Chromosome evolution in grass tribes *Aveneae*/*Poeae* (*Poaceae*): insights from karyotype structure and molecular phylogeny

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Abstract: Röser, M., Winterfeld, G., Döring, E. & Schneider, J. 2014: Chromosome evolution in grass tribes *Aveneae*/*Poeae* (*Poaceae*): insights from karyotype structure and molecular phylogeny. Schlechtendalia **28**: 1–21.

Current methods in chromosome analysis, especially the physical mapping of specific DNA probes to individual chromosomes, are useful tools for analyzing evolutionary processes in chromosome organization. They add significant new data to earlier studies based on chromosome counts or conventional karyotyping. This type of more detailed cytogenetic information is available to date for only 12 of the altogether 60–70 genera of the traditional grass tribe *Aveneae*. By far most studies dealt with the well-studied genus *Avena*, due to the agricultural significance of the cultivated oats. Information is less complete for the other genera, because some chromosome characters are not consistently known for all of them, which makes comparison difficult. Moreover, information is scattered in the literature and not easily accessible for systematic or phylogenetic work. Thus we summarized and reviewed these data on chromosome structure and organization, while leaving chromosome numbers out of focus, and evaluated or re-analyzed them in the light of an emerging phylogenetic framework leading to many changes in genus-level systematics of *Aveneae*/*Poeae*. In many instances the previously seemingly random distribution of chromosome characters is resolved as independent change within different phylogenetic lineages (parallelism), as seen, for example, in the numbers of nucleolar organizing regions, the presence of different satellite DNAs, or the distribution and composition of heterochromatin. Other aspects of chromosomal organization, such as the localization of the different ribosomal DNAs on particular chromosomes, are rather conserved through multiple speciation processes. Constraints in interphase nuclear architecture were invoked as likely explanation. Important progress thus has been made to identify the closest relatives of *Avena*, the most important crop plant genus. *Avena* fits the assemblage of genera, in which it is nested on molecular phylogenetic data, also in chromosomal characters (*Arrhenatherum*, *Helictotrichon* s. str. incl. *Pseudarrhenatherum*, *Tricholemma*). Polyploidy, long considered a driving factor in grass evolution turned out to be highly complicated process. As well-known for the genus *Avena*, chromosome structure and sequence analysis of ribosomal DNA concur also in examples from the genus *Helictochloa* by revealing most of its polyploids as allopolyploids, but there is sequence homogenization involving exchange of entire repeat units as well as stepwise, somewhat patchy change, while the corresponding chromosomal sites inherited from the parental genomes remain rather conserved. The value of detailed information on chromosome structure for future comparative studies on grass genomes across tribes and subfamilies is discussed.

Zusammenfassung: Röser, M., Winterfeld, G., Döring, E. & Schneider, J. 2014: Chromosomenevolution der Gräsertriben *Aveneae*/*Poeae* (*Poaceae*): Einblicke aus Sicht der Karyotypstruktur und molekularen Phylogenie. Schlechtendalia **28**: 1–21.

Gegenwärtige Methoden der Chromosomenanalyse, insbesondere die physische Kartierung von spezifischen DNA-Sonden in individuellen Chromosomen, sind nützliche Werkzeuge, um evolutionäre Vorgänge in der Chromosomenorganisation zu studieren. Sie ergänzen auf Chromosomenzählungen oder konventionellen Karyotyp-Analysen basierende frühere Studien um wichtige neue Daten. Derartig detaillierte cytogenetische Informationen sind bis heute nur für etwa 12 der insgesamt 60–70 Gattungen der traditionellen Gräser-Tribus Aveneae verfügbar. Die meisten Studien beschäftigten sich mit der gut untersuchten Gattung *Avena*, aufgrund der landwirtschaftlichen Bedeutung der kultivierten Saathafer. Weniger vollständig sind die Informationen über die anderen Gattungen, denn einige chromosomale Merkmale sind bei ihnen nicht durchwegs untersucht worden, was Vergleiche erschwert. Zudem sind die Daten relativ verstreut in der Literatur anzutreffen und nicht leicht für systematische oder phylogenetische Fragestellungen verfügbar. Aus diesen Gründen fassen wir diese Daten in einem Übersichtsartikel zur Chromosomenstruktur und -organisation zusammen und werten bzw. re-analysieren sie im Lichte sich entwickelnder neuer phylogenetischer Erkenntnisse, die zu vielfachen Änderungen in der Gattungssystematik der *Poeae*/*Aveneae* geführt haben. Daten über Chromosomenzahlen haben wir dabei weniger berücksichtigt. In vielen Fällen erwies sich scheinbar zufällige Verteilung chromosomaler Merkmale als mehrfach unabhängig voneinander erfolgte Veränderung in unterschiedlichen und voneinander unabhängigen phylogenetischen Linien (Parallelismus). Beispiel dafür sind die Zahl der Nukleolusorganisator-Regionen (NORs), das Vorkommen unterschiedlicher Satelliten-DNAs oder die Anordnung und Zusammensetzung des Heterochromatins. Andere Aspekte der Chromosomenorganisation, z.B. die Lokalisierung der unterschiedlichen ribosomalen DNAs in speziellen Chromosomen, sind evolutionär relativ konserviert und über multiple Artbildungsereignisse hinweg unverändert erhalten geblieben. Strukturelle Zwänge in der Organisation des Interphasekerns werden hierfür als wahrscheinlichste Erklärung diskutiert. Wesentliche Fortschritte wurden darin erzielt, die nächsten Verwandten von *Avena*, der wichtigsten Nutzpflanzengattung der *Aveneae*, zu identifizieren. *Avena* passt aufgrund ihrer chromosomalen Merkmale sehr gut in die Gruppe von Gattungen hinein, in der sie wegen molekular-phylogenetischer Daten gestellt wird (*Arrhenatherum*, *Helictotrichon* s. str. inkl. *Pseudarrhenatherum*, *Tricholemma*). Polyploidie, seit langem als wesentliche Antriebskraft der Evolution der Gräser betrachtet, erweist sich als höchst komplizierter Prozess. Wie bereits für die Gattung *Avena* bekannt, belegen Chromosomenstruktur und Sequenzanalysen der ribosomalen DNA in übereinstimmender Weise auch für Beispiele aus der Gattung *Helictochloa*, dass es sich bei den meisten Polyploiden

um Allopolyploide handelt. Hierbei kann eine Homogenisierung der unterschiedlichen parentalen DNA-Sequenzen eintreten, welche sowohl den Austausch vollständiger Wiederholungseinheiten (repeats) als auch kleinerer Abschnitte umfasst, während die zugehörigen chromosomalen Loci, die von den parentalen Genomen stammen, weitgehend konserviert bleiben. Die Bedeutung detaillierter Daten zur Chromosomenstruktur für zukünftige vergleichende Studien der Gräser-Genome über die Grenzen von Triben und Unterfamilien hinweg wird diskutiert.

Key words: *Avena*, *Avenula*, concerted evolution, cytogenetics, FISH, *Helictochloa*, *Helictotrichon*, polyploidy, ribosomal DNA, satellite DNA

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Introduction

Grasses as Crop Plants

Grasses are the most important plant family for mankind and in particular its foremost source of carbohydrates (Lieberei & Reisdorff 2012, FAO 2014). As such, understanding its evolution at the chromosomal level is of fundamental importance.

Cereals as crop plants come from no less than four different evolutionary lineages of grasses: 1) subf. *Erhartoideae* (rice, wild rice; *Oryza sativa*, *Zizania palustris*), 2) subf. *Pooideae* (wheat, barley, rye, oat; *Triticum* spp., *Hordeum vulgare*, *Secale cereale*, *Avena* spp.), 3) subf. *Panicoideae* (maize, sorghum, several genera of millets; *Zea mays*, *Sorghum bicolor*) and 4) subf. *Chloridoideae* (further millets as *Eragrostis tef*, *E.* spp. and species of *Setaria*).

As a common character, all of the cultivated species already date back to ancient, pre-historic selection and cultivation by humans in different continents of the world and since then split up into an enormous amount of different races, varieties or cultivars. Some genera contain several cultivated species (*Triticum*, *Avena*) involving different ploidy levels with a mostly allopolyploid and especially amphidiploid background, whereas others have only a single cultivated species (*Oryza*, *Hordeum*, *Secale*, *Sorghum*, *Zea*) with a constant chromosome number except aneusomatic breeding lines, artificially generated polyploids, etc. Additionally, hybrids between crop species of related genera have been firstly bred in the 20th century, e.g., between *Triticum* and *Secale* (×*Triticale*, etc.), and are becoming economically increasingly significant to date (Lieberei & Reisdorff 2012).

Highly important cereals, especially maize, rice and wheat, served during the past c. eight decades as one of the most important study objects and model systems in plant genetics, cytogenetics and plant physiology as they are suited for experimental study, due also to their short generation time as annuals. Many fundamental discoveries on inheritance, chromosome structure, order of genes in the chromosomes, gene regulation and epigenetic effects, transposable elements, or molecular organization of meiosis have been obtained with experiments on grasses, which are in focus also of comparative genome analysis by completed or progressing genome sequencing studies (rice, sorghum, maize).

Aveneae and Oat (*Avena sativa* and Other Species)

Chromosome research in the tribe *Aveneae* has focused on genus *Avena* which has about 30 species, all of which are native from the Near East and Ethiopia through the Mediterranean region to the islands of Macaronesia (Baum 1977; Baum & Fedak 1985a, b; Ladizinsky 1998). Species of *Avena* cultivated for grain harvesting are hexaploid *A. sativa* (genome composition ACD), to a lesser extent also diploid *A. strigosa* (A), tetraploid *A. abyssinica* (AB) and hexaploid *A. byzantina* (ACD). Hexaploid *Avena sativa* became introduced as a crop plant in all continents, but unintentionally also some species (e.g., *A. fatua* or *A. ludoviciana* with the same genomes ACD), which now rank among the world's most noxious weeds. Extensive cytogenetic studies in *Avena*, complemented by molecular and fingerprint studies have largely clarified the relations between the diploid species in *Avena* and aided to identify the origin of its polyploids (Rajhathy & Thomas 1974; Postoyko & Hutchinson 1986; Fominaya et al. 1988a, b; Linares et al. 1992, 1996, 1998; Thomas 1992; Jellen et al. 1993a, b, 1994; Chen & Armstrong 1994; Leggett & Markhand 1995; Leggett & Thomas 1995; Katsiotis et al. 1996, 1997, 2000; Irigoyen et al. 2001, 2002; Fu & Williams 2008 with references; Nikoloudakis & Katsiotis 2008;

Nikoloudakis et al. 2008; Peng et al. 2008 2010a, b; Shelukhina et al. 2008a, b; Morikawa & Nishihara 2009; Badaeva et al. 2010a).

All species of *Avena* are annual and inbreeding, except for the Algerian mountain endemic *A. macrostachya* which is perennial and outcrossing (Maire 1953, Saint-Yves 1931, Quézel & Santa 1962, Baum & Rajhathy 1976). This species thus was occasionally ascribed to the neighboring perennial genus *Helictotrichon* (Clayton & Renvoize 1986), but there is strong evidence from the fields of morphology, crossing experiments and molecular systematics to include it with genus *Avena* (Holub 1958; Baum 1968, 1977; Leggett 1990, 1991; Döring et al. 2007; Döring 2009). The karyotype structure of *A. macrostachya* is intriguing. It resembles that of perennial *Aveneae* genera, especially the recently described genus *Tricholemma* (Winterfeld 2006, Röser et al. 2009, Winterfeld et al. 2009a) and the A genome species of *Avena* with prevailingly metacentric chromosomes (Rajhathy & Thomas 1974, Loskutov 2008, Badaeva et al. 2010b), whereas hybrid meiosis, banding patterns and molecular characters point to rather close affiliation with C genome species of *Avena* (Leggett 1990, 1991; Pohler & Hoppe 1991; Leggett & Markhand 1995; Rodionov et al. 2005; Badaeva et al. 2010b).

Cytogenetics and Molecular Phylogenetics

Information on chromosome characters in the altogether c. 60–70 further genera of tribe *Aveneae* as traditionally delineated (cf. Tzvelev 1976, Clayton & Renvoize 1986, Watson & Dallwitz 1992) is rather scanty and much less detailed than for *Avena*. Exemplary counts of chromosome numbers are available for many genera (IPCN 2014) and were reviewed by Rodionov et al. (2007), but more detailed chromosome or molecular cytogenetic data have been obtained in only few (e.g., *Aira*, *Arrhenatherum*, *Avenella*, *Avenula*, *Deschampsia*, *Helictochloa*, *Helictotrichon*, *Phalaris*, *Pseudarrhenatherum*, *Tricholemma*, *Zingeria*; Albers 1980; Romero Zarco 1984, 1985a, b, c; Sauer & Heubl 1984; Röser 1989; Grebenstein 1992; Garcia-Suárez et al. 1997a, b; Li et al. 1997; Katsiotis et al. 2000; Röser et al. 2001; Mitchell et al. 2003; Kotseruba et al. 2003, 2010; Heinrichsmeier 2005; Winterfeld 2006; Winterfeld & Röser 2007a, b; Kim et al. 2009; Winterfeld et al. 2009a, b, 2011).

