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ORIGINAL PAPER

AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex

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Abstract For the first time amplified fragment length polymorphism (AFLP) fingerprinting is applied to classify tropical African *Festuca* species. Five afro-alpine narrow- and two afro-montane broad-leaved species from Uganda and Ethiopia are compared to ten European grass species. A principal coordinate analysis (PCoA) accounts for 62.5% with its first three coordinates. The PCoA and the neighbor-joining (NJ) distinguish the five narrow-leaved African *Festuca* species from all other species. The broad-leaved African *Festuca africana* and *Festuca simensis* are linked to the broad-leaved European species through *Festuca altissima* and *Festuca gigantea*, respectively. The narrow- and broad-leaved European species are separated as expected in the NJ. One narrow-leaved African alpine species recently described appears merged (i.e. *Festuca richardii* with *Festuca abyssinica*). We provide chromosome numbers for all seven Ugandan species and compare taxonomy and AFLP classification. Our most striking result is that the narrow-leaved African *Festuca* species are unique and not clustering with the narrow-leaved European species.

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Introduction

Members of the genus *Festuca* L. (including *Lolium* L. and *Vulpia* C. C. Gmel.) are perhaps the most economically important forage grasses in temperate and cold regions. Some species are important fodder for wild as well as domestic animals, and others are widely sown in lawns. In tropical Africa, *Festuca* is important as forage for wild animals and for soil cover preventing erosion in the mountains. Eight *Festuca* species are known in Uganda; six are narrow-leaved (*Festuca abyssinica*, *Festuca chodatiana*, *Festuca claytonii*, *Festuca elgonensis*, *Festuca pilgeri* and *Festuca richardii*) and two are broad-leaved (*Festuca africana* and *Festuca simensis*). These fescues grow in the African mountains and highlands at an altitudinal range of 1,800–4,700 m above sea level (1,830–4,300 m in Uganda). *F. abyssinica* has the widest altitude distribution (2,130–4,700 m), but is most abundant above 3,000 m. *F. africana*, *F. chodatiana* and *F. simensis* mainly occur below 3,000 m, whereas *F. claytonii*, *F. elgonensis*, *F. richardii* and *F. pilgeri* grow above 3,000 m. *F. elgonensis* is very rare and was not found during our sampling seasons in 2003 and 2004. *F. claytonii* is also a rare species in Uganda and is only known from restricted areas on Mt. Elgon.

The genus *Festuca* is not only very polymorphic (Jenkin 1959), but it is also known to form hybrids with *Lolium*, *Vulpia* and possibly *Bromus* (Clayton and Renvoize 1986; Watson and Dallwitz 1992). Some species such as *F. abyssinica* show a very large variation in a variety of traits (Launert 1971), making the definition of species boundaries based on morphology very difficult to determine. Traditionally, the taxonomy of *Festuca* is largely based on morphology and anatomy

and to a lesser degree on cytogenetics. Moreover, morphologically very similar plants with different ploidy levels have usually not been distinguished as separate taxa, such as in *F. rubra*. To date, chromosome numbers in tropical African fescues have been established for only a few species.

The systematics and phylogeny of *Festuca* have been described using DNA markers such as random amplified polymorphic DNA (Charmet et al. 1997; Fjellheim et al. 2001), restriction fragment length polymorphisms (Xu and Sleper 1994; Charmet et al. 1997), variation of internal transcribed spacers (Gaut et al. 2000; Torrecilla et al. 2003; Catalan et al. 2004) and restriction site variation of chloroplast DNA (Darbyshire and Warwick 1992). Amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) have also been used in *Festuca* diversity studies, breeding and genome mapping (Mian et al. 2002, 2005; Skibinska et al. 2002; Alm et al. 2003; Saha et al. 2005; Fjellheim and Rognli 2005a, b). However, the molecular studies have only concentrated on temperate, arctic and Mediterranean members of the genus, such as *F. ovina*, *F. pratensis* and *F. rubra*. None of the tropical African species have been investigated by DNA fingerprinting. We therefore focus on Ugandan material and describe the AFLP-based classification of seven Ugandan *Festuca* species in comparison with selected temperate species and some Ethiopian counterparts. We also provide chromosome numbers for seven African species; confirming Hedberg's (1957) counts for *F. abyssinica* and *F. pilgeri* whereas numbers for the remaining five species are reported here for the first time.

Materials and methods

Sampling procedures

Samples were collected from the mountains and highlands in eastern (Mt. Elgon), western (Rwenzori mountains) and southwestern (Mt. Gahinga, Mt. Muhavura, Echuya swamp and Bwindi forest) Uganda during 2003 and 2004. The sampled regions cover the known distribution of *Festuca* in Uganda. Three species were collected from the Semien Mountains in Ethiopia, nine from Norway and one from Denmark (Table 1).

