

Molecular cytogenetics of valuable Arctic and sub-Arctic pasture grass species from the Aveneae/Poeae tribe complex (Poaceae)

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Abstract

Background Grasslands in the Arctic tundra undergo irreversible degradation due to climatic changes and also over-exploitation and depletion of scarce resources. Comprehensive investigations of cytogenomic structures of valuable Arctic and sub-Arctic grassland species is essential for clarifying their genetic peculiarities and phylogenetic relationships and also successful developing new forage grass cultivars with high levels of adaptation, stable productivity and longevity. We performed molecular cytogenetic characterization of seven insufficiently studied pasture grass species from related genera *Alopecurus*, *Arctagrostis*, *Beckmannia*, *Deschampsia* and *Holcus* (*Poaceae*) which are the primary fodder resources in the Arctic tundra. Results For these species, integrated schematic habitat maps were constructed based on the available data on their distribution in Eurasia. The species karyotypes were examined with the use of DAPI-banding, fluorescence in situ hybridization with 35S rDNA, 5S rDNA and the (GTT)₉ microsatellite motif and also sequential rapid genomic in situ hybridization with genomic DNAs of *Deschampsia sukatschewii*, *Holcus lanatus* and *Deschampsia flexuosa*. Cytogenomic structures of the studied species were specified; peculiarities and common features of their genomes were revealed. Different chromosomal rearrangements were detected in *Beckmannia syzigachne*, *Deschampsia cespitosa* and *D. flexuosa*; B chromosomes with distinct DAPI-bands were observed in karyotypes of *D. cespitosa* and *H. lanatus*. Conclusions The peculiarities of distribution patterns of the examined chromosomal markers and also presence of common homologous DNA repeats in karyotypes of the studied species allowed us to verify their relationships. The obtained unique data on distribution areas and cytogenomic structures of the valuable Arctic and sub-Arctic pasture species are important for further genetic and biotechnological studies and also plant breeding progress.

Background

According to current molecular phylogenetic studies, the tribe *Poeae* R. Br. (*Pooideae*, *Poaceae*) now includes the formerly separate tribe *Aveneae* Dum. [1, 2]. The *Aveneae/Poeae* tribe complex comprises a large number of crops and forage plants which play an important role for humans and animals. As an example, economically important oat is the most ancient food supplies for humankind [3]. Lots of members of the *Aveneae/Poeae* tribe are polymorphic species with a wide geographical distribution, high morphological diversity and complicated taxonomy [1, 2, 4, 5]. Species of this tribe are highly tolerant to stressful and variable environmental conditions including extreme Arctic and Antarctic habitats [6-8]. In particular, *Deschampsia antarctica* E. Desv. is the only one species within the *Poaceae* family adapted to the harshest Antarctic environment [7]. Polar and subpolar ecotypes of perennial cereals from the related genera *Alopecurus*, *Arctagrostis*, *Beckmannia*, *Deschampsia* and *Holcus* are characterized by high biological value, stable productivity and longevity and used as native and/or introduced forage resources in the Arctic tundra [6, 7, 9-12]. Animal husbandry has always been the principal economic activities of the northern indigenous peoples (the Yakuts, Nenets, Evenki, etc.), and pasture grasses are the primary fodder resources in the Arctic tundra [10]. Currently, the unique Arctic ecosystem requires special attention as grasslands in the Arctic regions undergo irreversible degradation due to climatic changes and also

over-exploitation and depletion of scarce resources [10]. Sustainable grazing strategies can have a significant impact on rehabilitating the degraded grasslands. Also, introduction of native Arctic grassland species and also non-native cold-hardy ecotypes of perennial grasses with high levels of adaptation and seed productivity as well as *development* of new *valuable* cultivars from promising wild morphotypes could be the strategies for reducing feed costs and increasing feed efficiency in the Arctic and sub-Arctic regions [11,12]. Moreover, the investigation of the evolutionary changes occurred in their genomes (especially, under environmental stress factors) is an important aspect for clarifying the mechanisms contributing to abiotic stress tolerance and for further crop breeding strategies [13, 14].

Despite widespread occurrence and economic value of most cereals from the *Aveneae/Poeae* tribe, their genomes still remain insufficiently studied [14, 15]. Due to the agricultural significance, oat species have become the subject of complex investigation, and extensive molecular studies have largely clarified diversity and evolution of the genus *Avena* [16-20]. Phylogenetic studies in recent decades, mostly based on analyses of different DNA sequences from both plastid regions and nuclear ribosomal regions, have helped to clarify evolutionary relationships within the *Aveneae/Poeae* complex [4, 5, 15, 21-23]. However, the details of the phylogeny among many genera and suprageneric taxa of this diverse grass tribe still remain controversial [23]. Furthermore, the complicated breeding system, that is peculiar to evolution of major cereals from this tribe, included interspecific and intergeneric hybridization which was accompanied by the allopolyploid formation [24]. Moreover, environmental stress factors can lead to physiological stress followed by structural variations (chromosome rearrangements, mixoploidy and aneuploidy) in plant genomes [25-27]. Therefore, the investigation of genomes of such species should comprise molecular cytogenetic characterization [15, 23]. Particularly, physical mapping of specific DNA probes to individual chromosomes is a promising tool for analysis of chromosome organization and evolutionary processes in plant genomes [15]. Knowledge of the chromosome localization of *repetitive* DNA families is very useful in the assembly of genome sequences and their assignment to physical chromosomes [28].

Currently, detailed cytogenetic information is available for less than half of the altogether 60–70 genera of the *Aveneae/Poeae* [15, 29], and among them, the genus *Avena* is the most extensively studied [30-34]. Notably, in some widespread species of the genera *Helictotrichon* and *Deschampsia*, variations in the karyotype structure and ploidy status were revealed, which probably related to their genome plasticity [15, 27, 35-37]. Nevertheless, molecular cytogenetic data on most members of the *Aveneae/Poeae* tribe still remain rather limited, and for many of them, only chromosome numbers are currently available [38]. In particular, the karyotypes of many valuable pasture and forage grasses important for Arctic and sub-Arctic regions (including *Alopecurus aequalis* Sobol., *Alopecurus arundinaceus* Poir., *Arctagrostis latifolia* (R. Br.) Griseb., *Beckmannia syzigachne* (Steud.) Fernald, *Deschampsia cespitosa* (L.) P. Beauv., *Deschampsia flexuosa* (L.) Trin. (= *Avenella flexuosa* (L.) Drejer), *Deschampsia sukatschewii* (Popl.) Roshev. and *Holcus lanatus* L.), are still insufficiently cytogenetically characterized.

Objectives

In this study, we performed a molecular cytogenetic characterization of sub-Arctic and cold-hardy accessions of *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne*, *D. cespitosa*, *D. flexuosa* and *H. lanatus* in order to specify their ploidy status and karyotypic features and also reveal possible structural chromosome rearrangements important for further crop breeding strategies. For a comparative assessment of cytogenetic peculiarities and karyogenomic similarities within the studied species, we also characterized an accession of *Avena longiglumis* Dur. containing the most ancient oat A genome which also could be useful for development of new forage grass cultivars [12, 39-40].