The direction of evolutionary change is frequently hard to assess for chromosomal characters. Proposed "general" trends in the karyo-evolution of plants can often be read the other way round, may be opposite even in closely related taxa and changes have frequently rather quantitative than qualitative nature. For example, there is no unidirectional change in plant and animal evolution from symmetric to asymmetric karyotypes (i.e., chromosome sets with prevailingly metacentric to submetacentric versus subtelocentric to telocentric chromosomes), from small to large chromosomes, from low to high proportions of highly repetitive DNA (constitutive heterochromatin = C bands), or from small to large genomes (Levitsky 1931; Stebbins 1971; White 1973; Jones 1977, 1978; Greilhuber & Ehrendorfer 1988; Kenton et al. 1993; Greilhuber 1995; Briggs & Walters 1997; Leitch et al. 2005). Until recently it was argued there is one cytogenetic trait that never could be reverted, namely the change from diploidy to polyploidy, meaning the multiplication of chromosome sets. However, findings in *Arabidopsis thaliana* (with only $2n = 10$) or maize ($2n = 20$) show the opposite, as both have polyploid background and strongly restructured, entirely diploidized genomes (Swigonova et al. 2004; van de Peer & Meyer 2005).

Molecular phylogenetic studies proved very useful to interpret changes in chromosome and genome structure and can aid fundamentally to identify parallelism in chromosome characters or character changes and the evolutionary direction of change(s). Chromosome characters can be attached to a phylogenetic framework derived from a different set of characters and can be compared with it, which allows to identify and trace the sequence of different changes in chromosome structure along a likely phylogenetic branching of lineages and not only in terms of predefined assumptions on chromosome evolution. It is shown here that several seemingly identical characters in genomic or chromosome structure of Aveneae actually did not originate simultaneously and at the same time in the evolution of this tribe. For example, absence of certain satellite DNAs (satDNA) is shown as based on either primary absence or on secondary loss from the genomes (for example, in *Arrhenatherum elatius* relative to *Helictotrichon thorei*).

The same applies to monoploid chromosome sets with one nucleolar organising region (NO, NOR), which seemingly originated repeatedly from chromosome sets with two NORs (e.g., *Arrhenatherum* or in *Avena*, *Helictochloa*, *Helictotrichon*).

This report on chromosome evolution in *Aveneae* is mainly based on our results on cytogenetics and molecular organization of ribosomal DNAs (rDNA) and satDNAs, their occurrence and chromosomal disposition, and molecular phylogenetics of Aveneae (Grebenstein et al. 1998; Röser et al. 2001; Winterfeld 2006; Döring et al. 2007; Winterfeld & Röser 2007a, b; Döring 2009; Schneider et al. 2009, 2011, 2012; Winterfeld et al. 2009 a, b, 2011, 2012, 2014; Wölk & Röser 2014) analyzed together with many other studies providing detailed cytogenetic results on other genera or species, but with focus on the agriculturally important genus *Avena* (e.g., Rajhathy & Thomas 1974; Fominaya et al. 1988a, b; Grebenstein 1992; Grebenstein et al. 1995, 1996; Linares et al. 1992, 1996, 1998; Fominaya et al. 1995; Yang et al. 1999; Hayasaki et al. 2001; Irigoyen et al. 2001; Nikoloudakis et al. 2008; Shelukhina et al. 2008a, b; Badaeva et al. 2010a, b). Some of these investigations used only single or few species and have not been viewed before in the light of a phylogenetic framework on Aveneae, which is currently emerging. We further address some patterns in rDNA chromosomal localization, which are remarkably conserved in evolution and the complicated patterns of polyploid origin and allopolyploid speciation in *Aveneae*.

Results and Discussion

Satellite DNAs

SatDNAs are highly repeated non-coding motifs in the genomes, whose repeat units may consist of few (microsatellites) to ca. 100 (minisatellites) or some hundreds of base pairs (cf. Csink & Henikoff 1998, Sharma & Raina 2005, Hemleben et al. 2007, Mlinarec et al. 2009). For tribe *Aveneae*, a couple of large and complex satDNAs have been isolated to date by restriction enzyme digests of total genomic DNA and were characterized by sequence analyses. This was done within genus *Avena* to trace species relationships and the allopolyploid origin of cultivated oat, *A. sativa* (e.g., Linares et al. 1998, Li et al. 2000), but was also used for broader comparison of different genera within tribe *Aveneae* (Grebenstein 1992; Grebenstein et al. 1995, 1996; Winterfeld 2006; Winterfeld & Röser 2007a; Winterfeld et al. 2009a).

Occurrence and distribution of the different satDNAs within the subfamilies or tribes of grasses was previously rather inconclusive, but is becoming better understood with increasing molecular phylogenetic knowledge on the family. SatDNA CON1 (365 bp in length; Grebenstein et al. 1995, 1996; Alix et al. 1998) is seemingly widespread in the whole *Poaceae*, although its occurrence has not yet been tested for subfamily *Bambusoideae*, the more basal grass subfamilies and other families of order Poales as shown in Fig. 1.

Other satDNAs are confined to grass subfamily *Pooideae*, especially CON2 (562 bp), COM1 (345 bp) and COM2 (476 bp) and have most likely originated later in grass evolution than CON1 (Fig. 1). However, this is just one of two possible reconstructions: Following Fig.1, satDNAs CON2, COM1 and COM2 could have the same or similar age as CON1, assuming that the first were congruently lost in *Panicoideae* and *Chloridoideae* or their common ancestor and in *Ehrhartoideae*, in contrast to CON1 which remained preserved in all of these subfamilies. This solution would be equally parsimonious relative to the phylogenetic tree of Fig. 1.

Presence of the same satDNA is a reliable indicator of relatedness between different taxa, due to the fact that such complex sequence motifs are unlikely to have multiple origins in evolution. This is evident in grasses and known from many other groups of plants or animals (Koukalova et al. 2010). Absence of a satDNA, conversely, is equivocal, because it may rest on either *a priori* absence for a given taxon, its relatives and ancestors or, alternatively, is due to secondary loss, thus representing an automorphic character relative to the ancestors. Both patterns are hard to distinguish without a supported phylogenetic hypothesis.

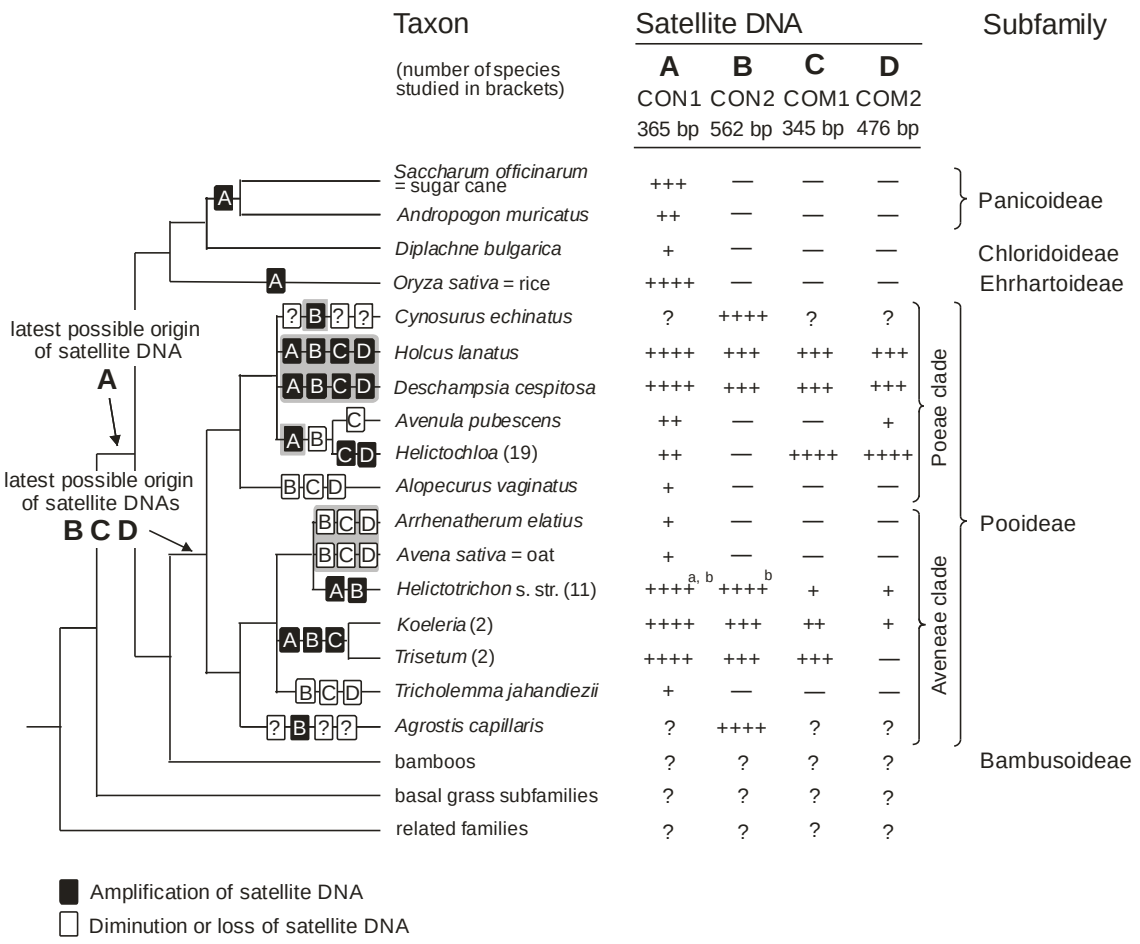


Fig. 1: Occurrence and distribution of satDNAs CON1, CON2, COM1, and COM2 in *Poaceae* compared with a simplified maximum parsimony tree based on chloroplast *matK* gene sequences. The symbol + indicates presence of a satDNA, ranging from weak (+) to strong signal intensity (++++) in Southern or dot blot hybridization and fluorescence *in situ* hybridization. Absence is denoted as — and missing data by a question mark. Including data from Grebenstein et al. (1996), Hilu et al. (1999), Winterfeld (2006), Döring et al. (2007), Winterfeld & Röser (2007a). [a] Except for *Helictotrichon desertorum* and *H. thorei* with satDNA CON1 and [b] *H. thorei* additionally with satDNA CON2 not present in high-copy number. Uncertain positions of character changes in tree polytomies, whether in parallel or only once in the common ancestor, are shaded.

Second, the origin of a satDNA is not necessarily contemporaneous with its amplification. Sequence motifs may persist in the genomes over long evolutionary times and many speciation processes and may be amplified eventually much later to high copy number (‘library hypothesis’; Salser et al. 1976, Ugarković 2008), eventually in lineages already separated from each other, meaning parallelism in a phylogenetic sense. The amplification pattern of satDNA CON1 in the subfamilies *Panicoideae* and *Ehrhartoideae* relative to *Chloridoideae* might be an example (Fig. 1). Differential amplification of satDNAs is even more striking in subfamily *Pooideae*, where amplification of satDNA COM2 occurred in parts of the *Poeae* lineage, but seemingly not in the *Aveneae* lineage, whereas COM1 became amplified in parts of both lineages, although to varying extent (Fig. 1).

Some of the genera tested so far share widely corresponding high-copy number inventories of satDNAs, for example, *Deschampsia* s. str. with *Holcus* or *Trisetum* with *Koeleria* (Fig. 1). This corroborates their intimate relationship, respectively, as suggested by morphological and molecular phylogenetic results (Clayton & Renvoize 1986; Grebenstein et al. 1998; Döring et al. 2007; Soreng et al. 2007; Quintanar et al. 2006, 2007; Döring 2009, Saarela et al. 2010).

The different representation of satDNAs in the genera *Avenula*, *Helictochloa*, *Helictotrichon* s. str. and *Tricholemma* (Figs. 1, 3) agrees with their taxonomic treatment as separate genera (Conti et al. 2005, Röser et al. 2009, Romero Zarco 2011), which is widely supported by

molecular phylogenetics (Grebenstein et al. 1998; Soreng & Davis 2000; Röser et al. 2001; Döring et al. 2007; Quintanar et al. 2007; Döring 2009; Schneider et al. 2009; Saarela et al. 2010; Wölk & Röser 2013, 2014). The previous concept of a broadly defined genus *Helictotrichon* including all these genera, eventually also *Avena macrostachya* and others (for different views see Holub 1962, 1976, 1980; Tzvelev 1976; Conert 1979–1998; Clayton & Renvoize 1986; Röser 1989, 1996, 1997; Lange 1995), thus is not corroborated by satDNA distribution (Figs. 1, 3).

