Phylogeographic history and taxonomy of some afro-alpine grasses assessed based on AFLPs and morphometry: *Deschampsia cespitosa, D. angusta* and *Koeleria capensis*

Catherine A. Masao[1,2#], Abel Gizaw[2,3], Rosalía Piñeiro[2], Felly M. Tusiime[2,4], Tigist Wondimu[2,3], Abdi A. Ahmed[2,5], Magnus Popp[2], Galina Gussarova[2,7] , Kåre A. Lye[6], Pantaleo Munishi[1], Sileshi Nemomissa[3*], Christian Brochmann[2*]

[1]Sokoine University of Agriculture, Department of Forest Biology, P.O. Box 3010, Morogoro, Tanzania
[2]National Centre for Biosystematics, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway
[3]Department of Plant Biology and Biodiversity Management, Addis Ababa University, P.O. Box 3434, Addis Ababa, Ethiopia
[4]College of Agriculture and Environmental Sciences, P.O. Box 7062, Makerere University, Kampala, Uganda
[5]East African Herbarium, National Museum of Kenya, P.O. Box 40658-00100, Nairobi, Kenya
[6]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, NO-1432 Ås, Norway
[7]Department of Botany, Faculty of Biology and Soil Sciences, St Petersburg State University, St Petersburg, 199034, Russia

*These authors share senior authorship

[#]**Corresponding author**: Catherine A. Masao; Institute of Resource Assessment, University of Dar es Salaam. **Email**: ndeutz@yahoo.com, Mobile: +255 784 598280,
Fax: +255-22-2410393

Abstract

Phylogeographic studies in the high mountains of Africa are hampered by the limited material available, resulting in insufficient knowledge of taxonomic variation within and among closely related species. Here we address genetic and morphological variation in three grass species, of which one (*Deschampsia angusta*) has been reported as narrowly endemic and vulnerable whereas *D. cespitosa* and *Koeleria capensis* are widely distributed also outside the afro-alpine region. We used Amplified Fragment Length Polymorphisms (AFLPs) to assess genetic structuring and diversity in material collected during recent field expeditions and included additional herbarium material in morphometric analyses. The plants identified as the endemic *D. angusta* were genetically very similar to those identified as *D. cespitosa* from the same mountain (Mt Ruwenzori), forming a single coherent genetic group in STRUCTURE analysis. The plants identified as *D. angusta* seem to represent extremes of continuous gradients of morphological variation within a single, variable species, *D. cespitosa*. We found that the afro-alpine material of *Deschampsia* consists of three genetically very distinct groups corresponding to the three mountains investigated, suggesting persistence in isolated afro-alpine refugia during one or more glacial cycles. In contrast, we found no clear genetic structure in *K. capensis*. This species harboured very little genetic diversity in all six mountain areas examined, and little genetic rarity except in the Ethiopian Simen Mts. This pattern may be explained by recent colonization of the afro-alpine region from a single source population or possibly by extensive recent gene flow combined with bottlenecks. We found however some differentiation between different *K. capensis* populations from Mt Kilimanjaro, corresponding to two described varieties. This study demonstrates the need for further taxonomic exploration of the enigmatic flora of the isolated afro-alpine 'sky islands' and highlights that different species may have conspicuously different phylogeographic histories.

Key words: AFLP, afro-alpine, *Deschampsia, Koeleria*, morphometry, phylogeography

Introduction

The unique plant diversity of the isolated high mountains of eastern Africa has attracted the attention of scholars of natural history for a long time (e.g. Richard 1847-1851; Engler 1892; Fries and Fries 1922; Hedberg 1951; 1957; Hedberg and Hedberg 1979), but the taxonomy of many afro-alpine plant groups remains insufficiently known. Many of these climatically harsh mountains are widely separated and steep, requiring carefully planned expensive and laborious expeditions for collection of material. The taxonomic exploration of the enigmatic afro-alpine flora has therefore been hampered by the limited material available for comparison of variation patterns, especially within and among closely related species, of which many have been described as endemic. The lack of material and insufficient taxonomic knowledge has hampered attempts to explore the phylogeographic history of the afro-alpine region, which has experienced dramatic environmental changes during the Pleistocene glacial cycles.

These mountains are often referred to as isolated biological 'sky islands', as the plants they harbour often have their closest relatives in remote areas of the world rather than in the surrounding 'sea' of lowlands (Hedberg 1957, 1986). The vegetation on these mountains, which mainly are of Miocene to late Pliocene volcanic origin, is divided into three zones: the lowermost afro-montane forest zone, the transitional ericaceous zone and the uppermost afro-alpine zone proper (Hedberg 1951; Griffiths 1993). Dispersal among the different mountains is traditionally thought to be very rare and suggested to be mediated by cyclones, except that plants of the montane forest zone also may have dispersed through forest bridges established between neighbouring mountains during the interglacials (Hedberg 1957, 1969, 1970; Koch et al. 2006). During the cold and dry glaciations, the afro-alpine and ericaceous zones may have been pushed down by ~1000 m and covered larger areas than today, whereas the extent of the afro-montane forest zone was reduced in low-altitude areas because of the expansion of open, drought-tolerant vegetation (Flenley 1979; Bonnefille 1995; deMenocal 1995; Gottelli et al. 2004). During the warm and more humid interglacials, the afro-alpine and ericaceous zones contracted to higher elevations whereas the afro-montane forest expanded and may have formed intermountain forest bridges (Mohammed and Bonnefille 1998; Ryner et al. 2006; Kebede et al. 2007; Voje et al. 2009). Thus, the Pleistocene climatic oscillations have resulted in repeated formation of highly fragmented habitats, with the species of the ericaceous and afro-alpine zones currently restricted to their isolated high mountain refugia (Hedberg 1970).

So far, only few studies have addressed the phylogeographic history of afro-montane and afro-alpine plants. Each of three montane forest endemics studied contains distinct genetic groups suggesting long-term isolation, but also show evidence for gradual expansion consistent with formation of montane forest bridges between mountains during interglacials (*Lobelia giberroa* Hemsl., Kebede et al. 2007; *Hagenia abyssinica* J.F.Gmel., Ayele et al. 2009; *Prunus africana* (Hook.f.) Kalkman, Kadu et al. 2011, 2013). In the widespread afro-alpine and arctic-alpine *Arabis alpina* L., plastid DNA sequence variation suggested that the afro-alpine region was colonized twice from the north by two divergent lineages (Koch et al. 2006; Assefa et al. 2007). The 'African lineage' probably immigrated several glacial cycles ago and is currently widespread and subdivided into two phylogeographic groups, suggesting long-term isolation in at least two refugia but also occasional long-distance dispersal (Assefa et al. 2007; Ehrich et al. 2007). The later-arriving 'Asian lineage' is still confined to some Ethiopian high mountains, closest to the Arabian Peninsula. Finally, AFLP and plastid DNA variation in two afro-alpine heather species suggested that their phylogeographic histories are

conspicuously different, although they are closely related and often co-occur in the same habitats (Gizaw et al.2013). The more high-altitudinal *Erica trimera* (Engl.) Beentje consisted of several distinct genetic groups, which likely have been isolated for a long time. In contrast, *E. arborea* L. showed little geographic structuring and seems either to have recently colonized the studied area from a single source population or to experience extensive gene flow.