The youngest leaves of individual plants (assumed to be free of fungal endophytes) were collected in the field and dried using silica gel. Three to five samples were collected from each population, ensuring spatial separation of at least 3 m between the samples when possible. Sample populations were chosen at different altitudes following transects up the mountains. Fifty-seven populations were sampled from Uganda and Ethiopia, and 67 populations were sampled in total. The European species served as controls for the African *Festuca* species. Narrow-leaved controls included accessions of *F. ovina* (type species of genus *Festuca*), *F. rubra*, *F. vivipara*, *Vulpia bromoides* and *V. myuros*. The broad-leaved controls included *F. altissima*, *F. arundinacea*, *F. gigantea*, *F. pratensis* and *Lolium multiflorum*. Herbarium vouchers were also collected and are kept at the Makerere University Herbarium (MHU; Table 1). Species identifications were made by the first author in the field and verified based on material at MHU and Kew Herbarium, on the floras ('Flora of Tropical East Africa and Flora of Ethiopia and Eritrea') and on Alexeev's taxonomy (Alexeev 1986, 1987). Sylvia Phillips (Kew) helped with some of the identifications.

DNA extraction and AFLP procedure

Silica-dried leaves were ground into a fine powder, and DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA). The quality and quantity of the undigested DNA was checked on 0.8% agarose gels together with λ DNA. A total of 167 accessions representing 17 species (Table 1) were included in the final analysis. The AFLP fingerprinting procedure followed the original procedures of Vos et al. (1995) with minor modifications described by Becker et al. (1995). In short, 400 ng DNA per sample was digested with 5 U *Eco*RI and 5 U *Mse*I for 2 h at 37 °C. *Eco*RI (GTC GTA GAC TGC GTA CC/AAT TGG TAC GCA GTC), and *Mse*I (GAC GAT GAG TCC TGA G/TAC TCA GGA CTC AT) adapters were ligated to the obtained DNA fragments during an incubation period of 3 h at 37 °C. The obtained restriction/ligation products were selectively PCR pre-amplified with the primers E01 (GAC TGC GTA CCA ATT CA) and M01 (GAT GAG TCC TGA GTA AA) at the temperature–time profile given by Vos et al. (1995). The resulting preamplification products were checked on 1% agarose gels and diluted 100-fold prior to the selective +3/+3 PCR amplification. The E-primers were labeled with radioactive γ-[^{33}P] before being used in the selective amplification, which followed the PCR profiles described by Becker et al. (1995). An equal volume of loading buffer (99% formamide, 10 mM EDTA pH 8, 1 mg/ml xylene cyanol FF, 1 mg/ml bromophenol blue) was added to the final PCR products, and the mix was denatured at 94 °C for 10 min and immediately put on ice. A 3 µl of the mix was loaded on 5% polyacrylamide (PAGE)