Still there is a rather complicated pattern of amplification and/or loss of satDNAs existing within *Helictotrichon* s. str. Amplification of satDNA CON2 (Figs. 1, 2f, 3) occurred already in

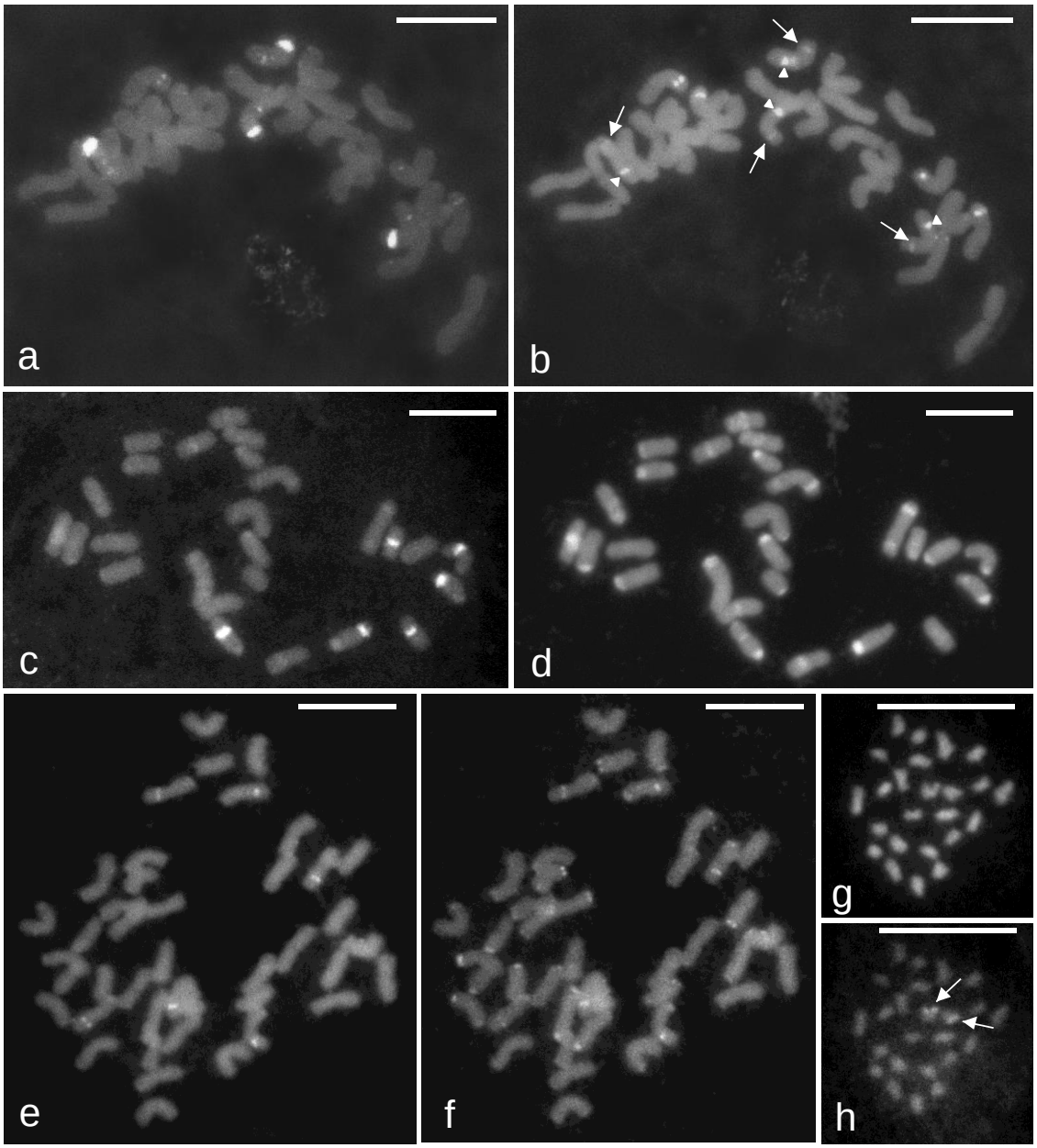


Fig. 2: Fluorescent *in situ* hybridization of specific DNA probes to chromosome preparations (FISH). **a** *Arrhenatherum elatius* (2*n* = 4x = 28) with four 18S–26S rDNA sites and **b** 5S rDNA sites either colocalized with the 18S–26S rDNA (arrows), or situated in the opposite chromosome arm of the same chromosome (arrowheads) or in chromosomes devoid of 18S–26S rDNA. **c** Allopolyploid and dysploid *Deschampsia cespitosa* (2*n* = 26) with four of its six 18S–26S rDNA sites and **d** some of its AT-rich heterochromatin bands localized in centromeric position, giving evidence of chromosome fusions. **e** *Helictotrichon sempervirens* (2*n* = 6*x* = 42) with six 5S rDNA sites. **f** All 5S rDNA sites of *H. sempervirens* are colocalized with bands of the satDNA CON2, which otherwise are abundantly present at the chromosome ends. **g** Chromosomes of *Danthoniastrum compactum* (2*n* = 24) of tiny size relative to the species of Aveneae and in **h** with two 18S–26S rDNA sites (arrowed). Scale bars: 10 µm.

the common ancestor of this group, but amplification of satDNA CON1 and the origin of extended AT-rich heterochromatin bands in the chromosomes followed later in evolution after divergence of *H. desertorum* (Fig. 3), a geographically isolated and ecologically deviating species growing under continental climate in the Eurasian steppe region (eastern Europe to Siberia). Occurrence of CON2 in high-copy number and of AT-rich heterochromatin bands thus is confined to the species-rich sister lineage of *H. desertorum*. This lineage is also differently distributed, namely in the Mediterranean and its adjacent high mountains (N Africa, Minor Asia, north to the Alps and the Carpathians). Based on molecular phylogenetic characters especially of the nuclear genome (5S and 18S–26S rDNA spacers; cf. Röser et al. 2001, Winterfeld et al. 2009a, Wölk & Röser 2014) the branching order of lineages within *Helictotrichon* is now rather well resolved, whereas all chloroplast DNA markers tested to date yielded only poor resolution (Döring 2009 and unpubl. data). *Helictotrichon* encompasses in the molecular phylogenies also the western Mediterranean traditional genus *Pseudarrhenatherum*, distributed along the Atlantic coast from France to Morocco. It is firmly nested within the Mediterranean-Alpine clade of *Helictotrichon* (*H. convolutum*, *H.* ×*krischae*, *H. parlatorei*, *H. sarracenorum*, *H. sedenense*, *H. setaceum*; Fig. 3) and shares the occurrence of AT-rich heterochromatin bands with it, but differs in the quantitative representation of the satDNAs in the genome and some structural characters of the chromosomes from the remainder of this clade (Figs. 3, 4c).

In view of the current molecular phylogenetic data we interpret these changes in *H. pallens* and *H. thorei* of the former genus *Pseudarrhenatherum* as autapomorphic and obtained secondarily (Fig. 3): diminution of satDNAs CON1 and CON2, loss of one NOR in the monoploid chromosome set of $x = 7$ and translocation of 5S rDNA bands into NO chromosomes (cf. below). Sequence organization of 5S rDNA additionally shows the deletion of 10 bp in the spacer of *Pseudarrhenatherum* relative to the remainder of *Helictotrichon* (Röser et al. 2001), while the other parts of this spacer are nearly identical. This deletion represents a further phylogenetically putatively autapomorphic change. The ancestor of this small lineage consisting of only two species (*H. thorei* and *H. pallens*) seemingly underwent a dramatic change in nuclear chromosomal and molecular organization.

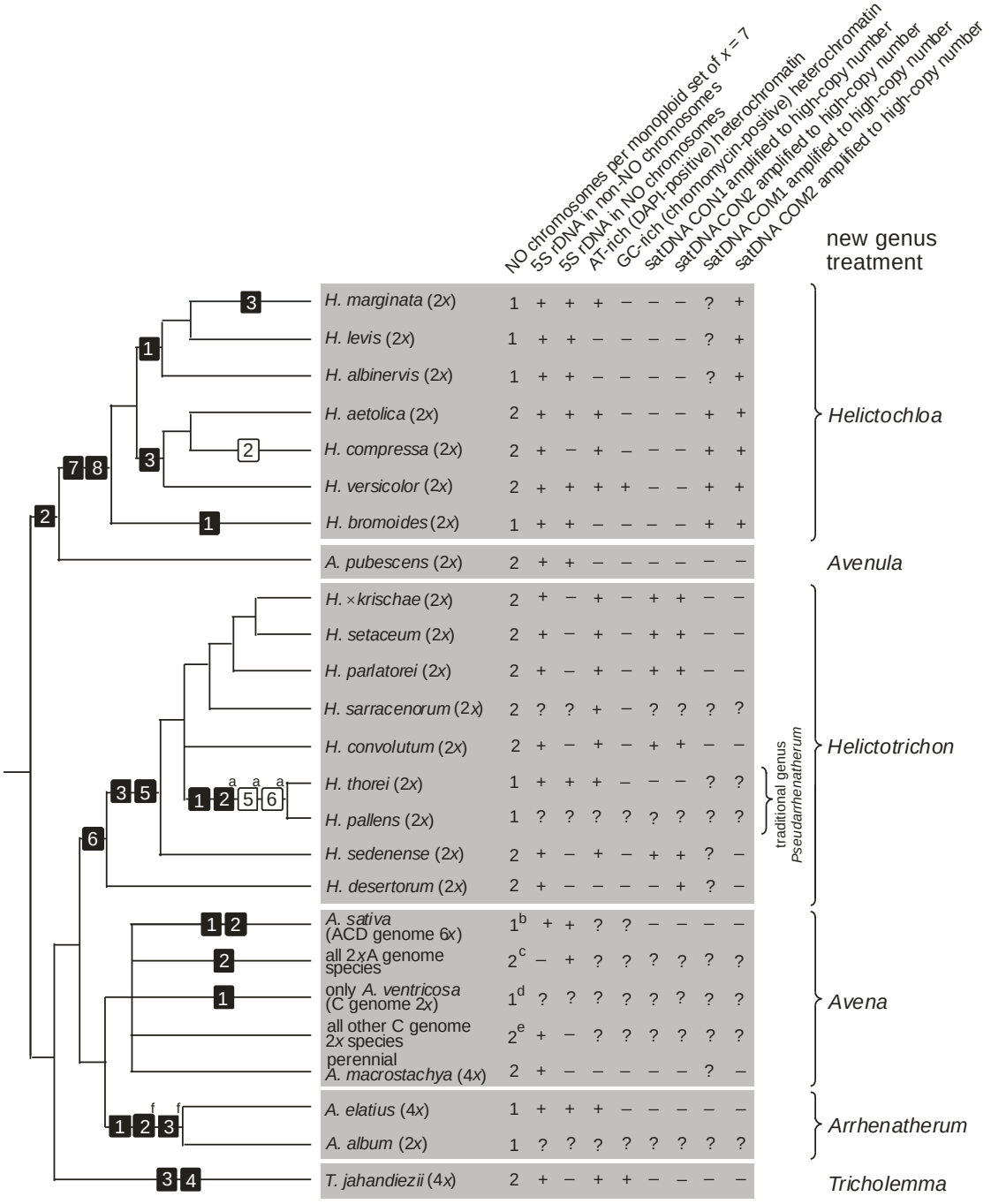
Nucleolar Organizing Regions

NORs belong to the ‘physical landmarks’ on chromosomes, frequently visible as constrictions in mitotic metaphase chromosomes, which are thus called ‘satellite chromosomes’. In the absence of such constrictions NORs remain undetected by conventional chromosome methods and can be demonstrated only by, for example, silver staining (Fig. 4 a–d) or DNA *in situ*-hybridizations using site-specific rDNA probes (Fig. 2a, c, h). The latter identifies also rDNA sites without nucleolar activity as found by silver impregnation (for discussion and references see Winterfeld & Röser 2007b).

The number of active NORs in taxa of *Aveneae* studied to date shows an only limited range of variation in contrast to previous, partly contradictory reports based exclusively on conventional chromosome staining. It is either one or two per monoploid chromosome set. Interestingly, the respective number of NORs is sometimes typical of larger groups of species such as genera or subgenera:

Mapping this character on the molecular phylogenetic tree for the taxa chromosomally studied (Fig. 3) shows that the plesiomorphic character state was most likely two NORs per monoploid chromosome set of $x = 7$ (Fig. 4a). For the taxa in question, this hypothesis is more parsimonious than assuming one NOR as original (Fig. 4b, c), although the latter would be sufficient in principle for the function of the nucleus and cell, as it is encountered in many other plant and animal organisms. The present reconstruction means also that two NORs present in most chromosome sets of genus *Avena* (oat) are not a special and secondarily obtained feature of this prevailingly annual and thus somewhat ‘derived’ genus. It is shared as a plesiomorphic character with most perennial genera of traditional *Aveneae* studied for this character (*Helictotrichon*, *Avenula*, *Tricholemma*) and also *Avena macrostachya*, the only perennial species of genus *Avena*.

