Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers

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Abstract

The genetic diversity of four indigenous Cameroonian cattle breeds was assessed using the Kenyan Boran breed as a reference breed. The Cameroonian cattle breeds were, Arab Shuwa, Ngaoundere Gudali, Namchi and White Fulani. A panel of 13 autosomal microsatellite loci was used. A total of 127 animals were genotyped, revealing 139 different alleles and 31 private alleles (21.3%). All the private alleles but two, allele 286 bp at ILSTS006 in the Arab Shuwa breed and allele 201 bp at ETH152 in the Ngaoundere Gudali- showed frequencies lower than 6 %. All the loci were polymorphic with a mean Polymorphic Information Content (PIC) of 0.75. Expected mean heterozygosity (Hex) ranged from 0.65-0.76. The mean observed heterozygosity (Ho) was higher in the Namchi. Only 6% of the total genetic variation could be attributed to the differences among the breeds. In all the populations, 80% of the overall loci do not deviate from the Hardy-Weinberg law. A high value of Number of effective migrants (Nem) (3.816) was found, combined to a low Fixation Index among individuals within populations ($F_{ST)}$ (7%), which indicates high migration. No close genetic relationship was found between the Kenyan Boran cattle and the Cameroonian indigenous cattle breeds. This study demonstrates that the genetic diversity of Cameroonian indigenous cattle seems to have been affected by gene flow between breeds. Therefore, adopting effective breeding management practices will facilitate the conservation of those breeds and preserve their special characteristics.

Keywords: diversity; gene flow; microsatellite; breeds; Cameroon

Introduction

The mammalian tribe Bovini (subfamily Bovinae, family Bovidae) contains all the most important of the world's larger domestic species. The domestication of these cattle and cattle-like taxa were among the most significant advances of the Neolithic transition (Lenstra & Bradley, 1999). Since then, Cattle have had a central role in the evolution of human cultures and are the most economically important of domesticated animal species. There are two major types, zebu (humped) and taurine (without humps), which are named as separate species (*Bos indicus* and *Bos taurus*), but which, due to complete inter-fertility are often considered as subspecies (Loftus et al., 1994*).

The history and biogeography of cattle populations in Africa represent a complex interaction of ecological, genetic and anthropological factors. The original indigenous cattle of Africa are universally considered to have been exclusively taurine. These populations are thought to have originated from migrations of early pastoralists from the Near East (Payne, 1991; Epstein, 1971). Recent archaeological evidence has, however, questioned this viewpoint, suggesting that the African aurochs (*Bos primigenius opisthonomus*) may have been domesticated independently somewhere on the African continent (Wendorf & Schild, 1994). Although, zebu cattle are thought to have been first introduced into Africa about 4000 years ago, they only started to become widespread about 700 AD with the Arabic migrations into North and East Africa. These recent migrations of zebu cattle pose a serious threat to the genetic integrity of valuable trypanotolerant populations of taurine cattle in the southern areas of West and Central Africa.

In Cameroon, various indigenous cattle breeds are specific to the agro-ecological areas (Messine et al., 1995). Cattle are raised without any selection or reliable breeding program and as a result, there is a big flow of genes between indigenous breeds (Tawah et al., 2007). It is now well known that the assessment of genetic diversity is a prerequisite for the management and conservation of these animal genetic resources (Cañon et al., 2001). Therefore it is essential to characterize these breeds either phenotypically or genetically to be able to keep their specificity before replacement or admixture occurs, and to design rational breeding strategies for their improvement and conservation. Furthermore, the large extent of male zebu influence on the African continent has clearly been demonstrated by Hanotte et al (2000) by the use of an indicine Y specific chromosome marker (INRA 124) but yet the distribution and frequency of autosomal markers between cattle breeds of Central Africa and East Africa has not been done.

Under such context, microsatellites were used to analyse the genetic structure and admixture existing between the Cameroonian indigenous cattle breeds and the Boran cattle of Kenya. Microsatellite markers are widely used to assess diversity within breeds, inbreeding levels, breeds differentiation, introgression or breed admixture (Freeman et al., 2005). However, in Cameroon, very few studies have been done so far (Ibeagha-Awemu and Erhardt, 2004). Microsatellite analysis of genetic diversity provides two distinct levels of information. In addition to allele frequency differences among populations, it also provides information about the cladistic relationships between alleles and group of alleles on the basis of differences in allelic repeat length. The aim of this work was to survey 13 microsatellite loci in four geographically distinct breeds of Cameroonian *Bos taurus* and *Bos indicus* cattle by describing the population genetics of these loci through standard genetic parameters and also estimating the relationships between them.

