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Genome scan of Kenyan *Themeda triandra* populations by AFLP markers reveals a complex genetic structure and hints for ongoing environmental selection

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ABSTRACT

Tropical and subtropical rangeland systems provide core ecosystem services for the welfare of human populations that rely on readiness and quality of forage resources. However, forage species are still widely overlooked by molecular biology studies. In the present study, we employ 366 AFLP markers to provide the first description of the genetic landscape of three Kenyan populations of *Themeda triandra* Forssk., a key wild grass forage species. By including Australian *T. triandra* accessions and other closely related species in a molecular phylogeny, we provide a first evaluation of the relationships existing between African and Australian germplasm. Genetic diversity, population genetic structure and recombination rates in Kenyan *T. triandra* populations were investigated in detail. GPS coordinates of each sampled population were used to retrieve meteorological data at specific locations, and environmental factors likely contributing to *T. triandra* genetic differentiation were taken into consideration using a correlative approach based on outlier loci distribution. The use of molecular markers unveiled some previously unknown aspects about the biology of *T. triandra*, namely: i) African and Australian *T. triandra* genotypes analyzed in this study are genetically undistinguishable, ii) sexual recombination of Kenyan *T. triandra* is likely to play a major role in its reproduction, and iii) environmental characteristics of the collection sites are correlated with the allelic distribution of a limited set of loci under selection.

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1. Introduction

The vast tropical and subtropical rangeland ecosystems of the world are experiencing severe alterations driven by climate change (Guenther et al., 1999; Parton et al., 1995; Tubiello et al., 2007). At the same time, and even more swiftly, land use in emerging countries is facing a dramatic change driven by the increase of meat consumption, referred to as the "livestock revolution" (Delgado et al., 1999, 2001). Such pressures modify rangeland mode of exploitation and pose a threat to the natural populations of perennial grasses that contribute in building up arid land resilience, a dam to drastic ecosystem shifts (Shaffer et al., 2001). Given that sufficient molecular diversity is present, natural populations can withstand these changes through plasticity and genetic changes (Hoffmann and Sgrò, 2011), yet often altering their historical geographical distribution (Parmesan and Yohe, 2003). As a result, pastures are displaced and degraded, and human populations relying on nomadic pastoralism see their cattle grazing areas' reduced, eventually leading to overgrazing and further pasture depletion (Jarvis, 1984). This unstable situation ultimately spirals into a condition of rural poverty at the base of violent micro-conflicts (Meier et al., 2007). A deeper understanding of agronomical biodiversity of key resources for human communities should thus be a primary objective.

Based on these premises our study focuses on one of the key wild grassy forage resources in rangeland ecosystems, *Themeda triandra* Forssk. *T. triandra* is a C_4 perennial grass distributed throughout many tropical and subtropical regions of the world, including tropical and temperate Asia, Australia, Africa and Turkey. *Themeda australis* (R. Br.) Stapf, which some authors identify as synonymous with Australian *T. triandra*, is also a forage resource mostly studied in regard to soil remediation, as a vegetation filter, and as a competitor for exotic invasive species. Because of its relatively high protein content within the *Poaceae* family, *Themeda* represents a valuable forage resource for cattle and wildlife especially in Africa and, in general, in dry grasslands (Dell'Acqua et al., 2013). Natural populations of *T. triandra* comprise diploid (2n = 20) and polyploid races (Liebenberg et al., 1993), which differ mainly in their mode of reproduction. Diploids reproduce mainly sexually (Woodland, 1964), tetraploids as facultative apomictics, and those with higher ploidy levels clonally as compelled apomictics (Birari, 1980). The balance between sexual and non-sexual reproduction

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in diploid and tetraploid *T. triandra* can also be influenced by abiotic factors such as day length and temperature (Evans and Knox, 1969). Similarly, rainfall contributes in shaping the *T. triandra* mode of reproduction (Van Rensburg et al., 1999) and influences its geographical distribution (Smith and Yeaton, 2005). Despite its socio-economic relevance in developed and developing countries, *T. triandra* has never been investigated with molecular tools. This would be a powerful approach to provide an up-to-date description of *T. triandra* in terms of diversity, mode of reproduction and adaptation within a specific environmental frame, a sensible step to push forward our comprehension of the dynamics underlying its geographical distribution.

Genetic approaches aimed at dissecting the biodiversity of species for which molecular characterization is lacking are not straightforward. In this regard, amplified fragment length polymorphisms (AFLPs; Vos et al., 1995) represent a fast and reliable tool to produce a large number of genome-wide genetic markers, thus rendering a detailed description of the extant genetic variability very useful in many molecular biology fields including population genetic studies (Mueller and Wolfenbarger, 1999). Though AFLP markers bear the limitation of the lack of a priori knowledge about their genomic location and their dominant nature, recent developments of statistical tools have proven them able to yield remarkable data on non-model species (Meudt and Clarke, 2007; Muluvi et al., 2003). This fostered AFLP genome scans as a solid alternative to whole-genome sequencing, providing substantial advances in population genetic studies when the latter is not feasible (Luikart et al., 2003). Their employment in an explicit geographical framework can shed light on genetic differentiation of natural populations both in terms of genome-wide diversity and past and current signatures of allelic displacement. Reproduction mode can also be addressed by the observation of the amount of genetic recombination occurring between ALFP markers in each population. None of these measures relies on the knowledge of markers' location, benefiting instead from AFLP data spanning the whole genome. The anonymous nature of AFLP can also be worked around by exploiting them as meta-signals of the functions played by genetic elements in genetic linkage with them. This makes AFLP useful in describing past and present adaptive processes affecting genotypes through the observation of outlier locus distribution among populations (Stinchcombe and Hoekstra, 2007). This approach is fruitfully exploited by landscape genetics (Manel, 2012), which exploits locus-based differentiation and climatic data to address the environmental processes shaping the genetic structure of populations (Holderegger and Wagner, 2008; Manel et al., 2003). Genetic differences among ecotypes can in fact be ascribed to locus-specific divergence (Bonin et al., 2006; Coyer et al., 2011), even in the presence of diffused gene flow. This follows from the concept of a porous genome, which entails a set of recognizable differences in a few loci over a background of diffused, low, neutral genetic variation (Gavrilets and Vose, 2005; Nosil et al., 2007). Albeit the identification of functional loci is not forthright when information on candidate genes is lacking, correlative approaches can be useful in identifying environmental forces shaping genomes in qualitative and quantitative terms.

Here we present the first molecular characterization of 71 *Themeda* genotypes, 65 of which are from three sites along a historical cattle migration route in Kenya. The first objective of this study was the evaluation of the current pattern of genetic variation and the description of the relationships existing between Kenyan *T. triandra* natural populations. By the inclusion of some Australian accessions, we also aimed at evaluating the genetic relationships occurring between specimens from two key *Themeda* distribution areas, Africa and Australia. We then focused on Kenyan accessions to provide the first molecular evaluation of the reproduction habits of *T. triandra*. This was meant especially to deliver an improved definition of the blurred line separating sexual and clonal reproduction in tetraploid karyotypes. Furthermore, we aimed at the identification of *T. triandra* ecotypes adapted to specific environments by correlating differences at genomic loci under selection to climatic characteristics of the sampling areas. The employment of AFLP markers provided a general picture of the extant genetic variation in Kenyan *T. triandra* within the broader frame of a historical transhumance path. The results of the phylogenetic analysis provided evidences of unexpected genetic uniformity between African and Australian *Themeda*. Kenyan *T. triandra*, reported tetraploid by a karyotypic analysis, showed unusual rates of sexual reproduction well higher than expected. Finally, molecular signatures of animal-driven displacement of genotypes and environmental selection at a genetic level provided the first evidence of genetic adaptation to temperature and rainfall conditions in Kenyan *T. triandra* genotypes.

2. Material and methods

2.1. Material collection and DNA extraction

T. triandra individuals (65) were collected during December 2005 in three broad regions located along a former Kenyan pastoral migration route: from north to south, i) Gallmann (11 samples) in Pokot District, ii) Delamare (28 samples) in the Rift Valley lake region, and iii) Campi Ya Kanzi (26 samples) between Mt. Kilimanjaro and the Chyulu hills. As the presence of *T. triandra* was generally sparse and never continuous, samples were collected in random plots by dividing the broader regions in smaller sampling areas (4 in Campi Ya Kanzi, 4 in Delamare and 2 in Gallmann): seeds and leaves from 4 to 8 individuals were sampled in each of the smaller areas. Seed bags and tissue vials were given a code representing their geographical origin (C = Campi Ya Kanzi; D = Delamare; G = Gallmann) and a sequential number as sample ID. The whole germplasm was grown to maturity at the Milan University Botanical Gardens. *T. triandra* seeds from Botswana (2 samples) were provided by the RGB Kew Millennium Seed Bank (Serial No. 0090711 and No. 0090630), while seeds from Australia (2 samples) were obtained from the Australian Botanic Garden of Canberra. *Themeda arguens* (L.). Hack. from Indonesia (2 samples) was obtained from Bogor Botanical Gardens. *Sorghum bicolor* (L.) Moench (1 sample) was provided by the Milan University Botanical Gardens. Genomic DNA was extracted from leaf tissues with GenElute plant genomic DNA miniprep kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. In total, 72 individuals were analyzed (Table 1).