There is thus some evidence that there may be more dispersal among the African mountain enclaves (Assefa et al. 2007; Ehrich et al. 2007; Gizaw et al. 2013) than traditionally thought (Hedberg 1957, 1969, 1970). It is also possible that isolation and differentiation within species is strongest in those restricted to higher altitudes (Gizaw et al. 2013), in agreement with the forest bridge hypothesis and the observation that endemism increases with altitude (Hedberg 1969). Here we further explore these questions by taking advantage of extensive new material collected during our field expeditions to most of the high mountains in Ethiopia and East Africa (Uganda, Tanzania, and Kenya) from 2007 to 2009, aimed at providing a better basis for taxonomic, phylogeographic, and phylogenetic studies of afro-alpine plants. In this paper we address the phylogeographic history and taxonomy of three afro-alpine grass species, of which *Deschampsia angusta* Staff & C. E. Hubb. has been reported as endemic to two mountains (Mt Ruwenzori in Uganda/Democratic Republic of the Congo, and Mt Elgon in Kenya), and the two others, *D. cespitosa* (L.) P. Beauv. and *Koeleria capensis* (Steud.) Nees, are more widespread both within and outside our study area and show complex and unresolved taxonomic variation.

Three species of *Deschampsia* have been reported from the East African and Ethiopian mountains (Hedberg 1957; Clayton 1970). In addition to the morphologically similar *D. angusta* and *D. cespitosa* studied here, *D. flexuosa* (L.) Trin. is widely distributed in the afro-alpine region (now segregated as *Avenella flexuosa* (L.) Drejer; Chiapella 2007). *Deschampsia cespitosa* is variable morphologically and globally widespread, showing remarkable ITS sequence differences between accessions from different geographic regions (Chiapella 2007; African material was not included in his study). The species belongs to a complex group with many taxa described at the species, subspecies and variety level, which are difficult to separate based on morphology and may have been prone to hybridization and polyploidization (Chiapella 2007; Chiapella et al. 2011). *Deschampsia angusta* is reported to be restricted to the upper parts of Mt Ruwenzori (where the type locality is situated) and Mt Elgon, growing along streams and lakes and only known from a few collections from a narrow altitudinal belt (3600-4300 m; Hedberg 1957; Clayton 1970; Magombo et al. 2004). It occurs in less than five localities and is classified as vulnerable in the IUCN Red List (Magombo et al. 2004). In eastern Africa, *D. cespitosa* occurs in the Ethiopian Highlands and in the East African mountains Ruwenzori, Elgon, Aberdare and Kilimanjaro, where it grows in bogs and on moist ground in grassland, often along lakes and streams, mainly in the ericaceous belt and the lower part of the alpine belt (altitudinal range 2750-4300 m; Hedberg 1957; Clayton 1970; Phillips 1995). The chromosome number of *D. angusta* is unknown. *Deschampsia cespitosa* has invariably been reported as diploid ($2n = 26$) in eastern Africa (counts from the Ethiopian Simen and Galama Mts, and from the East African Mt Ruwenzori and Mt Elgon; Hedberg and Hedberg 1977), whereas both diploids and tetraploids have been reported from other geographic areas (Chiapella et al. 2011; Elven 2011).

Both *Deschampsia angusta* and *D. caespitosa* are tall, tussock-forming perennials and morphologically similar except in minor floral characters. The key characters provided by Clayton (1970) include spikelet length (7-8 mm in *D. angusta* vs 4-6.5 mm in *D. cespitosa*)

and shape of the lower glume (linear to narrowly lanceolate in *D. angusta* vs narrowly oblong to narrowly elliptic in *D. cespitosa*). According to Clayton et al. (2006 onwards), *D. angusta* has long (7-8 mm), linear spikelets, a long (6-7.5 mm), linear lower glume that is 0.8-0.9 the length of the upper glume, a long (7-8 mm) upper glume that is 1.7-1.8 the length of the adjacent fertile lemma, and fertile lemmas that are 4-veined with a dentate, 2-fid and obtuse apex, whereas *D. cespitosa* has shorter (4-6 mm), lanceolate or oblong spikelets, a shorter (4-6 mm), lanceolate lower glume that are equally long as the upper glume, a shorter (4-6 mm) upper glume that is 1.2 the length of the adjacent fertile lemma, and fertile lemmas that are 5-veined with a erose or dentate, 4-fid and truncate apex. Morphological variation has also been reported within *D. cespitosa* in the afro-alpine region. Hedberg (1957) accepted two varieties, var. *latifolia* (Hochst. ex A. Rich.) Hook. fil., which he reported from Mt Ruwenzori (3450-3800 m), Mt Elgon, Mt Aberdare, Mt Kilimanjaro and Ethiopia, and var. *oliveri* C. E. Hubb., which he reported as endemic to Mt Ruwenzori and occurring between 3050 m and 4000 m. The two varieties are reported to be distinguished according to the site of insertion of the awn on the back of the lemma, but were not accepted by Clayton (1970).

Koeleria capensis is the only species of *Koeleria* reported from the afro-alpine region (Hedberg 1957 (as *K. gracilis* Pers.); Clayton 1970; Phillips 1995). It is a widely distributed afro-temperate species occurring over large parts of the continent. In our study area in eastern Africa, *K. capensis* is found at altitudes between 1800 m and 5300 m, growing in upland grassland and moorland and sometimes on dry open ground with little vegetation (Clayton 1970). The species is a densely tufted perennial with erect culms up to 0.8 m long. Its old leaf sheaths shred into stiff brown slivers forming a dense, conspicuous tuft around the base of the culm, and it has a linear to lanceolate, 4-15 cm long panicle and laterally compressed spikelets with 1-3 fertile florets (Clayton 1970). It is closely related to the temperate European *K. macrantha* (Ledeb.) Schult. (*K. cristata* (L.) Pers.; *K. gracilis* Pers.); the African and European plants were formerly regarded as conspecific. The two species cannot be separated by spikelet characters, but the decaying leaf sheaths of *K. macrantha* are broader and remain more soft and papery, and do not split into segments forming an erect brush-like tuft as in *K. capensis* (Clayton 1970; Phillips 1995). *Koeleria capensis* has been reported as diploid ($2n = 14$) in six eastern African mountain areas (the Ethiopian Simen, Galama, and Bale Mts, and the East African Mt Kenya, Mt Kilimanjaro, and Cherangani Hills), and one tetraploid count ($2n = 28$) has been reported from Mt Kilimanjaro (Hedberg and Hedberg 1977). Polyploidy is common in European *Koeleria* taxa (Pecinka et al. 2006). *Koeleria capensis* is a polymorphic species showing large variation in spikelet size, plant compactness, and stiffness and pubescence of the leaves (Phillips 1995). Several varieties have been described. Two of them were tentatively recognized by Hedberg (1957) as occurring in the afro-alpine region in East Africa: the Kilimanjaro endemic var. *supina* (Domin) Hedb. and the widespread var. *convoluta* (Hochst. ex Steud.) Hedb., also occurring on Mt Kilimanjaro. Var. *supina* is reported to be distinguished from the more common variety by its smaller anthers, linear (vs lanceolate) panicle, and leaves that are almost as long as the flowering culm (vs shorter in the common variety), but Clayton (1970) viewed it as 'not sharply segregated' and did not formally accept it. Var. *supina* has been recorded from 3600 m to 4700 m on Mt Kilimanjaro, and var. *convoluta* from 2150 m to 4750 m on the same mountain, apparently without ecological differences between them (Hedberg 1957).

