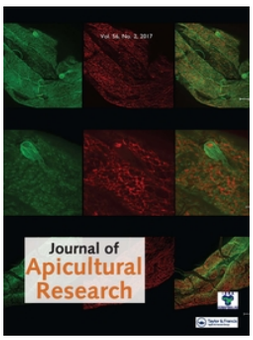

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ORIGINAL RESEARCH ARTICLE

Mitochondrial DNA (mtDNA) markers reveal low genetic variation and the presence of two honey bee races in Uganda's agro-ecological zones

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As plant pollinators, honey bees (*Apis mellifera)* play a very important role in agricultural and non agricultural crop production. In addition, honey bee products are of nutritional, medicinal, cosmetic and economic value to society. With the current changing trends in Uganda's farming systems, it is important to determine the level of genetic variation, and extent of differentiation in the honey bee population. This will be crucial for a successful honey bee management system for improved productivity and conservation of the honey bees in the different agro-ecological zones of Uganda. To achieve this, we investigated the genetic variation in honey bees from ten agro-ecological zones of Uganda by sequencing the intergenic region of the Mitochondrial DNA (mtDNA) COI-COII. We observed high haplotype diversity (Hd > 0.530) within a majority of the agro-ecological zones except the Southern Highland agro-ecological zone (Hd = 0.154). Both the population structure and the phylogenetic analyses revealed the presence of two honey bee races (*A. m. adansonii* and *A. m. scutellata*) in Uganda. *A. m. adansonii* is more widespread in the agro-ecological zones in the northern and eastern parts of Uganda while *A. m. scutellata* is more spread in the southern and western parts of the country. This is the first exhaustive population genetics research on bees in Uganda, and provides results that are useful to the beekeeping industry for effective management of the honey bee population. These results should be considered while planning beekeeping activities in the country.

Marcadores de ADN mitocondrial (mtDNA) revelan una baja variación genética y presencia de dos razas de abeja de la miel en las zonas agroecológicas de Uganda

Como polinizador, la abeja de la miel (*Apis mellifera)* juega un papel muy importante en la producción agrícola y no agrícola de cultivos. Además, los productos apícolas presentan valores nutritivos, medicinales, cosméticos y económicos para la sociedad. Con los cambios actuales en los sistemas agrícolas de Uganda, es importante determinar el nivel de variación genética, y grado de diferenciación en las poblaciones de abeja de la miel. Esto será crucial para un exitoso manejo de la abeja de la miel con el que mejorar tanto la productividad como la conservación de las abejas de la miel en las diferentes zonas agro-ecológicas de Uganda. Con el fin de lograrlo, hemos investigado la variación genética de la abeja de la miel en 10 zonas agro-ecológicas de Uganda secuenciando la región intergénica del ADN mitocondrial COI-COII. Observamos una alta diversidad de haplotipos (Hd > 0.530) en la mayoría de las zonas excepto en las zonas de alta montaña al sur del país (Hd = 0.154). Tanto la estructura poblacional como los análisis filogenéticos revelaron la presencia de dos razas de abeja de la miel (*A. m. adansonii* and *A. m. scutellata)* en Uganda. *A. m. adansonii* está más extendida en las zonas agroecológicas del norte y este de Uganda mientras que *A. m. scutellata* se encuentra más extendida al sur y oeste del país. Ésta es la primera investigación exhaustiva realizada sobre la genética de poblaciones de las abejas de Uganda, aportando resultados útiles para la industria apícola para el manejo efectivo de las poblaciones de abeja de la miel. Estos resultados deberían ser tenidos en cuenta a la hora de planear actividades apícolas en el país.

Keywords: agro-ecological zones; mtDNA; honey bees; intergenic region; bee population; beekeeping; *adansonii*; *scutellata*; genetic differentiation; molecular markers

Introduction

Honey bees (*Apis mellifera* L.) are essential pollinators of crops (Afik, Dag, Yeselson, Schaffer, & Shafir, 2010; Batra, 1995) and have been hunted for honey by ancient man who later started to keep them (beekeeping) to be sure of the honey harvest. They exhibit behavioral and morphological variations which provide the basis for sub-specific classification within their endemic range (Sheppard, Arias, Grech, & Meixner, 1997). Morphological characters have long been the traditional intra specific taxonomic method for honey bees (Barour, Tahar, & Baylac, 2011; Francoy et al., 2008; Ruttner, Kauhausen, & Koeniger, 1989; Ruttner, 1971; Charistos, Hatjina, Bouga, Mladenovic, & Maistros, 2014). However, molecular markers were recently identified and developed from the mitochondria DNA (mtDNA) and microsatellite loci of honey bees (Arias et al., 2006; Techer et al., 2015). Using these markers, genetic variations in the

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population of *A. mellifera* have been demonstrated (Cornuet & Garnery, 1991; Cornuet, Garnery, & Solignac, 1991; De La Rúa, Galián, Serrano, & Moritz, 2001; Franck et al., 2001; Garnery, Cornuet, & Solignac, 1992; Garnery, Vautrin, Cornuet, & Solignac, 1991; Haddad et al., 2009; Meixner et al., 2013).