2.2. Karyotype analysis

Seeds were germinated on moist sterilized filter paper with 16 h light/8 h dark at 22 °C. The ploidy level was evaluated by analyzing 5 roots for each specimen from Kenya, Indonesia, Botswana and Australia. Root apexes were treated with HCl 1 N (8 min), washed in distilled H_2O and stained using a DAPI solution (0.2 μg/μL). Samples were observed with an Axio Imager Zeiss microscope at 1000× enlargements, and chromosomes were counted in mitotic cells.

2.3. AFLP reactions

AFLP reactions were carried out by cleaving 250 ng of genomic DNA from each plant using 5 U of EcoRI and 5 U of MseI restriction enzymes (New England Biolabs, Ipswich, MA, USA) at 37 °C for 2 h in 20 mM of OnePhorAll buffer (GE Healthcare, Pittsburgh, USA), 5 mM of DTT and 2.5 ng/μL of BSA. Digestion products were subsequently ligated to Eco and Mse adapters, obtained by mixing an equimolar quantity of either EcoAdp1 and EcoAdp2 oligonucleotides (Eco adapter) or MseAdp1 and MseAdp2 (Mse adapter; Table 2). Before first use, the oligonucleotide solution was heated to 95 °C for 15 min and allowed to cool down at room temperature over a period of 30 min. Adapters were then stored at −20 °C until used. Ligation mixtures contained 1 U of T4 DNA ligase (Invitrogen, Carlsbad, CA, USA), 5 mM of DTT, 2.5 ng/μL of BSA, 0.2 mM of ATP, 50 pM of Mse adapter, 5 pM of Eco adapter and 250 ng of digested DNA in a final volume of 30 μL. The pre-amplification step was conducted in a total volume of 50 μL containing 2.5 μL of the ligation products,

Table 1
Sample pool composition with geographical origins in WGS84 coordinates.

Origin	Sampling area	Species	Sample code	N	Longitude	Latitude
Australia	New South Wales	*Themeda triandra*	Aus 01–02	**2**	~145	~−30
Botswana	Botswana	*Themeda triandra*	Bot 01–02	**2**	~23	~20
Indonesia	Bogor	*Themeda arguens*	Ind 01–02	**2**	~110	~−8
Italy	Milan	*Sorghum bicolor*	Sorg	1	9.2	45.467
Kenya	Campi Ya Kanzi_1	*Themeda triandra*	C 01–C 08	8	37.4352843	−2.51273
Kenya	Campi Ya Kanzi_2	*Themeda triandra*	C 09–C 15	6	37.3545316	−2.46047
Kenya	Campi Ya Kanzi_3	*Themeda triandra*	C 16–C 19	4	37.4933519	−2.40295
Kenya	Campi Ya Kanzi_4	*Themeda triandra*	C 20–C26	8	37.529069	−2.40473
Kenya	Delamare_1	*Themeda triandra*	D 01–D 08	8	36.2442136	−0.38167
Kenya	Delamare_2	*Themeda triandra*	D 09–D 14	6	36.1396796	−0.30398
Kenya	Delamare_3	*Themeda triandra*	D 15–D 20	6	36.0843124	−0.25471
Kenya	Delamare_4	*Themeda triandra*	D 21–D 28	8	36.1014486	−0.2226
Kenya	Gallmann_1	*Themeda triandra*	G 01–G 04	4	36.1875649	0.304213
Kenya	Gallmann_2	*Themeda triandra*	G 05–G 11	7	36.2471041	0.336595

0.2 mM of ATP, 1 U of GoTaq DNA polymerase (Promega, Fitchburg, WI, USA), 1 μM of E01 primer (Table 2), 1 μM of M02 primer (Table 2) and 1× GoTaq DNA polymerase buffer (Promega, Fitchburg, WI, USA). PCR amplification cycles were as follows: an initial denaturation step of 2 min at 95 °C followed by 12 cycles at 95 °C for 30 s, 65 °C for 30 s and 72 °C for 60 s. At each cycle the annealing temperature was decreased by 0.7 °C. The initial cycles were followed by 20 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. A final elongation step at 72 °C for 10 min was added.

Selective amplifications were carried out using E36/M49 and E41/M48 selective primers (Table 2) in a 20 μL reaction volume containing 0.4 U of GoTaq DNA polymerase (Promega, Fitchburg, WI, USA), 1× GoTaq reaction buffer (Promega, Fitchburg, WI, USA), 0.2 mM of dNTPs and 1 μM each of the selective primers (Table 2). PCR cycles started with an initial denaturation step at 95 °C for 2 min followed by 30 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for of 2 min. A final elongation step at 72 °C for 10 min was included.

Since E36 and E41 selective primers used for selective amplifications were 5′-labeled with 6-FAM fluorophore (Table 2) (Applied Biosystems, Foster City, USA), AFLP fragments were separated and analyzed using an ABI3730 DNA analyzer (Applied Biosystems, Foster City, USA). For that purpose, amplification products were diluted ten times and 2 μL of each dilution was mixed with 10 μL of deionized formamide and 0.15 μL of GeneScan 1200-LIZ internal size standard (Applied Biosystems, Foster City, USA). GeneMapper 4.1 software (Applied Biosystems, Foster City, USA) was employed to analyze electropherograms for manual scoring of polymorphic fragments. Quality control on AFLP data was realized by comparing the AFLP profiles obtained from 6 replicated plant samples, each obtained from independent DNA extractions. Quality control samples included one *S. bicolor*, one *T. arguens* and four *T. triandra* individuals. AFLP fingerprint profiles for each sample were converted into a dataset of binary data reporting polymorphisms as absence/presence (0/1) of a peak.

Table 2
List of primers utilized to carry out AFLP experiments.

Primer Name	Sequence (5′-3′)
EcoAdap1	CTCGTAGACTGCGTACC
EcoAdap2	AATTGGTACGCAGTC
MseAdap1	GACGATGAGTCCTGAG
MseAdap2	TACTCAGGACTCAT
Eco01	GACTGCGTACCAATTCA
Mse01	GATGAGTCCTGAGTAAC
E36	6FAM-GACTGCGTACCAATTCACC
M49	GATGAGTCCTGAGTAACAG
E41	6FAM-GACTGCGTACCAATTCACC
M48	GATGAGTCCTGAGTAACAC

2.4. Meteorological and environmental data

A commercial GPS device (precision ± 6 m) was used to mark the geographical coordinates for each sampling area. Sampling locations were imported in DIVA-GIS (Hijmans et al., 2001). Climatic data was derived from WorldClim (Hijmans et al., 2005), a set of global interpolated climate surfaces at a resolution of 2.5 min. In WorldClim, raw measurements of rainfall and temperature at monthly intervals are used to derive 19 BIOCLIM variables commonly used to model plant species' distribution. Thornthwaite's potential evapotranspiration (Thornthwaite, 1948) and field-measured altitude data were also associated to each sampling point. The set of environmental variables collected (Electronic Supplementary Material S1) was likely to include redundant information, thus a covariance-standardized principal component analysis (PCA) under Pearson's distances was used to summarize the environmental variables with SPSS statistics (IBM Corporation, 2011). This is intended to reduce the risk of increasing the background noise in the correlation analyses without a real gain in explanatory value (Beaumont et al., 2005). The first three principal components bearing most of the variability included in the original dataset were extracted, and a new Pearson's coefficient between the original variables and the derived principal components permitted to trace back their explanatory content, a common practice in ecological studies (Caccianiga et al., 2011).