Table 1 Species used in this study

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
F. abyssinica A. Rich.	$2n = 4x = 28$[b,c]	058E	1369	Elgon, Wagagai peak	4300	01°07.5′N, 34°31.5′E	Uganda
		071E, 072E	1374	Elgon, in the caldera	3870	01°09.0′N, 34°33.3′E	Uganda
		073E, 074E, 075E	1376*	Elgon, hot springs	3560		Uganda
		076E	1377	Elgon, in the caldera	3770		Uganda
		226E, 227E, 228E	1566	Elgon, bamboo forest	3050	01°10.6′N, 34°27.0′E	Uganda
		278E, 279E, 281E	1576	Elgon, Mongongo cave	3780	01°09.3′N, 34°30.8′E	Uganda
		290E	1581	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		291E, 292E	1582	Elgon, Piswa, Sasa trail	3740	01°09.0′N, 34°30.0′E	Uganda
		296E, 297E, 300E	1583	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		–	1660*	Elgon, above Sasa R. camp	2980	01°10.6′N, 34°26.9′E	Uganda
		–	1662*	Elgon, 1.5 km E of Sasa camp	2990	01°10.6′N, 34°26.9′E	Uganda
		–	1663*	Elgon, Sasa trail, Kimoro	3050	01°10.6′N, 34°27.0′E	Uganda
		–	1670*	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		113Vg, 114Vg	1406	Gahinga, montane forest	3070	01°22.9′S, 29°38.5′E	Uganda
		119Vg	1409	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		120Vg, 121Vg, 122Vg	1410	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		123Vg	1411	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		130Vg	1414	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		191Vg, 192Vg, 193Vg	1604	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		195Vg, 196Vg, 198Vg	1605	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		199Vg, 200Vg, 201Vg	1606	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		2*	–	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		146Vm, 147Vm, 148Vm	1592	Muhavura, forest	3080	01°22.3′S, 29°40.2′E	Uganda
		149Vm, 150Vm, 151Vm	1593	Muhavura, ericaceous zone	3800	01°22.0′S, 29°39.0′E	Uganda
		156Vm, 157Vm, 163Vm	1594	Muhavura, ericaceous zone	3800	01°22.0′S, 29°39.0′E	Uganda
		334T, 335T, 336T	1512	Semien Mts., Chenek	3530	13°15.0′N, 38°11.0′E	Ethiopia
F. chodatiana (St. Yves) Alexeev	$2n = 4x = 28$[b]	230E, 231E, 232E	1567	Elgon, bamboo forest	3070	01°10.6′N, 34°27.6′E	Uganda
		301E, 302E, 305E	1584	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		322E, 323E, 324E	1587	Elgon, montane forest	2710	01°10.4′N, 34°25.4′E	Uganda
		–	1671*	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		–	1687*	Elgon, Sasa trail	2750	01°10.5′N, 34°25.5′E	Uganda
		–	1689*	Elgon, Sasa trail	2600	01°10.5′N, 34°25.5′E	Uganda
		–	1695*	Elgon, Sasa trail	1770	01°10.5′N, 34°23.5′E	Uganda
		169Vg, 171Vg, 173Vg	1600	Gahinga, regenerating forest	2405	01°21.6′S, 29°37.4′E	Uganda
		182Vg, 183Vg, 184Vg	1602	Gahinga, regenerating forest	2610	01°22.0′S, 29°37.9′E	Uganda
		–	1696*	Gahinga, regenerating forest	2500	01°21.5′S, 29°37.0′E	Uganda
		137Vm, 138Vm, 139Vm	1590	Muhavura, regenerating forest	2620	01°21.8′S, 29°39.8′E	Uganda
		167Vm, 168Vm	1595	Muhavura, regenerating forest	2500	01°21.6′S, 29°39.8′E	Uganda
		210K, 211K, 212K	1609	Echuya, Kisoro, Kabale boarder	2280	01°15.4′S, 29°47.8′E	Uganda
		214B, 215B, 216B	1610	Bwindi forest, Ndeego	2220	01°06.5′S, 29°48.5′E	Uganda
		–	1717*	Bwindi forest, Ndeego	2220	01°06.5′S, 29°48.5′E	Uganda
F. claytonii Alexeev	$2n = 4x = 28$[b]	271E, 274E, 275E	1575*	Elgon, towards Wagagai	4140	01°08.7′N, 34°31.4′E	Uganda
		–	1678*	Elgon, towards Wagagai	4140	01°08.7′N, 34°31.4′E	Uganda

