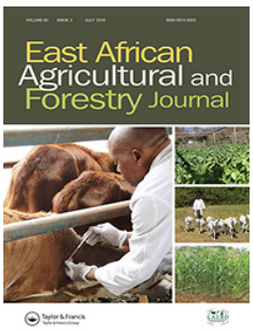

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Genetic diversity of endangered sandalwood (*Osyris lanceolata*) populations in Kenya using ISSR molecular markers

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ABSTRACT

Osyris lanceolata is an evergreen, drought tolerant tropical African tree species belonging to the family Santalaceae. It is endangered, owing to overexploitation for its essential oil used in cosmetics and pharmaceutical industries. The study aimed at determining: (1) the genetic diversity; and (2) the population genetic differentiation in seven key *O. lanceolata* populations, representing its natural distribution in Kenya. Genotype data for ISSR neutral molecular markers were generated for seven populations of *O. lanceolata*. The percentage of polymorphic loci (*P*), ranged from 51% (Wundanyi) to 82% (Gwasii), with a mean of 65%. The mean number of effective alleles (*Ne*) was 1.430, whereas the Shannon Information Index (*I*) mean was 0.263. Gwasii population was the most genetically diverse followed by Mt Elgon and least was Wundanyi. The coefficient of differentiation *Gst* was 0.343. Results of analysis of molecular variance (AMOVA) showed that most of the genetic variation (62%) in *O. lanceolata* resided within populations. Principal coordinate analysis (PCoA) analysis showed that Baringo population located in the Rift Valley was genetically distinct from the rest of the populations. In conclusion, Gwasii, Mt Elgon and Baringo populations should be delineated for *in situ* conservation, whereas selection for *ex situ* conservation should target good trees from all the populations.

KEYWORDS

conservation; effective alleles; endangered species; genetic diversity; genotype data; molecular variance; polymorphic loci

Introduction

Osyris lanceolata Hochst. and Steud. ex A. DC (African sandalwood) is a member of the family Santalaceae in the Order Santalales (Teklehaimanot et al. 2003; Mwang'ingo et al. 2007; Machua et al. 2009). It is drought tolerant, grows on poor sites mostly on rocky ridges, mountain slopes, margins of evergreen bushland, grasslands and thickets (Machua et al. 2009; IUCN 2013). The Kenyan populations are small and scattered along roads, farms, forest edges and bushlands. The species is a perennial one that can survive for more than 50 years in its natural stand. The Kenyan populations are likely to be more than 40 years old. Like other sandalwood trees species, *O. lanceolata* is

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dioecious and hemi-parasitic on the roots of host trees preferably *Rhus natalensis* (Krauss), *Dodonaea viscose* (L) Jacq, *Tecomaria capensis* (Thunb.) Lindl, *Catha edulis* (Vahl) Forssk. exEndl, *Apodytes dimidiate* E Mey exArn (Teklehaimanot et al. 2003; Mwang'ingo et al. 2007; Machua et al. 2009). The numbers of host species that can support the species seem limited. In Eastern Africa, the species occurs in Kenya, Uganda, Tanzania, Rwanda, Burundi, Eritrea and Somalia (Teklehaimanot et al. 2003; Mwang'ingo et al. 2007). Although in Southern Africa, it is found in Zambia, Malawi, Mozambique, Zimbabwe and South Africa (Mwang'ingo et al. 2007; IUCN 2013). Other members of Santalaceae family, though of different genera, are distributed in Australia (*Santalum spicatum, S. lanceolatum*), India and China (*S. album*) (Brand 1999; Hudson 2008; IUCN 2013) and Pacific Islands (*S. insulare*) (Butaud et al. 2005).

In Kenya, *O. lanceolata* is endangered and its survival threatened, owing to overexploitation for its valuable essential oils used for making perfumes, expensive cosmetics, medicines and drugs (Teklehaimanot et al. 2003; Mwang'ingo et al. 2007; Machua et al. 2009). This is because of high demand in developed countries for sandalwood products, occasioned by the reduction in supply from Australia and India, the major world suppliers (Mwang'ingo et al. 2003; Mwang'ingo et al. 2007). Illegal harvesters uproot the whole tree for its trunk and roots, which are believed to contain more oil per unit weight (Ruffo et al. 2002). Furthermore, preference is on female trees, which are believed to have higher oil content than males. This disrupts the species reproduction capacity and threatens its genetic pool (Ruffo et al. 2002; Mwang'ingo et al. 2007).

The situation is additionally worsened by the generally low natural regeneration, growth and development of *O. lanceolata* (Mwang'ingo et al. 2007; Kamondo et al. 2014). In addition, the species has low recruitment and experiences reproductive failures in its natural habitat (Mwang'ingo et al. 2 007; Kamondo et al. 2014; Mothogoane 2011). Information on the genetic diversity of *O. lanceolata* would therefore be essential for formulation of conservation strategies to mitigate the effects of reproductive failures, low regeneration and recruitment.

In Kenya, although the species has been placed under presidential protection (Government of Kenya, Legal Notice #3176 of 2007), so far no effort has been made to develop management strategies for sustainable utilisation and conservation of *O. lanceolata* germplasms, despite the fact that it is a source of livelihood for many people in areas where it grows naturally. Currently, there is no any existing information of the species genetic diversity status in Kenya. Genetic diversity is widely recognised as the key component for the long-term survival, dynamics and evolution of any tree species hence information on genetic status is an important component in management of genetic resources (Ramesha et al. 2007; Rhian and Michael 2008). Furthermore, the information on genetic diversity of plant species is a pre-requisite for the planning of *in situ* and *ex situ* conservation strategies (Holsinger and Gottlieb 1991; Chiveu et al. 2009; Rhian and Michael 2008).