Presence of only a single NOR in the monoploid set appears to represent a derived character state, which is somewhat unexpected in view of deviant previous reports for the taxa in question. It originated among the *Avena* diploids only in *A. ventricosa* (Fig. 3 with further explanation on *Avena* polyploids in the legend), is characteristic of the two tetraploid species of



Molecular and cytogenetic chromosome character states:

plesiomorphic	apomorphic	reversal
2 NO chromosomes per monoploid set	1 1 NO chromosome per set	
5S rDNA only in non-NO chromosomes	2 also in NO chromosomes	[2] only in non-NO chromosomes
absence of AT-rich heterochromatin	3 presence	
absence of GC-rich heterochromatin	4 presence	
satDNA CON1 present in low-copy number	5 amplification of CON1	[5] diminution or loss
satDNA CON2 present in low-copy number	6 amplification of CON2	[6] diminution or loss
satDNA COM1 present in low-copy number	7 amplification of COM1	
satDNA COM2 present in low-copy number	8 amplification of COM2	

Arrhenatherum studied to date (cf. Fig. 2a) and occurs also in some diploid species of the genera *Helictotrichon* and *Helictochloa* (Figs. 3, 4b). Within *Helictotrichon*, this character state is confined as a seemingly autapomorphic character to the species traditionally treated under genus *Pseudarrhenatherum* (*H. thorei* syn. *P. longifolium*, *H. pallens* syn. *P. pallens*; Figs. 3, 4c), whereas the other species of *Helictotrichon*, including its early-diverging *H. desertorum* and *H. sedenense* lineages, have two NORs (Figs. 3, 4a).

An occurrence of two NORs as plesiomorphic is less firmly established, however, for the large genus *Helictochloa* as delineated by Romero Zarco (2011), judging from its species studied to date (Fig. 3). This character state is characteristic of a central to southeastern European lineage composed of *A. compressa*, *A. aetolica* and *A. versicolor* (Fig. 3). The strictly western Mediterranean species *A. bromoides*, *A. albinervis*, *A. levis* and *A. marginata* share the occurrence of one NOR (Figs 3, 4b), however, they are not resolved as monophyletic by the current molecular phylogenetic data sets (cf. caption to Fig. 3), which show *A. bromoides* sister to the remainder of the genus. A transition from two to a single NOR thus has occurred in *Helictochloa* likely twofold in the west Mediterranean taxa, once in *A. bromoides* and a second time in the ancestor of the lineage of *A. albinerve*, *A. levis* and *A. marginata* (Fig. 3). The opposite pattern implying a transition from one, considering this as hypothetically original character state for *Helictochloa*, to two NORs would actually be equally parsimonious in Fig. 3. However, this pattern seems to be less likely, mainly due to the sister relation between *Helictochloa* and *Avenula*, the latter having again two NORs in the monoploid chromosome sets.

5S rDNA

The 5S rDNA is situated in the chromosomes of higher plants (ferns, seed plants) usually in loci separated from 18S–26S rDNA, although there are exceptions (Garcia et al. 2009). Both types of rDNA are required for ribosome biogenesis. 5S rDNA localizations, consisting of highly repeated gene and intergenic spacer units, are identifiable in chromosomes only by *in situ* hybridization. Among *Aveneae* studied to date for this character (Fig. 2), three patterns of chromosomal 5S rDNA disposition are encountered, which seem to represent a comparatively stable character of potential phylogenetic significance:

5S rDNA is localized in several genera and groups of closely related species exclusively in chromosomes without NORs (Fig. 3). Examples are the species of *Helictotrichon* except for *H. thorei* (syn. *P. longifolium* from traditional genus *Pseudarrhenatherum*), *Tricholemma jahandiezii* and perennial *Avena macrostachya*. The same pattern is characteristic of the diploid *Avena* species with the C genome (*A. clauda*, *A. eriantha*; cf. Fig. 3 with further details in the caption, Linares at al. 1996, Shelukhina et al. 2008b) and the species of *Agrostis*, *Deschampsia* and *Holcus* investigated to date (Winterfeld 2006, Winterfeld & Röser 2007a, b). The exception of traditional *Pseudarrhenatherum* is again interpreted as an autapomorphic character change (Fig. 3; cf. above).

Fig. 3: Most parsimonious reconstruction of chromosome character changes according to a simplified maximum parsimony dendrogram based on combined analyses of chloroplast *matK* and nuclear ITS1–5.8S gene–ITS2 and 5S rDNA sequences with *Bromus erectus* (tribe *Bromeae*) as outgroup. Symbols + and – denote presence or absence of a character. Missing data are indicated by a question mark. Chromosome characters partly from Rajhathy & Thomas (1974), Romero Zarco (1985b, c), Linares et al. (1992, 1996, 1998), Grebenstein et al. (1996), Yang et al. (1999), Irigoyen et al. (2001), Winterfeld (2006), Döring et al. (2007), Nikoloudakis et al. (2008), Shelukhina (2008a, b), Winterfeld et al. (2009a, b, 2012, 2014), Badaeva et al., (2010a, b). [a] Tentatively attached to this branch, though tested only for *Helictotrichon thorei*. [b] This average value results from suppression of NORs: 6*x* ACD *Avena sativa* (2*n* = 42) has six NO chromosomes, four of which belong to the D genome and two to the A genome (Linares et al. 1998; Irigoyen et al. 2002). [c] With additional minor sites of 18S–26S rDNA in ACD and AB polyploids (Fominaya et al. 1995; Linares et al. 1996; Yang et al. 1999; Hayasaki et al. 2001; Badaeva et al. 2010b). [d] This finding of Rajhathy & Thomas (1974) was corroborated later by silver staining (Fominaya et al. 1988a). [e] With additional minor, but nucleolar inactive sites of 18S–26S rDNA (Fominaya et al. 1988a). [f] Tentatively attached to this branch, though tested only for *Arrhenatherum elatius.*

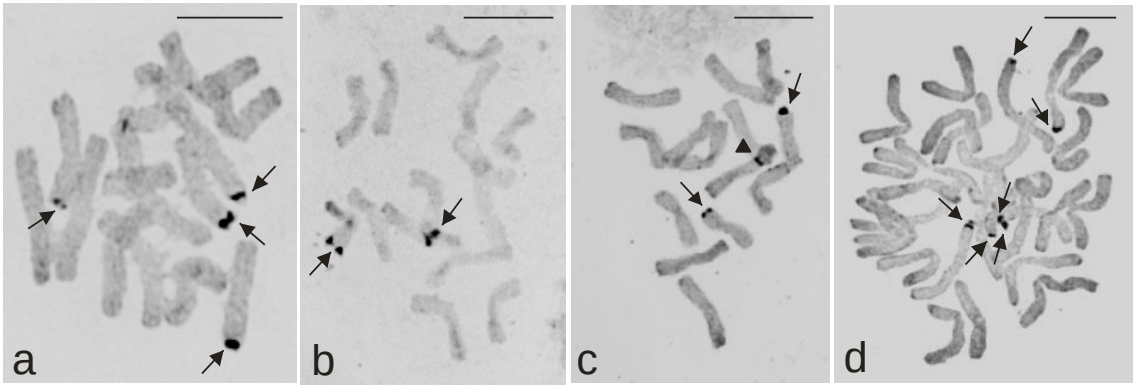


Fig. 4: Silver-impregnated preparations of metaphase chromosomes demonstrating nucleolar activity of the respective 18S–26S rDNA sites (arrowed). **a** *Helictotrichon desertorum* (2*n* = 14) with four NORs. **b** *Helictochloa albinervis* (2*n* = 14) with two NORs. **c** *Helictotrichon thorei* (2*n* = 14 + 1B) with two NORs. **d** Allopolyploid *Helictochloa cincinnata* (2*n* = 4*x* = 28) with six NORs. The arrowhead in c points to a supernumerary chromosome (B chromosome) carrying a NOR. Scale bars: 5 µm.

Other groups contain 5S rDNA sites simultaneously in chromosomes with and without NORs, for example, the species of *Helictochloa* except for *A. compressa*, *Avenula pubescens* or *Helictotrichon thorei* (syn. *Pseudarrhenatherum longifolium*) as an exception within the genus *Helictotrichon* (Figs. 2a–b, 3 and legends). This type of distribution is encountered also in other genera of *Aveneae*, such as the species of *Amphibromus*, *Ammophila*, *Trisetum* and *Koeleria* studied to date (Winterfeld 2006, Winterfeld & Röser 2007b). The occurrence of 5S rDNA loci exclusively in non-NO chromosomes found in *Helictochloa compressa* represents an obviously autapomorphic character state of this species within the whole genus *Helictochloa* (Fig. 3). This is based on the argument that 1) all other diploids of *Helictochloa* investigated to date share the presence of 5S rDNA simultaneously in non-NO and NO chromosomes (Fig. 3; Winterfeld & Röser 2007a, b, unpubl. data), 2) *A. compressa* diverges at one of the terminal nodes in the molecular phylogenetic tree of this genus and 3) chromosomes of *A. compressa* deviate from that of other diploids of the *Helictochloa* lineage also in further characters such as heterochromatin proportions and the positions of NORs. This points to overall strong structural rearrangements in the *A. compressa* genome relative to its congeners (Winterfeld & Röser 2007a). Although not traceable in detail, we assume that all 5S rDNA sites were translocated to non-NO chromosomes only secondarily in *A. compressa*.

The third pattern of rDNA arrangement, i.e., localization of 5S rDNA exclusively in NO chromosomes has not been found to date in perennial *Aveneae*, but it was recently detected in the A genome species of *Avena* (Shelukhina et al. 2008a, cf. Linares et al. 1996) and additionally appears to represent a consistent character of this assemblage (*A. canariensis*, *A. damascaena*, *A. longiglumis*, *A. strigosa*). In comparison with the other 5S rDNA localizations found in *Aveneae*, the unique arrangement in the A genome species of *Avena* thus is highly significant as a phylogenetically potentially synapomorphic character of the A genome species group in *Avena*.

The original character state of 5S rDNA chromosomal localization in the chromosomes of *Aveneae* (character 2 in Fig. 3) is difficult to reconstruct from the limited set of taxa studied (Winterfeld et al. 2009a). It is most likely absence of 5S rDNA from NO chromosomes, followed by an apomorphic change in the common ancestor of the *Helictochloa*/*Avenula* lineage and a reversal in only a single species of *Helictochloa*, i.e., *A. compressa* (Fig. 3). The plesiomorphic character state persisted more widely in the second major lineage, i.e., in *Tricholemma jahandiezii*, *Avena macrostachya*, the C genome diploid species of annual *Avena* and most of *Helictotrichon* including its early-diverging species *H. desertorum* and *H. sedenense* (Fig. 3). 5S rDNA was translocated into NO chromosomes in this major lineage most likely three times independently, namely in *Arrhenatherum* (cf. Fig. 2b) and in *Helictotrichon thorei*, both of which still have 5S rDNA bands in non-NO chromosomes (Fig. 3 and legend). In the A genome diploids of *Avena* all non-NO chromosomes are entirely devoid of 5S rDNA

bands, phylogenetically interpreted here as a secondary translocation of all 5S rDNA bands entirely into NO chromosomes characteristic of this lineage.

Translocation of 5S rDNA bands from non-NO to NO chromosomes was not linked with the loss of one NOR per monoploid chromosome set encountered in *Arrhenatherum* and *Helictotrichon thorei*, respectively and occurred in the A genome diploids of *Avena* without change of the likely plesiomorphic number of NORs, which is still preserved in these species (two per monoploid chromosome set).

Variability in chromosomal loci of 5S rDNA

The number of 5S rDNA sites does not follow a general pattern, judging from the comparatively few taxa of *Aveneae* studied to date for this character. The number of sites is frequently four in monoploid chromosome sets of $x = 7$, more rarely two. Slightly higher numbers are encountered occasionally in single specimens of otherwise 'orthodox' species and are seemingly caused by sporadic chromosome mutations reported, e.g., in *Helictotrichon convolutum* (Winterfeld 2006).

The number of 5S rDNA sites thus is in fundamental agreement with the number of two or four NORs in the respective chromosome sets. However, there is no arithmetically absolutely strict correspondence in the number of both rDNA sites:

1) Species with two NORs per monoploid chromosome set usually have also two 5S rDNA sites in the chromosomes. This is seen in the C and A genome diploid species of *Avena* (Linares et al. 1996; Shelukhina et al. 2008a, b), in *Tricholemma jahandiezii*, in most species of *Helictotrichon* except for *H. desertorum*, in *Avenula pubescens* and in the lineage of species with two NORs within genus *Helictochloa* (*A. aetolica*, *A. versicolor*; Fig. 3) except for *A. compressa*. Among these two-NOR species, *Helictotrichon desertorum* has only a single 5S rDNA site, *H. setaceum* has two or one. *Helictochloa compressa* deviates by the exceptional occurrence of altogether three 5S rDNA sites (Winterfeld 2006, Winterfeld & Röser 2007b), which is possibly linked with other chromosomal re-organizations encountered in this latter species (see above).

