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Genome Size and GC Content Evolution of *Festuca*: Ancestral Expansion and Subsequent Reduction

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• *Background and Aims* Plant evolution is well known to be frequently associated with remarkable changes in genome size and composition; however, the knowledge of long-term evolutionary dynamics of these processes still remains very limited. Here a study is made of the fine dynamics of quantitative genome evolution in *Festuca* (fescue), the largest genus in Poaceae (grasses).
• *Methods* Using flow cytometry (PI, DAPI), measurements were made of DNA content (2C-value), monoploid genome size (Cx-value), average chromosome size (C/*n*-value) and cytosine + guanine (GC) content of 101 *Festuca* taxa and 14 of their close relatives. The results were compared with the existing phylogeny based on ITS and *trn*L-F sequences.
• *Key Results* The divergence of the fescue lineage from related Poeae was predated by about a 2-fold monoploid genome and chromosome size enlargement, and apparent GC content enrichment. The backward reduction of these parameters, running parallel in both main evolutionary lineages of fine-leaved and broad-leaved fescues, appears to diverge among the existing species groups. The most dramatic reductions are associated with the most recently and rapidly evolving groups which, in combination with recent intraspecific genome size variability, indicate that the reduction process is probably ongoing and evolutionarily young. This dynamics may be a consequence of GC-rich retrotransposon proliferation and removal. Polyploids derived from parents with a large genome size and high GC content (mostly allopolyploids) had smaller Cx- and C/*n*-values and only slightly deviated from parental GC content, whereas polyploids derived from parents with small genome and low GC content (mostly autopolyploids) generally had a markedly increased GC content and slightly higher Cx- and C/*n*-values.
• *Conclusions* The present study indicates the high potential of general quantitative characters of the genome for understanding the long-term processes of genome evolution, testing evolutionary hypotheses and their usefulness for large-scale genomic projects. Taken together, the results suggest that there is an evolutionary advantage for small genomes in *Festuca*.

Key words: *Festuca*, fescue, grasses, genome size evolution, chromosome size, base composition, GC content, polyploidy, phylogeny, retrotransposon dynamics, flow cytometry.

INTRODUCTION

Beyond polyploidy, genome size differentiation is one of the most important evolutionary processes in plants, and a large number of recent studies have documented significant differences in genome size associated with evolution of taxa at different taxonomic levels, groups of different ecological specialization or geographical origin (e.g. Bureš *et al.*, 2004; Caetano-Anollés, 2005; Leitch *et al.* 2005; Price *et al.*, 2005; Johnston *et al.*, 2005; Závesky *et al.*, 2005; Bancheva and Greilhuber, 2006; Weiss-Schneeweiss *et al.*, 2006). Monoploid genome size, the total DNA content divided by the ploidy level (Cx-value; Greilhuber *et al.*, 2005), is an important general characteristic of genomes. It is especially useful for comparing genomes of taxa within polyploid complexes.

Another general parameter of the genome is the percentage of guanine and cytosine nucleotides in the genome (GC content). The GC content may reflect significant compositional features of the genome, as indicated by several studies in human and other vertebrates (Zoubak *et al.*, 1996; Bernardi, 2000*a*,*b*) and in Prokaryota (Nishio *et al.*, 2003; Musto *et al.*, 2004; Basak and Ghosh, 2005). The GC content also clearly differs among some plant families (Barow and Meister, 2002) and among the nearly completed sequences of Arabidopsis, maize and rice (Arabidopsis Genome Initiative, 2000; Meyers *et al.*, 2001; International Rice Genome Sequencing Project, 2005). However, until now, the GC content has been reported for only about 215 species (Meister and Barow, 2007), and its role in plant evolution, especially in lower taxonomic groups, is still unknown.

The differences found in monoploid genome size and GC content among monophyletic groups in plants provide an opportunity to use both attributes in evolutionary and phylogenetic studies, which until now have been based solely on the sequence data of very limited portions of genomes. Recent molecular studies bring particular advances in the understanding of mechanisms for genome size diversification and genome evolution of some model taxa; however, detailed long-term dynamics of these processes remain still only limitedly known. Advances in this area may come from detailed genome size studies in genera in which the genome size and composition data may be

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associated with both the results of fine phylogenetic studies and comparative genomic projects (Gregory, 2005).

Grasses (Poaceae, Gramineae) represent such a model group for these studies. Grasses include nearly 11 000 species (Clayton *et al.*, 2002 onwards; http://www.kew.org/data/grasses-db.html) and are the most widespread plant family, dominating in many biomes over the entire globe. Numerous species, such as rice, maize, wheat, barley, sorghum, rye-grass, meadow fescue and tall fescue, are very important human food sources or forage crops and recently have been targets of detailed comparative genomic studies (Gaut, 2002; Feuillet and Keller, 2002; Paterson *et al.*, 2005; Ammiraju *et al.*, 2006).

The evolution of grasses was accompanied by frequent and repeated genome size gain and loss (Kellogg, 1998; Gaut, 2002; Kellogg and Bennetzen, 2004; Caetano-Anollés, 2005) and DNA base composition changes (King and Ingrouille, 1987). To date an approx. 64-fold difference in monoploid genome size (Angiosperm DNA C-value database; Bennett and Leitch, 2004; http://www.rbgkew.org.uk/cval/homepage.html) and 6·2 % variation in GC content are found in grasses; ranging from 41·0 % in *Setaria woodii* to 47·2 % in *Zea mays* (cf. Meister and Barow, 2007).

Fescue (*Festuca* L., Pooideae, Poeae) is the most species-rich and highly diversified genus of grasses. It comprises about 600 species, which are perennial and dominates nearly worldwide in various types of dry, steppe, mountain and alpine grasslands or meadows; some species grow also in forests; some are cultivated as crops or ornamentals (Clayton *et al.*, 2002 onwards). About 70 % of species are polyploid (up to 12*x*); diploids are restricted mostly to Eurasia (Hunziker and Stebbins, 1987; Dubcovsky and Martínez, 1992; Šmarda and Stančík, 2006), an assumed primary diversification centre of the genus with the highest species diversity (Catalán *et al.*, 2004). The monoploid genome sizes range in *Festuca* from 1·58 to 4·03 pg (Angiosperm DNA C-value database, with some corrections of ploidy level; Bennett and Leitch, 2004) and seems to be useful for infrageneric classification (Šmarda, 2006; Loureiro *et al.*, 2007).

Within the tribe Poeae, *Fetuca* belongs to the well-supported subtribe Loliinae. The sister groups of the subtribe Loliinae (Fig. 3) represent subtribes Dactylidinae + Cynosurinae (Catalán *et al.*, 2004). *Festuca* is formed by two main evolutionary lines (Fig. 3), broad-leaved and fine-leaved species (Torrecilla and Catalán, 2002; Catalán *et al.*, 2004; Torrecilla *et al.*, 2004). The main evolutionary lineages of broad-leaved fescues are assumed to have evolved at the same time (Catalán *et al.*, 2004). One of the derived lineages is represented by *Festuca* subgen. *Schedonorus*, which gave rise to the genus *Lolium* (Pasakinskiene *et al.*, 1998; Torecilla and Catalán, 2002; Catalán *et al.*, 2004). Within fine-leaved fescues, sections *Eskia* and *Dimorphae* possess basal positions, while sections *Festuca* and *Aulaxyper* represents derived groups, both being the most rapidly evolving and species-rich groups. The members of sections *Festuca* and *Aulaxyper* are also assumed to be ancestral to numerous Mediterranean annuals treated as separate genera, e.g. *Vulpia*, *Castellia*, *Cutandia*, *Ctenopsis*, *Micropyrum*, *Wangenheimia* and *Psilurus* (Catalán *et al.*, 2004; Torrecilla *et al.*, 2004). Over 250 species included within sections *Festuca* and *Aulaxyper* used to be divided into species groups according to important morphological characters, which, however, vary among authors. The present low cover of their species-diversity with molecular markers, high intra-specific sequence diversity (Gaut *et al.*, 2000) and reticular relationships does not allow a reliable and detailed synthetic view on their evolution to be presented yet.

Here, an analysis of monoploid genome size (Cx-value), average chromosome size (C/*n*-value) and GC content of Eurasian fescues and their close relatives is completed. The data are compared with the existing phylogeny based on ITS and *trn*L-F sequences and used to outline a possible scenario for genome evolution within this group. It is intended to evaluate the impact of phylogenetic history, polyploidy and speciation rate on Cx- and C/*n*-values and GC content variation, as well as to discuss the main mechanisms responsible for the observed Cx- and C/*n*-values and GC content variability.