2.5. Diversity and statistical analyses

The AFLP binary dataset deriving from the 72 *Themeda* samples was used to build a phylogeny with SplitsTree 4.12.6 (Huson, 1998). An UPGMA tree was constructed from Jaccard's similarity coefficient (Jaccard, 1908), a routine deemed appropriate in AFLP studies (Laborda et al., 2005). Jaccard's similarity is in fact based on shared status of markers, which can originate only by a sequence match among samples. Since the method does not consider differences, it avoids the homoplasy bias potentially introduced by the absence of DNA fragments that might originate by a number of different sequences. Bootstrapping procedure with 1000 replicates was used to assess phylogenetic tree robustness. All of the subsequent analyses were restricted to Kenyan *T. triandra* genotypes. PCA analysis was conducted using GenAlEx 6.0 (Peakall and Smouse, 2006) on the pairwise matrix of genetic distances calculated between Kenyan individuals. The first three principal coordinates accounting for local genetic variation were extracted and superimposed to the sampling map with a graphic editor. Population structure of Kenyan *T. triandra* was studied using a heuristic method based on Bayesian clustering algorithms implemented in Structure software (Falush et al., 2003; Pritchard et al., 2000). Due to the facultative apomictic reproduction habit of this species, hence to the uncertain amount of genetic recombination, some deviation from Hardy–Weinberg (H–W) equilibrium was expected (Marshall and Weir,

1979; Overart and Asmussen, 1998). To overcome the possible effects of apomixis, the analysis was carried out using both the "admixture" and the "no admixture" models implemented in Structure software. In both cases the rationale of the clustering method is the allocation of each genotype to subpopulations in which deviation from H–W equilibrium is minimized. The no "admixture" model differs from the "no admixture" model in that it implies that individuals come solely from one of the *K* populations, thus being appropriate for the analysis of discrete clusters at the cost of a certain loss in flexibility in data analysis (Pritchard et al., 2000). Burn-in length and number of MCMC repetitions were set to 1.5×10^4, testing a number of *K* clusters from 1 to 10 with twenty iterations each. Standard settings for dominant data were used (Falush et al., 2007), while the most probable number of *K* clusters was determined using the derivative method proposed by Evanno et al. (2005). Recombination events in the AFLP dataset were evaluated by calculating matrix incompatibility (MI) (Van der Hulst et al., 2000, 2003). The rationale of this analysis is to ascertain whether clonal or sexual reproduction is compatible with the pattern of all possible biallelic pairs of AFLP markers. If apomorphic and plesiomorphic alleles for a pair of markers are present in a population, asexual reproduction would originate in three out of four allelic combinations. Conversely, up to four possible allelic combinations are possible under sexual reproduction. MI counts were calculated using a PICA 4.0 software (Wilkinson, 2001). Differences in incompatibility counts were evaluated by comparing the matrix of the complete dataset of AFLP profiles with the one lacking a specific *T. triandra* individual at a time, so as to quantify the contribution of each genotype to the total MI (Chapman et al., 2003). Genomic loci under selection (i.e., outlier loci) were investigated under the assumption that loci with uneven distribution are expected to mark the highest genetic differentiation between populations, therefore being more informative than neutral alleles in terms of selection detection (Storz, 2005). Mcheza software (Antao and Beaumont, 2011) implements an F_{st}-outlier discovery method specifically designed for dominant markers derived from the DFDIST algorithm (Pérez Figueroa et al., 2010; Zhivotovsky, 1999). An F_{st} null sampling distribution is generated upon neutral expectations from a pairwise comparison between populations, and it is used to identify loci with peculiarly high or low F_{st} values. In order to be more confident about the selection signal expressed by outlier loci, populations were compared in pairs and outlier loci detected in at least two comparisons were the only variables retained (Tollenaere et al., 2010). It is in fact unlikely for divergence caused by type I errors or factors else than selection to coincidentally act on the same locus in more than one population (Nosil et al., 2009). To be more conservative, the outlier list was evaluated after the removal of loci with a frequency of the rarest allele of at least 5%.

A Mantel test (Mantel, 1967) was performed on Kenyan samples: this is a matrix-based permutation test useful in evaluating the bias introduced in genetic diversity by uneven geographical sampling. It was noted that the reduction of manifold variables to a pairwise distance matrix is a practice that hinders statistical power and increases false positives (Harmon and Glor, 2010). In the present study this might apply to molecular data but not to geographical distances, that must be treated as such. This poses Mantel test as a solid choice for geographic bias evaluation in landscape genetic practices. GenAlEx 6.0 was used to perform the analysis and test the output with 9999 permutations. Finally, outlier loci in Kenyan populations were employed as dependent variables in a canonical correspondence analysis (CCA) (Ter Braak, 1986). The three principal components deriving from the environmental variation characterizing the sampling areas were used as independent variables. The CCA method allows the identification of highly correlated linear combinations from two source matrixes, typically comparing environmental data with species distribution information (Ter Braak, 1987), and less frequently with genetic diversity data (Angers et al., 2002; Brouat et al., 2004). Here we consider individuals as sites, and alleles at outlier loci as objects. As climatic data refer to *T. triandra* individuals, the presence or absence of an AFLP amplification fragment in a given outlier locus is directly correlated with climatic data. XlStat 2012 software (Copyright Addinsoft 1995–2012) was used to extract the two most significant correlation axes running 1000 permutations to evaluate the statistical significance of the CCA results.

3. Results

3.1. Karyotype and molecular diversity analyses

Karyotype analysis was carried out at a 1000× magnification. Chromosomes appeared small (about 1 μm wide and 2–5 μm long), with a haploid number of n = 10 for all examined accessions. African and Australian *T. triandra* samples investigated in this study were all tetraploids (2n = 40), while *T. arguens* presented a diploid chromosome set (2n = 20) (Electronic Supplementary Material S2).

As the AFLP technique was never applied to *Themeda* before, an assessment of AFLP marker reliability was carried out by replicating a limited number of samples. The quality check showed that all peaks having a relative fluorescent unit (RFU) larger than 1500 were completely reproducible and consequently this threshold was adopted to identify AFLP markers from background fluorescence noise and from weaker, unreliable signals.

In total, 366 polymorphic AFLP loci were used to produce the UPGMA phylogram shown in Fig. 1. *S. bicolor* was used as outgroup. *T. arguens* accessions appeared to be the most diverse of the dataset. All *T. triandra* samples are separated from these two taxa with high bootstrap values, then the differences in the phylogram rapidly become less pronounced making impossible to differentiate African and Australian genotypes. Low bootstrap values depict what should be considered as a politomic arrangement of the geographical separation of *T. triandra* samples. Only few accessions from Gallmann and Campi Ya Kanzi group with high confidence in the distal portion of the phylogram. The relationships between Kenyan genotypes both in geographic and genetic terms are maybe better depicted by PCA (Fig. 2), whose first three components account for 68.8% of the total genetic variability present in the dataset. The first component allows the separation of the Campi Ya Kanzi samples from the others, confirming the solid cluster observed in the phylogram (bar marker in Fig. 1). The second principal component separates most of the genotypes from those from Gallmann, which also cluster peripherally to the main Kenyan clade in the phylogenetic analysis. The results obtained by PCA report that the geographic origin of genotypes does not explicitly account for their genetic relationship, even though the southern and northern extremes of the collection transect host some peculiar genotypes.

3.2. Population structure of T. triandra

Bayesian clustering supports the presence of three cryptic genetic clusters among the Kenyan populations. Both the "admixture" model (Fig. 3) and the "no admixture" model (data not shown) report a clustering which corroborates the results obtained from the PCA analysis reported in Section 3.1. Though deviation from H–W equilibrium was expected under the assumption of facultative apomictic populations, the convergence of both models suggested that AFLP fingerprinting of the African *T. triandra* populations is compatible with a significant amount of genetic recombination. The largest cluster (cluster 1) includes genotypes from all sampled areas. The intermediate group (cluster 2) is composed mainly of Campi Ya Kanzi genotypes, some of which are partially mixed to cluster 1, and a single genotype from Delamare (D06). The smallest cluster (cluster 3) comprises few genotypes from the Gallmann area and some from Delamare, again sharing some similarities with cluster 1.

3.3. Recombination within T. triandra

Matrix incompatibility (MI) contribution for each genotype was calculated to establish the presence of recombination