2) Chromosome sets with a single NOR have either one (*Helictochloa albinervis*, *A. levis*, *A. marginata*; cf. Winterfeld 2006, Winterfeld & Röser 2007b) or two 5S rDNA sites (e.g., *Phalaris coerulescens*; Li et al. 1997). In *Helictochloa bromoides* (Fig. 3) and *Helictotrichon thorei* unstably one or two sites are encountered (Winterfeld 2006). Thus there is no firm correlation between the number of both rDNA sites in the single-NOR species.

3) Most high polyploids, but already some tetraploids do not show any correlation between the number of NORs and 5S rDNA sites of the chromosome sets they contain. Examples would be the autotetrapolyploids *Avena macrostachya* with altogether eight NORs (~ two per chromosome set) and 12 5S rDNA sites and *Arrhenatherum elatius* with only four NORs (~ one per set; Fig. 2a) and altogether 14 5S rDNA sites (Fig. 2b). Chromosomal localizations of 'additional' 5S rDNA sites in such polyploids rest on mostly typical chromosome mutations such as duplication of loci and pericentric inversions including break-points situated within 5S rDNA bands (Winterfeld & Röser 2007b). These changes at the cytogenetic level appear to belong to the processes discussed in terms of genomic stabilization and chromosomal 'adjustment' of polyploids (cf. Wendel 2000).

Other polyploids of *Aveneae* do not show major restructuring of their parental genomes on the new polyploid background. This is seen even in allopolyploids, facing the 'genomic shock' of simultaneously hybridity and polyploidy. A striking example is allopolyploid 4*x Helictochloa cincinnata* with altogether six NORs (Fig. 4d), two of which stem from *A. bromoides* or a genomically close relative and four from a species of the two-NOR branch of this genus (Fig. 3), but not identical with any of its known extant species (see below). The number of 5S rDNA sites in *A. cincinnata* accessions is eight to nine, thus reflecting an overall conservation of 5S rDNA sites of its parental genomes with most likely two 5S rDNA sites in each of it. Neither autopolyploidy nor allopolyploidy thus are necessarily linked with changes in chromosomal localization or multiplication of 5S rDNA sites (cf. Winterfeld et al. 2009b for further discussion).

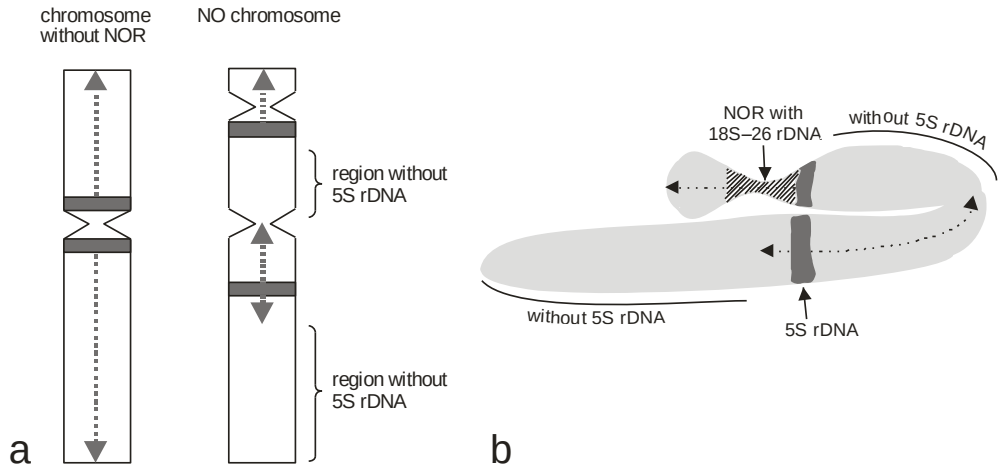


Fig. 5: Variability and constraints in the chromosomal localizations of 5S rDNA relative to 18S–26S rDNA bands (NORs). **a** 5S rDNA bands are localized in chromosomes without NORs in any position between the centromere and the telomeres (left), but occur in NO chromosomes only adjacent to the 18S–26S rDNA sites or distally to it, or in the opposite chromosome arm in proximal chromosome regions (right). In the chromosome arm with the 18S–26S rDNA, the chromosome regions proximal to it are devoid of 5S rDNA. If 5S rDNA occurs in the opposite arm, the chromosome segment distal the distance between the centromere and the 18S–26S rDNA is devoid of 5S rDNA. **b** Schematic representation of anaphase movement in a chromosome containing simultaneously a NOR and 5S rDNA. The chromosome regions containing 5S rDNA bands (dotted lines) are expected to be spatially closer to the nucleolus formed in the following interphase than the chromosome regions in which 5S rDNA is absent. Modified from Winterfeld & Röser (2007b).

Constraints in the Arrangement of 5S rDNA

The localization of 5S rDNA sites is highly variable in chromosomes without NORs, their position ranging then from near telomeric to near centromeric, though with a prevalence at intercalary sites of either the long or the short chromosome arm (Fig. 5a). 5S rDNA sites located within NO chromosomes, by contrast, are much less varied in chromosomal disposition and are confined to special chromosomal regions. Their position depends on the respective position of the NOR within the same chromosome (Fig. 5a and legend; for further discussion see Winterfeld & Röser 2007b). Due to this constraint, interpreted as based on interphase nucleus organization (Fig. 5b and legend), different types of arrangement of 5S rDNA in the chromosomes with NORs are not *a priori* a reliable taxonomic character, because most 5S rDNA positions found in NO chromosomes of *Aveneae* can be converted into each other by well-known and frequently occurring chromosome mutations as inversions by either involving or excluding the 18S–26S rDNA sites (see Fig. 2b; Winterfeld & Röser 2007b, Winterfeld et al. 2009a). The general pattern of spatial dependence of 5S rDNA from 18S–26S rDNA sites in NO chromosomes is kept also in polyploids of *Aveneae* despite otherwise structural rearrangement in the chromosomes (Winterfeld 2006 and unpubl. data), thus additionally excluding a general taxonomic significance of the particular 5S rDNA localizations in NO chromosomes.

5S rDNA is sometimes colocalized in *Aveneae* with either 18S–26S rDNA (Fig. 2a–b) or, more frequently, with satDNA CON2 (Fig. 2e–f). The first pattern, we assume, has nothing to do with an insertion of the 5S rDNA gene into 18S–5.8S–26S repeat stretches (see Garcia et al. 2009) and represents only an intermingled occurrence of both repetitive DNAs at the same chromosome site. The second pattern of colocalization of 5S rDNA with bands of satDNA CON2 is frequent in species of genus *Helictotrichon*, but is encountered also, for example, in *Koeleria cristata* or *Cynosurus cristatus*. In each of the latter species all CON2 bands are even localized in 5S rDNA sites (Winterfeld 2006). However, satDNA CON2 did not originate from 5S rDNA such as found for satDNAs of rice (Dong et al. 1998), because both sequences have an homology of only c. 50% (Winterfeld & Röser 2007a). It is likely that CON2 repeats are interspersed in all of these instances within the 5S rDNA, but this needs to be tested experimentally.

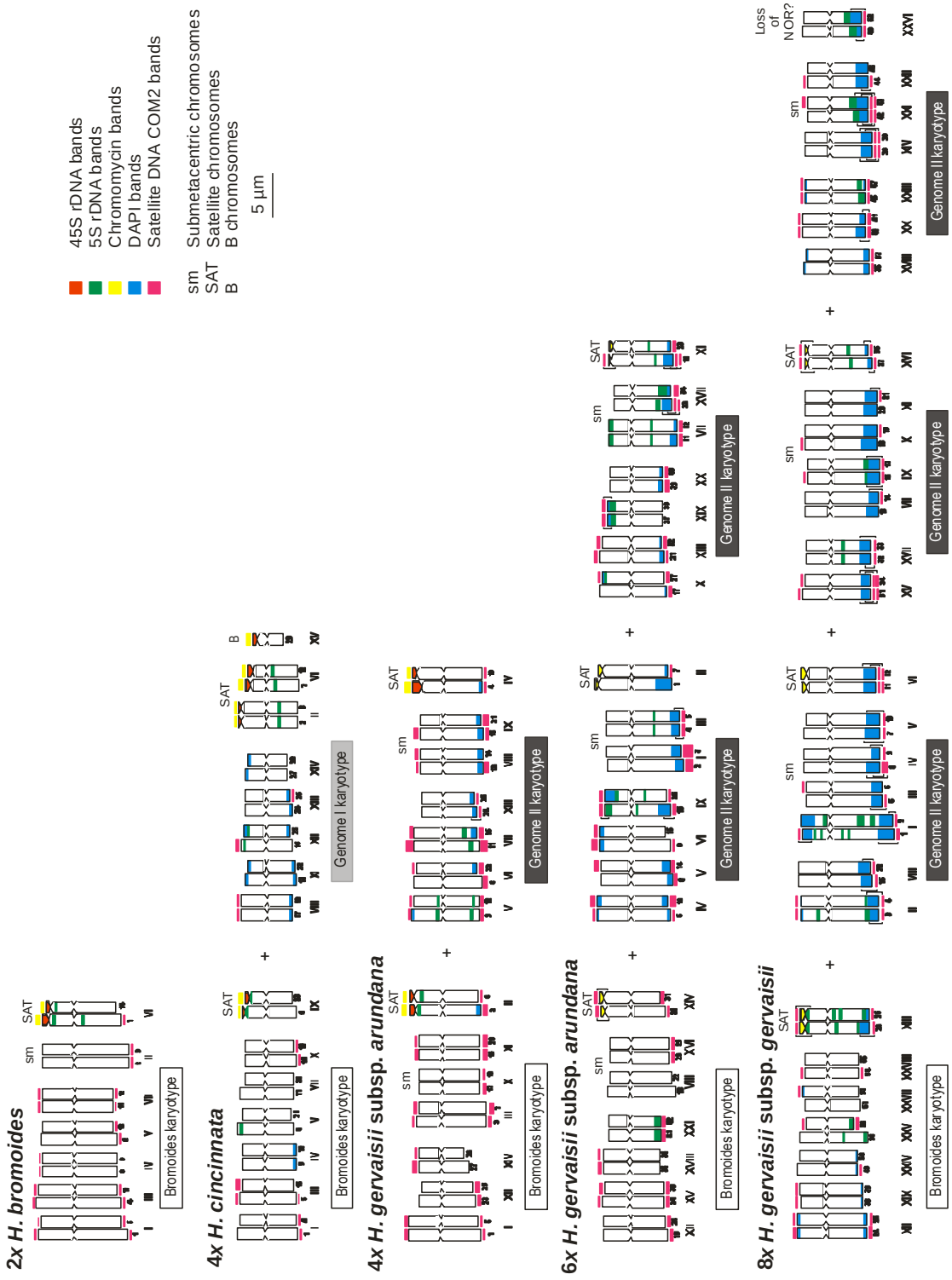


Fig. 6: Chromosome idiograms of 2*x*–8*x* taxa of the *Helictochloa bromoides* polyploid complex arranged as sets of two monoploid genomes, with localization of 18S–26S, 5S rDNAs and satDNA COM2 bands analyzed by FISH, and staining with the AT or GC base pair-specific fluorescent dyes DAPI and chromomycin, respectively. Bands arranged above or below the chromosomes are located subtelomeric, unless an intercalary position is indicated by a square bracket at the side of a chromosome. Modified from Winterfeld et al. (2009b).

Speciation and Polyploidy

Grasses rank among the families of angiosperms with generally high rates of polyploidy. Already in the pre-genomic era the proportion of polyploids was estimated as higher than 80% (de Wet 1987). Origin of polyploids, derived either via auto- or allopolyploidy, is cytogenetically usually much easier to trace in annual grasses than in perennials. This is due to the fact that annuals tend to have more strongly differentiated chromosomes (size, shape, etc.) within their chromosome sets (e.g., *Avena*, *Aira*; Rajhathy & Thomas 1974, Heinrichsmeier 2005), facilitating also to identify such chromosome sets in polyploids. Chromosomes of perennials mostly look like each other in conventional staining methods, making it difficult to trace the origin of their polyploids.

A combined molecular cytogenetic and DNA sequence-based approach for a species complex of Mediterranean perennials of genus *Helictochloa* (*A. bromoides* species group comprising *A. bromoides* s. str. and at least six further species) with comparatively weak morphological differences between the individual species reveals that all polyploids studied to date are allopolyploids, i.e. they contain different genomes in combination. Notably, following the broad species concept for these grasses employed till the 1980s with only a single collective species acknowledged, viz. *A. bromoides* s. lat., polyploidy in this species complex would have erroneously become interpreted a 'typical instance' of infraspecific autopolyploidy.

The *A. bromoides* species group contains only one extant diploid species, i.e. widespread western Mediterranean *A. bromoides* s. str., and a high number of polyploids up to 18*x* with partly narrow geographical distribution in the Mediterranean. The most widespread polyploid is 4*x A. cincinnata* distributed in the southern Mediterranean (N Africa and Sicily). Its karyotype points to allopolyploidy as seen already from the presence of six NO chromosomes (Fig. 4d), which rests on a combination of chromosomes sets with one and two NORs, which are known to occur within this genus (see above). Chromosome structure investigated by further cytogenetic markers reveals that the genome of *A. bromoides* or a highly similar one is present in *A. cincinnata*, whereas the second, termed Genome I in Fig. 6, is not known from other diploids of *Helictochloa* studied to date (Winterfeld et al. 2009b).