MATERIALS AND METHODS

Plants

Plants originated predominantly from the wild collections of the authors cultivated in the garden of the Faculty of Education and the Faculty of Science, Masaryk University, Brno, Czech Republic, and in the Botanical Garden 'Giardino dei Semplici', Florence University, Florence, Italy. Some Siberian fescues and representatives of the annual Mediterranean relatives were grown in Brno from seed collections by the authors and from seeds provided by the Palermo Botanical Garden, Italy. Some living samples of Portuguese fescues were kindly provided by J. Loureiro and P. Silveira. The origin of the samples is shown in Supplementary Information. The herbarium vouchers of the samples investigated are stored at the herbaria of the Masaryk University in Brno (BRNU), University of Florence (FI), University of Pavia (PAV) and University of Aveiro (AVE).

Genome size and GC content estimations

Genome size and GC content were measured by flow cytometry at the Institute of Botany and Zoology, Masaryk University. Measurements were conducted on two flow cytometers using two different fluorochromes: Cy Flow SL (Partec, Germany) – intercalating propidium iodide (PI) for absolute DNA content estimations; and PA-1 (Partec) – an AT-specific 4′,6-diamidino-2-phenylindole (DAPI) for the calculation of AT : CG ratio and GC content. A two-step procedure (Otto, 1990) was used for sample preparation. A piece of tiller leaf was chopped using a sharp razor blade together with an internal standard in a Petri dish containing 1 mL Otto I buffer (0·1 M citric acid, 0·5 % Tween 20). An additional 1 mL Otto I buffer was added. The crude nuclear suspension was filtered through 50-μm nylon mesh. The filtered suspension was divided into two sample tubes to which either 1 mL of Otto II buffer (0·4 M $Na_2HPO_4 \cdot 12H_2O$) supplemented

with DAPI, or 1 mL of Otto II buffer with PI + RNase was added. The final concentration of PI and RNase was 50 $\mu g\,mL^{-1}$. The concentration of DAPI was 2·0 $\mu g\,mL^{-1}$. All samples were measured simultaneously on both flow cytometers with *Pisum sativum* 'Ctirad' as the primary internal standard (2C DNA content = 9·09 pg; Doležel *et al.*, 1998; GC content = 38·5 %; Barow and Meister, 2002). When sample peaks overlapped with the peak of the primary standard either in the measurement with PI or DAPI, an alternative internal standard of diploid sample F1229 of *Festuca pallens* ($2n = 14$; Šmarda and Kočí, 2003; 2C DNA = 5·059 pg; this work) was used. In that case, results were consequently recalculated to the primary standard *Pisum sativum* based on the estimated ratio of both standards (F1229/*Pisum* = 0·5565 for PI; F1229/*Pisum* = 0·3914 for DAPI). All measurements were repeated three times and the results were averaged. The average coefficient of variance of all peaks in the measurements with PI was 3·48 % and 1·51 % with DAPI.

Monoploid genome size (Cx-value) was calculated according to Greilhuber *et al.* (2005) as the absolute 2C DNA content (2C-value) of the sample divided by the ploidy level. It is proposed here to include also the average chromosome size (C/*n*-value), calculated analogically by dividing the somatic total DNA content (2C) by somatic chromosome number ($2n$). As the basic chromosome number ($x = 7$) is the same for all the species and genera analysed, Cx-value and C/*n*-value are linearly correlated and together are referred to as 'Cx- and C/*n*-values' in the text.

When selecting plants for measurements, plants with previously documented chromosome numbers were preferred. In plants where chromosome numbers were not directly known, or any other counted plant was not available for comparison, the most common number reported in the literature, preferably from the same or a geographically close region, was used (for detailed reference list see Supplementary Information). In six taxa that had ambiguous chromosome counts or in 15 taxa that have not yet been investigated karyologically, ploidy level and the chromosome number were approximated from a comparison with genome sizes of the closest relatives.

The GC content was determined from comparison of parallel measurements with the two different fluorochromes, intercalating PI and base-specific DAPI. For the calculation, the 'Dye Factor', the proportion of the sample : standard fluorescence ratio with base-specific DAPI to the sample : standard fluorescence ratio with base-unspecific, intercalating PI, was used (Barow and Meister, 2002: eqn 6). The exact values of the GC content were calculated according to Barow and Meister (2002: eqns 7 and 8) using the mathematical approximation, *regula falsi* method, calculated in an automated Excel sheet available at http://www.sci.muni.cz/botany/systemgr/Data/Festuca/ATGCFlow.xls. The binding length of DAPI was calculated as equal to 4 according to Barow and Meister (2002).

Statistical treatment

Within the whole dataset, the Cx- and C/*n*-values and GC content of diploids and polyploids were compared by Student's *t*-test. The respective species pairs used for direct diploid–polyploid comparison are indicated in Supplementary Information. The effect of polyploidy on Cx- and C/*n*-values and GC content within six groups (*F. amethystina* group; *Festuca* sect. *Eskia*; *F. ovina* + *pallens* + *glauca* + *laevigata* cluster; *F. valesiaca* + *brevipila* cluster; *Festuca* sect. *Bovinae*; *Vulpia* sect. *Vulpia*) was evaluated by factorial ANOVA. The overall correlation of GC content with monoploid genome size in the dataset and in the data of Ammiraju *et al.* (2006, tab. 4) was tested by Pearson parametric correlation. The descriptive statistics, statistical tests and graphs were calculated in the Statistica 7·1 program (http://www. statsoft.com).

RESULTS

In total, absolute DNA content, monoploid genome size, average chromosome size and GC content of 129 samples from 115 taxa were completed (Table 1). Within the fine-leaved and broad-leaved fescues, monoploid genome size and average chromosome size varied about 2·52-fold: monoploid genome size ranged from 1·94 pg in the diploid *Festuca arvernensis* to 4·89 pg in the diploid *Festuca drymeia*; GC content varied by 3·8 %, from 42·6 % in the diploid *Vulpia bromoides* to 46·4 % in the diploid *Festuca alpestris*. Taking into account other Poeae relatives, monoploid genome size varied 3·14-fold (including the smallest genomes of the diploid *Sclerochloa dura*, Poinae), and the GC content differed by 3·9 % (including the most GC-poor tetraploid, *Dactylis polygama*).

Although Cx-value and GC content were correlated ($r = 0{\cdot}60$; $P < 0{\cdot}001$), these parameters clearly differed among taxa of all the taxonomic levels analysed. Considerable differences were noted among subtribes and genera, as well as among closely related species groups. Plotting Cx- and C/*n*-values versus GC content, several clear clusters representing individual evolutionary lineages and species groups were obtained (Figs 1 and 2). The mutual relations of species groups within Loliineae (Figs 1 and 3) agree well with the present phylogeny of the group based on ITS and *trn*L-F sequence data (Catalán *et al.*, 2004, fig. 3A, maximum parsimony method). Both basal groups of fine-leaved and broad-leaved fescues have apparently high Cx- and C/*n*-values and GC content compared with the other related genera, which are placed in the left bottom of the diagram (Figs 1 and 4). Within the evolutionarily derived groups, Cx- and C/*n*-values and GC content considerably decrease. In broad-leaved fescues, the Cx- and C/*n*-values of the most extreme *Festuca* subgen. *Drymanthele* is about twice as high, and the genome contains about 3 % more GC than members of the sister clade including subtribes Dactylidinae and Cynosurinae. An apparent reduction in Cx- and C/*n*-values and GC content appears in the *Festuca* subgen. *Schedonorus* clade, and a further decrease can be seen in its derived lineage represented by genus *Lolium* (Figs 1, 3 and 4). Within the fine-leaved fescues of *Festuca* subgen. *Festuca*, the basal sections *Eskia* and *Dimorphae* have the highest Cx- and C/*n*-values and GC content. As in the broad-leaved clade, the derived groups of fine-leaved