The genetic diversity of plant populations has been assessed using morphological characterisation, but later complemented by the use of isozymes (Dangasuk et al. 1997; Dangasuk and Gudu 2000). However, these methods have proven to be slow and inadequate to determine the genetic diversity among various plant populations (Xiao-ru and Alfred 2001). Recently, molecular techniques have been applied to detect genetic variability in various plant species (Porth and El-Kassaby 2014). Some of these widely

applied molecular markers includes; Restriction Fragment Length Polymorphism (RFLP) (Botstein et al. 1980), Amplified Fragment Length Polymorphism (AFLP) (Vos et al. 1995), Random Amplified Polymorphic DNA (RAPD) (Williams et al. 1990), Simple Sequence Repeats (SSR) and Inter-Simple Sequence Repeats (ISSR) (Zietkiewicz et al. 1994; Awasthi et al. 2004), Single Nucleotide Polymorphism (SNP) (Nasu et al. 2002).

As a PCR-based marker, ISSR, is applicable to organisms from diverse genera and because of the large number of primers available for analyses, it provides good overall genome coverage (Williams et al. 1990; Zietkiewicz et al. 1994). They show dominant inheritance and are useful in detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome and evolve rapidly (Gupta et al. 2008; Ansari et al. 2012). Furthermore, they are easier to analyse (Ramesha et al. 2007; Patel et al. 2016) and unlike SSR markers, ISSR markers do not require prior knowledge of the target sequences flanking the repeat regions (Yang et al. 2012; Gaafar et al. 2014; Žukauskienė et al. 2014; Panda et al. 2015). The ISSR makers are neutral, have higher annealing temperature and longer sequence and hence are accurate and able to deduce variations in a species with wide geographical habitat variation (Deshpande et al. 2001; Ramesha et al. 2007).

The objectives of this study were to determine: (1) the extent of genetic diversity, and (2) the level of genetic differentiation among the Kenyan populations of *O. lanceolata* using ISSR molecular markers in order to recommend a conservation strategy for the species.

Materials and methods

Sample collections

Seven distinct *Osyris lanceolata* populations, namely Gwasii, Baringo, Mt Elgon, Kitui, Wundanyi, Meru and Mau representing the natural distribution area of the species in Kenya were sampled (Figure 1 and Table 1). Leaf samples were collected from up to 30 adult trees per population were dried on silica gel in plastic Ziploc bags and stored at −70° C until DNA extraction.

DNA extractions

Nuclear DNA was extracted following the CTAB (cetyl-trimethyl-ammonium-bromide) protocol described by FAO/IAEA (2002) with some modifications. Five grams of dry leaf samples were ground into powder using a steel ball mill grinder (model Retsch MM400, Hamburg Germany).

Six hundred µl of 1.5% freshly prepared and preheated (65 °C) CTAB extraction buffer (CTAB 1 ml, PVP 2 ml, NaCl 2.8 ml, EDTA 0.4 ml, Tris 1 ml (100 mMTris-HCl, pH 8.3; 83 mM acetic acid), Mecarpto-ethanol 0.0 5 ml, Water 2.75 ml) was added to the leaf powder. The mixture was vortexed gently for 1 minute then incubated in water bath at 65 °C for 1 hour with frequent gentle inversion of the tubes after every 20 minutes. The mixture was left for 4 minutes to cool to room temperature and 600 µl of chloroform: isoamyl alcohol (24: 1) added to it and mixed by gentle inversion to allow complete denaturation of the proteins in the suspension.

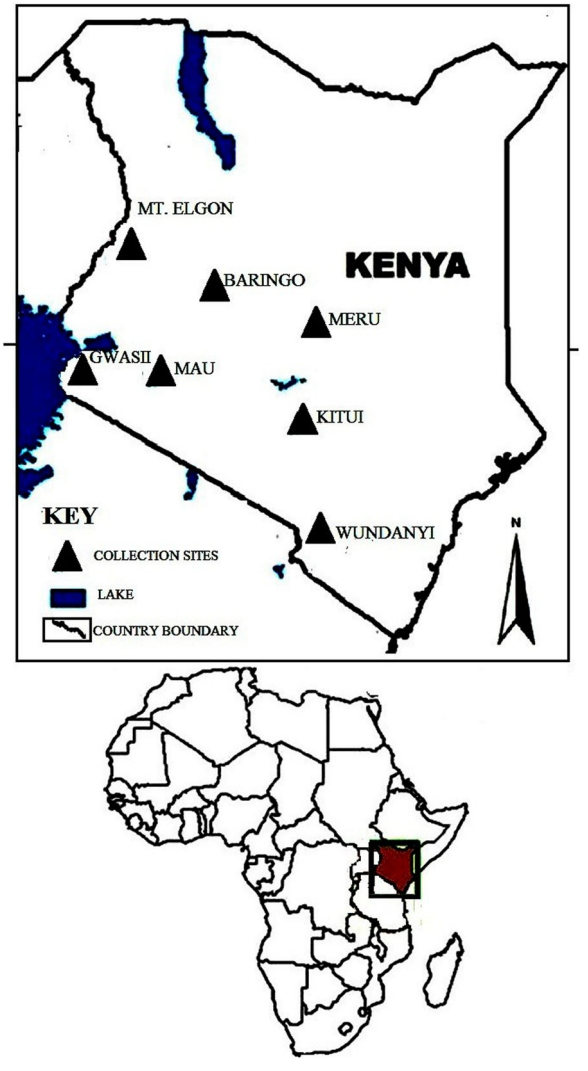

Figure 1. Location of the seven sampled populations of *Osyris lanceolata* in Kenya.