Sequence analyses of cloned ITS1–5.8S–ITS2 rDNA repeat units corroborate the presence of the *A. bromoides* repeat type (sequence CIN3 in Fig. 7) in 4*x A. cincinnata*, together with a prevailing repeat hitherto not known from diploids (the other repeats with acronym CIN in Fig. 7) stemming from the two-NOR genome present in *A. cincinnata* (Fig. 6). Other polyploids of the *A. bromoides* species group studied so far, i.e., 4*x*–8*x* plants of *A. gervaisii* of the western Mediterranean, show the presence of a further genome (Genome II in Fig. 6) carrying an ITS1–5.8S–ITS2 rDNA repeat type (with acronyms GEA and GER in Fig. 7) different from that encountered in *A. cincinnata*. The typical Bromoides repeat type is not preserved in any of the cytotypes of polyploid *H. gervaisii* examined, but there are repeats with mosaic character in combining sequence motifs of the Bromoides and Genome II repeats (e.g., repeats GAR5, GER4, GER5 in Fig. 7). There are signs of ongoing sequence homogenization between the different parental genomes in these allopolyploid grasses (see Winterfeld et al. 2009b for details), comparable to examples of allopolyploids studied in other plant families (e.g., *Gossypium*, *Nicotiana*, *Tragopogon*; Wendel et al. 1995; Lim et al. 2004, 2008; Kovařík et al. 2005).

One of the most surprising findings, however, is the strong physical conservation of NO sites from the parental genomes in the chromosomes of these allopolyploids of *Helictochloa* despite strong changes in the rDNA repeats they harbor. Second, change at the sequence level in this repetitive ITS1–5.8S–ITS2 rDNA is not exclusively due to genetic mechanisms as gene conversion or unequal crossing over, but is somewhat 'patchy' by involving stepwise molecular exchanges (see Winterfeld et al. 2009b, 2012, 2014 for details).

Unorthodox Patterns?

Deschampsia cespitosa (2*n* = 26) ranks among the species with a monoploid chromosome set of apparently either *x* = 13 or *x* = 7. The first would be exceptional (although likewise found in the related monotypic genus *Scribneria*; see Schneider et al. 2012) within *Aveneae*, which usually

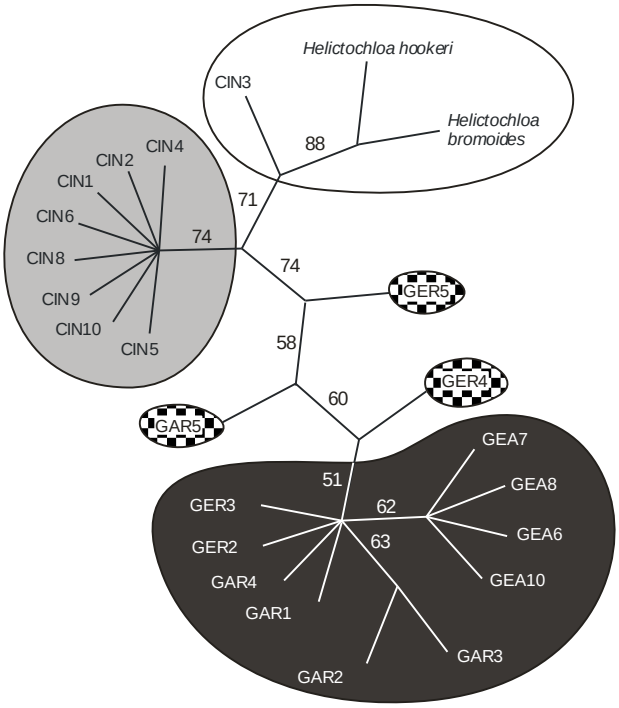


Fig. 7: Unrooted maximum parsimony dendrogram of cloned nuclear rDNA ITS1–5.8S gene–ITS2 sequences in 2*x Helictochloa bromoides*, 4*x A. cincinnata* (CIN), 4*x A. gervaisii* subsp. *arundana* (GAR), 6*x* subsp. *arundana* (GEA), 8*x* subsp. *gervaisii* (GER) and a sequence of *A. hookeri*. Clusters with the sequence type of the Bromoides genome are unshaded, clusters of sequences from Genome I appear in light and that from Genome II in dark shading. Recombinant 'mosaic' or 'chimerical' sequences in *A. gervaisii* subsp. *gervaisii* and subsp. *arundana* with sequence motifs of the Bromoides sequence type have chequer-board pattern. Bootstrap values above 50 are indicated. Modified from Winterfeld et al. (2009b).

have $x = 7$, whereas it is rather typical of early-diverging tribes within the entire subfamily *Pooideae*. The latter would imply polyploidy with a dysploid chromosome number change either before or after polyploidization. Such polyploid-dysploid origin of *D. cespitosa* is very likely, based on the uncommon occurrence of 18S–26S rDNA sites close to the centromere in some of its chromosomes (Fig. 2c) and additionally the bands of AT-rich heterochromation found in intercalary chromosome positions (Fig. 2d), both pointing to Robertsonian chromosome fusions. The chromosome complement of polyploid-dysploid *D. cespitosa* thus seems to be derived from a monoploid set of originally $x = 7$ with comparatively large chromosomes typical of the *Aveneae* lineage.

Danthoniastrum compactum, sometimes considered as belonging to tribe *Aveneae* (e.g., Clayton & Renvoize 1986), also has a comparable high chromosome number of $2n = 24$ (Fig. 2g–h; Winterfeld 2006), though erroneously reported before as $2n = 14$ (Kožuharov & Petrova 1991). The minute chromosome sizes in *D. compactum*, together with the presence of only a single pair of NO chromosomes in the diploid chromosome complement point to a misplacement of this genus in tribe *Aveneae* and its likely affiliation with early-diverging lineages of the entire subfamily *Pooideae*. This has been corroborated by molecular phylogenetics, showing *Danthoniastrum* nested along with other genera among within the the earliest branches of this subfamily, much in contrast to *Deschampsia* (Döring et al. 2007; Schneider et al. 2009, 2011).

Outlook

Combining DNA-based molecular phylogenetic approaches with classical and molecular cytogenetics has enabled many new insights on grass genome evolution, but studies of this kind are in general quite rare, even within major crop plant genera. The preliminary results presented on some *Aveneae* point to rather unexpected genomic and chromosomal relations between genera or groups of species previously considered as rather disparate. This is especially well visible in genus *Avena* (oat) whose closest extant relatives are becoming increasingly better identified. Ongoing progress in identifying the major and minor lineages of grasses (subfamilies, tribes, groups of genera, etc.) based on molecular phylogenetics complement previous and exclusively morphology-based treatments. It has strongly impacted the view on chromosome number evolution in grasses (Hilu 2004), but the molecular cytogenetic background of such change is still incompletely resolved even within comparatively small

taxonomic groups such as the few genera of *Aveneae* discussed here. Gain, amplification, change, or loss of sequences, together with a capability to restructure the karyotype within comparatively short evolutionary time spans belong to the main features seen in the chromosomal change within *Aveneae*, yet their role is far from being understood in the complex evolution of grass genomes.

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References

Albers, F. 1980: Vergleichende Karyologie der Gräser-Subtriben Aristaveninae und Airinae (Poaceae-Aveneae). Plant Systematics and Evolution **136**: 137–167.

Alix, K., Baurens, F.-C., Paulet, F., Glaszmann, J.-C. & d'Hont, A. 1998: Isolation and characterization of a satellite DNA family in the *Saccharum* complex. Genome **41**: 854–864.

Badaeva, E. D., Shelukhina, O. Y., Goryunova, S. V., Loskutov, I. G. & Pukhalskiy, V. A. 2010a: Phylogenetic relationships of tetraploid AB-genome *Avena* species evaluated by means of cytogenetic (C-banding and FISH) and RAPD analyses. Journal of Botany (2010): Article ID 742307. doi:10.1155/2010/742307.

Badaeva, E. D., Shelukhina, O. Y., Diederichsen, A., Loskutov, I. G. & Pukhalskiy, V. A. 2010b: Comparative cytogenetic analysis of *Avena macrostachya* and diploid C-genome *Avena* species. Genome **53**: 125–137.

Baum, B. R. 1968: Delimitation of the genus *Avena* (Gramineae). Canadian Journal of Botany **46**: 121–132.

Baum, B. R. 1977: Oats: wild and cultivated. A monograph of the genus *Avena* L. (Poaceae). Canada Department of Agriculture, Ottawa.

Baum, B. R. & Fedak, G. 1985a: *Avena atlantica*, a new diploid species of the oat genus from Morocco. Canadian Journal of Botany **63**: 1057–1060.

Baum, B. R. & Fedak, G. 1985b: A new tetraploid species of *Avena* discovered in Morocco. Canadian Journal of Botany **63**: 1379–1385.

Baum, B. R. & Rajhathy, T. 1976: A study of *Avena macrostachya*. Canadian Journal of Botany **54**: 2434–2439.

Briggs, D. & Walters, S. M. 1997: Plant Variation and Evolution. Third edition. Cambridge University Press, Cambridge.

Chen, Q. & Armstrong, K. 1994: Genomic *in situ* hybridization in *Avena sativa*. Genome **37**: 607–612.

Clayton, W. D. & Renvoize, S. A. 1986: Genera Graminum. Grasses of the World. HMSO, London.

Conert, H. J. 1979–1998: Poaceae (Echte Gräser oder Süßgräser). Pp. 1–898. In Conert, H. J., Jäger, E. J. Schultze-Motel, W., Wagenitz, G. & Weber, H. E. (Eds.): Illustrierte Flora von Mitteleuropa. Third edition. Vol. 1, part 3. Parey Buchverlag, Berlin.

Conti, F., Abbate, G., Alessandrini, A. & Blasi, C. 2005: An annotated checklist of the Italian vascular flora. Ministerio dell'Ambiente, Roma.

Csink, A. & Henikoff, S. 1998: Something from nothing: the evolution and utility of satellite repeats. Trends in Genetics **14**: 200–204.

de Wet, J. M. J. 1987: Hybridization and polyploidy in the Poaceae. Pp. 188–194. In Soderstrom, T. R., Hilu, K. W., Campbell, C. S. & Barkworth, M. E. (Eds.): Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

Dong, F., Miller, J. T., Jackson, S. A., Wang, G. L., Ronald, P. C. & Jiang, J. M. 1998: Rice (*Oryza sativa*) centromeric regions consist of complex DNA. Proceedings of the National Academy of Sciences of the United States of America **95**: 8135–8140.

Döring, E. 2009: Molekulare Phylogenie der Hafer-Gräser (Poaceae: Pooideae: Aveneae). Ph. D. thesis, University Halle-Wittenberg, Halle. http://digital.bibliothek.uni-halle.de/hs/content/titleinfo/177571.

Döring, E., Schneider, J., Hilu, K. W. & Röser, M. 2007: Phylogenetic relationships in the Aveneae/Poeae complex (Pooideae, Poaceae). Kew Bulletin **62**: 407–424.

FAO 2014: FAO Statistics division. http://faostat3.fao.org/faostat-gateway/go/to/home/E.

Fominaya, A., Hueros, G., Loarce, Y. & Ferrer, E. 1995: Chromosomal distribution of a repeated DNA sequence from C genome heterochromatin and the identification of a new ribosomal DNA locus in the *Avena* genus. Genome **38**: 548–557.

Fominaya, A., Vega, C., & Ferrer, E. 1988a: Giemsa C-banded karyotypes of *Avena* species. Genome **30**: 627–632.

Fominaya, A., Vega, C., & Ferrer, E. 1988b: C-banding and nucleolar activity of tetraploid *Avena* species. Genome **30**: 633–638.

Fu, Y.-B. & Williams, D. J. 2008: AFLP variation in 25 *Avena* species. Theoretical and Applied Genetics **117**: 333–342.

Garcia, S., Lim, K. Y., Chester, M., Garnatje, T., Pellicer, J., Vallès, J., Leitch, A. R. & Kovařík, A. 2009: Linkage of 35S and 5S rRNA genes in *Artemisia* (family Asteraceae): first evidence from angiosperms. Chromosoma **118**: 85–97.

Garcia-Suárez, R., Alonso-Blanco, C., Fernández-Carvajal, M. C., Fernández-Prieto, J. A., Roca, A. & Giraldez, R. 1997a: Diversity and systematics of *Deschampsia sensu lato* (Poaceae), inferred from karyotypes, protein electrophoresis, total genomic DNA hybridization and chloroplast DNA analysis. Plant Systematics and Evolution **205**: 99–110.

Garcia-Suárez, R., Alvarez, E., Fernández-Prieto, J. A., Fernández-Carvajal, M. C. & Giraldez, R. 1997b: Diversity of *Avenella* Drejer (Gramineae) inferred from micromorphology, cytogenetic analysis, isozyme assays and *in situ* hybridization. Botanical Journal of the Linnean Society **125**: 309–318.

