 J Life Sci, 4(2): 97-105 (2012)

Genetic Variation of Kenyan Populations of *Warburgia ugandensis*, an Important East African Highlands Medicinal Tree Species

Alice Muchugi[1,2], Roland Kindt[1], Geoffrey M. Muluvi[2], Edward Muge[1], Hillary Kipruto[1]and Ramni H. Jamnadass[1]

[1] World Agroforestry Centre (ICRAF). P.O. Box 30677 00100 Nairobi, Kenya
[2]Department of Biochemistry and Biotechnology, Kenyatta University, PO. Box 43844 00100 Nairobi, Kenya

KEYWORDS African Medicinal Species. AFLP. Genetic Variation. Kenyan Rift Valley

ABSTRACT *Warburgia ugandensis* is an important medicinal tree species in Eastern Africa used to treat several ailments. Wild populations are under great threat due to unsustainable harvesting for medicines and indiscriminate felling of trees for timber and firewood. There is an urgent need for developing and implementing conservation strategies of this species and information on genetic structure is a crucial input. Analysis of molecular variance (AMOVA), which employed 141 AFLP markers revealed most genetic variation to be among individuals within populations (59%, $P< 0.0001$), but variation among populations (41%, $P< 0.0001$) was highly significant as well. Constrained ordination analysis illustrating the relationship among populations showed a clear distinction between *W. ugandensis* from Uganda and western Kenya and other *W. ugandensis* populations. No correspondence was shown in some cases on pair-wise genetic distances and geographic distances among populations. These findings suggested that conservation strategies for the species in Kenya should place relatively more emphasis on the revealed genetic structuring within the country.

INTRODUCTION

The World Health Organisation estimates that 80% of the world's population rely on traditional herbal remedies (WHO 2002). However, over-harvesting and poor harvesting techniques of plants with medicinal value, as well as encroachment of their natural habitat, threaten their survival and conservation. *Warburgia ugandensis* is one of such species. It is widely used by the local communities to cure several ailments such as such as stomach ache, constipation, toothache, common cold, cough, fever, muscle pains, weak joints, candidiasis, measles and malaria, (Beentje 1994; Kokwaro 1976). It is also used in treating livestock diseases, for example, trypanasomiasis (Kioy et al. 1990; Olila et al. 2001). Stem and root barks as well as leafy twigs are harvested and their raw extract used singly or in combination with other herbal plants.

The species extracts have high antibacterial (Olila et al. 2001; Wube et al. 2005), antifungal (Taniguchi and Kubo 1993) and molluscidal (Kubo et al. 1983). Important phytochemicals isolated from *W. ugandensis* extracts are mainly drimane sesquiterpenes such as muzigadial, ugandensial, polgodial, mukadial, warburganal, ugadensial and ugadensiolide (Kioy et al, 1990; Kubo et al. 2005; Olila et al. 2001; Taniguchi and Kubo 1993). With the positive results *in vivo* and *in vitro* trials against economically important tropical diseases such as malaria (Muregi et al. 2003) and measles (Olila et al. 2001), there is great potential for *W. ugandensis* pharmaceutical products in the commercial markets. In Kenya and Uganda, a few herbalists have started packaging *W. ugandensis* extracts for the local markets (www.naturally africanplatform). Extracts from a species in the same genus that has a wider distribution in southern African countries, *W. Salutaris*, are commercially processed in South Africa and marketed internationally at highly competitive prices (Botha et al. 2004).

Considering the existing competition for arable land and forests, the main way of increasing tree cover in sub-Saharan Africa is by cultivating trees on farms. This not only ensures a domestic supply of tree products and conservation of the species, but it also provides farmers with extra income when they sell some of the tree products. A key factor that governs the adoption of planting of agroforestry species is the prospective products they provide since high value trees with a ready market are likely to be planted by farmers, whereas other species of less

Address for correspondence:
Alice Muchugi,
Department of Biochemistry and Biotechnology,
Kenyatta University
PO Box 43844-00100 Nairobi, Kenya
E-mail: amuchugi@gmail.com

defined use are not. To enhance the *W. ugandensis* conservation and utilisation, planting of the species on farms is being encouraged. A prerequisite for the efficient use of genetic resources in any planting program is a detailed understanding of the extent and distribution of genetic variation available within a species. A preliminary study involving the species of genus *Warburgia* showed significant genetic differentiation within and among *W. ugandensis* populations (Muchugi et al. 2008) and unique clustering in the ordination analysis linked to the population geographical position with relation to the eastern arm of the Rift Valley. This study therefore carried further analysis to assess the genetic structuring among *W. ugandensis* populations from Kenya and two reference populations from Tanzania and Uganda, with a primary objective of determining more efficient management strategies for the species at national and regional levels.

