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Genetic Structure of *Prunus africana* Rosaceae (Hook.f.) Kalkm. in East Africa, as Inferred from Nuclear and Chloroplast DNA

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ABSTRACT

Prunus africana (Hook.f.) Kalkml., the African cherry, is an evergreen climax vegetation tree species typically reaching 25–30 m in height and occurs primarily in montane and submontane forests. In this study, the genetic structure of *P. africana* was analyzed using a coding chloroplast DNA region; Megakaryocyte-Associated Tyrosine Kinase Gene (MATK) and nuclear ribosomal internal transcribed spacer (nrITS) with 63 individuals in East Africa. This study detected low levels of genetic diversity as revealed by nucleotide diversity in nrDNA (π = 0.00529) and cpDNA (0.00448). As revealed by the results of AMOVA analysis, genetic differentiation for cpDNA (F_{ST} = 0.0275) was obviously lower than for nrDNA data (F_{ST} = 0.237) in *P. africana*. Gene flow among populations based on nrDNA data (*Nm* = 2.641) was significantly higher than that based on cpDNA (*Nm* = 0.82). Mantel test revealed a significant correlation between genetic and geographic distances for cpDNA ($r^2 = 3.0 \times 10^{-5}$) and nrDNA ($r^2 = 7.0 \times 10^{-5}$). Demographic history analyses based on pair-wise nucleotide sequence mismatch distributions revealed that only the Kakamega population was in mutation-drift disequilibrium. Tajima's *D* neutrality test, however, revealed significant signatures of recent population expansion in only the Kakamega population (*D* = –1.85646; *P* < 0.05). This study therefore proposes immediate *ex situ and in situ* conservation of *P. africana* populations in Mabira and Elgon forests coupled with *in situ* conservation of Budongo, Kakamega and Monduli forests.

Keywords: African cherry, demographic history, haplotype, population diversity
Abbreviations: A**MOVA**, analyses of molecular variance; **cpDNA**, chloroplast DNA; **FST**, genetic differentiation among populations; **h**, haplotype diversity; **hT**, total genetic diversity; **ITS**, Internal transcribed spacer; **MATK**, megakaryocyte-associated tyrosine kinase; **Nm**, gene flow among populations;; **nrDNA**, nuclear DNA; **PCA**, Principal Components Analysis; **π**, nucleotide diversity

INTRODUCTION

Genetic diversity represents the heritable variation within and between populations of organisms, and in the context of this paper, among populations of *Prunus africana* species. This pool of genetic variation within an inter-mating population is the basis for selection as well as for plant improvement. Thus, conservation of this plant genetic diversity is essential for present and future human well-being. Diversity patterns inferred by such studies can have important implications for forest conservation and management (Newton *et al*. 1999).

Prunus africana (Hook.f.) Kalkml., the African cherry, is an evergreen climax vegetation tree species typically reaching 25–30 m in height and occurs primarily in montane and submontane forests (Beentje 1994; Hall *et al.* 2000). *P. africana* is the only species of the genus *Prunus* native to Africa. The fruit (drupe) resembles a cherry when ripe. It is distributed mostly along the eastern side of Africa from Ethiopia to South Africa, though there are populations in several West African countries and in the Comoros and Madagascar (Hall *et al*. 2000). Seeds of *P. africana* germinate well under shady conditions but require light gaps to survive beyond the sapling stage (Tsingalia 1989; Kiama and Kiyiapi 2001; Nzilani 2002). The tree is valued for its medicinal extract from its bark for the treatment of benign prostatic hyperplasia, a common condition in elderly men. Diversity of other uses of this tree include making of handles for axes and hoes, firewood and poles (Cunningham *et al*. 1997). The overexploitation has sparked conservation concerns (Cunningham and Mbenkum 1993).

Raising *Prunus* through tissue culture techniques has therefore been suggested to accelerate tree production (Sunderland and Nkefor 1996; Dawson 1997; Dawson *et al.* 2000). The transfer of germplasm from the wild into on farm niches helps to preserve valuable genetic resources particularly if attention is paid to the origin and diversity of cultivated material. In addition, cultivation of endangered forests species takes pressure off their natural resource base, thus promoting the conservation of natural forests (Engles *et al.* 2001).

