles of polyploidization (e.g., Jiao et al. 2011; Soltis et al. 2014; Wendel et al. 2018) although the exact number and timing of these events are still disputed (Ruprecht et al. 2017). From analyzing the whole-genome data of major lineages, Jiao et al. (2011) infer at least two events of ancient whole-genome duplications to have occurred about 319 and 192 million years ago; this is long before the divergence of monocots and eudicots. Further duplications within various lineages provided raw material for plant evolution, which makes the study of its relationship with lineage diversification essential (Weiss-Schneeweiss and Schneeweiss 2013).

Grasses (Poaceae) are among those groups that have experienced ancient WGDs (Jiao et al. 2011) affecting the entire family, and further ancient or more recent polyploidizations in several lineages such as those in *Andropogon*

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gerardii Vitman (Keeler and Davis 1999), in the genera *Hordeum* L. (Eilam et al. 2009), as well as in *Zea* L. and *Triticum* L. (Kellogg 2015), have played a major role in evolution. The genus *Deschampsia* is another group experiencing polyploidization followed by chromosomal rearrangements (Amosova et al. 2017), as evidenced also by different ploidy levels, chromosome numbers (Amosova et al. 2015, 2017) and a high variation in genome size (1C from 5 to 5.5 pg) for diploids (Bennett et al. 1982; Murray et al. 2005; Pascual-Díaz et al. 2020). However, we found only one value for a tetraploid plant (1C = 9 pg) reported by Bennett et al. (1982). The basic chromosome number of the genus ($x = 13$) differs from other members of the core Pooideae (mostly $x = 7$), suggesting polyploidy incidence predating the diversification of the genus. In the traditional narrow sense, with exclusion of taxa formerly ascribed to *Deyeuxia* Clarion ex P.Beauv (Saarela et al. 2017) and *Scribneria* Hack (Soreng et al. 2015), *Deschampsia* comprises ca. 30 species of mostly perennials and a few South American annual species (Chiapella and Zuloaga 2010). Hybridization and reticulate evolution are common in this group of grasses (Wölk and Röser 2017) and might obscure phylogenetic relationships that are traditionally based on only a few selected molecular markers.

Deschampsia cespitosa (L.) P.Beauv., the tufted hair grass, is the most common species of the genus, found in all continents, with a disjunct distribution among the northern hemisphere landmasses, southern South America, Australasia and South Africa. *Deschampsia cespitosa* is a tussock-forming, wind-pollinated, self-incompatible grass, occurring in habitats with moderate to high moisture in a cold-temperate climate. This grass has established populations in similar habitats in regions separated by thousands of kilometers, where appropriate ecological conditions are present.

Taxonomic challenges

The high morphological variation of *Deschampsia cespitosa* has led to a confusing taxonomy in the northern hemisphere (e.g., Böcher et al. 1968; Porsild and Cody 1980) where several Central European populations in particular ecological settings have been treated as narrow endemic species (Conert 1987; Lauber and Wagner, 1998), or subspecies (Chiapella 2000). Intergradation and hybridization between geographic variants that may be considered subspecies were assumed by Clarke (1978). Taxa from Russia and eastern Asia were also treated either as different species (Probatova 1985; Czerepanov 1995; Tzvelev and Probatova 2012) or as infraspecific taxa by Chiapella and Probatova (2003). Several endemic taxa were described by Tzvelev et al. (2015) and Tzvelev and Probatova (2019). In contrast, the lumping treatment in the Flora of China (Wu and Phillips 2006) recognizes only two species (*D. koelerioides* Regel and *D. cespitosa*) with four subspecies of *D. cespitosa*. The occurrence of pseudovivipary or facultative pseudovivipary has further complicated systematic approaches. With this form of clonal reproduction, the entire spikelet is transformed into a small plantlet. Because of the unclear taxonomy, especially in the northern hemisphere, some authors (e.g., Kawano 1963; Rothera and Davy 1986) preferred to use the informal rank of *D. cespitosa* complex which includes all morphologically similar varieties, subspecies and regional species.

Karyological variation

Deschampsia cespitosa has been studied in various regional samples (e.g., Kawano 1963; Albers 1980; Garcia-Suarez et al. 1997; Murray et al. 2005; Amosova et al. 2017). Eurasian accessions of *D. cespitosa* were found to be mostly diploid with $2n = 26$ (Kawano 1963; Albers 1975, 1980; Garcia-Suarez et al. 1997; Dobes and Vitek 2000) with occasional reports of $2n = 28$ (Kawano 1963) or $2n = 26 + 1B$ (Marhold et al. 2007). Aneuploidy was reported for both the diploids (Kawano 1963) and the polyploids (tetraploids or occasionally also triploids; Kawano 1963; Albers 1980; Hedberg 1958). Deviating numbers such as $2n = 41$, 49, 50 (Löve and Löve 1975; Albers 1980) and $2n = 42$ (Sokolovskaya and Probatova 1975) have also occasionally been reported for the polyploids. In a detailed survey of ploidy levels in populations of *D. cespitosa* across Great Britain, both diploids and tetraploids ($2n = 26$ and $2n = 52$, respectively) were reported by Rothera and Davy (1986). This survey revealed that tetraploids are the predominant cytotype on the island. The presence of diploids and polyploids has also been documented in several of the taxa of different ranks related to *D. cespitosa* recognized from Russia (Chiapella and Probatova 2003; Tzvelev and Probatova 2019). It has been hypothesized that morphological variation may be correlated with ploidy levels (Chiapella and Probatova 2003).

Thus, *Deschampsia cespitosa* provides an interesting system to study the role of polyploidy in the context of biogeographical and habitat features. Using flow cytometry (FCM), this paper reports the genome size and ploidy-level variation of multiple populations of *D. cespitosa* to (i) identify biogeographic patterns related to genome size and ploidy levels; (ii) examine to which extent ploidy is related to specific environments and asexual reproduction.

Materials and methods

Plant material

Plant leaves of 129 populations of *Deschampsia cespitosa* and two related species for FCM were collected in the field and dried under standard herbarium conditions. Tillers were

also transplanted to the Botanical Garden of the University of Vienna (HBV) to provide the source of fresh leaf tissue for genome size measurements and root tips for chromosome counts. Plantlets of pseudoviviparous origin were also used for genome size measurement and grown in petri dishes for collecting root tips. Finally, fresh material was used for only a few samples from Lake Constance and for a small number of Austrian samples. The majority of samples for genome size measurement, however, was taken from the dried vouchers collected during the field trips. In a pilot test, we have followed the performance of fresh and dried leaf samples by measuring after several intervals over 5 months (data in Online Resource 1). The vast majority of our samples was stored between 3 and 4 months before processing. Vouchers of the samples are deposited in the herbaria WU and CSH (for duplicates of Chinese samples).

With the broad concept of *Deschampsia cespitosa*, we follow here Chiapella and Probatova (2003) and further relevant sources (Clarke 1980; Chiapella 2000; Wu and Phillips 2006) as none covers the entire investigated area (see Online Resource 2). We were not able to collect the newly described endemic taxa by Tzvelev and Probatova (2019). Regarding Europe, we are aware of several variants given subspecific or specific rank (Clarke 1980; Conert 1987; Chiapella 2000) or regarded as evolutionary distinct regional units (Peintinger et al. 2012) or narrowly distributed neo-endemics (Heydel et al. 2017). However, for the purpose of this large-scale study we could not collect all those variants that in part may reflect ecotypical differentiation. Following Peintinger et al. (2012), we have separated the *D. cespitosa* subsp. *rhenana* (Gremli) Kerguélen and *D. cespitosa* subsp. *littoralis* (Gaudin) K.Richter.