Several races of honey bees have been reported in Africa (Hall, Zettel-Nalen, & Ellis, 2005). The most widely distributed race of African honey bees is *A. m. scutellata* (Hall et al., 2005) also termed the "killer bees" referring to Africanized honey bees in America (Roe, 2000). Segeren (2004) and Eisa and Roth (2008) described *A. m. scutellata* and *A. m. adansonii* as honey bees with similar behavior. *Apis m. scutellata* is found mostly in the savannahs of East, Central and South Africa (Eisa & Roth, 2008; Raina & Kimbu, 2005). On the other hand, *A. m. adansonii* is found in West Africa (Segeren, 2004) and Central to East Africa (Franck et al., 2001).

In order to investigate the genetic diversity in honey bees, highly specific COI-COII intergenic mtDNA region has been used as a genetic marker (Arturo & Segura, 2000; Cornuet & Garnery, 1991; Lee et al., 2016; Tan, Qu, Wang, Liu, & Engel, 2016). Indeed, genetic variation studies by Cornuet and Garnery (1991) reported three major lineages of *A. mellifera*: lineage A: (African genotypes) comprising *adansonii, scutellata, capensis* and *monticola* races; lineage M: (*mellifera* genotypes) comprising *mellifera, liberica, major* races and lineage C: (*Carnica* genotypes) comprising *ligustica, carnica* and *caucasica* races. In Uganda, *A. mellifera* is the only honey bee species reported (UEPB & the sector counterpart team, 2005) and it is speculated that three races: *A. m. scutellata, A. m. adansonii* and *A. m. monticola* are present (Hepburn, Radloff, & Oghiakhe, 2000; Radloff & Hepburn, 2000; Kajobe, 2008).

Beekeeping in Uganda is on the rise, and is mainly practiced by rural smallholder farmers. This activity is practiced in all the agro-ecological zones (AEZs) of Uganda and it was estimated that there were 759,000 bee hives in Uganda by 2008 (MAAIF, UBOS., 2010), 87.3% of which were local bee hives. The AEZs have varying ecological conditions. To a large extent, the beekeeping community in Uganda has practical information regarding beehive management. The current beekeeping activity could benefit from information regarding the honey bee genetic variation, extent of genetic differentiation and evolution in the different AEZs. This will be useful in formulating guidelines towards developing honey bee colonies for selection for improved honey production and bee conservation. This study therefore, sought to investigate the genetic variation, differentiation and evolutionary history of *A. mellifera* in Uganda and answer the question 'which races of *A. mellifera* exist in Uganda?'

Materials and methods

Sample collection

Honey bee samples were collected from ten (10) AEZs (AEZs) of Uganda (Figure 1) as described in Kajobe et al. (2009), Kajobe et al. (2010), Kajobe, Kato, Otim, Kasangaki, and Abila (2016) and Kasangaki et al. (2015). Between fourteen (14) and twenty-nine (29) honey bee colonies were sampled from four selected sites (apiaries) from each AEZ. To avoid collecting flying bees

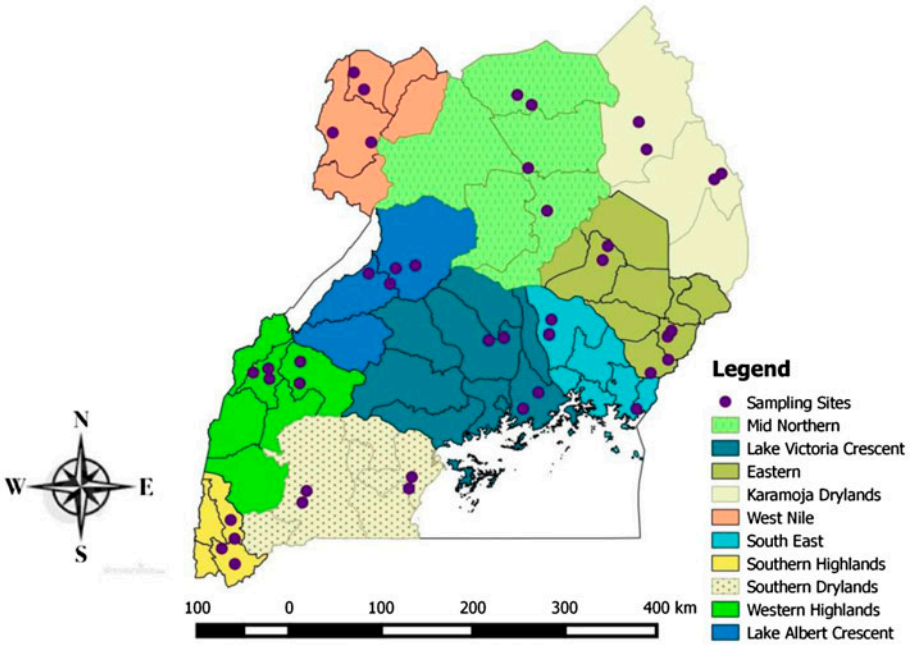

Figure 1. Location of study sites in the agro-ecological zones of Uganda.