Knowing the degree of genetic variation is of fundamental importance for species' conservation (Barrett and Kohn 1991; Ellstrand and Elam 1993; Gilpin and Soule 1986; Hamrick and Godt 1996a; Karron 1997; Lande 1999). There are factors such as species' life-history, biogeography, and gene flow into the population that could also play critical roles in determining the current genetic composition of populations (Hamrick and Godt 1996a, 1996b; Holsinger *et al*. 1999). Therefore, understanding genetic factors that contribute to extinction risks for particular species is critically important for their conservation (Godt and Hamrick 1998; Hamrick and Godt 1996a). The extent of genetic variation in a species and its distribution among populations (or other entities of subdivision) is determined by a large number of factors, including the mating system, the demographic history, the effective population size, and the extent of gene flow by, e.g., migration or seed dispersal between populations. At each level, the genetic variants are united through interbreeding at some time point in the past (Weising *et al.* 2005). By analyzing the amount of variation and its partitioning over these hierarchical levels, important conclusions about the biology of a species can be drawn. Therefore, it was the aim of this study to evaluate the genetic diversity of *P. africana* from forests of different protection status in Eastern Africa.

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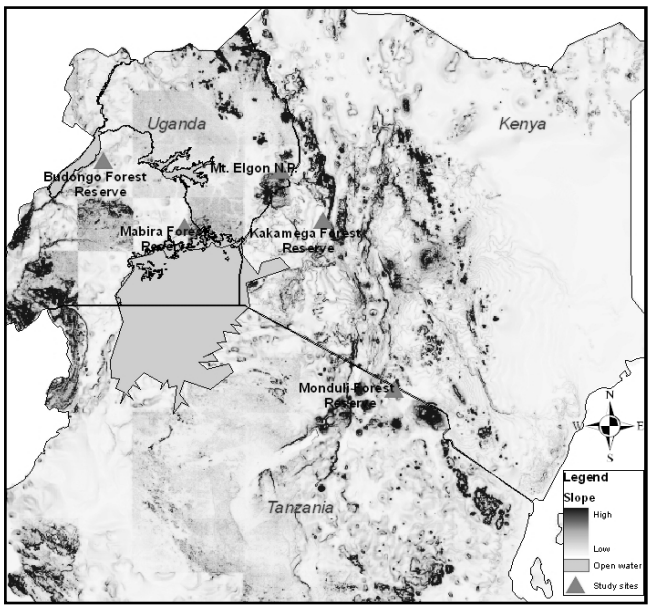

Fig. 1 Locations of *Prunus africana* populations sampled for this study.

MATERIALS AND METHODS

Population sampling

From July to September 2009, wild populations of *P. africana* were sampled within five forests in East Africa; Mabira, Budongo, Elgon, Kakamega and Moduli forests (**Fig. 1**), using random sampling. Random sampling is a sampling technique where a group of subjects (a sample) is selected for study from a larger group (a population). Each individual is chosen entirely by chance and each member of the population has a known, but possibly non-equal, chance of being included in the sample (Easton and McColl 1997). In each of the five populations; 16 individuals were sampled from Mabira, 5 individuals from Budongo, 7 individuals from Elgon, 26 individuals from Kakamega and 8 individuals from Monduli forests. Leaves were collected from individual trees at least 15-20 m apart to increase the possibility of detecting the potential variations within each population. Fresh leaves were collected, pressed and dried between blotters. A 1 × 1 cm^3 leaf area was crushed. Genomic DNA was extracted using Innu Speed Plant DNA kit. DNA quality and quantity were assessed on a spectrophotometer by absorbance (BioPhotometer, Eppendorf, Hamburg, Germany).

