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# Genetic Variability and Relationship of Camel (*Camelus dromedarius*) Populations in Ethiopia as Evidenced by Microsatellites Analysis

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## አህፅሮት

የዚህ ጥናት ዓላማ በስድስት የኢትዮጵያ የግመል ዝርያዎች ላይ ያተኮረ ሲሆን (አሚባራ፣ጅግጅጋ፣ ገለብ፣ ሁር፣ ሚሌ እና ሊበን) በእያንዳንዱ የግመል ዝርያ ውስጥ ያለው የዘር ህዋስ ስፋትና እርስ በርስ ያላቸውን ግንኙነት ማየት ነው። ለዚህም ይረዳ ዘንድ በ114 የተለየዩ ዝርያ ካላቸው ግመሎች (ከያንዳንዱ ዝርያ ከ17-24) የደም ናሙና ተሰብስቧል። በተገኘው የጥናት ውጤት መሠረት የኢትዮጵያ ግመሎች በአማካይ በአንድ የዘረመል ግንድ ጥንድ ላይ በአሀሉ ወቅት 0.55 (55 ከመቶ) የተለያዩ የዘረመል ቅንጣቶች ሲኖሩ ወደፊት የግመል ዘር መረጣ እና ማሻሻያ ከተደረገ እስከ 0.73 (73ከመቶ) ሊደርስ ይችላል። ይህም ውጤት በዓለም ላይ በተለያዩ አገራት ካሉት ከአብዛኞቹ የግመል ዝርያዎች በላይ ነው። በስድስቱ የግመል ዝርያ ውስጥ በአጠቃላይ 52 የየራሳቸው የዘር ህዋስ ቅንጣት ሲኖራቸው ከዚህ ውስጥ የሊበን (ሱማሌ) ግመል 15ቱን እና የአሚባራ (አፋር) ግመል 14 የዘር ህዋስ ቅንጣት ሲኖራቸው ሌ ሎቹ ከ2-7 አላቸው። የእነኝህ የዘር ህዋስ ቅንጣት በአንድ ዝርያ ውስጥ በብዛት መኖር የዘር ህዋሶቹ ከአካባቢው ጋር ያላቸው መስተጋበር፣ መላመድ እና የግመል ዘር ህዋሰን ለመጠበቅ መረጃ ይሰጣል። በኢትዮጵያ ግመሎች መካከል ያለው ልዩነት 8 ከመቶ ሲሆን በእያንዳንዱ የግመል ዝርያ ውስጥ 24.52ከመቶ እንዲሁም በእያንዳንዱ ግመል ውስጥ 67.41ከመቶ ይሆናል ይህም የተሻለ የዘር ህዋስ ስፋትን ያሰየናል። የኢትዮጵያ ግመሎች በአጠ ቃላይ 24 ከመቶ በሚሆን አማካኝ መጠን የቅርብ ዝምድና ካላቸው ግመሎች ጋር ተዳቅለዋል ይህም ከፍተኛ መጠን ኢንብሪዲንግ ያሳየናል። ሌላው ይህ ጥናት ያሳየው ምንም እንኳን መቀላቀላቸው ያነሰ ቢሆንም የጅግጅጋ ግመል አፋር ከሚገኘው የሚሌ ግመል ጋር ሲቀራረብ ሱማሌ ክልል ጉዴ ዞን የሚገኘው ጌለብ የሚባለው ግመል አፋር ካለው አሚባራ ግመል ጋር ይቀራረባል። በተጨማሪ ሸበሌ ዞን እና አካባቢው የሚገኙት ጌለብና ሁር እንዲሁም ሞያሌና አካባቢው የሚገኘው ሊበን የተባለው ግመል የተለያዩ መሆናቸውን አሳይቷል። በአጠቃላይ የኢትዮጵያ ግመሎች ሰፊ የዘር ህዋስ ሲኖራቸው ነገር ግን የቅርብ ዝምድና ያላቸው ግመሎች እርስ በእርሳቸው እየተዳቀሉ በመሆኑና አንዳንድ የዘር ህዋስ ቅንጣት በተደጋጋሚ በመሸታና በድርቅ እየጠፉ ስለሆነ ዝርያን የማሻሻል እና የመጠበቅ ኘሮግራም ተነድፎ ወደ ተግባር ሊገባ ይገባል።

# Abstract

*This study was carried out to assess the genetic diversities and population structure of six camel populations of Ethiopia. Blood samples were collected from 114 camels (17-24 per population) and genotyped using10 camel microsatellite marker loci. The result revealed high genetic diversities in Ethiopian camel populations with average observed and expected heterozygosity, total number of alleles (TNA), mean number of alleles (MNA) and effective number of alleles of 0.55, 0.73,153, 6.8 (0.36) and 4.47(0.23), respectively. Among the 52 private alleles in the six Ethiopian camel populations, 31 of them were occurring at a frequency of ≥5%. It was also found that most of the variations in Ethiopian camels were attributed to within population variation (92%) while 8% was explained by between populations variation. Even though there was high heterozygosity, high inbreeding coefficient was detected in Ethiopian camel populations. From the sex camel populations 34.1,* 33.4 and 28.6% alleles of Gelleb, Amibara and Liben camel populations respectively affected by inbreeding. *The between population differentiation was ($F_{ST}$=0.11(±0.01) indicating moderate population differentiation. The neighbor-joining tree and structure analysis show that the Ethiopian camel populations were clustered into four subgroups. The Afar camels were grouped into two together with the Ethiopian Somali camels (Jigjiga with Mille and Gelleb with Amibara) and the two Ogaden camels (Liben and Hoor) are separated into two subgroups indicating that some of the Afar and the Somali camels were admixed. Therefore, even though high hetrozygosity within population and moderate genetic differentiation between populations were observed, the presence of high inbreeding coefficient may affect hetrozygosity in the overall populations.*


# Introduction

According to the estimate of World Watch List of Domestic Animal Diversity, there is a loss of 1 to 2 breeds every week among the domesticated populations of animals (FAO 2003). In Africa, genetic diversity of farm animals is under threat due to recurrent drought, diseases, conflicts and migration. Most programs in livestock improvement in Africa have resorted to crossbreeding indigenous breeds with imported breeds or directly replacing the indigenous genotypes (Wollny 2003). However, the documentation of losses due to such practices remains very poor. Therefore, documentation of diversity of local genetic resources is needed to set new strategies for livestock conservation (Hanotte and Jianlin 2005). Livestock conservation activities include documentation of existing genetic resources such as phenotypic characteristics, performance, cultural importance and genetic uniqueness (Alvarez et al. 2009). Studies of genetic diversity in domestic animals are based on evaluation of the genetic variation within breeds and genetic relationships among them (Tapio et al. 2005).