from other colonies, adult worker honey bees were picked from inside the bee hives. The samples were preserved in 95% ethanol and kept at 4 °C for subsequent molecular analyses. Quantum GIS software was used to place the geo-referenced study sites on the map of Uganda and to calculate the geographic distances between the agro-ecological zones.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the thorax of the honey bee samples using Quick-gDNA MiniPrep and w/Zymo-Spin™ IIC Columns (Capped) extraction kits, following the manufacturers' protocols, with slight modifications (overnight incubation of the crushed tissue samples in extraction buffer at room temperature). The extracted DNA was stored at −20 °C. The mtDNA COI-COII gene fragment was PCR-amplified using primers E2 – (5′-GGCAGAATAAGTGCATTG-3′) and H2 – (5′-CAATATCATTGATGACC-3′) (Garnery et al., 1991; Garnery et al., 1992). The PCR reactions contained a 20 µl reaction mixture of 2X DreamTaq™ Green PCR Master Mix, 10 µM of both primers above, 1 µl containing DNA template and 5 µl of nuclease-free water. Amplification was conducted through an initial denaturation at 94 °C for 2 min followed by 35 cycles (denaturation at 94 °C for 45 s, annealing at 55 °C for 45°s and extension at 72 °C for 45°s) and then a final extension at 72 °C for 5 min using a PxE 0.2 Thermocycler (Thermo Electron Corporation). Aliquots of 5 µl of the amplified products were separated using 1% agarose gel stained with 0.5 µg/ml Ethidium bromide and visualized under UV light. The PCR amplicons were purified using GeneJET™ PCR Purification Kit following the manufacturer's protocol.

Sequencing was done using BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems) on an ABI 3730xl DNA Analyzer, at Macrogen Europe centre. Sequences were cleaned using CodonCode Aligner (www.codoncode.com) and aligned in ClustalX2 software (Thompson, Higgins, & Gibson, 1994).

Sequence analysis

The sequences were investigated for characters such as length, segregation site, number of haplotypes and singletons. Haplotype diversity (Hd) and nucleotide diversity (π) (Nei, 1987; Nei & Miller, 1990) were analyzed using DnaSP version 5.10 (Rozas, Sanchez-DelBarrio, Messeguer, & Rozas, 2003). Significance was assessed with 1000 permutations. The partitioning of the genetic diversity within and among AEZs was evaluated using the analysis of molecular variance (AMOVA) as implemented in Arlequin 3.5 (Excoffier, Laval, & Schneider, 2005). We performed a nested analysis of variance (AMOVA) framework to partition the total amount of genetic differentiation between AEZs (Excoffier, Smouse, & Quattro, 1992), and produced Φ-statistics that measure the similarity of pairs of haplotypes in each hierarchical level of the analysis, relative to pairs drawn from the pool of sequences in the higher hierarchical level. Significance of the Φ-statistics was tested by permutating the haplotypes among the corresponding AEZs, and recalculating the statistics to obtain their null distributions (Excoffier et al., 1992).

To obtain pairwise estimates of genetic differentiation we computed Φ_{ST} values among ecological zones using Arlequin 3.5 with 1,000 random permutations. We used Φ_{ST} because it also accounts for the evolutionary relatedness of the mtDNA haplotypes. To test the correlation between genetic distances and geographic distances, we used linear regression in SPSS (Landau & Everitt, 2004).

Genetic differentiation among the 10 AEZs was evaluated with and without spatial information using the Bayesian approach implemented in BAPS 6 (Corander & Tang, 2007). All molecular data collected from a particular AEZ were used to obtain the population posterior distribution of haplotype frequencies. The genetic structure was calculated assuming a priori that the structure within a particular AEZ depends on the neighboring AEZs. In the spatial model, geographical coordinates of a central point within each AEZ were assigned to the individuals to be clustered. The best partition was visualized using a Voronoi tessellation as implemented in BAPS. The relationship between haplotype diversity and the AEZs was visualized using the program GenGIS (Parks et al., 2009).

To investigate the evolutionary relationships of the honey bee mtDNA haplotypes in Uganda, parsimony networks at both species and race levels were constructed using TCS software (Clement, Posada, & Crandall, 2000). Representative sequences of haplogroups were interrogated in NCBI using MEGA BLAST and the respective groupings named according to the homologies.

Results

Genetic diversity

Analyses were based on a 501 bp fragment from the COI-COII mtDNA intergenic region, for 167 honey bee sequences. These sequences were all similar to the African haplotypes of honey bees; 88 A1C *adansonii* haplotypes (Zambia), 8 A26 *adansonii* haplotypes (Namibia) and 71 A4 *scutellata* haplotypes (South Africa) (Franck et al., 2001). These were represented by thirty-three haplotypes with thirty-two segregation sites identified (Table 1); whereby Haplotype 4 (Hap4) was the most predominant (53 sequences) followed by Hap2 (28 sequences) and Hap1 (21 sequences). The number of haplotypes within AEZ varied considerably from 2 in Southern Highlands (SH) to 13 in Eastern (E) AEZs. On average, haplotype diversity was very high (Hd = 0.850). Apart from SH AEZ where haplotype diversity was very low (Hd = 0.154), haplotype diversity ranged from 0.538 in Southern Drylands (SD), to 0.945 in Lake Albert

Table 1. Haplotype distributions among the 10 agro-ecological zones of Uganda, based on mitochondrial COI-CO II sequence data: 1st column: Haplotype code name (Hap1–Hap24); 2nd column: segregating sites in each haplotype, numbers on top of 2nd column are the variable sites, dots represent identical nucleotides to the ones for Hap1. The location code names (column 3–12) are those shown in Table 2. The last column shows the frequency of each haplotype in the whole mitochondrial CO II sequence data.