MATERIALS AND METHOD

Genomic DNA was isolated from leaf material taken from 77 individuals sampled from seven populations of *W. ugandensis* (Fig. 1, Table 1). AFLP analysis was carried following the standard procedures described in Vos et al. (1995). Data generated was analysed using the *GENALEX 6.2* (Peakall and Smouse 2006) to give genetic diversity and genetic distance measures which were used to derive distance-based constrained ordination (Legendre and Anderson 1999) was used to show correlation among populations. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was analysed using the *Arlequin 3.1* (Schneider et al. 1997) package. Further analysis was carried out to assess the relationship between the species geographic location and genetic diversity using the stepping stone model.

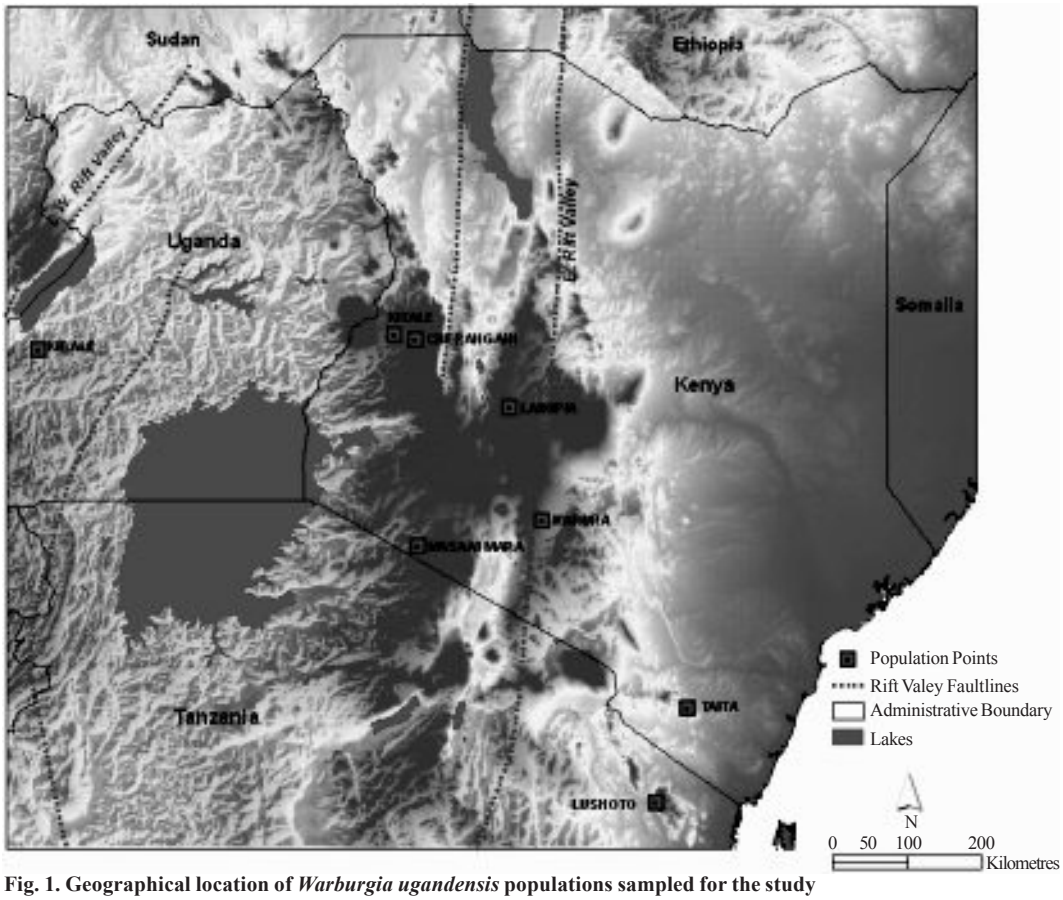

Fig. 1. Geographical location of *Warburgia ugandensis* populations sampled for the study

Table 1: Mean diversity estimates (*H*) for eight populations of *Warburgia ugandensis* generated from 141 AFLP markers for 77 individuals sampled from Kenya, Tanzania and Uganda. 'Position' denotes the population geographic position with relation to the Kenyan arm of the Rift Valley; east within or west. The percentage polymorphic loci (% loci) and sample size (*N*) are also shown

Country	*Population name*	*Position*	*Sample size (N)*	*H (SE)*
Kenya	Karura	east	10	0.157 (0.016)
	Kitale	west	10	0.161 (0.016)
	Cherangani	west	10	0.126 (0.015)
	Laikipia	within	10	0.163 (0.016)
	Masai Mara	within	10	0.128 (0.015)
	Taita	east	7	0.120 (0.014)
Tanzania	Lushoto	east	10	0.135 (0.015)
Uganda	Kibale	west	10	0.168 (0.015)