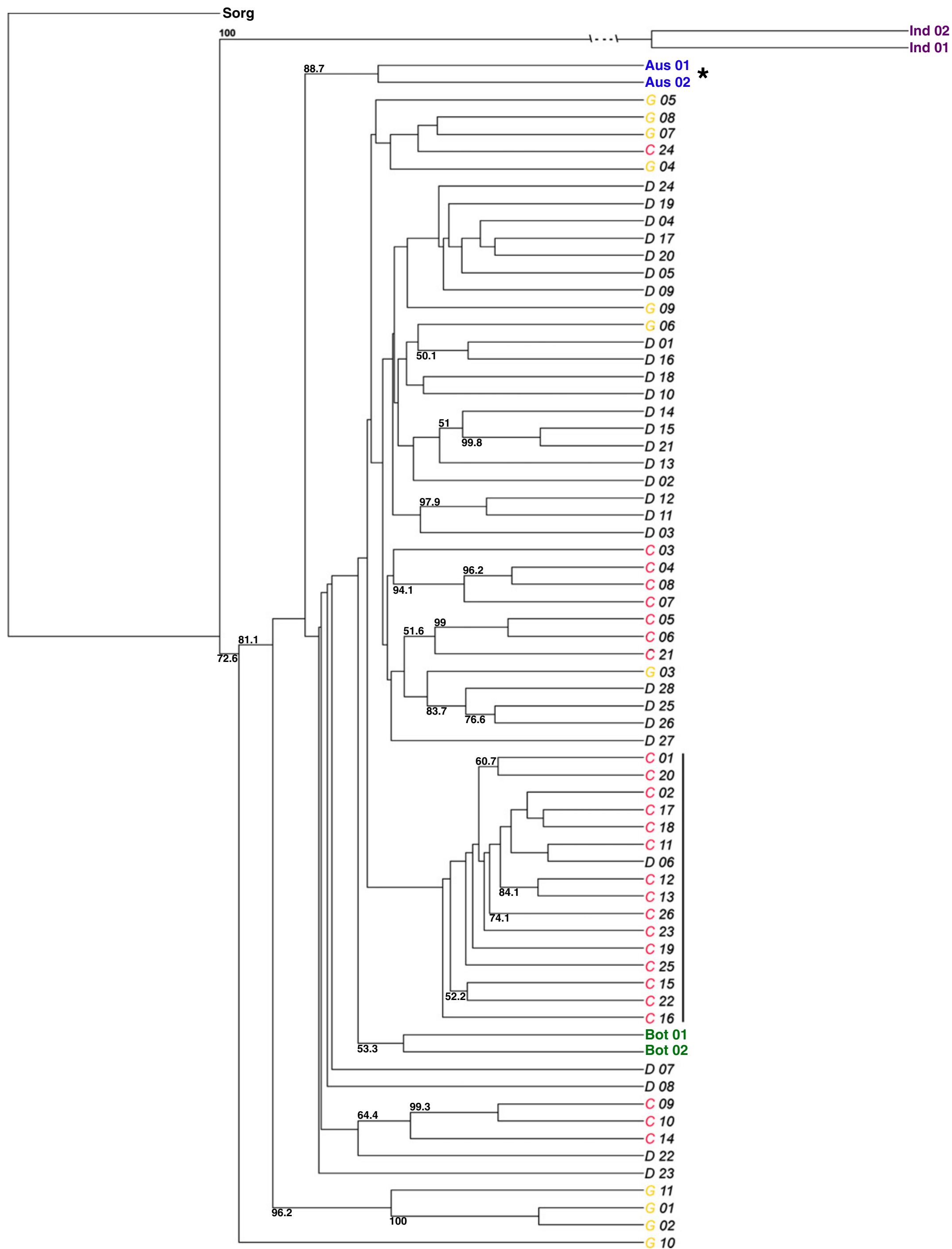

Fig. 1. UPGMA phylogram of the genotypes analyzed in this study based on the Jaccard similarity index. Bootstrap values above 50 are shown. *Sorghum bicolor* (Sorg) and *Themeda arguens* (Ind) encompass all Botswanan (Bot 01–02) and Kenyan (C 01–26, D 01–28, G 01–11) genotypes. Australian *Themeda triandra* (Aus; *) is also mixed with African genotypes. Geographic group separation is often supported by low bootstrap values, suggesting low differentiation among populations. Genotypes G 03–06 lay outside the African and Australian pool. The line marker points to a clade markedly separated from the surrounding genotypes.

while observing whether the individuals had different reproductive strategies. All three sampling areas gave similar outcomes: individuals, independent from their geographic origin, equally contributed to MI, with great individual-based variability. The plot of MI count variation in relation to genotype depletion appears as a smooth decreasing curve (Fig. 4), indicating that all individuals generate comparable amounts of incompatibility counts.

3.4. Outlier locus analysis

The three Kenyan populations were compared to identify loci deviating from the expected equilibrium assumptions in order to identify selection signatures within the AFLP marker dataset. A set of 27 loci out of the 366 AFLP loci studied (7.4%) emerged as outliers ($p < 0.05$) in at least two of the three possible comparisons between the sampling areas. Among the outlier loci, only those having an allele frequency of

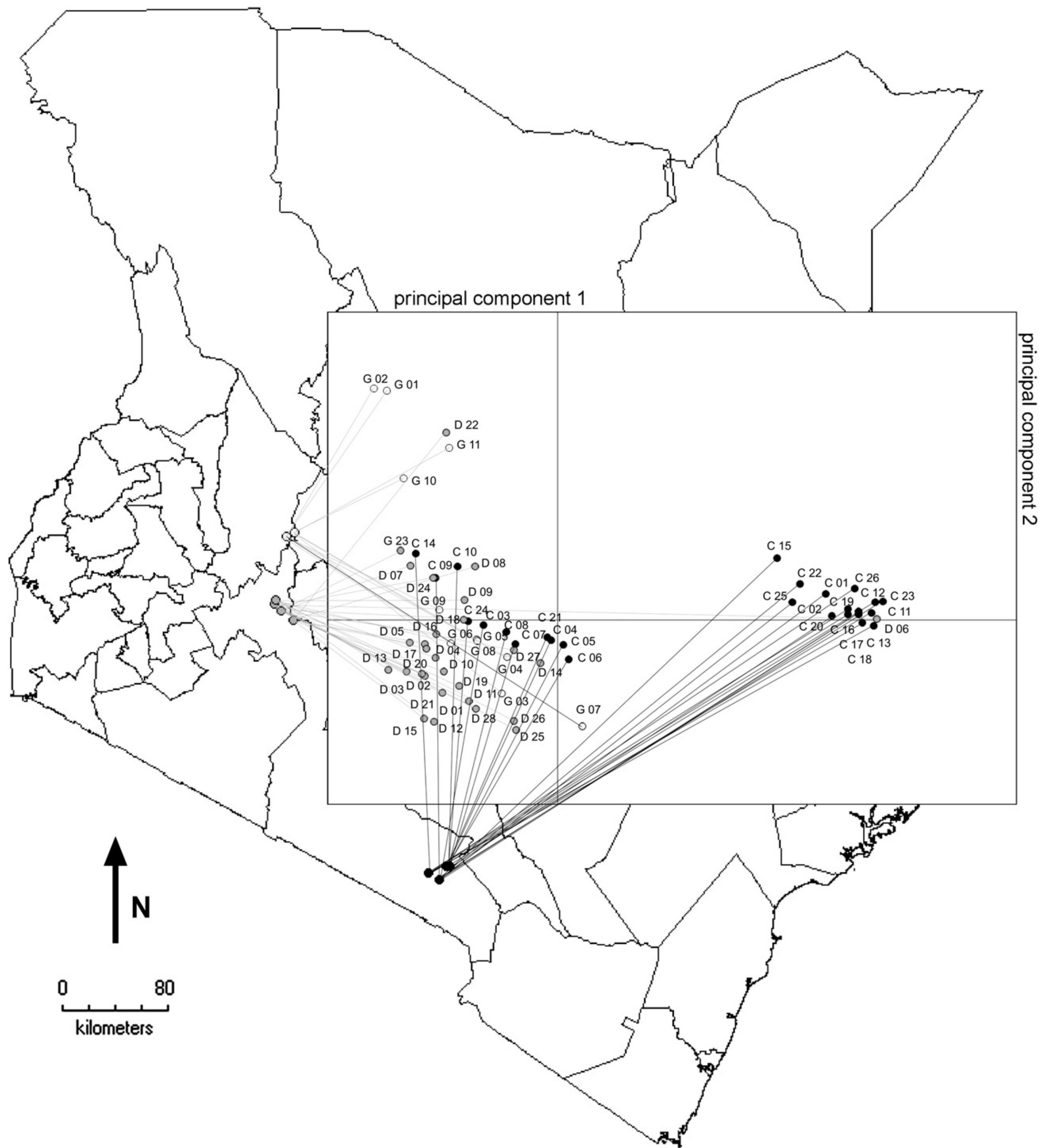

Fig. 2. PCA clustering of the genotypes analyzed in this study revealed that the pattern of genomic variation is not always consistent with the geographical sampling of the individuals. Dots on the map represent individual sampling areas (white for Gallmann, gray for Delamare and black for Campi Ya Kanzi), sample names are the same as in Fig. 1. The widest cloud of genotypes located around the center of the PCA space is comprised by individuals coming from all of the sampling areas, and separated by component 1 from part of Campi Ya Kanzi genotypes and by component 2 by few Gallmann genotypes.

the rarest allele higher than 0.05 were considered, resulting in a set of 13 loci putatively under selection (3.5% of all loci) (Fig. 5).

