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Characterization of some satellite DNA families in *Deschampsia antarctica* (Poaceae)

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Abstract *Deschampsia antactica* E. Desv. is one of the only two native vascular plants of Antarctica, having a disjunct distribution with South America. Its presence in different environmental conditions turns it into an interesting evolution model, particularly for genomic evolutionary studies. The repetitive DNA is a genome component that cause important changes in genome size and chromosome organization, and therefore, its variation is very important in group's delimitation. Some tandem repetitive DNA sequences, known as satellite DNA (satDNAs) are shared between many groups of Poaceae (e.g., of these are the CON1, CON2, COM1, and COM2 sequences) highlighting its evolutionary component. This study aims to identify, classify, and characterize repetitive elements in the *D. antarctica* genome by clustering analysis of genome sequences, focusing on the CON1, CON2, COM1, and COM2. Repetitive DNA represented about 73.3% of the *D. antarctica* genome. All studied populations presented loci for the studied satDNAs but the distribution pattern showed differences that seem to be related to the geographic distribution. The analysis of CON/COM sequences in *D. antarctica* contributes to the understanding of these elements in Poaceae genomes and highlights the importance of changes in chromosome organization of repetitive DNA in populations with fragmented geographical distribution. The distribution of such chromosome changes may both reflect the process of colonization of *D. antarctica* in Antarctica and explain some evolutionary processes of differentiation in *Deschampsia* species complex in the Patagonia, which is still unresolved with other DNA sequences.

Keywords *Deschampsia antarctica* · Repetitive DNA · Satellite DNA · Chromosomes

Introduction

Deschampsia antarctica (Antarctic hairgrass) is a perennial grass distributed from northern Patagonia (35°S) to the Antarctica (68°43′S) (Komarkova et al. 1985, 1990; Chiapella and Zuloaga 2010). Unlike the Arctic, the Antarctic flora is composed of just two native vascular plants, with *D. antarctica* being one of them (Moore 1970). This scarce Antarctic flora generates many unanswered questions till date about its origin and evolution (Mosyakin et al. 2007; Parnikoza et al. 2007a). The Antarctic hairgrass has developed morphological and physiological adaptations to extreme environmental conditions (Vieira and Mantovani 1995; Alberdi et al. 2002; Chwedorzewska 2006; Parnikoza et al. 2007b), such as resistance to cold, freezing, and high irradiance, or photosynthetic capacity at low temperatures (Montiel et al. 1999; Ruhland and Day 2000; Bravo et al. 2001; Alberdi et al. 2002; Gidekel et al. 2003; John et al. 2009). These features make it a valuable resource for identifying mechanisms of genome evolution.

The species of *Deschampsia* have a large genome size, from 4.98 to 9 pg (1C) (Murray et al. 2005; Bennett and Leitch 2012); the C value of 4.975 pg was reported for *D. antarctica* (Bennett et al. 1982), indicating a genome size of 4.87 Gpb (Dolezel et al. 2003). The chromosome number reported for *D. antarctica* in Antarctica

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is $2n = 26$ ($x = 13$), with a karyotype composition of 10m + 6sm + 8st + 2t (Moore 1970). Cells with irregular numbers of chromosomes could be a product of failure mitotic segregation (Cardone et al. 2008) or increased genome instability (Navrotska et al. 2014). Even mixoploidy was reported for an Antarctic plant (Amosova et al. 2015). A Patagonian polyploid population (4×) was found at lower latitude, possibly related to hybridization events with different cytotypes or even other sympatric native species of the genus (González et al. 2016). Most of the genetic studies in *D. antarctica* showed low genetic variability in Antarctic populations (Holderegger et al. 2003; van de Wouw et al. 2008; Fasanella et al. 2017).

Genome size variation may reflect different evolutionary strategies in speciation (Guerra 1988; Stergianou 1989; Levin 2002; Feitoza and Guerra 2011). Repetitive DNA is one of the main components of the genome of higher plants (between 50 and 90%), mostly transposable elements (retroelements and transposons) and tandem repeats (satellite DNA), and is considered one of the principal factors responsible for size variation (Mehrotra and Goyal 2014). This rapid evolution and constant homogenization ("concerted evolution") (Hemleben et al. 2007) give rise to sequences that are species- or genome-specific (Lane Rayburn and Gill 1986; Metzlaff et al. 1986; Reddy et al. 1993; King 1995; Alix et al. 1998). The molecular drive process plays a role in the evolution of satellite DNA by the generation and spread of new sequence variants that are later fixed in populations by sexual reproduction (Dover 1986). This process generates divergence between species (or reproductive groups), leading to species-specific satellite DNA (satDNA) (López-Flores and Garrido-Ramos 2012). The importance of repetitive DNA in the genome of *Deschampsia* has been not well studied. SatDNA sequences, CON1, CON2, COM1, and COM2 families, were isolated from the genus *Helictotrichon* (Grebenstein et al. 1995) and detected in *Deschampsia cespitosa* genome and other grasses (Grebenstein et al. 1996) by Southern blot techniques.