RESULTS

The AFLP analysis using four primer combinations; *Eco*RI+ACA/*Mse*I+CAA, *Eco*RI+ACT/*Mse*I+CAT, *Eco*RI+ACT/*Mse*I+CAG and *Eco*RI+AGC/*Mse*I+CAG, gave a total of 141 polymorphic markers. Frequencies of allele products generated with AFLP markers were calculated and used to estimate the genetic diversity, *H,* within populations (Table 1). Results showed that the populations sampled were quite diverse with the Kibale population from Uganda having the highest diversity estimate (H = 0.168). Kenyan Masai Mara population showed the lowest diversity estimate (H = 0.120). Analysis of molecular variance (AMOVA) showed that most of the genetic variation (59%) was retained within the populations (Table 2). Further structured AMOVA analysis with populations structured with reference to the Rift Valley revealed this regional differentiation accounting for 35% of genetic variation. Constrained ordination analyses (Fig. 2) showed clear split between individuals from Kitale and Cherangani populations from Kenya and Ugandan Kibale population on the right side quadrants, from the other four Kenyan populations (Karura, Laikipia, Masai Mara and Taita) and the Tanzanian population from Lushoto, which were in the left side quadrants. The Kenyan Cherangani population Kitale and Ugandan Kibale populations differentiated clearly, with each population falling in its own quadrant on the left side. Clear grouping was also evident in the Kenyan Taita and Tanzanian Lushoto populations in the lower part of the left side quadrant while Karura, Laikipia and Masai Mara (all from Kenya) were on the upper part of the left side quadrant.

An assessment of the relationship between pair- wise genetic distance and geographic distance among populations indicated that genetic distances from the focal population clearly had non-linear relationships with geographic distances (Figs. 3 and 4, Table 3). The genetic distance did not always increase with geographic distance; for example, the investigation with Cherangani as the focal population and the Nei genetic distance showed that genetic distance decreased from the Karura stepping stone population (genetic distance in between Cherangani and Karura = 0.319) to the Kibale next stepping stone (Nei genetic distance in between Cherangani and Kibale = 0.146), whereas the geographic distance from the focal population increased from 295 to 509 km (Fig. 3, Table 3). Indeed several genetic distances increased at a greater rate than the change in geographic distance (Table 3, *numbers indicated in bold)* while in others genetic distances decreased at a greater rate than the change in geographic distance (Table 3, *underlined numbers)*. Taking the earlier example, the change in genetic distance between the Karura and Kibale stepping stones was -1.19 ([0.146 – 0.319] / 0.319), which corresponds to a larger absolute change in distance than the change in geographic distance of 0.72

Table 2: Analysis of molecular variance (AMOVA) based on 141 AFLP markers for 77 individuals sampled from Kenya, Tanzania and Uganda. Nested analysis was undertaken on population regional basis with respect to the Kenyan arm of the Rift Valley (east, within and west). Degrees of freedom (df), sum of squares (SS) mean squared deviation (MSDs) and the % variances are shown

Source of variation	*d.f.*	*SS*	*MSD*	*% of total variance*	*P value*
Among all populations	7	677.79	96.83	41.0	0.0001
Among individuals within populations	69	876.34	12.70	59.0	0.0001
Among regions	2	380.44	190.22	23.0	0.0001
Among populations within regions	5	297.34	59.47	22.0	0.0001
Among individuals within populations	69	876.34	12.7	56.0	0.0012

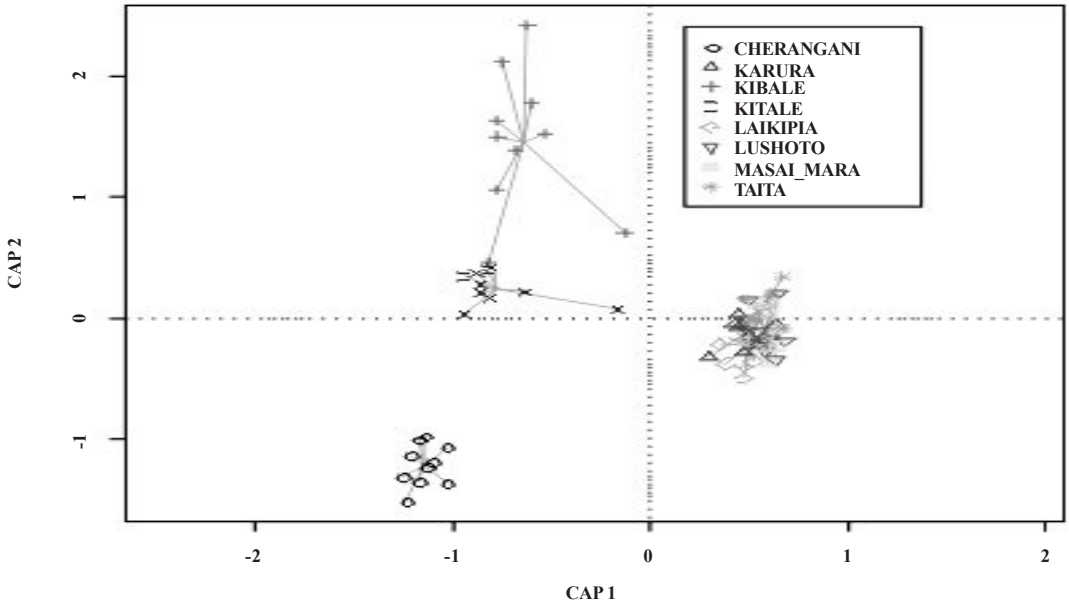

Fig. 2. Ordination diagram for eight populations of *Warburgia ugandensis* generated with Jaccard distance-based redundancy analysis using longitude and latitude of individual trees as explanatory variables with 141 AFLP markers. These two constrained ordination axes explain 16.6% of squared Jaccard distances (P = 0.001 based on 1000 permutations). Spiderplots summarise the distribution of individuals of the same population

([509 - 295] / 295). For each focal population, the number of instances where genetic distance changed significantly in between two stepping stone populations was counted and then classified changes in genetic distances in terms of changes between stepping stones that occurred in the same geographic region (Table 4) and changes that occurred in between geographic regions (Table 5).