DNA isolation, PCR amplification and DNA sequencing

In total, 10 – 20 mg of fresh air dried plant leaf tissue from five forests (**Fig. 1**) were taken and disrupted in a mixer mill (MM2, Retsch, Germany), using 3 – 4 iron balls of 3 mm diameter per sample. The powdered tissue was then portioned to 2 mg portions in 2 ml reaction tubes (Sarstedt, Germany) using a precision gauge (R200D, Sartorius, Germany). Sample preparation and DNA extractions were carried out in a contaminant free environment. Total genomic DNA was extracted using InnuPREP Plant DNA kit (Analytik Jena AG, Jena, Germany). DNA concentration was assessed using a spectrophotometer by absorbance (BioPhotometer, Eppendorf, Hamburg, Germany). Working stocks of DNA were then prepared based on estimates, and stored in 0.1 × TE buffer. After preliminary screening, polymorphism was observed in the coding region and the internal transcribed spacer (ITS). These were amplified via polymerase chain reactions (PCRs) using primers; MatK-Kim-f reverse (5′-cgtacagtactttgtgtttta-3′) and ITS-4R reverse (5′-CACCACCACCACRC-3′).

DNA amplifications were carried out in reaction volumes of 25.0 µl containing 2.0 µl DNA, 0.4 µM of primer (100 µM/µL), 0.2 mM of each 2 mmol/L dNTP (BIO-39028, Bioline Germany), 1 µL 5 × buffer (BIO-39028, Bioline Germany), 0.5 µL *Taq* Polymerase (5 U/µL, Mango*Taq*^{TM}) and 0.8 µg/µl, water (ddH_2O) up to 50 µl. PCR was carried out in an Eppendorf Mastercycler gradient that was programmed for one cycle of 1 min at 94°C followed by 35 cycles of 30 sec at 94°C, 20 sec at 56°C and 1 min at 72°C, 4 min at 95°C followed by 35 cycles of 40 sec at 95°C, 40 sec at 56°C and 1 min at 72°C and with a cycle of 1 min at 72°C. PCR products were purified using the Genillustra kit (GE Healthcare, city, USA).

Sequencing was done using the same primers as used for PCR amplification by a commercial sequencing provider (GATC Biotech, Germany). The DNA sequences were edited manually based on the chromatograms and aligned by Clustal X (Thompson *et al*. 1997).

Data analysis

1. Chloroplast DNA (cpDNA)

Haplotypes of nuclear gene were inferred via "haplotype subtraction" (Clark 1990; Zhou *et al*. 2007). Haplotype diversity (h) and nucleotide diversity (n) were calculated for each population (*h*S, πS) and at the species level (*h*T, πT) using DNASP (version 4.0; Rozas *et al*. 2003). Gene flow (*N*m) (Hudson *et al*. 1992) among populations was also calculated by DNASP (Librado and Rozas 2009). Analyses of molecular variance (AMOVAs) were used to calculate genetic variance components and their significance levels among populations and within populations by ARLEQUIN (version 3.1; Excoffier and Lischer 2010). To see if the obtained cpDNA and nrDNA sequences satisfied the assumption of neutrality, we calculated Tajima's *D* (Tajima 1989) and Fu and Li's *D** (Fu and Li 1993) for the entire species and groups of populations, using DNASP ver.4.90.1 (Librado and Rozas 2009). Statistical significance of *D* and *D** was estimated with coalescent simulations as implemented in this program. In general, significant negative departures of these statistics from zero indicate deviation from neutrality, but might also be taken as evidence of recent demographic expansions or population bottlenecks when markers are otherwise assumed to be independent of selection (Tajima 1989; Fu 1997). To further infer demographic processes, we explicitly tested the null hypotheses of a spatial expansion and of a pure demographic expansion in DNASP by comparing observed and expected distributions of pairwise sequence differences (mismatch distributions). For both cpDNA and nrDNA data, Mantel tests were carried out to investigate the correlation between genetic and geographic distances. A Principal Component Analysis (PCA) was carried out, using GenAlEx 6.4 (Peakall and Smouse 2006).

2. Nuclear DNA (nrDNA)

Percent identity and G: C content was calculated with Bioedit. Sequence diversity within a subpopulation was calculated as π, which is the average pairwise difference between sequences from within a taxon (Tajima 1983). The standard deviation of π over both the stochastic and sampling process is given in Nei (1975). We included both indels and ambiguous sites (except 'N's) in the calculation of π and its standard deviation.