fescues, sections *Festuca* and *Aulaxyper*, have smaller genomes and lower GC content. The Cx- and C/*n*-values and GC content pattern within these two sections are shown in detail in Fig. 2. *Festuca* sect. *Aulaxyper* appears as four distinct lineages. The *Festuca amethystina* group possesses a very separate position with the highest Cx- and C/*n*-values and GC content. The tetraploid *F. heterophylla* and the diploids of the *F. violacea* morphological group also occupy isolated positions. The rest of high polyploid taxa of the *F. rubra* and *F.trichophylla* group form one cluster (*F. rubra* + *trichophylla*) in the bottom left part of the diagram. Compared with *F.* sect. *Aulaxyper*, *F.* sect. *Festuca* is more homogeneous in its Cx- and C/*n*-values and GC content pattern. Only three clusters, representing the main morphological groups, are partly separated. Alpine fescues of the *F. halleri* group have generally the largest and the most GC-rich genomes. The *Festuca valesiaca* and *F. brevipila* morphological groups were very similar and form a compact cluster (*F. valesiaca* + *brevipila*) in the middle of the Cx- and C/*n*-values and GC content diagram. Other species are dispersed regularly over the whole *Festuca* sect. *Festuca* cluster and are included in one polymorphic cluster (*F. ovina* + *pallens* + *glauca* + *laevigata*). The main difference in *Vulpia* is found between the diploids and the polyploids. The diploid species of *Vulpia* form a separate group together with *Castellia tuberculosa*, another Mediterranean annual; the *Vulpia* polyploids are very close to the polyploid taxa of the *F. rubra* + *trichophylla* cluster, *Festuca* sect. *Aulaxyper* (Figs 1 and 2).

Within the entire dataset, polyploids had significantly lower Cx- and C/*n*-values compared with diploids ($P < 0{\cdot}001$). This was because polyploids are much more common in the derived evolutionary groups of fine-leaved fescues that generally have lower Cx- and C/*n*-values than basal, predominantly diploid groups. No difference was found in GC content of polyploids and diploids ($P > 0{\cdot}05$). The effect of polyploidy within separate species groups became insignificant for Cx- and C/*n*-values ($P > 0{\cdot}05$) and was only marginally significant for GC content ($P = 0{\cdot}023$). Although the Cx- and C/*n*-values in polyploids were lower within *Festuca* sect. *Bovinae* and genus *Vulpia*, a reverse trend appeared in the *Festuca* sect. *Eskia*. Polyploids in all groups had a very similar to a slightly higher GC content compared with diploids; the statistical significance of this parameter was caused mainly by a shift towards higher GC content in polyploid taxa of *Vulpia* sect. *Vulpia*.

As by comparison of distant taxa within a section or a wider species group the effect of polyploidy on Cx- and C/*n*-values and GC content may be biased, the core effect of polyploidy on Cx- and C/*n*-values and GC content was analysed by only comparing pairs of closely related diploid and polyploid taxa. This comparison revealed two different patterns (Fig. 5): (1) polyploids derived from a parent with a large genome size and high GC content had smaller Cx- and C/*n*-values and only slightly deviated from the parental GC content; (2) polyploids derived from parents with a small genome and low GC content generally had a markedly increased GC content and slightly higher Cx- and C/*n*-values.

DISCUSSION

Evolutionary, phylogenetic and taxonomic implications

*Ancient Cx- and C/*n*-values and GC content expansion and subsequent reduction.* The pattern observed among separate sections and species in Cx- and C/*n*-values and GC content corresponded well with their phylogenetical relationships based on ITS and *trn*L-F sequence data (Catalán *et al.*, 2004; Fig. 3). The comparison of flow cytometry data with the present phylogeny also reveals the long-term cyclic character of Cx- and C/*n*-values and GC content evolution in fescues. Both basal fine-leaved and basal broad-leaved fescues actually had apparently high Cx- and C/*n*-values and GC content compared with the other related Poeae genera (Fig. 4), indicating that their divergence must have been associated with a process effectively increasing their genome size and GC content. In the most extreme case, *Festuca* subgen. *Drymanthele*, Cx- and C/*n*-values were about twice as high, and the genome was about 3 % GC richer than in subtribes Dactylidinae and Cynosurinae, sister to *Festuca* genus (Fig. 3). Similarly high Cx- and C/*n*-values and GC content in the basal groups of both fine-leaved and broad-leaved fescues indicate that this increase was probably due to a unique, ancient event preceding their divergence. Following the ancient Cx- and C/*n*-values expansion and GC enrichment, the fescue genome must have undergone substantial reverse reduction running parallel in both broad-leaved and fine-leaved fescues, and numerous species groups appear to separate during this reduction process. The most dramatic reductions of Cx- and C/*n*-values and GC content were particularly associated with the derived evolutionary lineages. Within the broad-leaved fescues, a high reduction appeared in *Festuca* subgen. *Schedonorus*, and a further decrease was apparent in its derived lineage, *Lolium*. Within fine-leaved fescues, a marked decrease of Cx- and C/*n*-values and GC content was found in sections *Festuca* and *Aulaxyper*, and a further reduction accompanied the evolution of *Vulpia*, probably a derived lineage of *Festuca* sect. *Aulaxyper* (Ainscough *et al.*, 1986; Torrecilla *et al.*, 2004), in which Cx- and C/*n*-values and GC content were similar to those of related Poeae. As indicated by the high substitution rate of ITS and *trn*L-F sequences (Torrecilla *et al.*, 2004) and high intra-specific ITS sequence diversity (Gaut *et al.*, 2000), *Festuca* sect. *Festuca*, *F.* sect. *Aulaxyper*, and the genus *Vulpia* belong to the most rapidly evolving groups, and the reduction process seems to be closely associated with the rapid genome evolution within these groups. Some taxa of these groups also exhibit considerable intra-specific genome size variability (Šmarda, 2006; Šmarda and Bureš, 2006; Šmarda *et al.*, 2007) which, together with the observations described above, indicates that that the reduction process is probably ongoing and evolutionarily recent.

Phylogenetic and taxonomic consequences. The differences in Cx- and C/*n*-values and GC content pattern found

TABLE 1. *Ploidy levels, infrageneric classification, 2C DNA content (2C-value), monoploid genome sizes (Cx-value), average chromosome sizes (C/*n*-value) and GC contents (GC) of the samples analysed*