The study of repetitive DNA has been facilitated significantly since the introduction of next generation sequencing (NGS) technologies (Margulies et al. 2005). The power of NGS, which can generate up to gigabases of sequence data in a single run, has presented new opportunities for the study of these elements in plant genomes (Macas et al. 2007). The clustering procedure of genome sequence reads, based on similarity, has been improved recently by employing graph-based methods (Novák et al. 2010). This approach has been efficient in the identification and characterization of repeat genome elements in several organisms (Aversano et al. 2015; Derks et al. 2015; Wolf et al. 2015; Tiwari et al. 2015; Wang et al. 2016). Thus, this work aims to identify and classify repetitive elements in the *D. antarctica* genome by sequence read clustering, and to recognize and characterize the sequence and chromosomal localization of the CON1, CON2, COM1, and COM2 families in Antarctic and Patagonian populations of this species.

Materials and methods

Plant material

Samples of six populations of *D. antarctica* were collected from Antarctica and Patagonia (Table 4) and kept in pots in culture chambers at 14 °C. The vouchers were included in the collection of the herbarium of the Botanical Museum of Córdoba, Argentina (CORD).

Next generation sequencing, pre-processing data, and clustering analysis

The genome sequence was taken from NCBI (SRX465632) (Lee et al. 2014). Sequencing was performed on an Illumina HiSeq 2000 by Korea Polar Research Institute, using 153 M of 101 bp paired-end reads (30 Gb), representing about 3.14× of coverage of *D. antarctica* genome. The reads were filtered by quality (Quality cut-off value: 20 and Percent of bases in sequence that must have quality equal to/higher than the cut-off value: 90). The similarity-based clustering analysis was performed in RepeatExplorer (Novák et al. 2010, 2013), with a read similarity cut-off of 90% over at least 55%. Reads within clusters were assembled into contigs and clusters containing satellite repeats were identified based on graphs. The monomers reconstruction of satellite repeats were generated using k-mer analysis of unassembled short reads with Tandem Repeat Analysis (TAREAN) tool of the RepeatExplorer server (Novák et al. 2017). The putative satellite repeats were compared to the already described CON1, CON2, COM1, and COM2 families described by Grebenstein et al. (1996) (Accession numbers: Z68761-Z68765, Z68772-Z68775, Z68777-Z68779, Z68783-Z68786). Subrepeat units within the isolated satDNA were studied using Tandem Repeats Finder (Benson 1999), with default parameters. Sequence graphical representations were generated from multiple sequence alignment with WebLogo (Crooks et al. 2004).

DNA extraction, primer design and PCR for satellite DNA of CON1, CON2, and COM2 families

Genomic DNA was isolated from dry material in silica gel following the CTAB II methods (Weising et al. 2005). Satellite monomers CON1, CON2, and COM2, used as probes in FISH, were amplified with specific primers designed from satellite DNA identified in cluster analyses (Table 1).

Table 1 Primer list used for to amplify DNA satellite of CON1 and COM2 family in *D. antarctica*. Tm: melting temperature

Primer	Repeat family	sequence	Tm (°C)
DaCON1-F	CON1	GTTAGGGTGGATAGTTTAGATGATTCC	56.7
DaCON1-R	CON1	TTTCTATGAGTCATTTCCGTCTTAACC	55.2
DaCON2-F	CON2	AACAGTGCCTAGGTGGTTAGAGCC	59.1
DaCON2-R	CON2	CATGGAATGTTTTTGGCGATGAGCC	57.7
DaCOM2-F	COM2	TGGGAAGTCGTAAAAACTCGTCG	55.3
DaCOM2-R	COM2	ATAATCCTGTACCGGACGTGAGAG	57.4

The satellite DNA fragments were obtained by PCR. The PCR mix contained 1 µl of template DNA (25 ng), 0.5 U Taq DNA polymerase (Genbiotech), 1 µl 10× Taq buffer, 0.25 mM of each dNTP, and 0.5 uM of each primer in a total volume of 10 µl. PCR program consisted of 35 cycles, each with 1 min denaturation at 94 °C, 1 min annealing 55–57 °C (depending on the primer pair used), 1 min extension at 72 °C, and a final extension of 10 min. Amplification products were separated by electrophoresis on a 1% agarose gel, stained with Syber Safe (Invitrogen, Eugene, OR, USA), and visualized with UV transilluminator.

Molecular cytogenetic techniques

Mitotic chromosomes were prepared from root meristems. The roots were pretreated with 2 mM 8-hydroxyquinoline at 14 °C for 4–6 h and fixed in ethanol/acetic acid (3:1, v:v). The tissues were digested with Pectinex enzyme solution (Novozimes) and squashed in 45% acetic acid. Preparations were frozen in liquid nitrogen to remove the coverslip. Fluorescence in situ hybridization (FISH) was used to detect satellite DNA sequences and ribosomal DNA, following the protocols by Schwarzacher and Heslop-Harrison (2000), with minor modifications. To satDNA hybridization, we used DNA fragments obtained by PCR. To rDNA hybridization, we used p*Ta*71 recombinant plasmid with 18-5.8-26S and 5S rDNA fragments obtained by PCR. The probes were labeled with biotin (Bionick, Invitrogen) or digoxigenin (DIG Nick translation mix, Roche), and the detection was made with Avidin-FITC (Sigma Aldrich) and AntiDIG-Rhodamine (Roche). Chromosome metaphases were photographed using an Olympus BX61 microscope coupled with monochrome camera and Cytovision software (Leica Biosystems).