On the analysis where other populations were sorted out as stepping stones from each focal population based on their geographic distance, significant changes were not expected for stepping stone populations that occurred within the same geographic region,. However, Kitale and Cherangani (two populations occurring closely on the western side of the Rift Valley) showed significant changes (Table 4). For focal populations from the west (Kibale) and from the east (Taita and Lushoto), Cherangani had a significantly larger genetic distance than Kitale. For focal populations from the Rift Valley, Cherangani was significantly closer to Laikipia, whereas Kitale was significantly closer to the Masai Mara. Another odd pattern occurred where the Masai Mara was significantly closer to Taita (East) than to Lushoto (East).

DISCUSSION

Although population densities of *W. ugandensis* trees in the sampled populations were quite low in the ecological survey, genetic diversity was high within these populations when compared to other molecular studies on other indigenous tree species within the same region (Dawson and Powell 1999; Kadu et al. 2006; Lengeek et al. 2006; Muchugi et al. 2006). This was contrary to the expectations as low population densities have been linked to reduction in population heterozygosity and gene diversity (Dusan 1992), which is attributed to restricted maternal mother trees and genetic drift. This is because mating between related individuals (inbreeding) accelerates the reduction in heterozygosity by reducing the effective population size (Hamrick and Godt 1989). However, in this case, the low population is recent (occasioned by felling of mature trees) and the observed high genetic diversity is resulting from remnant trees of the previous wide distribution.

The high genetic diversity is important for the species adaptation to changing user requirements in different environments. In collection of *W. ugandensis* germplasm material for plant-

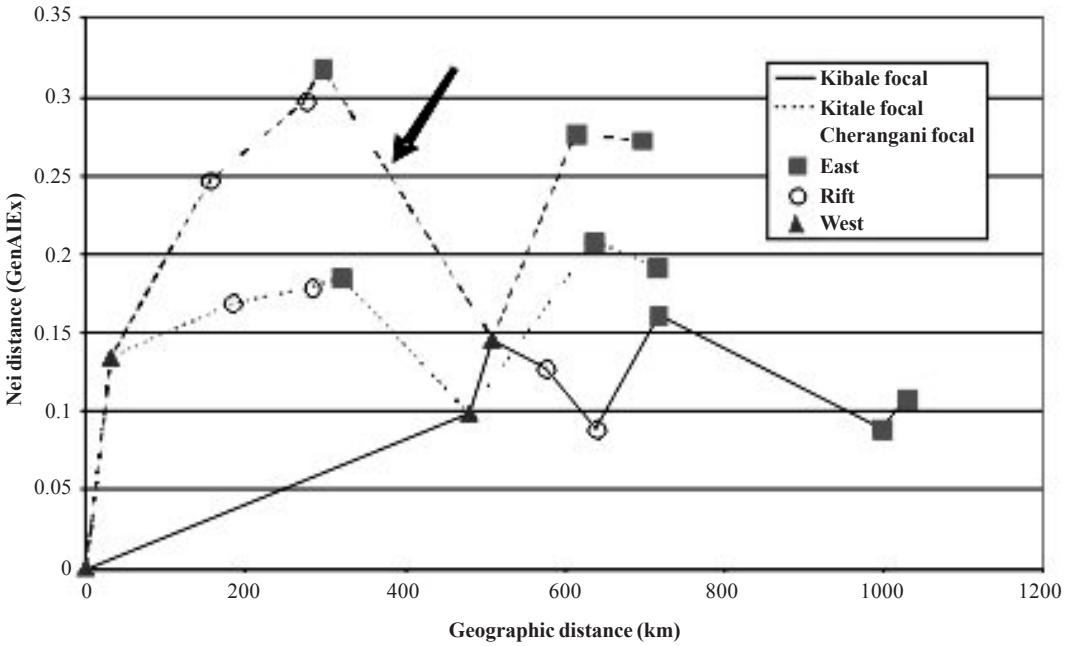

Fig. 3. Change in the Nei genetic distance from a focal population west of the Rift Valley in respect to geographic distance. The arrow indicates a significant decrease in genetic distance from Karura to Kibale for the investigation for the Cherangani focal population. Populations are indicated by symbols, whereas investigations from the same focal population are connected by the same line type