Additionally, we have included in this investigation two rare species related to *D. cespitosa*, namely *D. koelerioides* Regel, which was also considered a subspecies of the former by Tzvelev (1976), and *D. argentea* Lowe from Macaronesia which may be an endemic derivative of *D. cespitosa*. These samples were not included in the statistical analyses.

Genome size measurements using flow cytometry (FCM)

Twenty-five milligrams of fresh or dried leaf tissue was co-chopped (Galbraith et al. 1983) along with fresh standard leaf material (*Solanum pseudocapsicum* L., 1C = 1.295 pg; Temsch et al. 2010) in Otto's buffer I (Otto et al. 1981) using a sharp razor blade. The resulting nuclear isolate was filtered through a 30-µm nylon mesh. Subsequently, double-stranded RNA was removed by a half an hour treatment with RNase A (Sigma-Aldrich, USA) at 37 °C. Afterward, Otto's buffer II (Otto et al. 1981) that contained 50 mg/L propidium iodide (PI; AppliChem GmbH, Germany) was added. The preparations were allowed to incubate before measurement for at least 1 h in the refrigerator or overnight. The samples were measured using a flow cytometer CyFlow ML or CyFlow space (both Sysmex Partec GmbH, Germany), equipped with a diode-pumped laser (532 nm, 100 mW, Cobolt AB, Sweden). The 1C values of each sample were calculated in respect of a linear relationship between the mean fluorescence intensity (FI) of the G1 nuclei population of the standard and the samples. $1C\ value_{sample} = mean\ FI_{sample\ G1}/mean\ FI_{standard\ G1} * 1C\text{-}value_{standard}$. From 3,333 up to 10,000 particles were measured per preparation. Means and standard deviations for several runs are given in Table 1. For visualization of different genome sizes, squared decimal size classes were used with genome sizes < 4.1 pg combined in a single class. The DNA ploidy estimated from the FCM data was calibrated by chromosome counts in one tetraploid and two diploid samples.

Chromosome number analyses and Feulgen densitometry (FDM)

Feulgen densitometry (FDM) was used for chromosome number analyses in order to verify the ploidy level in two diploid and one tetraploid individuals (genome size data not given, Table 1 contains only FCM data of these samples). Root tips were harvested from healthy plantlets of pseudoviviparous plants grown in petri dishes and from samples of seminiferous (seed bearing) plants grown in HBV. For chromosome number analyses, selected root tip meristems were pre-treated with 0.002 M 8-hydroxyquinoline in darkness for 2.5 h at room temperature and 2.5 h at 4 °C, fixed in methanol/acetic acid (3:1) overnight and stored at − 20 °C until use. The fixed root tips were washed six times in distilled water together with fixed root tips from an internal standard and hydrolyzed in 5 N HCl in an ultra-thermostatic water bath (Model LTD6, Grant Instruments Ltd., Cambridge, Barrington, England) at 20.0 °C for 60 min. The hydrochloric acid was removed by three washings with distilled water, followed by the staining step with Schiff's reagent (Merck, Darmstadt, Germany) for 1.5 h at room temperature under light protection. The samples were subsequently washed six times with SO_2 water (0.02 M potassium metabisulfite dissolved in 0.01 N HCl) over a time period of 45 min. Each root tip was squashed in 45% acetic acid on a slide under a cover slip. After removal of the cover slip, the slides were shortly fixed with ethanol (96%) and finally air-dried.

The preparations were analyzed using the AxioPlan light microscope (Carl Zeiss, Vienna, Austria) equipped with a CCD black–white camera. Images of the metaphase plates were captured using ZEN software (Carl Zeiss, Vienna, Austria). Genome size was estimated from telophases of the objects and the standard in order to unambiguously assign the ploidy level to the genome size estimates on the basis of single root tips. Therefore, the integrated optical density

Table 1 The studied *Deschampsia* populations, their holoploid and monoploid genome size values (1C and 1Cx, pg), ploidy level, collection site including latitude, longitude and altitude

Nr	Country	Prov/State/County	Latitude	Longitude	Altitude (m)	1C (pg)	S.D.	n runs	1Cx (pg)	DNA Ploidy	Mat (d/f)	Repr (s/v)	Hab class	Subsp.	Sample ID
1	ARM	Tawusch	40.835930	44.884380	2110	4.3228		1	4.3228	2x	d	s	Wet	cespitosa	228
2	AUT	Steiermark	47.425556	13.615278	1170	4.3954	0.1355	7	4.3954	2x	f	s	Wet	cespitosa	1
3	AUT	Steiermark	47.373056	14.481111	1230	4.5234	0.0476	7	4.5234	2x	f	s	Wet	cespitosa	2
4	AUT	Niederösterreich	47.915556	16.050983	395	4.3671	0.0872	4	4.3671	2x	d	s	Wet	cespitosa	12
5	AUT	Steiermark	47.634278	13.854167	732	4.3732		1	4.3732	2x	d	s	Wet	cespitosa	15
6	AUT	Niederösterreich	47.718889	15.761111	1610	4.3769		1	4.3769	2x	d	s	Grass	cespitosa	17
7	AUT	Niederösterreich	48.948889	15.220833	620	4.4279	0.3190	2	4.4279	2x	d	s	Wet	cespitosa	24
8	AUT	Steiermark	47.355333	15.481781	1230	4.4067		1	4.4067	2x	d	s	Forest	cespitosa	48
9	AUT	Steiermark	47.348531	15.480414	1200	4.3018	0.0956	4	4.3018	2x	d	s	Wet	cespitosa	49
10	AUT	Wien	48.192889	16.447633	160	4.4773	0.0542	11	4.4773	2x	d	s	Wet	cespitosa	166
11	AUT	Vorarlberg	47.506670	9.710280	400	8.7420		1	4.3710	4x	f	v	Wet	rhenana	990
12	AUT	Niederösterreich	47.994167	15.958611	900	4.6660	0.0038	3	4.6660	2x	d	s	Forest	cespitosa	994
13	AUT	Wien	48.193055	16.382777	170	4.3741	0.0203	3	4.3741	2x*	f	s	Ruderal	cespitosa	995
14	AUT	Niederösterreich	47.856914	16.091500	520	4.2522	0.0123	3	4.2522	2x	d	s	Ruderal	cespitosa	998a
15	AUT	Niederösterreich	47.856914	16.091500	520	8.6279		1	4.3140	4x	d	s	Ruderal	cespitosa	998b
16	CHE	Graubünden	46.671383	9.562867	947	4.4629		1	4.4629	2x	d	s	Wet	cespitosa	6
17	CHE	Graubünden	46.473400	9.721300	2233	4.3969		1	4.3969	2x	d	s	Grass	cespitosa	7
18	CHE	Graubünden	46.406433	9.704583	1797	4.5192	0.0555	4	4.5192	2x	d	s	Wet	cespitosa	8
19	CHE	Graubünden	46.273467	10.109500	962	4.5061		1	4.5061	2x	d	s	Wet	cespitosa	9
20	CHE	Canton Vaud	46.665278	6.324167	1000	8.9732	0.1377	9	4.4866	4x*	d	s	Wet	littoralis	154
21	CHE	Canton Thurgau	47.655278	8.954444	400	8.7241	0.0262	6	4.3621	4x	d	v	Wet	rhenana	156
22	CHE	Canton Thurgau	47.632778	9.239167	400	8.7402	0.0629	12	4.3701	4x	f	v	Wet	rhenana	157
23	CHE	Canton Vaud	46.620470	6.247861	1000	9.0399	0.1271	7	4.5200	4x	d	s	Wet	littoralis	216
24	CHN	Xinjiang	43.807780	87.998138	1959	4.7274		1	4.7274	2x	d	s	Wet	pamirica	190
25	CHN	Xinjiang	43.119061	86.989190	2705	5.1957		1	5.1957	2x	d	s	Wet	pamirica	191
26	CHN	Qinghai	37.128991	101.568463	2663	4.7153		1	4.7153	2x	d	s	Wet	orientalis	194
27	CHN	Qinghai	37.996606	100.927149	3628	4.5269		1	4.5269	2x	d	s	Wet	orientalis**	197
28	CHN	Qinghai	38.097691	100.194161	3260	4.9147		1	4.9147	2x	d	s	Ruderal	orientalis	198
29	CHN	Qinghai	37.271870	99.898515	3234	4.9910		1	4.9910	2x	d	s	Ruderal	orientalis	200
30	CHN	Qinghai	37.035230	98.661912	3510	5.4375		1	5.4375	2x	d	s	Wet	orientalis**	201
31	CHN	Qinghai	35.831190	99.829300	3801	4.9024		1	4.9024	2x	d	s	Grass	orientalis**	202
32	CHN	Qinghai	35.500085	99.791060	3673	4.8808		1	4.8808	2x	d	s	Wet	orientalis**	203
33	CHN	Sichuan	29.882158	102.019095	3639	5.3777	0.0068	3	5.3777	2x	d	s	Wet	orientalis	204
34	CHN	Sichuan	29.991143	101.878902	3221	5.1003		1	5.1003	2x	d	s	Wet	orientalis	205
35	CHN	Sichuan	30.014773	101.860549	3557	5.1003		1	5.1003	2x	d	s	Wet	orientalis**	206
36	CHN	Sichuan	30.076194	101.805183	4292	5.0146		1	5.0146	2x	d	s	Grass	orientalis**	207