The camel (*Camelus dromedarius*) represents an important economic resource in many arid areas across several countries. Camels are most numerous in the arid areas of Africa particularly in the arid lowlands of Eastern Africa with estimated population of 17 million

from 28 million camels in the World (FAO 2014). The camel population of Ethiopia estimated to be 4.5 million (Shapiro et al. 2015).

Utilization of camel in Ethiopia is important and different camel populations are specialized for milk and meat traditionally. In the past, very few phenotypic (Yosef et al. 2014) and genetic (Pamela 2012) (blood samples were taken only from Borena camels in the southern periphery of the country) characterization of Ethiopian camels were conducted. Conservation of genetic diversity is important for long-term genetic improvement to meet the requirement of growing population and unforeseen challenges arising through changing production systems and agro-climatic conditions. Hence, characterization of breeds is the first step in conservation program (Toro et al. 2006).

Camel genetic characterization is of major importance to establish a proper management that considers the distribution of genetic variability between and within different populations on different regions, and the identification of the genetic groups that constitute reservoirs of genetic variability. Now, it is more important than ever to understand the genetic basis underlying camel phenotypic traits and physiological features for selective breeding, adaptation to hot environments in the context of global climate change and increasing desertification.

In this regard, genetic polymorphisms are playing an increasingly important role as genetic markers in many species of animals and camels are no exception. Microsatellites are currently the markers of choice for the molecular characterization of livestock genetic resources. To date several microsatellite loci have been characterized in domestic Bactrian camels (*Camelus bactrianus*) (Chuluunbat et al. 2014) and dromedary (Mehta et al. 2007). As in other domestic animal species, microsatellites in camels are highly polymorphic, and enough informative markers exist to carry out diversity studies (Mburu et al. 2003; Schulz et al. 2005).

With this background, this study was undertaken with the objective to characterize genetic diversity of Ethiopian camel populations and distinguish their relationships.

# Material and Methods

## Molecular Characterization

### Blood sample collection and storage

Based on the traditional classification by the local people, and from previous study Yosef et al. (2014) on their morphologically distinctiveness, and geographical location six Ethiopian camel populations have been studied - Mille and Amibara from Afar region and Hoor, Gelleb, Liben and Jigjiga from Somali region. Blood samples have been taken from 25-31 unrelated camels (to reduce relatedness blood of one camel was collected from each village) of each population by puncturing the jugular vein and drops of blood have been collected into EDTA coated vacutainer tubes (Becton Dickinson, USA) from the

respective breeding tracts. The blood samples were stored in a refrigerator at -20 °C until DNA extraction.

## DNA extraction and microsatellite markers

Genomic DNA was extracted using Jetquick DNA purification kits (Thermo Fisher Scientific corporation, Invitrogen) following the manufacture's recommended protocol. Samples were diluted in elution buffer based on the amount of DNA visible on the 1% agarose gel and stored at –20 °C. From each population, 17-24 (Table 3) unrelated camels (the blood was taken one per village and all taken from different village) were genotyped for ten microsatellites loci in a total of 114 individuals.(Table 1). The microsatellite loci were selected based on their polymorphic nature and easiness of scoring.

PCR master mixes were prepared forward primers modified by an end labeling with fluorescent dyes (6-FAMTM, VIC®, NEDTM, PET®) at the 5' end. Each reaction consisted of water, DNA, primers (0.06 nM of forward primer, 0.6 nM of reverse primer dNTPs (30 mM each), 1X PCR buffer [200 mM of Tris–HCl (pH 8.4), 500 mM of KCl], BSA (0.4 ug/ll), $MgCl_2$ (between 1.5 and 3 mM, according to the locus) and Platinum® Taq DNA Polymerase [(0.3 U); Invitrogen] and 10 ng of template DNA (from each individual per population). Genomic DNA was amplified by polymerase chain reaction (PCR) for 17 autosomal microsatellite loci (Table 1). Samples were amplified in a Dual 96-Well GeneAmp® PCR System or on ABI veriti (Applied Biosystems) thermo-cyclers under the following conditions: initial denaturation at 95 °C for 10 min and 95°C for 30s in one cycle followed by 29 cycles of 1min at 64 °C and 72°C for 35s; the third stage was 16 cycle in 58°C for 1 min and 72°C in 30s; the fourth stage 10 cycle in 95 for 30s, 54 for 35s and 72 for 30 and in the last elongation stage we used 1 cycle in 72 for 20min and a final elongation cycle for 12 mn. The PCR products were checked in 2% agarose gel stained with GelRed™. In general, the 17 markers were checked for 7 camel populations. Finally, seven markers and one camel population (Shinille) were removed from the analysis due to low polymorphic character and poor DNA quality respectively (Table1).

## Microsatellite genotyping and scoring

After amplifying the DNA for seven (7) populations, seventeen (17) microsatellite and 135 individuals the products were sent for genotyping. The result of the genotype data was scored using peak scanner software, version1. After excluding the less polymorphic markers, population and individuals that produced low data, six (6) populations, ten (10) polymorphic markers and 114 (17-24 per population as indicated in Table 3) individuals were used in the analysis.

## Statistical analyses

Fisher exact test for deviations from Hardy-Weinberg equilibrium (HWE) was performed using GenAlex version 6.5. Unbiased estimates of exact P-values were obtained by the MARKOV CHAIN MONTE CARLO (MCMC) algorithm of (Guo and Thompson 1992) using 10,000 dememorization steps, 500 batches and 5000 iterations per batch. Expected heterozygosity (Gene diversity) ($H_E$), observed heterozygosity (*Ho*), total number of allele (TNA) and mean number of alleles (MNA) were determined across all loci and population

following (Nei's 1987) using the same program and the results obtained were verified using MICROSATELLITE TOOLKIT (Park 2001).