Table 1. Collection site data of *Osyris lanceolata* populations in Kenya used in this study.

Population	Longitude	Latitude	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)
Gwasii	34°10′ E	00°30′ S	1 600.7	1 100.0	26.0
Mt Elgon	34°43′ E	00°10′ N	2 007.4	1 280.0	18.5
Baringo	35°68′ E	00°50′ N	2 040.0	635.0	25.0
Kitui	38°00′ E	01°21′ S	1 185.4	775.0	25.0
Meru	37°34′ E	00°00′ N	1 427.7	1 440.0	20.7
Mau	35°16′ E	00°36′ S	2 288.0	1 025.0	21.0
Wundanyi	37°39′ E	01°10′ S	1 319.9	650.0	23.0

The mixture was centrifuged using a Hermle-microcentrifuge (model Eppendorf centrifuge 5804R, Hamburg Germany) at 13 000 rpm at 5 °C for 10 minutes. Five hundred µl of the upper, supernatant phase was transferred into fresh sterile Eppendorf tubes

marked accordingly using 1 000 µl micropipette, and 2/3 volume of isopropanol added and mixed gently by inversion to precipitate the total nucleic acids. The mixture was then refrigerated at −20 °C for 30 minutes to allow the extracted DNA to precipitate.

The precipitated DNA was centrifuged at 13 000 rpm for 10 minutes and the supernatant discarded leaving DNA pellets at the bottom of the tube. The pellets were washed twice in 70% ethanol. The ethanol was decanted and the DNA pellets air dried for 3 hours. The pellets were re-suspended in 100 µl of TE buffer and incubated overnight in a water bath at 55 °C to dissolve.

DNA standardization and quantification

The extracted DNA was quantified using a bio-photometer (Hamburg Germany). A total of fifteen ISSR primers were used for screening for utility (Table 2).

ISSR-PCR amplification

The PCR thermo-cycler was programmed as follows: one cycle of 94 °C for five minutes (Hot start step); 40 cycles at 94 °C for 30 seconds (denaturation step); a range between 45 and 47 °C (depending on the specific primer's annealing temperature) for 45 seconds (template annealing temperature step); 72 °C for 2 minutes (polymerization step); and a final extension phase of 10 minutes at 72 °C (Williams et al. 1990).

Out of the 15 primers, six primers were selected (808, 809, 810, 811, 818 and 825). These showed clear banding patterns, sufficient variation and high reproducibility after rechecking the banding (Tables 1 and 2) PCR-ISSR amplification for the seven populations, as shown in Table 2.

ISSR amplification was performed in a 25 µl volume containing 21 µl PCR mix, 3 µl primer and 1 µl DNA. The final tube volume had concentration of each dNTP 200 µM in 10 mM Tris-HCL buffer (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM $MgCl_2$; 2.5 mM each of dATP, dCTP, dTTP, dGTP; 0.5 µm primer; DNA and 0.3 U Taq polymerase. The PCR products were then electrophoresed in a 2.5% agarose gel for 2 hours and stained in ethidium bromide (0.8 mg ml^{-1}). The gels were then visualised under UV light and photographed using Kodak camera (Kodak MI ^{MT}). The presence of DNA bands were compared with the standard 100 bp DNA mass ladder. Amplification was repeated in the standardised conditions above and 90% banding similarity accepted for scoring. Only well-stained visible bands were scored as binary data in MS Excel sheet for analysis.

Table 2. Osyris lanceolat*a* ISSR primer oligonucleotide sequences and annealing temperature Tm (°C).

Primer Code	Primer sequence 5′→3′	No of fragments amplified	No of polymorphic fragments	Percentage of polymorphic fragments (%)
808	AGAGAGAGAGAGAGAGC	13	3	23.1
809	AGAGAGAGAGAGAGAGG	16	6	37.5
810	GAGAGAGAGAGAGAGAT	15	5	33.3
811	ACACACACACACACACC	15	5	33.3
818	CACACACACACACACAG	20	10	50.0
825	ACACACACACACACACT	17	7	41.2
Mean		16	6	36.4

Data analysis

Only well-stained visible polymorphic bands were scored for presence (1) or absence (0) (Wendel and Weeden 1989). The binary matrix data file created was configured as an input for the data analysis. The percentage of polymorphic loci (*P*),analysis of molecular variance (AMOVA), Shannon Information Index (*I*), number of effective allele (*Ne*), and coefficient of differentiation (*Gst*) were derived using PopGene version 1.32 (Yeh et al. 2000). The genetic distance (*D*) and the principal coordinates analysis (PCoA) were derived using GenALEx software 1.61 (Peakall and Smouse 2012). A dendrogram based on Nei's unbiased genetic distance (Nei 1978) was generated using MEGA 4 software (Tamura et al. 2007).