Species	Ploidy level (*x*)*	Origin†	Clade‡	Subgenus§	Section¶	Species group#	2C-value (pg)	Cx-value (pg)	C/*n*-value (pg)	GC content (%)
Subtribe Cynosurinae Fr.										
Cynosurus cristatus L.	2[d]	Cz	–	–	–	–	6·095	3·047	0·435	42·66
Subtribe Dactylidinae Stapf.										
Dactylis glomerata L.	4[d]	Cz	–	–	–	–	9·042	2·260	0·323	43·53
D. polygama Horv.	2[d]	Cz	–	–	–	–	4·525	2·262	0·323	42·53
Subtribe Loliinae Dumort.										
Castellia tuberculosa (Moris) Bor	2[g]	It	C	–	–	–	6·313	3·156	0·451	42·93
Festuca acuminata Gaudin	2[d]	It	F	Fes	Esk	–	6·576	3·288	0·470	45·61
F. airoides Lam.	2[d]	F	F	Fes	Fes	Ovi	4·882	2·441	0·349	44·38
F. airoides Lam.	2[d]	Bu	F	Fes	Fes	Ovi	4·939	2·469	0·353	44·33
F. alpestris Roem. et Schult.	2[a]	It	F	Fes	Esk	–	8·892	4·446	0·635	46·41
F. alpina s.l. (alfrediana Foggi et Signorini)	2[b]	It	F	Fes	Aul	Hal	4·587	2·294	0·328	45·43
F. alpina Suter subsp. *alpina*	2[b]	A	F	Fes	Aul	Hal	4·388	2·194	0·313	44·89
F. alpina Suter subsp. *alpina*	2[b]	Sk	F	Fes	Aul	Hal	4·250	2·125	0·304	45·05
F. altaica Trin.	4[d]	Ru	B	Leu	Bre	–	14·996	3·749	0·536	45·34
F. altissima All.	2[e]	Cz	B	Dry	–	–	8·939	4·470	0·639	45·71
F. amethystina L. subsp. *amethystina*	4[b]	Cz	F	Fes	Aul	Am	12·971	3·243	0·463	45·03
F. arundinacea Schreb. subsp. *arundinacea*	6[e]	Cr	B	Sch	Bov	–	17·218	2·870	0·410	44·49
F. arundinacea subsp. *uechtritziana* (Wiesb.) Hegi	6[g]	It	B	Fes	Bov	–	16·978	2·830	0·404	44·44
F. arvernensis Auquier, Kerguélen et Markgr.-Dann. subsp. *arvernensis*	2[f]	F?	F	Fes	Fes	Lvg	3 887	1·943	0·214	44·09
F. arvernensis subsp. *costei* (St-Yves) Auquier et Kerguélen	4[a]	It	F	Fes	Fes	Lvg	9·098	2·274	0·325	44·90
F. auquieri Kerguélen	4[d]	F	F	Fes	Fes	Pal	9·460	2·365	0·338	44·69
F. balcanica (Acht.) Markgr.-Dann. subsp. *balcanica*	2[g]	Bu	F	Fes	Esk	–	7·412	3·706	0·529	46·02
F. billyii Kerguélen et Plonka	6[e]	F	F	Fes	Fes	Lvg	13·356	2·226	0·318	44·12
F. bosniaca Kumm. et Sendtn. subsp. *bosniaca*	2[g]	Cr	F	Fes	Esk	–	7·397	3·698	0·528	45·94
F. bosniaca subsp. *pirinica* (Acht.) Markgr.-Dann.	2[g]	Bu	F	Fes	Esk	–	6·982	3·491	0·499	45·96
F. brevipila R.Tracey	6[c]	A	F	Fes	Fes	Bre	14·088	2·348	0·335	44·79
F. brigantina (Markgr.-Dann.) Markgr.-Dann.	8[a]	Pt	F	Fes	Fes	Ovi	20·122	2·515	0·359	43·88
F. callieri (Hack.) Markgr.	4[e]	Rm	F	Fes	Fes	Bre	9·770	2·443	0·349	44·82
F. calva (Hack.) K.Richt.	2[g]	Sl	F	Fes	Esk	–	7·300	3·650	0·521	45·68
F. carnuntina R.Tracey	6[c]	A	F	Fes	Fes	Val	13·940	2·323	0·332	44·38
F. cinerea Vill.	4[b]	F	F	Fes	Fes	Gla	9·962	2·491	0·356	44·69
F. circummediterranea Patzke *s.l.*	4[b]	It	F	Fes	Fes	Lvg	9·979	2·495	0·356	44·37
F. circummediterranea Patzke	2[a]	It	F	Fes	Fes	Lvg	5·433	2·717	0·388	44·23
F. csikhegyensis Simonk.	4[a]	Ge	F	Fes	Fes	Pal	9·450	2·363	0·338	45·17
F. dalmatica (Hack.) K.Richt..	4[d]	Hu	F	Fes	Fes	Val	9·915	2·479	0·354	45·07
F. degenii (St-Yves) Markgr.-Dann.	4[d]	F	F	Fes	Fes	Gla	10·068	2·517	0·360	44·53
F. drymeia Mert. et Koch	2[d]	Cz	B	Dry	–	–	9·781	4·890	0·699	45·44
F. × *duernsteinensis* Vetter	4[b]	Sk	F	Fes	Fes	Ovi	10·037	2·509	0·358	44·67
F. durandoi var. *livida* (Hack.) Rivas Ponce et Cebolla	4[b]	Pt	B	Fes	Sub	–	14·662	3·666	0·524	45·85
F. duriotagana Franco et Rocha Afonso	10[a]	Pt	F	Fes	Aul	Rub	20·283	2·028	0·290	43·95
F. duvalii (St-Yves) Stohr	4[d]	Ge	F	Fes	Fes	Val	10·144	2·536	0·362	45·00
F. eggleri R.Tracey	2[d]	A	F	Fes	Fes	Ovi	4·857	2·428	0·347	44·14
F. exaltata C.Presl	2[g]	It	B	Dry	–	–	9·725	4·862	0·695	45·64
F. extremiorientalis Ohwi	4[e]	Ru	B	Sub	Par	–	13·467	3·367	0·481	45·57
F. filiformis Pourr.	2[b]	It	F	Fes	Fes	Ovi	5·013	2·507	0·358	44·15
F. gamisansii subsp. *aethaliae* Signorini et Foggi	10[a]	It	F	Fes	Fes	Lvg	24·080	2·408	0·344	44·59
F. gautieri subsp. *scoparia* (A. Kern. et Hack.) Kerguélen	2[d]	orn	F	Fes	Esk	–	6·116	3·058	0·437	45·82
F. gigantea (L.) Vill.	6[e]	Cz	B	Sch	Pla	–	20·752	3·459	0·494	43·68

Continued

TABLE 1. *Continued*

Species	Ploidy level (*x*)*	Origin†	Clade‡	Subgenus§	Section¶	Species group#	2C-value (pg)	Cx-value (pg)	C/*n*-value (pg)	GC content (%)
F. glauca Vill.	6[d]	Sp	F	Fes	Fes	Gla	14·215	2·369	0·338	44·06
F. guestfalica Reichenb.	4[b]	Cz	F	Fes	Fes	Ovi	9·780	2·445	0·349	44·64
F. guinochetii (Bidault) S.Arndt.	10[d]	It	F	Fes	Fes	Lvg	22·918	2·292	0·327	44·26
F. guinochetii (Bidault) S.Arndt.	10[d]	It	F	Fes	Fes	Lvg	22·896	2·290	0·327	44·07
F. hallerii All.	2[e]	It	F	Fes	Aul	Hal	5·040	2·520	0·360	44·94
F. hallerii All.	2[e]	It	F	Fes	Aul	Hal	5·405	2·703	0·386	44·36
F. heteromalla Pourr.	8[f]	A	F	Fes	Aul	Rub	16·386	2·048	0·293	43·63
F. heteropachys (St-Yves) Auquier	4[e]	F	F	Fes	Fes	Ovi	9·837	2·459	0·351	44·69
F. heterophylla Lam.	4[e]	It	F	Fes	Aul	Het	11·322	2·830	0·404	44·05
F. hirtovaginata (Acht.) Markgr.-Dann.	6[g]	Bu	F	Fes	Fes	Lvg	13·978	2·330	0·333	44·07
F. humifusa Brullo & R.Guarino	2[a]	It	F	Fes	Fes	Lvg	5·234	2·617	0·374	44·50
F. inops De Not.	2[b]	F	F	Fes	Fes	Gla	4·679	2·340	0·334	43·82
F. inops De Not.	2[b]	It	F	Fes	Fes	Pal	4·677	2·339	0·334	43·90
F. inops De Not.	2[b]	It	F	Fes	Fes	Pal	4·870	2·435	0·348	44·17
F. laevigata Gaudin	8[a]	It	F	Fes	Fes	Lvg	18·604	2·325	0·332	44·28
F. laevigata Gaudin	8[b]	It	F	Fes	Fes	Lvg	18·709	2·339	0·334	44·03
F. laxa Host	4[e]	Sl	F	Fes	Dim	–	12·934	3·234	0·462	45·84
F. lemanii Bast.	6[d]	Ge	F	Fes	Fes	Ovi	13·960	2·327	0·332	44·41
F. malyschevii E.B.Alexeev	2[g]	Ru	F	Fes	Aul	Am	6·986	3·493	0·499	45·01
F. nigrescens Lam. subsp. *nigrescens*	6[b]	Cz	F	Fes	Aul	Rub	13·307	2·218	0·317	43·90
F. nigrescens Lam. subsp. *nigrescens*	6[a]	F	F	Fes	Aul	Tri	13·105	2·184	0·312	44·20
F. nigrescens subsp. *microphylla* (St-Yves) Markgr.-Dann.	6[e]	It	F	Fes	Aul	Rub	13·225	2·204	0·315	43·96
F. norica (Hack.) K.Richt.	2[d]	It	F	Fes	Aul	Am	6·172	3·086	0·441	45·12
F. ovina L.	2[b]	It	F	Fes	Fes	Ovi	4·825	2·412	0·345	43·84
F. pallens Host	2[a]	Sk	F	Fes	Fes	Pal	5·059	2·529	0·361	45·05
F. paniculata (L.) Schinz et Thell. subsp. *paniculata*	2[d]	It	B	Fes	Sub	–	7·646	3·823	0·546	46·15
F. picturata Pils	2[d]	A	F	Fes	Aul	Vio	5·809	2·905	0·415	44·44
F. pirinica Markgr.-Dann.	2[d]	Bu	F	Fes	Aul	Hal	5·254	2·627	0·375	45·02
F. polesica Zapał.	2[d]	Po	F	Fes	Fes	Pal	5·196	2·598	0·371	44·44
F. pratensis Huds. subsp. *pratensis*	2[e]	Cz	B	Sch	Bov	–	6·472	3·236	0·462	44·38
F. psammophila subsp. *dominii* (Krajina) P.Šmarda	2[a]	Cz	F	Fes	Fes	Pal	4·918	2·459	0·351	44·37
F. psammophila (Čelak.) Fritsch subsp. *psammophila*	2[b]	Po	F	Fes	Fes	Pal	5·187	2·594	0·371	44·68
F. pseudodalmatica Domin	4[b]	Sk	F	Fes	Fes	Val	9·652	2·413	0·345	44·83
F. pseudovaginata Penksza	4[e]	Hu	F	Fes	Fes	Pal	9·841	2·460	0·351	45·13
F. pseudovaria subsp. *winnebachensis* (Wallosek) J.Müller	6[e]	It	F	Fes	Esk	–	18·959	3·160	0·451	46·05
F. pseudovina Wiesb.	2[d]	Hu	F	Fes	Fes	Val	4·412	2·206	0·315	44·35
F. pumila Vill.	2[f]	A	F	Fes	Esk	–	6·561	3·281	0·469	45·95
F. pumila Vill.	2[f]	It	F	Fes	Esk	–	6·911	3·455	0·494	46·04
F. riccerii Foggi et Gr.Rossi	6[g]	It	F	Fes	Fes	Ovi	14·052	2·342	0·335	44·53
F. riloensis (Hayek) Markgr.-Dann.	2[d]	Bu	F	Fes	Aul	Hal	5·670	2·835	0·405	44·85
F. robustifolia Markgr.-Dann.	10[a]	It	F	Fes	Fes	Gla	22·286	2·229	0·318	44·16
F. rubra L. subsp. *rubra*	6[f]	Rm	F	Fes	Aul	Rub	13·684	2·281	0·326	44·07
F. rubra subsp. *juncea* (Hack.) K.Richt	8[f]	Cz	F	Fes	Aul	Rub	16·971	2·121	0·303	43·99
F. rubra subsp. *pruinosa* (Hack.) Piper	6[a]	Pt	F	Fes	Aul	Rub	12·885	2·147	0·307	43·77
F. rupicaprina (Hack.) A.Kern.	2[d]	A	F	Fes	Aul	Hal	4·857	2·428	0·347	44·71
F. rupicaprina (Hack.) A.Kern.	2[e]	It	F	Fes	Aul	Hal	4·965	2·483	0·355	45·03
F. rupicola Heuff.	6[a]	Cz	F	Fes	Fes	Val	14·180	2·363	0·338	44·50
F. rupicola Heuff.	6[b]	Ru	F	Fes	Fes	Val	14·536	2·423	0·346	44·69
F. saxatilis Schur	6[e]	Rm	F	Fes	Fes	Val	14·362	2·394	0·342	44·74
F. staroplaninica Velčev	6[g]	Bu	F	Fes	Fes	Bre	13·833	2·305	0·329	44·01