Table 1 (continued)

Nr	Country	Prov/State/County	Latitude	Longitude	Altitude (m)	1C (pg)	S.D.	n runs	1Cx (pg)	DNA Ploidy	Mat (d/f)	Repr (s/v)	Hab class	Subsp.	Sample ID
37	CHN	Sichuan	31.731949	100.735661	3908	5.3232		1	5.3232	2x	d	s	Ruderal	orientalis**	209
38	CHN	Sichuan	31.708748	102.312484	4010	5.0215		1	5.0215	2x	d	s	Ruderal	orientalis**	210
39	CHN	Sichuan	30.279167	99.553056	4642	5.1593		1	5.1593	2x	d	s	Wet	cespitosa s.l.	214
40	CZE	Severomoravsky	49.819153	17.666156	440	4.2363		1	4.2363	2x	d	s	Wet	cespitosa	29
41	DEU	Bayern	48.872083	13.047889	753	4.3616		1	4.3616	2x	f	s	Wet	cespitosa	3
42	DEU	Bayern	48.018300	11.141825	533	4.5704	0.1649	6	4.5704	2x	f	s	Forest	cespitosa	4
43	DEU	Baden-Württemberg	47.686817	9.086317	395	4.3220	0.0760	6	4.3220	2x	d	s	Wet	cespitosa	5
44	DEU	Schleswig-Holstein	53.618611	13.616944	60	4.3278		1	4.3278	2x	d	s	Forest	cespitosa	18
45	DEU	Thüringen	50.809444	10.407778	540	3.9323		1	3.9323	2x	d	s	Forest	cespitosa	19
46	DEU	Nordrhein-Westfalen	50.514722	6.285833	540	4.0363		1	4.0363	2x	d	s	Ruderal	cespitosa	20
47	DEU	Sachsen-Anhalt	51.748333	10.935000	420	3.7543		1	3.7543	2x	d	s	Forest	cespitosa	21
48	DEU	Schleswig-Holstein	54.093056	10.094167	30	4.5338		1	4.5338	2x	d	s	Forest	cespitosa	22
49	DEU	Baden-Württemberg	47.679169	9.322386	450	4.5940	0.0591	7	4.5940	2x*	f	s	Ruderal	cespitosa	37
50	DEU	Baden-Württemberg	47.670556	9.213889	400	8.7546	0.1823	10	4.3773	4x	d	v	Wet	rhenana	152
51	DEU	Baden-Württemberg	47.747500	9.143056	400	8.4793	0.3500	9	4.2397	4x	d	v	Wet	rhenana	153
52	DEU	Bayern	47.561111	9.644167	400	8.6444		1	4.3222	4x	f	v	Wet	rhenana	996
53	DEU	Baden-Württemberg	47.669722	9.329167	400	8.8603	0.0459	3	4.4302	4x	f	v	Wet	rhenana	997
54	DEU	Baden-Württemberg	47.703056	9.043611	400	8.7967	0.1694	5	4.3984	4x	f	v	Wet	rhenana	999
55	ESP	Castilla y León	42.855970	− 6.817722	1700	7.9426	0.0633	3	3.9713	4x	d	s	Wet	cespitosa	94
56	ESP	Ávila. Piedrahita	40.423170	− 5.293222	?	4.2178	0.0065	3	4.2178	2x	d	s	Wet	cespitosa	95
57	EST	Ida-Viru County	59.004167	27.360556	40	4.3729	0.0079	3	4.3729	2x	d	s	Forest	cespitosa	50
58	EST	Ida-Viru County	58.981490	27.183890	40	4.3863	0.0551	3	4.3863	2x	d	s	Forest	cespitosa	142
59	FIN	Etelä-Suomen	60.343889	25.603528	8	4.3677		1	4.3677	2x	d	s	Forest	cespitosa	39
60	FIN	Etelä-Savo	61.672528	27.276694	88	4.2418		1	4.2418	2x	d	s	Ruderal	cespitosa	40
61	FIN	Lapland	65.616306	26.718333	134	4.5808		1	4.5808	2x	d	s	Forest	cespitosa	41
62	FIN	Lapland	67.973283	23.681267	256	4.3201		1	4.3201	2x	d	s	Forest	cespitosa	42
63	FIN	Enotekiö	69.060944	20.770000	494	4.4937	0.0263	4	4.4937	2x	d	s	Ruderal	cespitosa	43
64	FIN	Enotekiö	69.089222	20.782000	595	4.0615	0.0748	5	4.0615	2x	d	s	Wet	cespitosa	44
65	FIN	Northern Ostrobothnia	65.028944	25.446111	2	4.5265		1	4.5265	2x	d	s	Wet	cespitosa	45

Table 1 (continued)