Results

Genetic diversity

The six ISSR primers used produced 96 fragments, with size ranging from 150 to 1 000 base pairs. A total of 36 polymorphic bands were produced, with anaverage of the 6 polymorphic loci. The largest number of polymorphic bands were amplified by primer 818 (10 bands) followed by primer 825 (7 bands). The least (3 bands) were amplified by primer 808 (Table 2).

The number of observed alleles (*Na*) ranged from Wundanyi (1.229) to Gwasii (1.667), with a mean of 1.429. The number of effective alleles (*Ne*) value ranged from 1.229 (Wundanyi) to 1.575 (Gwasii), with a mean of 1.430. The Shannon Information Index (*I*) ranged from 0.232 for Kitui to 0.446 for the Gwasii populations, with a mean of 0.365. The percentage of polymorphic loci (*P*%) ranged from 51% in Wundanyi to 82% in the Gwasii populations, with a mean of 65% (Table 3).

Population genetic differentiation

An analysis of molecular variance (AMOVA), which indicated that majority of the genetic variation was partitioned within populations (62%) compared with among populations (Table 4). Similar results were obtained for the estimated coefficient of differentiation (*Gst*), which was 0.3429 ($p = 0.001$), indicating that 34% of the total variation existed among the populations, whereas the majority of the variations (66%) resided within the populations (Table 5).

Table 3. Genetic diversity indices of seven *Osyris lanceolata* populations generated.

Population	*n*	*Na*	*Ne*	*I*	*P*
Gwasii	30	1.667	1.575	0.446	82.29
Mt Elgon	30	1.635	1.552	0.372	78.13
Mau	30	1.375	1.405	0.307	62.50
Baringo	30	1.417	1.364	0.441	60.42
Kitui	20	1.302	1.427	0.232	59.38
Meru	20	1.375	1.401	0.307	59.38
Wundanyi	18	1.229	1.229	0.338	51.04
		1.429	1.430	0.365	64.73

n = population size; *Na* = observed alleles; *Ne* = number of effective alleles; *I* = Shannon Information Index; *P* = percentage of polymorphic loci

Table 4. AMOVA for seven populations of *Osyris lanceolata,* based on variation at ISSR loci.

Source of variations	df	SS	MS	Estimated variance	%
Among populations	6	1 241.291	206.882	7.698	37.9
Within populations	171	2 146.889	12.555	12.555	62.1
Total	177	3 388.180		20.253	100

Table 5. Coefficient of differentiation *Gst* in *Osyris lanceolata* across different loci using ISSR.

Primer	Number of loci	Ht	Hs	*Gst*
Primer 808	13	0.3911	0.2039	0.4714
Primer 809	16	0.3559	0.2713	0.2384
Primer 810	15	0.3605	0.2230	0.3680
Primer 811	15	0.4523	0.2884	0.3636
Primer 818	20	0.3369	0.2431	0.2696
Primer 825	17	0.3788	0.2487	0.3283
Mean	96	0.3765	0.2474	0.3429

Ht – genetic diversity, Hs – average genetic diversity within subpopulations, *Gst* – coefficient differentiation

Unbiased genetic distance between the seven populations ranged from 0.124 between the Gwasii and Mau populations to 0.333 between the Baringo and Kitui populations. The populations had a mean genetic distance of 0.217. The Mau, Mt Elgon and Gwasii populations appeared to be closer to each other, with a genetic distance ranging from 0.124 to 0.185. The Baringo population was the most distinct, and differentiated from the rest of the populations, with a genetic distance ranging from 0.234 to 0.333. Wundanyi followed it, with a genetic distance ranging from 0.216 to 0.247. Similarly, the Baringo population was differentiated by a dendrogram generated in MEGA 4 software, based on Nei's unbiased genetic distance (Nei 1978) (Figure 3 and Table 6).

The relationship among individuals and populations is summarised in the PCoA scatter diagram. This analysis indicated a genetic overlap among six populations, whereas the Baringo population was distinctively separate from the rest (Figure 2).

Discussion

Genetic variation of Osyris lanceolata among seven Kenyan populations

The number of effective alleles (*Ne*) observed in this study ranged from 1.229 in Wundanyi to 1.575 in Gwasii, with a mean of 1.430. This is lower than the *Ne* (1.86) observed from

Table 6. Nei's unbiased genetic distance of *Osyris lanceolata* populations in Kenya.

Population	Gwasii	Baringo	Mt Elgon	Mau	Wundanyi	Kitui	Meru
Gwasii	0						
Baringo	0.254 (351.00)	0					
Mt Elgon	0.129 (201.14)	0.259 (152.18)	0				
Mau	0.124 (211.21)	0.234 (128.80)	0.158 (254.45)	0			
Wundanyi	0.216 (561.15)	0.247 (521.38)	0.241 (660.39)	0.222 (401.35)	0		
Kitui	0.189 (435.71)	0.333 (325.26)	0.212 (475.61)	0.175 (231.756)	0.294 (228.66)	0	
Meru	0.147 (395.71)	0.290 (217.28)	0.183 (366.05)	0.185 (192.80)	0.212 (391.79)	0.245 (163.32)	0
Mean	0.177	0.270	0.197	0.183	0.231	0.234	0.2241

Numbers in parentheses are kilometres (km)

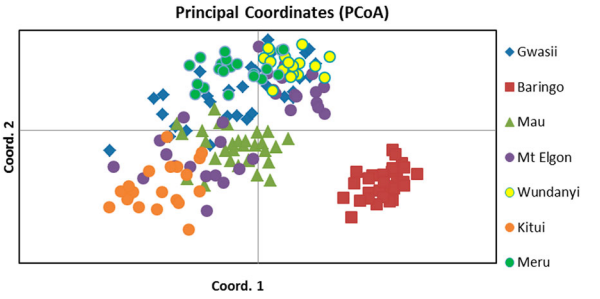

Figure 2. The principle coordinates analysis of 178 *Osyris lanceolata* individuals.