Continued

TABLE 1. *Continued*

Species	Ploidy level (*x*)*	Origin†	Clade‡	Subgenus§	Section¶	Species group#	2C-value (pg)	Cx-value (pg)	C/*n*-value (pg)	GC content (%)
F. staroplaninica Velčev	6[g]	Bu	F	Fes	Fes	Bre	13·200	2·200	0·314	43·57
F. stenantha (Hack.) K.Richt.	2[d]	A	F	Fes	Aul	Hal	5·387	2·693	0·385	44·91
F. stricta Host subsp. *stricta*	6[c]	A	F	Fes	Fes	Bre	14·050	2·342	0·335	44·60
F. stricta subsp. *bauzanina* Pils	8[d]	It	F	Fes	Fes	Bre	18·484	2·310	0·330	44·54
F. summilusitana Franco et Rocha Afonso	10[a]	Pt	F	Fes	Fes	Gla	21·830	2·183	0·312	44·34
F. supina Schur	4[e]	Rm	F	Fes	Fes	Ovi	9·917	2·479	0·354	45·12
F. tatrae (Csakó) Degen	2[d]	Sk	F	Fes	Aul	Am	6·998	3·499	0·500	45·09
F. cf. *taurica* (Hack.) Trautv.	2[g]	Bu	F	Fes	Fes	Val	4·687	2·343	0·335	44·3
F. trichophylla (Gaudin) K.Richt. subsp. *trichophylla*	6[e]	It	F	Fes	Aul	Tri	13·205	2·201	0·314	43·81
F. trichophylla subsp. *asperifolia* (St.-Yves) Al-Bermani	6[g]	It	F	Fes	Aul	Tri	13·106	2·184	0·312	44·09
F. tristis Krylov et Ivanitzky	4[g]	Ru	B	Leu	Bre	–	12·917	3·229	0·461	46·38
F. vaginata Willd.	2[b]	Hu	F	Fes	Fes	Pal	4·901	2·450	0·350	44·56
F. valesiaca Gaudin	2[c]	Po	F	Fes	Fes	Val	4·553	2·276	0·325	44·17
F. valida (Uechtr.) Penzés subsp. *valida*	4[d]	Bu	F	Fes	Esk	–	18·029	4·507	0·644	46·13
F. valida (Uechtr.) Penzés subsp. *valida*	4[d]	Bu	F	Fes	Esk	–	17·780	4·445	0·635	46·05
F. versicolor subsp. *brachystachys* (Hack.) Markgr.-Dann	2[c]	A	F	Fes	Esk	–	7·728	3·864	0·552	46·17
F. versicolor subsp. *pallidula* (Hack.) Markgr.-Dann.	2[g]	A	F	Fes	Esk	–	7·111	3·555	0·508	46·00
F. versicolor Tausch subsp. *versicolor*	2[b]	Cz	F	Fes	Esk	–	7·343	3·672	0·525	45·65
F. violacea subsp. *italica* Foggi, Gr.Rossi et Signorini	2[b]	It	F	Fes	Aul	Vio	5·135	2·568	0·367	44·29
F. vivipara (L.) Sm.	4[f]	No	F	Fes	Fes	Ovi	9·922	2·481	0·354	45·14
F. wagneri (Degen, Thaisz et Flatt) Degen, Thaisz et Flatt	4[d]	Hu	F	Fes	Fes	Bre	9·494	2·373	0·339	44·60
F. xanthina Roem. et Schult.	2[d]	Rm	F	Fes	Esk	–	7·980	3·990	0·570	45·46
Lolium multiflorum Lam.	2[e]	Cz	B	–	–	–	5·442	2·721	0·389	43·28
L. perenne L.	2[e]	Cz	B	–	–	–	5·511	2·756	0·394	43·46.
L. rigidum Gaudin	2[e]	It	B	–	–	–	5·494	2·747	0·392	43·61
L. temulentum L.	2[e]	It	B	–	–	–	5·717	2·858	0·408	43·51
Vulpia bromoides (L.) S.F.Gray	2[e]	It	F	–	Vul	–	5·861	2·930	0·419	42·59
V. ciliata Dumort.	4[e]	It	F	–	Vul	–	8·289	2·072	0·296	43·35
V. myuros (L.) C.C.Gmel.	6[e]	It	F	–	Vul	–	13·783	2·297	0·328	43·94
V. myuros (L.) C.C.Gmel.	6[d]	Cz	F	–	Vul	–	13·718	2·286	0·327	43·75
V. sicula (C. Presl) Link	2[d]	It	F	–	Lor	–	5·899	2·949	0·421	42·80
Subtribe Poinae Dumort.										
Poa trivialis L.	2[e]	Cz	–	–	–	–	3·324	1·662	0·237	43·89
Sclerochloa dura (L.) P.Beauv.	2[e]	Cz	–	–	–	–	3·117	1·558	0·223	44·01

See Supplementary Information for extended measurement results, details to sample origin, taxonomic classification and references to ploidy level data.

* Superscript letters in ploidy levels indicate the relevance of ploidy level data, as follows. [a, b] Direct comparison with counted plant: [a], the counted plant was measured; [b], the results were compared with another counted plant. [c–e] DNA content of a sample agrees with the expected chromosome number reported in literature from [c] the same locality, [d] geographically close region (within the state, or up to ± 200 km around) or [e] geographically far region. [f, g] Ploidy level derived from comparison of DNA content of related taxa because [f]several sources report different chromosome counts or [g]no chromosome data are available.