Table 1. Characteristics of microsatellite primers used

| Primer | Primer sequence (5' -> 3') forward/reverse | Annealing temperature °C | Accession number | Allele range** | Diversity studies |
|---|---|---|---|---|---|
| CMS15 | AAATACTTAAAGGTTCCCAGA<br>TTGTAAACTAAAGCCAGAAAG | 55 | AF329151 | 138–146A<br>140–146L<br>140–159B<br>121–144D | R1 |
| CMS25 | GATCCTCCTGCGTTCTTATT<br>CTAGCCTTTGATTGGAGCAT | 58 | AF380345 | 93–118A<br>93–95L<br>118–128B<br>93–102D | R1 |
| CMS121 | CAAGAGAACTGGTGAGGATTTTC<br>AGTTGATAAAAATACAGCTGGAA<br>AG | 60 | AF329159 | 128–157A<br>128–151L<br>151–159B<br>147–166D | R1 |
| CVRL01 | GAAGAGGTTGGGGCACTAC<br>CAGGCAGATATCCATTGAA | 55 | AF217601 | Polymorphic A<br>188-253B<br>196-253D | R5,R6 |
| CVRL05 | CCTTGGACCTCCTTGCTCTG<br>GCCACTGGTCCCTGTCATT | 60 | AF217602 | Polymorphic A<br>148-174B<br>155-176D | R3,R5.R6 |
| CVRL06 | TTTTAAAAATTCTGACCAGGAGT<br>CTG<br>CATAATAGCCAAAACATGGAAAC<br>AAC | 60 | AF217606 | Polymorphic A<br>185-205B<br>196-203D | R3,R5.R6 |
| LCA66 | GTGCAGCGTCCAAATAGTCA<br>CCAGCATCGTCCAGTATTCA | 50-58 | AF091125 | 220-262A+L<br>212-242B<br>240-244D | R2,R3,R8 |
| VOLP32 | GTGATCGGAATGGCTTGAAA<br>CAGCGAGCACCTGAAAGAA | 55 | AF305234 | 192-247A<br>256-262B<br>256-262D | R2, R3, R6, R7, R9 |
| YWLL 44 | CTCAACAATGCTAGACCTTGG<br>GAGAACACAGGCTGGTGAATA | 55-60 | | 86-120A+L<br>101-117B<br>90-114D | R2, R3, R4, R6,R9 |
| YWLL 59 | TGTGCAGGAGTTAGGTGTA<br>CCATGTCTCTGAAGCTCTGGA | 55-58 | | 96-136A+L<br>109-135B<br>109-111D | R2, R4, R6 |

** Unassigned but believed to be all autosomal, ** A: alpaca (Lama pacos), L: llama (Lama glama), B: Bactrian camel (Camelus bactrianus), D: dromedary camel (Camelus dromedarius).*

F-statistics ($F_{IS}$, $F_{IT}$, and $F_{ST}$) for each locus and overall loci were calculated using the variance-based method of (Weir and Cockerhan 1984). Significance levels were tested using FSTAT version 2.9.3 (Goudet 2001) with 1000 randomizations. Pairwise Reynolds' linearized distances between populations (Reynolds et al. 1983) were calculated from allele frequencies following the procedure described by (Nei et al. 1983) using GenAlEx version 6.5 (Peakall and Smouse 2006) and POPULATIONS (Langella 1999). Analysis of molecular variance (AMOVA) was performed using Arlequin version 3.1 (Excoffier *et al*.

2005) to study the distribution of the components of variance within and among the Ethiopian camel populations. A Neighbour Joining( NJ) tree based on CSE distances was constructed by using the "NEIBHBOR" routine as implemented in the Phylip version 3.695 software package (Felsenstein 2013). The pattern of population structure and detection of probable introgression was visualized using a Bayesian model based clustering method implemented in STRUCTURE software, Version 2.2 (Pritchard et al. 2000). For this, the admixture model with correlated allele frequencies was used. A burning period of 500,000 was used followed by 1,000,000 Markov Chain Monte Carlo (MCMC) replications for data collection for K=1 to K=6. For each K value, ten replicates were run. This procedure clusters individuals into populations and estimates the proportion of membership in each population for each individual (Falush et al. 2003). Mean and standard deviation of likelihood values were calculated for each K and plotted together with (Evanno et al. 2005) statistic by using the STRUCTURE HARVESTER on-line option (Earl and vonHoldt 2012). For genetic bottle neck analysis wilcoxon tests and IAM,TPM and SMM model were used.

# Results and Discussion

## Genetic diversity

All the ten-microsatellite loci primer-pairs were found to be polymorphic. The total number of alleles (*Na*), mean effective number of alleles (*Ne*) and observed (*Ho*) and expected ($H_E$) heterozygosity estimated for the six populations is given in Table 2. The number of alleles per locus ranged from 2 to 14 (Table 2). The loci CVRL6 and LCA66 have two alleles each and were the least polymorphic while loci CMS15 and CMS121 were highly polymorphic with 14 alleles each. The Mean Number of Alleles (MNA) in the six camel populations ranged from 4.80-8.00 with average of 6.80. The largest MNA was found in the Liben (MNA=8.00) while the lowest was in the Jigjiga camel population (MNA = 4.80) (Table 3). A total of 153 alleles were detected among the 114 camels by applying the 10 loci, of which 52 were designated as private alleles (Table 4). Out of these private alleles, 15 were observed in Liben, 14 in Amibara, seven each in Geleb, Hoor and Mille, and two in Jigjiga populations. Of these 52 alleles, 31 of them were occurring at a frequency of ≥5%. These include 10 alleles in Amibara, five in Gelleb, three in Hoor, two in Jigjiga, and seven in Liben and four in Mille camel populations. These private alleles were found in seven loci for Amibara and Liben camel, five loci of Gelleb and Hoor camels and two and four loci of Jigjiga and Mille camel populations, respectively.

The number of alleles per locus observed in the present study was higher compared to other previous studies on different camel populations of India (Mehta et al. 2007) which is 2-6, Saudi camel populations (Al-Swalim et al. 2009; Mohmoud et al. 2012) with a mean of 6.2 and (Ahmed 2010) for Tunisian camel with the number of alleles per locus range 2-7. On the other hand, (Schulz et al. 2010) reported higher number of alleles (2 to 22) per locus in the Canarian camels. The presence of large number of alleles in the Ethiopian camel populations indicates that Ethiopian camel populations have high genetic variation within population.

The MNA in the present study was higher than the values reported for Kenyan, Pakistan, Saudi Arabian camel populations with MNA of 4.22, 4.77 and 4.83, respectively (Mburu et al. 2003). The MNA in this study was also higher than the MNA found in African camels of Botswana (4.08), Namibia (3.41) and South Africa (4.42) but lower than the MNA in Malvi and Bikaneri of Indian camels. Breeds with a low MNA have low genetic variation due to genetic isolation, historical population bottlenecks or founder effects. A high MNA implies great allelic diversity which could have been influenced by cross breeding or admixture.