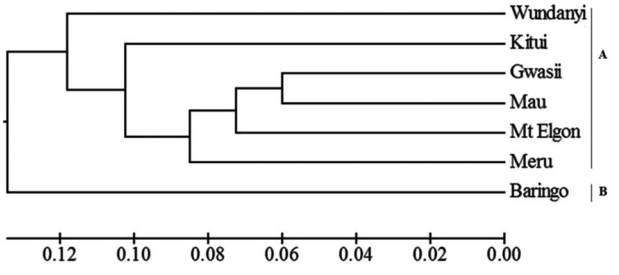

Figure 3. Dendrogram based on the Nei unbiased genetic distance in 178 *Osyris lanceolata* individuals.

A. senegal, a tropical tree that shares the same habitat with *O. lanceolata* using ISSR markers (Chiveu et al. 2009). However, it was comparable with the *Ne* obtained from other tropical tree species, such as *Jatropha curcas* Linnaeus (*Ne* = 1.407), using ISSR makers (Gupta et al. 2008) and *Michelia coriacea* (Hung T Chang & BL Chen) Figlar (*Ne* = 1.460; Zhao et al. 2012). using ISSR markers. Incidentally, contrary to the small population sizes and their fragmentation, the mean number of observed alleles (*Na* = 1.429) is closely similar to the mean number of effective alleles (*Ne* = 1.430). This seems to imply that the major factors endangering the survival of *O. lanceolata* in Kenya are ecological and anthropogenic (e.g. habitat specialisation, fragmentation and overexploitation) rather than genetic factors (Wu et al. 2014). The Shannon Information Index (*I*), which is a measure of the degree of variation within populations, in this study ranged from Kitui (0.232) to Gwasii (0.446), with a mean of 0.365 for the species. This is comparable with the 0.374 obtained in *J. curcas* populations in India based on ISSR markers, which was considered high (Gupta et al. 2008). In contrast, it was less than what was obtained for *M. coriacea* (0.436) (Zhao et al. 2012), for *Argania spinosa* (L.) Skeels in Morocco (0.460) (Naima et al. 2015) and the 0.430 for *Pongamia pinnata* (L.) Pierre a tropical tree species in Brazil (Rout et al. 2009) all using ISSR markers. The percentage of polymorphic loci (*P*) obtained in this study ranged from Wundanyi (51%) to Gwasii (82%), with an overall mean of 65%, which was comparatively similar to the 64% found in *S. spicatum* an Australia tree species of the same family (Byrne et al. 2003), but lower than the 76% for *S. album* in India, 87% for *A. senegal* in Kenya (Chiveu et al. 2009; Patel et al. 2016), the 96% for *M. coriacea* in China (Zhao et al. 2012), the 77% for *J. curcas* genotypes in India (Gupta et al. 2008), the 94% for *P. pinnata* in Brazil (Rout et al. 2009), and the 79% for *A. spinosa* in Morocco (Naima et al. 2015) all using ISSR

markers. It may be concluded that comparatively the level of polymorphism obtained in this study at species level is moderately high. This was supported by the fact that out of the seven populations only two had high levels of polymorphism (Gwasii 82% and Mt Elgon 78%). The remaining five had moderately high polymorphism ranging from 51% in Wundanyi to 62% in Mau.

The distribution of the genetic index values among the populations does not show a clear relationship with environmental parameters, such as altitude, mean annual rainfall and mean annual temperatures. This is in agreement with Hamrick and Godt (1989), who observed that geographic range was not significantly associated with variation among populations or population differentiation. The pattern of genetic variation as portrayed by the genetic indices seems to be influenced by the observed population density and size. For example, Gwasii and Mt. Elgon had large and high-density populations of *O. lanceolata*, whereas Wundanyi had the smallest population with scattered individual trees, to the extent that only 18 trees were sampled out of the target of 30 trees. A scenario similar to the one observed in Wundanyi was encountered in Kitui and Meru, where only 20 trees out of a target of 30 per population were sampled. The density of the Baringo and Mau populations was intermediate between that of the Mt. Elgon and Kitui populations, which agrees with the finding of Hamrick (1984). Tropical tree populations, particularly those that occur at high density, maintain a high level of genetic density. Low effective population sizes lead to a loss of genetic diversity, because of genetic drift (Hamrick 1984; Wu et al. 2014).

Population differentiation in Osyris lanceolata

The Nei (1978) genetic distance (*D*) showed that the Baringo population, with a mean genetic distance of 0.270, was genetically distinct from the rest of the populations. Overall, the mean genetic distance of 0.215 was indicative of a high level of genetic differentiation between the populations of *O. lanceolata*. Sebbenn et al. (2008) found that long-term and selective logging resulted in loss of alleles and genotypes, which increased the genetic distance. Gwasii was the least differentiated of the populations, with a mean genetic distance of 0.177.