† Origin/locality: A, Austria; Bu, Bulgaria; Cr, Croatia; Cz, Czech Republic; F, France; Ge, Germany; Hu, Hungary; It, Italy; No, Norway; Po, Poland; Pt, Portugal; Rm, Romania; Ru, Russia; Sl, Slovenia; Sk, Slovakia; Sp, Spain; orn, ornamental plant.

‡ Main clades of fescues and fescue-like grasses based on *trn*L-F and ITS sequences according to Catalán *et al.* (2004): B, broad-leaved clade; C, *Castellia*; F, fine-leaved clade (see Fig. 3 for further details).

§ Subgenera (only for *Festuca* genus): Dry, *Festuca* subgen. *Drymanthele* V.I.Krecz. et Bobrov; Fes, *Festuca* subgen. *Festuca*; Leu, *Festuca* subgen. *Leucopoa* (Griesb.) Tzvel.; Sch, *Festuca* subgen. *Schedonorus* (P.Beauv.) Peterm.; Sub, *Festuca* subgen. *Subulatae* (Tzvel.) E.B.Alexeev.

¶ Sections (only for *Festuca* and *Vulpia* genera): Aul, *Festuca* sect. *Aulaxyper* Dumort.; Bov, *Festuca* sect. *Bovinae* (Anderss.) Hack.; Bre, *Festuca* sect. *Breviaristatae* Krivot.; Dim, *Festuca* sect. *Dimorphae* Joch. Müller et Catalán; Esk, *Festuca* sect. *Eskia* Willk.; Fes, *Festuca* L. sect. *Festuca*; Lor, *Vulpia* sect. *Loretia* (Duval-Jouve) Boiss.; Par, *Festuca* sect. *Parviglumae* S.Aiken et X.Chen; Pla, *Festuca* sect. *Plantynia* (Dum.) Tzvel; Sub, *Festuca* sect. *Subbulbosae* (Nyman) Hack.; Vul, *Vulpia* Gmelin sect. *Vulpia*.

Morphological species groups (only for *Festuca* sect. *Festuca* and *F.* sect *Aulaxyper*): Ame, Amethystina; Bre, Brevipila; Gla, Glauca; Hal, Halleri; Het, Heterophylla; Lvg, Laevigata; Ovi, Ovina; Pal, Pallens; Rub, Rubra; Tri, Trichophylla; Val, Valesiaca; Vio, Violacea.

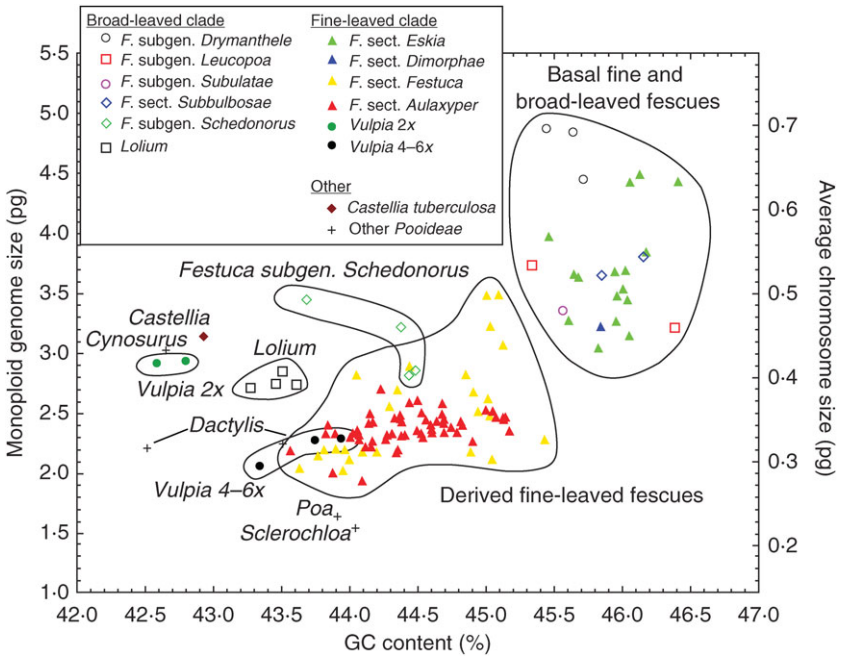

FIG. 1. A comparison of monoploid genome size and average chromosome size with GC content of the taxa analysed.

among several species and taxa groups as well as known trends of their evolution show that it is possible to use these parameters for the delimitation of taxa, and formulation of and testing evolutionary hypotheses. Preliminary results from an analysis of Cx- and C/*n*-values and GC content with easily accessible methods also offer an opportunity to survey main evolutionary processes in large species groups, which is essential for appropriate sample

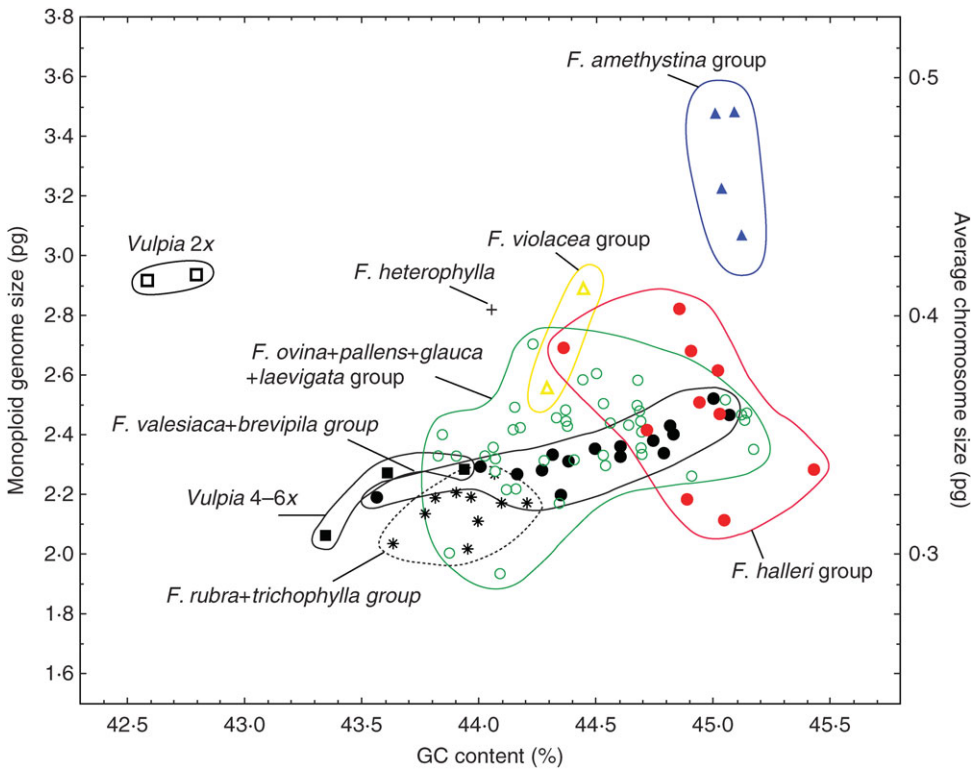

FIG. 2. A comparison of monoploid genome size and average chromosome size with GC content within derived groups of the fine-leaved fescue clade, genus *Vulpia*, *Festuca* sect. *Festuca*, and *F.* sect. *Aulaxyper*.