RESULTS

cpDNA diversity and population structure

Chloroplast DNA (cpDNA) regions sequenced in *P. africana* (63 individuals, 5 populations) showed a consensus sequence length of 578 bp after alignment. The mean haplotype diversity and nucleotide diversity was $h = 0.24153$ and $\pi = 4.48 \times 10^{-3}$, respectively. Gene flow among populations based on combined cpDNA data was ($Nm = 0.82$). Nucleotide diversity (π) among 5 populations ranged from 0 to 9.42×10^{-3} and haplotype diversity (*h*) varied between 0 and 0.163. Highest nucleotide and haplotype diversity was in Kakamega, followed by Mabira while there was no diversity in Budongo, Elgon and Monduli (**Table 1**).

Based on these polymorphisms, five cpDNA haplotypes (A-E) were identified across the material surveyed (**Table 1**). Neighbor joining tree of haplotypes revealed that out of the five haplotypes, haplotype D was distantly related to the

Table 1 Details of population locations, sample size, cpDNA and nrDNA variation of *Prunus africana* sampled in East Africa. Sample size (N) is indicated for cpDNA and nrDNA analysis separately; *h*: haplotype diversity, π: nucleotide diversity.

Population	Sample size	Longitude (E)	Latitude (N)	Elevation (m)	cpDNA			nrDNA		
					Haplotype	$\pi T \times 10^{-3}$	h	Haplotype	$\pi T \times 10^{-3}$	h
Mabira	17	32° 56'21"	0°27'78"	1,072-1,183	A, B	1.76	0.118	H1, H2, H3, H4, H5, H6	3.38	0.77206
Budongo	5	31°28'46"	1°42'62"	1,065-1,100	C	0.00	0.000	H2, H3, H7, H8	10.27	0.7000
Elgon	7	34°40'65"	1°31'62"	2,103-2,380	A	0.00	0.000	H2 H9, H10, H11, H12	4.25	0.85714
Kakamega	26	34°86'17"	0°35'18"	1,560-1,630	A, D, E	9.42	0.163	H1, H2, H3, H4, H11, H13, H14, H15, H16, H17	4.47	0.72923
Monduli	8	36°42'88"	-3°24'89"	2,057-2,140	A	0.00	0.000	H3, H12, H18, H19	3.91	0.64286
Species mean	63				5	4.48	0.24153	19	5.29	0.81055

cpDNA haplotypes; A, B, C, D and E and are 5 in total. nrDNA haplotypes; H1 to H19 and are 19 in total.

Table 2 Analysis of molecular variance (AMOVA) for populations of *P. africana* based on cpDNA and nrDNA data.

Source of variation	d. f.	SSD	Variance component	Percentage of variation	FST
cpDNA					0.275%
Among populations	4	6.557	0.03525	2.75	
Within populations	56	69.804	1.24650	97.25	
	60	76.361	1.28175		
nrDNA					23.698%
Among populations	4	34.854	0.59711	23.70	
Within populations	58	111.511	1.92260	76.30	
	62	146.365	2.51971	100.00	

(d.f., degree of freedom; SSD, sum of squares, FST, Fixation index); $^{**}P < 0.001$

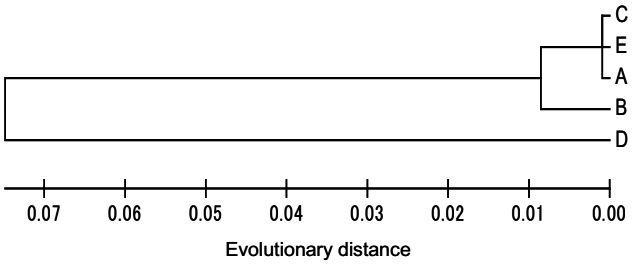

Fig. 2 Neighbor joining clustering of five cpDNA haplotypes of *P. africana*. A, B, C, D, E are haplotypes.

other haplotypes. The distant relationship portrays that the haplotype evolved first compared to the recent evolution of the four (**Fig. 2**), probably implying that it is the ancestral stock for chloroplast DNA (**Fig. 2**). However, Maximum parsimony analysis did not reveal any parsimony informative sites in the sequences.