Here we analyze genetic variation using ALFP markers and morphological variation in key characters to assess the phylogeographic history and taxonomy of these three grass

species in the isolated high mountains in Ethiopia and East Africa. In particular, we address (i) whether the rare endemic *D. angusta* is genetically and morphologically distinct from the widespread *D. cespitosa*, justifying its recognition as a separate taxon and inclusion in the IUCN Red List, (ii) whether discernable genetic groups exist within *D. cespitosa* and *K. capensis*, and, if so, whether these groups correspond to previously reported infraspecific taxa, (iii) whether the different taxa have similar phylogeographic histories, and (iv) the relative importance of long-term isolation in different mountains versus dispersal among mountains for shaping phylogeographic patterns in the highly fragmented afro-alpine environment.

Materials and methods

Materials

Fresh young leaf material of *D. cespitosa, D. angusta,* and *K. capensis* was collected in the field from seven mountain systems in tropical East Africa (Kenya, Uganda, and Tanzania) and Ethiopia between 2007 and 2009 (Fig. 1; Appendix 1). Whenever possible, five individual plants representing one population were collected from 100 m x 100 m plots (one in each corner and one in the centre), and several widely separated populations were collected in each mountain. We were only able to find a single population that was identified to the rare endemic *D. angusta* based on Clayton (1970). We were able to collect *D. cespitosa* from three of the five mountain systems from where it has been reported (lacking Mt Elgon and the Aberdare Mts). A total of eight populations of *Deschampsia cespitosa*, one population of *D. angusta*, and 24 populations of *K. capensis* were collected. Leaf samples were dried in silica gel. Three voucher specimens from each population are deposited in the following herbaria: one in the Natural History Museum, University of Oslo (O); one in the National Herbarium of Ethiopia, Addis Ababa University (ETH), and the third voucher was deposited according to country of collection, i.e., East African Herbarium (EA), Kenya; Makerere University Herbarium (MHU), Uganda; and National Herbarium of Tanzania (NHT). Additional material for the morphometric analyses was obtained from the herbaria ETH, EA, MHU, and NHT (Appendix 2).

DNA extraction and AFLP fingerprinting

Total genomic DNA was extracted from the silica-gel-dried leaves using MoleStrips™ Plant DNA Kit with an automated GeneMole® extraction system following the manufacturer's instructions (Mole Genetics AS, Lysaker, Norway) with the following modifications. Leaf tissue was mechanically grounded in 2.0 mL tubes with two tungsten carbide beads for c. 2 min at 15 Hz in a mixer mill (MM301, Retsch GmbH & Co., Haan, Germany), 300 µL of lysis buffer was added to the crushed material, vortexed, centrifuged for 20 sec at 3400 Hz, incubated for 15 min at 65°C, and centrifuged at 14000 Hz for 3 min. Two hundred microliters of the lysate was loaded on the GeneMole® robot.

The AFLP protocol was optimized according to Gaudeul et al. (2000) except that the reaction mixture for the restriction ligation stage was incubated for 3 h; our reaction volumes in the polymerase chain reaction (PCR) were reduced by 50% following Kebede et al. (2007); we used 30 pre-PCR cycles instead of 25 and 13 selective PCR cycles instead of 12 (cf. Gaudeul et al. 2000); for each individual, 2.0 µL 6-FAM, 2.0 µL VIC and 3.0 µL NED labeled selective PCR products were mixed with 11.7 µL formamide and 0.3 µL GENESCAN ROX 500 size standard and run on an ABI 3100 sequencer (Applied Biosystems). For each of *Deschampsia cespitosa* and *Koeleria capensis*, 12 primers were tested on two samples from different geographic regions. The primer combinations resulting in high numbers of clear and well separated polymorphic bands were chosen for the final analysis. Three primer pairs (*EcoRI*-AGA-(6FAM)/*Msel*-CTG, *EcoRI*-AGG-(VIC)/*Msel*–CAT, and *EcoRI*-ACC-(NED)/*Msel*-CAT) were chosen for *D. cespitosa* and *D. angusta* and two (*EcoRI*-ATG-

(6FAM)/*Msel*-CGA, and *EcoRI*-ACA-(VIC)/*Msel*-CAC) for *K. capensis* (fluorescent dye in parentheses). For each individual, 2.0 µL 6-FAM, 2.0 µL VIC, and 3.0 µL NED labeled selective PCR products were mixed with 11.7 µL HiDi formamide and 0.3 µL GENESCAN ROX 500 size standard (Applied Biosystems, Foster City, CA, USA) and run on ABI 3100 sequencer (Applied Biosystems). The raw data for each primer combination were collected and analyzed using ABI prism GENESCAN version 3.7 analysis software (Applied Biosystems). Thereafter, the data were imported to GeneMapper version 4.0 (Applied Biosystems), and AFLP bands in the size range 50-500 base pairs (bp) were automatically scored as present (1) or absent (0). Reproducibility of the AFLP markers (error rate) was calculated according to Bonin et al. (2004). Approximately 10% of the samples from each of the two data sets (*Deschampsia* spp. and *Koeleria capensis*) were extracted twice and the whole AFLP procedure was repeated for the duplicated samples independently and finally scored as part of the entire data set for each species. A total of 31 plants of *Deschampsia* spp. (2 of *D. angusta* and 29 of *D. cespitosa*), and 93 plants of *Koeleria capensis* were successfully genotyped (Appendix 1).

AFLP data analyses

Genetic similarity among AFLP phenotypes was quantified using Dice's coefficient of similarity in NTSYS-PC version 2.02 (Rohlf 1990) and the pairwise relationships were graphically represented in principal coordinate analyses (PCoAs). Neighbor-joining (NJ) analyses were performed based on Nei and Li (1979) genetic distance using the software TREECON 1.3b (Van de Peer and De Wachter 1994). The trees were midpoint rooted and support for branches was estimated with 1000 bootstrap replicates. Genetic structure was examined by genetic mixture analysis using the STRUCTURE program v. 2.3.3 (Pritchard et al. 2000). The analysis was carried out at the Bioportal, University of Oslo (http://www.bioportal.uio.no) for K = 1-10 and 10 replicates per K, a burn-in period of 2×10^5 and 10^6 iterations. We used the recessive allele model to take into account the dominant nature of AFLP data (Falush et al. 2007). The admixture model with correlated allele frequencies were assumed for the analysis of each of the two data sets separately. The best value of K was chosen based on two criteria: the estimated posterior log probability of the data, $L(K)$, and the stability of assignment patterns across runs. Whenever $L(K)$ continued to grow slightly with increasing values of K, the most likely number of clusters was determined also by taking into account the rate of change in the probability between successive Ks, ΔK (Evanno et al. 2005). Analyses of Molecular Variance (AMOVAs) were performed to investigate population genetic structuring and differentiation at different hierarchical levels using ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Genetic diversity was estimated for each species, for each mountain system, for each sampling locality (population) within mountain systems and for the identified genetic (STRUCTURE) groups. The 95% confidence intervals (CI estimated with 1000 bootstrap replicates; Table 1 and Appendix 1) of the genetic diversity estimates were calculated to assess possible effects of sample size variation. We used the R-script AFLPdat (Ehrich 2006) to estimate the proportion of polymorphic markers (P) and Nei's gene diversity (D; corresponding to the average proportion of pair-wise differences between AFLP profiles, i.e. phenotypes; Kosman 2003). Genetic rarity for each mountain system and for each population was estimated as frequency-down-weighted marker values (DW or rarity) following Schönswetter and Tribsch (2005), using AFLPdat. Data conversions and

preparations of input files for most of the analyses were performed using the R-script AFLPdat (Ehrich 2006).