Nr	Country	Prov/State/County	Latitude	Longitude	Altitude (m)	1C (pg)	S.D.	n runs	1Cx (pg)	DNA Ploidy	Mat (d/f)	Repr (s/v)	Hab class	Subsp.	Sample ID
66	FIN	Central Ostrobothnia	63.817194	22.978056	2	4.0070		1	4.0070	2x	d	s	Forest	cespitosa	46
67	FRA	Auvergne. Massif Central	45.066486	4.079469	1100	8.4467		1	4.2234	4x	d	s	Ruderal	cespitosa	31
68	FRA	Auvergne. Massif Central	45.070831	4.126953	1200	8.0231	0.0651	3	4.0116	4x	d	s	Ruderal	cespitosa	32
69	FRA	Rhone Alpes. Vercors	45.071469	5.494672	730	4.2972		1	4.2972	2x	d	s	Wet	cespitosa	33
70	FRA	Rhone Alpes. M. Vanoise	45.388144	6.567428	1400	4.3943		1	4.3943	2x	d	s	Wet	cespitosa	34
71	FRA	Haut-Jura	46.496972	6.083319	1010	4.5695	0.1122	7	4.5695	2x	d	s	Forest	cespitosa	35
72	GBR	England. North Yorkshire	54.245978	– 0.682961	140	8.5221	0.2794	4	4.2611	4x	d	s	Ruderal	cespitosa	14
73	GBR	Scottland. Ross & Cromarty	57.600000	– 4.951189	125	4.2978	0.0831	4	4.2978	2x	d	s	Ruderal	cespitosa	13a
74	GBR	Scottland. Ross & Cromarty	54.245978	– 0.682961	140	8.2434	0.0425	4	4.1217	4x	d	s	Ruderal	cespitosa	13b
75	HRV	Zagorje	45.947222	15.825000		4.3362		1	4.3362	2x	d	s	Wet	cespitosa	141
76	ISL	Western Region	64.600556	– 22.019444	35	4.5419	0.1443	11	4.5419	2x	d	s	Grass	cespitosa	96
77	ISL	Western Region	64.877222	– 23.685833	200	8.5727	0.0112	3	4.2864	4x	d	v	Grass	alpina	97
78	ISL	Western Region	64.877222	– 23.685833	150	4.4486	0.0153	3	4.4486	2x	d	s	Ruderal	cespitosa	98
79	ISL	Western Region	65.550278	– 24.352778	5	4.4427	0.0070	3	4.4427	2x	d	s	Ruderal	cespitosa	100
80	ISL	Northeastern Region	65.595833	– 17.177500	260	4.4680	0.0071	3	4.4680	2x	d	s	Forest	cespitosa	105
81	ISL	Northeastern Region	66.048333	– 17.345000	20	4.5668	0.0064	3	4.5668	2x	d	s	Ruderal	cespitosa	106
82	ISL	Southern Region	63.891944	– 21.364444	10	4.2975	0.0646	9	4.2975	2x	d	s	Ruderal	cespitosa	109
83	ISL	Northeastern Region	65.669390	– 15.095570	155	4.5180	0.0141	3	4.5180	2x	d	s	Grass	cespitosa	115
84	ISL	Northeastern Region	66.361740	– 14.947700	11	4.4531	0.0084	3	4.4531	2x	d	s	Grass	cespitosa	116
85	ISL	Northeastern Region	66.361740	– 14.947700	11	8.3636	0.0201	3	4.1818	4x	d	v	Wet	alpina	117
86	ISL	Northeastern Region	65.512222	– 18.602778	290	4.4406	0.0585	4	4.4406	2x	d	s	Grass	cespitosa	108a
87	ISL	Northeastern Region	65.512222	– 18.602778	290	8.3278	0.0202	3	4.1639	4x	d	v	Wet	alpina	108b

Table 1 (continued)

Nr	Country	Prov/State/County	Latitude	Longitude	Altitude (m)	1C (pg)	S.D.	n runs	1Cx (pg)	DNA Ploidy	Mat (d/f)	Repr (s/v)	Hab class	Subsp.	Sample ID
88	ISL	Northeastern Region	65.512222	− 18.602778	290	6.6817	0.0727	4	4.4545	3x	d	s	Wet	cesp X alp	108c
89	ITA	Bolzano	46.770067	11.966483	1079	4.4418		1	4.4418	2x	f	s	Forest	cespitosa	10
90	ITA	Udine	46.496567	13.690333	857	4.3915		1	4.3915	2x	f	s	Forest	cespitosa	11
91	ITA	Toscana. Pistoia	44.126389	10.633611	1560	8.6328		1	4.3164	4x	d	s	Grass	cespitosa	23
92	KGZ	Osh Region	39.490520	72.911420	3560	4.9631		1	4.9631	2x	d	s	Wet	cespitosa s.l.	227
93	KOR	Cheju Island	33.361872	126.517726	1680	4.2285	0.2336	6	4.2285	2x	d	s	Forest	cespitosa	36
94	MKD	Municip Struga	41.260000	20.531944		8.4416	0.0801	3	4.2208	4x	d	s	Wet	cespitosa	144
95	NOR	Viken	60.123611	10.374722	200	4.4086	0.1026	4	4.4086	2x	d	s	Grass	cespitosa	158
96	POL	Sulecyn County	52.292317	15.058283	90	4.3018		1	4.3018	2x	d	s	Ruderal	cespitosa	26
97	POL	Magura Witowska	49.311581	19.831108	830	4.0800		1	4.0800	2x	d	s	Forest	cespitosa	28
98	POL	Lublin. Chelm County	51.143056	23.311389	210	3.8728		1	3.8728	2x	d	s	Ruderal	cespitosa	76
99	RUS	Tyumen Region	57.105000	66.106389	70	3.9999	0.0104	2	3.9999	2x	d	s	Forest	cespitosa	78
100	RUS	Sverdlovsk Region	57.146111	60.215556	290	4.3737	0.0793	3	4.3737	2x	d	s	Grass	cespitosa	82
101	RUS	Sverdlovsk Region	59.598333	69.300000	30	4.3794	0.0106	3	4.3794	2x	d	s	Forest	cespitosa	84
102	RUS	Leningrad Region	60.649710	33.116310	10	4.3696	0.0376	3	4.3696	2x	d	s	Forest	cespitosa	87
103	RUS	Sakhalin Region	48.225640	142.541242	30	4.2112	0.0327	3	4.2112	2x	d	s	Ruderal	cespitosa s.l.	88
104	RUS	Tver Region	57.777660	35.223740	160	4.3066		1	4.3066	2x	d	s	Grass	cespitosa	89
105	RUS	Moscow Region	55.091185	37.501470	180	4.5834		1	4.5834	2x	d	s	Ruderal	cespitosa	121
106	RUS	Murmansk Region	67.860000	34.420000	380	4.4595		1	4.4595	2x	d	s	Ruderal	cespitosa	122
107	RUS	Karelia	66.301960	33.286260	0	4.7046		1	4.7046	2x	d	s	Grass	cespitosa	131
108	RUS	Krasnodar Region	44.070500	39.703830	1500	4.5176		1	4.5176	2x	d	s	Ruderal	cespitosa	134
109	RUS	Taymir Region	69.003944	91.008389	48	8.4955		1	4.2478	4x	d	s	Wet	cespitosa	149
110	RUS	Yakutia	70.714660	127.417820	8	4.5432		1	4.5432	2x	d	s	Wet	obensis	217
111	RUS	Yakutia	71.847340	126.909180	220	8.4735		1	4.2368	4x	d	s	Wet	obensis	218
112	RUS	Yakutia	72.001590	129.109330	10	4.6751	0.2681	3	4.6751	2x	d	s	Ruderal	submutica	219
113	RUS	Yakutia	71.563930	128.760910	30	8.4726		1	4.2363	4x	d	s	Wet	submutica	220
114	RUS	Yakutia	66.775500	123.371460	30	4.5629		1	4.5629	2x	d	s	Wet	submutica	221
115	RUS	Burjatja	52.909170	108.160600	450	4.9784		1	4.9784	2x	d	s	Wet	turczaninowii	222
116	RUS	Irkutskaja Region	53.398330	107.438300	450	4.7819		1	4.7819	2x	d	s	Wet	turczaninowii	223
117	RUS	Chukotka	66.115440	− 170.522000	30	4.7392		1	4.7392	2x	d	s	Wet	obensis	235
118	RUS	Chukotka	64.714900	− 174.090500	30	4.8121		1	4.8121	2x	d	s	Wet	beringensis	236
119	RUS	Novgorod Region	57.082310	30.751470	90	4.4866		1	4.4866	2x	d	s	Wet	cespitosa s.l.	237
120	RUS	Kostroma Region	58.349722	42.216111	130	4.2465	0.0184	2	4.2465	2x	d	s	Wet	cespitosa s.l.	239

Table 1 (continued)