The average effective number of alleles in this study was 65.7% of the observed number of alleles (Table 3), suggesting a large number of alleles at higher frequency. This result was much higher than that of Saudi camels where the average effective number of alleles from the observed number of alleles is 46.8% (Mohmoud et al. 2012). The mean effective number of alleles in this study was higher than the values reported for the African, Arabian and Indian camel populations (Mburu et al. 2003; Banerjee et al. 2012).

The observed hetrozygosity per population ranged from 0.44 (Gelleb) to 0.66 (Jigjiga) and the expected hetrozygosity ranged from 0.70 (Jigjiga) to 0.77 (Gelleb) (Table 3). Average $H_O$ and $H_E$ were 0.55 and 0.73, respectively. Generally, the observed hetrozygosity is lower than the expected hetrozygosity in all populations which indicates the opportunity to increase hetrozygosity.

The Ethiopian populations displayed higher Ho (0.55) compared to the Tunisian camels ($H_O$ = 0.46) (Ahmed et al. 2010) and higher than that observed in Australian camels (0.45) (Spencer and Woolnough 2010). The result of the present study is comparable to that of Schulz et al. (2010) who noted observed hetrozygosity of 0.552 in the Arabian camels. Likewise, (Vijh et al. 2007) observed hetrozygosity values of 0.580, 0.570, 0.560 and 0.600 for Bikaneri, Jaisalmeri, Kutchi and Mewari camel populations of India, respectively which is similar to that observed for Ethiopian camel population. The $H_E$ value in the present camel population was higher than that of Arabian camel populations, which exhibited 0.59-0.64 (Mohmoud et al. 2012), of Kenyan camels which exhibited 0.54 (Mburu et al. 2003) and that of Indian Jaisalmeri and Kachichi camels with $H_E$ of 0.53 and 0.54, respectively (Gautam et al. 2004; Mahta et al. 2007). The extent of observed and expected hetrozygosity values recorded for Ethiopian camels were contrary to the results reported by (Banerjee et al. 2012) which concluded that the Ho and He values increase in the direction from Africa through the Middle East to the Far East.

Hetrozygosity is a measure of genetic variation within a population. High hetrozygosity values for a breed may be due to long-term natural selection for adaptation, to the mixed nature of the breeds or to historic mixing of strains of different populations. A low level of hetrozygosity may be due to isolation with the subsequent loss of unexploited genetic potential. Locus hetrozygosity is related to the polymorphic nature of each locus. A high level of average hetrozygosity at a locus could be expected to correlate with high levels of genetic variation at loci with critical importance for adaptive response to environmental changes (Kotzé and Muller 1994). Hence, the presence of high-$H_E$ values in the Ethiopian camel populations indicates the presence of high genetic variation within the populations.

Table 2: Number of alleles and hetrozygosity per locus and population

| Population | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Amibara | | | | | Gelleb | | | | | Hoor | | | | |
| | NA | Ne | Ho | He | Hnb | NA | Ne | Ho | He | Hnb | NA | Ne | Ho | He | Hnb |
| YW44 | 7.00 | 3.92 | 0.60 | 0.75 | 0.77 | 8.00 | 4.91 | 0.53 | 0.80 | 0.82 | 4.00 | 2.62 | 0.82 | 0.62 | 0.64 |
| CV6 | 7.00 | 3.33 | 0.63 | 0.70 | 0.72 | 6.00 | 2.98 | 0.47 | 0.66 | 0.68 | 5.00 | 2.13 | 0.50 | 0.53 | 0.55 |
| VOL32 | 3.00 | 2.99 | 0.50 | 0.67 | 0.70 | 4.00 | 2.99 | 0.36 | 0.66 | 0.69 | 4.00 | 2.20 | 0.18 | 0.55 | 0.57 |
| CMS15 | 7.00 | 4.35 | 0.72 | 0.77 | 0.79 | 11.00 | 7.12 | 0.93 | 0.86 | 0.89 | 14.00 | 9.48 | 0.88 | 0.89 | 0.92 |
| YW59 | 6.00 | 4.03 | 0.58 | 0.75 | 0.77 | 9.00 | 3.88 | 0.61 | 0.74 | 0.76 | 6.00 | 3.27 | 0.53 | 0.69 | 0.72 |
| CMS25 | 8.00 | 4.27 | 0.55 | 0.77 | 0.79 | 9.00 | 7.81 | 0.39 | 0.87 | 0.90 | 8.00 | 6.68 | 0.56 | 0.85 | 0.88 |
| CMS121 | 14.00 | 8.42 | 0.72 | 0.88 | 0.91 | 11.00 | 7.05 | 0.47 | 0.86 | 0.88 | 8.00 | 5.26 | 0.88 | 0.81 | 0.83 |
| CV1 | 10.00 | 4.84 | 0.13 | 0.79 | 0.82 | 5.00 | 3.54 | 0.33 | 0.72 | 0.74 | 6.00 | 4.76 | 0.69 | 0.79 | 0.82 |
| CV5 | 10.00 | 7.23 | 0.27 | 0.86 | 0.89 | 9.00 | 5.95 | 0.30 | 0.83 | 0.86 | 9.00 | 6.72 | 0.84 | 0.85 | 0.88 |
| LCA66 | 2.00 | 1.98 | 0.06 | 0.49 | 0.52 | 3.00 | 2.95 | 0.04 | 0.66 | 0.69 | 7.00 | 5.36 | 0.67 | 0.81 | 0.84 |
| Mean | 7.40(1.09) | | | | | 7.50(0.89) | | | | | 7.10(0.94) | | | | |
| Population | | | | | | | | | | | | | | | |
| Locus | JIGJIGA | | | | | LIBAN | | | | | MILLE | | | | |
| | NA | Ne | Ho | He | Hnb | NA | Ne | Ho | He | Hnb | NA | Ne | Ho | He | Hnb |
| YW44 | 7.00 | 4.52 | 0.71 | 0.78 | 0.80 | 10.00 | 4.12 | 0.41 | 0.76 | 0.78 | 8.00 | 6.92 | 0.69 | 0.86 | 0.88 |
| CV6 | 2.00 | 1.67 | 0.56 | 0.40 | 0.41 | 4.00 | 1.41 | 0.33 | 0.29 | 0.29 | 3.00 | 1.77 | 0.33 | 0.43 | 0.45 |
| VOL32 | 4.00 | 2.99 | 0.56 | 0.67 | 0.68 | 6.00 | 4.84 | 0.42 | 0.79 | 0.81 | 4.00 | 2.60 | 0.50 | 0.62 | 0.64 |
| CMS15 | 4.00 | 3.11 | 0.65 | 0.68 | 0.70 | 13.00 | 6.08 | 0.47 | 0.84 | 0.86 | 9.00 | 7.00 | 0.79 | 0.86 | 0.89 |
| YW59 | 4.00 | 3.83 | 0.78 | 0.74 | 0.76 | 5.00 | 3.19 | 0.42 | 0.69 | 0.70 | 4.00 | 2.73 | 0.56 | 0.63 | 0.65 |
| CMS25 | 5.00 | 4.53 | 0.44 | 0.78 | 0.80 | 10.00 | 4.86 | 0.55 | 0.79 | 0.81 | 6.00 | 4.79 | 0.60 | 0.79 | 0.82 |
| CMS121 | 4.00 | 3.56 | 0.72 | 0.72 | 0.74 | 7.00 | 3.88 | 0.63 | 0.74 | 0.76 | 7.00 | 4.60 | 0.67 | 0.78 | 0.81 |
| CV1 | 8.00 | 4.82 | 0.77 | 0.79 | 0.82 | 8.00 | 4.72 | 0.55 | 0.79 | 0.81 | 9.00 | 6.28 | 0.71 | 0.84 | 0.87 |
| CV5 | 6.00 | 4.50 | 0.56 | 0.78 | 0.80 | 8.00 | 5.56 | 0.55 | 0.82 | 0.84 | 4.00 | 3.13 | 0.28 | 0.68 | 0.71 |
| LCA66 | 4.00 | 3.26 | 0.89 | 0.69 | 0.71 | 9.00 | 6.50 | 0.73 | 0.85 | 0.86 | 6.00 | 3.16 | 0.69 | 0.68 | 0.71 |
| Mean | 4.80(0.55) | | | | | 8.00(0.84) | | | | | 6.00(0.69) | | | | |