Table 1 continued

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
F. pilgeri St. Yves	$2n = 4x = 28$[b,c]	033E, 034E	1365	Elgon, above Jackson's pool	4100	01°08.6′N, 34°31.3′E	Uganda
		255E, 256E, 258E	1572	Elgon, near Jackson's pool	3910	01°08.9′N, 34°30.6′E	Uganda
		264E, 265E, 267E	1574	Elgon, above Jackson's pool	4010	01°08.7′N, 34°31.0′E	Uganda
		–	1675*	Elgon, above Jackson's pool	4000	01°08.7′N, 34°31.0′E	Uganda
F. richardii Alexeev	$2n = 4x = 28$[b]	020E, 021E, 022E	1359	Elgon, above Mude camp	3900	01°08.9′N, 34°30.6′E	Uganda
		023E	1361	Elgon, above Jackson's pool	3965	01°08.8′N, 34°30.8′E	Uganda
		239E, 241E, 242E	1569*	Elgon, alpine grassland	3310	01°10.4′N, 34°28.3′E	Uganda
		244E, 245E, 248E	1570	Elgon, alpine grassland	3470	01°10.0′N, 34°29.0′E	Uganda
		249E, 250E, 251E	1571*	Elgon, alpine grassland	3730	01°09.5′N, 34°30.0′E	Uganda
		259E, 260E, 261E	1573*	Elgon, above Jackson's pool	4010	01°08.7′N, 34°31.0′E	Uganda
		288E	1579	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		289E	1580	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		–	1674*	Elgon, above Jackson's pool	4000	01°08.7′N, 34°31.0′E	Uganda
F. africana (Hack.) Clayton	$2n = 10x = 70$[b]	309E, 310E, 313E	1585	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		186Vg, 188Vg, 190Vg	1603	Gahinga, *Hypericum* forest	3170	01°22.9′S, 29°38.5′E	Uganda
		219B, 220B, 222B	1612	Bwindi forest	2230	01°05.6′S, 29°48.7′E	Uganda
		089R	1394	Rwenzori Mts., Bwamba Pass	2430	01°40.0′N, 30°08.0′E	Uganda
		090R, 091R, 092R	1395	Rwenzori Mts., Bwamba Pass	2600		Uganda
		af2*	–	Elgon, Sasa trail	2500	01°10.5′N, 34°25.0′E	Uganda
		af3*, af4*	–	Bwindi, along forest road	2550	01°05.5′S, 29°48.0′E	Uganda
F. simensis A. Rich.	$2n = 4x = 28$[b]	234E, 235E, 237E	1568	Elgon, bamboo forest	3210	01°10.5′N, 34°27.9′E	Uganda
		282E, 283E, 286E	1577*	Elgon, Mongongo cave	3780	01°09.3′N, 34°30.8′E	Uganda
		317E, 318E, 319E	1586	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		–	1664*	Elgon, Sasa trail	2800	01°10.0′N, 34°26.0′E	Uganda
		174Vg, 176Vg, 177Vg	1601	Gahinga, regenerating forest	2490	01°21.7′S, 29°37.7′E	Uganda
		131Vm, 133Vm, 134Vm	1589	Muhavura regenerating forest	2530	01°21.6′S, 29°39.8′E	Uganda
		141Vm, 142Vm, 143Vm	1591	Muhavura, montane forest	2990	01°22.2′S, 29°40.1′E	Uganda
		–	1698*	Muhavura regenerating forest	2330	01°21.5′S, 29°39.5′E	Uganda
		204K, 206K, 207K	1608	Echuya, Kabale, Kisoro boarder	2280	01°15.4′S, 29°47.8′E	Uganda
		096B, 097B, 098B	1398	Bwindi forest, Ndeego	2550	01°05.5′S, 29°48.5′E	Uganda
		–	1715*	Bwindi, along forest road	2550	01°05.5′S, 29°48.0′E	Uganda
		088R	1390	Rwenzori Mts., Bwamba Pass	2100		Uganda
		331T, 332T, 333T	1511	Semien Mts., Chenek	3530	13°15.0′S, 38°11.0′E	Ethiopia
F. ovina L.	$2n = 2x = 14$[d], $2n = 4x = 28$[e]	342N, 343N	1500	Besstrond	980		Norway
F. rubra L.	$2n = 6x = 42$[d]	345N, 346N	1501	Moss	0.5		Norway
F. vivipara L.	$2n = 3x = 21$[f], $2n = 4x = 28$[f]	360N, 362N	1506	Besstrond	1020		Norway
F. altissima All.	$2n = 2x = 14$[g]	357N, 359N	1505	Moss	120		Norway
F. arundinacea Schreber	$2n = 6x = 42$[h]	363N, 364N	1507	Rygge	0.5		Norway
F. gigantea (L.) Vill.	$2n = 6x = 42$[g]	352N, 353N	1503	Asker	150		Norway
F. pratensis Hudson	$2n = 2x = 14$[h]	354N, 355N	1504	Ås, Breivoll	0.5		Norway
Lolium multiflorum Lam.	$2n = 2x = 14$[i]	348N, 350N	1502	Ås	95		Norway
Vulpia bromoides (L.) S. F. Gray	$2n = 2x = 14$[j]	337N, 338N	1499	Rennesøy	5		Norway

Table 1 continued

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
V. myuros (L.) C. C. Gmelin	$2n = 6x = 42$[k]	328T, 329T, 330T 340D, 341D	1510 27761	Semien Mts., Chenek Himmelbjerget	3530 120		Ethiopia Denmark

Accessions marked with *asterisk* (*) were used for chromosome counting, and those without lab numbers were not used in the AFLP analysis. All accessions with lab numbers were used in the AFLP analysis. Voucher specimens are kept at the Makerere University Herbarium (MHU)

[a] Meters above sea level

[b] Reported in this study

[c] Hedberg (1957)

[d] Arohonka (1982)

[e] Malakhova and Markova (1994)

[f] Salvesen (1986)

[g] Lövkvist and Hultgård (1999)

[h] Jenczewski and Alix (2004)

[i] Spies et al. (1999)

[j] Morton (1993)

[k] Barker and Stace (1984)

gels. Electrophoresis was at 80 W for 1.5 h, with one and two times TBE in the upper and lower chambers, respectively. The gels were fixed in 10% acetic acid for 30 min, dried and exposed to X-ray film for 26–75 h depending on radiation intensity and then developed with an AGFA Curix 60. Five primer combinations were used: (1) E34/M37 (*Eco*RI+AAT/*Mse*I+ACG), (2) E39/M37 (*Eco*RI+AGA/*Mse*I+ACG), (3) E35/M36 (*Eco*RI+ACA/*Mse*I+ACC), (4) E37/M40 (*Eco*RI+ACG/*Mse*I+AGC) and (5) E37/M38 (*Eco*RI+ACG/*Mse*I+ACT).