	Haplotype segregation sites	Haplotype frequency per agro-ecological zone										Total
	1122222 3333333344 4444444444 44											
	4781913349 0227889922 3777778899 99	MN	E	SE	VC	SD	SH	WH	AC	WN	KD	
Haplotype	3955085779 7451792856 8456898907 89	15	26	19	14	19	13	18	11	17	15	167
Hap1	AAAGGACGGA AATAATGTTC GGTTAATATC TT	5	4	1	1	2	–	4	1	2	1	**21**
Hap2	G…… ……. ……. ..	4	3	4	2	1	–	–	2	6	6	**28**
Hap3	……. ……T ……. ..	1	3	–	–	–	–	1	1	4	1	**11**
Hap4	……. ……. .AGAT.AGAT AA	1	4	6	3	13	12	9	2	1	2	**53**
Hap5	..G……. ……. ……. ..	1	1	3	2	–	–	–	1	–	–	**8**
Hap6	..G……. ….G….. .AGAT.AGAT AA	1	–	–	–	–	–	–	–	–	–	**1**
Hap7	G…..T… ……. ……. ..	1	2	1	–	–	–	–	–	1	–	**5**
Hap8	G…A….. ……. ……. ..	1	–	–	–	–	–	–	–	–	–	**1**
Hap9	……T… ……. ……. ..	–	1	–	–	–	–	–	–	–	–	**1**
Hap10	……G ……T ……. ..	–	3	–	–	–	–	–	–	–	–	**3**
Hap11	……. …….A.. .AGAT.AGAT AA	–	1	–	–	–	–	–	–	–	–	**1**
Hap12	..G……. ……. .AGAT.AGAT AA	–	1	1	2	–	–	1	–	–	–	**5**
Hap13	……. ..A……. .AGAT.AGAT AA	–	1	–	–	–	–	–	2	–	–	**3**
Hap14	..G……. …..GA… .AGAT.AGAT AA	–	1	–	–	–	–	–	–	–	–	**1**
Hap15	…..G…. ……. ……. ..	–	1	–	–	–	–	–	–	–	–	**1**
Hap16	……. ……. …..T…. ..	–	–	1	–	–	–	–	–	–	–	**1**
Hap17	……. ……..AT ……. ..	–	–	1	–	–	–	–	–	–	1	**2**
Hap18	…….A.. ……. ……. ..	–	–	1	–	–	–	–	–	–	–	**1**
Hap19	…A…… ……. .AGAT.AGAT AA	–	–	–	1	1	–	–	–	–	–	**2**
Hap20	……. ……T .AGAT.AGAT AA	–	–	–	1	–	–	2	–	–	1	**4**
Hap21	……. .TA……T .AGAT.AGAT AA	–	–	–	1	–	–	–	–	–	–	**1**
Hap22	……. .T…….T .AGAT.AGAT AA	–	–	–	1	–	–	–	–	–	–	**1**
Hap23	..G……. .TA……. .AGAT.AGAT AA	–	–	–	–	1	–	–	–	–	–	**1**
Hap24	..GA…… ……A ……. ..	–	–	–	–	1	–	–	–	–	–	**1**
Hap25	……. .TA……. .AGAT.AGAT AA	–	–	–	–	–	1	–	–	–	–	**1**
Hap26	…A…… ……. ……. ..	–	–	–	–	–	–	1	–	–	–	**1**
Hap27	……..A. ……. ……. ..	–	–	–	–	–	–	–	1	1	–	**2**
Hap28	……. T…… ……. ..	–	–	–	–	–	–	–	1	–	–	**1**
Hap29	……. ……. .A…….. ..	–	–	–	–	–	–	–	–	1	–	**1**
Hap30	……. …T…… ……. ..	–	–	–	–	–	–	–	–	1	–	**1**
Hap31	……. ……. AAGAT.AGAT AA	–	–	–	–	–	–	–	–	–	1	**1**
Hap32	……. ……. .AGAT.AGAT .A	–	–	–	–	–	–	–	–	–	1	**1**
Hap33	.C…….. ……. .AGAT.AGAT AA	–	–	–	–	–	–	–	–	–	1	**1**

Table 2. Genetic diversity measures for honey bees from agro-ecological zones of Uganda calculated from 167 mtDNA COI-COII sequences.

Sites	Code	N	H	Hd	π
Mid north	MN	15	8	0.848	0.00753
East	E	26	13	0.932	0.01226
South east	SE	19	9	0.860	0.01237
LV crescent	VC	14	9	0.934	0.01309
South dry	SD	19	6	0.538	0.00864
South high	SH	13	2	0.154	0.00061
West high	WH	18	6	0.719	0.01042
LA crescent	AC	11	8	0.945	0.01292
West Nile	WN	17	8	0.838	0.00505
Karamoja dry	KD	15	9	0.848	0.01270
ALL		167	33	0.850	0.01240

Notes: N = Number of sequences; H = number of haplotypes; Hd = haplotype diversity; π = nucleotide diversity.

Crescent (AC) (Table 2). On the contrary, nucleotide diversity was very low ranging from 0.00061 in SH to 0.01309 in Lake Victoria Crescent (VC) AEZs.