Methods

Plant material

Seeds of polar and subpolar accessions of *A. arundinaceus* (144, Magadan region, RF: 59°34' N; 150°48' E), *A. latifolia* (85, Magadan region, RF: 59°34' N; 150°48' E), *B. syzigachne* (93, Magadan region, RF: 59°34' N; 150°48' E), *D. cespitosa* (688, Vologda region, RF: 59°13' N; 39°54' E), *D. flexuosa* (598, Republic of Karelia, RF: 63°49' N; 33°00' E) and a cold-hardy accessions of *D. sukatschewii* (78, Altai Mountains, 1400 m above mean sea level (mamsl), RF: 48°45' N; 89°36' E) were received from the Laboratory of genetic resources of fodder plants, Federal Williams Research Center of Forage Production and Agroecology, Lobnya, Moscow region, RF. Seeds of *A. longiglumis* (k-1811, Morocco) were received from the Department of genetic resources of oat, rye and barley, Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), St. Petersburg, RF. Cold-hardy accessions of *A. aequalis* (Alt11-309, natural populations, Altai Mountains, 1200 mamsl, RF: 48°45' N; 89°36' E) and *H. lanatus* (T-115, natural populations, Caucasus Mountains, 1400 mamsl, RF: 43°46'38" N; 41°55'00" E) were collected and identified by Dr. E.O. Punina, Laboratory of Biosystematics and Cytology, Komarov Botanical Institute, RAS, St. Petersburg, RF. Collection of these wild plant samples and experimental research conducted on these materials was performed in accordance with the legal regulations of Komarov Botanical Institute, RAS, St. Petersburg, RF approved by the Ministry of Science and Higher Education of the RF.

Fixation

Seeds of the studied species were germinated on moistened filter paper in Petri dishes, and then the plants were grown in a greenhouse at 15°C. For cell cycle synchronization and accumulation of mitotic divisions, root tips were incubated at 0°C (in ice water) for 24 hours and then fixed in the ethanol: glacial acetic acid fixative (3:1) at room temperature for 48 h. After that, they were stored at -20°C before use.

Chromosome spread preparation

The fixed root tips were stained with 1% acetocarmine solution in 45% acetic acid for 40 min, the tip caps with root meristem were cut on the object-plate, macerated in a drop of 45% acetic acid and covered with cover slips. Then squashed chromosome preparations were made. After freezing in liquid nitrogen, the cover slips were removed; the preparations were dehydrated and stored in 96% ethanol at -20°C before use.

FISH procedure

Following probes were used for FISH:

1. pTa71 containing a 9 kb long DNA sequence of common wheat including 18S-5.8S-26S (35S) rDNA [41];
2. pTa794 containing a 420 bp long DNA sequence of wheat including 5S rDNA [42].

These DNA probes were labelled directly with fluorochromes SpectrumAqua, SpectrumGreen and/or SpectrumRed (Abbott Molecular, Wiesbaden, Germany) by nick translation according to manufacturers' protocols.

3. The oligo-(GTT)₉ probe, labelled at the 3'-end with fluorescein-12-dUTP (Roche Diagnostics, Mannheim, Germany), was synthesized using a synthesizer ABI 394 (Applied BioSystems, Redwood City, USA).

FISH assays were carried out according to Muravenko et al. [43]. After overnight hybridization, the slides were washed twice with 0.1xSSC at 44°C for 10 min, twice with 2xSSC at 44°C for 5 min followed by a 5-min wash in 2xSSC and three washes in PBS for 3 min each at room temperature. Then the slides were dehydrated through a graded ethanol series and air dried. The oligo-(GTT)₉ probe labelled with fluorescein was detected using anti-fluorescein/Oregon green, rabbit IgG fraction, Alexa Fluor 488 conjugate (Molecular Probes, Oregon, USA).

DAPI staining

After FISH, chromosome preparations were stained with 0.1 µg/ml DAPI (4',6-diamidino-2-phenylindole) (Serva, Heidelberg, Germany) in Vectashield mounting medium (Vector laboratories, Peterborough, UK).

Rapid GISH procedure

Genomic DNAs of *D. sukatschewii*, *H. lanatus* and *D. flexuosa* were isolated from green leaves using CTAB (cetyltrimethylammonium bromide) standard protocol with minor modifications [44]. The genomic

DNAs were labelled directly with SpectrumAqua or SpectrumRed (Abbott Molecular, Wiesbaden, Germany) by nick translation according to the manufacturer's instructions.

After the FISH procedure and documentation of the hybridization patterns, the cover glasses were removed from chromosome slides, the slides were washed twice in deionized water for 10 min, dehydrated in the ethanol series (70%, 85%, 96%) for 3 min in each and air dried. Then a rapid GISH procedure was conducted on the same slides as described previously [27].

Chromosome analysis

The chromosome slides were examined using Olympus BX61 epifluorescence microscope (Olympus, Tokyo, Japan) combined with a monochrome CCD camera (Cool Snap, Roper Scientific Inc., Tucson, USA). The captured images were sequentially collected in grayscale channels, pseudocoloured and then processed with Adobe Photoshop 10.0 (Adobe Systems Inc., Birmingham, USA) and VideoTest-FISH 2.1 (IstaVideotest, St. Petersburg, Russia) software programs. At least five plants of each accession and fifteen metaphase plates from each sample were analyzed. In the karyotypes, the identification of chromosomes was performed based on their morphological similarities as well as revealed molecular cytogenetic markers. In the species karyograms of *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *A. longiglumis*, *B. syzigachne* and *H. lanatus*, the chromosome pairs were set in the decreasing order of size. The cytological numerical designation of *D. cespitosa* and *D. flexuosa* was according to Amosova et al. [37].

Results

Species distribution areas

For *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne*, *D. cespitosa*, *D. flexuosa*, *D. sukatschewii* and *H. lanatus*, we constructed integrated schematic maps of their distribution in the northern, central and eastern parts of Eurasia based on the analysis of currently available data [2, 6, 45-52] (Fig. 1). In these regions, *A. latifolia* is distributed from the Arctic Archipelago of Svalbard to Chukotka Peninsula including the Archipelago of Novaya Zemlya (75° N). Circumboreal-polar species, *A. aequalis*, *A. arundinaceus* and *D. cespitosa* are widespread in the Eurasian continent from the Arctic Scandinavia to the Far East regions and Chukotka Peninsula. Northern populations of *H. lanatus* and *D. flexuosa* occur in the Arctic Scandinavia and Kola Peninsula and also in Kamchatka Peninsula and Far East regions. *B. syzigachne* is widespread in the Eurasian continent from the Volga river basin to Chukotka Peninsula and *D. sukatschewii* is distributed in all northern regions of Siberia and Chukotka Peninsula (Fig. 1).

Karyotype structure

The accessions of the studied grass species presented diploid $2n = 14$ (*A. aequalis*, *A. longiglumis*, *B. syzigachne* and *H. lanatus*) and also tetraploid $2n = 28$ (*A. arundinaceus* and *D. flexuosa*) karyotypes with the basic chromosome number $x = 7$ except for *D. cespitosa* having $2n = 26$ chromosomes. Besides, in karyotypes of *D. cespitosa* and *H. lanatus*, 0-3 supernumerary small chromosomes with uncertain morphology were observed together with chromosomes of the basic set (Figs 2-5).

The karyotypes of *A. aequalis*, *A. longiglumis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne*, *D. flexuosa* and *H. lanatus* contain metacentric and submetacentric chromosomes which were not very different in size and morphology (Figs 2-5). Chromosomes in the *D. cespitosa* karyotype could be subdivided into four groups: metacentric, submetacentric, subtelocentric and telocentric according to centromeric positions and morphology (Figs 2-5).

FISH mapping of the (GTT)₉ oligonucleotide, 35S and 5S rDNA

FISH analysis revealed different patterns of chromosomal distributions of oligo-(GTT)₉, 35S and 5S rDNA probes in karyotypes of the studied grass species.

In karyotypes of the studied *A. aequalis* accession, FISH analysis (performed for the first time) revealed large 35S rDNA hybridization sites in the secondary constriction regions (short arms) of chromosome 6. Sites of 5S rDNA were detected in the proximal regions of chromosome 1 (long arms, double sites) and 4 (short arms). Small (GTT)₉ sites were revealed in the subtelomere regions of chromosome 2 (short arms) and 6 (long arms) (Figs 2, 4).