AMOVA revealed that the greatest percentage of variation 97.25% was distributed within populations as compared to 2.75% among populations (**Table 2**). Significant Mantel test though negative was detected for cpDNA at the species-wide range ($r^2 = 3.0 \times 10^{-5}$). Tajima's *D* and Fu and Li's *D** statistics for deviation from neutrality were not significant for each geographic population and the whole species (all $P > 0.10$). Furthermore, the observed mismatch distributions of haplotypes from the whole species was not different significantly from mismatches expected under models of both spatial and sudden demographic expansion ($r = 0.3742$). Chloroplast DNA also revealed a high level of intermingling with two individuals far away. The first three components of PCA explained 86.93, 9.19 and 1.6% of the total variation.

Nuclear (nrDNA) diversity and population structure

The aligned sequences of the ITS were 456 bp in length. The haplotype diversity and nucleotide diversity were $hT = 0.8106$, $\pi T = 5.29 \times 10^{-3}$. Nucleotide diversity ($\pi$) among 5 populations ranged from 3.38×10^{-3} to 10.27×10^{-3} and haplotype diversity (*h*) varied between 0.643 and 0.857 (**Table 1**). Highest nucleotide diversity was found in Budongo where as haplotype diversity was highest in Elgon (**Table 1**). There were 19 haplotypes (H1-19, **Table 1**). The sequences of *P. africana* samples have been deposited in GenBank database under accession numbers (JN412001-JN412062).

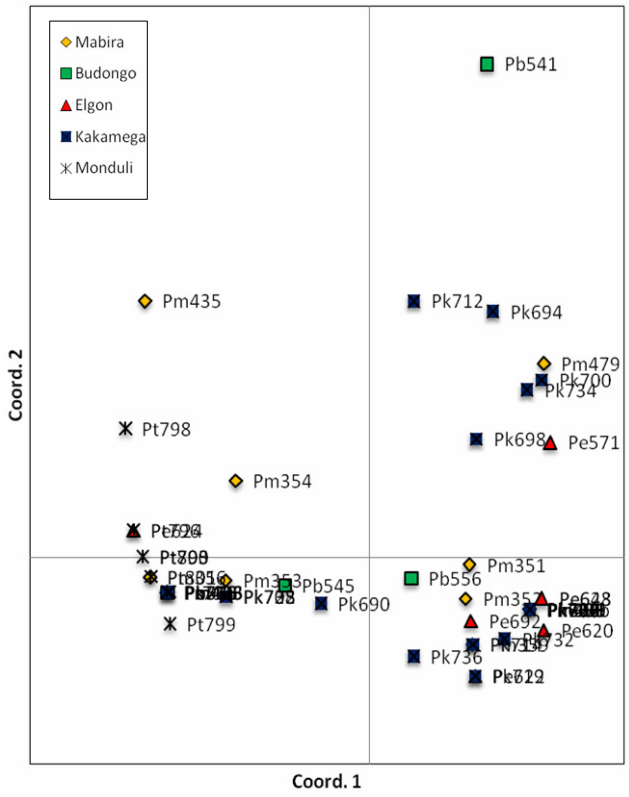

Fig. 3 Principal coordinates analysis representing relationships among 61 individuals from five populations of *Prunus africana* using nrDNA. Pm (*P. africana* from Mabira population), Pb (*P. africana* from Budongo population), Pe (*P. africana* from Elgon population), Pk (*P. africana* from Kakamega population) and Pt (*P. africana* from Monduli population).

Gene flow among populations based on nrDNA ($Nm = 2.641$) was obviously higher than that based on cpDNA. Maximum Parsimony tree of haplotypes revealed that there was no distinct or outstanding group; there was intermingling of haplotypes from populations. Branches corresponding to partitions reproduced in less than 50% trees are collapsed.