3.5. Eleven AFLP loci show statistical relation with environmental variation

Mantel test showed a low linear correlation between geographic and genetic distances ($R^2 = 0.069$, $p < 0.001$), therefore excluding the presence of a strong sampling bias in the molecular dataset. As expected, when checked for redundancy by Pearson's correlation analysis, several of the 21 environmental variables showed high correlation levels. For that reason, PCA analysis was used to reduce the number of variables to be considered. PCA grouping of environmental variables resulted in the identification of three new variables accounting for 83.3%, 8.44% and 3.13% of the original variation, thus altogether explaining about 94.9% of the total environmental variability considered. A Pearson's correlation test between the new PCA variables and the original ones allowed establishing the relationship between the new and the old variables (Electronic Supplementary Material S3). The first PCA variable (PC 1) positively accounts for potential evapotranspiration, for BIOCLIM variables Bio 1, 4, 5, 6, 8, 9, 10 and 11 (temperature indexes), and Bio 13, 15 and 18 (rainfall seasonality). PC 1 is negatively correlated with altitude, with Bio 2, 3 and 7 (temperature range indexes), and Bio 12, 14, 16, 17

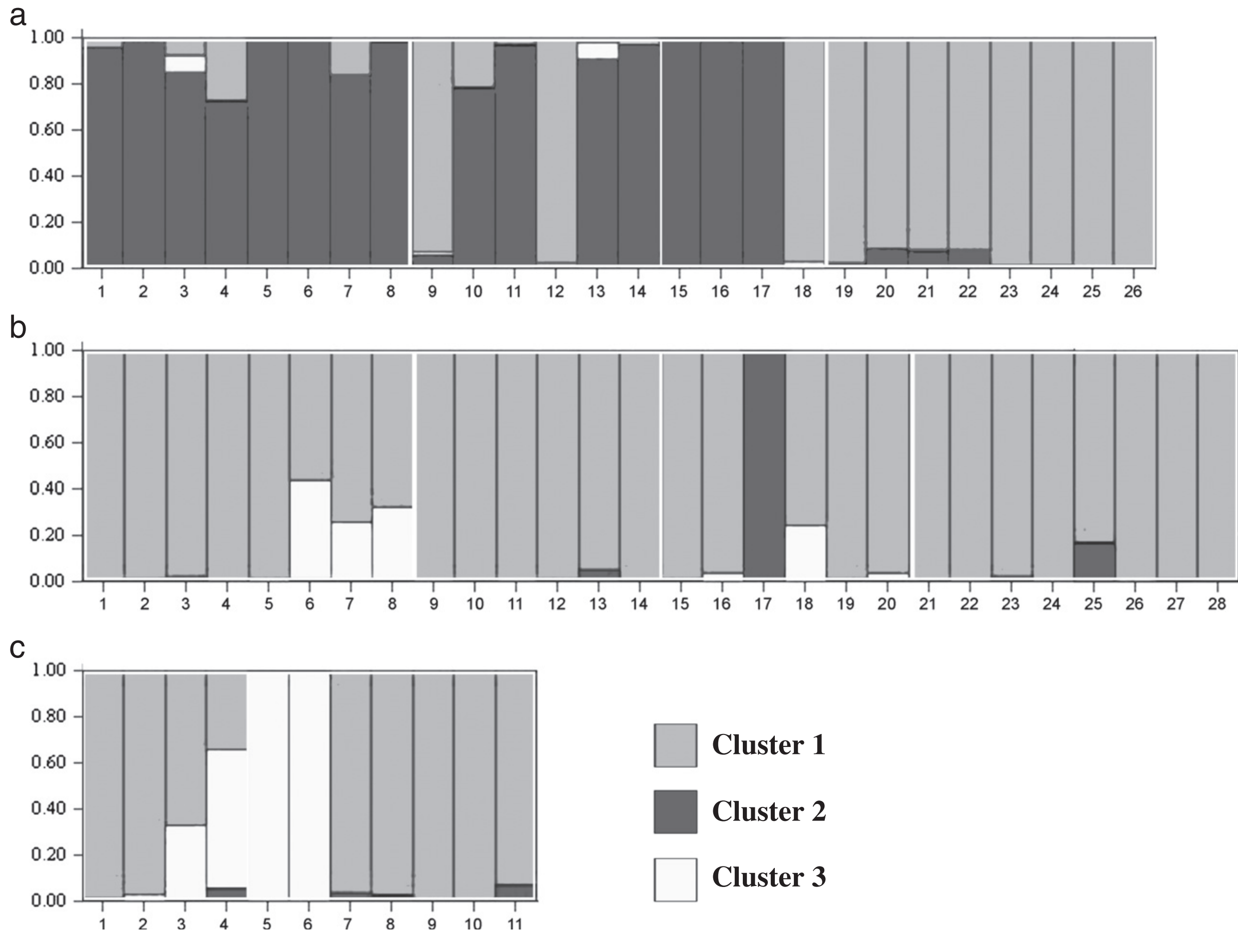

Fig. 3. Genotype clustering after Bayesian analysis for cryptic population structure with K = 3. a) Campi Ya Kanzi, b) Delamare and c) Gallmann; white frames split the broader areas in individual sampling areas. Each genotype is represented by a vertical line partitioned into K segments whose length is proportional to the individual coefficients of membership in the K clusters (clusters 1–3, in legend). The three genetic clusters are consistently present at the same time in the Delamare region only.

and 19 (precipitation indexes). The second principal component (PC 2), of much lower significance, is related to the original Bio 12, 13, 16 and 18 variables (precipitation of the wettest month and quarter) and correlates inversely with Bio 7 (temperature range). Finally, the third principal component (PC 3) is negatively correlated with Bio 18 (precipitation during the warmest quarter). Therefore, the environmental variables for the area under examination describe altogether an environmental polarization for altitude and rainfall opposed to higher temperatures and evapotranspiration. The CCA revealed a high degree of correlation between the set of outlier loci and the first three variables of environmental PCA ($p < 0.001$). Taken together, the first two CCA axes accounted for 94.98% of the inertia (Fig. 6). The outlier loci scattered at the center of the plot (loci 19, 76) seem to be unaffected by the environmental variables taken in account. The first principal component is positively related with loci 13, 129, 135, 137 and 163, and negatively with loci 55, 78, 133, 291, 318 and 321. PC 2 and PC 3 contribute mostly on the vertical CCA axis, which bears lower explanatory value.

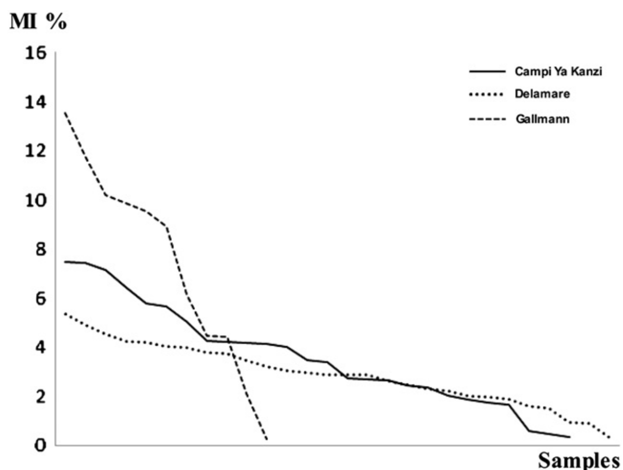

Fig. 4. Matrix incompatibility count in relation to most incompatible genotypes' recurrent deletion for each population. On the horizontal axis the increasing amount of genotypes was successively removed from the dataset and on the vertical axis the amount of incompatibility, reducing at each depletion. Differences in curve slopes are due to different amounts of individuals; the decrease is smooth throughout.

4. Discussion

4.1. Intercontinental genetic uniformity?

AFLP are known to be a reliable tool for intra-genera phylogeny, often producing results comparable with those obtained through traditional sequencing approaches (Hodkinson et al., 2000; Koopman, 2005) especially in low-divergence, intra-genera scenarios (García-Pereira et al., 2010; Near and Keck, 2012), where the multilocus nature of AFLP makes them superior to single-locus markers (El-Rabey et al., 2002). For these reasons we were surprised by the occurrence of a strong genetic similarity between African and Australian *T. triandra* (Fig. 1). The origin of the genus *Themeda* is currently to be traced to Asia, where most of the *Themeda* species are present. Also because the *Themeda* presence in Australia was already reported as *T. australis* in

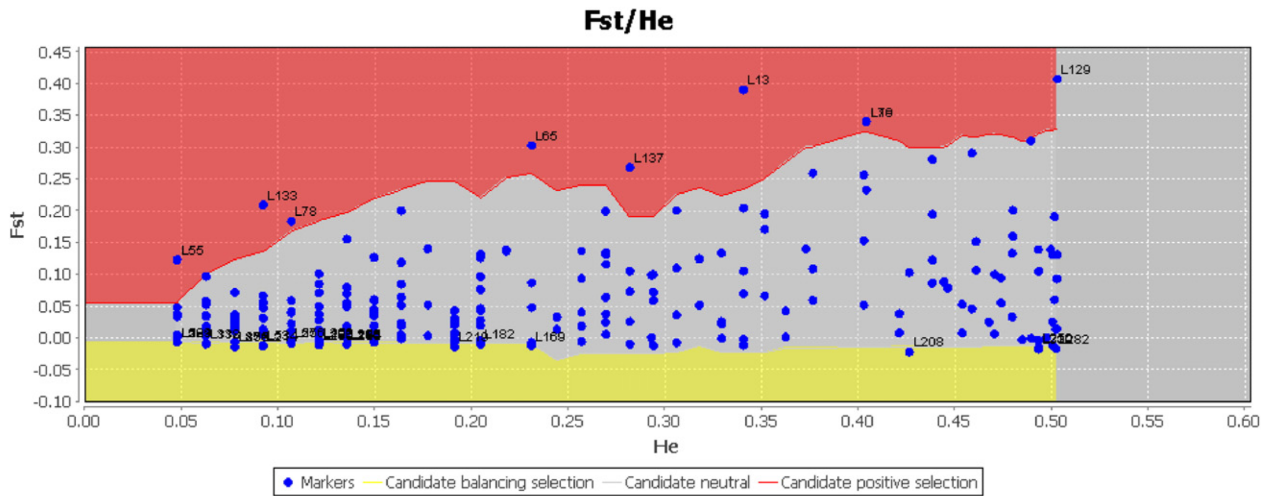

Fig. 5. Outlier locus analysis output, showing each of the 366 loci in regard to heterozygosity (He) and F_{st}. The upper line marks the threshold for divergent outlier marker calling. Increasing He determines higher F_{st} values falling inside the expectation of neutrality hypothesis.