*NA=Number of alleles; Ne=Effective number of alleles; Ho=Observed hetrozygosity; He=Expected hetrozygosity; Hnb= Unbiased expected hetrozygosity; numbers in parenthesis are standard errors.*

Table 3. Estimates of average and effective number of alleles and hetrozygosity (±SE) per population

| Population | N | TNA | Number of allele per population | Effective number of allele per population | Hetrozygosity | | |
|---|---|---|---|---|---|---|---|
| | | | | | Observed | Expected | Un-biased expected |
| Amibara | 19 | 74 | 7.40 (1.09) | 4.54 (0.61) | 0.48 (0.08) | 0.74 (0.04) | 0.77 (0.03) |
| Gelleb | 18 | 75 | 7.50 (0.90) | 4.92 (0.61) | 0.44 (0.07) | 0.77 (0.03) | 0.79 (0.03) |
| Hoor | 18 | 71 | 7.10 (0.94) | 4.85 (0.75) | 0.65 (0.06) | 0.74 (0.04) | 0.76 (0.04) |
| Jigjiga | 17 | 48 | 4.80 (0.55) | 3.68 (0.31) | 0.66 (0.04) | 0.70 (0.03) | 0.72 (0.04) |
| Liban | 24 | 80 | 8.00 (0.84) | 4.52 (0.47) | 0.50 (0.03) | 0.73 (0.05) | 0.75 (0.05) |
| Mille | 18 | 60 | 6.00 (0.70) | 4.30 (0.60) | 0.58 (0.05) | 0.72 (0.04) | 0.74 (0.04) |
| Mean (±SE) | | | 6.80 (0.36) | 4.47 (0.23) | 0.55 (0.03) | 0.73 (0.02) | 0.76 (0.02) |

Table 4. Population-specific private alleles (frequencies in parenthesis) identified overall loci for the six camel populations

| Population | | | | | | |
|---|---|---|---|---|---|---|
| Locus used | Afar, zone 3 | Afar, zone 1 | Shebelle | | Fafen | Liben |
| | Amibara | Mille | Gelleb | Hoor | Jigjiga | Liben |
| | Loci and alleles (frequency) | Loci and alleles (frequency) | Loci and alleles (frequency) | Loci and alleles (frequency) | Loci and alleles (frequency) | Loci and alleles (frequency) |
| CMS15 | CVRL6Ra 162 (0.079) | YWLL44a 136(0.16) | CMS15a 138(0.07) | CMS15a 258(0.03) | CVRL1Ra 342(0.06) | YWLL44a 114(0.05) |
| CMS25 | YWLL59a 120(0.08) | CMS15a 134(0.21) | CMS15a 176(0.07) | CMS15a 274(0.01) | CVRL5Ra 142(0.08) | YWLL44a 116(0.16) |
| CMS121 | CMS25a 134(0.03) | CMS15a 140(0.04) | YWLL59a 122(0.03) | YWLL59a 132(0.06) | | CVRL6Ra 176(0.02) |
| CVRL01 | CMS121a 156(0.03) | CMS15a 142(0.07) | YWLL59a 134(0.09) | CMS25a 108(0.11) | | CMS15a 116(0.06) |
| CVRL05 | CMS121a 178(0.03) | CMS15a 146(0.07) | CMS25a 142(0.11) | CVRL1Ra 314(0.15) | | CMS15a 160(0.06) |
| CVRL06 | CMS121a 180(0.06) | CVRL1Ra 344(0.03) | CMS121a 152(0.03) | CVRL5Ra 136(0.03) | | CMS15a 166(0.32) |
| LCA66 | CVRL1Ra 202(0.07) | LCA66a 236(0.03) | CVRL1Ra 236(0.07) | CVRL5Ra 160(0.03) | | CMS15a 168(0.03) |
| VOLP32 | CVRL1Ra 208(0.13) | | | | | CMS15a 172(0.09) |
| YWLL 44 | CVRL1Ra 212(0.07) | | | | | CMS25a 116(0.07) |
| YWLL 59 | CVRL1Ra 214(0.03) | | | | | CMS25a 140(0.02) |
| | CVRL1Ra 232(0.07) | | | | | CMS121a 186(0.02) |
| | CVRL1Ra 234(0.07) | | | | | CMS121a 192(0.02) |
| | CVRL5Ra 182(0.07) | | | | | CVRL1Ra 326(0.02) |
| | LCA66a 222(0.44) | | | | | LCA66a 244(0.02) |
| | | | | | | LCA66a 262(0.11) |