Morphometric analyses

Both our own herbarium material (Appendix 1) and previously collected, well-developed herbarium specimens (the identity of most of this material had previously been verified by experts, see Appendix 2) were used for morphometric analyses. Five measurements were made of each specimen for each character (if possible), and the individual mean values were used in further analyses. All width measurements are maximum widths. We selected characters emphasized in the literature to distinguish between taxa. For *Deschampsia*, we measured seven primary characters and calculated three ratio characters (Table 3) selected based on Clayton (1970) and Clayton et al. (2006 onwards), representing characters proposed to separate the endemic *D. angusta* from *D. cespitosa* and one character (position of the insertion of the awn on the lemma) suggested to separate the two varieties of *D. cespitosa* (cf. Hedberg 1957). We excluded spikelet width, as we found this character to vary with degree of spikelet maturity. The length of the lemma was measured without the awn. Initially, the awn was scored as inserted in the basal 1/3 (0), medium 1/3 (1) or upper 1/3 (2) part of the lemma, but as we found no variation (all except a single awn obtained the 0 score; cf. Results), we measured some of the material for distance (mm) from the point of insertion to the lemma base (cf. Table 3). For *Koeleria capensis*, we measured four primary characters and calculated two ratio characters (Table 4) tentatively suggested by Hedberg (1957) to separate two varieties. Only basal leaves were measured. We carried out calculations and performed nonparametric statistical testing (Mann-Whitney U test) and multivariate analysis (Principal Component Analysis, PCA) of the *Deschampsia* data using IBM® SPSS® Statistics v 21.

Results

Deschampsia - genetic variation

The final dataset contained 31 plants, representing one population referred to *D. angusta* and eight populations referred to *D. cespitosa*. Of the totally 171 fragments retained in the final matrix, 91.2% were polymorphic. Reproducibility was 97.9%. In the STRUCTURE analysis, $L(K)$ increased rapidly at $K = 2$ and then continued to grow gradually except for a decrease at $K = 5$, until it reached the highest value at $K = 6$. The assignment of individuals to $K > 2$ showed however inconsistent results among replicated runs. The rate of change in the probability between successive Ks, ΔK, clearly identified $K = 2$ as the most likely number of genetic groups, one containing the plants from Mt Ruwenzori and the other containing the plants from Mt Kilimanjaro and the Bale Mts (Fig. 2).

Both the NJ tree and the PCoA revealed three distinct groups corresponding to the three mountain areas, Mt Ruwenzori (100% bootstrap support), Mt Kilimanjaro (71%), and the Bale Mts (89%; Fig. 2). The populations from the Bale Mts and Mt Kilimanjaro appeared to be most similar to each other (99% bootstrap support in the NJ) and were separated from the Mt Ruwenzori populations along the first axis in the PCoA plot, which extracted 26.4% of the total variation, in accordance with the STRUCTURE analysis. The populations from the Bale Mts were separated from those from Mt Kilimanjaro along the second axis (11.1% of the variation).

The non-hierarchical AMOVA assigned most of the overall genetic variation (59.48%) to variation within populations (Table 2). One hierarchical AMOVA assigned 40.34% of the variation to variation among mountains and 8.06% to variation among populations within mountains. In another hierarchical AMOVA, 35.64% of the variation was attributed to variation between the two genetic groups identified in STRUCTURE (Table 2). Notably, the plants identified as the endemic *D. angusta* were not distinct from those identified as *D. cespitosa* based on AFLPs. They grouped with the Mt Ruwenzori populations of *D. cespitosa* in all analyses (Fig. 2). In an AMOVA, no significant proportion of the total variation was assigned to differences among the two species ($P = 0.1202$), in contrast to the high and significant percentage (35.53%) assigned to differences among populations (Table 2).

The total gene diversity pooled over all genotyped individuals was $D = 0.197$. The mean within-population gene diversity was 0.125 ($SD = 0.034$) and the mean proportion of polymorphic loci (P) was 19.75% (range 12.28-32.16%; Appendix 1). Genetic diversity pooled by mountains differed among mountains, with most diversity in Mt Ruwenzori ($D = 0.163$, $P = 50.88\%$) and least diversity in Mt Kilimanjaro ($D = 0.082$, $P = 14.62\%$, Table 1; Fig. 1). The 95% confidence intervals of D ranged from 0.029 to 0.234 (Table 1 and Appendix 1). Genetic rarity (DW) was quite similar in different mountains, ranging from 4.65 to 5.34.

Deschampsia - morphological variation

The total morphological variation observed in the afro-alpine material of *Deschampsia* was consistent with that described in the literature, but we found no clear discontinuities that could be used for consistent separation of different taxa (Table 3, Fig. 4). Rather, the variation in all characters seemed to be more or less continuous, with the specimens identified as *D. angusta*

tending to show extreme character values representing small and arbitrarily delimited parts of the total gradients of variation. Although the mean values of the specimens identified to the two different species were statistically significantly different in most of the characters, the total range and mean ± SD variation always overlapped. In the ordination analysis (PCA, Fig. 4), upper and lower glume width and length, spikelet length, upper glume length/lemma length ratio, and lower glume length/width ratio were most strongly correlated with Principal Component (PC) 1, whereas lower glume length/width ratio, upper glume length/lemma length ratio, lower glume length/upper glume length ratio, and various length characters were most strongly correlated with PC 2. The first two PCs explained 88.3% of the total variation.

We observed morphological differences between the two specimens we collected at the same site in Ruwenzori, tentatively identified as *D. angusta*, and also included in the AFLP analysis (population UG 2262, individual numbers 1 and 2; marked in Fig. 4 a-c). Whereas one of them showed typical '*angusta*' traits, some of the characters of the other plant were '*cespitosa*'-like, suggesting high intrapopulational variation. Notably, these two plants had very similar AFLP genotypes and both were clearly assigned to the Ruwenzori genetic group in the STRUCTURE analysis, together with all Ruwenzori plants identified as *D. cespitosa* (Fig. 2).

We did not observe distinct morphological differences between three genetic groups identified in the AFLP analysis (Figs 2, 4). The variation in position of the insertion of the awn on the lemma was examined to assess whether two varieties of *D. cespitosa* could be distinguished based on this character. Hedberg (1957) reported both of them to occur in Ruwenzori, one of them as endemic (var. *oliveri*) and the other (var. *latifolia*) to occur also in other afro-alpine mountains. We observed some variation in this character in the total material, with the awn on the average inserted farther from the base of the lemma in *D. angusta* (0.8 ± 0.20 mm) than in *D. cespitosa* (0.4 ± 0.32 mm; note that their lemma lengths are similar; Table 3). However, in a single specimen identified as *D. cespitosa* from Ruwenzori (UG 2528-2), we observed four spikelets with lemmas with typical insertion position (0.5 mm) and a single spikelet with a lemma with the awn inserted 1.6 mm from the base, demonstrating that occasional deviation in this character may occur within individual plants.