the 18th century by no less than James Cook, the most likely hypothesis entails a separated radiation of African and Australian *Themeda* species from Asia. Yet, Australian *Themeda* samples share enough physiological and morphological similarities with their African counterpart that sometimes are considered as the same species, ensuing confusion (APNI, 2013; Dell'Acqua et al., 2013; Groves et al., 2003). It is not clear whether this confusion is due to *T. australis* itself or to its co-occurrence with *T. triandra* in the Australian territory. The molecular data hereby presented, although preliminary, push towards the latter hypothesis. This could be explained by a reunion of the two *Themeda* species in Australian territory, possibly driven by camel trades occurring during the 19th century. *T. triandra* fruits have awns that may cling to animal furs, favoring epizoochoric seed dispersal. If this is the case, African *T. triandra* genotypes exist

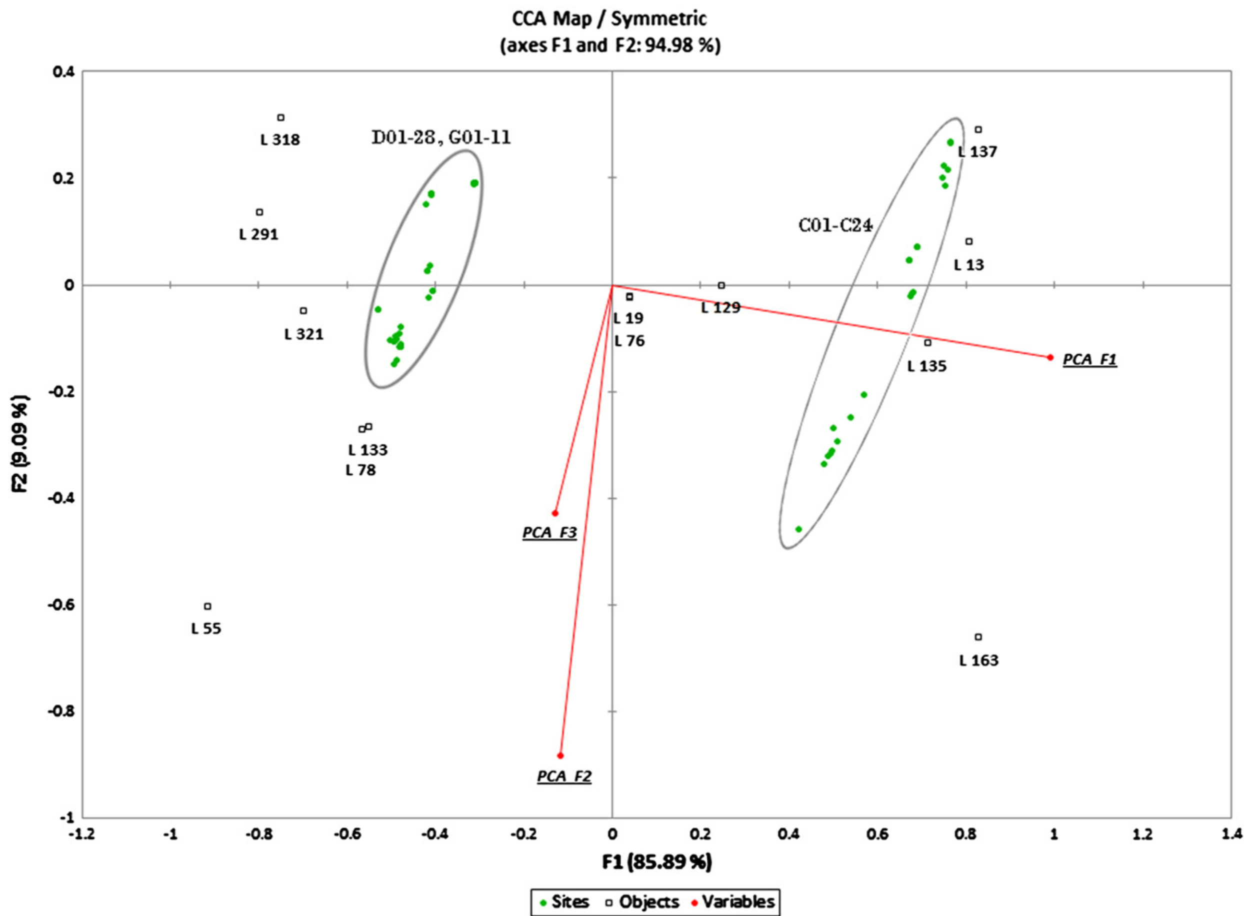

Fig. 6. Tri-plot for CCA analysis. Squares represent outlier loci and dots represent sampling sites (individuals). Vectors deriving from the PCA summary of environmental data depart from the center of the graph and depict the forces driving locus selection. On the right of the graph is the circle entangling Campi Ya Kanzi genotypes. Delamare and Gallmann genotypes are grouped on the opposite side. The horizontal axis holds the highest explanatory value, with 85.89 of the inertia.

in Australia, and the results hereby presented are the first molecular evidence showing genomic uniformity in *Themeda* across continents. The question then becomes, where is James Cook's *T. australis*? Certainly, a deeper sampling is needed to have a clearer picture of *Themeda* diversity in Australia: this, however, is beyond the objectives of the present study. Here we show molecular evidence that the Australian *T. triandra* genotypes analyzed are completely indistinguishable from African *T. triandra* (Fig. 1). Based on these observations, we suggest that African genotypes of *T. triandra* might have been recently introduced into Australia. The opposite migration route seems less likely, as humans and human-related animals mainly moved from Africa to Australia. We cannot exclude that *T. australis* is also present in Australia: further studies including more Australian samples are required to provide a definitive answer to this question.

T. triandra diversity in Kenya may also involve seed dispersal. *T. triandra* ripens during the dry season, when cattle and wildlife migrate from the southern territories to the greener northern pastures (FEWS, 2014). Such animal migration can account for the distribution of genotypes observed in Campi Ya Kanzi, Delamare, and Gallmann (Fig. 2), justifying the widespread presence of cluster 1 (Fig. 3) in all three sampling areas. Starting from the mid-1900s, however, the historical migration route connecting those areas, and generally the Kenyan drylands, underwent severe fragmentation owing to a shift from a traditional open range grazing system to a more sedentary agriculture (ASARECA, 2011; Fall, 2000; Munyasi et al., 2012). Consequently, what historically functioned as a channel fostering *T. triandra* seed displacement witnessed a gradual reduction of its importance owing to the limited animal movements. Even though further studies are in order to quantify the displacement potential of *T. triandra* in relation to animal migrations, our results may depict the remnants of a migration corridor that recently faded, eventually leading to genetic isolates. While the ubiquitous genetic cluster 1 suggests historical movement of genotypes, two genetic clusters (2, 3) remain spatially polarized in Campi Ya Kanzi and Gallmann, respectively. Such a scenario likely derives from the existence of a genetic background where recombination is common on top of which localized selection pressures are taking place. These aspects will be treated in more detail in Section 4.3.

4.2. Recombination signatures

All the analytical approaches employed in our study reveal the presence of a significant extent of genetic admixture (Figs. 1, 2 and 3). The phylogenetic analysis (Fig. 1) shows individuals connected by long terminal branches with small distances and weak nodes. Such structure is typical when recombination is widespread among samples (Schierup and Hein, 2000). When reproduction does not imply sexual recombination, the lack of genetic recombination would result in a phylogenetic tree where samples are tightly clustered according to their geographical origin. On the contrary, our data confirm the existence of gene flow counteracting populations' differentiation at large geographical scales. Such picture is corroborated by surprisingly high rates of recombination estimated by the incompatibility counts on the AFLP data (Fig. 4). The observed MI, in fact, confirms high recombination rates (Mes, 1998): while the magnitude of the differences among populations in incompatibility counts can be attributed to different sizes in sample pools; the way in which MI varies is to be considered informative (Van der Hulst et al., 2000). Our results show that the number of incompatibility counts is larger between individuals than between populations, hence excludes the existence of a geographical pattern of reproduction strategy. MI analysis can sometimes be misleading, especially when no incompatibility counts are detected. This is due to the fact that the physical position and the mutual relations of AFLP markers are unknown, thus the absence of some combinations can be due to the tight linkage of markers rather than the absence of recombination. Conversely, incompatibility counts can only be generated when recombination occurs between pairs of markers. Our data support the latter, more confident, hypothesis: more than one generation of sexual reproduction connects most of the considered genotypes. The gradual decrease in MI counts obtained by the deletion of the most incompatible genotypes clearly indicates that recombination rates are comparable for all genotypes and in all collection areas. Reproduction within the considered populations must be therefore considered mostly sexual. The investigated Kenyan populations of *T. triandra* are tetraploid and sexually reproducing, showing higher recombination rates than expected (Dell'Acqua et al., 2013). We deem these unexpected findings to be due to the unclear classification and behavior of diploid and tetraploid accessions of *T. triandra* in regard to apomixis, a phenomenon which in *Themeda*, as in other angiosperms, is often influenced by environmental factors (Evans and Knox, 1969; Nogler, 1984).