## Genetic differentiation

### F-statistics and genetic variation among populations

The mean estimate of F statistics were $F_{IS}$=0.242, $F_{IT}$ 0.322 and $F_{ST}$=0.11 (Table 5). The $F_{IS}$ index from Hardy-Weinberg equilibrium analysis indicated 33.4, 34.1, 12.4, 3.4, 28.6 and 19.6% of inbreeding level in Amibara, Gelleb, Hoor, Jigjiga, Liben and Mille camel populations, respectively. Alleles of Gelleb camel population from Shebelle zone of Somali region was majorly affected by inbreeding followed by Amibara camel from zone 3 of Afar region and Liben camel of Somali region. The $F_{ST}$ among the six Ethiopian camel populations is indicated in Table 6. The $F_{ST}$ value that is the relative measure of gene differentiation among populations was 0.11. The mean pair-wise $F_{ST}$ estimates were significantly different from zero. Pair-wise $F_{ST}$ estimates revealed a genetic differentiation between 0.03-0.11, which was significantly different from zero ($p<0.05$).

The $F_{IS}$, $F_{IT}$ and $F_{ST}$ value in this study was higher than that of Tunisian camels (Ahmed et al. 2010) but the $F_{IS}$ value was lower than Bikaneri, Jaisalmeri, kutchi and Doubie of Indian camels (Banerjee et al. 2012). The high $F_{IS}$ value in the Ethiopian camel population may indicate the presence of high inbreeding effect which leads to decreased hetrozygosity within population.

The mean differentiation index ($F_{ST}$) value in the present study (0.11±0.01**)** was higher than the values reported by (Mburu et al. 2003) for all dromedary populations (Kenyan, Middle East and Pakistan together) with the value of 0.056 and Kenyan dromedaries (Turkana, Randelle and Gabra) (0.009). The $F_{ST}$ value of Ethiopian camel populations was comparable to that of the Tunisian camel populations (Ahmed et al. 2010). Hence, the relatively higher $F_{ST}$ value (0.11) among the Ethiopian camel populations indicates the presence of moderate genetic differentiation. Different studies noted that the $F_{ST}$ value of populations should be greater than zero for the populations to exhibit genetic differentiation. According to (Hedrick 1999), differentiation is not negligible if $F_{ST}$ is 0.05 or less. However, the interpretation of the magnitude of $F_{ST}$ is more complex than simple reference to this quantitative guide. The $F_{ST}$ values can be considerably lower for modern hyper variable markers characterized by many alleles than for genetic markers with very few alleles. Therefore, in modern population genetic procedures a more important question is whether we can detect significant genetic differentiation ($F_{ST} > 0$) or not and whether this differentiation is biologically meaningful. Thus, this study revealing the moderate $F_{ST}$ in the Ethiopian camel populations, which is higher than most camel populations in other regions, may be considered an indication of the ancestral variation in the Ethiopian camel populations.

All F-statistic parameters ($F_{IS}$ = 0.242(0.04), $F_{IT}$ = 0.322(0.04) and $F_{ST}$ = 0.105(0.01)) were significantly different from zero ($P < 0.01$). The within individual and population inbreeding estimate, which represents the nonrandom union of gametes and deviation from HWE, revealed that most of the genotypes with several loci deviated from HWE with significant difference ($P < 0.01$). If mean within-subpopulation ($F_{IS}$) diversity is much greater than between-subpopulation ($F_{ST}$) diversity, it is believed that the

subpopulations show low differentiation (Lewontin 1972; Nie 1973). The across population differentiation ($F_{IT}$) of this study was comparable to the Tunisian ( 9%) and Indian camel populations (8.2%) (Vijh et al. 2007; Ahmed et al. 2010) but much higher than that of Kenyan camel populations (0.009%) (Mburu et al. 2003). Observed and expected hetrozygosity at different loci can be used to estimate the extent of inbreeding. A large value reflects the existence of a large number of heterozygote genotypes and less of homozygote genotypes. A small value indicates the occurrence of homozygote genotypes at a higher proportion than the heterozygote genotypes. In this study, some of the loci deviated from HWE and it shows the impact of inbreeding on the hetrozygosity of loci and genotypes. The possible causes may be the utilization of specific breeding male camel in the population at a non-random mating and the occurrence of genetic bottleneck in the recent past.

Table 5. Result of F-statistics (±SE) for each locus in the six camel populations obtained by jackknifing

| Locus | $F_{IS}$ (inbreeding coefficient) | $F_{IT}$ (individual inbreeding within the total population) | $F_{ST}$ (overall genetic divergence) |
|---|---|---|---|
| YWLL44a | 0.23 | 0.23 | 0.09 |
| CVRL6Ra | 0.23 | 0.23 | 0.18 |
| VOLP32a | 0.46 | 0.46 | 0.14 |
| CMS15a | 0.20 | 0.20 | 0.11 |
| YWLL59a | 0.23 | 0.23 | 0.06 |
| CMS25a | 0.40 | 0.40 | 0.06 |
| CMS121a | 0.19 | 0.19 | 0.05 |
| CVRL1Ra | 0.42 | 0.42 | 0.14 |
| CVRL5Ra | 0.46 | 0.46 | 0.07 |
| LCA66a | 0.41 | 0.41 | 0.161 |
| Overall mean(±SE) | 0.24(0.04) | 0.32(0.04) | 0.11(0.01) |

Table 6. Genetic divergence ( $F_{ST}$ ) in pair wise comparisons between six Ethiopian camel populations

| Population | Amibara | Gelleb | Hoor | Jigjiga | Liben | Mille |
|---|---|---|---|---|---|---|
| Amibara | 0.000 | | | | | |
| Gelleb | 0.069 | 0.000 | | | | |
| Hoor | 0.095 | 0.058 | 0.000 | | | |
| Jigjiga | 0.110 | 0.058 | 0.044 | 0.000 | | |
| Liben | 0.106 | 0.060 | 0.042 | 0.042 | 0.000 | |
| Mille | 0.111 | 0.061 | 0.030 | 0.029 | 0.037 | 0.000 |

## Breed assignment

The breed assignment was calculated with frequency estimate of leave one out. The lowest type I error was seen in the Jigjiga population where 94.3% of the individuals were correctly classified. All the other populations showed a moderate to high accuracy of breed designation from 66.7 to 84.2% of the animals correctly assigned to the source population. Interestingly, some Mille camels were assigned to Jigjiga and Hoor camel populations. However, no animals were assigned between the Mille and Amibara populations of Afar region.