AMOVA revealed that the greatest percentage of variation 76.3% was distributed within populations as compared to 23.7% among populations (**Table 2**). Significant

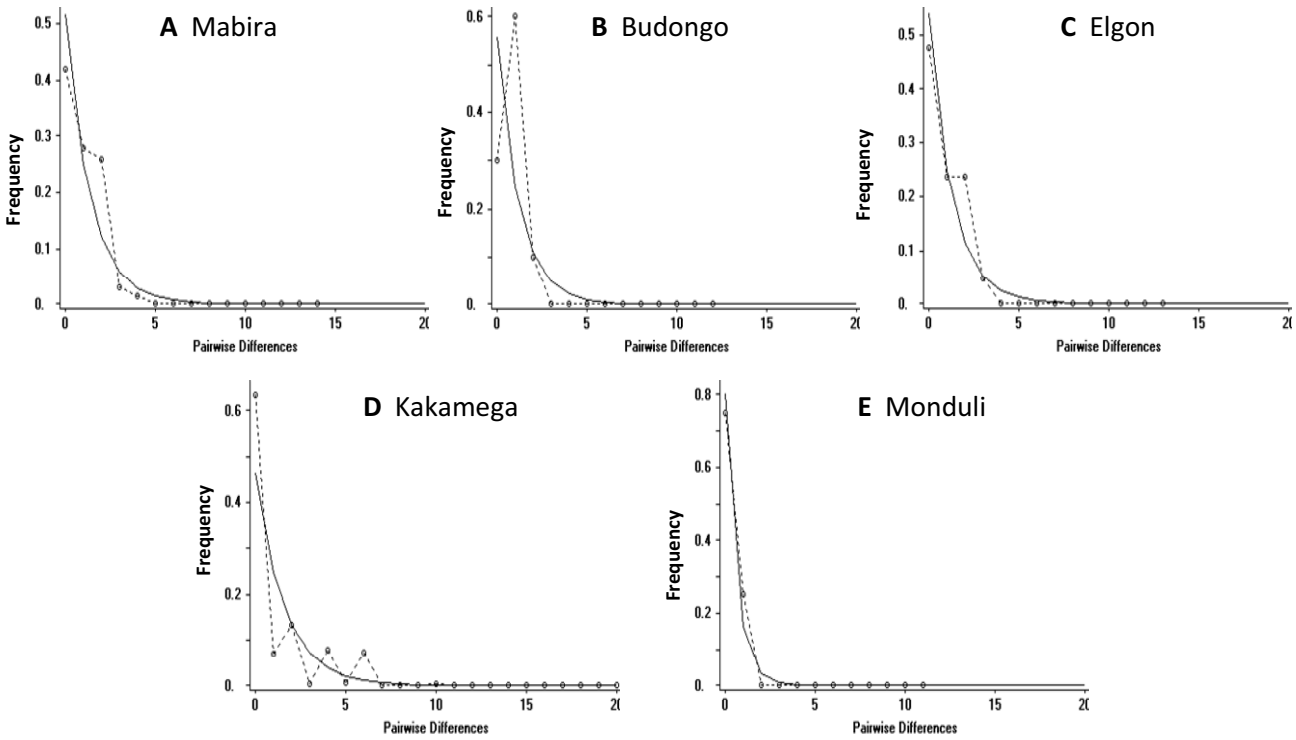

Fig. 4 Observed and expected pair-wise mismatch distributions in the five *Prunus africana* populations under the sudden population expansion model. The number of pair-wise nucleotide differences between haplotypes is represented on the abscissa whereas their frequencies are represented on the ordinate axis.

Table 3 Demographic history summary statistics of the five *Prunus africana* populations using nrDNA.

Population	SSD	*D* (P-value)	F_s (*P*-value)
Mabira	0.941	-0.62592 (> 0.10)	-1.493 (0.123)
Budongo	0.800	-0.97256 (> 0.10)	-0.829 (0.244)
Elgon	0.857	-1.35841 (> 0.10)	-0.237 (0.299)
Kakamega	1.160	-1.85646 (< 0.05)*	-1.462 (0.115)
Monduli	0.250	-1.05482 (> 0.10)	-0.182 (0.354)
Total sample	0.766	-2.78761 (< 0.05)	-5.095 (< 0.005)

*$P < 0.05$; SSD = sum of squared differences, D = Tajima's statistic, F_S = Fu's statistic.

Mantel test though negative was detected for cpDNA at the species-wide range ($r^2 = 7.0 \times 10^{-5}$). Tajima's D and Fu and Li's D* statistics for deviation from neutrality were not significant for each geographic population and the whole species; D = -2.068248 (all $P > 0.10$). Furthermore, the observed mismatch distributions of haplotypes from the whole species was different significantly from mismatches expected under models of both spatial and sudden demographic expansion ($r = 0.0204$).