Koeleria - genetic variation

The final dataset contained 93 plants representing 24 populations. Of the totally 458 fragments retained in the final matrix, 59.4% were polymorphic. Reproducibility was 97.4%.

The STRUCTURE analysis of the total data set revealed a lack of genetic structure in *K. capensis* in the afro-alpine region. The mean value of $L(K)$ increased rapidly to $K = 2$ and then it leveled off at $K = 7$ (Fig. 3). Then it dropped at $K = 8$ and $K = 9$ before increasing again at $K = 10$. The rate of change in the probability between successive Ks, ΔK, clearly identified $K = 7$ (Fig. 3) as the most likely number of genetic groups. However, there was a lack of strong assignment patterns to one of the seven groups among individual plants, many of them showed admixture among the different groups, and there was no clear geographic patterns in the assignments. We also examined the assignment patterns for $K = 2$ and did neither find these reasonable; the majority of individuals (~70%) strongly assigned to the same of the two groups and each of the remaining plants symmetrically assigned to both of them. Thus, our

material of *Koeleria capensis* appeared to lack genetic structuring and rather seem to represent a single large panmictic population.

This finding was consistent with the results of the PCoA and NJ analyses (Fig. 3). The first PCoA axis extracted 13.5% of the total variation and separated three of the eight Mt Kilimanjaro populations from the remaining populations. These three divergent Mt Kilimanjaro populations also formed a cluster in the NJ tree with 69% bootstrap support (Fig. 3). The remaining populations were more or less intermixed in the NJ tree and along the second and third axes in the PCoA (Fig. 3).

The non-hierarchical AMOVA of the *K. capensis* data set assigned two third of the overall genetic variation (66.47%) to variation within populations (Table 2). In a hierarchical AMOVA, 15.09% of the total variation was found among the six sampled mountains and 20.42% of the variation among populations within mountains.

The total gene diversity pooled over all genotyped individuals was $D = 0.059$. The mean within-population gene diversity was 0.042 ($SD = 0.022$) and the mean proportion of polymorphic loci was 7.27% (range 3.28-19.43%; Appendix 1). Genetic diversity pooled by mountain showed very similar values among the six mountains, with slightly more diversity in the Simen Mts ($D = 0.062$) and slightly less in Mt Elgon ($D = 0.044$) and Mt Kilimanjaro ($D = 0.047$; Table 1). The 95% confidence intervals (CI) of D ranged from 0.010 to 0.127 (Table 1 and Appendix 1). Genetic rarity showed somewhat more variation, with the highest value in the Simen Mts (DW = 6.53) and the lowest in Mt Elgon (DW = 2.40).

Koeleria - morphological variation

We found considerable but continuous variation in the two ratio characters suggested by Hedberg (1957) to distinguish between two varieties in the afro-alpine material of *Koeleria capensis*; panicle shape (expressed as panicle width:length) and culm length relative to length of the basal leaves (culm length:leaf length; Fig. 5, Table 4). Some specimens, most of them from Kilimanjaro, grouped in the lower left of the scatter diagram (Fig. 5) and thus corresponded to the Kilimanjaro endemic var. *supina* as recognized by Hedberg by its linear panicles and leaves almost as long as the culm. Other specimens, including some from Kilimanjaro, corresponded to Hedberg's widespread (also reported from Kilimanjaro) var. *convoluta* by having more lanceolate panicles and culms that were longer than the leaves (Fig. 5). Interestingly, although this morphometric variation in Kilimanjaro appeared to be continuous, it seemed to correspond to the more distinct genetic variation observed in the PCoA and NJ analyses (Fig. 3). The genotyped specimens belonging to the compact group to the right in the PCoA plot and in the 69% bootstrap cluster in the NJ tree in Fig. 3 grouped in the lower left of the scatter diagram in Fig. 5, thus corresponding to var. *supina* in morphology.

Discussion

Our genetic and morphological analyses of new material from the widely separated African mountains have provided new insights into the history and taxonomy of the afro-alpine representatives of two grass genera. We were only able to locate a single population in the field that morphologically could be referred to *Deschampsia angusta*, a species classified as very rare and vulnerable by the IUCN and as endemic to two mountains. However, our results suggest that this population is not genetically distinct from those of the widespread *D. cespitosa*, and that these and other specimens identified as *D. angusta* seem to represent extremes of continuous gradients of morphological variation. We rather found that the afro-alpine material of *Deschampsia* consists of genetically distinct groups, suggesting persistence in isolation for a long time in different mountain areas. In contrast, we found no clear genetic structure in *Koeleria capensis*, suggesting that this species has either experienced extensive recent intermountain gene flow or colonized the entire investigated area much more recently than *D. cespitosa*.

In the morphometric analysis of our own and other afro-alpine material of *Deschampsia*, we found no clear discontinuities that could be used for consistent separation of different taxa (Table 3, Fig. 4). We found the total range of variation to be mainly consistent with that reported in the literature (Clayton 1970; Clayton et al. 2006 onwards), but any attempt to delimit distinct species along these variation gradients seems necessarily to be arbitrary (see e.g. Fig. 4). We do not put emphasis on our finding of statistically significant differences between the specimens identified as *D. angusta* and those identified as *D. cespitosa*, since selecting points from one extreme of a variation gradient can lead to significant differences also in the absence of a discontinuity that can be used as selection criterion. Interestingly, we found quite distinct differences in the reported key characters between specimens collected at a single site, suggesting that variation from '*angusta*-like' to '*cespitosa*-like' traits can be found within local populations. In our AFLP analysis, all plants of *Deschampsia* from Mt Ruwenzori (from where *D. angusta* originally was described) formed a single coherent genetic group, suggesting that these plants belong to a single species (Fig. 2). We neither found consistent variation in position of the insertion of the awn on the lemma corresponding to the two varieties described of *D. cespitosa*; it is possible that these varieties were described based on occasionally deviating awns as we observed within a single plant. We cannot exclude the possibility that some of the morphological variation observed in the afro-alpine material of *Deschampsia* (Fig. 4) reflects variation in one or a few genes affecting lengths and shapes of floral parts, but this variation seems nevertheless to be taxonomically insignificant. Based on our combined morphological and genetic analyses, we therefore suggest to recognize only a single variable species of *Deschampsia* in the afro-alpine region, *D. cespitosa*, without any intraspecific taxa.

A similar conclusion was made by Chiapella et al. (2011) in their study of the *D. cespitosa* complex (including several described taxa) in North America, based on 55 morphological characters and sequences of ITS and one plastid marker. They concluded that their overall evidence points to existence of only a single variable species, *D. cespitosa*, and that "the characters used to define these taxa might have overemphasized the recognition of entities that are essentially part of a continuous gradient of variation in North American *D. cespitosa*". Notably, their list of characters included most of those reported to distinguish *D. angusta* from *D. cespitosa* in the afro-alpine region, and the variation they observed in e.g.

glume length and width in the North American material was similar to that observed in our material (Fig. 1 in Chiapella et al. 2011).