Materials and Methods

Study Animals

A total of 107 blood samples -collected on FTA® cards- from four different breeds from Cameroon were analyzed. The breeds were: Arab Shuwa (n = 24), Ngaoundere Gudali (n = 36), Namchi (n = 25) and White Fulani (n = 22). All these samples were collected from several representative herds and breeding stations located in the Adamaoua region, North and Far-north regions of Cameroon. According to their spatial location, the breeds were divided into populations: Namchi into Namchi Ngaoundere and Namchi Poli; White Fulani into White Fulani Ngaoundere and White Fulani Garoua. DNA was extracted from FTA® cards according to a modified protocol of Smith & Burgoyne (2004). Additionally, 20 samples from Boran were collected in Kenya to be used as reference (samples).

Microsatellite Marker Typing

Out of 30 Markers, Genotype of thirteen microsatellite loci were successfully amplified by PCR (BM1815, BM1824, CSRM60, CSSM66, ETH104, ETH152, HAUT27, HEL9, HEL5, ILSTS006, INRA063, TGLA122). The selection of markers was based on the FAO panel (http://www.projects.roslin.ac.uk./). The PCR analysis of microsatellites was carried out in a volume of 10 µl using fluorescently labeled PCR primers. The former reaction

was made up of: 10nM dNTP's, Taq polymerase (5 U/l) and primers (10 µM each), 10 X PCR buffer and 20 ng/µl of DNA. So the final concentration of the primers was 0.2 M. PCR reactions were performed in 96-well microtitre plates and were carried out in a Gene Amp PCR System 900 (Applied Biosystems, Weiteterstadt, Germany). Thermocycling conditions were: 3 min at 94°C followed by a first round of 10 cycles of 45 s at 94°C, 1 min at 5 °C above the annealing temperatures with a decrease of 0.5 °C every minute and 1 min at 72 °C. This last step was followed by a second round of 30 cycles of 45 s at 94°C, 1 min at the annealing temperature and 2 min at 72 °C. The last elongation step was prolonged to 20 min. PCR products were separated by electrophoresis in 1.8 % agarose gel in TBE buffer. Each pooled sample (representing 1.5 µl of each PCR product) was heated to 95 °C for 3, in after being added in 9 µl of a mixture of internal standard size (GeneScan ™ 500 LIZ®, Applied Biosystems) and formamide (representing 16 µl of LIZ and 1 ml of formamide). DNA fragment sizing was performed using an automated DNA sequencer (ABI PRISM ® 3730). The Genemapper version 4.1 was used to determine the fragment sizes in base pairs.

Data analysis

Genetic variability was measured by estimating observed (Ho) and expected (He) heterozygosities (Nei, 1978) as well as the total number of alleles (TNA), the Mean Number of alleles (MNA), the Number of effective migrants (Nem), the number of private alleles (Na, alleles found in only one breed). The Polymorphic Information Content (PIC), the unbiased F-statistics (Wright, 1951) and the Analysis of Molecular Variance (AMOVA) were determined using GenAlex software version 6.3 (Peakall and Smouse, 2006) and Power Marker. Pairwise F_{ST} (proportion of genetic variability due to population sub-structuring) values among pairs of populations were computed for all populations using GenAlex software version 6.3. Genetic relationship were also explored by Principal Component Analysis (PCA) using the GeneAlex.

Results

A summary of the amount of genetic variation found within and between population samples is presented in Table 1. In total, 139 alleles were found for all loci, with an average of 10.69. The locus with the lowest number of alleles was INRA 063 with 6 alleles while the locus with the highest number of alleles was CSSM66 with 17. At the level of populations, the least number of alleles (n= 9) was found in White Fulani Ngaoundere, while the highest (n=22) was found in Arab Shuwa. The mean PIC was 0.75. There was 31 distinct private alleles (22.3 %) which were mainly found in Boran cattle (n=14). Among the indigenous cattle of Cameroon, the highest number of private alleles (n=7) was found with Arab Shuwa, while the Namchi Ngaoundere presented no private allele. However, the Cameroonian indigenous breeds-with the exception of allele 286 bp at ILSTS006 in the Arab Shuwa breed and allele 201 bp at ETH 152 in the Ngaoundere Gudali breed- all the others private alleles had frequencies lower than 6 %.