Grebenstein, B. 1992: Anatomische, karyologische und molekularbiologische Untersuchungen an mediterranen, nordanatolischen und kaukasischen Sippen der Gattung *Helictotrichon* Besser ex Schultes and Schultes (Poaceae). Ph. D. thesis, University Tübingen, Tübingen.

Grebenstein, B., Grebenstein, O., Sauer, W. & Hemleben, V. 1995: Characterization of a highly repeated DNA component of perennial oats (*Helictotrichon*, Poaceae) with sequence similarity to a A-genome-specific satellite DNA of rice (*Oryza*). Theoretical and Applied Genetics **90**: 1101–1105.

Grebenstein, B., Grebenstein, O., Sauer, W. & Hemleben, V. 1996: Distribution and complex organization of satellite DNA sequences in Aveneae species. Genome **39**: 1045–1050.

Grebenstein, B., Röser, M., Sauer, W. & Hemleben, V. 1998: Molecular phylogenetic relationships in Aveneae (Poaceae) species and other grasses as inferred from ITS1 and ITS2 rDNA sequences. Plant Systematics and Evolution **213**: 133–150.

Greilhuber, J. 1995: Chromosomes of the monocotyledons (general aspects). Pp. 379–414. In Rudall, P. J. Cribb, P. J., Cutler, D. F. & Humphries, C. J. (Eds.): Monocotyledons: Systematics and Evolution. Kew, Royal Botanic Gardens.

Greilhuber, J. & Ehrendorfer, F. 1988: Karyological approaches to plant taxonomy. Pp. 289–297. In ISI Atlas of Science: Animal and Plant Sciences. Vol. 1. Institute for Scientific Information, Philadelphia.

Hayasaki, M., Morikawa, T. & Leggett, J. M. 2001: Intraspecific variation of 18S–5.8S–26S rDNA sites revealed by FISH and RFLP in wild oat, *Avena agadiriana*. Genes & Genetic Systems **76**: 9–14.

Heinrichsmeier, N. 2005: Phylogenetische Untersuchungen an Arten der Gräser-Gattung *Aira* L. anhand karyologischer und molekularbiologischer Methoden sowie *in situ*-Hybridisierungen. Ph. D. thesis, University Münster, Münster. http://nbn-resolving.de/urn:nbn:de:hbz:6-34649489497.

Hemleben, V., Kovařík, A., Torres-Ruiz, R. A., Volkov, R. A. & Beridze, T. 2007: Plant highly repeated satellite DNA: molecular evolution, distribution and use for identification of hybrids. Systematics and Biodiversity **5**: 277–289.

Hilu, K. W. 2004: Phylogenetics and chromosomal evolution in the Poaceae (grasses). Australian Journal of Botany **52**: 13–22.

Hilu, K. W., Alice, L. A. & Liang, H. 1999: Phylogeny of *Poaceae* inferred from *matK* sequences. Annals of the Missouri Botanical Garden **86**: 835–851.

Holub, J. 1958: Bemerkungen zur Taxonomie der Gattung *Helictotrichon* Bess. Pp. 101–133. In Klaštersky, I. (Ed.): Philipp Maxmilian Opiz und seine Bedeutung für die Pflanzentaxonomie. Tschechoslowakische Akademie der Wissenschaften, Praha.

Holub, J. 1962: Ein Beitrag zur Abgrenzung der Gattungen in der Tribus Aveneae, die Gattung *Avenochloa* Holub. Acta Horti Botanici Pragensis (1962): 75–86.

Holub, J. 1976: A newly adopted restriction of illegitimity in generic names and its consequence for *Avenochloa* Holub 1962. Folia Geobotanica et Phytotaxonomica **11**: 281–300.

Holub, J. 1980: *Helictotrichon* Besser; *Helictochloa* (Dumort.) Dumort.; *Danthoniastrum* (J. Holub) J. Holub; *Arrhenatherum* (Beauv.) Beauv.; *Pseudarrhenatherum* Rouy. Pp. 208–217. In Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (Eds.): Flora Europaea. Vol. 5. Cambridge University Press, Cambridge.

IPCN = Index to plant chromosome numbers 2014: http://www.tropicos.org/Project/IPCN.

Irigoyen, M. L., Linares, C., Ferrer, E. & Fominaya, A. 2002: Fluorescence *in situ* hybridization mapping of *Avena sativa* L. Cv. SunII and its monosomic lines using cloned repetitive DNA sequences. Genome **45**: 1230–1237.

Irigoyen, M. L., Loarce, Y., Linares, C. & Ferrer, E. 2001: Discrimination of the closely related A and B genomes in AABB tetraploid species of *Avena*. Theoretical and Applied Genetics **103**: 1160–1166.

Jellen, E. N., Gill, B. S. & Cox, T. S. 1994: Genomic *in situ* hybridization differentiates between A/D- and C-genome chromatin and detects intergenomic translocations in polyploid oat species (genus *Avena*). Genome **37**: 613–618.

Jellen, E. N., Phillips, R. L. & Rines, H. W. 1993a: C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome **36**: 1129–1137.

Jellen, E. N., Rooney, W. L., Phillips, R. L., & Rines, H. W. 1993b: Characterization of the hexaploid oat *Avena byzantina* C. Koch cv. Kanota monosomic series using C-banding and RFLPs. Genome **36**: 962–970.

Jones, K. 1977: The role of Robertsonian change in karyotype evolution in higher plants. Chromosomes Today **6**: 121–129.

Jones, K. 1978: Aspects of chromosome evolution in higher plants. Pp. 119–194: In Woolhouse, H. W. (Ed.): Advances in Botanical Research. Vol. 6. Academic Press, London.

Katsiotis, A., Hagidimitriou, M. & Heslop-Harrison, J. S. 1997: The close relationship between the A and B genomes in *Avena* L. (Poaceae) determined by molecular cytogenetic analysis of total genomic, tandemly and dispersed repetitive DNA sequences. Annals of Botany **79**: 103–109.

Katsiotis, A., Loukas, M. & Heslop-Harrison, J. S. 2000: Repetitive DNA, genome and species relationships in *Avena* and *Arrhenatherum* (Poaceae). Annals of Botany **86**: 1135–1142.

Katsiotis, A., Schmidt, T. & Heslop-Harrison, J. S. 1996: Chromosomal and genomic organization of TY1-copia-like retrotransponson sequences in the genus *Avena*. Genome **39**: 410–417.

Kenton, A., Parokonny, A. S., Bennett, S. T. & Bennett, M. D. 1993: Does genome organization influence speciation? A reappraisal of karyotype studies in evolutionary biology. Pp. 189–206. In Lees, D. R. & Edwards, D. (Eds.): Evolutionary Patterns and Processes. Academic Press, London.

Kim, E. S., Bolsheva, N. L., Samatadze, T. E., Nosov, N. N., Nosova, I. V., Zelenin, A. V., Punina, A. A., Muravenko, O. V. & Rodionov, A. V. 2009: The unique genome of two-chromosome grasses *Zingeria* and *Colpodium*, its origin, and evolution. Russian Journal of Genetics **45**: 1329–1337.

Kotseruba, V., Gernand, D., Meister, A. & Houben, A. 2003: Uniparental loss of ribosomal DNA in the allotetraploid grass *Zingeria trichopoda* (2*n* = 8). Genome **46**: 156–163.

Kotseruba, V., Pistrick, K., Blattner, F. R., Kumke, K., Weiss, O., Rutten, T., Fuchs, J., Endo, T., Nasuda, S., Ghukasyan, A. & Houben, A. 2010: The evolution of the hexaploid grass *Zingeria kochii* (Mez) Tzvel. (2*n* = 12) was accompanied by complex hybridization and uniparental loss of ribosomal DNA. Molecular Phylogenetics and Evolution **56**: 146–155.

Koukalova, B., Moraes, A. P. & Renny-Byfield, S. 2010: Fall and rise of satellite repeats in allopolyploids of *Nicotiana* over c. 5 million years. New Phytologist **186**: 148–160.

Kovařík, A., Pires, J. C., Leitch, A. R., Lim, K. Y., Sherwood, A. M., Matyášek, R., Rocca, J., Soltis, D. E. & Soltis, P. S. 2005: Rapid concerted evolution of nuclear ribosomal DNA in two *Tragopogon* allopolyploids of recent and recurrent origin. Genetics **169**: 931–944.

Kožuharov, S. I. & Petrova, A. V. 1991: Chromosome numbers of Bulgarian angiosperms. Fitologiya **39**: 72–77.

Ladizinsky, G. 1998: A new species of *Avena* from Sicily, possibly the tetraploid progenitor of hexaploid oats. Genetic Resources and Crop Evolution **45**: 263–269

Lange, D. 1995: Untersuchungen zur Systematik und Taxonomie der Gattung *Helictotrichon* Besser ex J. A. Schultes and J. H. Schultes (Poaceae) in Südosteuropa und Vorderasien. Bibliotheca Botanica **144**: 1–238.

Leggett, J. M. 1990: A new triploid hybrid between *Avena eriantha* and *A. macrostachya*. Cereal Research Communications **18**: 97–101.

Leggett, J. M. 1991: Further hybrids involving the perennial autotetraploid oat *Avena macrostachya*. Genome **35**: 273–275.

Leggett, J. M. & Markhand, G. S. 1995: The genomic structure of *Avena* revealed by GISH. Pp. 133–139. In Brandham, P. E. & Bennett, M. D. (Eds.): Kew Chromosome Conference IV. Royal Botanic Gardens, Kew.

Leggett, J. M. & Thomas, H. 1995: Oat evolution and cytogenetics. Pp. 121–149. In Welch, W. (Ed.): The Oat Crop: Production and Utilization. Chapman and Hall, London.

Leitch, I. J., Soltis, D. E., Soltis, P. S., & Bennett, M. D. 2005: Evolution of DNA amounts across land plants (Embryophyta). Annals of Botany **95**: 207–217.

Levitsky, G. A. 1931: The karyotype in systematics. Bulletin of Applied Botany, of Genetics and Plant Breeding **27**: 182–240.

Lieberei, R. & Reisdorff, C. 2012: Nutzpflanzenkunde. Eighth edition. Georg Thieme Verlag, Stuttgart, New York.

Li, C.-D., Rossnagel, B. G. & Scoles, G. J. 2000: Tracing the phylogeny of the hexaploid oat *Avena sativa* with satellite DNAs. Crop Science **40**: 1755–1763.

Li, X., Guo, R., Pedersen, C., Hayman, D. & Langridge, P. 1997: Physical localization of rRNA genes by two-colour fluorescent *in-situ* hybridization and sequence analysis of the 5S rRNA gene in *Phalaris coerulescens*. Hereditas **126**: 289–294.

Lim, K. Y., Matyášek, R., Kovařík, A. & Leitch, A. 2004: Genome evolution in allotetraploid *Nicotiana*. Biological Journal of the Linnean Society **82**: 599–606.

Lim, K. Y., Soltis, D. E., Soltis, P. S., Tate, J., Matyášek, R., Srubarova, H., Kovařík, A., Pires, C., Xiong, Z. & Leitch, A. R. 2008: Rapid chromosome evolution in recently formed polyploids in *Tragopogon* (Asteraceae). PLoS ONE **3**(10), e3353.

Linares, C., Vega, C., Ferrer, E. & Fominaya, A. 1992: Identification of C-banded chromosomes in meiosis and the analysis of nucleolar activity in *Avena byzantina* C. Koch cv. 'Kanota'. Theoretical and Applied Genetics **83**: 650–654.

Linares, C., Ferrer, E. & Fominaya, A. 1998: Discrimination of the closely related A and D genomes of the hexaploid oat *Avena sativa* L. Proceedings of the National Academy of Sciences of the United States of America **95**: 12450–12455.

Linares, C., González, J., Ferrer, E. & Fominaya, A. 1996: The use of double-fluorescence *in situ* hybridization to physically map the positions of 5S rDNA genes in relation to the chromosomal location of 18S–5.8S–26S rDNA and a C genome-specific DNA sequence in the genus *Avena*. Genome **39**: 535–542.

Loskutov, I. G. 2008: On evolutionary pathways of *Avena* species. Genetic Resources and Crop Evolution **55**: 211–220.

Maire, R. 1953: Flore de l'Afrique du Nord. Vol. 2. Lechevalier, Paris.

Mitchell, C. C., Parkinson, S. E., Baker, T. J. & Jellen, E. N. 2003: C-banding and localization of 18S–5.8S–26S rDNA in tall oatgrass species. Crop Science **43**: 32–36.

Mlinarec, J., Chester, M., Siljak-Yakovlev, S., Papeš, D., Leitch, A. R. & Besendorfer, V. 2009: Molecular structure and chromosome distribution of three repetitive DNA families in *Anemone hortensis* L. (Ranunculaceae). Chromosome Research **17**: 331–346.

Morikawa, T. & Nishihara, M. 2009: Genomic and polyploid evolution in genus *Avena* as revealed by RFLPs of repeated DNA sequences. Genes & Genetic Systems **84**: 199–208.