Results

All of the reads were pre-processed (quality filtered and interlaced); and a fasta file with 1457770 reads (coverage of 0.03×) was used as input in the cluster analysis with Repeatexplorer. Of the total, 1088137 reads were grouped in 35676 clusters (Fig. 1). Clusters corresponding with chloroplast DNA were excluded of the analysis, thus the percent of nuclear repetitive DNA correspond with 73% of

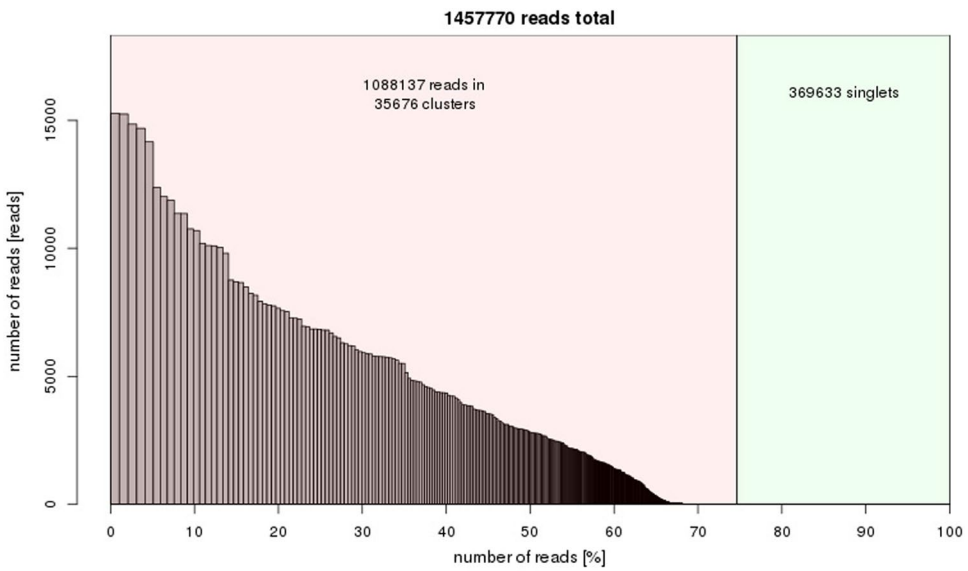


Fig. 1 Clustering results of *D. antarctica* genome from Repeat Explorer analysis. The *X*-axis represents the percentage of the genome covered by the cluster. The *Y*-axis represents the number of NGS reads in each cluster (bars)

genome. Retrotransposons were the most abundant components, representing 51.44% of the genome, with an important proportion of *Gypsy* (21.93%) with *Chromo*, *Ogre*, and *Athila* groups, followed by *Copia* elements (19.82%) with *Maximus*, *Tork*, *Angela*, *Tar*, and *Ivana* groups present. Retroelements are followed by single copy DNA (26.67%) and transposons (3.50%). Satellite DNA and rDNA only represented 0.78 and 0.13% of the genome, respectively (Table 2).

Tandem Repeat Analyzer of Repeatexplorer clusterized 1457770 reads. The analysis found 14 putative tandem repeat element clusters of high confidence and 12 of low confidence. Three clusters of the first group showed high similarity with satellite families CON1, CON2, and COM2 (clusters 256, 281, and 193, respectively). No cluster showed similarity to satellite COM1.

Table 2 Repeat elements composition of *D. antarctica* genome estimated from cluster analysis of Repeatexplorer. Inside the Retrotransposons and Transposons categories the main elements detected are detailed

Repeat Name	Genome proportion (%)	No. clusters
Retrotransposons	51.44	
Copia	19.82	59
Gypsy	21.93	84
Line	0.07	2
Unspecified	9.63	23
Transposons	3.50	
Cacta	3.02	12
Mutator	0.23	2
Pif Harbinger	0.03	1
Unspecified	0.21	2
DNA satellite	0.78	8
rDNA 45S/5S	0.13	6
Unknown	8.40	108
Small uncharacterized clusters	9.14	
DNA single	26.67	

Cluster 256 (*Da*CON1) represented 0.024% of the genome, with satellite probability of 98%, consensus sequence with 366 bp long, 43.4% GC, and 74% of similarity with CON1 satellite descripted in *Helictotrichon convolutum* (Figs. 2a, 3a). Cluster 281 (*Da*CON2) represented 0.015% of genome with satellite probability of 99%, consensus sequence with 563 bp long, 43.9% GC, and 78% of similarity with the CON2 in *H. convolutum* (Figs. 2b, 3b). Cluster 193 (*Da*COM2) represented 0.087% of genome with satellite probability of 88%, consensus sequence with 355 bp long, 49.0% GC, and 58% of similarity with COM2 descripted in *Helictotrichon compressum* (Figs. 2c, 3c; Table 3). As in *Helictotrichon* (Grebenstein et al. 1996), COM2 in *D. antarctica* seems to have sub-structural domains, with sub-repeats of 117–119 bp and a similarity of 70–72% (Fig. 4). CON1 and CON2 did not show sub-repeat structure according to the parameters used.