Estimates of Ho and He for all loci are presented in table 1.The mean Ho was highest in the Namchi Ngaoundere..Mean Ho and He values at microsatellite loci were similar for breeds in the zebu group (all the breeds but the Namchi) -Ho values ranged from 0.718 with the White Fulani Ngaoundere to 0.785 with the Boran and White Fulani Garoua ; He ranged from 0.653 with the Boran to 0.735with the Arab Shuwa.

Table1. Estimates (±SD) of genetic parameters from Cameroonian and Boran cattle breeds

Populations	N[1]	MNA[2]	Ho[3]	He[4]	Na[5]
Arab Shuwa	24	7.2 (0.6)	0.777	0.735	7
Boran	20	4.8 (0.3)	0.785	0.653	14
Ngaoundere Gudali	36	6.7 (0.6)	0.755	0.726	5
Namchi Poli	15	6.7 (0.5)	0.704	0.750	2
Namchi Ngaoundere	12	5.4 (0.4)	0.791	0.697	0
White Fulani Ngaoundere	10	6.1 (0.6)	0.718	0.727	2
White Fulani Garoua	12	5.8 (0.5)	0.785	0.704	1

[1] Number of individuals samples; [2]Mean number of alleles; [3] Observed heterozygosity; [4] Expected heterozygosity; [5]Number of private alleles within analysed breeds; Standard deviations are given in parenthesis.

The Hardy-Weinberg Equilibrium (HWE) tested against all loci and populations is shown in table 2. Eighty percent (80 %) of the overall loci do not deviate from the Hardy-Weinberg law.Except the White Fulani Ngaoundere, all the populations had at least two loci deviating from HWE and the maximum values of 4 and 5 loci deviated from HWE was reached for the Boran and the Ngaoundere Gudali respectively.

Table 2 Tests for Hardy-Weinberg Equilibrum among the Cameroonian and Boran cattle breeds

Locus	Arab Shuwa	Boran	Goudali Ngaoundere	Namchi Poli	Namchi Ngaoundere	White Fulani Ngaoundere	White Fulani Garoua
BM1818	$0{,}456^{ns}$	$0{,}071^{ns}$	$0{,}858^{ns}$	$0{,}980^{ns}$	$0{,}051^{ns}$	$0{,}310^{ns}$	$0{,}999^{ns}$
BM1824	$0{,}134^{ns}$	$0{,}259^{ns}$	$0{,}641^{ns}$	$0{,}875^{ns}$	$0{,}485^{ns}$	$0{,}789^{ns}$	$0{,}273^{ns}$
CSRM60	$0{,}558^{ns}$	$0{,}602^{ns}$	$0{,}893^{ns}$	$0{,}256^{ns}$	$0{,}984^{ns}$	$0{,}496^{ns}$	$0{,}489^{ns}$
CSSM66	$0{,}598^{ns}$	$0{,}794^{ns}$	$0{,}038^{ns}$	$0{,}003^{**}$	$0{,}152^{ns}$	$0{,}472^{ns}$	$0{,}040^{*}$
ETH104	$0{,}929^{ns}$	$0{,}000^{***}$	$0{,}342^{ns}$	$0{,}624^{ns}$	$0{,}034^{*}$	$0{,}705^{ns}$	$0{,}211^{ns}$
ETH152	$0{,}587^{ns}$	$0{,}583^{ns}$	$0{,}032^{ns}$	$0{,}605^{ns}$	$0{,}363^{ns}$	$0{,}013^{*}$	$0{,}075^{ns}$
HAUT 24	$0{,}041^{*}$	$0{,}016^{*}$	$0{,}027^{ns}$	$0{,}000^{***}$	$0{,}255$	$0{,}609^{ns}$	$0{,}161^{ns}$
HAUT 27	$0{,}117^{ns}$	$0{,}166^{ns}$	$0{,}926^{ns}$	$0{,}374^{ns}$	$0{,}874^{ns}$	$0{,}490^{ns}$	$0{,}270^{ns}$
HEL9	$0{,}562^{ns}$	$0{,}347^{ns}$	$0{,}663^{ns}$	$0{,}606^{ns}$	$0{,}345^{ns}$	$0{,}696^{ns}$	$0{,}372^{ns}$
HEL51	$0{,}007^{**}$	$0{,}444^{ns}$	$0{,}005^{**}$	$0{,}248^{ns}$	$0{,}005^{**}$	$0{,}263^{ns}$	$0{,}049^{*}$
ILSTS006	$0{,}027^{*}$	$0{,}012^{*}$	$0{,}123^{ns}$	$0{,}086^{ns}$	$0{,}268^{ns}$	$0{,}869^{ns}$	$0{,}812^{ns}$
INRA063	$0{,}266^{ns}$	$0{,}003^{**}$	$0{,}496^{ns}$	$0{,}009^{**}$	$0{,}841^{ns}$	$0{,}970^{ns}$	$0{,}697^{ns}$
TGLA122	$0{,}177^{ns}$	$0{,}996^{ns}$	$0{,}000^{***}$	$0{,}098^{ns}$	$0{,}629^{ns}$	$0{,}235^{ns}$	$0{,}000^{**}$