The haplotypes are distributed in the AEZs as shown in Figure 2, with HAP4 being the most common haplotype (32.0%; Table 1) having majority of individuals (63.6%; Table 1). Interestingly, only two haplotypes were got from the 14 individuals collected from SH AEZ, of which one (HAP25; Table 1) is a singleton (i.e., haplotype seen only once in a group of samples), and the rest (13 individuals) were HAP4. While the singleton could be indicative of an expanding population, the distribution of HAP4 suggests a northward spread of this haplotype. Contrary, the second most common haplotype (HAP2; Table 1) which was less frequent (16.8%) than HAP4 was found in only 8 of 10 AEZs, and majority of individuals (42.8%) were obtained from the AEZs in the Northern part of the country (21.4% from West Nile (WN); 21.4% from Karamoja Drylands (KD)). The distribution of HAP2 diminishes towards the AEZs in the central and southern parts of Uganda, and finally not found in SH AEZ, suggesting a southward spread. Additionally, in the AEZs to the south of Uganda, HAP2 was only got from the SD, suggesting

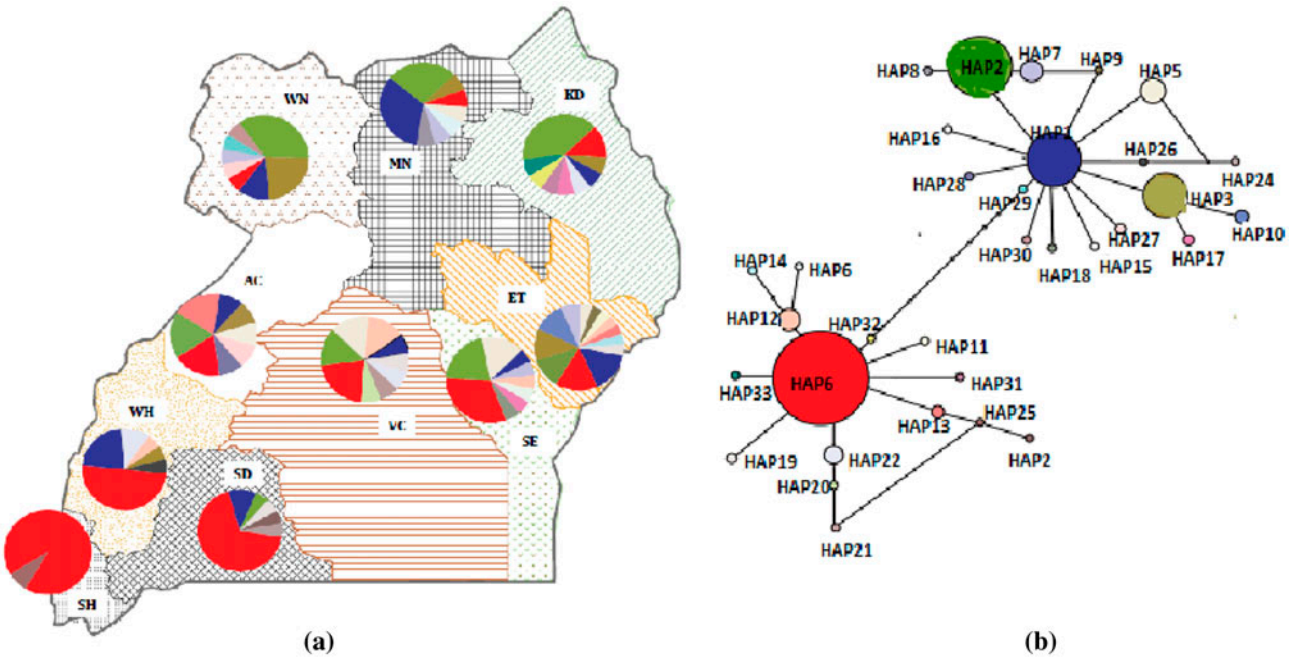

Figure 2. (a) Haplotype distribution map and; (b) Parsimony network of all the 33 haplotypes.
Notes: Size of the ball represents the haplotype frequency and the colors represent the different haplotypes. The very small white balls represent missing haplotypes.

Table 3. Pairwise differentiation estimates of mtDNA Φst for the agro-ecological zones of Uganda.

	MN	E	SE	VC	SD	SH	WH	AC	WN	KD
MN	0.00000									
E	0.07392	0.00000								
SE	−0.02731	0.16293	0.00000							
VC	0.02364	−0.02386	**0.08779**	0.00000						
SD	0.05318	−0.04355	**0.15035**	−0.02753	0.00000					
SH	**0.31922**	0.05456	**0.42905**	0.12141	0.06578	0.00000				
WH	**0.50798**	**0.21817**	**0.60241**	**0.28877**	**0.23108**	−0.00454	0.00000			
AC	**0.77681**	**0.50594**	**0.84933**	**0.52458**	**0.50239**	**0.24724**	0.09814	0.00000		
WN	0.04995	−0.05976	**0.15197**	−0.04740	−0.06218	0.05219	**0.22960**	**0.55455**	0.00000	
KD	**0.37650**	0.09198	**0.47849**	**0.15998**	0.10900	−0.04716	−0.01530	**0.22874**	0.09469	0.00000

Notes: Bold figures show significant differentiation at $p \leq 0.05$.
MN = Mid North; SH = Southern Highlands; SE = South Eastern; E = Eastern; WH = Western Highlands; AC = Lake Albert Crescent; VC = Lake Victoria Crescent; WN = West Nile; SD = Southern Drylands; KD = Karamoja Drylands.

that this haplotype could be associated with drylands. Unlike HAP4 and HAP2, the third most frequent haplotype, HAP1 (Table 1; Figure 2) has a relatively even distribution across the country (19.0% from E; 19.0% from WH). A private haplotype, HAP10 was found in the E AEZ (3; Table 1).