Data analysis

AFLP fragments were manually scored as present (1) or absent (0). Because a high number of markers were generated per gel, only the clearest AFLPs were scored. Because of the selective amplification (Vos et al. 1995), each primer combination yields a unique, independent and randomly distributed set of fragments. Thus, each primer combination is an independent replication, and when these give similar results (as observed here), they can be merged and seen as a solid basis for a phenetic DNA marker-based analysis. Spreadsheet data matrices were prepared for each of the five primer combinations from which similarity matrices were computed using DICE derived distances. The five distance matrices obtained and the combined data set were analyzed by principal coordinate analysis (PCoA) using NTSYSpc 2.11f (Rohlf 2000). Neighbor-joining (NJ, Saitou and Nei 1987) and bootstrapping (1000 times) was done with TREECON (Van de Peer and De Wachter 1994). Normalized Mantel tests (Mantel 1967) were computed with the MxComp option of NTSYSpc.

Squash preparations and chromosome counts

Seeds collected from the same plants/populations sampled for leaf material or from nearby populations were germinated in Petri dishes at room temperature for 5–11 days, depending on the species. When about 1–1.5 cm long, the roots were cut and pretreated in ice water for 24 h. The pretreated roots were then fixed in fresh Carnoy's solution I (one acetic acid:three absolute ethanol) for about 3 days at room temperature and then stored at 4 °C. The roots were stained in 1% acetocarmine for at least 15 min and then heated until the acetocarmine started to boil. Using a razor blade, the root tip was cut off, placed in a drop of 1% acetocarmine on a glass slide, covered with a cover slip and the material was gently loosened by taping on the cover slip using a lancet needle. The loosened material

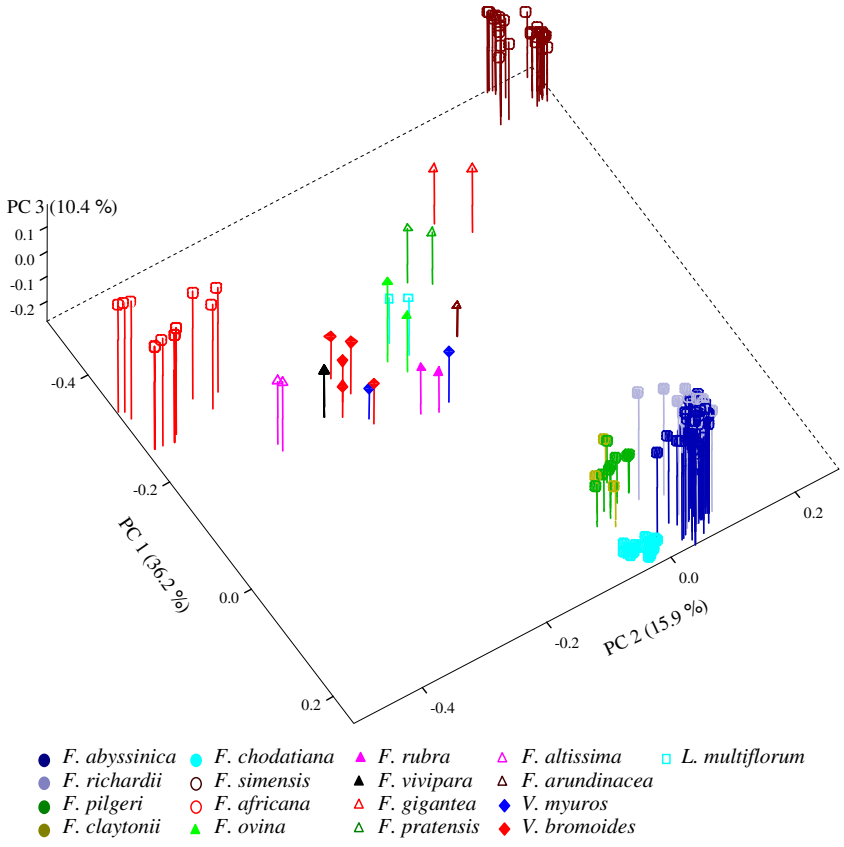

Fig. 1 Principal coordinate analysis of 167 *Festuca* accessions derived from 616 polymorph AFLPs. *Rings* represent the African fescues; *filled rings* are the narrow-leaved African fescues whereas the *empty rings* are the broad-leaved species. *Triangles* represent the European fescues; *filled triangles* are the narrow-leaved European fescues whereas the *empty triangles* are the broad-leaved species. *Diamonds* represent *Vulpia* species and squares represent *Lolium*. The first three coordinates (PC1, PC2 and PC3) account for a cumulative value of 62.5%