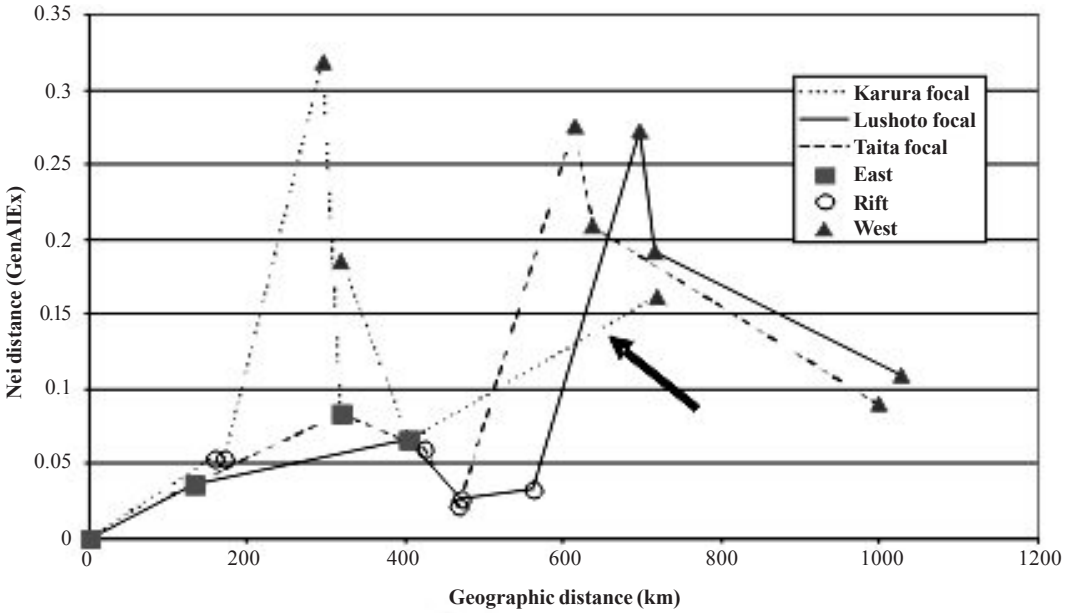

Fig. 4. Change in the Nei genetic distance from a focal population east of the Rift Valley in respect to geographic distance Graphs for the focal populations from the. The arrow indicates a non-significant increase in genetic distance from Lushoto to Kibale from the Karura focal population. Populations are indicated by symbols, whereas investigations from the same focal population are connected by the same line type

Table 3: Geographic and pairwise genetic distances among populations from each focal population and the other populations are sorted as stepping stones. Distances in bold showed a significant increase in genetic distance compared to the previous stepping stone population, whereas distances that were underlined indicate a significant decrease in genetic distance

Pairwise distance	*Stepping stone populations*	*Focal population (abbreviation)*							
		CHE	*KIB*	*KIT*	*LAI*	*MAS*	*KAR*	*LUS*	*TAI*
Geographic	Cherangani	0	509	31	155	274	295	696	613
	Kibale	509	0	479	640	575	717	1028	998
	Kitale	31	479	0	184	283	319	716	637
	Laikipia	155	640	184	0	221	157	561	467
	Masai Mara	274	575	283	221	0	171	469	424
	Karura	295	717	319	157	171	0	405	318
	Lushoto	696	1028	716	561	469	405	0	132
	Taita	613	998	637	467	424	318	132	0
Nei distance	Cherangani	0.000	**0.146**	0.135	0.247	**0.297**	**0.319**	**0.273**	**0.276**
	Kibale	0.146	0.000	0.099	**0.089**	**0.128**	0.162	0.109	0.089
	Kitale	0.135	0.099	0.000	**0.169**	0.179	**0.186**	0.192	0.208
	Laikipia	0.247	0.089	0.169	0.000	0.036	0.054	**0.033**	0.021
	Masai Mara	0.297	0.128	0.179	0.036	0.000	0.053	0.026	0.060
	Karura	0.319	**0.162**	0.186	0.054	0.053	0.000	0.066	0.083
	Lushoto	**0.273**	**0.109**	0.192	**0.033**	0.026	0.066	0.000	0.037
	Taita	0.276	0.089	**0.208**	0.021	0.060	0.083	0.037	0.000
Average Jaccard	Cherangani	0.337	**0.723**	0.570	0.826	**0.818**	0.789	**0.829**	0.881
	Kibale	0.723	0.618	0.665	**0.827**	**0.806**	0.786	0.817	0.860
	Kitale	0.570	0.665	0.352	**0.745**	0.709	**0.669**	0.747	0.819
	Laikipia	0.826	0.827	0.745	0.534	0.589	0.561	0.616	0.670
	Masai_Mara	0.818	0.806	0.709	0.589	0.406	0.511	0.523	0.650
	Karura	0.789	0.786	0.669	0.561	0.511	0.375	0.556	0.647
	Lushoto	0.829	0.817	0.747	0.616	0.523	0.556	0.460	0.658
	Taita	0.881	0.860	0.819	0.670	0.650	0.647	0.658	0.540
Number of homo-geneous loci	Cherangani	84	36	48	30	36	28	33	33
	Kibale	36	51	33	25	27	22	27	26
	Kitale	48	33	70	33	**40**	38	36	**37**
	Laikipia	30	25	33	70	55	52	53	55
	Masai_Mara	36	27	40	**55**	84	57	**67**	62
	Karura	28	22	38	**52**	57	74	53	54
	Lushoto	33	27	36	53	67	53	81	62
	Taita	33	26	37	55	62	**54**	62	82