ns=not significant, * P<0.05, ** P<0.01, *** P<0.0

The analysis of Molecular Variance demonstrated that 6 % of the total variation was due to differences among populations, 7 % among individuals within populations and 87 % accounted for differences within individuals (Table 3).

Table 3: Analysis of Molecular Variance among Cameroonian indigenous cattle of breeds

Source of variation	Sums of squares	Variance components	Percentage variation (%)	F-statistics over all loci
Among population	109.731	0.324	6	
Among individuals	645.075	0.379	7	$F_{IS}=0.076$
Within populations				$F_{ST}=0.061$
Within individuals	590.000	4.574	87	$F_{IT}=0.133$
Total	1344.806	5.277		

All the F-statistics are positive which means a deficit in heterozygosity at the level of the population structure. Results of F-statistics showed on average a deficit of heterozygotes (F_{IS}) of 7.6 % (P<0.001) for each of the analysed breeds and 13.3% (P<0.001) in the whole population (F_{IT}).

The average Nem value was found to be 3.816, showing a large exchange of genes between the populations. Table 4 shows a pair wise population matrix of Nei's unbiased genetic distance between the breeds. The highest genetic distance was found between Boran and Namchi Poli (0.155), while the least genetic distance was found between Arab Shuwa and White Fulani Garoua (0.010).If we consider only the indigenous breeds of Cameroon, the highest genetic distance was found between Namchi Poli and Ngaoundere Gudali (0.071).In general, the distances between indigenous cattle breeds found in Cameroon are not high.

Table 4. Pairwise population matrix of Nei unbiaised genetic distance between Cameroonian and Boran cattle breeds estimated from 13 microsatellites.

	Arab Shuwa	Boran	Ngaoundere Gudali	Namchi Poli	Namchi Wakwa	White Fulani Ngaoundere	White Fulani Garoua
Arab Shuwa	0,000						
Boran	0,121	0,000					
Ngaoundere Gudali	0,019	0,127	0,000				
Namchi Poli	0,052	0,155	0,071	0,000			
Namchi Ngaoundere	0,023	0,142	0,030	0,058	0,000		
White Fulani Ngaoundere	0,036	0,137	0,027	0,033	0,033	0,000	
White Fulani Garoua	0,010	0,124	0,034	0,048	0,028	0,053	0,000