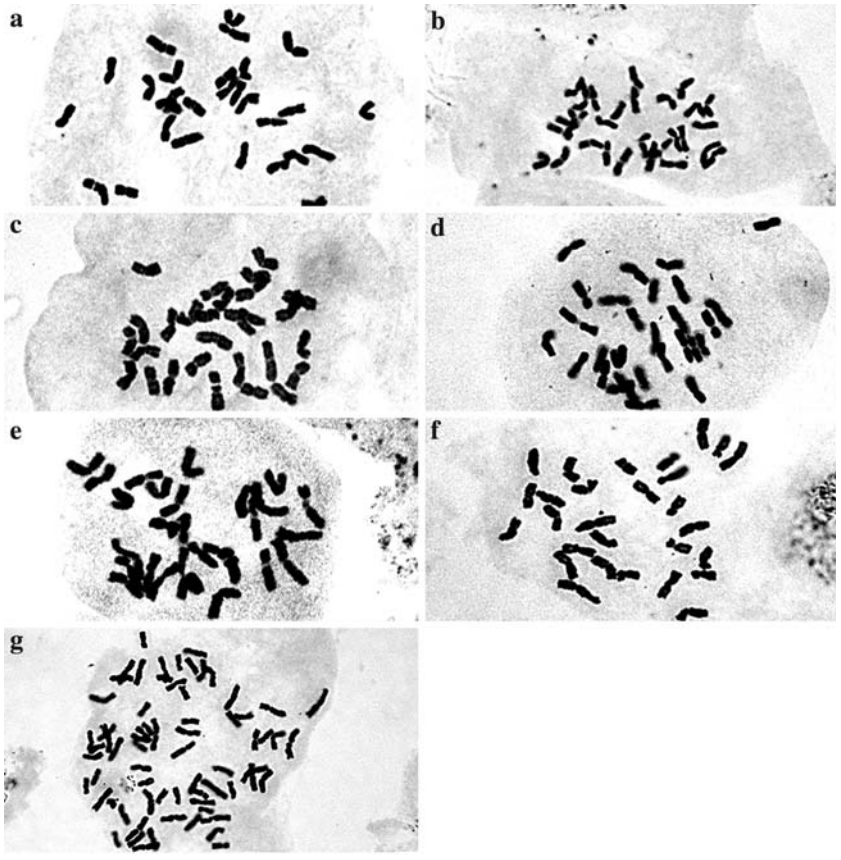

Fig. 2 Mitotic metaphase chromosomes stained with acetocarmine: **a** *Festuca abyssinica*; **b** *F. richardii*; **c** *F. pilgeri*; **d** *F. claytonii*; **e** *F. chodatiana*; **f** *F. simensis*; **g** *F. africana*

was heated to a point just below boiling and quickly squashed. It was generally difficult to get good chromosome preparations; hard squashing was found to be best. Mitotic metaphase chromosomes were viewed and counted under a phase-contrast microscope. Photographs were taken using a SPOT CCD camera and enhanced using Adobe Photoshop version 7. Ten roots were counted per accession analyzed (Table 1).

Results

The field collection was done in two consecutive years and targeted to get representative samples of all eight reported species from Uganda. The number of accessions for the individual species was variable due to the unequal frequency of occurrence of the species in the field. We therefore obtained few accessions for the rare East African endemics (*F. claytonii* and *F. pilgeri*). The endemic *F. elgonensis*, the eighth species, was not found. Table 1 shows the accessions of the species used for DNA analysis.

Each of the five AFLP primer combinations gave more than 100 polymorphic bands (i.e. 124 AFLPs with primer combination E34/M37, 119 with E39/M37, 116 with E35/M36, 122 with E37/M40 and 135 with E37/M38) and resulted in almost identical NJs and PCoAs (not shown). Mantel tests gave values ≥0.9 for the underlying data matrices (not shown). Therefore, the primer combinations were merged, and the resulting data set comprises 616 AFLPs and 167 accessions.