In karyotypes of the studied *A. longiglumis* accession, 35S rDNA loci were revealed in the secondary constriction regions (short arms) of two chromosomes 1 and 6. Sites of 5S rDNA were detected in the proximal regions of both arms of chromosome 6. The oligo-(GTT)₉ probe produced signals on five chromosome pairs. Distinct signals were observed in the pericentromeric regions of chromosomes 2, 3 and 7. Besides, a number of minor sites were observed in chromosome 3 (along the short arms and in the terminal region of the long arms) and chromosome 5 (along the long arms) (Figs 2, 4).

In karyotypes of the studied *B. syzigachne* accession, FISH analysis (performed for the first time) revealed 35S rDNA loci in the secondary constriction regions (short arms) of two chromosomes 2 and 3. 5S rDNA sites were observed in the subtelomeric region of the short arm (in one homolog) and in the proximal region of the long arms of chromosome 1, in the distal region of the short arms of chromosome 2 and in the distal region of the long arm of one homolog of chromosome 6. One small (GTT)₉ site was detected in the distal region of the short arms of chromosome 1 (Figs 2, 4).

In karyotypes of the studied *H. lanatus* accession, FISH analysis (performed for the first time) revealed 35S rDNA loci in the secondary constriction regions (short arms) of chromosomes 1 and 4. Sites of 5S

rDNA were detected in the proximal region of the long arms of chromosome 1. A small (GTT)₉ site was observed in the distal region of the short arms of chromosome 3 (Figs 2, 4).

In karyotypes of the studied *A. arundinaceus* accession, FISH analysis (performed for the first time) revealed large 35S rDNA sites in the secondary constriction regions (short arms) of four chromosomes 1, 3, 4 and 10. Sites of 5S rDNA were visualized in the proximal region of the short (double sites) and long arms of chromosome 1, in the proximal region of the long arms of chromosome 2 (double sites) and in the short arms of chromosome 4 (in colocalization with 35S rDNA sites). Distinct (GTT)₉ sites were detected in the pericentromeric regions of chromosomes 6 and 11 (Figs 3, 5).

In karyotypes of the studied *A. latifolia* accession, FISH analysis (performed for the first time) revealed large 35S rDNA sites in the secondary constriction regions (short arms) of chromosomes 1, 4 and 5. Sites of 5S rDNA were visualized in the proximal and distal regions of the long arms of chromosome 1 and also in the distal regions of the long arms of chromosome 6. A small (GTT)₉ site was detected in the distal regions of the long arms of chromosomes 4 (Figs 3, 5).

In karyotypes of the studied *D. cespitosa* accession, 35S rDNA loci were located in the secondary constriction regions (short arms) of three chromosome pairs (5, 6 and 9). 5S rDNA sites were observed on chromosome 1 (in the proximal regions of both short and long arms), chromosome 3 (in the distal region of the long arms of both homologs and in the proximal region of the short arm of one homolog), chromosome 7 (in the proximal region of the long arms), chromosomes 9 (in the proximal region of the long arm of one homolog) and chromosome 10 (in the proximal region of the long arm of one homolog). A small (GTT)₉ site was revealed in the proximal region of the long arms of chromosome 1 (Figs 3, 5).

In karyotypes of the *D. flexuosa* accession, 35S rDNA loci were detected in the secondary constriction regions (short arms) of chromosomes 5 and 9. Also, a small 35S rDNA locus was detected in the pericentromeric region of one homolog of chromosome 10. Large 5S rDNA sites were detected in the proximal region of the long arms of chromosome 5 and in the proximal region of the short arms of chromosome 12. Besides, minor sites were revealed in the proximal regions of the long arms of chromosome 7 and 8. Small (GTT)₉ sites were detected in the subtelomeric regions of the long arms of chromosomes 12 and 14 (Figs 3, 5).

Chromosomal markers revealed by rapid GISH

A rapid GISH procedure with total genomic DNAs of *H. lanatus* and *D. sukatschewii* as DNA probes was performed on chromosomes of *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *A. longiglumis*, *B. syzigachne*, *D. cespitosa* and *D. flexuosa*. On chromosomes of *H. lanatus*, a rapid GISH procedure was performed with the use of total genomic DNAs of *D. flexuosa* and *D. sukatschewii* as DNA probes.

As a result, multiple clustered hybridization signals (rapid GISH markers) of *H. lanatus* were revealed in different positions on chromosomes of *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne* and *D. cespitosa*. Dispersed and few clustered hybridization signals of *H. lanatus* were observed along the chromosomes of *D. flexuosa*. Few small clustered (coinciding with the localization of 35S rDNA sites) and dispersed hybridization signals of *H. lanatus* were observed along the chromosomes of *A. longiglumis* (Figs 2-5).

On chromosomes of *H. lanatus*, the rapid GISH analysis revealed few small clustered and dispersed hybridization signals of *D. flexuosa* (Figs 2, 4).

Multiple clustered hybridization signals of *D. sukatschewii* were revealed in different positions on chromosomes of *A. aequalis*, *A. arundinaceus*, *B. syzigachne* and *D. cespitosa*. Besides, few small hybridization signals of *D. sukatschewii* were detected on chromosomes of *H. lanatus* and *A. latifolia*. Dispersed and small clustered hybridization signals of *D. sukatschewii* were observed along the chromosomes of *D. flexuosa*. Dispersed signals of *D. sukatschewii* were observed along the chromosomes of *A. longiglumis* (Figs 2-5).

DAPI-banding analysis

Chromosome staining with DAPI performed after FISH procedures revealed DAPI-banding patterns specific to the studied species accessions. Large (intense) DAPI-bands were observed in karyotypes of *A. arundinaceus*, *A. latifolia*, *D. cespitosa*, *D. flexuosa* and *H. lanatus* (mostly, in the pericentromeric and subtelomeric regions of chromosomes) while in karyotypes of the other studied species accessions, only small or dispersed bands were detected. B chromosomes found in karyotypes of *D. cespitosa* and *H. lanatus* possessed distinct DAPI-bands in distal regions (Figs 4, 5).

Karyotype analysis

Based on chromosomal morphology, DAPI-banding patterns, distribution of the oligo-(GTT)₉, 35S and 5S rDNA probes as well as rapid GISH markers, chromosomes in karyotypes of the studied species were identified and the species karyograms were constructed (Figs 4, 5). The comparison of patterns of distribution of the examined molecular cytogenetic markers allowed us to reveal different chromosomal rearrangements in karyotypes of *B. syzigachne*, *D. cespitosa* and *D. flexuosa* (detailed in Figs 4, 5).

Discussion

Within the *Aveneae/Poeae* tribe complex, most grass species were shown to have a basic chromosome number $x = 7$, though other basic chromosome numbers have also been found (<http://www.tropicos.org/project/ipcn>). Besides, a natural polyploid series as well as variation in ploidy

level were revealed [4, 15, 27, 35]. Polyploidy is widespread in grass species, and it considered to play an important role in the evolution of vascular plants [53, 54]. The speciation within the *Aveneae/Poeae* tribe complex has been accompanied with episodes of polyploidy and intergeneric hybridization between the representatives of this tribe (especially, having hybrid or/and rearranged genomes) resulted in appearance of allopolyploid species [55-58]. Polyploidization events often seem to be associated with increases in vigor followed by adaptation of newly formed polyploids to novel conditions [55-57], so polyploids are able to colonize larger geographic ranges and/or occur in more habitats than related diploids [54]. The genera *Alopecurus*, *Arctagrostis*, *Beckmania*, *Deschampsia* and *Holcus* comprise widespread polyploid species and/or polymorphic forms which are highly tolerant to stressful and/or variable environmental conditions [6, 8-10, 55, 59]. The species *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne*, *D. cespitosa*, *D. flexuosa*, *D. sukatschewii* and *H. lanatus* are predominant grasses within the pastures of the Arctic and sub-Arctic regions [6, 10, 11]. However, only scattered data on their distribution in the vast territory of Eurasia is currently available. In the present study, we constructed the integrated schematic maps of their occurrence in the northern, central and eastern parts of Eurasia based on the currently available data [2, 6, 45-52]. These maps indicate the vast areas with multi-species occurrence and also the regions (predominantly in the Far North and the Far East) where only certain grass species are now distributed. In these regions, shortage in forage resources could be recovered through the introduction of the other Arctic grassland species with high levels of adaptation and seed productivity as well as developing new *valuable* cultivars with the use of promising wild morphotypes [11-13].