Nikoloudakis, N. & Katsiotis, A. 2008: The origin of C-genome and cytoplasm of *Avena* polyploids. Theoretical and Applied Genetics **117**: 273–281.

Nikoloudakis, N., Skaracis, G. & Katsiotis, G. 2008: Evolutionary insights inferred by molecular analysis of the ITS1–5.8S–ITS2 and IGS *Avena* sp. sequences. Molecular Phylogenetics and Evolution **46**: 102–115.

Peng, Y.-Y., Wei, Y.-M., Baum, B. R. & Zheng, Y.-L. 2008: Molecular diversity of 5S rRNA gene and genomic relationships in the genus *Avena* (Poaceae: Aveneae). Genome **51**: 137–154.

Peng, Y.-Y., Baum, B. R., Ren, C.-Z., Jiang, Q.-T., Chen, G.-Y., Zheng, Y.-L. & Wei, Y.-M. 2010a: The evolution pattern of rDNA ITS in *Avena* and phylogenetic relationship of the *Avena* species (Poaceae: Aveneae). Hereditas **147**: 183–204.

Peng, Y.-Y., Wei, Y.-M., Baum, B. R., Yan, Z.-H., Lan, X-J., Dai, S.-F., & Zheng, Y.-L. 2010b: Phylogenetic inferences in *Avena* based on analysis of FL intron2 sequences. Theoretical and Applied Genetics **121**: 985–1000.

Pohler, W. & Hoppe, H.-D. 1991: Homeology between the chromosomes of *Avena macrostachya* and the *Avena* C genome. Plant Breeding **106**: 250–253.

Postoyko, J. & Hutchinson, J. 1986: The identification of *Avena* chromosomes by means of C-banding. Pp. 50–51. In Lawes, D. A. & Thomas, H. (Eds.): Proceedings of the Second International Oat Conference. M. Nijhoff Publishers, Dordrecht.

Quézel, P. & Santa, S. 1962: Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales. Vol. 1. Centre Nationale de la Recherche Scientifique, Paris.

Quintanar, A., Castroviejo, S. & Catalán P. 2007: Phylogeny of the tribe Aveneae (Pooideae, Poaceae) inferred from plastid *trnT–F* and nuclear ITS sequences. American Journal of Botany **94**: 1554–1569.

Quintanar, A., Catalán, P. & Castroviejo, S. 2006: Adscription of *Parafestuca albida* (Lowe) E. B. Alexeev to *Koeleria* Pers. Taxon **55**: 664–670.

Rajhathy, T. & Thomas, H. 1974: Cytogenetics of oats (*Avena* L.). Miscellaneous Publications of the Genetics Society of Canada **2**: 1–90.

Rodionov, A. V., Kim, E. S., Punina, E. O., Machs, E. M., Tyupa, N. B. & Nosov, N. N. 2007: Evolution of chromosome numbers in the tribes Aveneae and Poeae inferred from comparative analysis of the internal transcribed spacers ITS1 and ITS2 of nuclear 45S rRNA genes. Botanicheskii Žhurnal **92**: 57–71.

Rodionov, A. V., Tyupa, N. B., Kim, E. S., Machs, E. M., & Loskutov, I. G. 2005: Genomic configuration of the autotetraploid oat species *Avena macrostachya* inferred from comparative analysis of ITS1 and ITS2 sequences: on the oat karyotype evolution during the early events of the *Avena* species divergence. Russian Journal of Genetics **41**: 518–528.

Romero Zarco, C. 1984: Revisión taxonómica del género *Helictochloa* (Dumort.) Dumort. en la Península Ibérica e Islas Baleares. Lagascalia **13**: 39–146.
Romero Zarco, C. 1985a: Revisión del género *Helictotrichon* Bess. ex Schultes and Schultes fil. (Gramineae) en la Península Ibérica. II. Estudios experimentales. Anales del Jardín Botánico de Madrid **42**: 133–154.
Romero Zarco, C. 1985b: Estudio taxonómico del género *Pseudarrhenatherum* Rouy (Gramineae) en la Península Ibérica. Lagascalia **13**: 255–273.
Romero Zarco, C. 1985c: Revisión del género *Arrhenatherum* Beauv. (Gramineae) en la Península Ibérica. Acta Botanica Malacitana **10**: 123–154.
Romero Zarco, C. 2011: *Helictochloa* Romero Zarco (Poaceae), a new genus of oat grass. Candollea **66**: 87–103.
Röser, M. 1989: Karyologische, systematische und chorologische Untersuchungen an der Gattung *Helictotrichon* Besser ex Schultes and Schultes im westlichen Mittelmeergebiet. Dissertationes Botanicae **145**: 1–250.
Röser, M. 1996: Ecogeography of the grass genus *Helictotrichon* (Poaceae: Aveneae) in the Mediterranean and adjacent regions. Plant Systematics and Evolution **203**: 181–281.
Röser, M. 1997: Patterns of diversification in Mediterranean oat grasses (Poaceae: Aveneae). Lagascalia **19**: 101–120.
Röser, M., Döring, E., Winterfeld, G. & Schneider, J. 2009: Generic realignments in the grass tribe Aveneae (Poaceae). Schlechtendalia **19**: 27–38.
Röser, M., Winterfeld, G., Grebenstein, B. & Hemleben, V. 2001: Molecular diversity and physical mapping of 5S rDNA in wild and cultivated oat grasses (Poaceae: Aveneae). Molecular Phylogenetics and Evolution **21**: 198–217.
Saarela, J. M., Liu, Q., Peterson, P. M., Soreng, R. J. & Paszko, B. 2010: Phylogenetics of the grass 'Aveneae-Type plastid DNA clade' (Poaceae: Pooideae, Poeae) based on plastid and nuclear ribosomal DNA sequence data. Pp. 557–587. In Seberg. O., Petersen, G., Barfod, A. F. & Davis, J. I. (Eds.): Diversity, Phylogeny, and eEolution in the Monocotyledons. Aarhus: Aarhus University Press.
Saint-Yves, A. 1931: Contribution à l'étude des *Avena* sect. *Avenastrum* (Eurasie et région méditeranéenne). Candollea **4**: 353–504.
Salser, W., Bowen, S., Browne, D., el-Adli, F., Fedoroff, N., Fry, K., Heindell, H., Paddock, G., Poon, R., Wallace, B. & Whitcome, P. 1976: Investigation of the organization of mammalian chromosomes at the DNA sequence level. Federation Proceedings **35**: 23–35.
Sauer, W. & Heubl, G. W. 1984: Beiträge zur Kenntnis ausdauernder Wildhafer: Karyotypanalysen an west- und osteuropäischen sowie an alpinen Wildhaferarten der Gattung *Helictochloa* (Dumort.) Dumort. (Poaceae). Phyton; Annales Rei Botanicae **24**: 193–223.
Schneider, J., Döring, E., Hilu, K. W. & Röser, M. 2009: Phylogenetic structure of the grass subfamily Pooideae based on comparison of plastid *matK* gene–3'*trnK* exon and nuclear ITS sequences. Taxon **58**: 405–424.
Schneider, J., Winterfeld, G., Hoffmann, M. H. & Röser, M. 2011: Duthieeae, a new tribe of grasses (Poaceae) identified among the early diverging lineages of subfamily Pooideae: molecular phylogenetics, morphological delineation, cytogenetics and biogeography. Systematics and Biodiversity **9**: 27–44.
Schneider, J., Winterfeld, G. & Röser, M. 2012: Polyphyly of the grass tribe Hainardieae (Poaceae: Pooideae): identification of its different lineages based on molecular phylogenetics, including morphological and cytogenetic characteristics. Organisms Diversity and Evolution **12**: 113–132.
Sharma, S. & Raina, S. N. 2005: Organization and evolution of highly repeated satellite DNA sequences in plant chromosomes. Cytogenetic and Genome Research **109**: 15–26.
Shelukhina, O. Y., Badaeva, E. D., Brezhneva, T., A., Loskutov, I. G. & Pukhalsky, V. A. 2008a: Comparative analysis of diploid species of *Avena* L. using cytogenetic and biochemical markers: *Avena canariensis* Baum et Fedak and *A. longiglumis* Dur. Russian Journal of Genetics **44**: 694–701.
Shelukhina, O. Y., Badaeva, E. D., Brezhneva, T., A., Loskutov, I. G. & Pukhalsky, V. A. 2008b: Comparative analysis of diploid species of *Avena* L. using cytogenetic and biochemical markers: *Avena pilosa* M. B. and *A. clauda* Dur. Russian Journal of Genetics **44**: 1087–1091.
Soreng, R. J. & Davis, J. I. 2000: Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: misclassification versus reticulation. Pp. 61–74. In Jacobs, S. W. L. & Everett, J. (Eds.): Grasses: Systematics and Evolution. CSIRO, Melbourne.
Soreng, R. J., Davis, J. I. & Voionmaa, M. A. 2007: A phylogenetic analysis of Poaceae tribe Poeae *sensu latu* based on morphological characters and sequence data from three plastid-encoded genes: evidence for reticulation, and a new classification of the tribe. Kew Bulletin **62**: 425–454.
Stebbins, G. L. 1971: Chromosomal Evolution in Higher Plants. Edward Arnold, London.

Swigonova, Z., Lai, J., Ma, J., Ramakrishna, W., Llaca, V., Bennetzen, J. L. & Messing, J. 2004: On the tetraploid origin of the maize genome. Comparative and Functional Genomics **5**: 281–284.

Thomas, H. 1992: Cytogenetics of *Avena*. Pp. 473–507. In Marshall, H. G. & Sorrells, M. E. (Eds.): Oat Science and Technology. Monograph 33, Agronomy Series. ASA and CSSA, Madison.

Tzvelev, N. N. 1976: Grasses of the Soviet Union. Vols. 1 and 2. Nauka, Leningrad. [Cited from the English translation 1984. A. A. Balkema, Rotterdam.]

Ugarković Đ. 2008: Satellite DNA libraries and centromere evolution. The Open Evolution Journal **2**: 1–6.

van de Peer, Y. & Meyer, A. 2005: Large scale gene and ancient genome duplications. Pp. 330–368. In Gregory, T. R. (Ed.): The Evolution of the Genome. Elsevier, Amsterdam.

Watson, L. & Dallwitz, M. J. 1992: The Grass Genera of the World. Revised edition. CAB International, Wallingford, U.K.

Wendel, J. F. 2000: Genome evolution in polyploids. Plant Molecular Biology **42**: 225–249.

Wendel, J. F., Schnabel, A. & Seelanan, T. 1995: Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proceedings of the National Academy of Sciences of the United States of America **92**: 280–284.

White, M. J. D. 1973: Animal cytology and evolution. Third edition. Cambridge University Press, Cambridge.

Winterfeld, G. 2006: Molekular-cytogenetische Untersuchungen an Hafergräsern (Aveneae) und anderen Poaceae. Stapfia **86**: 1–170.

Winterfeld, G., Döring, E. & Röser, M. 2009a: Chromosome evolution in wild oat grasses (Aveneae) revealed by molecular phylogeny. Genome **52**: 361–380.

Winterfeld, G., Perner, K. & Röser, M. 2011: Genome composition and origin of the polyploid Aegean grass *Helictochloa agropyroides* (Poaceae) Journal of Biogeography **38**: 727–741.

Winterfeld, G. & Röser, M. 2007a: Chromosomal localization and evolution of satellite DNAs and heterochromatin in grasses (Poaceae), especially tribe Aveneae. Plant Systematics and Evolution **264**: 75–100.

Winterfeld, G. & Röser, M. 2007b: Disposition of ribosomal DNAs in the chromosomes of perennial oats (Poaceae: Aveneae). Botanical Journal of the Linnean Society **155**: 193–210.

Winterfeld, G., Schneider, J., Perner, K. & Röser, M. 2012: Origin of highly polyploids: different pathways of auto- and allopolyploidy in 12–18*x* species of *Avenula* (Poaceae). International Journal of Plant Sciences **173**: 1–14.

Winterfeld, G., Schneider, J., Perner, K. & Röser, M. 2014: Polyploidy and hybridization as main factors of speciation: complex reticulate evolution within the grass genus *Helictochloa*. Cytogenetic and Genome Research **142**: 204–225.

Winterfeld, G., Schneider, J. & Röser, M. 2009b: Allopolyploid origin of Mediterranean species in *Helictotrichon* (Poaceae) and its consequences for karyotype repatterning and homogenisation of rDNA repeat units. Systematics and Biodiversity **7**: 277–295.

Wölk, A. & Röser, M. 2013: The new genus *Trisetopsis* and new combinations in oat-like grasses (Poaceae). Schlechtendalia **25**: 57–61.

Wölk, A. & Röser, M. 2014: Polyploid evolution, intercontinental biogeographical relationships and morphology of the newly described African oat genus *Trisetopsis* (Poaceae). Taxon, in press.

Yang, Q., Hanson, L., Bennett, M. D. & Leitch, I. J. 1999: Genome structure and evolution in the allohexaploid weed *Avena fatua* L. Genome **42**: 512–518.

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