According to FISH, the three satDNA families had hybridization signal in all populations. Satellite CON1 in Antarctic locations, both 25 de Mayo Island (2772) and Antarctic Peninsula (2782), presented 4 loci in terminal position (on short arm of chromosome pair # 4 and on long arm of chromosome pair #13). Patagonia populations presented 6 CON1 loci in diploids and 4 in tetraploids, with a variable pattern: population 880 keeps the Antarctic patron plus a terminal locus on short arm of #3. Locations 877 and 852 presented the same patron, without the loci of the pairs #13 and #3, and pair #4 with double terminal loci. The tetraploids (845) showed only the double terminal loci in the metacentric pair #7 (similar to #4 in diploids). SatDNA CON2 is present on the long arm of chromosome pair #12 in all populations. SatDNA COM2 presented conspicuous and dispersed blocks, mainly in terminal region

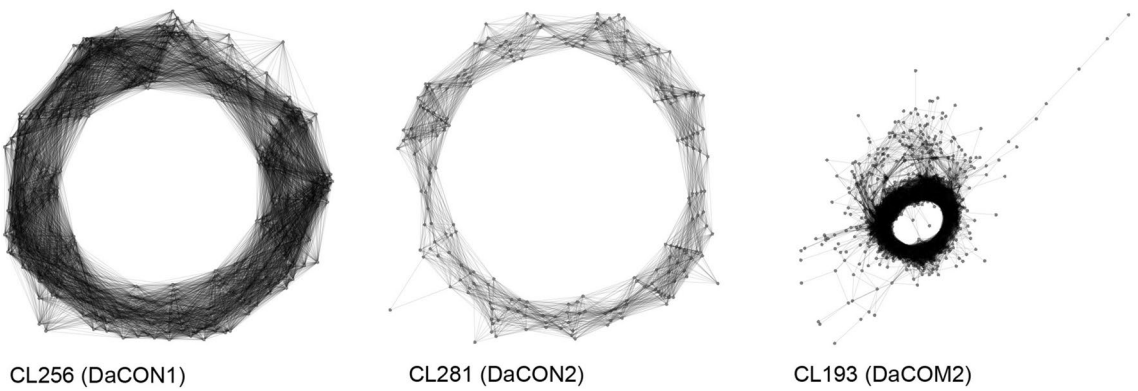


Fig. 2 Graph of the clusters homologous to CON/COM satellite DNA families in of *Deschampsia antarctica* genome. Points represent NGS reads and bridges represent similarity between such reads. The circular forms is because the graphic correspond with tandem repetitive DNA (Novák et al. 2010)

of diverse chromosomes, with variation between populations (Figs. 5, 6). These three satNA families in *D. antarctica* are in DAPI bands observed after FISH application, previously reported (González et al. 2016).

Discussion

In Poaceae, genome size varies from 0.25 to 26 pg (1C) and such variation is mainly attributed to differences in the amount of repetitive DNA (Bennett and Leitch 2012). Repetitive DNA is an important component in several taxa of Poaceae and is frequently regarded as a reflection of the evolutionary history of species and species groups. The homology of repetitive sequences found in different Poaceae lineages has been explained as resulting from a set of ancestral repeats diverging among those lineages (Hemleben et al. 2007). Repetitive DNA sequences were especially studied in species of economic interest, such as barley (Brandes et al. 1995), maize (Jiang et al. 1996; Lamb et al. 2007), rice (Grebenstein et al. 1995), rye (Evtushenko and Vershinin 2010), and wheat (Vershinin et al. 1995).

The genome size of *D. antarctica* is 4.975 pg (1C value) (Bennett et al. 1982) and the common chromosome number is $2n = 26$ (Amosova et al. 2015; González et al. 2016). In the clustering analysis using RepeatExplorer server, with a genome coverage of 0.03×, we detected that about 73% of the *D. antarctica* genome is composed of repetitive DNA. Several retrotransposon families represent almost 40% (*Copia* and *Gypsy*) of genome, with less than 1% being satDNA. This repetitive DNA richness is highly variable in Poaceae, as previously observed in other grasses using similar methods (Ištvánek et al. 2014; Křivánková et al. 2017).