We found that the studied accessions presented normal diploid ($2n = 2x = 14$, *A. aequalis*, *A. longiglumis*, *B. syzigachne* and *H. lanatus*) and tetraploid ($2n = 4x = 28$, *A. arundinaceus*, *A. latifolia* and *D. flexuosa*) karyotypes with the typical for cereals basic chromosome number $x = 7$ except for the paleopolyploid *D. cespitosa* having $2n = 4x = 26$ chromosomes. Our findings agreed with the cytological data on these species reported earlier [35, 37, 60]. Despite the stressful natural environmental conditions (low temperatures, strong winds, low participations, high ultraviolet radiation, etc.), aneuploid karyotypes were not revealed in the accessions examined in this study. At the same time, different ploidy levels including di-, tri-, tetra- and mixoploid cytotypes had earlier been described in several taxa of the almost cosmopolitan *Deschampsia* genus which probably were related to the genome plasticity of this species [27, 37, 60].

In karyotypes of the accessions of the most widespread species *D. cespitosa* and *H. lanatus*, we detected supernumerary B chromosomes (Bs). In *D. cespitosa*, these supernumerous chromosomes were observed earlier [37], while in *H. lanatus*, they were revealed for the first time. Bs are dispensable components of the genome exhibiting non-Mendelian inheritance which are found in all eukaryotic phyla and are thought to stand for a specific type of selfish DNA [61]. The molecular mechanism and a functional role of Bs still remain unclear; though their appearance in a karyotype is associated with genome instability [62]. Particularly, the appearance of Bs in karyotypes of *D. cespitosa* and *H. lanatus* could be induced by environmental stress factors as a correlation between the presence of Bs in a karyotype and environmental conditions was described earlier [63-64].

After polyploidization or under the environmental stress, different genetic alterations could occur (structural chromosome rearrangements, aneuploidy, aneusomy, direct changes in the DNA sequence (point mutations), loss of duplicated genes and gene conversion) [57]. In *Poaceae*, repetitive DNA sequences are a major genomic component which have high evolution rates and can play an important role in speciation processes [65]. Knowledge of cytogenetic positions of specific repetitive sequences (chromosomal markers) provides information on genome structure differences which is important for analysis of the structural evolution of plant chromosomes [66]. Particularly, in karyotypes of vascular plants, DAPI staining, performed after FISH or GISH procedures, reveals AT-rich heterochromatin which consists largely of highly repetitive ('satellite') DNA as strongly stained bands [67]. In karyotypes of the studied species, different patterns of AT-heterochromatin distribution (DAPI-banding) were revealed. Clustered localization of highly repetitive DNA sequences (large distinct DAPI-bands) was observed on chromosomes of *A. arundinaceus*, *A. latifolia*, *D. cespitosa*, *D. flexuosa* and *H. lanatus* whereas in karyotypes of the other species, including *A. longiglumis*, we detected small or dispersed DAPI-bands. The similar nature of chromosomal *distribution* of constitutive heterochromatin was earlier described for different *Avena* species with the use of C-banding technique [30-32]. In the present study, the revealed patterns of DAPI-banding were specific to the examined species and were used for chromosome identification.

Physical mapping of ribosomal 35S and 5S DNA on chromosomes of diploid and polyploid plant species provides information on the structural evolution of the chromosomes carrying these sequences [28, 29, 68], whereas nucleotide similarity among diploid and polyploid rDNA copies reveals some of their phylogenetic and genomic relationships [69]. In this study, the comparative karyotypic analysis of the diploid and tetraploid accessions of *Alopecurus* (*A. aequalis* and *A. arundinaceus*) revealed chromosomes with similar morphology and distribution patterns of 35S rDNA, 5S rDNA and DAPI-bands (e.g., chromosome 1 in *A. aequalis* and chromosome 2 in *A. arundinaceus*; chromosome 6 in *A. aequalis* and chromosome 10 in *A. arundinaceus*) which could be related to the allopolyploid origin of the *A. arundinaceus* genome. Interestingly, in both *Alopecurus arundinaceus* and *Avena longiglumis* (Al genome) karyotypes, we indicated one pair of chromosomes with the similar pattern of multiple rDNAs localization (large terminal 35S rDNA and distal 5S rDNA sites in the short arm and also interstitial 5S rDNA loci in the long arm). It has been shown that the genus *Avena* comprises species with diploid ($2n = 2x = 14$) A and C genomes which have structural variants (for A genomes: As, Al, Ad, Ap and Ac; for C genome: Cv and Cp), tetraploid ($2n = 4x = 28$) AB and AC genomes and hexaploid ($2n = 6x = 42$) ACD genomes. Also, it was assumed that A genome could be a possible progenitor of B and D genomes [39-40]. The above-mentioned chromosome pair with multiple 35S and 5S rDNA localization was earlier described in diploid and polyploid *Avena* species with different types of the A genome [30-34], and it could be inherited from a common progenitor at a remote period. In karyotypes of *A. latifolia*, *H. lanatus* and *D. flexuosa*, we observed a large chromosome carrying a distal 35S rDNA site in the short arm and interstitial 5S rDNA loci in the long arm. Such an occurrence of multiple rDNA sites localized in specific chromosomes may have value in chromosome identification and elucidation of evolutionary relationships and also delineation of possible break point sites [29, 70].

Currently, microsatellite DNA sequences are widely used as FISH probes for cytogenetic studies as they are major components of many plant genomes [33, 34, 71, 72]. Particularly, it has been recently determined that FISH with the oligo-GTT probe produces six constant signals located in the pericentromeric regions of three chromosome pairs of diploid A genome *Avena* species with minor interspecies differences in signal intensity [34]. These data indicate genomic variations among AA species and agree with the results of C-banding analysis [30-32] and Southern hybridization [73]. In the *A. longiglumis* accession studied here, FISH with the oligo-(GTT)₉ probe revealed not only the six cluster signals mentioned above but also a number of minor signals which demonstrated intraspecific variability in chromosomal distribution of this microsatellite motif. Interestingly, on chromosomes of two species, *Avena longiglumis* and *Alopecurus arundinaceus*, clustered (GTT)₉ signals were detected in the pericentromeric regions. In karyotypes of the other studied species, only small distal or subtelomere (GTT)₉ sites were observed. This agrees with the molecular phylogenetic and cytogenetic data reported earlier [5, 34] and could be related to distant relationships between these species.