The first three components of PCA explained 82.3% of the variation; 62.98, 10.02 and 9.30% (**Fig. 3**). PCA revealed that there was intermingling of individuals from different populations.

Demographic history of the populations

The mismatch frequency spectra for the five populations are shown in **Fig. 4A-E**. The observed unimodal mismatch frequency distributions of most of the populations (Mabira, Budongo, Elgon and Monduli) sampled showed excesses of mutations that appeared in only a few individuals and a deficiency of mutations shared by many individuals in the population. This is a scenario in conformity with the recent expansion model. On the other hand, the mismatch distribution of the Kakamega population (**Fig. 4D**) was bimodal and fitted poorly with the corresponding distribution expected under the recent expansion model.

Tajima's D neutrality test, revealed significant signatures of recent population expansion in only the Kakamega population; ($D = -1.85646$; $P < 0.05$). However, Fu's Fs test and SSD statistics were in contrast not significant (**Table 3**). There was no population with mismatch frequency spectra significantly deviated from what would be expected under the sudden expansion model (**Table 3**).

DISCUSSION

Genetic diversity

The natural populations of *P. africana* have decreased tremendously as a result of the overexploitation of the species for its medicinal extract, yet little is known about its genetic diversity. The results of using both cpDNA and nrDNA indicate that there are low levels of genetic diversity in all 5 investigated natural populations of *P. africana* as revealed by nucleotide diversity within nrDNA and cpDNA. Total levels of cpDNA haplotype diversity in *P. africana* (hT = 0.24153) were lower than other seed plants for maternally inherited markers (Huang *et al*. 2002; Gao *et al*. 2007). Still total cpDNA diversity was lower than for *P. africana* in Africa (hs = 0.886; Kadu *et al.* 2010), and comparable to *Fragaria* species all in family Rosaceae; range of (0.28 to 0.78; Wambui 2010). However, this value is comparable to the mean population diversity (hs = 0.234; Kadu *et al.* 2010).

For nrDNA, species-wide levels of haplotype diversity in *P. africana* (hT = 0.81055) were significantly high by comparison with other woody plant species with similar life history traits and geographical range reviewed by Hamrick *et al*. (1992) (mean hT = 0.211, for out crossing, animal-pollinated species). However, haplotype diversity was lower than for *Ficus carica* L. hT = 0.983 (Baraket *et al*. 2009). Generally, geographic distribution, breeding system and size of population are all associated with genetic diversity in plant species (Hamrick and Godt 1989). Species with out-crossing and mixed breeding have significantly higher diversity than species with self-crossing, geographically widespread species usually have higher genetic diversity than limited distributions, large size of population have higher than small diversity (Hamrick and Godt 1989; Ham-

rick *et al*. 1992; Hamrick and Godt 1996; Nybom 2004). As a widespread species in East Africa, *P. africana* is an out crossing insect pollinated species with small population sizes. Although, their population size decrease dramatically, the detected responses in genetic diversity may be due to overharvesting of the species from the wild.

Widespread tree species generally have high levels of genetic diversity overall (Hamrick and Godt 1989); like *Eucalyptus* species (Moran and Hopper 1987; Moran 1992). As with most tree species the majority of genetic diversity occurs within populations (**Table 2**). Among the populations investigated for cpDNA, Kakamega (h = 0.163, $\pi = 9.42\times10^{-3}$) had the highest haplotype diversity and nucleotide diversity; for nrDNA, Elgon (h = 0.85714), had the highest haplotype diversity while Budongo ($\pi = 10.27 \times 10^{-3}$) had the highest nucleotide diversity. Mabira has the least nucleotide diversity ($\pi = 3.38 \times 10^{-3}$) yet it is under high pressure from the surrounding human populations.

Overall, *P. africana* contains low levels of variation within populations.