Nr	Country	Prov/State/County	Latitude	Longitude	Altitude (m)	1C (pg)	S.D.	n runs	1Cx (pg)	DNA Ploidy	Mat (d/f)	Repr (s/v)	Hab class	Subsp.	Sample ID
121	RUS	Voronezh Region	51.195250	40.308080	100	4.0687		1	4.0687	2x	d	s	Forest	cespitosa	241
122	SVK	Mala Fatra	49.253836	19.045972	550	3.9494		1	3.9494	2x	d	s	Forest	cespitosa	27
123	SWE	Skane	55.713330	13.306110	50	4.0507	0.0244	3	4.0507	2x	d	s	Forest	cespitosa	114
124	UKR	Chernivtsi Region	48.291840	25.876510		4.2246		1	4.2246	2x	d	s	Grass	cespitosa	25
125	UKR	Zarkapattia Region	48.150800	24.357580	1680	4.2715		1	4.2715	2x	d	s	Grass	cespitosa	111
Related taxa													*Species*		
126	CHN	Xinjiang	43.117684	86.848150	3516	5.2352		1	5.2352	2x	d	s	Wet	koelerioides	192
127	CHN	Xinjiang	43.110661	86.841308	3528	4.9385		1	4.9385	2x	d	s	Wet	koelerioides	193
128	CHN	Qinghai	38.021690	100.232778	3939	4.8637		1	4.8637	2x	d	s	Wet	koelerioides	199
129	PRT	Madeira	32.746667	−16.937500	1660	4.7538	0.0898	9	4.7538	2x	d	s	Forest	argentea	52

If more then one run per population was performed, mean values along with standard deviation (S.D.) are indicated. Country codes according to ISO 3166 standard: *AUT* Austria, *ARM* Armenia, *CHE* Switzerland, *CHN* China, *CZE* Czech republic, *DEU* Germany, *ESP* Spain, *EST* Estonia, *FRA* France, *FIN* Finland, *GBR* Great Britain, *HRV* Croatia, *ITA* Italy, *ISL* Iceland, *KGZ* Kyrgyzstan, *KOR* South Korea, *NOR* Norway, *MKD* North Macedonia, *POL* Poland, *PRT* Portugal, *RUS* Russian Federation, *SWE* Sweden, *SVK* Slovakia, *UKR* The Ukraine; sample (Mat): dry (d), fresh (f); reproduction (Repr): seminiferous (s), pseudoviviparous (v); habitat class (Habclass: forest/ruderal/wetland/grassland)

*DNA ploidy confirmed by chromosome count

**Approaching *D. koelerioides* in panicle shape (more contracted) and color (due to mostly golden glumes)

(IOD) was calculated from each nucleus by using the cell image and retrieval system (CIRES, Kontron, Germany) and the genome size estimates were calculated from the linear relationship between the IODs of the objects and the standard, respectively.

Biogeography, habitat and reproductive system

We have divided the entire *Deschampsia cespitosa* samples into two regions (Europe and Asia) separated by the 60th degree longitude (Urals). Longitudinal classes covering 10 degrees were applied for visualization of genome size variation related to geography. Four habitat classes (Habclass) based on descriptive sampling data were distinguished: forest (including clearings); grass (meadows, pastures); ruderal (wasteland, roadsides); and wet (wet meadows, lake shores, riverbanks, riparian gravel). Plants were collected in reproductive phase (flowering or with proliferous spikelets), or the reproductive system was determined from monitoring records (observed by MP).

Statistical analysis

SPSS statistics 25.0 (IBM, Armonk, New York, USA) was used for the analyses and visualization (box plots, bar charts and scatter diagrams) of the overall genome size variation, the variation within the ploidy levels, and its association with habitat classes. Nonparametric options were used to test for differences in sample parameters (Kruskal–Wallis tests). A linear multivariate regression was used to evaluate the effects of altitude, habitat and bioclimatic factors on the genome size of diploid plants. We extracted 19 bioclimatic variables of climate data for each sample location using ArcGIS 10.0.4 from the WorldClim database (Fick and Hijmans 2017; https://worldclim.org/data/worldclim21.html), which are the average for the years 1970–2000 at a resolution of 5 min. We ran pairwise correlation analyses among the 19 bioclimatic variables (BIO1-BIO19) and choose nine with low correlation ($R < 0.8$) which are BIO1 (annual mean temperature), BIO2 (mean diurnal range), BIO3 (isothermality), BIO4 (temperature seasonality), BIO5 (max temperature of warmest month), BIO8 (mean temperature of wettest quarter), BIO9 (mean temperature of driest quarter), BIO12 (annual precipitation) and BIO18 (precipitation of warmest quarter). The AIC was employed to determine the best model in stepwise backward multiple regression. The partial effects of each dependent variable in the best model are shown in partial plots according to Grace et al. (2016).

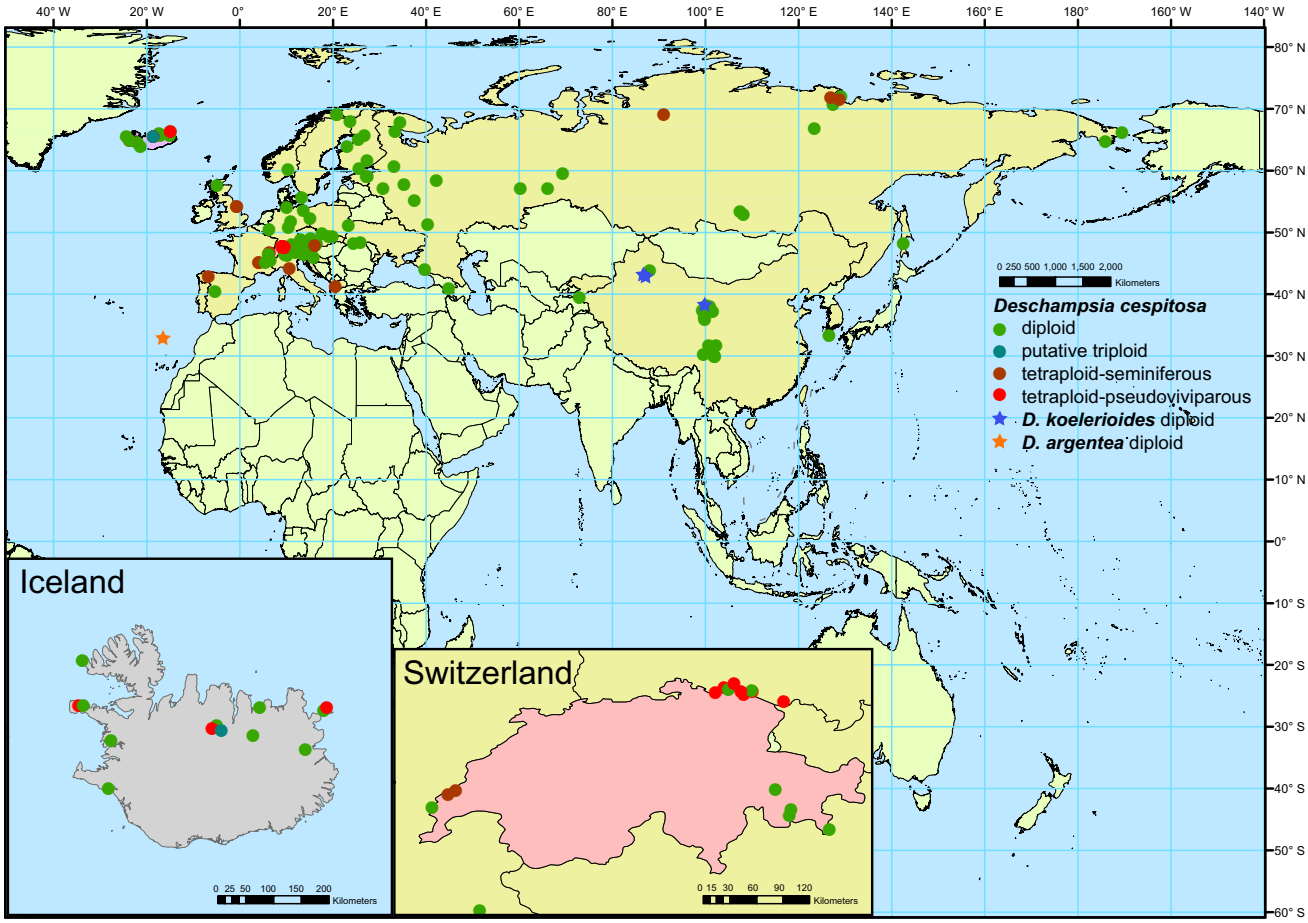

Fig. 1 Map showing the distribution of all *Deschampsia* samples in Europe and Asia. The map insets depict two regions with dense sampling in Iceland and Switzerland and the region around Lake Constance (including Germany and Austria). The symbols are explained in the map