The neighbor-joining dendrogram constructed for the 6 camel populations was observed in Figure 1. It shows the Liben camel population separated first. The Hoor and the ancestor of Mille and Jigjiga camels separated next. Then Mille, Jigjiga, finally Amibara, and Gelleb camel populations were separated.

The extent of gene flow in the six camel populations in the present study with the highest gene flow (Nm=8.23) was observed between Jigjiga and Mille populations, whereas the lowest (Nm=2.00) was observed between Mille and Amibara. This result was much lower than obtained among Saudi Arabian camel populations (14.5 up to 39) (Mohamoud et al. 2012). The study (Michael and David 1999) indicated that Nm less than 10 was considered as low flow of gene between or among populations. Even though the gene flow among the six camel populations was low the relative high gene flow between Jigjiga and Mille camel populations (8.23) needs further study.

The results of this study on breed assignment was comparable with that of (Mburu et al. 2003) who found moderate to high accuracy of breed designation in the Saudi Arabia and Pakistan camel populations with 68 and 81%, respectively, of the animals correctly assigned to the source population. On the other hand, there was a much higher rate of misclassification for the Kenyan dromedaries with the proportion of individuals correctly assigned only ranging from 39 to 48%. When the Kenyan dromedaries were treated as two groups, the Somali and Gabbra–Rendille–Turkana populations, 62% of the former and 76% of the latter were assigned to their respective populations. Breed assignment accuracy was low, especially in the Kenyan dromedaries. The poor success in correct breed assignment could be attributed to the weak genetic differentiation and gene flow between populations. The result of breed assignment in this study confirms that the genetic variation among the six Ethiopian camel populations is moderate.

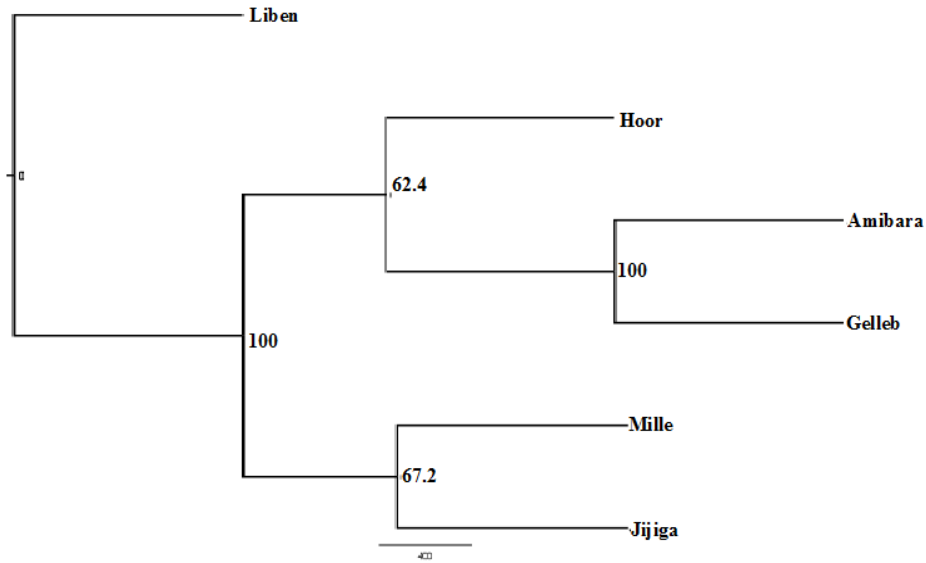


Figure 1. Genetic relationships among the six Ethiopian camel populations,
Neighbor-Joining tree of genetic distances based on estimated membership coefficients.

## Population genetic admixture and structuring

The level of population genetic admixture was shown in Figure 2. Six independent runs of Gibbs sampling were performed and a meaningful genetic admixture and population structure was obtained.

At K=1, all populations were homogenous and did not form any substructure. At K=2, Jigjiga Liben and Mile were clustered into a single group but Amibara clustered into a separate group (Figure 3). However, the remaining two populations had indicated some degree of introgression microsatellite. At K=3, there was high degree of population genetic admixture between populations and it can be clustered in to Amibara as first group, Jigjiga and Mille clustered in the second group, Hoor and Liben as third group and Gelleb as fourth group. Therefore, the most likely cluster and accurate membership was obtained at K=3 and this provided a relatively meaningful explanation of population genetic structure and level of admixture in the Ethiopian camel populations.

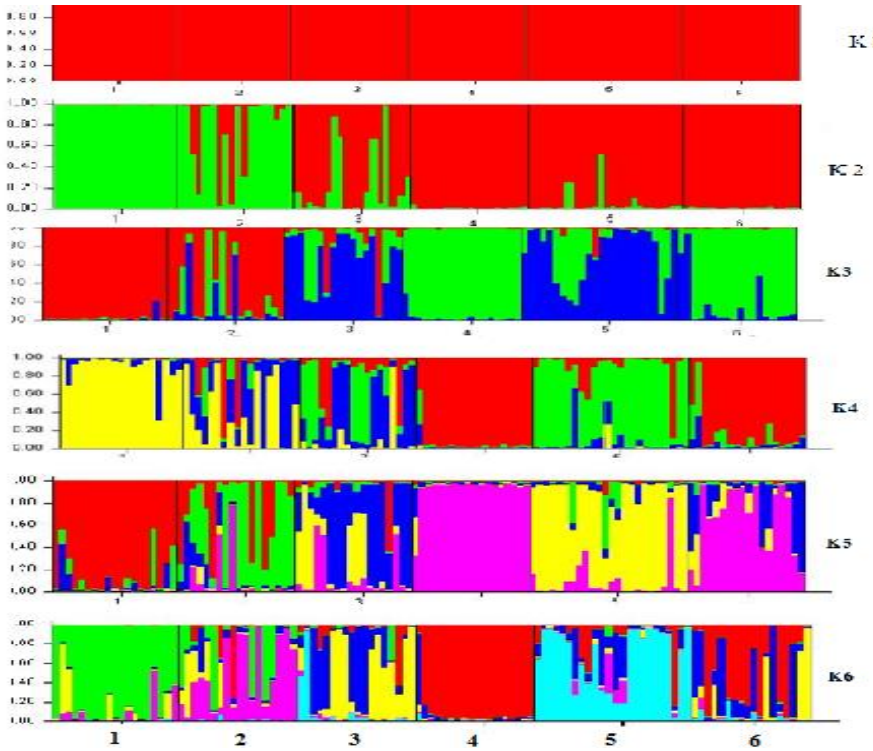


Figure 2. Genetic structure and memberships for six camel populations of Ethiopia based on 6 assumed clusters, K. Each individual population was represented by numbers from 1 to 6 as indicated in the legend. Legend: 1=Amibara; 2=Gelleb; 3=Hoor; 4= Jigjiga; 5=Liben; 6= Mille