Genetic structure

nrDNA data demonstrated significantly population differentiation within *P. africana* (F_{ST} = 0.23698), this was due to the fixation of particular haplotypes in single populations. In contrast to the significant population genetic structure obtained with cpDNA, there was a moderate level of genetic differentiation for cpDNA haplotype (F_{ST} = 0.275). A high pollen flow among populations was considered to be the explanation for the high population differentiation in *P. africana*. NrDNA differentiation was lower compared to *Angelica palustris* (Apiaceae) Φ_{ST} = 0.34 (Dittbrenner *et al*. 2005), *Gentiana germanica* Φ_{ST} = 0.42 (Fisher and Matthiens 1998), *Dictamnus albus* Φ_{ST} = 0.62 (Hensen and Oberprieler 2005) and *Pulsatilla vulgaris* Φ_{ST} = 1.22 (Hensen *et al*. 2005).

Tajima's *D* statistic together with the multimodal mismatch distribution also suggested that population expansion did not occur but instead, population reduction revealed by the negative values. Maximum parsimony tree of nrDNA haplotypes revealed that there was no distinctive group; haplotypes occurred in more than one collection site, with haplotype 19 forming the ancestral stock for nrDNA. Neighbor-Joining of cpDNA revealed that also there is no distinct group, with Haplotype D forming the ancestral stock for cpDNA. Although, the pollen flow was very strong compared to seed flow, the gene flow might play a main role in nearby populations.

The PCA resolved the five populations into mainly three differentiated groups which were not correlated with geography of the sampling sites. This was in support of the Maximum Parsimony analysis which did not reveal a distinct group. Lack of evidence for correlation between genetic and geographic distances was further supported by a Mantel test. The results indicate that distance is not responsible for the genetic structure of the species. The breeding system (1) maintains the genetic diversity of individuals and populations, and (2) promotes gene flow. The Nm value (Nm = 2.641) actually indicates a high rate of gene flow among populations. Apportion of genetic diversity of plant species usually reflects their breeding system (Hamrick and Godt 1996).

Population demography

Tajima's and Fu's tests for neutrality of mutations used in this study have been reported to be unable to disentangle the effects of true selective departures from neutrality (such as those caused by purifying selection and genetic hitchhiking) from those due to demographic processes such as population expansion on mismatch distribution profiles. For example, both statistics can be significantly negative due to purifying selection, genetic hitchhiking or population expansion while both of them can be significantly positive under scenarios of population bottlenecks and balancing selection at linked loci (Tajma 1989a, 1899b). However, because the data used in this study are derived from the selectively neutral non-coding region of the nuclear genome (control region), it is highly unlikely that the observed significant deviations from neutrality are due to selection. The high haplotype diversity, low nucleotide diversity, unimodal mismatch distributions, and significantly negative values of Tajman's *D*s statistic (see **Table 1, Fig. 4, Table 3**) all support a recent population expansion from a smaller founder population as the most plausible explanation for the observed significant deviations from neutrality in the Kakamega populations. The observation that population expansion in the Kakamega population was detected by Tajima's *D* statistic but not Fu's *F*s attests to the fact that Tajima's *D* statistic is a more powerful tool for detecting deviations from neutrality when testing for population expansion and genetic hitchhiking in populations.

CONCLUSION

This study has established that there is low gene diversity in both cpDNA (π = 0.0045) and nrDNA (π = 0.0053) of *P. africana* within East Africa. It also points out the uniqueness of the species within the Budongo population as exemplified within haplotype D within cpDNA.

GUIDELINES FOR CONSERVATION

The estimate of genetic diversity and population genetic structure provide a basis for conservation and utilization of *P. africana.* It will help us in determining what to conserve and how to conserve this species. In this study, the results show that there are low levels of gene diversity in *P. africana.* The low levels of gene diversity make *P. africana* prone to genetic erosion. This implies that soon, populations of *P africana* will not be viable, leading to extinction of the species if the present populations are not conserved. Due to this fact there is an urgent need to conserve the current populations of *P. africana* both in situ and exsitu. *In situ* conservation is first recommended to protect its original habitat from further destruction. Out of the five populations, Mabira is critically endangered because of habitats loss. Therefore, it should be protected immediately. For *ex situ* conservation, we suggest that the population which has high gene diversity should be selected as the conservation area. Elgon and Kakamega populations with high gene diversity are the best candidates for *ex situ* conservation. Also the Budongo population should be conserved since it is different in terms of cpDNA.

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