Analysis of molecular variance (AMOVA) showed that overall genetic variation within AEZs was large (74.1%), but variation among AEZs was comparatively low (20.3%), which is indicative of some genetic divergence among AEZs. This is further supported by the distribution of haplotypes among the AEZs (Figure 2, Tables 1 and 2). This is similar to the overall levels of genetic divergence inferred from the pairwise Φ_{ST} values (Table 3). Among AEZs these values ranged from −0.00454 between populations from SH and WH, both located in southwestern Uganda, to relatively high and statistically significant values ($\Phi_{ST} = 0.849$, $p < 0.05$) between AC and South Eastern (SE) AEZs, which are located at the extreme ends of Central Uganda. While samples from MN were significantly distinct from SH and WH ($\Phi_{ST} > 0.05$, $p < 0.05$), also samples from WN were distinct from SD and WH ($\Phi_{ST} > 0.05$, $p < 0.05$), suggesting that honey bees from the south may be genetically distinct from those from the northern AEZs. This is further supported by the predominance of HAP4 in the south, the predominance of HAP2 in the north and the AMOVA results. Surprisingly, samples from both AC and SE are also significantly differentiated from samples from both Northern and Southern Uganda AEZs ($\Phi_{ST} > 0.05$, $p < 0.05$), suggesting that central Uganda is a hybrid zone for northern and southern

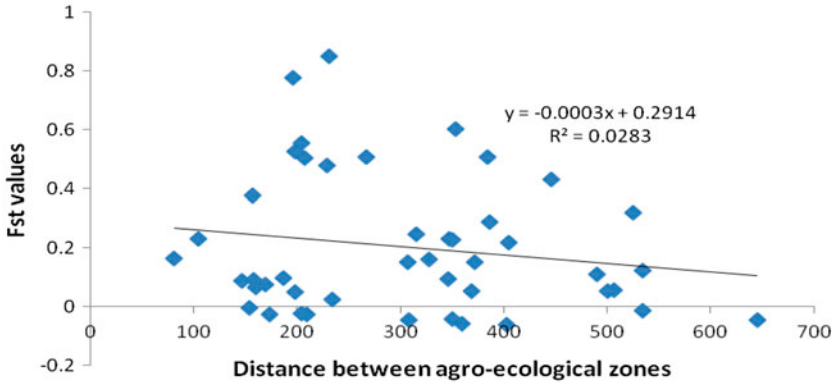

Figure 3. Relationship between the genetic distances of the haplotypes and geographic distances between the agro-ecological zones.

haplotypes, after all AEZs in central Uganda exhibit high haplotype diversity and share haplotypes with samples from both northern and southern AEZs. On the other hand, the differentiation of AC and SE samples from other samples, coupled with high frequency of singletons in these AEZs could indicate introduction of honey bees from other parts of the world to Central Uganda. This is further supported by the retrieval of a private haplotype from South Eastern ecological zone. Though not significant, there was some similarity between samples from far apart agro-ecological zones. For example, MN located in the north and VC Ecological zones (Φ_{ST} = 0.024, $p > 0.05$); SD and VC (Φ_{ST} = 0.027, $p > 0.05$), suggesting some gene flow across the country. Both sharing of haplotypes among even distant AEZs and linear regression (R^2 = 0.0283; p = 0.269; Figure 3) showing a negative correlation between genetic and geographic distances also support this gene flow. The BAPS analysis of the genetic differentiation of the honey bee population in the agro-ecological zones of Uganda showed existence of two genetic structures as represented by red and green colors in Figure 4.

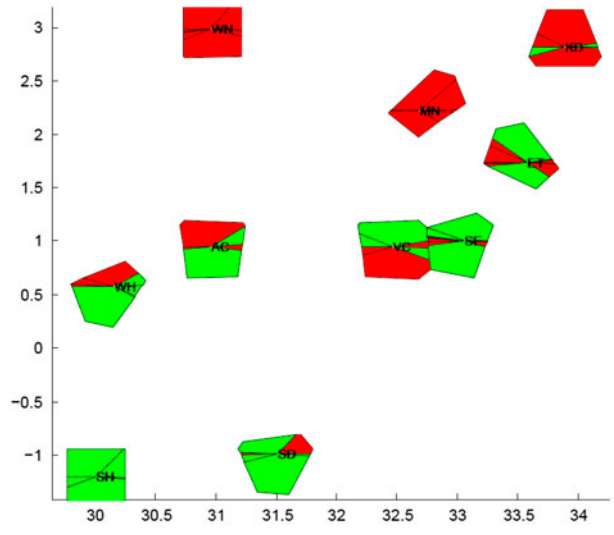

Figure 4. Population structure of the honey bees in the agro-ecological zones of Ugnada.
Notes: The colors represent the population clusters.