Results

Genome size variation and ploidy

The geographical distribution of the entire sample is shown on the map in Fig. 1. The genome size (1C) ranged from 3.754 to 5.438 pg in diploid and from 7.943 to 8.9732 pg in tetraploid *Deschampsia cespitosa* (Fig. 2). We report here also the genome size of 4.864–5.235 pg found in *D. koelerioides* and 4.753 pg for *D. argentea*. Genome size data and DNA ploidy of the investigated population samples as revealed by the FCM measurements are provided in Table 1. Mitotic metaphase chromosomes for a diploid plant ($2n = 26$, 1C = 4.374 pg) of population 13 and a tetraploid plant ($2n = 52$, 1C = 8.973 pg) of population 20 are shown in Online Resource 3. The genome size of one individual of 6.682 pg suggested a putative DNA triploid, sampled in a mixed population (therefore, different numbers 86–88 in Table 1) of seminiferous and pseudoviviparous plants. Genome size (1C, pg) clearly varied between diploids, tetraploids and the putative triploid (Fig. 2). The monoploid genome sizes (1Cx, pg) showed geographically structured variation within the diploids, and between some diploids and all tetraploids, but not within tetraploids (Table 1, Fig. 3).

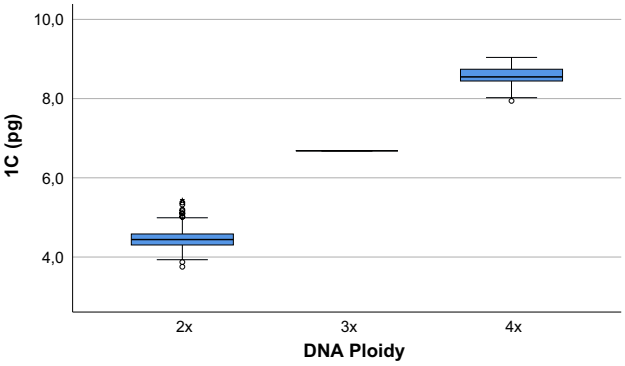

Fig. 2 Variation in genome size of *Deschampsia cespitosa* (1C, pg) in diploids ($n = 100$), tetraploids ($n = 24$; seminiferous and pseudoviviparous) and the putative triploid ($n = 1$)

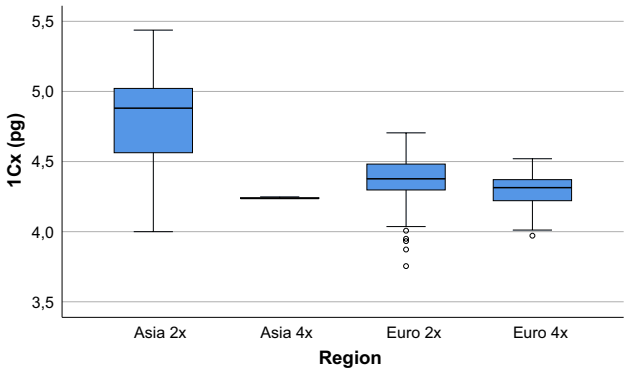

Fig. 3 Variation in monoploid genome size of *Deschampsia cespitosa* (Cx, pg) between diploids (n(Asia) = 29; n(Europe) = 71) and tetraploids (n(Asia) = 3; n(Europe) = 21)

Biogeographic patterns and environmental determinants of genome sizes

Monoploid genome sizes (Cx, pg) of Asian (median = 4.881) and European (median = 4.377) diploid populations (Fig. 3) were significantly different ($H = 41.441$, $P < 0.001$). Similarly, Asian diploids and all European tetraploids (median = 4.314) were significantly ($H = 58.007$, $P < 0.001$) different. There was a weak although nonsignificant tendency toward smaller genome sizes (i.e., down-sizing) in the European tetraploids compared to the diploids of the same region. The sample of 4x Asia was too small ($n = 3$) for any meaningful statistics. Variation in monoploid genome size from Western Europe to eastern Asia along longitude classes of 10 degree is shown in Online Resource 4.

While the tetraploid pseudoviviparous variants are clustered in Iceland and around Lake Constance (insets in Fig. 1), the distribution of the tetraploid seminiferous plants did not show a distinct geographic pattern. We found them in southern and Western Europe as well as in Arctic Siberia. No tetraploid seminiferous plants were found in northern Europe and in remaining Asia.

The genome size variation of the entire European and Asian sample of diploid plants was best explained by a model containing altitude, BIO5 (max temperature of warmest month) and BIO12 (annual precipitation). The altitude had a positive effect ($R_\partial = 0.53$, $P < 0.001$), and BIO5 ($R_\partial = -0.26$, $P < 0.001$) as well as BIO12 ($R_\partial = -0.25$, $P = 0.011$) had a negative effect on genome size (GS increment/decrement) shown in the partial plots (Fig. 4). The same analysis did not find any significant effect on genome size within the European diploids. European plants with small genomes (1C < 4.1 pg) were confined to low and moderate altitudes (< 830 m) mainly in temperate latitudes (49–52° N) of Central Europe, while plants with large genomes were found in the whole range of altitudes being scattered across all the studied area in Europe (Fig. 5a, b).

Ploidy in relation to ecology and reproduction

Frequencies of diploids and tetraploids considering their reproduction mode were analyzed in the four habitat types (Fig. 6). Both diploids and tetraploids showed a preference for wet habitats. Diploids were found in all environment types, whereas tetraploids were not found in forests. Seminiferous plants were either diploid or tetraploid, while pseudoviviparous plants were only tetraploid. Separating the tetraploids by habitat class, reproduction mode and region indicated the clear preference (91%) of pseudoviviparous plants for wet habitats, while a high proportion (50%) of the nonviviparous western tetraploid plants was found in ruderal habitats (Online Resource 5).