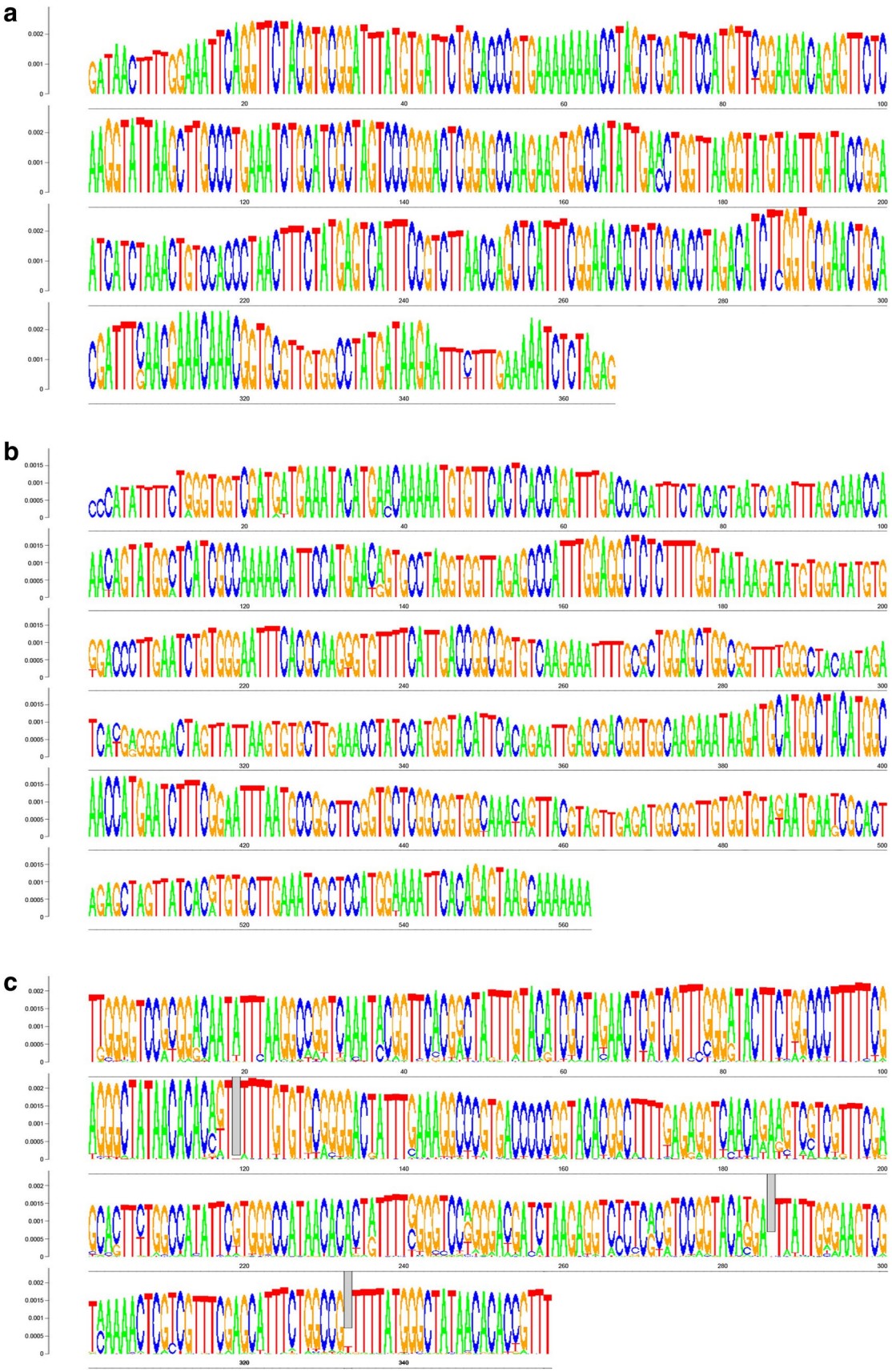
Previously, some satDNAs were isolated in the tribe Poeae using restriction enzymes and their sequences suggest a common origin in several satDNA families. Four families of satDNA were isolated with restriction enzymes in *Helictotrichon*: CON1 (365 bp) and CON2 (562 bp) in *H. convolutum*, and COM1 (346 bp) and COM2 (476 bp) in *H. compressum*. These components were also detected in the terminal heterochromatin of many taxa of Poaceae (Grebenstein et al. 1995; Winterfeld and Röser 2007). The CON1 satDNA is the most dispersed in Poaceae, and its origin was proposed in the common ancestor of Pooideae, Chloridoideae, Ehrhartoideae, Panicoideae (Röser et al. 2014). However, CON2, COM1, and COM2 are restricted to the subfamily Pooideae, and were detected in the tribe Poeae and some species of tribes Andropogoneae and Oryzeae (Grebenstein et al. 1995). *Holcus* has monomers related to CON1 but composed of only 190 bp and *Koeleria* has monomers related to COM1 composed of only 180 bp. This variation in size of the monomers suggests that, in the evolutionary history of satDNA families, loss of part of the monomer may occur.

These four satDNA families were detected in *D. cespitosa* by southern blot (Grebenstein et al. 1996); however, only CON1, CON2, and COM2 were found in *D. antarctica* genome using bioinformatic methods. Our results indicate that the COM1 element was lost in *D. antarctica*. Although CON1 is thought to be ancestral in the subfamily Pooideae, this satDNA is absent in several genera of the Poeae clade (Röser et al. 2014). The "library hypothesis" holds that the occurrence of species-specific satDNA profiles is the result of differential amplification or contraction within a pool of sequences of an ancestral genome (Fry and Salser 1977; Plohl et al. 2012). Different motifs may persist in the genomes over long evolutionary times and can be amplified eventually (Ugarković 2008). Thus, the COM1 satDNA sequence may persist in the *D. antarctica* genome, but not as a highly repetitive element. Further phylogenetic studies with species closely related to *D. antarctica* will be able to solve at which point the COM1 satellite was lost in the evolution of this genus.

The divergent evolution of satDNA can give rise to reproductively isolated individual groups and seem to be related to species divergence (Plohl et al. 2012), since the presence and distribution of satDNA is important for the recognition of homologous chromosome pairs and recombination (Bostock 1980; Schwarzacher 2003; Mehrotra and Goyal 2014). The similarity in monomer sizes and the sequences between *D. antarctica* and *Helictotrichon* suggest that CON1 and CON2 would be more conserved than COM2 (and probably COM1) (Tables 3, 4).