The results of the PCA on the allele frequencies of 13 microsatellite markers on all breeds are presented in figure 1. Three groups were each evident on the basis of the 1st (25.22%) and 2nd (21.3%) PC values and clearly portray the magnitude of divergence between them. The three groups under the first PC were the Boran, a first zebu/taurine group constitute in a very large majority with Namchi Poli, followed by some Arab Shuwa and White Fulani.Under the 2nd PC,White Fulani/Namchi Poli, Namchi Poli/Boran/Arab Shuwa and Arab Shuwa/Ngaoundere Gudali/White Fulani Ngaoundere/Namchi Ngaoundere. The separation between the west African zebu (Boran) and Cameroon indigenous cattle was clear under the first PC and between Namchi Poli and the rest of the Cameroonian indigenous breeds under the second PC.The Cameroonian indigenous breeds but Namchi Poli, on the basis of both PC values occupied a position midway between the two extremes, Boran and Namchi Poli.
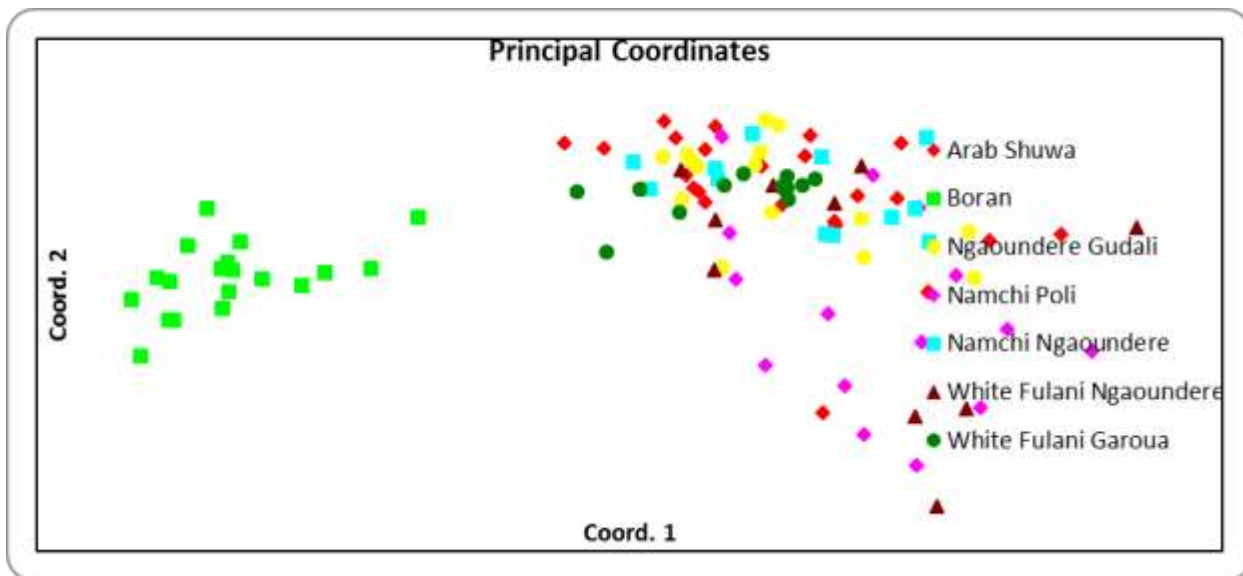

Figure 1 PCA on the allele frequencies of 13 microsatellite markers Cameroonian and Boran cattle breeds.

Discussion

We used 13 FAO microsatellites to study population genetics structure of local central Africa zebu and taurine breeds. This number is less than that used by Berthouly et al. (2008), Ibeagha-Awemu and Erhardt (2006) (n=20), MacHugh et al. (1998), Moazami-Goudarzi et al.(1997)(n=17). It is in the same range of that used by Bessa et al.(2009)(n=13), MacHugh et al.(1994)(n=12). However, with a mean PIC of 0.75, the microsatellites used in this study are highly informative and can therefore give reliable informations on genetic diversity and population structure of breeds. Given that according to Ya-Bo et al. (2006), Vanhala et al.(1998) or Vaiman et al. (1994), loci are highly informative when PIC>0.5, reasonably informative when 0.25<PIC<0.5 and slightly informative when PIC<0.25. All the loci studied here are highly informative, with values ranging from 0.59 to 0.87. The mean PIC value obtained is higher than the 0.6 generated by Moazami-Goudarzi et al. (1997) in ten Europeans cattle breeds or the 0.59 given Chaudhari et al. (2009) in India. High PIC values were also seen in the taurine and indicus breeds investigated earlier using microsatellites (Khumar et al., 2003; Metta et al., 2004; Mukesh et al., 2004; Pandey et al., 2006). In total, 139 alleles were found overall loci with an average of 10.69.The average number of alleles per marker obtained in this study (n=10.69) is in the same range of that reported by Ibeagha-Awemu et al. (2004) for the local Cameroonian and Nigerian breeds (n=11.05).At least six alleles were detected for each microsatellite locus in all breeds. This is in agreement with the selective standard of the microsatellite given by FAO (2004).The mean number of alleles per locus (MNA) obtained in this study (from 5.4 to 7.2) are in the same range of values given by Bessa et al. (from 5.9 to 6.3)(2009) in cattle from Mozambique. However, the MNA observed over a range of loci for different populations is considered to be a reasonable indicator of genetic variation with the provisos that the populations are at mutation-drift equilibrium and that the sample is more or less the same for each population (Nei, 1987). Consequently, in this study, we might have introduced a sample bias, given that all the populations were not in the same size.

The Namchi Ngaoundere is found to present no private alleles, which is a sign of high gene flow between this population and the others as Gudali, located in the same geographic area. All the Boran alleles were privates, meaning that there is no gene flow between the East African zebu Boran and the Cameroonian indigenous cattle breeds.