In our genetic analysis of *Deschampsia*, we found distinct genetic differentiation corresponding to the three mountain areas investigated: the East African Mt Ruwenzori and Mt Kilimanjaro and the Ethiopian Bale Mts (Figs. 1, 2; Table 2). The most distinct division was observed between Mt Ruwenzori, which is situated along the western branch of the Rift system, and the eastern Rift Mountains Kilimanjaro and Bale. The highest genetic diversity and rarity were found in the Ruwenzori populations, but genetic rarity was pronounced and fairly similar in all three areas (DW = 4.65-5.34; Table 1), suggesting that each of them have been isolated at least during the last glacial cycle. The lower diversity in Mt Kilimanjaro may thus have been caused by refugial bottlenecking rather than by recent colonization by a few founder individuals. We were not able to find *Deschampsia* during our field work in the two other mountains from where it has been reported to occur (Mt Elgon and Aberdare Mts). Inclusion of material from these mountains would however not have changed the result that the three currently analyzed mountains contain very divergent genotypes. The Elgon and Aberdare plants might represent additional distinct genetic groups or bridge the clear genetic structuring observed here, but this would not affect the conclusions of 1) the species has a long history in each of the three mountains analyzed here and 2) no recent dispersal has occurred among those three mountains.

Koeleria capensis appears to have experienced a phylogeographic history in the afro-alpine region that is conspicuously different from that inferred for *Deschampsia cespitosa*. Whereas only 15.09% of the AFLP variation in *K. capensis* was attributed to variation among mountains in the AMOVA analysis (Table 2), the corresponding amount was 40.34% in *D. cespitosa*, suggesting a much shorter history or extensive dispersal of *K. capensis*. In *K. capensis*, our STRUCTURE analysis did not reveal any meaningful genetic grouping, consistent with the lack of geographic structure in the NJ and PCoA analyses (Fig. 3), where the AFLP phenotypes from different mountains were to a large degree intermingled. Furthermore, this species harboured very little genetic diversity in all six mountain areas examined (D = 0.044-0.062; Table 1), and little genetic rarity (DW = 2.40-3.81) except in the Ethiopian Simen Mts (DW = 6.53). This result suggests recent colonization of our study area from a single source population, or possibly extensive recent intermountain dispersal combined with bottlenecking in small populations (Figs. 1, 3). Our results seem most consistent with a scenario involving colonization of each mountain by a few founder individuals, probably following long-distance dispersal during or after the last glaciation. The higher rarity and slightly higher diversity observed in the Simen Mts might indicate that the remaining mountains were colonized from northern Ethiopia. However, further insights into the colonization history of *K. capensis* necessitate more extensive sampling, since this species is widespread in Africa.

We found however some genetic differentiation among different *Koeleria* populations from one of the mountains (Kilimanjaro) that may correlate with Hedberg's (1957) report of morphological variation in this mountain. Some of the plants from Kilimanjaro formed a quite distinct group both in the NJ and PCoA analyses (Fig. 3), corresponding to the Kilimanjaro endemic var. *supina* in our morphological analysis (Fig. 5). Although we analyzed only a few individual plants from Mt Kilimanjaro both for morphology and AFLPs, the results may indicate that it is reasonable to recognize two taxa in the afro-alpine material of *Koleria capensis*, but only at the variety level (as var. *supina* and var. *convoluta*, in agreement with

Hedberg) since the morphological variation appears continuous (Fig. 5). Further studies including determination of ploidy levels are however necessary, since the species is widespread also outside our study area and since variation in ploidy level also has been reported (Hedberg and Hedberg 1977). Whereas only diploid chromosome numbers have been reported from other mountains, both diploid and tetraploid numbers have been reported from Mt Kilimanjaro, and it is possible that the variation we observed correlates with ploidy level variation.

The conspicuously different histories inferred for *D. cespitosa* and *K. capensis* in the afro-alpine region might be based on different dispersal abilities. However, *D. cespitosa* is believed to be able to disperse over large distances in the Arctic, both via seeds and pollen (Chiapella et al. 2011). It is possible that the differences rather are associated with differences in population sizes and ecological requirements. Whereas *D. cespitosa* typically occurs in high-altitude, locally moist sites in tropical East Africa and Ethiopia (altitudinal range 2750-4300 m) and has limited geographic distribution (Hedberg 1957; Clayton 1970; Phillips 1995), *K. capensis* is more widespread in the afro-alpine region and commonly occurs in drier sites, also in the montane forest belt (altitudinal range 1800-5300 m; Clayton 1970). *Koeleria capensis* might have higher probability for long-distance dispersal than *D. cespitosa* because of higher seed production in larger populations. The abundant habitats available for *K. capensis* may also have increased its probability of successful establishment after long-distance dispersals. In addition, by being able to grow at lower altitudes and especially in drier areas than *D. cespitosa*, dispersal of *K. capensis* might have been facilitated by stepwise intermountain migration across dry lowland areas during colder periods (cf. Hedberg 1969). Interestingly, we recently found similar differences between the two afro-alpine heather species *Erica trimera* and *E. arborea*, of which the more high-altitudinal *E. trimera* consisted of several distinct genetic groups that likely have been isolated for a long time, whereas the more low-altitudinal *E. arborea* showed very little geographic structuring (Gizaw et al. 2013).

Deschampsia cespitosa showed an overall genetic diversity of $D = 0.197$ in the afro-alpine region (Appendix 1), which is less than the averages reported for long-lived perennial (0.25), outcrossing (0.27), and late successional (0.30) plant taxa (Nybom 2004). We observed much less diversity in afro-alpine *K. capensis* ($D = 0.059$), a much lower value than the averages reported by Nybom (2004). The low diversity in this species may be caused by repeated founder effects during recent colonization of our study area, possibly from a source population situated outside it.

We found that the reported endemic *D. angusta* probably should be regarded as conspecific with the variable *D. cespitosa* and thus should be considered to be removed from the IUCN Red List. However, we found that afro-alpine *D. cespitosa* contains three distinct genetic groups that appear to have been isolated for a long time and may warrant recognition in conservation. Genetically distinct populations are often suggested as candidates for conservation to prevent the loss of unique genetic variants (Kebede et al. 2007; Geleta et al. 2008). We therefore propose to treat the identified three groups corresponding to Mt Kilimanjaro, Mt Ruwenzori, and the Bale Mts (Fig. 1) as evolutionarily significant units (ESUs; Moritz 1994). The ESU concept was developed to provide a rational basis for prioritizing populations for conservation efforts, given that resources are limited and that existing taxonomy may not adequately reflect underlying genetic diversity (Moritz 1994). It is also possible that the populations of *D. cespitosa* previously reported from Mt Elgon and the

Aberdare Mts, which we were not able to include in our analyses, also represent distinct genetic entities and thus separate ESUs.

Acknowledgements

We thank the Tanzanian National Parks Authority (TANAPA), Uganda Wildlife Authority, Bale Mountains National Park and Simen Mountains National Park in Ethiopia, and National Museums of Kenya for permission to conduct field work. We are grateful to the staff at the following institutions for help with curation of our specimens: National Herbarium - Addis Ababa University (ETH), Natural History Museum - University of Oslo (O), the East African Herbarium - National Museum of Kenya (EAH), Makerere University Herbarium (MHU), Sokoine University of Agriculture (SUA) and National Herbarium of Tanzania (NHT); and to the editor and two anonymous reviewers for valuable suggestions to improve the paper. This study was funded by the Norwegian Programme for Development, Research and Higher Education (NUFU) project 2007/10058 (AFROALP-II - Afroalpine 'sky islands': genetic versus taxonomic biodiversity, climate change, and conservation) headed by S. Nemomissa and C. Brochmann.