The occurrence of CON/COM families in AT-rich bands (González et al. 2016) is common in Aveneae (Winterfeld and Röser 2007), but other unknown families could be present in these heterochromatin regions. As in other grasses, various families of repetitive DNA occur in the subterminal regions of chromosomes and constitute the so called telomere-associated sequences or TASs (Vershinin et al. 1995; Sharma and Raina 2005). AT-rich terminal repeated sequences are frequent in plant genomes (Flavell 1986; Kubis et al. 1998; Sanmiguel and Bennetzen 1998). Of the studied satDNA families, 60% are AT-rich sequences, and satDNA size usually varies between 135–195 and 315–375 pb, corresponding to mono- and dinucleosomes (Macas et al. 2002; Sharma and Raina 2005). These common features between taxa suggest still unknown functions of satDNA in the genome structure. The satDNA could provide structural genetic codes for the chromatin packing (Trifonov 1989) and to favor the transition between telomeric domains and internal chromosomal regions (Sýkorová et al. 2001).

Between the three isolated elements, COM2 is the most abundant in *D. antarctica* as seen by proportion in cluster analysis and number of FISH signals. Our sequence analysis


a
b
c

◂**Fig. 3** Graphical representation of the sequences of best-scored monomer of each satellite DNA obtained by TAREAN (Novák et al. 2017). The relative sizes of the letters indicate their frequency in the consensus sequence. **a** CL256 (DaCON1); **b** CL281 (DaCON2); and **c** CL193 (DaCOM2)

suggests that COM2 in *D. antarctica* has 355 bp, with three sub-repeats of approximately 118 bp. *Helictotrichon* COM2 has 474 bp composed of four 120 bp sub-repeats. Thus, this satDNA has remained during divergence of these species, but with sub-structural changes.

The localization pattern of satDNA CON2 is associated with 5S rDNA distribution in the genera *Helictotrichon*, *Koeleria,* and *Cynosurus*; however, the low homology between monomers (c. 50%) suggests an independent origin (Winterfeld and Röser 2007). The localization pattern of satDNA CON2 in *D. antarctica* is different of localization pattern of 5S rDNA, highlighting the rapid evolutionary dynamics of the repetitive DNA sites in Aveneae species.

Antarctic and Patagonian populations showed differences in number and position of the CON1 and COM2 signals. These variations could be a consequence of differential amplification in different regions of the genome, and may be related to the geographic distribution of populations (Plohl 2005; Emadzade et al. 2014). The similarity of rDNA and satDNA patterns between populations of *D. antarctica* exhibits some relationship with the geographical distribution. Thus, Antarctic populations 2772 and 2782 exhibit almost the same pattern. This can be related to the hypothesis of a single or few events of colonization of Antarctica from the same South American population (González et al. 2016; Fasanella et al. in press). However, more Antarctic populations need to be studied to verify the homogeneity of these elements in Antarctic genomes. Further studies of repetitive elements of *D. antarctica* (included dispersed and tandem) can provide valuable information about the process of colonization of Antarctica from Patagonia.

The variability of satDNA patterns between populations can be a reflection of time and degree of isolation between them. If the evolution rate of repetitive DNA is high, as

Table 3 Characterization of satellite DNA families CON1, CON2, and COM2 in *D. antarctica* genome

	Cl256 (*Da*CON1)	Cl281 (*Da*CON2)	Cl193 (*Da*COM2)
Length (bp)	366	563	355
AT-richness (%)	56	56	51
Helictotrichon similarity (%)	74	78	58
Satellite probability	0.98	0.99	0.88
% Genome	0.024	0.015	0.087

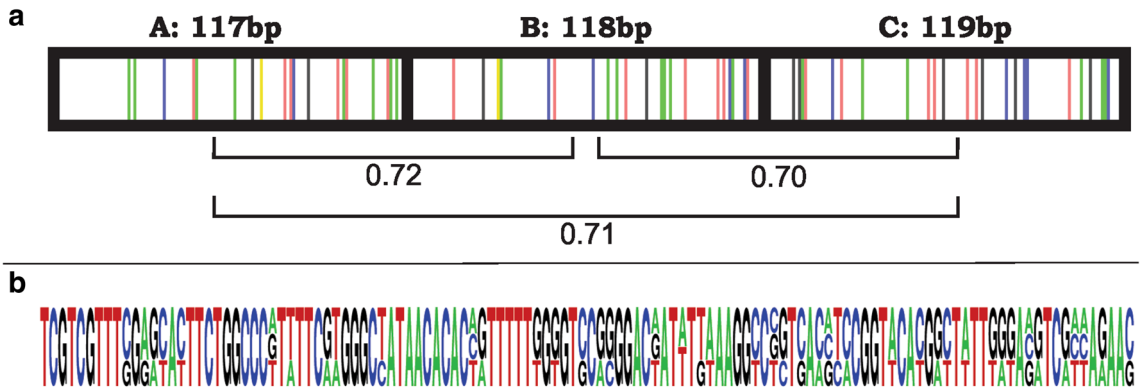


Fig. 4 COM2 satellite DNA substructure. **a** Sub-repeats components of COM2 are represented as rectangles. Vertical color bars represent differences respect to consensus sequence, both mismatches and indels (*yellow*). Similarity proportions between sub-repeats are shown. **b** Graphical representation of the consensus sequence of the three sub-repeats. The sizes of the letters indicate their proportion in the consensus sequence. (Color figure online)