The PCoA cluster in this study discriminated the *O. lanceolata* populations into two broad clusters, which have been affected by such factors as genetic drift, breeding systems, population sizes, gene flow, evolutionary history and natural selection, but not by geographic proximity. Cluster A consists of the Gwasii, Mau, Mt Elgon, Meru, Wundanyi and Kitui populations. In this cluster, the Wundanyi and Kitui populations occur at relatively lower altitudes (1 185.4–1 319.9 m asl) and in conditions of low mean annual rainfall (650–775 mm) and high mean annual temperatures (23–25 °C). These populations are small in size and occur in semi-arid areas. Incidentally, they contain the least amount genetic polymorphism (51.04–59.38%). The Gwasii, Mau, Mt Elgon and Meru populations in the same cluster occur at higher altitudes (1 427.7–2 288 m asl), where there is a high mean annual rainfall (1 025–1 440 mm) and relatively lower mean annual temperatures (18.5–26 °C). They are located in the mountainous areas of Kenya and have denser populations and the highest genetic polymorphism (59.38–82.29%). Cluster B consist of the Baringo population only. This population occurs at a high altitude (2 040 m asl) in an area with the lowest mean annual rainfall (635 mm)

and high mean annual temperature (25 °C). This population is located within the Rift Valley and apparently is physically isolated from the rest and has a moderately high genetic polymorphism (60.42%). The uniqueness of the Baringo population concurs with what was reported for *A. senegal* from Baringo by Chiveu et al. (2008 and 2009), using RAPD and ISSR markers.

Based on an analysis of molecular variance (AMOVA), most of the genetic variation in *O. lanceolata* resides within the populations (62%). This agrees with the findings of Loveless and Hamrick (1984) for predominantly outcrossing plant species and is consistent with results obtained for other tropical tree species, such as *Acacia senegal* 95% (Chiveu et al. 2009), *Acacia tortilis* 76% (Olong'otie 1992) and *J. curcas* 69% (Gupta et al. 2008). However, the lower value of 62% observed for *O. lanceolata*, could be attributed to the smaller sizes of its populations, which consist largely of scattered individual trees in the wild. This could likely result in plasticity of the outcrossing mating system of the species, accordingly affecting the within population genetic diversity (Zhang et al. 2009). Similarly, the coefficient of differentiation *Gst* of 0.3429 observed suggests that 34% of the variation resides among populations and 66% resides within population, which concurs with the AMOVA results.

Overall, the data showed a high genetic differentiation among the Kenyan *O. lanceolata* populations. Reasons that have been advanced for higher levels of population differentiation in tropical tree species include small population sizes with lower densities, more widely scattered populations, that reduces gene flow and increases genetic drift, and greater spatial variation in natural selection pressure (Bawa 1986; Liengsiri et al. 1995). Other factors associated with high degree of genetic differentiation among populations are mating systems and evolutionary and life history factors (Hogbin and Peakal 1999; Zhao et al. 2012). Currently, most of the *O. lanceolata* populations in Kenya are of small sizes and consist of scattered individual trees on rocky and/or mountainous sites, which establish barriers for gene flow between them. In addition, habitat loss and fragmentation, overexploitation of the species and introduction of exotic species in the natural environment where *O. lanceolata* occurs has led to subdivision of the populations into smaller units and its imposed barriers to gene flow therefore leading to greater differentiation. Compared with widespread taxa, many endangered species, such as *O. lanceolata,* might become genetically differentiated, owing to their small population sizes. This accords with the findings of Bottin et al. (2005) for *Santalum austrocaledonicum* (Vieill.), a sandalwood endemic to New Caledonia and Vanuatu.

Limitations of the study

This study used ISSR dominant markers, which might not bring out the deviations in the Hardy–Weinberg equilibrium, and consequently the level of population inbreeding cannot be evaluated exclusively. Exclusive genetic differentiation observed in the population is reliable considering the estimates by both AMOVA and *Gst* closeness. However, inbreeding level could not be conclusively estimated based on the dominant markers used in this study. The study concentrated on the genetic aspect and failed to consider quantitative genetic information on the distribution of variation of the species. Variations in growth, phenology and drought resistance among other quantitative genetic information would have been important in guiding the species conservation strategies.

Conclusions and recommendations

Osyris lanceolata populations in Kenya showed a moderate-high genetic diversity. The Gwasii population had the highest genetic diversity by all criteria used and was followed by Mt Elgon. The least genetically diverse was Wundanyi population with the rest being intermediate. Most of the genetic variation was found to reside within than among populations. The Baringo population is completely differentiated and isolated from the rest of the six populations. The seven Kenyan populations of *O. lanceolata* were differentiated into two clusters according to their genetic relationships as opposed to geographic proximity. The Meru, Kitui, Wundanyi and Baringo populations are small and restricted populations and do not share alleles between themselves, which is indicative of isolation, reduced gene flow and genetic drift. The results of this study therefore suggest strongly that the Gwasii and Mt Elgon populations, which have the highest levels of genetic diversity, should be delineated and managed as *in situ* conservation areas. An *ex situ* conservation program should be immediately initiated, targeting healthy trees from all the populations. This should ensure conservation of full array of genetic diversity within the species and restoration of the genetic potential necessary for its survival, adaptation and evolution. A study on the growth, phenology and drought resistance variation in the *O. lanceolata* population is required.

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References

Ansari SA, Narayanan C, Wali SA, Kumar R, Shukla N, Rahangdale SK. 2012. ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* L.f.) populations. Ann. For. Res. 55(1):11–23.

Awasthi KA, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J. 2004. Genetic diversity and relationship in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. BMC Genet 5:1.