According to current molecular phylogenetic studies, the studied here grass species (except *A. longiglumis*) are included in chloroplast group 2 (*Poaeae* type) which is subdivided into two clades comprising genera 1) *Avenella*, *Deschampsia*, *Holcus* and 2) *Alopecurus*, *Arctagrostis*, *Beckmannia* [1]. In the present study, we used a rapid GISH approach to reveal common homologous DNA repeats in karyotypes of the studied species groups. We have previously reported that a rapid GISH procedure with genomic DNA of *D. cespitosa* revealed multiple large hybridization signals on chromosomes of *D. sukatschewii* (confirming their close relationships). We have also found that *D. sukatschewii* genome was rich in AT-heterochromatin [37]. Besides, the species from both *Deschampsia* and *Holcus* contain common DNA repeats CON1, CON2, COM1 and COM2 which are widespread in *Poaceae* [15]. In the rapid GISH assays performed in the present study, we used labelled genomic DNAs of *D. sukatschewii* and *H. lanatus* (chloroplast group 2) to reveal common DNA repeats on most studied species except *H. lanatus*. For *H. lanatus* chromosomes, we used genomic DNAs of *D. sukatschewii* and *D. flexuosa* as *D. flexuosa* differed karyotypically from *D. sukatschewii* and *D. cespitosa* [37]. The performed rapid GISH analysis showed that species from both chloroplast groups possessed common DNA repeated sequences as clustered hybridization signals of genomic DNAs of both *H. lanatus* and *D. sukatschewii* were revealed in different positions on chromosomes of *A. aequalis*, *A. arundinaceus*, *B. syzigachne*, *D. cespitosa* and *D. flexuosa*. On chromosomes of *A. latifolia*, more hybridization signals of genomic DNA of *H. lanatus* were detected (which therefore, indicated more genome similarities between these species) compared to genomic DNA of *D. sukatschewii*. At the same time, on chromosomes of *A. longiglumis*, which belongs to chloroplast group 1 (*Aveneae* type) [1, 5], we mostly observed dispersed hybridization signals of genomic DNAs of both *H. lanatus* and *D. sukatschewii*. Thus, our findings generally agree with the molecular phylogenetic data reported earlier [1, 5].

The genus *Beckmannia* comprises two perennial species, *B. eruciformis* and *B. syzigachne*. This genus has been subjected to several taxonomic revisions. Based on morphology, *Beckmannia* has been assigned to tribes *Phalarideae*, *Chloriideae*, *Beckmanniinae*, and finally *Aveneae* (subtribe *Alopecurinae*)

[74]. Until recently, molecular phylogenetic studies have not separated *Beckmannia* in a distinct lineage, tentatively leaving it within subtribe *Alopecurinae* [75-77] though support for potential separating *Beckmannia* and *Alopecurus* from each other has been provided [75]. Finally, however, Soreng et al. [1] has placed the genera *Alopecurus* and *Beckmannia* in two different subtribes (*Alopecurinae* and *Beckmanniina*). In support of these recent data, the molecular cytogenetic analysis performed in the present study did not reveal any karyotypic similarities between *B. syzigachne* and both diploid and tetraploid *Alopecurus* accessions. Nevertheless, further investigations of *Beckmannia* species based on different chromosomal and molecular markers are necessary to clarify the phylogenetic position of *Beckmannia* within the *Aveneae/Poeae* tribe.

Distribution patterns of the examined molecular cytogenetic markers in the studied accessions of *D. cespitosa* and *D. flexuosa* agreed with our previous results obtained for non-polar accessions [37]. Nevertheless, differences in number and size of some DAPI-bands as well as in localization of several chromosomal markers (mainly due to chromosomal rearrangements) were also observed. Molecular cytogenetic analysis of these species showed that their karyotypes differed significantly from each other. These results agreed with our previous findings indicating that *D. flexuosa* also had basic karyotypic differences with *D. antarctica*, *D. danthonioides*, *D. elongata*, *D. sukatschewii* and *D. parvula* [37]. According to the phylogenetic analyses inferred from nuclear ITS and plastid trnL sequence data, *D. flexuosa* is regarded a better suited to the genus *Avenella* [78]. However, rapid GISH assays with labelled genomic DNAs of both *D. sukatschewii* (in the present study) and *D. cespitosa* [37] detected clustered signals on chromosomes of *D. flexuosa* indicating the presence of common homologous highly repeated DNA sequences in their genomes.

It should be noticed that phylogenetic position of the genus *Deschampsia* within the family *Poaceae* is still controversial. According to Soreng et al. [1], *Deschampsia*, *Holcus* and *Vahlodea* were classified in the subtribe *Holcinae* which is not, however, monophyletic because *Holcus* and *Vahlodea* do not form a clade with *Deschampsia* in the plastid and nuclear ribosomal DNA trees [21, 22, 79]. In a parallel classification of grasses, *Holcinae* is treated as a synonym of *Airinae* [4]. Some recent authors render the genus *Deschampsia* paraphyletic [80] or consider that *Deschampsia* would be better treated in its own monotypic subtribe [23]. Our findings agree with the last of these observations as the comparative molecular cytogenetic analysis of *Deschampsia* species and *Holcus lanatus* did not reveal similarities in distribution patterns of the studied chromosomal markers.

The analysis of distribution of the examined markers in karyotypes of *B. syzigachne*, *D. cespitosa* and *D. flexuosa* revealed different chromosomal rearrangements. The presence of numerous chromosomal rearrangements in plant genomes is considered to be related with speciation events [66, 81]. Accordingly, the process of genome evolution in these taxa could include chromosomal reorganization (chromosome interchanges, inversions, translocations) of the initial parental genomes.

Thus, the comparative molecular cytogenetic analysis of valuable Arctic and sub-Arctic grassland species from the related genera revealed structural differences and also similar features in their karyotypes. The

obtained results can be a basis for the further genetic and biotechnological studies.

Conclusion

The results of the present study provide unique information on distribution areas and cytogenomic structures of valuable Arctic and sub-Arctic pasture grass species from related genera of the Aveneae/Poeae tribe. The peculiarities of distribution patterns of the examined chromosomal markers and also presence of common homologous DNA repeats in karyotypes of the studied species allowed us to verify their relationships. The revealed karyotypic similarities between *Alopecurus aequalis* and *Alopecurus arundinaceus* indicated the allopolyploid origin of the *A. arundinaceus* genome. The karyotype of *Beckmannia syzigachne* differed greatly from the studied *Alopecurus* accessions in support of the recent molecular phylogenetic studies which have placed the genera *Alopecurus* and *Beckmannia* into different subtribes within the Poeae tribe. The comparative molecular cytogenetic analysis of *Deschampsia* species and *Holcus lanatus* confirmed the recent molecular phylogenetic data which suggested not be classified *Deschampsia* and *Holcus* in one subtribe. Our findings are important for further genetic and biotechnological studies and also plant breeding progress.

Abbreviations

AFLP: Amplified fragment length polymorphism

PCR: Polymerase chain reaction

SNP: Single nucleotide polymorphism

FISH: fluorescence *in situ* hybridization

GISH: genomic *in situ* hybridization

Declarations

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Author's Contributions

The present study was conceived and designed by AVA, OVM and SAZ. AVA, OVM, SAZ, OYY, TES and LG carried out the experiments. AVA, OVM, AVR, SAZ, OYY, TES and LG analyzed the obtained data. AVR, EOP and IGL provided plant materials. All authors participated in preparing and writing the manuscript. AVA, OVM, SAZ and AVR performed the analysis with constructive discussions. All authors have read and approved the final manuscript. AVA and SAZ contributed equally to this work.