References

Assefa A, Ehrich D, Taberlet P, Nemomissa S, Brochmann C (2007) Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine *Arabis alpina*. Heredity 99:33-142

Ayele TB, Gailing O, Umer M, Finkeldey R (2009) Chloroplast DNA haplotype diversity and postglacial recolonization of *Hagenia abyssinica* (Bruce) J.F. Gmel. in Ethiopia. Plant Syst Evol 280:175-185

Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13:3261–3273

Bonnefille R (1995) A reassessment of the Plio-Pleistocene pollen record of East Africa. In: Vrba ES, Denton GH, Partridge TC, Burckle LH (eds) Paleoclimate and evolution with emphasis on human origins. Yale University Press, New Haven, pp 299-310

Chiapella JO (2007) A molecular phylogenetic study of *Deschampsia* (Poaceae: Aveneae) inferred from nuclear ITS and plastid *trn*L sequence data: support for the recognition of *Avenella* and *Vahlodea*. Taxon 56:55-64

Chiapella JO, DeBoer VL, Amico GC, Kuhl JC (2011) A morphological and molecular study in the *Deschampsia cespitosa* complex (Poaceae; Poeae; Airinae) in northern North America. Am J Bot 98:1366-1380

Clayton WD (1970) Gramineae I. In: Milne-Redhead E, Polhill RM (eds) Flora of Tropical East Africa. Crown Agents, London, pp 1-176

Clayton WD, Vorontsova MS, Harman KT, Williamson, H (2006 onwards) GrassBase. The Online World Grass Flora. http://www.kew.org/data/grasses-db.html. Accessed 28 November 2012

deMenocal PB (1995) Plio-Pleistocene African climate. Science 270:53-59

Ehrich D (2006) AFLPdat: a collection of R functions for convenient handling of AFLP data. Mol Ecol Notes 6:603-604

Ehrich D, Gaudeul M, Assefa A, Koch MA, Mummenhoff K, Nemomissa S, Brochmann C (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. Mol Ecol 16:2542-2559

Elven R (2011) Annotated Checklist of the Panarctic Flora (PAF). Vascular plants. http://nhm2.uio.no/paf/. Accessed 5th December 2012

Engler A (1892) Uber die Hochgebirgsflora des tropischen Afrika. Berlin, Germany: Verlag der Konigl. Akademie der Wissenschaften

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611-2620

Excoffier L, Lischer HEL (2010) Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564-567

Falush D, Stephens M, Pritchard J (2007) Inference of population structure using multilocus genotype data: dominant markers and null allele. Mol Ecol Notes 7:574-578

Flenley J (1979) The Equatorial Rain Forest: A Geological History. Butterworth, London

Fries RE, Fries TCE (1922) Die Riesen-Lobelien Afrikas. Sven Bot Tidskr 16:383-416

Gaudeul M, Taberlet P, Till-Bottraud I (2000) Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol Ecol 9:1625-1637

Geleta M, Bryngelsson T, Bekele E, Dagne K (2008) Assessment of genetic diversity of Guizotia abyssinica (L.f.) Cass. (Asteraceae) from Ethiopia using amplified fragment length polymormophism. Plant Genet Resour 6: 41 - 51.

Gizaw A, Kebede M, Nemomissa S, Ehrich D, Bekele B, Mirré V, Popp M, Brochmann C (2013) Phylogeography of the heathers *Erica arborea* and *E. trimera* in the afro-alpine 'sky islands' inferred from AFLPs and plastid DNA sequences. Flora 208:453-463.

Gottelli D, Marino J, Sillero-Zubiri C, Funk SM (2004) The effect of the last glacial age on speciation and population genetic structure of the endangered Ethiopian wolf (*Canis simensis*). Mol Ecol 13:2275-2286

Griffiths CJ (1993) The geological evolution of East Africa. In: Lovett JC, Wasser SK (eds) Biogeography and Ecology of the Rain Forests of Eastern Africa. Cambridge University Press, Cambridge, pp 19-23

Hedberg O (1951) Vegetation belts of the East African mountains. Sven Bot Tidskr 45:140-202

Hedberg O (1957) Afroalpine vascular plants. a taxonomic revision. Symb Bot Ups 15:1-411

Hedberg O (1969) Evolution and speciation in a tropical high mountain flora. Bot J Linn Soc 1:135-148

Hedberg O (1970) Evolution of the afroalpine flora. Biotropica 2:16-23

Hedberg O (1986) Origin of the afroalpine flora. In: Vuilleumier F, Monastero M (eds) High Altitude Tropical Biogeography. Oxford University Press, New York, pp 443-468

Hedberg I, Hedberg O (1977) Chromosome numbers of afroalpine and afromontane angiosperms. Bot Not 130:1-24

Hedberg I, Hedberg O (1979) Tropical-alpine life-forms of vascular plants. Oikos 33:297-307

Kadu CA, Schueler S, Konrad H, Muluvi GM, Eyog-Matig O, Muchugi A, Williams VL, Ramamonjisoa L, Kapinga C, Foahom B, Katsvanga C, Hafashimana D, Obama C, Geburek T (2011) Phylogeography of the Afromontane *Prunus africana* reveals a former migration corridor between East and West African highlands. Mol Ecol 20:165-178

Kadu CA, Konrad H, Schueler S, Muluvi GM, Eyog-Matig O, Muchugi A, Williams VL, Ramamonjisoa L, Kapinga C, Foahom B, Katsvanga C, Hafashimana D, Obama C, Geburek T (2013) Divergent pattern of nuclear genetic diversity across the range of the Afromontane *Prunus africana* mirrors variable climate of African highlands. Ann Bot 111:47-60

Kebede M, Ehrich D, Taberlet P, Nemomissa S, Brochmann C (2007) Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. Mol Ecol 16:1233-1243

Koch MA, Kiefer C, Ehrich D, Vogel J, Brochmann C, Mummenhoff K (2006) Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (Brassicaceae). Mol Ecol 15:825-839

Kosman E (2003) Nei's gene diversity and the index of average differences are identical measures of diversity within populations. Plant Pathol 52:533-535

Magombo ZLK, Mbeiza Mutekanga N, Ndiritu GG (2004) Freshwater Biodiversity Assessment workshop (Uganda. Dec' 2003), *Deschampsia angusta*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. http://www.iucnredlist.org Accessed 25 November 2012

Mohammed MU, Bonnefille R (1998) A late Holocene pollen record from a highland peak at Tamsaa, Bale Mountains, South Ethiopia. Global Planet Change 16-17:121–129

Moritz C (1994) Defining 'evolutionary significant units' for conservation. Trends Ecol Evol 9:373-375

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143-1155

Pecinka A, Suchankova P, Lysak MA, Travnicek B, Dolezel J (2006) Nuclear DNA content variation among Central European *Koeleria* taxa. Ann Bot 98:117-122