Two honey bee races were identified through MEGA BLAST against the sequences at the NCBI Database i.e., *A. m. adansonii* and *A. m. scutellata* (Reference numbers: FJ477987, FJ477986 and FJ477990). Figure 5 shows the evolutionary relationships among the 33 haplotypes. At species level, the network (Figure 5A) shows two haplogroups separated by seven mutational steps. The most common haplotype (HAP4) is located internally in one haplogroup, with the other haplotypes arising from it, suggesting that HAP4 is the ancestral haplotype of this haplogroup. The second haplogroup has three major haplotypes HAP3, HAP2 AND HAP1, which is located internally and possibly ancestral to this haplogroup. Overall, the network shows relatively low levels of sequence divergence among haplotypes and a high frequency of singletons, a pattern suggesting recent divergence and possibly population expansion. Interestingly, no haplotypes were shared among races (Figure 5B and 5C), each honey bee race exhibited different haplotypes. *A. m. adansoni* exhibited two haplogroups separated by eight mutational steps, with HAP1 (Figure 5B) located internally in the larger haplogroup, and having one haplotype HAP24 separated from the haplogroup by one mutational step. On the contrary, *A. m. scutelata* was characterized by one haplogroup (Figure 5C) and most of the haplotypes in this haplogroup were recovered from ecological zones in Southern Uganda.

Discussion

Until today, mtDNA sequencing remains the most effective and accurate method for studying honey bee population variability (Cornuet & Garnery, 1991; Cornuet et al., 1991; Achou et al., 2015). Here we investigated the population genetic diversity of honey bees from different AEZs of Uganda using the mtDNA COI-COII markers. We observed high genetic variability in the

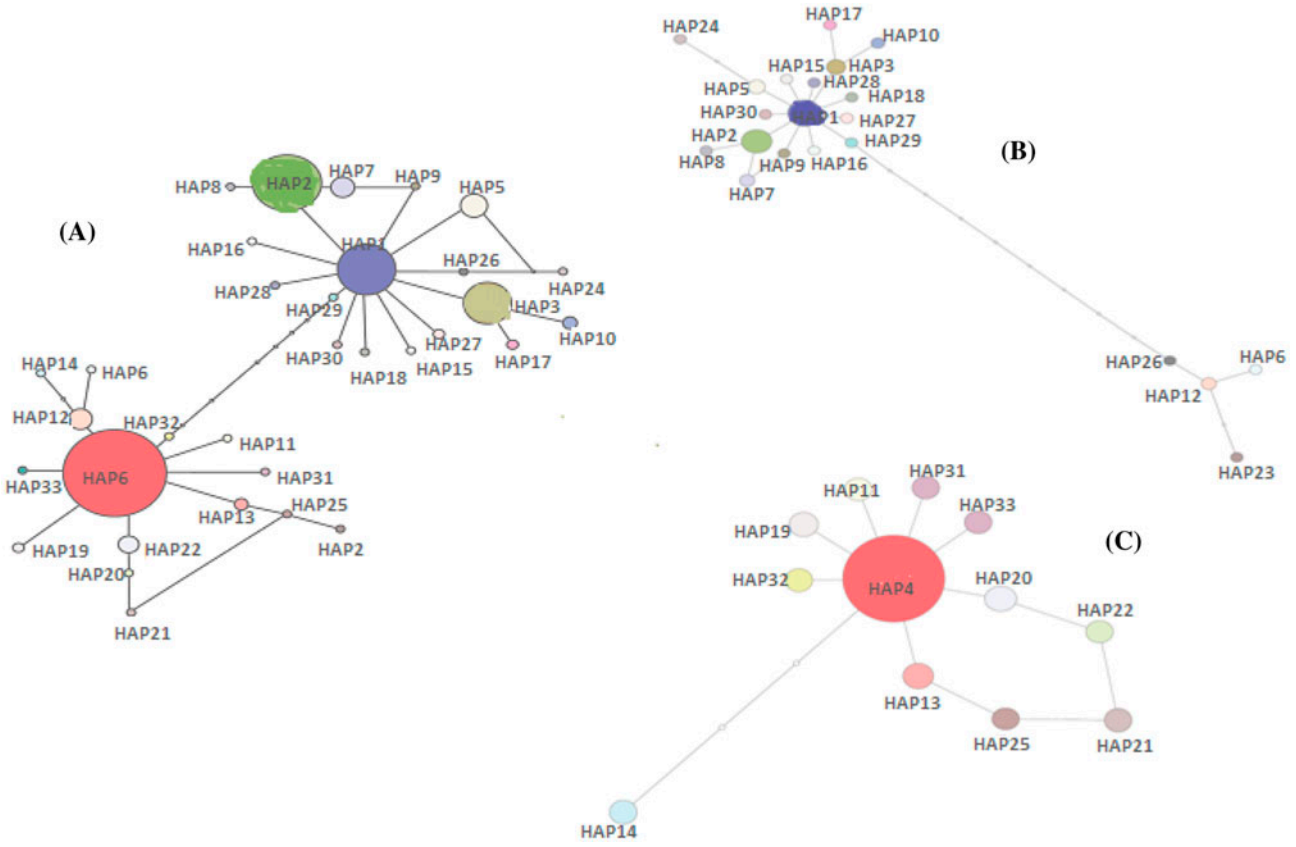

Figure 5. Parsimony network. Size of the ball represents the haplotype frequency and the colors represent the different haplotypes.
Notes: The very small white balls represent missing haplotypes: (A) All 33 haplotype; (B) *A. m. adansonii* haplotypes; (C) *A. m. scutellata* haplotypes.