Ethics approval and consent to participate

This study including the plant sample collection and experimental research conducted on these materials was according to the federal law on environmental protection approved by the Council of the Russian Federation.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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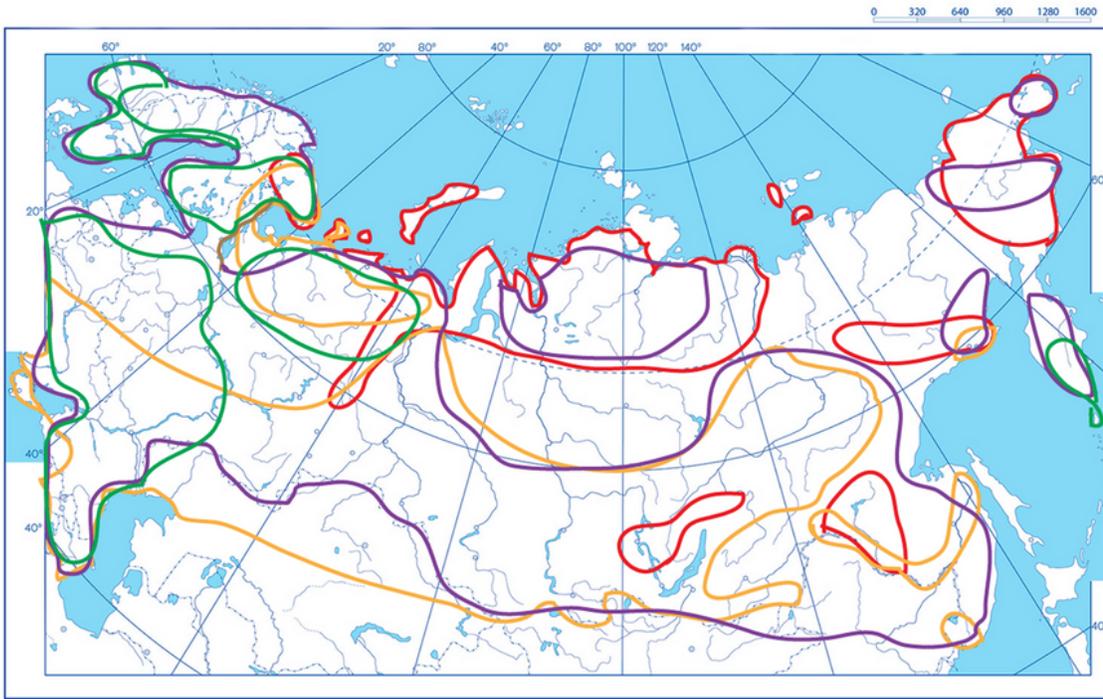
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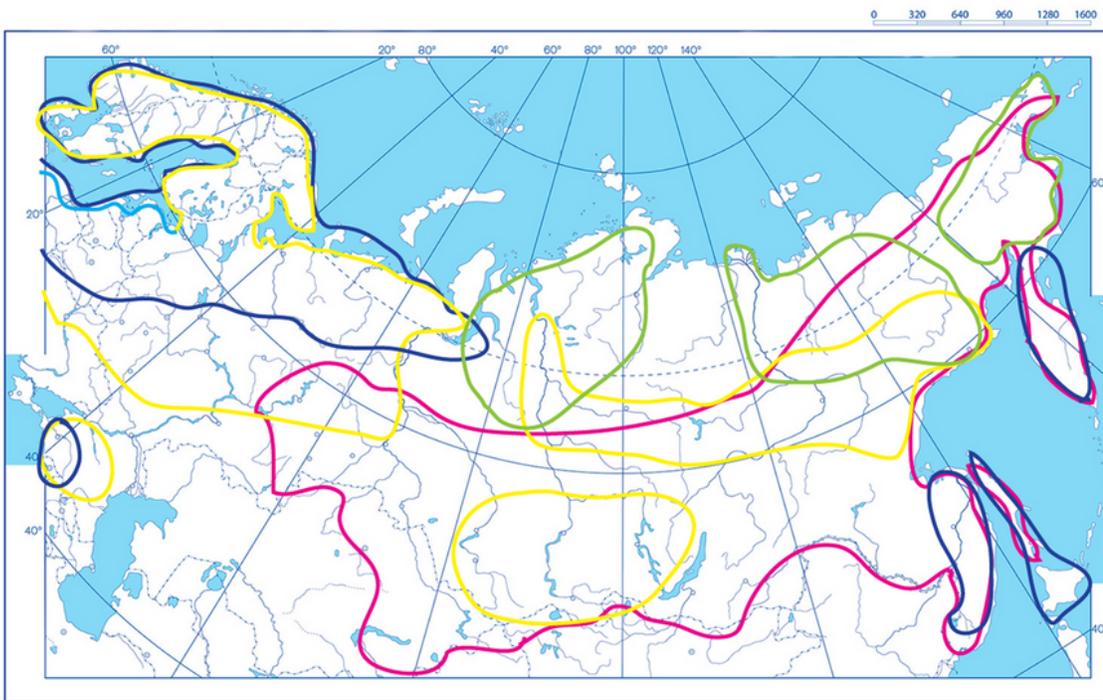
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Figures



■ *Arctagrostis latifolia* ■ *Alopecurus arundinaceus* ■ *Alopecurus aequalis* ■ *Holcus lanatus*



■ *Beckmannia syzigachne* ■ *Deschampsia cespitosa* ■ *Deschampsia flexuosa* ■ *Deschampsia sukatschewii*

Figure 1

Integrated schematic habitat maps showing distribution of *A. aequalis*, *A. arundinaceus*, *A. latifolia*, *B. syzigachne*, *D. cespitosa*, *D. flexuosa*, *D. sukatschewii* and *H. lanatus* within the northern, central and eastern parts of Eurasia. The species names and correspondent colours of the lines indicating the boundaries of the species occurrence are specified under the maps.