Phillips S (1995) Poaceae (Gramineae). In: Hedberg I, Edwards S (eds) Flora of Ethiopia and Eritrea, vol. 7. Addis Ababa: The National Herbarium, Addis Ababa University and Department of Systematic Botany, Uppsala University, Addis Abab and Uppsala, pp 33-38

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Richard A (1847-1851) Tentamen Florae Abyssinicae. Paris: Arthus Bertrand. Reprinted by Uppsala University, 1982

Rohlf F (2000) NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System. Version 2. 11a. Exeter Software, Setauket (NY)

Ryner MA, Bonnefille R, Holmgren K, Muzuka A (2006) Vegetation changes in Empakaai Crater, northern Tanzania, at 14 000–9 300 cal yr BP. Rev Palaeobot Palynol 140:163-174

Schönswetter P, Tribsch A (2005) Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). Taxon 54:725-732

Van de Peer Y, De Wachter R, (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Appl Biosci 10:569-570

Voje KL, Hemp C, Flagstad O, Saetre GP, Stenseth NC (2009) Climatic change as an engine for speciation in flightless *Orthoptera* species inhabiting African mountains. Mol Ecol 18:93-108

Table Captions

Table 1 Average gene diversity and genetic rarity based on AFLP data for populations of *Deschampsia cespitosa* s.lat. and *Koeleria capensis*, pooled by mountains and by genetic (STRUCTURE) groups. Country: ET - Ethiopia, KN - Kenya, TZ - Tanzania, UG - Uganda; *n* - number of individuals successfully analyzed; *P* - percentage of polymorphic loci; DW - frequency-down-weighted marker value as a measure of genetic rarity; *D* - gene diversity; CI - 95% confidence interval

Table 2 Analyses of molecular variance (AMOVAs) based on AFLP data for nine populations of *Deschampsia cespitosa* s.lat. (i.e. including *D. angusta*) and 24 populations of *Koeleria capensis*. *P*-values were estimated in a permutation test (1000 permutations)

Table 3 Morphological variation in *Deschampsia cespitosa* s. lat. (including *D. angusta*). For each character in each specimen, five measurements (if possible) were made and a mean value was calculated and used to calculate the grand mean for each putative species. Lemma length was measured without the awn. N - number of measurements, N_{ind} - number of individual specimens measured. Measurements in mm. Significant differences ($p < 0.001$) according to Mann-Whitney U tests are indicated in bold.

Table 4 Morphological variation in *Koeleria capensis*. For each character in each specimen, five measurements (if possible) were made and a mean value was calculated and used to calculate the grand mean for each area. N - number of measurements, N_{ind} - number of individual specimens measured. Measurements in mm. PanicleL – Panicle length; PanicleW – Panicle width, maximum; CulmL – Culm length excluding panicle; BLeafL – Length of basal leaf; PanicleWLratio – Panicle width/ Panicle length; CulmBLeafratio – Culm length/ Length of basal leaf.

Figure Captions

Fig. 1 Sampling sites, AFLP-based estimates of genetic diversity (*D*) and frequency-down-weighted marker value (DW) as a measure of genetic rarity in *Deschampsia cespitosa* s.lat. (i.e. including *D. angusta*) and *Koeleria capensis*. The size of the circles is proportional to within-mountain gene diversity and the shading reflects genetic rarity in each mountain. Different circle sizes and shadings (from white – minimum to black – maximum) represent one quartile each of the distribution of *D* and DW, respectively. The stippled lines represent the Great Rift Valley system

Fig. 2 Mid-point rooted neighbour-joining tree based on Nei and Li's (1979) distance (left, with bootstrap values above 50% estimated from 1000 replicates), detection of the number of groups (*K*) in STRUCTURE analysis (top right; mean value of the log probability of the data, $L(K)$, and the rate of change in the probability between successive runs, (ΔK; Evanno et al.

2005) as a function of *K* ranging from 1 to 10), and Principal Coordinates Analysis (PCoA; bottom right) based on Dice's coefficient of similarity among the AFLP phenotypes observed in 31 plants (nine populations) of *Deschampsia cespitosa* s.lat. Filled and open symbols correspond to the two genetic groups inferred by STRUCTURE

Fig. 3 Mid-point rooted neighbour-joining tree based on Nei and Li's (1979) distance (left, with bootstrap values above 50% given for the main branches, estimated from 1000 replicates), detection of the number of groups (*K*) in STRUCTURE analysis (top right: mean value of the log probability of the data, *L*(*K*), and the rate of change in the probability between successive runs, (Δ*K*; Evanno et al. 2005) as a function of *K* ranging from 1 to 10), and Principal Coordinates Analysis (PCoA; right, middle and bottom) based on Dice's coefficient of similarity among the AFLP phenotypes observed in the 93 plants (24 populations) of *Koeleria capensis*

Fig. 4 Morphological variation among individual specimens of *Deschampsia cespitosa* s. lat. (including *D. angusta*). For each character in each specimen, a mean value was calculated based on five measurements (if available). The two specimens identified as *D. angusta* in our own material and genotyped with AFLPs (see Fig. 2) were collected at the same site and are here indicated with their population/individual number (UG 2262-1 and UG 2262-2). Other specimens identified as *D. angusta* are indicated with their collection number (cf. Appendix 2). a - scatter diagram showing variation in lower glume length vs width. b - scatter diagram showing variation in upper glume length vs width. c - ordination (PCA) plot depicting variation among individuals based on eight characters. d - the corresponding ordination plot showing correlation between individual characters and principal components (cf. Table 3)

Fig. 5 Scatter diagram showing morphological variation among individual specimens of *Koeleria capensis*, based on two characters suggested to separate the Kilimanjaro endemic var. *supina* from the widespread var. *convoluta* by Hedberg (1957; who also reported the latter from Kilimanjaro). For each character in each specimen, a mean value was calculated based on five measurements (if available). Specimens from Kilimanjaro that also were genotyped using AFLPs are indicated as c (belonging to the compact group to the right in the PCoA plot and in the 69% bootstrap cluster in the NJ tree in Fig. 3) or s (belonging to the scattered 'group' to the left in the PCoA and scattered in the NJ tree in Fig. 3). Specimens placed in the lower left of the scatter diagram had linear panicles (as opposed to more lanceolate ones) combined with basal leaves that were more or less as long as the culm (as opposed to basal leaves that were shorter than the culm), thus corresponding to var. *supine*)

Appendix Captions

Appendix 1 Geographic origin, DNA bank ID of the DNA bank database of the Natural History Museum, University of Oslo (O), AFLP-based gene diversity and genetic rarity in populations of *Deschampsia* spp. and *Koeleria capensis* sampled during our own field work. *n* - number of individuals successfully analyzed; country: ET - Ethiopia, KN - Kenya, TZ - Tanzania, UG - Uganda; *P* - percentage of polymorphic loci; DW - frequency-down-weighted marker value as a measure of genetic rarity; *D* - gene diversity; *SD* - standard deviation; CI - 95% confidence interval

Appendix 2 Geographic origin and voucher information for the additional herbarium material used for morphometric analyses (ETH - National Herbarium of Ethiopia, Addis Ababa University; EA - East African Herbarium, Kenya; MHU - Makerere University Herbarium, Uganda; NHT - National Herbarium of Tanzania)