The PCoA obtained is shown in Fig. 1. Principal coordinate (PC) 1 accounts for 36.2% and mostly separates the narrow-leaved African species (represented by 103 accessions) from the remaining 64 accessions. PC 2 and 3 (accounting for 15.9 and 10.4%, respectively) provide further differentiation: the narrow-leaved African species with broad enveloping glumes about 3/4 as long as the spikelet, i.e. *F. abyssinica* and *F. richardii*, are intermixed and separate from the rest of the narrow-leaved African species that have short and narrow glumes only up to 1/2 the spikelet length. The later category of the narrow-leaved African species divides into species with acicular leaves (*F. claytonii* and *F. pilgeri*, which are intermixed) versus *F. chodatiana* with flat or folded leaves. These African narrow-leaved species are tetraploid (Fig. 2). The decaploid (see Fig. 2) *F. africana* and the tetraploid (see Fig. 2) *F. simensis* are also distinct. The ten European control species (each represented by two accessions, except for *Vulpia bromoides*, which is represented by five) are spread in the centre.

An NJ tree (based on individual accessions due to the unequal number of accessions per species) was done (Fig. 3) and the main differentiation separating narrow-leaved African species from the remaining species is again obtained and supported by a bootstrap value of 100%. Also, among European species, *F. gigantea* is the closest to the African *F. simensis* and *F. altissima* is the closest to the African *F. africana*. All broad-leaved species of *Festuca* (including *Lolium*) appear together on the NJ tree (Fig. 3), yet are separated from each other by bootstrap values of 100%. The same holds true for the European narrow-leaved species of *Festuca* (including *Vulpia*). A distinction between all *F. claytonii* versus all *F. pilgeri accessions* is obtained (bootstrap value 100%) but not for all *F. abyssinica* versus *F. richardii*.

Discussion

African fescue diversity

This being the first analysis of tropical African fescues using DNA markers, we anticipated results similar to those from earlier molecular investigations of European fescues, which support the old classical morpho-anatomical separation into a 'narrow-leaved' and a 'broad-leaved' clade (Charmet et al. 1997; Gaut et al. 2000; Fjellheim et al. 2001; Torrecilla and Catalan 2002; Torrecilla et al. 2003, 2004; Catalan et al. 2004). We found that the narrow-leaved African species are well separated from the broad-leaved African species, but not merged with the narrow-leaved European species. In fact, the first PC alone clearly separated the narrow-leaved African species from the remaining species. This wide separation of the narrow-leaved African species should be addressed from a taxonomic point of view with respect to the hierarchical placement of this group within the genus *Festuca*. The broad-leaved African *F. africana* and *F. simensis* are also distinct from the European fescues, but they are to some degree linked to the broad-leaved European species through *F. altissima* and *F. gigantea* (see below).

Morphology versus DNA classification

The taxonomic distinction (as described in the 'Flora of Tropical East Africa') between *F. abyssinica*, *F. chodatiana* and *F. pilgeri* is in full accordance with the distinction obtained by AFLP fingerprinting. All three species are tetraploid.

F. richardii (also $2n = 2x = 28$, reported here), a species recently separated from *F. abyssinica* by Alexeev (1987), is intermixed with *F. abyssinica* in the DNA analyses. The two species mostly show overlapping morphological traits in important characters, such as panicle shape, spikelet size and shape, and size and texture of the glumes. Distinctions are noted in habit (culms decumbent in *F. abyssinica* and erect in *F. richardii*) and leaf structure (1–3 mm wide, involute, filiform or acicular in *F. abyssinica*, and about 0.5 mm wide, conduplicate and acicular in *F. richardii*). Phillips (1995) described *F. richardii* as "a high mountain segregate from the *F. abyssinica* complex, whose specific status rests mainly on its slightly different leaf-anatomy." The distribution of the sclerenchyma tissue is emphasized by taxonomists to support this species distinction, but the results presented by Alexeev (1986, 1987) and Phillips (1995) are conflicting. We suggest following our AFLP data and re-merging *F. richardii* with *F. abyssinica*.

The second incongruence with Alexeev occurs when the AFLPs do not separate *F. claytonii* (also $2n = 2x = 28$) and *F. pilgeri* with the PCoA. However, our NJ indicated such AFLP differentiation (Fig. 3). Again, the two species are mostly similar morphologically but can also be separated. *F. claytonii* is a generally a small plant with soft leaves and an open panicle, whereas *F. pilgeri* is a bigger plant with stiff leaves and a contracted panicle. Whether these morphological differences justify species distinction is uncertain, but since the NJ split of these two species is well supported by bootstrapping and since we have preliminary cytogenetic data (M. Namaganda et al. unpublished) indicating some differentiation, we conclude that Alexeev's classification is justified.