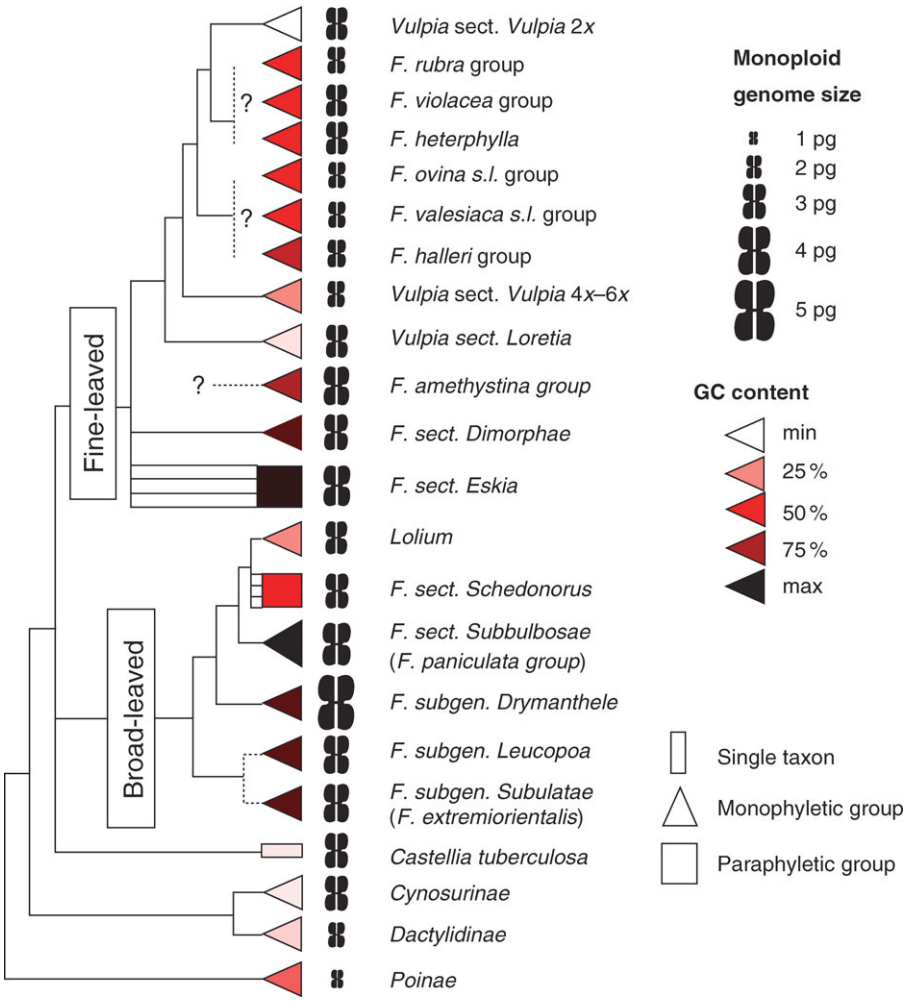

FIG. 3. The evolution of monoploid genome size and GC content within Loliinae. Reduction of both parameters in the derived groups of both main clades of fine- and broad-leaved fescues is of particular interest. Based on the consensus ITS and *trn*L-F tree by Catalán *et al.* (2004; modified).

selection for future large-scale genomic projects, as has been already shown in *Oryza* and *Gossypium* (Hawkins *et al.*, 2006; Piegu *et al.*, 2006).

The high Cx- and C/*n*-values and GC content in basal fine-leaved fescues, sections *Eskia* and *Dimorphae*, are associated with the particular presence of the broad-leaved syndrome (flat leaves, sclerenchyma girders, extravaginal shoots and convolute to supervolute vernation; cf. Catalán *et al.*, 2004), which is otherwise typical of all broad-leaved fescues and all other Poeae. The loss of this syndrome, formation of fine, narrow leaves, and the reduction in Cx- and C/*n*-values and GC content seem to follow the evolution of 'modern' fine-leaved fescues of sections *Festuca* and *Aulaxyper*.

The partial differentiation of the three main clusters of *Festuca* sect. *Festuca* in Cx- and C/*n*-values and CG content pattern (Fig. 2) gives additional support to our hypothesis that they might have developed at different evolutionary centres: (1) the *F. halleri* group in the alpine zone of the Alps and related south European high mountains; (2) the *F. valesiaca* + *brevipila* cluster in steppes and mountains of southern Siberia and the Middle East; and (3) the *F. ovina* + *pallens* + *glauca* + *laevigata* cluster in steppes and mountains of the southern and south-western Mediterranean.

Festuca amethystina may have a basal position within *Festuca* sect. *Aulaxyper* as indicated by the partial presence of the broad-leaved syndrome (sclerenchyma girders), and considerably higher Cx- and C/*n*-values and GC content, which are similar to basal fine- and broad-leaved fescues (Figs 1 and 2). The main gradient in the rest of the taxa of *Festuca* sect. *Aulaxyper* seems to be between diploids and polyploids, similarly as in *Vulpia*. The difference between *Vulpia* diploids and polyploids agrees with the main differences found in the ITS and *trn*L-F sequences (Catalán *et al.*, 2004; Torrecilla *et al.*, 2004), but it disagrees with the morphological data. Stace (2005) considered this to be a result of allopolyploidy and reticular evolution that may also have served as a reason for the decrease in Cx- and C/*n*-values typical of allopolyploids observed (see below).

Evolutionary implications. Studying highly diversified genera of Macaronesian flora, Suda *et al.* (2005) argued

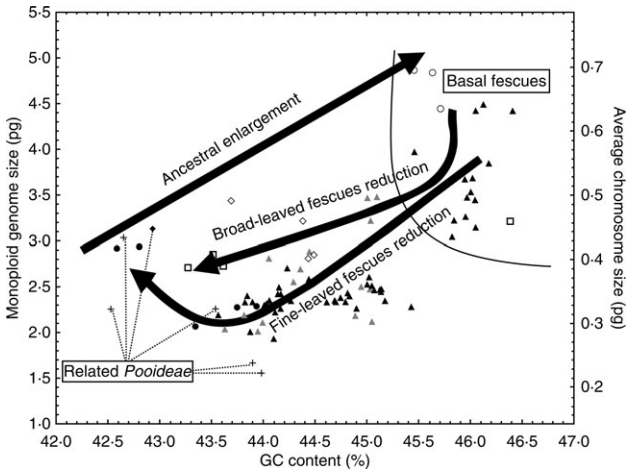

FIG. 4. The possible scenario of monoploid genome size (Cx-value), average chromosome size (C/*n*-value), and GC content evolution in *Festuca*. The divergence of basal fescues from the related Poeae was preceded by about a 2-fold increase in Cx- and C/*n*-values and considerable GC content enrichment. The subsequent reduction in GC content and Cx- and C/*n*-values, running parallel in both main evolutionary lineages of fine-leaved and broad-leaved fescues, appears to diverge among the existing species groups. Symbols correspond to those used in Fig. 1.

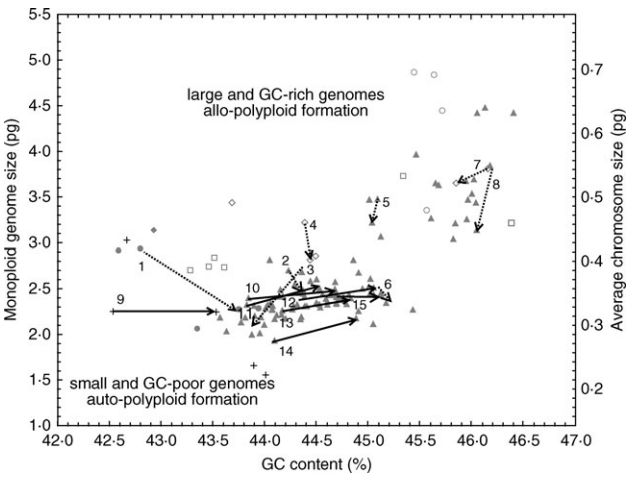

FIG. 5. A comparison of monoploid genome size, average chromosome size and GC content evolution in close diploid–polyploid species pairs or taxa groups. The arrows run from diploid to polyploid. The two different trends observed (different types of arrow) may indicate the limitation of genome evolution of polyploids by parental monoploid genome size or by allopolyploid (interrupted arrows) versus autopolyploid (solid arrows) formation of polyploids. Symbols correspond to those used in Fig. 1. Pairs: 1, *Vulpia* (2*x*) → *Vulpia* (4–6*x*); 2, *Festuca circummediterranea* (2*x*) → *F. circummediterranea s.l.* (4*x*); 3, *F. violacea* group (2*x*) → *F. rubra* group (6–10*x*); 4, *F. pratensis* → *F. arundinacea*; 5, *F. tatrae* → *F. amethystina*; 6, *F. pallens* → *F. csikhegyensis*; 7, *F. paniculata* → *F. durandoi*; 8, *F. versicolor* subsp. *brachystachys* → *F. pseudovaria* subsp. *winnebachensis*; 9, *Dactylis polygama* → *D. glomerata*; 10, *F. ovina* → *F. guestfalica*; 11, *F. inops* → *F. degenii*; 12. *F. airoides* → *F. supina*; 13, *F. valesiaca* → *F. pseudodalmatica*; 14, *F. arvernensis* subsp. *arvernensis* → *F. arvernensis* subsp. *costei*; 15, *F. vaginata* → *F. pseudovaginata*.

for the advantage of smaller genome in rapid adaptive evolution. Similarly, Knight *et al.* (2005) argued that large genomes have evolutionary and ecological constraints and showed that genera with small genomes are likely to be species-richer and over-represented in extreme environmental conditions. These hypotheses correspond well with the situation found in *Festuca* where the smallest and very GC-poor genomes are found in sections *Festuca* and *Aulaxyper*, which are (*a*) the most rapidly diverging fescue groups (Gaut *et al.*, 2000) harbouring the most of species-diversity found within the genus, and (*b*) groups that have large ecological amplitude with numerous species dominating in extreme habitats of dry xeric steppes and alpine grasslands.