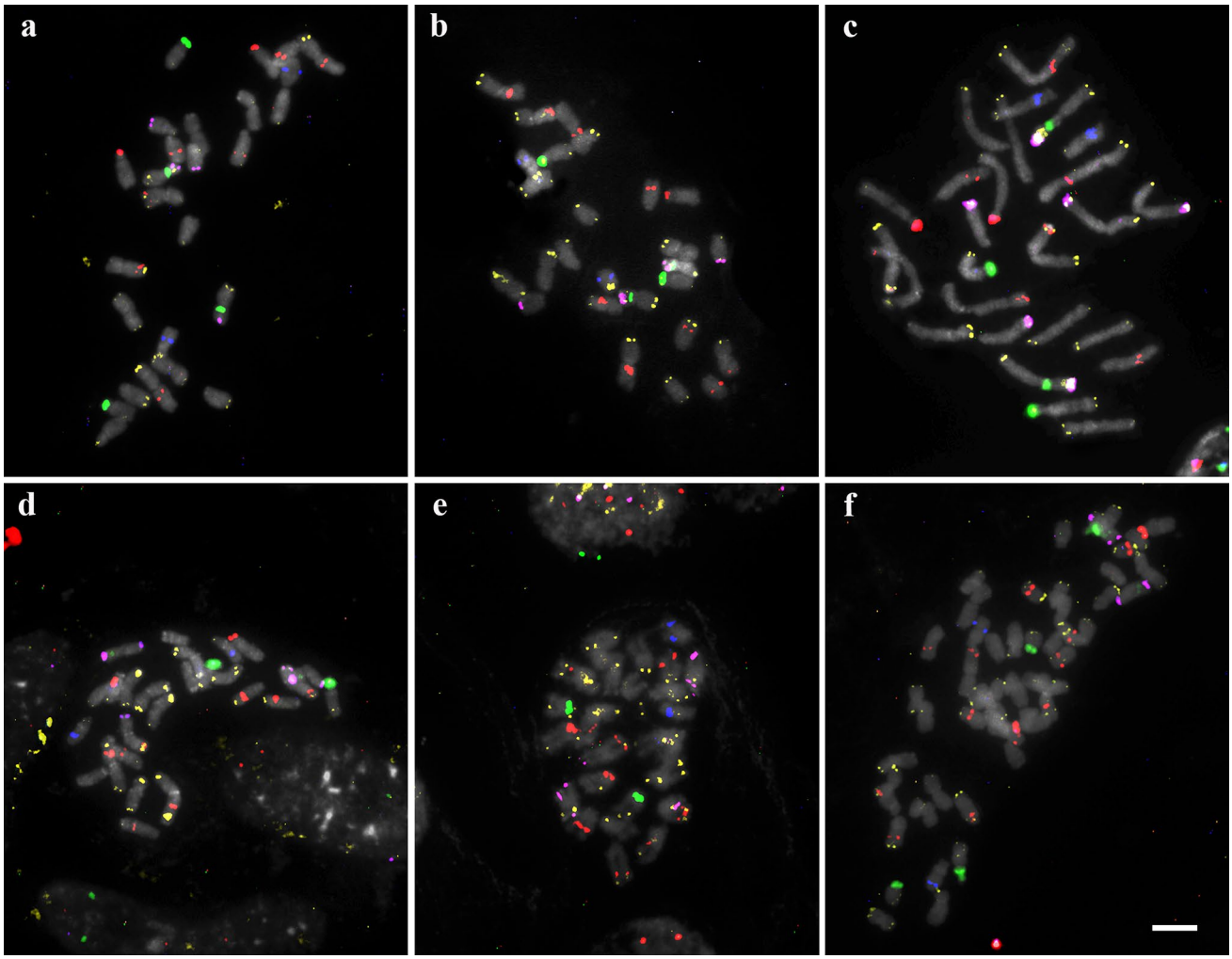


Fig. 5 Fluorescence in situ hybridization of satellite DNA and ribosomal DNA in *Deschampsia antarctica* populations. **a** 2772, **b** 2782, **c** 880, **d** 877, **e** 852, **f** 845. Probes: CON1 (*violet*), CON2 (*blue*), COM2 (*yellow*), 45S rDNA (*green*), and 5S rDNA (*red*). *Scale* 5 µm. (Color figure online)

has been proposed (Grebenstein et al. 1996; Vittorazzi et al. 2014; Garrido-Ramos 2015), the variation observed in satDNA pattern could have facilitated reproductive isolation between *D. antarctica* populations because of the failure in homologous chromosome recognition. The secondary contact between previously isolated populations, e.g., during ancient marine ingressions (Premoli et al. 2012) could be related to existence of polyploidy observed in some *D. antarctica* populations (González et al. 2016). Studies of the reproductive isolation of populations are required to verify this hypothesis.