## AMOVA and bottleneck test

Analysis of molecular variance indicated that the Ethiopian camel populations had 24.52 and 67.41% of variation within population and within individual, respectively. Between

populations, variation was only 8.06% (Figure 3). The AMOVA result in this study was similar with the variation among Ethiopian domestic donkey populations of 92% (Kefena et al. 2013) but the result was different from Ethiopian cattle breeds that accounts among breed genetic differences for 0.04%. Genetic diversity is in fact important to allow genetic improvement and facilitate rapid adaptation to changing environments and breeding objectives (Notter 1999). Hence, within population variations could be used as a raw material for selection and further genetic improvements. In this regard, the study of (Yang et al. 2015) explained that mutation rates are elevated in individuals with higher overall heterozygosity, particularly in regions close to heterozygous sites and regions in which there are high rates of DNA exchange between chromosomes (recombination). Hence, a positive-feedback loop, whereby high levels of molecular variation in an individual facilitate the production of more variation.

Results of bottleneck analysis were given in Table 7. In Jigjiga camel population, three of the models (IAM, TPM and SMM) indicated presences of genetic bottleneck in the recent past ($p < 0.05$). Even though the other two models were in discordance with IAM model, of the three mutation model IAM model in Wilcoxon test and sign test indicated occurrence of genetic bottleneck ($P<0.05$) in Amibara, Gelleb, Hoor and Mille camel populations whereas, Liben camel population indicated no genetic bottleneck ($P > 0.05$) in the recent past.

In this study, mean number of allele per population, mean effective number of allele per population and breed-specific alleles of Jigjiga camel population were lower than other camel populations. This may be due to population bottleneck through disease, migration or natural disaster in the past. In population bottleneck, the study by (Nei 1987) described that after a reduction of effective population size, both the number of alleles and their hetrozygosity drop, but allele diversity drops more rapidly because the rare alleles are rapidly lost, but their loss has only a weak influence on hetrozygosity. Theory predicts that population bottlenecks are followed by a loss of allelic richness and gene diversity until a new equilibrium is reached or diversity recovers through population growth (Nei et al. 1975). For highly variable loci, the rarest alleles are expected to disappear quickly, whereas gene diversity will be lost more slowly (Leberg 1992). Therefore, there is a transitory excess of gene diversity after the bottleneck relative to the value expected based on the number of alleles in the population. Study has shown that a loss in genetic diversity is commonly associated with decreases in fitness, both at the individual and population levels (Grueber et al. 2008). This can occur through inbreeding depression or increases in the genetic load of populations (Hedrick and Fredrickson 2010).

Thus, populations with low genetic variation also have reduced evolutionary potential (Fisher 1958). That is, populations with decreased genetic diversity will be less likely to adapt to future environmental changes.

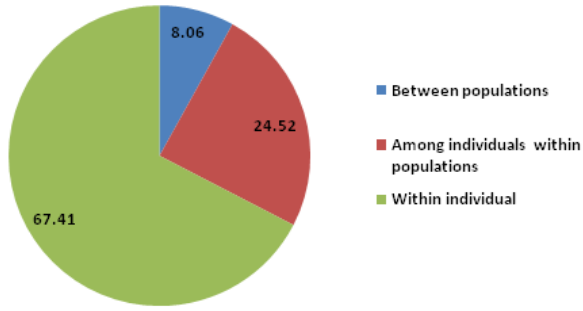


Figure 3. Analysis of Molecular Variance (AMOVA) on Ethiopian camel populations

Table 7. Bottleneck analysis result for the six camel populations

| Population | Mutation model | WILCOXON TEST | SIGN TEST |
|---|---|---|---|
| | | Probability (two tails for H excess and deficiency) | Probability |
| Amibara | IAM* | 0.00684 | 0.04047 |
| | TPM* | 0.32227 | 0.60368 |
| | SMM* | 0.49219 | 0.19409 |
| Gelleb | IAM | 0.00293 | 0.04338 |
| | TPM | 0.13086 | 0.16157 |
| | SMM | 0.92188 | 0.19636 |
| Hoor | IAM | 0.00488 | 0.04592 |
| | TPM | 0.16016 | 0.36582 |
| | SMM | 1.00000 | 0.60810 |
| Jigjiga | IAM | 0.00098 | 0.00371 |
| | TPM | 0.00098 | 0.00420 |
| | SMM | 0.00293 | 0.03635 |
| Liben | IAM | 0.23242 | 0.37948 |
| | TPM | 0.76953 | 0.62889 |
| | SMM | 0.19336 | 0.17158 |
| Mille | IAM | 0.00098 | 0.00444 |
| | TPM | 0.00488 | 0.16215 |
| | SMM | 0.19336 | 0.14498 |

**IAM: Infinite alleles model, *SMM: Stepwise mutation model, *TPM: Two phase model*

# Conclusion

The result of the present study suggest that compared to other camel population, the six Ethiopian camel population in this study have high genetic variation within population which is a raw material for genetic selection. The estimate of genetic differentiation among the six Ethiopian camel populations indicated that differentiation among these populations is moderate; this may show the existence of variations in their ancestors and low gene flow. Most of the six Ethiopian camel populations included in this study were affected by inbreeding.

From this study the following recommendations are forwarded:

- Genetic diversity studies should be undertaken for the remaining camel populations of Ethiopia;
- Appropriate breeding and conservation strategies should be implemented to maintain the genetic diversity and to reduce the inbreeding effect in the Ethiopian camel populations; and
- Accuracy of population structuring could be increased by using more sample size and marker loci.

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