Bawa SK. 1986. Breeding of tropical hardwoods: an evaluation of underlying bases, current status and future prospects. In: Burley J and Styles BT (Ed.). Tropical trees: variation, breeding and conservation. London: Academic Press. pp 43–59.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32(3):314–331.

Bottin L, Verhaegen D, Tassin J, Olivieri I, Vaillant A, Bouvet JM. 2005. Genetic diversity and population structure of an insular tree *Santalum austrocaledonicum* in New Caledonian archipelago. *Mol Ecol* 14:1979–1989.

Brand JE. 1999. Ecology of sandalwood (*S. spicatum*) near Payne Find and Manzies, Western Australia, size structure and dry-sided stems. *Rangeland J* 21(2):220–228.

Butaud JF, Rives F, Verhaegen D, Bouvet JM. 2005. Phylogeography of Eastern Polynesian sandalwood (*Santalum insulare*), an endangered tree species from the Pacific: a study based on chloroplast microsatellites. Laboratory of Natural Products Chemistry, University of French Polynesia, *TahFren Polyn* 32(10):1763–1774.

Byrne M, MacDonald B, Broadhurst L, Brand J. 2003. Regional genetic differentiation in Western Australian sandalwood (*Santalum spicatum*) as revealed by nuclear RFLP analysis. *Theor Appl Gen* 107(7):1208–1214.

Chiveu CJ, Dangasuk OG, Omunyin ME, Wachira FN. 2008. Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd. revealed by combined RAPD and ISSR markers. Afr J Biotech 7(14):2333–2340.

Chiveu CJ, Dangasuk OG, Omunyin ME, Wachira FN. 2009. Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd. based on ISSR markers. Silv Gen 58(1–6):22–28.

Dangasuk OG, Gudu S. 2000. Allozyme variation in 16 natural populations of *Faidhebia albida* (Del.) A. Chev. Hereditas 133:133–145.

Dangasuk OG, Seurei P, Gudu S. 1997. Genetic variation in seed and seedling traits in 12 African provenances of *Faidherbia albida* (Del.) A. Chev at Lodwar, Kenya. Agrofor Syst 37:133–141.

Deshpande AU, Apte GS, Bahulikar RA, Lagu MD, Kulkarni BG, Suresh HS, et al. 2001. Genetic diversity across natural populations of three montane plant species from the Western Ghats, India, revealed by inter-simple sequence repeats (ISSR. Mol Ecol 10:2397–2408.

FAO/IAEA. 2002. Mutant germplasm characterization using molecular markers. A manual prepared by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Vienna: International Atomic Energy Agency (IAEA)

Gaafar ARZ, Al-Qurainy F, Khan S. 2014. Assessment of genetic diversity in the endangered populations of *Breonadia salicina* (Rubiaceae) growing in the kingdom of Saudi Arabia using ISSRmarkers. BMC Genet 15(109):1–10.

Government of Kenya. 2007. Legal notice number 3176 of 2007.

Gupta S, Srivastava M, Mishra GP, Naik PK, Chauhan RS, Tiwari SK, Kumar M, Singh R. 2008. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. Afr J Biotechnol 7(23):4230–4243.

Hamrick JL. 1984. The distribution of genetic variation within and among natural plant populations. In: Shonewald-Cox CM, Chambers SM, MacBryde B, Thomas L (Eds) Genetics and Conservation, Sunderland, MA. Sinauer, pp 43–63.

Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. In: Brown AHD, Cleg MT, Kahle AL, Weir BS (Eds). Plant population genetics, breeding and genetic resources. Massachusetts: Sinauer. pp 1–19.

Hamrick JL, Godt MJW. 1990. Plant population genetics, breeding and genetic resources. Sunderland, Massachusetts: Sinauer Associates, Inc. pp 43–63.

Hogbin PM, Peakall R. 1999. Evaluation of the contribution of genetic research to the management of the endangered plant Zieriaprostrata ConserBiol 13:514–522.

Holsinger KE, Gottlieb LD. 1991. Conservation of rare and endangered plants: principles and prospects. Genetics and conservation of rare plants New York: Oxford University Press. pp 195–208.

Hudson P. 2008. Kununurra could become World's biggest producer of Indian sandalwood. http://www.abc.net.au/rural/wa/content/2006/s2244847.htm.

IUCN. 2013. IUCN Red List categories and criteria: version 3.1. IUCN Species Survival Commission, Gland. http://jr.iucnredlist.org/documents/redlist_cats_crit_en.pdf [Accessed 28 Dec 2013].

Kamondo B, Giathi G, Osore C, Machua J, Kagunyu L, Wafula A, et al. 2014. Growing of East African Sandalwood. Guidelines for Growers Nairobi: KEFRI.

Liengsiri C, Yeh HC, Boyle TJB. 1995. Isoenzyme analysis of tropical forest tree *Pterocarpus macrocarpus* Kurz. in Thailand. For Ecol Manag 74:13–22.

Machua J, Kamondo B, Mwangi L, Giathi G, Chahilu O. 2009. A useful parasite. Miti 3:30–31.

Mothogoane M S. 2011. *Osyris lanceolata* Hocst. and Steud. South African National Biodiversity Institute (SANBI) Report. www.plantzafrica.com/plantnop/osyrislanc.html. [Accessed 02 April 2016].