Table 4: Changes in genetic distance between two stepping stone populations from the same region for population genetic distances (Pop, 9 tests), average distances between individuals (Ind, 9 tests) and number of loci that were homozygous (Loc, 3 tests). Bold figures indicate situations were more than 50% of tests resulted in significant changes

Group	*Previous stepping stone*	*Stepping stone*	*Focal*	*Pop*		*Ind*		*Loc*	
				Pos	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*
West	Kitale	Cherangani	Kibale	**9**	0	**5**	0	0	0
	Kitale	Kibale	Lushoto	0	4	0	0	0	0
	Kitale	Kibale	Taita	0	0	0	0	0	0
	Cherangani	Kitale	Masai Mara	0	**9**	0	**9**	0	1
	Cherangani	Kitale	Taita	0	**8**	0	**8**	0	1
	Cherangani	Kitale	Lushoto	0	**9**	0	**9**	0	0
	Cherangani	Kitale	Laikipia	**9**	0	**7**	0	**2**	0
Rift	Masai Mara	Laikipia	Taita	1	2	1	2	0	0
	Masai Mara	Laikipia	Kibale	0	**6**	0	1	0	0
	Masai Mara	Laikipia	Lushoto	**7**	0	0	0	0	0
	Laikipia	Masai Mara	Karura	4	1	1	3	0	0
	Laikipia	Masai Mara	Kitale	0	0	0	0	0	0
	Laikipia	Masai Mara	Cherangani	0	0	0	0	0	0
East	Taita	Karura	Lushoto	0	0	0	0	0	0
	Taita	Lushoto	Masai Mara	0	**9**	0	**6**	0	0
	Taita	Lushoto	Kitale	0	0	0	2	0	0
	Taita	Lushoto	Kibale	**6**	0	**5**	1	0	0
	Taita	Lushoto	Laikipia	4	0	0	0	0	0
	Taita	Lushoto	Cherangani	0	0	1	0	0	0
	Lushoto	Karura	Taita	0	0	0	0	0	0
	Karura	Taita	Kibale	0	4	0	0	0	0

Table 5: Changes in genetic distance between two stepping stone populations from different regions derived from population genetic distances (Pop, 9 tests), average distances between individuals (Ind, 9 tests) and number of loci that were homozygous (Loc, 3 tests). Bold figures indicate situations were more than 50% of tests resulted in significant changes

Boundary	*Previous Stepping Stone*	*Stepping stone*	*Focal*	*Pop*		*Ind*		*Loc*	
				Pos	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*
E-W	Cherangani	Karura	Laikipia	0	**9**	0	**9**	0	**3**
	Cherangani	Taita	Karura	0	**9**	0	**8**	0	**2**
	Karura	Kibale	Kitale	0	**6**	0	0	0	0
	Karura	Kibale	Cherangani	0	**5**	0	0	0	0
	Kibale	Taita	Cherangani	**9**	0	3	0	0	0
	Kibale	Taita	Kitale	**7**	0	1	0	0	0
	Kitale	Lushoto	Karura	0	**9**	0	2	0	0
	Kitale	Taita	Masai Mara	0	**9**	0	2	0	0
	Lushoto	Kibale	Karura	0	0	0	0	0	0
	Lushoto	Kibale	Laikipia	**9**	0	**8**	0	**2**	0
	Lushoto	Kibale	Masai Mara	**9**	0	**8**	0	**2**	0
	Taita	Kitale	Karura	**9**	0	**7**	2	**2**	1
E-R	Karura	Laikipia	Masai Mara	0	4	0	0	0	0
	Karura	Masai Mara	Lushoto	0	**9**	0	2	0	**2**
	Karura	Masai Mara	Taita	0	0	0	0	0	0
	Laikipia	Karura	Kibale	**7**	0	0	1	0	0
	Masai Mara	Karura	Kitale	0	0	0	2	0	0
	Masai Mara	Karura	Cherangani	0	0	0	0	0	0
	Masai Mara	Taita	Laikipia	0	1	0	0	0	0
W-R	Cherangani	Laikipia	Kitale	0	0	0	0	0	0
	Cherangani	Masai Mara	Kibale	4	3	1	0	0	0
	Kitale	Laikipia	Cherangani	0	0	0	0	0	0
	Kitale	Masai Mara	Laikipia	0	**9**	0	**8**	0	**2**
	Laikipia	Cherangani	Lushoto	**9**	0	**7**	0	0	0
	Laikipia	Cherangani	Masai Mara	**9**	0	**7**	0	0	0
	Laikipia	Cherangani	Taita	**5**	0	**5**	0	0	0
	Masai Mara	Cherangani	Karura	**6**	0	0	0	0	0

ing programs, proper sampling should aim at maximizing conservation of the high intra-population genetic diversity found in the natural populations. Distribution of planting material with high genetic base will cushion subsequent generations of founder effects (Simons et al. 1993). For all categories of AMOVA analyses in both markers, results showed most of the genetic variations being retained within individuals among sampled populations rather than among populations. This is quite in agreement with previous finding where tropical woody perennials were found to maintain most of the genetic variation within populations (Hamrick et al. 1992). Focusing on a few populations for conservation purposes at national level would therefore capture the genetic differentiation.