This study highlights the importance of changes in chromosome organization based on non-random differential amplification of repetitive DNA units in populations with

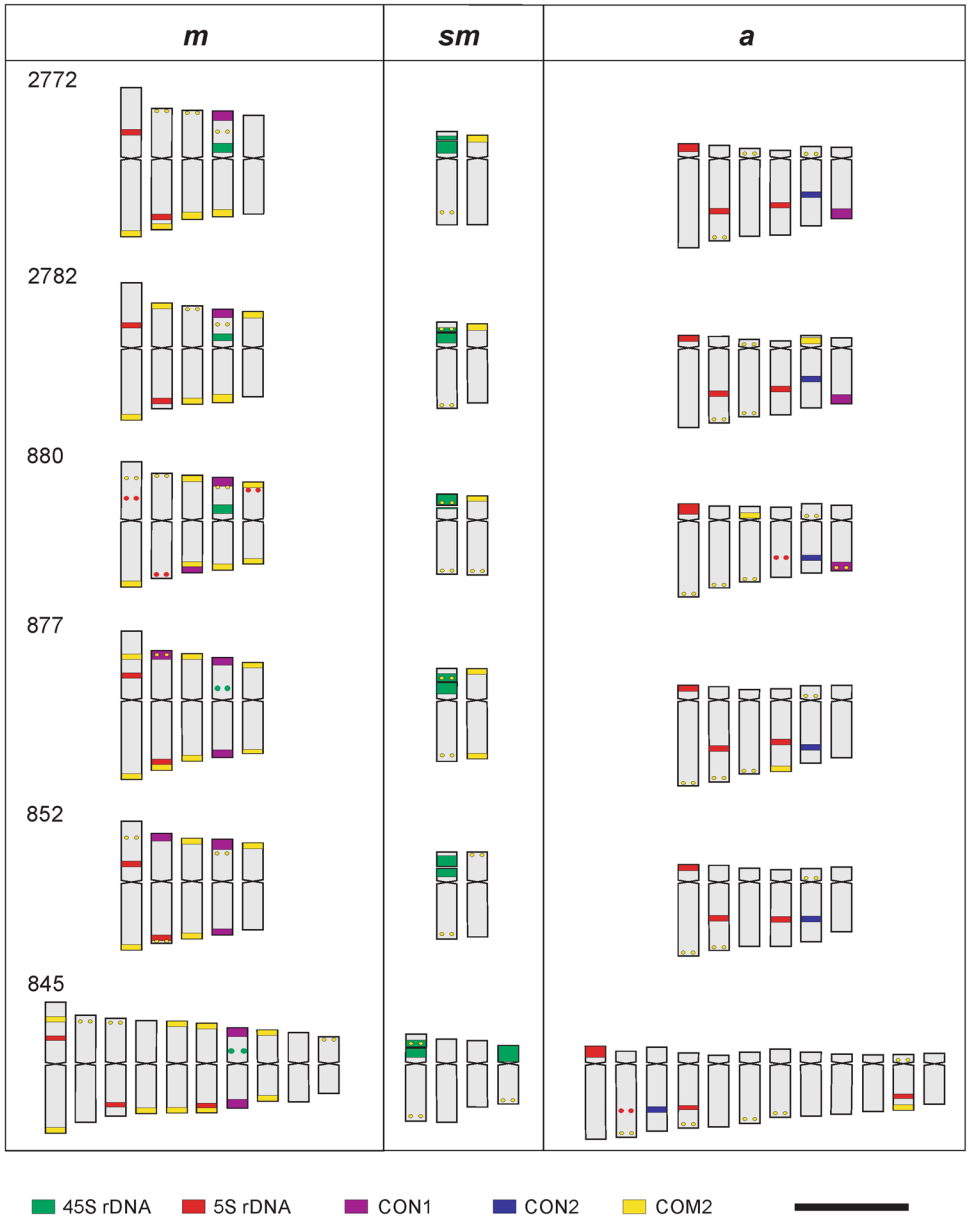


Fig. 6 Idiograms of *Deschampsia antarctica* populations, showing satellite DNA and ribosomal DNA. Chromosomes are organized in first order by morphology and in second order by length. The rectangles represent constant hybridization signs and the circles represent the weak and the variable hybridization signs. *Scale* 5 µm. (Color figure online)

Table 4 Description of collection number and collector, location, chromosome number, and satDNA loci number for each population of *D. antarctica*. All vouchers were deposited in CORD herbarium

Voucher	Location	2*n*	CON1	CON2	COM2
Chiapella et al. 2782	Argentine Antarctic Sector, Antarctic Peninsula	26	4	2	24
Chiapella et al. 2772	Argentine Antarctic Sector, 25 de Mayo Island	26	4	2	28
Urdampilleta et al. 880	Santa Cruz, Argentina	26	6	2	38
Urdampilleta et al. 877	Santa Cruz, Argentina	26	6	2	34
Urdampilleta et al. 852	Chubut, Argentina	26	6	2	22
Urdampilleta et al. 845	Chubut, Argentina	52	4	2	42

fragmented geographical distribution. The distribution of

such chromosome structural changes may both reflect the process of colonization of *D antarctica* in Antarctica and explain some evolutionary processes of differentiation in *Deschampsia* species complex in the Patagonia, still unresolved with other DNA sequences (Chiapella 2007; González et al. 2016).

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