4.3. Environmental selection

The proportion of loci detected as outliers (7.4%) is comparable with previous findings, generally placing it below 10% (Stinchcombe and Hoekstra, 2007), and is consistent with the hypothesis of a porous genome (Gavrilets and Vose, 2005). The aim of the outlier detection approach is not that of describing or identifying the function of the involved loci, as AFLP markers are random genetic markers and do not bear such information. Indeed, nucleotide substitutions at the base of AFLP polymorphisms often occur in non-coding DNA regions, which can undergo indirect selection through selective sweeps (Andolfatto, 2001). Therefore, the information deriving from CCA analysis on outlier loci must be considered as a meta-signal indicative of the presence of adaptive loci in genetic linkage with AFLP markers. The climatic variables considered in the present study provided a good explanation for the allele segregation of outlier loci: even if BIOCLIM variables cannot be considered as the only ones with some importance, they were useful in revealing the presence of *T. triandra* ecotypes related to rainfall, temperature and evapotranspiration in close natural populations. *T. triandra* accessions collected in different environmental conditions, thus, are not merely differentiated at a phenotypic level, but indeed show some degree of genetic adaptation. The outcome of the Mantel test for correlation between geographic and genetic distances supports the existence of a real selection signature, rather than simple geographic differentiation. In Fig. 6, a rainfall/temperature gradient separates outlier loci in two groups further expanded by PC 2 and PC 3, which weakly account for precipitation strength. Other abiotic and biotic stresses are likely to exert a selective pressure on *T. triandra* natural populations: among those are large tree canopies, herbivory (Treydte et al., 2009), and fire regimes (Johnson and Matchett, 2001). Though these factors might be explicitly addressed by further studies, they are likely influenced by rainfall and temperatures in the complex and interconnected environmental system. CCA through environmental PCA is a modular method that can embrace a virtually unlimited set of variables. We do not expect that adding new variables would change the general picture of the outlier loci strongly related to the environmental gradient surveyed. Conversely, this could contribute to a better description of the loci poorly correlated with PCA coordinates, thus located at the center of the graph in Fig. 6. As AFLP cannot provide a functional description of genomic loci, the outcome of the CCA should be considered the evidence of environment selecting *T. triandra* ecotypes. The sampling areas under study necessarily represent only a part of the environmental gradient for all these traits, but according to the results presented in Section 3.5 we expect environmental selection to operate across the whole *T. triandra* distribution areas in Eastern Africa. In this context, the peculiar genetic types detected at the extremes of the transect (clusters 2 and 3 in Fig. 3) should not be considered *T. triandra* vanguard expansions towards new ecological niches but rather, and together with cluster 1, part of a broader collection of *T. triandra* ecotypes genetically adapted to different conditions across a cline of ecological variation.

4.4. Conclusions

The fruitful protection and the wise exploitation of rangelands require a deeper understanding of the dynamics shaping the genetic structure of natural populations of plant resources. When applied to neglected forage species, molecular approaches might provide new insights useful in reconsidering their biology in a conservation perspective: the results presented above report some *T. triandra* traits that might be of use for devising sustainable efforts to rangeland conservation. Kenyan polyploid *T. triandra* populations show signatures of high recombination and environmental adaptation driven by local climates. Selected *T. triandra* ecotypes coming from areas identified in this and further studies might be used to improve resilience of impoverishing rangelands through targeted sowing of genotypes adapted to drought or other environmental stresses. Owing to the existence of a widespread sexual reproduction, the further characterization of genomic loci involved in climatic adaptation might also lead to marker-assisted breeding efforts in *T. triandra* tetraploid individuals to improve the genetic makeup of populations posed at risk by climate change. Hints of genetic uniformity between African and Australian individuals also ask for further studies untangling the intercontinental relationships among *T. triandra* populations. If a strong genetic link exists between the two main areas of distribution of *T. triandra*, an exchange of germplasm as well as of conservation and management practices might also ensue. This work represents the first tile of a much wider picture: further studies extending the surveyed area are required to increase the knowledge about *T. triandra* genetic diversity and to move towards modern conservation approaches for this important forage species.