Both the ordination and cluster analysis showed that the Kitale populations from Kenya clustered together with the Kibale population from Uganda rather than with other geographically closer Kenyan populations. A similar grouping was observed among Kenyan populations of *Prunus africana* (Muchugi et al. 2006). This study further confirmed the implied theories of African floral evolution (White 1983), which considers western Kenya as the most eastern remnant of the Guineo-Congolian phytochoria while eastern Kenya populations fall within the Somalia-Masai centre of endemism. Such genetic differentiation reflects the different evolutionary history of the species in the different ecological niches combined with different gene dispersal mechanisms (White 1983). Previous work by Martinelli et al. (1986) showed that at a macrogeographic scale across Africa, the composition *P. africana* bark extract depends on the origin of material. Accordingly, phytochemical analyses on active ingredients in *W. ugandensis* extracts associated with the herbal therapy may show similar diversity between these grouping and may influence the selection of superior material for domestication. It is therefore important that conservation of the species in Kenya must inevitably consider anthropogenic impacts on *W. ugandensis* germplasm distribution as the species cultivation increases.

A regular decrease of genetic similarity with increasing geographic distance was predicted by the theory of isolation by distance and by the stepping stone model (Cox and Durrett 2002) under the assumption that movement connected with mating is usually restricted to short distances. Results from the analysis of geographic and pairwise genetic distances among populations from each focal population and the other populations are sorted as stepping stones showed interesting patterns. In case that stepping stone population was from the same region as the focal population, a significant decrease in genetic distance was expected while in case that the previous stepping stone population was from the same region as the focal population, then a significant increase in genetic distance was expected. This pattern was observed in most cases which explained further the genetic differentiation shown by AMOVA and constrained ordination analysis. However two odd patterns were identified. The first odd pattern showed that the genetic distance decreased in between Karura and the Masai Mara for the focal population of Lushoto, whereas an increase was expected since Lushoto and Karura both occur on the eastern side of the Rift Valley. The second odd pattern was obtained for the change in distance from Cherangani to the Masai Mara with the Kibale focal population as four population genetic distances confirmed an increase (as expected since Cherangani and Kibale belong to the same region), whereas three population genetic distances confirmed a decrease. The seven cases investigated did not confirm significant change. For example, there was no significant change from the Karura-Lushoto genetic distance to the Karura-Kibale genetic distance whereas had a significant decrease was expected as Karura and Lushoto occur on the east whereas Kibale occurs on the west. Checking for the Nei genetic distance showed that the Karura-Lushoto distance was 0.066 and the Karura-Kibale genetic distance was 0.162 (Table 3). However, for these populations, the change in genetic distance (0.59) was smaller than the change in geographic distance (0.77) so that the test concluded that there was no significant change.

CONCLUSION

Warburgia ugandensis populations in Kenya display significant regional genetic differentiation with respect to Rift Valley. These results confirm earlier suggestion that other evolutionary factors rather than geographic distances are responsible for the genetic differentiation within the *W. ugandensis* populations in Kenya.

RECOMMENDATIONS

Conservation strategies of *W. ugandensis* in Kenya must put into consideration the genetic disjunction displayed by the populations across the Rift Valley so that unique regional germplasm is not lost through over exploitation. In addition, there is need to assess the active ingredients in *W. ugandensis* extracts associated with the herbal therapy across the species range in Kenya to establish whether it will positive correlation to the genetic disjunction.

ACKNOWLEDGEMENTS

Funding for this research was provided by International Foundation for Agricultural Research (IFAR) and the core financial support from European Commission and USAID to the World Agroforestry Centre (ICRAF).

REFERENCES

Beentje HJ 1994. *Kenya Trees, Shrubs and Lianas*. Nairobi, Kenya: National Museums of Kenya.

Botha J, Witkowski ETF, Shackleton CM 2004. The impact of commercial harvesting on *Warburgia salutaris* (pepper-bark tree) in Mpumalanga, South Africa. *Biodiversity and Conservation,* 13: 1675-1698.

Cox JT, Durrett R 2002. The stepping stone model: New formulas expose old myths. *The Annals of Applied Probability,* 12(4): 1348–1377.

Dawson IK, Powell W 1999. Genetic variation in the Afromontane tree *Prunus africana*, An endangered medicinal species. *Molecular Ecology,* 8: 151-156.

Doyle JJ, Doyle JL 1987. CTAB DNA extraction in plants. *Phytochemical Bulletin,* 19: 11-15.

Dusan G 1992. Effects of origin on the genetic diversity of Norway spruce (*Picea abies* Karst) populations. *Forest Ecology and Management,* 54: 215-223.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondria DNA restriction data. *Genetics,* 131: 479-491.