The broad-leaved African fescues *F. simensis* and *F. africana* are more similar to the European species of *Festuca*, *Lolium* and *Vulpia* than they are to the narrow-leaved African fescues. *F. simensis* is most closely related to the European *F. gigantea*, and this

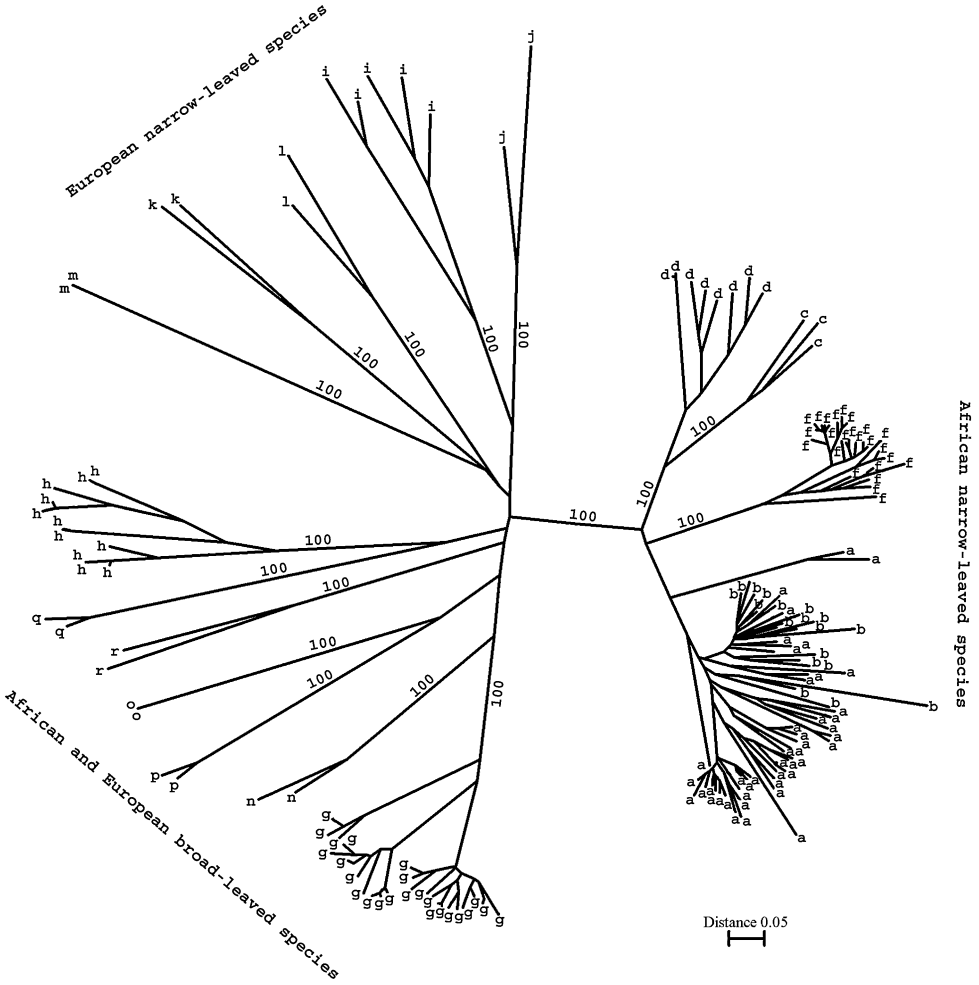

Fig. 3 Neighbor-joining of the whole data set: 167 *Festuca* accessions analyzed with 616 AFLPs. *Letters* at the end of each branch indicate the taxonomic classification of the respective accession: **a** *F. abyssinica*; **b** *F. richardii*; **c** *F. claytonii*; **d** *F. pilgeri*; **f** *F. chodatiana*; **g** *F. simensis*; **h** *F. africana*; **i** *Vulpia bromoides*; **j** *V. myuros*; **k** *F. ovina*; **l** *F. rubra*; **m** *F. vivipara*; **n** *F. gigantean*; **o** *F. arundinacea*; **p** *F. pratensis*; **q** *F. altissima*; **r** *Lolium multiflorum*. The *bar* represents a distance of 0.05. Bootstrapping values of 100% are only reported on those branches leading to species

agrees with the morphology (Clayton 1970). However, *F. simensis* is tetraploid, whereas *F. gigantea* is hexaploid ($2n = 6x = 42$, Lövkvist and Hultgård 1999). *F. africana* is most similar to *F. altissima* and this also corresponds to morphology. Both species are forest dwellers, lack auricles and have transverse veinlets in the leaves, but *F. africana* is decaploid whereas *F. altissima* is diploid.

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