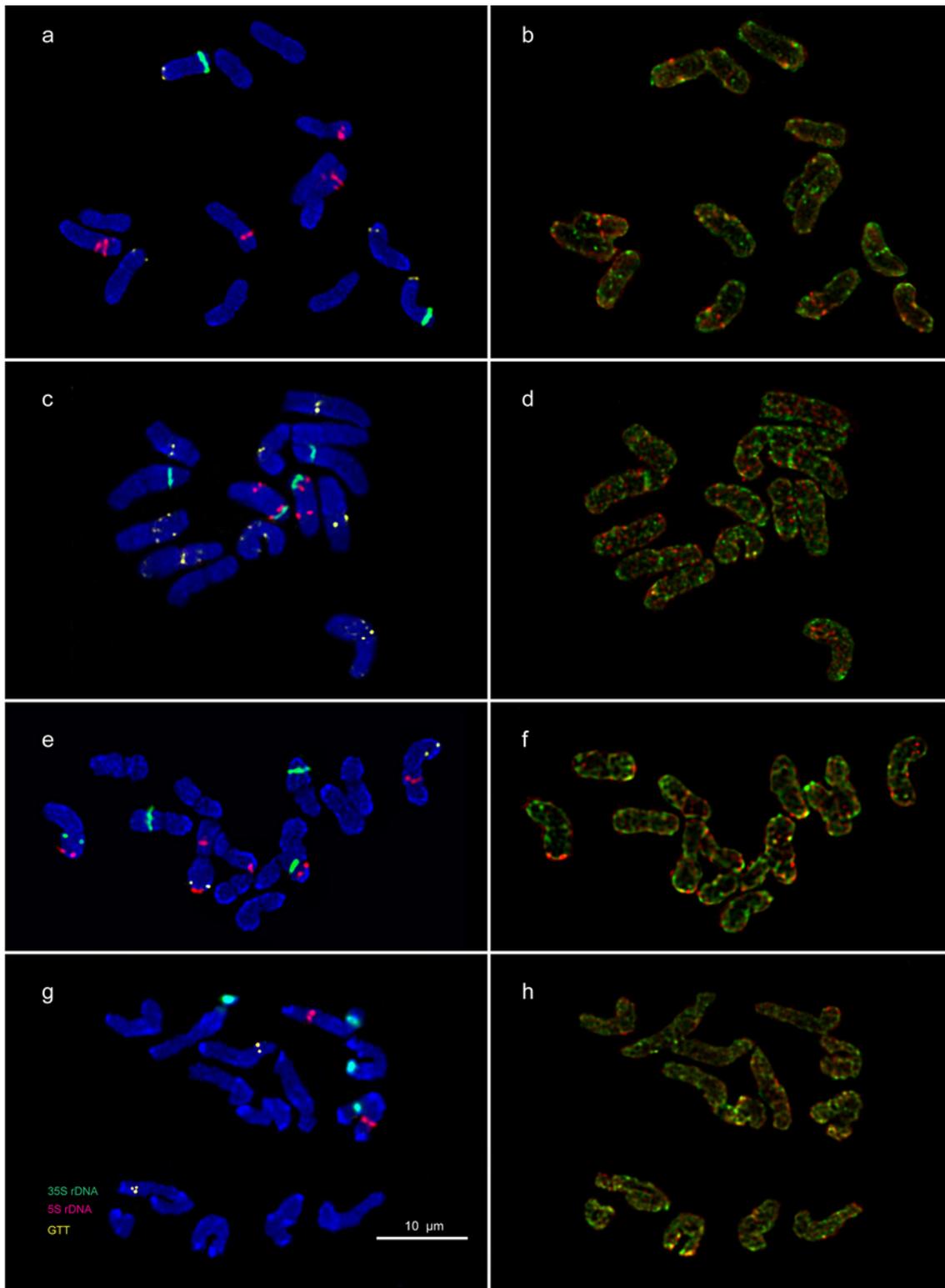


Figure 2

Chromosome spreads of the studied diploid species. (a, b) *A. aequalis*, (c, d) *A. longiglumis*, (e, f) *B. syzigachne* and (g, h) *H. lanatus*. Merged fluorescent images after multicolour FISH with 35S rDNA (green), 5S rDNA (red), oligo-(GTT)9 (yellow) (a, c, e, g) and sequential rapid GISH with genomic DNAs of *D. sukatschewii* (red) and *H. lanatus* (green) (b, d, f) and also *D. sukatschewii* (red) and *D. flexuosa* (green) (h). DAPI chromosome staining - blue. Scale bar - 10 μm.

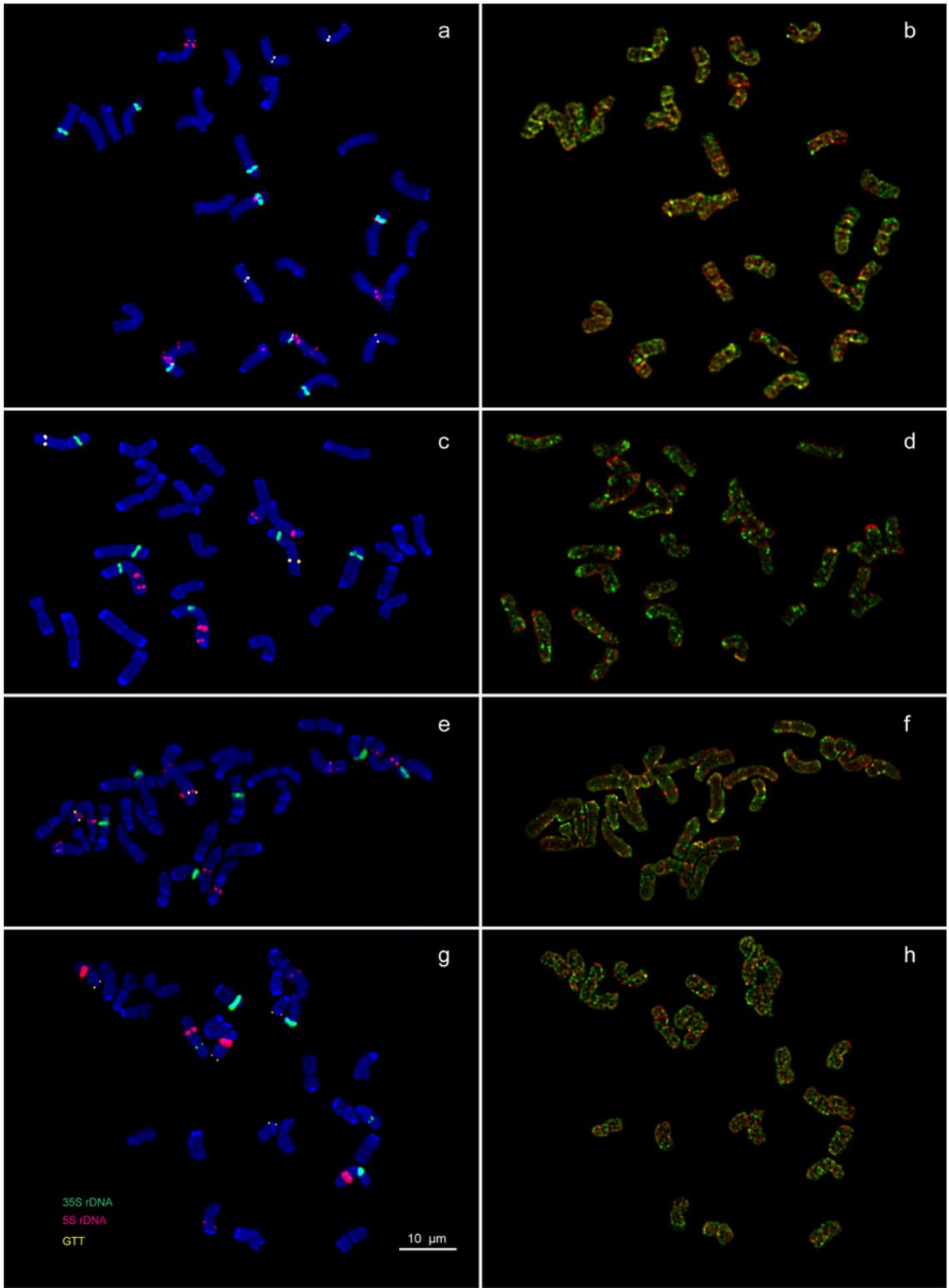


Figure 3

Chromosome spreads of the studied tetraploid species. (a, b) *A. arundinaceus*, (c, d) *A. latifolia*, (e, f) *D. cespitosa* and (g, h) *D. flexuosa*. Merged fluorescent images after multicolour FISH with 35S rDNA (green), 5S rDNA (red), oligo-(GTT)₉ (yellow) (a, c, e, g) and sequential rapid GISH with genomic DNAs of *D. sukatschewii* (red) and *H. lanatus* (green) (b, d, f, h). DAPI chromosome staining - blue. Scale bar - 10 µm.