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References

Andolfatto, P., 2001. Adaptive hitchhiking effects on genome variability. Current Opinion in Genetics and Development 11 (6), 635–641.
Angers, B., Magnan, P., Plante, M., Bernatchez, L., 2002. Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). Molecular Ecology 8 (6), 1043–1053.
Antao, T., Beaumont, M.A., 2011. Mcheza: a workbench to detect selection using dominant markers. Bioinformatics 27 (12), 1717–1718.
APNI, 2013. Australian Plant Name Index, at Australian National Botanic Gardens. Available online at http://www.anbg.gov.au/cgi-bin/apni.
ASARECA, 2011. Drylands development in Eastern Africa: the policy context and recommendations. ASARECA Policy Analysis and Advocacy Programme Electronic Newsletter. 14(24).
Beaumont, L.J., Hughes, L., Poulsen, M., 2005. Predicting species distributions: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions. Ecological Modelling 186 (2), 251–270.
Birari, S.P., 1980. Apomixis and sexuality in *Themeda* Forssk. at different ploidy levels (gramineae). Genetica 54, 133–139.
Bonin, A., Taberlet, P., Miaud, C., Pompanon, F., 2006. Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). Molecular Biology and Evolution 23 (4), 773–783.
Brouat, C., Chevallier, H., Meusnier, S., Noblecourt, T., Rasplus, J.Y., 2004. Specialization and habitat: spatial and environmental effects on abundance and genetic diversity of forest generalist and specialist *Carabus* species. Molecular Ecology 13 (7), 1815–1826.
Caccianiga, M., Andreis, C., Diolaiuti, G., D'Agata, C., Mihalcea, C., Smiraglia, C., 2011. Alpine debris-covered glaciers as a habitat for plant life. The Holocene 21 (6), 1011–1020.
Chapman, H., Robson, B., Pearson, M.L., 2003. Population genetic structure of a colonising, triploid weed, *Hieracium lepidulum*. Heredity 92 (3), 182–188.
IBM Corporation, 2011. IBM SPSS Statistics for Windows, Version 20.0. IBM Corp, Armonk, NY.
Coyer, J.A., Hoarau, G., Pearson, G., Mota, C., Jüterbock, A., Alpermann, T., Uwe, J., Olsen, J.L., 2011. Genomic scans detect signatures of selection along a salinity gradient in populations of the intertidal seaweed *Fucus serratus* on a 12 km scale. Marine Genomics 4 (1), 41–49.
Delgado, C.L., Rosegrant, M.W., Steinfeld, H., Ehui, S., Courboi, C., 1999. The coming livestock revolution. Choices 14, 40–44.
Delgado, C.L., Rosegrant, M.W., Meijer, S., 2001. Livestock to 2020: the revolution continues. Annual Meetings of the International Agricultural Trade Research Consortium (IATRC), Auckland, New Zealand, January 18–19, 2001.
Dell'Acqua, M., Gomarasca, S., Porro, A., Bocchi, S., 2013. A tropical grass resource for pasture improvement and landscape management: *Themeda triandra* Forssk. Grass and Forage Science 68, 205–215.
El-Rabey, H.A., Badr, A., Schäfer-Pregl, R., Martin, W., Salamini, F., 2002. Speciation and species separation in *Hordeum* L. (Poaceae) resolved by discontinuous molecular markers. Plant Biology 4, 567–575.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14, 2611–2620.
Evans, L.T., Knox, R.B., 1969. Environmental control of reproduction in *Themeda australis*. Australian Journal of Botany 17, 375–389.
Fall, A., 2000. Makueni district profile: livestock management, 1990–1998. Drylands Research Working Paper—Drylands Research, 8.
Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7 (4), 574–578.
FEWS, 2014. Kenya Seasonal Calendar and Critical Events Timeline. Available online at http://www.fews.net/east-africa/kenya.
García-Pereira, M.J., Caballero, A., Quesada, H., 2010. Evaluating the relationship between evolutionary divergence and phylogenetic accuracy in AFLP data sets. Molecular Biology and Evolution 27 (5), 988–1000.
Gavrilets, S., Vose, A., 2005. Dynamic patterns of adaptive radiation. Proceedings of the National Academy of Sciences of the United States of America 102 (50), 18040–18045.
Groves, R.H., Austin, M.P., Kaye, P.E., 2003. Competition between Australian native and introduced grasses along a nutrient gradient. Austral Ecology 28 (5), 491–498.
Guenther, A., Archer, S., Greenberg, J., Harley, P., Helmig, D., Klinger, L., Vierling, L., Wildermuth, M., Zimmermann, P., Zitzer, S., 1999. Biogenic hydrocarbon emissions and landcover/climate change in a subtropical savanna. Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere 24 (6), 659–667.
Harmon, L.J., Glor, E., 2010. Poor statistical performance of the Mantel test in phylogenetic comparative analyses. Evolution 64 (7), 2173–2178.
Hijmans, R.J., Guarino, L., Cruz, M., Rojas, E., 2001. Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genetic Resources Newsletter 127, 15–19.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965–1978.
Hodkinson, T.R., Renvoize, S.A., Chonghaile, G.N., Stapleton, C.M., Chase, M.W., 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). Journal of Plant Research 113 (3), 259–269.
Hoffmann, A.A., Sgrò, C.M., 2011. Climate change and evolutionary adaptation. Nature 470, 479–485.
Holderegger, R., Wagner, H.H., 2008. Landscape genetics. Bioscience 58 (3), 199–207.
Huson, D.H., 1998. SplitsTree: analyzing and visualizing evolutionary data. Bioinformatics 14 (1), 68–73.
Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bulletin Société Vaudoise des Sciences Naturelles 44, 223–270.
Jarvis, L.S., 1984. Overgrazing and Land Degradation in Africa: The Need and the Scope for Government Control of Livestock Numbers. International Livestock Centre for Africa (ILCA) (October).
Johnson, L.C., Matchett, J.R., 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82, 3377–3389.
Koopman, W.J., 2005. Phylogenetic signal in AFLP data sets. Systematic Biology 54 (2), 197–217.
Laborda, P.R., Oliveira, K.M., Garcia, A.A.F., Paterniani, M.E.A.G.Z., de Souza, A.P., 2005. Tropical maize germplasm: what can we say about its genetic diversity in the light of molecular markers? Theoretical and Applied Genetics 111 (7), 1288–1299.
Liebenberg, H., Lubbinge, J., Fossey, A., 1993. Cytotaxonomic studies in *Themeda triandra* Forssk.: IV. A population cytogenetic study of a contact zone between tetraploid and hexaploid populations. South African Journal of Botany 59 (3), 305–310.
Luikart, G., England, P.R., Tallmon, D., Jordan, S., Taberlet, P., 2003. The power and promise of population genomics: from genotyping to genome typing. Nature Reviews Genetics 4 (12), 981–994.
Manel, S., 2012. Adaptive genetic variation on the landscape: methods and cases. Annual Reviews of Ecology, Evolution, and Systematics 43 (1).
Manel, S., Schwartz, M.K., Luikart, G., Taberlet, P., 2003. Landscape genetics: combining landscape ecology and population genetics. Ecology and Evolution 18 (4), 189–197.
Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27 (2), 209–220.
Marshall, D.R., Weir, B.S., 1979. Maintenance of genetic variation in apomictic plant populations: I. Single locus models. Heredity 42 (2), 159–172.
Meier, P., Bond, D., Bond, J., 2007. Environmental influences on pastoral conflict in the Horn of Africa. Political Geography 26, 716–735.
Mes, T.H.M., 1998. Character compatibility of molecular markers to distinguish asexual and sexual reproduction. Molecular Ecology 7, 1719–1727.

Meudt, H.M., Clarke, A.C., 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends in Plant Science 12 (3), 106–117.
Mueller, U.G., Wolfenbarger, L.L., 1999. AFLP genotyping and fingerprinting. Trends in Ecology and Evolution 14 (10), 389–394.
Muluvi, G.M., Sprent, J.I., Soranzo, N., Provan, J., Odee, D., Folkard, G., McNicol, J.W., Powell, W., 2003. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Molecular Ecology 8 (3), 463–470.
Munyasi, J.W., Gitunu, A.M.M., Manyeki, J.K., Muthiani, E.N., Nyamwaro, S.O., 2012. Non-traditional land-use practices in the pastoral Maasai region in Loitokitok district of Kajiado county, Kenya. Agricultural Extension and Rural Development 428.
Near, T.J., Keck, B.P., 2012. AFLPs do not support deep phylogenetic relationships among darters (Teleostei: Percidae: Etheostomatinae). Heredity 108 (6), 647–648.
Nogler, G.A., 1984. Gametophytic apomixis. In: Johri, B.M. (Ed.), Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 475–518.
Nosil, P., Egan, S.P., Funk, D.J., 2007. Heterogeneous genomic differentiation between walking stick ecotypes: “isolation by adaptation” and multiple roles for divergent selection. Evolution 62 (2), 316–336.
Nosil, P., Funk, D.J., Ortiz Barrientos, D., 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology 18 (3), 375–402.
Overart, R.D., Asmussen, M.A., 1998. Genetic diversity at a single locus under viability selection ad facultative apomixis: equilibrium structure and deviations for Hardy–Weinberg frequencies. Genetics 148, 2029–2039.
Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421 (2), 37–42.
Parton, W.J., Scurlock, J.M.O., Ojima, D.S., Schimel, D.S., Hall, D.O., 1995. Sopergram Group Members, 1995. Impact of climate change on grassland production and soil carbon worldwide. Global Change Biology 1, 13–22.
Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6 (1), 288–295.
Pérez Figueroa, A., García Pereira, M.J., Saura, M., Rolán Alvarez, E., Caballero, A., 2010. Comparing three different methods to detect selective loci using dominant markers. Journal of Evolutionary Biology 23 (10), 2267–2276.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Schierup, M.H., Hein, J., 2000. Consequences of recombination on traditional phylogenetic analysis. Genetics 156, 879–891.
Shaffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596.
Smith, F.R., Yeaton, R.I., 2005. Mean annual rainfall and the population growth (1993–2002) and potential distribution of *Themeda triandra* in the shrubby vegetation of the southern Nama-Karoo. South African Journal of Botany 71, 117–121.
Stinchcombe, J.R., Hoekstra, H.E., 2007. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. Heredity 100 (2), 158–170.
Storz, J.F., 2005. Using genome scans of DNA polymorphism to infer adaptive population divergence. Molecular Ecology 14 (3), 671–688.
Ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.
Ter Braak, C.J.F., 1987. The analysis of vegetation–environment relationships by canonical correspondence analysis. Ecology 69 (1–3), 69–77.
Thornthwaite, C.W., 1948. An approach toward a rational classification of climate. Geographical Review 38 (1), 55–94.
Tollenaere, C., Duplantier, J.M., Rahalison, L., Ranjalahy, M., Brouat, C., 2010. AFLP genome scan in the black rat (*Rattus rattus*) from Madagascar: detecting genetic markers undergoing plague mediated selection. Molecular Ecology 20 (5), 1026–1038.
Treydte, A.C., Grant, C.C., Jeltsch, F., 2009. Tree size and herbivory determine below-canopy grass quality and species composition in savannahs. Biodiversity and Conservation 18 (14), 3989–4002.
Tubiello, F.N., Soussana, J., Howden, S.M., 2007. Crop and pasture response to climate change. Proceedings of the National Academy of Sciences 104 (50), 19686–19690.
Van der Hulst, R.G.M., Mes, T.H.M., Den Nijs, J.C.M., Bachmann, K., 2000. Amplified fragment length polymorphism (AFLP) markers reveal that population structure of triploid dandelions (*Taraxacum officinalis*) exhibits both clonality and recombination. Molecular Ecology 9, 1–8.
Van der Hulst, R.G.M., Mes, T.H.M., Falque, M., Stam, P., Den Nijs, J.C.M., Bachmann, K., 2003. Genetic structure of a population sample of apomictic dandelions. Heredity 90, 326–335.
Van Rensburg, S.J., Stock, W.D., Bond, W.J., 1999. Ecological significance of ecotypic variation in *Themeda triandra* Forssk. In: Freudenberger, D. (Ed.), People and Rangelands: Building the Future. Proceedings of the VI International Rangeland Congress, Townsville, Qld., Australia, pp. 938–939.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23 (21), 4407–4414.
Wilkinson, M., 2001. PICA 4.0: Software and Documentation. Department of ZoologyThe Natural History Museum, London, UK.
Woodland, P.S., 1964. The floral morphology and embryology of *Themeda australis* (R. Br.) Stapf. Australian Journal of Botany 12 (2), 157–172.
Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Molecular Ecology 8, 907–913.