Mwang'ingo PL, Teklehaimanot Z, Hall JB, Zilihona JE. 2007. Sex distribution, reproductive biology and regeneration in the dioecious species *Osyris lanceolata* (African sandalwood) in Tanzania. Tanz J For Nat Conser 76(1):118–133.

Mwang'ingo PL, Teklehaimanot Z, Hall JB, Lulandala LLL. 2003. African sandalwood (*Osyris lanceolata*): resource assessment and quality variation among populations in Tanzania. South Afr For J 199(1):77–88.

Naima AA, Msanda F, El-Mousadik A. 2015. Genetic diversity of the endangered argan tree (*Argania spinosa* L.) (Sapotaceae) revealed by ISSR analysis. Basic Res J Agric Sci Rev 4 (46):176–186.

Nasu S, Suzuki J, Ohta R, Hasegawa K, Yui R, Kitazawa N, Monna L, Minobe Y. 2002. Search for and analysis of single nucleotide polymorphism (SNP) in rice (*Oryza sativa, Orizaru figogon*) and establishment of SNP markers. DNA Res 9:163–171.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Genetics 70:3321–3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nei M, Roychoudhury AK. 1974. Sampling variances of heterozygosity and genetic distance. Genetics. 76:379–390.

Olong'otie PAS. 1992. Population structure and breeding systems in *Acacia tortilis* (Forsk) Hayne. PhD thesis. Oxford University, UK.

Panda S, Naik D, Kamble A. 2015. Population structure and genetic diversity of the perennial medicinal shrub Plumbago. *AoB PLANTS 7*: plv048.

Patel DM, Fougat RS, Sakure AA, Kumar S, Kumar M, Mistry JG. 2016. Detection of genetic variation in sandalwood using various DNA markers. Biotech 6:55.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinform 28:2537–2539.

Porth I, El-Kassaby YA. 2014. Assessment of the genetic diversity in forest tree populations using molecular markers. Diver 6:283–295.

Ramesha BT, Ravikanth G, Rao MN, Ganeshaiah KN, Shaanker RU. 2007. Genetic structure of the rattan *Calamus thwaitesii* in core, buffer and peripheral regions of three protected areas in central Western Ghats, India: do protected areas serve as refugia for genetic resources of economically important plants? J Gen 86(1):9–18.

Rhian JS, Michael FF. 2008. An introduction to plant conservation genetics. Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey. TW93DS. http://www.ccw.gov.uk/landscape–wildlife/habitats–species/terrestrial/species/floweringplants/plantconservation-genetics.Aspx. [Accessed 26 January 2008].

Rout GR, Sahoo DP, Aparajita S. 2009. Studies on inter and intra-population variability of *Pongamia pinnata*: a bioenergy legume tree. Crop Breed Appl Biotech 9:268–273.

Ruffo CK, Binie A, Tengas B. 2002. Edible wild plants of Tanzania. RELMA technical handbook series 27. Regional Land Management unit (RELMA) Swedish International Development Agency (SIDA), Nairobi Kenya.

Sebbenn AM, Degen B, Azevedo VCR, Silva MD, de Lacerda AEB, Ciampi AY, et al. 2008. Modelling the long term impact of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. For Ecol Manag 254:335–349.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599.

Teklehaimanot Z, Mwag'ingo PL, Mugasha AG, Ruffo CK. 2003. Influence of the origin of stem cutting, season of collection and auxin application on the vegetative propagation of African sandalwood (*Osyris lanceolata*) in Tanzania. South Afr For J 201:13–14.

Vos PR, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, et al. 1995. AFLP a new technique for finger printing. Nucleic Acids Res 23(21):4407–4414.

Wendel JF, Weeden NF. 1989. Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (Eds) Isozymes in plant biology. London: Chapman and Hall. pp 46–73.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified methods to plant systematic and evolutionary biology. In: Soltis PS, Soltis, DE, Doyle JJ (Eds) Molecular systematic of plants: DNA sequencing. New York: Kluwer. pp 43–86.

Wu FQ, Shen SK, Zhang XJ, Wang YH, Sun WB. 2014. Genetic diversity and population structure of an extremely endangered species: the world's largest Rhododendron. AoB Plants 7:1–9.

Xiao-ru W, Alfred ES. 2001. Molecular markers in population genetics of forest trees. Scand J For Res 16:199–220.

Yang J, Yang J, Yang M, Qing H. 2012. Genetic diversity of *Caragana* species of the Ordos Plateau in China. Plant Syst Evol 298(4):801–809.

Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan M. 2000. PopGenee Version 1.32. Microsoft windows based free software for population genetics analysis. Molecular Biology and Biotechnology Centre, University of Alberta and Center for International Forestry Research.

Zhang JF, Kimatu JN, Guo WL, Liu B. 2009. Habitat fragmentation causes rapid genetic differentiation and homogenization in natural plant populations - A case study in *Leymuschinensis*. Afr J Biotech 8(150):3440–3447.

Zhao XF, Ma YP, Sun WB, Wen XY, Milne R. 2012. High genetic diversity and low differentiation of *Michelia coriacea* (Magnoliaceae), a critically endangered endemic in Southeast Yunnan, China. Int J Mol Sci 13:4396–4411.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genom 20(2):176–183.

Žukauskienė J, Paulauskas A, Varkulevičienė J, Maršelienė R, Gliaudelytė V. 2014. Genetic Diversity of Five Different Lily (*Lilium* L.) Species in Lithuania Revealed by ISSR Markers. Am J Plant Sci. 5:2741–2747.