Hamrick JL, Godt MW 1989. Allozyme diversity in plant species. In: ADH Brown, MT Clegg, AL Kahler, B Weir (Eds.): *Plant Population Genetics, Breeding and Genetic Resources*. Sunderland Massachusetts: Sinauer Press, pp. 43-63.

Hamrick JL, Godt MW, Sherman-Broyles SL 1992. Factors influencing genetic diversity in woody plants species. *New Forests,* 6: 95-124.

Haraguchi H 1998. Mode of action of antimicrobial substances in plants. *Recent Research Developments in Agricultural and Food Chemistry,* 2: 259-268.

Kadu CAC, Imbuga M, Jamnadass R, Dawson IK 2006. Genetic management of indigenous fruit trees in southern Africa: A case study of *Sclerocarya birrea* based on chloroplast and nuclear variation. *South African Journal of Botany*, 72: 42-427.

Kioy D, Gray AI, Waterman PG 1990. A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of *Warburgia ugandensis* and *S. stuhlmannii*. *Phytochemistry,* 29(11): 3535-3538.

Kokwaro JO 1976. *Medicinal Plants of East Africa*. Nairobi, Kenya: East African Literature Bureau.

Kubo I, Fujita K, Lee SH, Joung Ha T 2005. Antibacterial activity of Polygodial. *Phytotherapy Research,* 19: 1013–1017.

Legendre P, Anderson MJ 1999. Distance-based redundancy analysis: Testing multi-species responses in multi-factorial ecological experiments. *Ecological Monographs,* 69(1): 1-24.

Lengkeek AG, Muchugi AM, Agufa CAC, Ahenda JO, Dawson IK 2006. Comparing genetic diversity in agroforestry systems with natural forest: A case study of the important timber tree *Vitex fischeri* in central Kenya. *Agroforestry Systems,* 67: 293-300.

Martinelli EM, Seragli R, Pifferi G 1986. Characterisation of *Pygeum africanum* bark extracts by high-resolution gas chromatography with computer assistance. *Journal of High Resolution Chromatography and Chromatography,* 9: 106-110.

Muchugi A, Lengkeek AG , Kadu CAC, Muluvi GM, Njagi ENM, Dawson IK 2006. Genetic variation in the threatened medicinal tree *Prunus africana* Cameroon and Kenya: Implications for current management and evolutionary history. *South African Journal of Botany,* 72: 498-506.

Muchugi A, Muluvi GM, Kindt R, Kadu CAC, Simons AJ, Jamnadass RH 2008. Genetic structuring of important medicinal species of genus *Warburgia* as revealed by AFLP analysis. *Trees Genetics and Genome,* 4: 787-795.

Muregi WF, Njagi ENM, Ndiege IO, Chabbra SC, Langat-Thorwa C, Njue E, Omar SA, Mkoji GM 2003. In vivo anti-malarial activity of some Kenyan plants and their effects in combination with chloroquine. *Journal of Ethnopharmacology,* 84(2-3): 237-241.

Olila D, Olwa-Odyek B, Opuda-Asibo J 2001. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*. *African Health Sciences,* 1(2): 66-72.

Peakall R, Smouse PE 2006. GENALEX 6: Genetic analysis in Excel: Population genetic software for teaching and research. *Molecular Ecology,* 6(1): 288-295.

Schneider S, Kueffer JM, Roessli D, Excoffier L 2000. *Arlequin ver. 1.1: A Software for Population Genetic Data Analysis.* Switzerland: University of Geneva.

Simons AJ, MacQueen DJ, Stewart JL 1993. Strategic concepts in the domestication of non-industrial trees. In: RRB Leakey, AC Newton (Eds.): *Tropical Trees: The Potential for Domestication and the Rebuilding of Forest Resource* London, UK: HMSO, pp. 91-102.

Sneath PHA, Sokal RR 1973. *Numerical Taxonomy*. San Francisco, USA.: Freeman.

Taniguchi M, Kubo I 1993. Ethnobotanical drug discovery based on medicine men's trials in the African savannah: Screening of East African plants for anti-microbial activity II. *Journal of Natural Products,* 56: 1539-1546.

Vos P, Hogers R, Bleecker M, Reijans V, Lee T, Hornes M, Freijters A, Pot J, Peleman J, Zabeau M 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acid Research,* 23: 4407-4414.

White F 1983. The Vegetation of Africa: A Descriptive Memoir to Accompany the UNESCO/AETFAT/UNSO Vegetation Map of Africa by F. White. *Natural Resources Research Report XX*. Paris, France: UNESCO.

WHO (World Health Organisation) 2002. Traditional Medicine Strategy 2002–2005. From <www.who.int/medicines/library/trm/trm_strat_eng.pdf> (Retrieved 15 July, 2007).

Wube AA, Bucar F, Gibbons S, Kaleab A 2005 Sesquiterpenes from *Warburgia ugandensis* and their antimyco-bacterial activity. *Phytochemistry,* 66: 2309–2315.