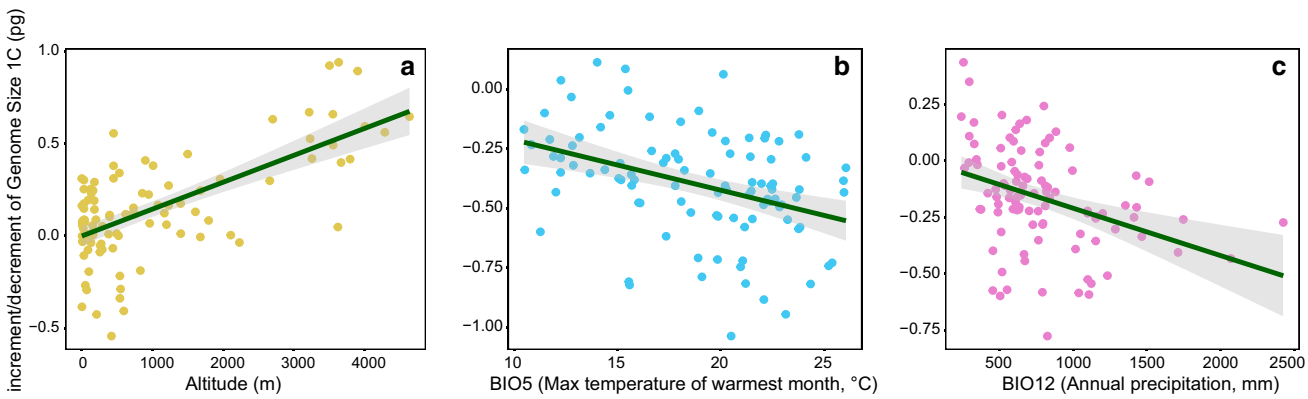

Fig. 4 Plots showing partial effects of predictors on genome size of diploid *Deschampsia cespitosa* revealed by a multivariate linear regression of **a** altitude, **b** BIO5 (max. temperature of warmest month), and **c** BIO12 (annual precipitation). Shaded areas represent 95% confidence intervals

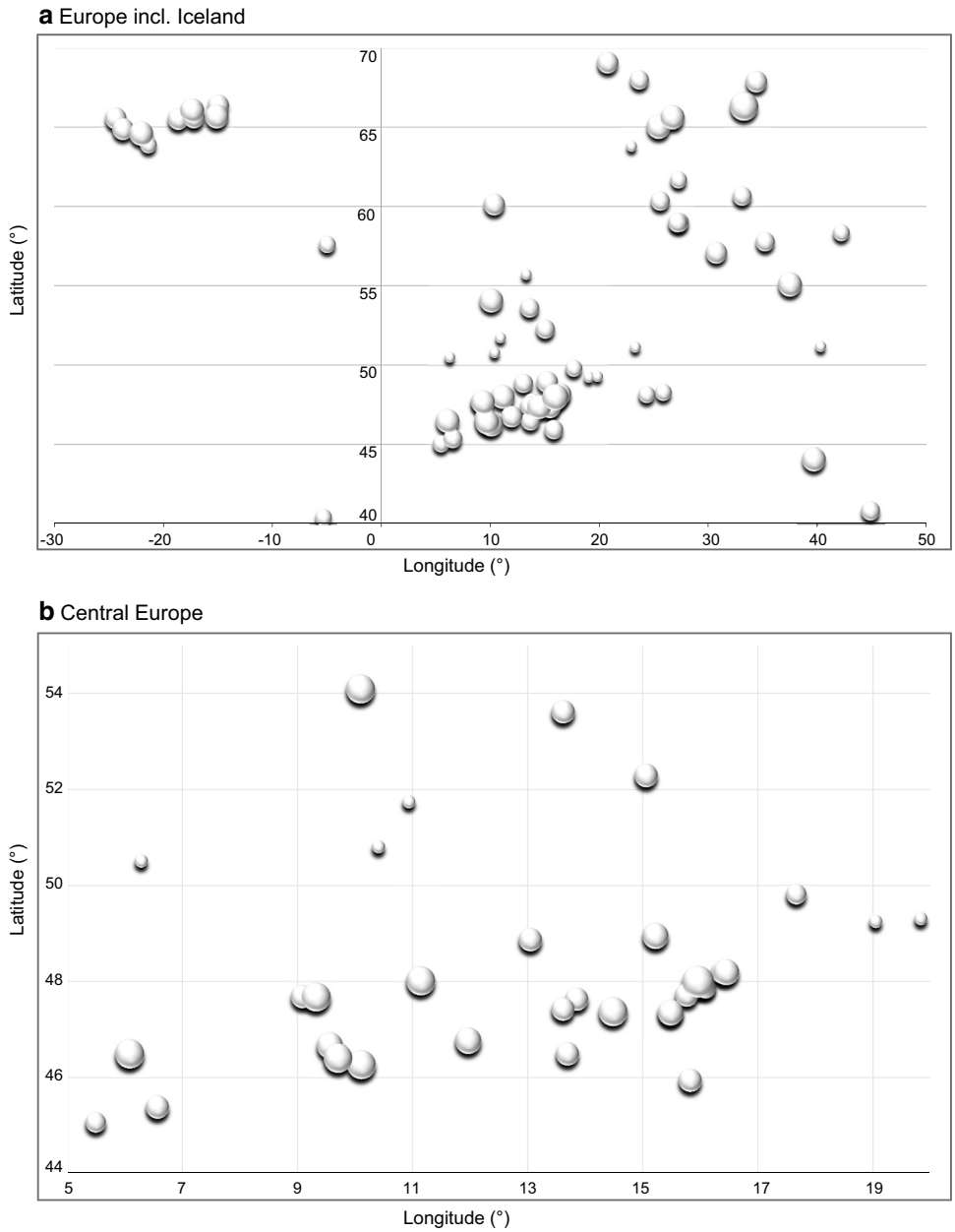

Fig. 5 Scatter plots showing different genome size classes of diploid *Deschampsia cespitosa* in relation to geographical longitude (*x*-axis) and latitude (*y*-axis)

Discussion

Distribution patterns and ecology of *Deschampsia cespitosa* in relation to ploidy levels and genome size

This study confirmed that seminiferous *Deschampsia cespitosa* is predominantly diploid in Eurasia. In addition, it revealed a pattern of variation in genome size related to biogeography between diploid European and Asian plants. Several tetraploid populations were found, but their number was too low to draw more general conclusions on their distribution apart from the fact that the tetraploid seminiferous plants tended to occur more often in disturbed habitats in Southern and Western Europe and in wet habitats in East Asia, whereas tetraploid pseudoviviparous plants were restricted to periglacial or high-latitude regions and special environments. Diploid *D. cespitosa* was found in all environments but exhibited a preference for wet habitats. Polyploids were reported to be more common than diploids on the British Isles (Rothera and Davy 1986), and although there was no evidence that they were better adapted to cold environments, they were putatively associated with more disturbed habitats. Given the wide distribution of Subarctic and Arctic variants of diploid *D. cespitosa* (this paper; Kawano 1963; Tzvelev and Probatova 2019) and