GC content and genome size variability

It is assumed that the reason why the ancient Cx- and C/*n*-values and GC content increase in basal fescues is that it may have been caused by GC-rich retrotransposon proliferation. Polyploidization or hybridization are unlikely as (*a*) diploids are common in both basal and derived fescues groups and (*b*) the present linkage map of meadow fescue (*Festuca pratensis*) is highly collinear with all model grasses, showing only a minimum of duplicated segments (Alm *et al.*, 2003), which regularly accompany diploidized polyploid genomes (Ahn and Tanksley, 1993; Gaut and Doebley, 1997; Ilic *et al.*, 2003) or homoploid hybrids (Rieseberg *et al.*, 1995). Different mechanisms of transposable element proliferation and deletion are considered major reasons for the present genome size variability in grasses (SanMiguel and Bennetzen, 1998; Li *et al.*, 2004) as well as in angiosperms as a whole (Kumar and Bennetzen, 1999; Bennetzen, 2002; Bennetzen *et al.*, 2005; Vitte and Panaud, 2005). Transposable elements form a large portion of grass genomes (Meyers *et al.*, 2001; Li *et al.*, 2004; Messing *et al.*, 2004; Haberer *et al.*, 2005; Messing and Dooner, 2006; Paux *et al.*, 2006), even in the smallest grass genome of rice (about 35 % of the genome; International Rice Genome Sequencing Project, 2005). Similarly to polyploidy, massive retrotransposon amplifications may rapidly increase genome size during a relatively short evolutionary period (SanMiguel and Bennetzen, 1998; SanMiguel *et al.*, 1998; Bennetzen *et al.*, 2005; Hawkins *et al.*, 2006; Piegu *et al.*, 2006). The genome of *Oryza australiensis* was increased >2-fold during the last three million years by a massive amplification of three retrotransposons, RIRE1, Kangourou and Wallabi (Piegu *et al.*, 2006), recently accounting for about 60 % of its genome. GC contents of RIRE1, Kangourou and Wallabi retrotransposons (Noma *et al.*, 1997; Piegu *et al.*, 2006) are 44·6 %, 50·0 % and 50·9 %, respectively, which is considerably more, compared with the average GC content of the whole genome (43·6 %) or even genes (45·3 %) of the related *Oryza sativa* (International Rice Genome Sequencing Project, 2005). The assumed increase in GC content of *O. australiensis* was recently confirmed by partial sequencing of its genome within *Oryza* Map Alignment Project (Ammiraju *et al.*, 2006). We believe that the retrotransposon-driven evolution of the *Oryza* genus (Uozu *et al.*, 1997; Ma *et al.*, 2004; Ammiraju *et al.*, 2006) may be analogous to the evolution of fescues, and that the ancient Cx- and C/

n-values and GC content expansion and reverse reduction (Fig. 4) may reflect a long-term dynamics of GC-rich retrotransposon proliferation and removal. This assumption serves also as a possible solution for a considerable correlation of Cx- and C/*n*-values with GC content, which was similarly to *Festuca* found also by comparing the GC contents and monoploid genome sizes in 12 wild rice species (cf. data of Ammiraju *et al.*, 2006, tab. 4; $r = 0{,}91$; $P < 0{\cdot}001$).

The 42·5–46·4 % GC content found in fescues and related genera is in accordance with results from studies in other grasses, indicating that the GC content of grasses is apparently the highest within the whole of the angiosperms and gymnosperms (Carels and Bernardi, 2000; Barow and Meister, 2002; Kuhl *et al.*, 2004; Meister and Barow, 2007). One of the reasons for a higher GC content in grasses may be explained by the presence of grass genes that are extremely GC rich compared with other angiosperms (Carels and Bernardi, 2000; Meyers *et al.*, 2001; Wong *et al.*, 2002; Kuhl *et al.*, 2004) and use therefore a modified codon coding (Kuhl *et al.*, 2004). The fact that the GC content of genes positively correlates with the GC content of its surrounding, forming a GC-rich isochore structures (Bernardi, 2000*a*; Eyre-Walker and Hurst, 2001; Zhang and Zhang, 2004), further explains the potential impact of a gene's GC content on the overall genome GC content, in spite of the relatively low density of genes in most plant genomes. Beyond the phylogenetic differences in gene structure, the reasons for the GC content variability in grasses may be found further (*a*) in the composition and amount of transposable elements that can strongly vary in GC content; e.g. from about 28–34 % in MITE elements, common in rice, to over 60 % in Huck type elements in maize (Meyers *et al.*, 2001; Turcotte *et al.*, 2001), or (*b*) by differences in the proportion of coding and non-coding DNA, as genes and gene-rich regions are generally much more GC rich than non-coding ones (cf. Arabidopsis Genome Initiative, 2000; Meyers *et al.*, 2001; International Rice Genome Sequencing Project, 2005).

Diploid–polyploid comparison

The two kinds of polyploid formation (Fig. 5) seem not to be phylogenetically conditioned, as similar trends occur in advanced fine-leaved fescue groups and closely related genus *Dactylis*. Beyond the limitation of polyploid Cx- and C/*n*-values and GC content by parental genome constitution, the reason for these two different patterns gives also allopolyploid versus autopolyploid formation of polyploids (Fig. 5; patterns 1 and 2, respectively).

In some taxa (groups) examined here, polyploids originating from diploids with large and GC-rich genomes may be assumed to be allopolyploids. This assumption is supported by the qualitative morphological differences within compared pairs. In the diploid *F. pratensis* and hexaploid *F. arundinacea*, the allopolyploid origin of the latter was proved also by the rDNA restriction pattern (Pasakinskiene *et al.*, 1998). The putative hybrid origin of some polyploid *Vulpia* species is discussed by Stace (2005) and a recent hybrid origin of polyploid taxa of the *F. rubra* group is hypothesized by Kerguélen and Plonka (1989). The decrease in Cx- and C/*n* values in allopolyploids observed here agrees with the genome downsizing and rapid non-genic DNA elimination documented in wheat hybrids, assumed to be the general mechanism of successful allopolyploid formation (Feldman *et al.*, 1997; Ozkan *et al.*, 2001, 2003; Shaked *et al.*, 2001).

The diploid–polyploid pairs with small and GC-poor genomes are morphologically closer than those of diploid–polyploid pairs with large genomes. In the *F. inops–degenii*, *F. airoides–supina*, *F. ovina–guestfalica* and *F. valesiaca–pseudodalmatica* pairs, polyploids differ only in some quantitative morphological characteristics from diploids, and in the latter two pairs, mixed populations are frequently found (P. Šmarda, unpubl. res.). It is likely that these pairs reflect a diploid–autopolyploid relationship. Experimental evidence for the autotetraploid origin of *Dactylis glomerata* from France was reported by Lumaret *et al.* (1989) and a diploid-autotetraploid relationship may also be suspected for the *Dactylis polygama–glomerata* pair in the present study. Although it is not clear whether all the pairs discussed represent diploid–autopolyploid relationships, autopolyploidy may explain the relatively small Cx- and C/*n*-value deviation in the polyploids observed here. It is unlikely that autopolyploidy itself results in large shifts in the proportion of bases, and it is assumed that these shifts are a consequence of another process. A small increase in genome size and the positive shift in GC content in autopolyploids may be explained, for instance, by post-hybridization amplification of GC-rich transposable elements, assumed above to be the main reason for the large-scale variation in Cx- and C/*n*-values and GC content within the whole *Festuca* genus.

SUPPLEMENTARY INFORMATION

Supplementary Information is available online at http://aob.oxfordjournals.org/ and consists of a table that includes all details and results for genome size, chromosome size and GC content measurements, selection of taxa in diploid–polyploid comparisons, as well as taxonomical classifications of samples, sources of chromosome number data, and the geographic origin of the samples.

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