honey bee mtDNA within all the AEZs but low variability in the mtDNA among the AEZ. This was clearly demonstrated by the high haplotype diversity of the honey bees in each AEZ. However, there was observed low nucleotide diversity. These low levels of nucleotide diversity may be due to relatively recent reduction in population size (Tan, Warrit, & Smith, 2007) or recent colonization events. On the other hand, the high haplotype diversity indicates differences in demographic dynamics among the AEZs (Yin & Ji, 2013). Franck et al. (2001) associated the low genetic differentiation among African honey bees due to their high absconding and swarming tendencies (migratory behavior). This is a behavior typical of *A. m. adansonii* and *A. m. scutellata* (Hepburn & Radloff, 1998), the only races of honey bees recorded in all the AEZs of Uganda. Similar results were obtained by Ivanova et al. (2010) and Kamrani, Pirany, Hashemi, and Kamrani (2012) respectively from the analysis of mtDNA of *A. mellifera* populations from Bulgaria and the North West of Iran. The high number of haplotypes obtained (33) is a clear indicator of high variability within the COI-COII intergenic region (Alburaki, Moulin, Legout, Alburaki, & Garnery, 2011). This strongly explains why there are several haplotypes within the honey bees in Uganda. This rhymes well with the diverse ecological conditions of the AEZs of Uganda (MAAIF, UBOS., 2010).

A weak differentiation (F_{ST} = 0.25954, $p < 0.01$) was observed among the honey bee populations in the AEZs of Uganda (low F_{ST} values) as most F_{ST} values were not significant indicating that there is significant gene flow among the honey bee populations in Uganda. These kind of results have been reported in the population structure study of North African honey bees, a phenomenon linked to seasonal swarming (Chahbar et al., 2013). Furthermore, genetic comparison studies on the *Bombus ignitius* population structure of Asia also showed long-time gene pool homogenization (Shao et al., 2004). Moritz, Cornuet, Kryger, Garnery, and Hepburn (1994) suggested that adjacent populations or races feed in to a mtDNA pool which might be the cause of low genetic differentiation in AEZs of Uganda even though only two honey bee races exist. There is growing consensus that genetic diversity is a key factor both at population and colony level for organisms to cope with increasing changing environmental conditions (Pinto et al., 2014). It is reasonable to speculate that the low genetic variability of honey bees is due to the insignificant changes in the environmental conditions within the AEZs of Uganda.

Synonymous to Garnery et al. (1992), our findings further show limited highly polymorphic ancestral population and no long term isolation of the populations. This is in agreement with a study by Ilyasov, Kutuev, Poskryakov, Petukhov, and Nikolenko (2011) on *A. m. mellifera* from the Russian Ural and west European populations, in which possibilities of migration was ascertained explaining the low genetic differentiation between the honey bee populations there.

Our study clearly demonstrated the presence of only two honey bee races in Uganda; *A. m. adansoni* and *A. m. scutellata*. Interestingly, this does not agree with the previous belief that three honey bee races (*A. m. monticola, A. m. adansoni* and *A. m. scutellata*) are present in Uganda (Radloff & Hepburn, 2000; Hepburn et al., 2000; Kajobe, 2008). This finding raises several questions on what could have happened to the *A. m. monticola* honey bee race. Could this be a case evolution toward extinction? In this highly changing climatic condition, it is possible that this honey bee species has succumbed to the environmental pressure or was simply not found within the areas sampled. Consistent with reports by (Hepburn et al., 2000), our data predominantly showed presence of *A. m. scutellata*, a highland race in the SH, WH and SD AEZs. On the other hand, *A. m. adansonii* was identified as more predominant in the MN, WN, KD, E and SE AEZs of Uganda.

However, the parsimony network differentiates the honey bee haplotypes into three clades arising from the three main haplotypes (Hap4, Hap2 and Hap1). These data show no clear geographic distance (Tunca & Kence, 2011) among the AEZs of Uganda to induce genetic structure in honey bee populations (Figure 3). This implied that geographic distance only accounted for 2.83% of the honey bee population variability within the AEZs of Uganda.

This study found low genetic differentiation among the honey bee populations in the AEZs of Uganda. Only two races of honey bees were recorded in Uganda and are distributed throughout the country except in the SH where only *A. m. scutellata* is present. The low genetic variations in the honey bee populations among the AEZs of Uganda means that the honey bees can be used for breeding programs in the AEZs to improve productivity in Uganda. There is a south-north spread of *A. m. scutellata* and a north-south spread of *A. m. adansonii* in Uganda. A beekeeping policy should be put in place in order to monitor and maintain the genetic integrity of the honey bees in Uganda since any rushed bee breeding may lead to loss of vital genetic traits which are not yet documented. This is important as the honey bees are adapted to the different AEZs and their performance can only be improved through management. More studies using other molecular markers should be conducted in order to detect the sequence composition and try to investigate what could have happened to *A. m. monticola* which was thought to exist in the country.

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Disclosure statement

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