Levels of gene diversity were very similar for all breeds, suggesting that there are no appreciable differences in the level of genetic variability among Cameroonian breeds. The amounts of genetic diversity in these breeds were comparable to or higher than those reported for other breeds in Mozambique (Bessa et al., 2009), India (Chaudhari et al., 2009); or West Africa (Moazami-Goudarzi et al., 2001).These high levels of gene diversity can be explain by a combination of their hybridized status and the absence of selection for any particular trait. They can also suggest a high level of gene flow between the breeds due to the system of management. Takezaki & Nei (1996) pointed out that the average heterozygosity must be between 0.3 and 0.8 in a breed, to be useful marker for measuring genetic variation.The present results are for mean heterozygosity are within this range.

Heterozygote deficiency analysis revealed that all the populations were showing at least one locus deviating from the HWE. Two microsatellites, HEL 5 and HAUT 24, were in heterozygotes deficit for 50 % of the populations studied. However, Ibeagha- Awemu and Erhardt (2004) observed that on 9 zebu breeds from their study, 7 were not in HWE for HEL 5. This deviation can be as a result of the presence of nul alleles segregating at these loci (Ede and Crawford, 1995). Therefore some heterozygotes are genotyped as homozygotes.On the other hand, the deficiency of heterozygotes among indigenous cattle populations is an indicator of inbreeding among cattle breeds or the occurrence of population substructure, which is called Wahlund effect (Nei, 1987). In this study, although the verification of the presence of null alleles has not been done, a high level of inbreeding should be the major reason of this deviation with regards to the pastoral system. This observed deficiency of heterozygotes could also be due to non-random sampling. This is confirmed by the inbreeding coefficient F_{IS} which is significantly positive. This coefficient expresses the departure from randomness in the mating. When $F_{IS}>0$, the inbreeding exceeds the level expected under random mating, implying that mating among more closely related parents than the average is predominant, or the population is partitioned into subpopulations and mating is less restricted within each subpopulation (Nomura et al., 2001).

Wright's F-statistics provide important insights into evolutionary processes that influence the structure of genetic variation within and among populations, and they are most widely used descriptive statistics in population and evolutionary genetics (Holsinger & Weir, 2009).According to Hart & Clark (1997), F_{ST} measures the heterozygote deficit relative to its expectation under HWE. For the interpretation of F_{ST}, it has been suggested that the a value lying in the range 0-0.05 indicates little genetic differentiation; a value between 0.05 and 0.15, moderate differentiation; a value between 0.15 and 0.25, great differentiation; and a value above 0.25, very great genetic differentiation (Wright, 1978; Balloux and Lugon-Moulin, 2002).In our study, 6.1% of the total genetic variation

was caused by differentiation among subpopulations. Therefore, there is a moderate genetic differentiation among the various populations studied. The figure is lower than the 7 % of the total genetic variability (mean $F_{ST}=0.07$) reported by Cañon et al. (2001) among local European beef cattle breeds and much more higher than the 1.6% given by Ibeagha-Awemu and Erhardt (2006) among Red Bororo and White Fulani cattle breeds of Nigeria and Cameroon.However, the same value was found among 12 African Bos indicus and Bos taurus cattle breeds (Ibeagha-Awemu and Erhardt, 2004).

In this study, we found a high value of the number of effective migrants Nem (3.816).An important factor promoting a high percentage of shared genotype is the movement of different breeds to the southern regions of Cameroon during the drier periods of the year. To confirm this, this high Nem value is combined to a low F_{ST} (6%), which indicates high migration (Bossart and Powell, 1998).

Genetic distances were first used in population genetics to provide a single quantitative measure of differences in two or more sets of allele frequencies (Slatkin, 1985). By using the pairwise F_{ST} values, we found that the highest distance was between Namchi Poli and Ngaoundere Gudali while the least was found between Arab Shuwa and White Fulani Garoua. This can be explained by their systems of managements. The Fulani people use to cross their breeds with local ones when they arrive somewhere in other to have crossbreeds with more adaptative characteristics to their environment (Hanotte et al., 2000). In the contrary, the Namchi Poli and the Ngaoundere Gudali are breed relatively far away from the others. But this last has started to be cross with White Fulani Ngaoundere, despite of the recommendations of the government.

Conclusion

The genetic diversity of Cameroonian indigenous cattle breeds seems to have been affected by gene flow and therefore little differentiation exists among them. However, it appears that they retain some of their genetic identity. No existing gene flow has been found between these breeds and the Boran breed of East Africa. Controlling gene flow between Cameroonian indigenous cattle breeds by adopting effective breeding and management practices will facilitate the conservation of historical breeds and preserve special characteristics of each breed.

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