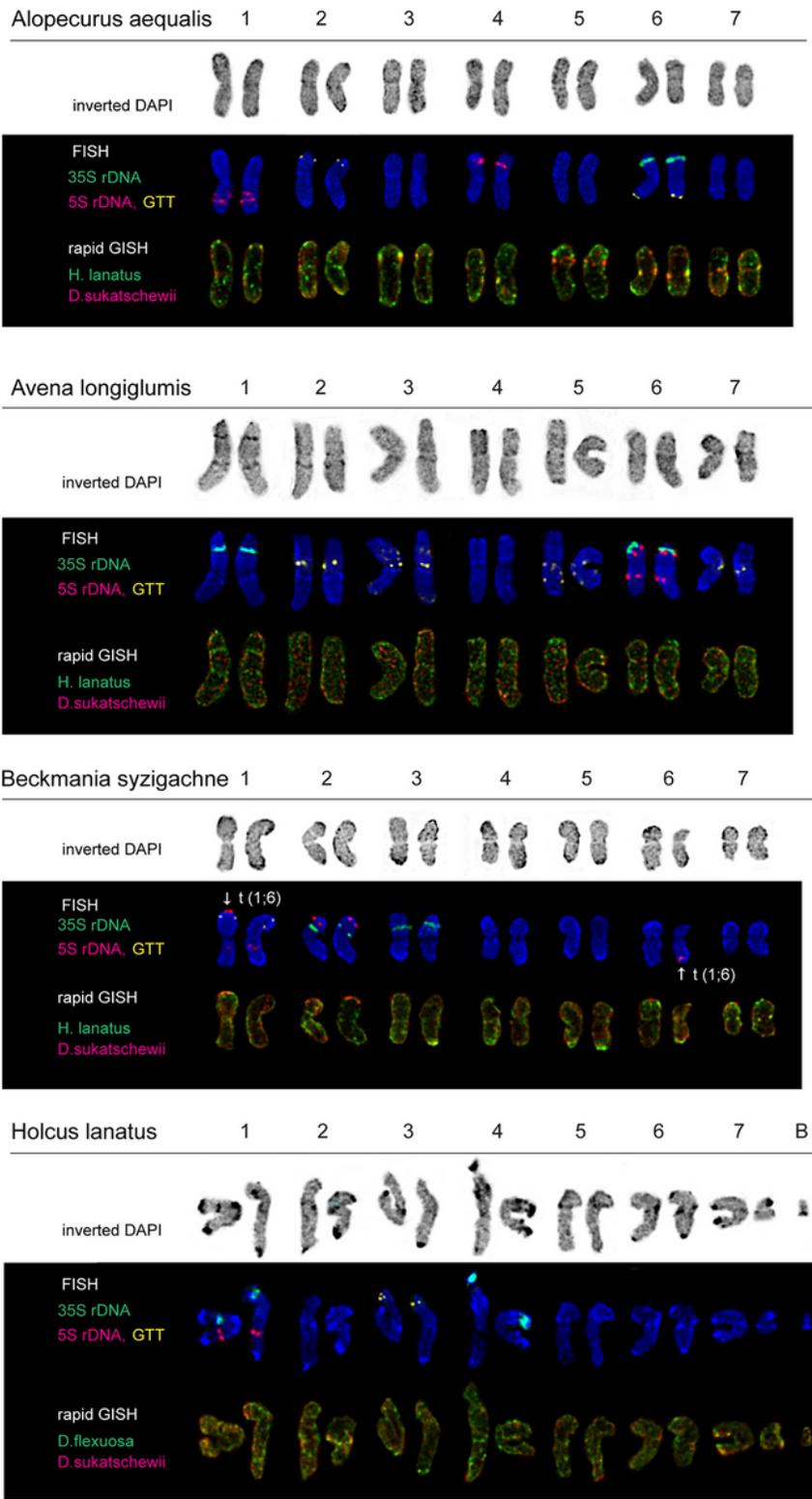
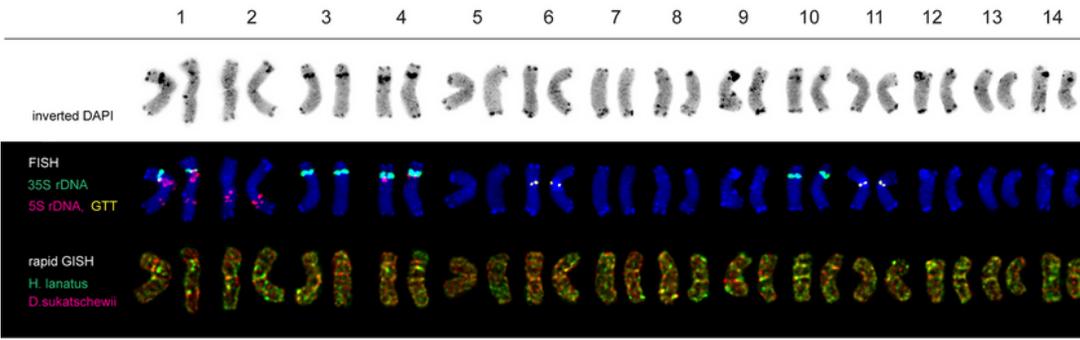


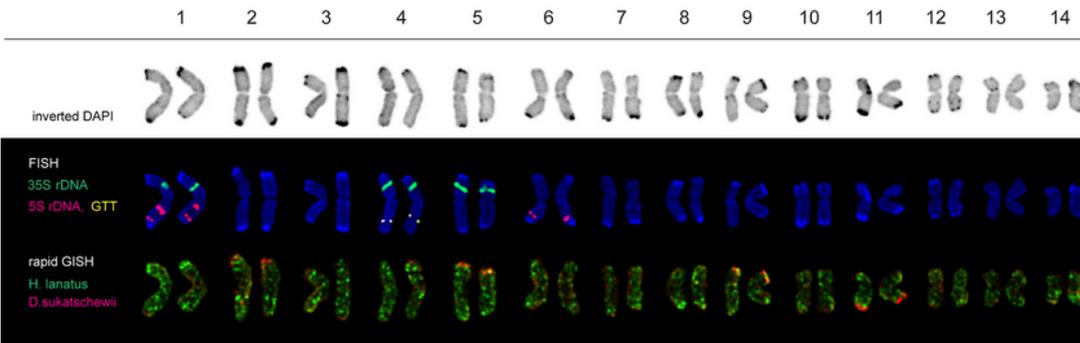
Figure 4

Karyotypes of *A. aequalis*, *A. longiglumis*, *B. syzigachne* and *H. lanatus*. Karyograms of the metaphase plates shown in Fig 2 after DAPI-banding (inverted images), FISH with the oligo-(GTT)₉, 35S and 5S rDNA probes and also rapid GISH with genomic DNAs of *D. flexuosa*, *D. sukatschewii* and *H. lanatus*. The correspondent probes and their pseudo-colours are specified in the left. Arrows point to chromosome rearrangements. B - B chromosomes.

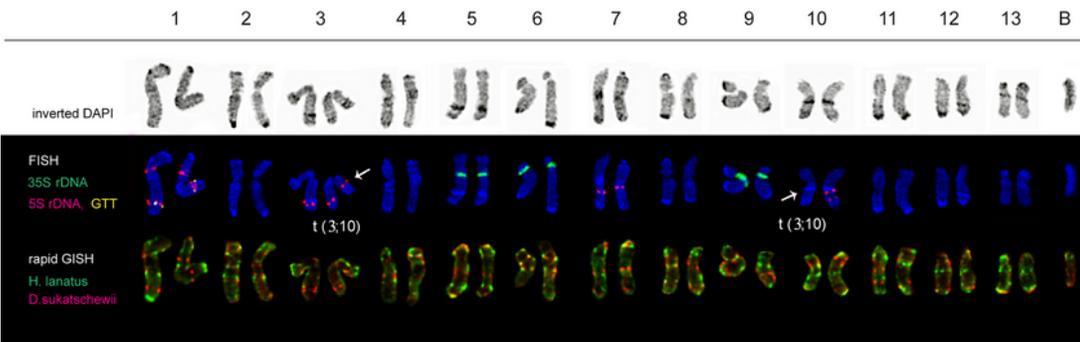
Alopecurus arundinaceus



Arctagrostis latifolia



Deschampsia caespitosa



Deschampsia flexuosa

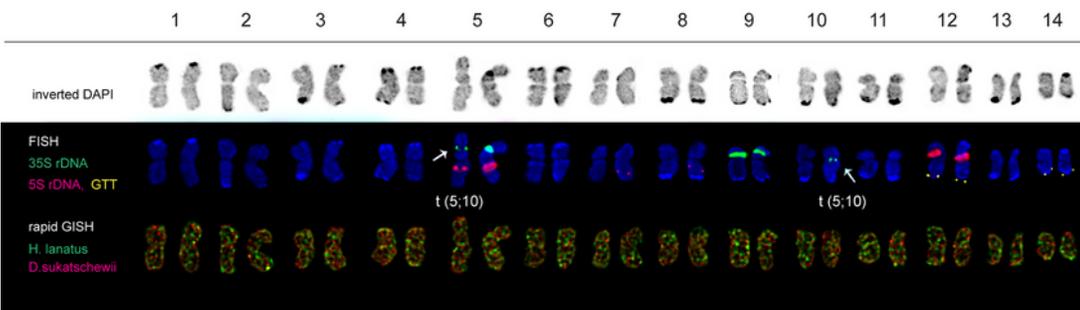


Figure 5

Karyotypes of *A. arundinaceus*, *A. latifolia*, *D. caespitosa* and *D. flexuosa*. Karyograms of the metaphase plates shown in Fig 3 after DAPI-banding (inverted images), FISH with the oligo-(GTT)₉, 35S and 5S rDNA probes and also rapid GISH with genomic DNAs of *H. lanatus* and *D. sukatschewii*. The correspondent probes and their pseudo-colours are specified in the left. Arrows point to chromosome rearrangements. B - B chromosomes.