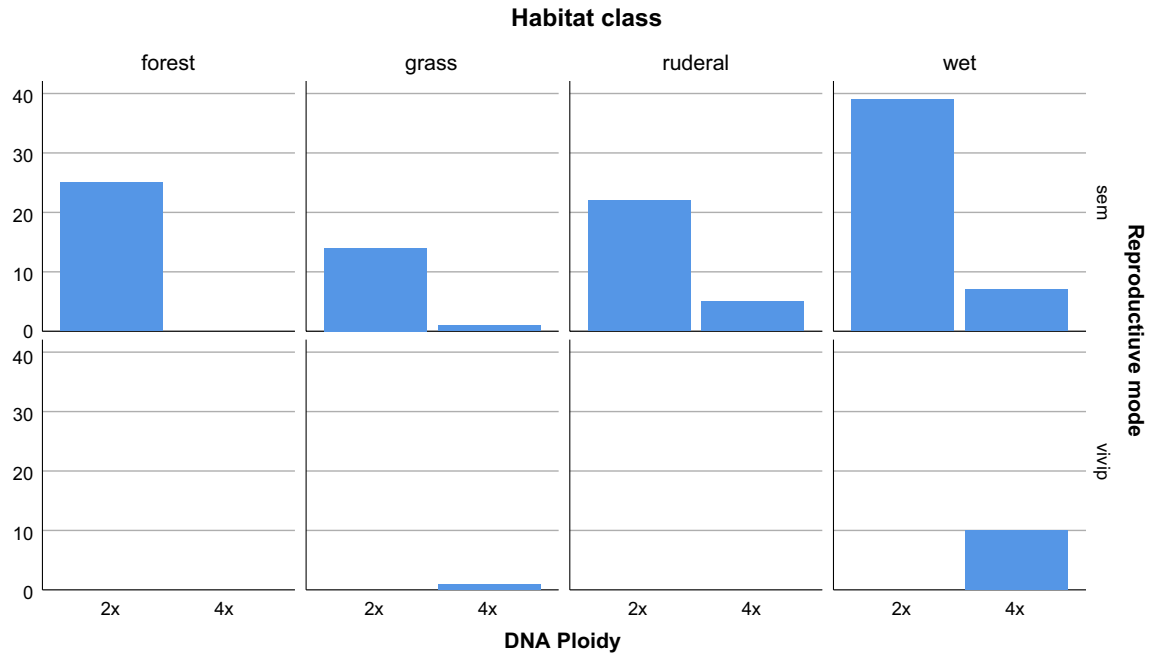

Fig. 6 Numbers of diploid and polyploid *Deschampsia cespitosa* populations separated by reproduction mode (sem = seminiferous; vivip = pseudoviviparous) in four habitat classes

the predominance of diploid *D. antarctica* E.Desv on the Antarctic Peninsula (González et al. 2016; Pascual-Díaz et al. 2020), it is unlikely that temperature alone affected the ploidy levels. Polyploids of various plant groups in arctic regions were hypothesized to be more successful than diploids in post-glacial (re-)colonization (Brochmann et al. 2004). Such polyploids were, however, often found to be of allopolyploid origin, and their success was hypothesized to result from effects of fixed heterozygosity. We do not know whether *D. cespitosa* tetraploids are of auto- or allopolyploid origin. Further genetic and molecular phylogenetic analyses are needed to infer their origin.

The tetraploid and pseudoviviparous variants/subspecies of our sample were found in lake shore habitats or close to water courses in cold environments. Peintinger et al. (2012) considered the subsp. *rhenana* around Lake Constance a periglacial relict endemic with special adaptations in its reproduction (facultative pseudovivipary) to flooding and/or very harsh conditions in glacial periods. The other tetraploid and pseudoviviparous subsp. *alpina* (L.) Tzvelev occurring in the mountains of northern Europe (Clarke 1980) was thought to result from several independent polyploidization events of diploid lineages of *D. cespitosa* with the potential of reproducing by pseudovivipary (Hedberg 1958).

The C values found for diploid *Deschampsia cespitosa* were mostly lower than the genome sizes reported for the closely related *D. antarctica* (1C(pg) between 5.30 and 5.36: Pascual-Díaz et al. 2020). The trend toward higher genome size in lower (southern) latitudes observed for the diploid Asian *D. cespitosa* samples resulted from the higher genome size values of the Chinese samples collected mainly in high altitudes between 3500 and 4000 m. Correlations of genome size and altitude in other plant groups were found to be group-specific: They were negative in wild relatives of *Zea mays* L. (Poaceae; Laurie and Bennet 1985; Bilinski et al. 2018) and in *Arachis duranensis* Krapov & W.C.Greg (Fabaceae; Temsch and Greilhuber 2001) and positive in *Lagenaria siceraria* (Molina) Standl (Cucurbitaceae: Achigan-Dako 2008), and no correlation was inferred for *Sesleria albicans* Kit. ex Schult (Poaceae; Lysak et al. 2000). The association between high genome size, low maximum temperature of the warmest month and low annual precipitation is plausible concerning the moderate temperature. Difficult to interpret is the effect of low precipitation on the genome size; however, Fig. 4c shows a high variation between < 500 and 1000 mm that is obviously not well processed by the statistical model. A general caveat is that the coarse grid climate data may not well represent the specific conditions at the collection sites. This concerns especially the Chinese samples (considered different subspecies) as we found *Deschampsia* mostly close to water courses in the high Chinese mountain ranges (Z.X., H.S., J.G.: pers. obs.). Other investigations found a negative correlation in Liliaceae between genome size and precipitation seasonality (Carta and Peruzzi 2016). Jakob et al. (2004) found disparate genome size patterns in different lineages of *Hordeum marinum* L. (Poaceae) in association with climatic variables. They concluded that phylogenetic constraints might be more important than ecological determinants. The

presence of cryptic evolutionary lineages connected to different vegetation history across Europe was inferred to be responsible for the complex patterns of genome size variation within morphologically similar groups of *Picris hieracioides* L. (Asteraceae; Slovák et al. 2009). This can likely be also the case in *D. cespitosa* in Europe and on a large continental scale, especially that many of the regional variants have been classified as distinct subspecies (e. g., Conert 1987; Chiapella and Probatova 2003).

No evidence for genome downsizing was found in Europe by comparing monoploid genome sizes of diploids and tetraploids. In Asia, the tetraploid sample was too small for any conclusions. The only significant difference in monoploid genome size was found between the Asian diploids and all other European samples. It may likely represent two different geographically and thus genetically distinct lineages. Further genetic analyses will allow for more insight into this observed pattern.

Ploidy and reproductive mode (seed producing versus pseudovivipary)

All pseudoviviparous plants investigated in our study had a genome size indicative of DNA tetraploidy. A reproductive switch to pseudovivipary in wild populations was often inferred to be associated with polyploidy and specific environmental conditions (Hedberg 1958; Sarapultsev 2001). Experimentally induced environmental stress (short-time daylight) was reported to trigger a switch from seminiferous to pseudoviviparous reproduction in *D. cespitosa* (Nygren 1949); however, the association with polyploidy was not tested. There is only a scarce record of diploid and pseudoviviparous variants of *Deschampsia*. An experimentally induced switch to this reproductive mode was observed in diploid European accessions transplanted to transects in California (Lawrence 1945). Another diploid plant collected in the wild with pseudoviviparous reproduction was reported by Hedberg (1958). A few diploid chromosome numbers were also reported for samples assigned to the usually tetraploid and pseudoviviparous northern variant (subsp. *alpina*), however, without reference to the reproductive mode (Kawano 1963). One of these records refers to a diploid chromosome number in a seminiferous accession under the name *D. alpina* Roem. ex Schult which was hypothesized to be a potentially slender variety of *D. cespitosa* s. str. (Nygren 1949).

Conclusions

Seminiferous *Deschampsia cespitosa* s.l. is mostly diploid throughout Eurasia. However, a high variation (CV% = 7.24) in genome size was found among the diploid populations across the geographic west–east gradient in Eurasia. Genome size was significantly lower in European accessions than in the Asian ones. Tetraploids of seminiferous plants were often found on disturbed habitats, albeit without any geographically or ecologically consistent pattern in their distribution. Pseudovivipary was found to be specific only to polyploids and associated with habitats of high moisture.

Information on Electronic Supplementary Material

Online Resource 1. Performance test of two *Deschampsia cespitosa* samples in FCM analysis during five months.
Online Resource 2. Classification of *Deschampsia* in Europe and Asia.
Online Resource 3. Mitotic metaphase chromosomes of *Deschampsia cespitosa*.
Online Resource 4. Regional variation in monoploid genome size of *Deschampsia cespitosa*.
Online Resource 5. Polyploids of *Deschampsia cespitosa* in relation to reproduction mode, habitat class, and region.

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Author contributions All authors contributed to the study conception and design. Material collection and preparation were performed by JG, ZX, PV, MP, PW, IS, HS and JOC. Analyses in the laboratory were performed by EMT and HWS, and data analyses were performed by EMT, JG, ZX, HS and PV. The first draft of the manuscript was written by JG and EMT, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

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

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