Genetic diversity and zebu genes introgression in cattle population along the coastal region of the Bight of Benin

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Summary

Genetic diversity and Zebu genetic introgression have been assessed in five subpopulations of cattle along the coastal region of Togo, Benin and Nigeria using 15 autosomal and one Y- specific microsatellite markers. Mean observed heterozygosity (Ho) ranges from 0.55 to 0.61 and the mean number of alleles (MNA) from 5.47 to 6.47. Genetic differentiation indexes (F_{st}), were significant between the five subpopulations ($P< 0.01$). Some possible population diagnostic alleles are identified with allele 254 at locus ILSTS033 and allele 182 at locus ILSTS005 found only in the population from Togo with frequencies of 5.41% and 12.82% respectively. Allele 226 of locus ILSTS103 is fixed in the Togolese population (100%) and almost fixed (98.75%) in the Benin-Valley population. Y chromosome analysis reveals male Zebu introgression in all five populations with a frequency of indicine Y chromosome ranging from 37.5% in Benin-Valley and Benin Plateau East to 100% for Benin Plateau West. Admixture analysis using the programme STRUCTURE (k = 2) confirms phenotypic observations suggesting different level of taurine background and therefore Zebu introgression amongst the populations. Within populations, variations in levels of Zebu admixture between herds were also detected. Whereas the valley population from Benin shows low level of Zebu introgression, it is the population from Benin Plateau East which is the purest.

Résumé

La diversité génétique et l'introgression de gènes de zébu dans la population de bovins le long de la côte du golfe de Guinée allant du Togo au Nigeria ont été évaluées à partir de 15 marqueurs microsatellites autosomes et un marqueur spécifique du chromosome Y. La moyenne des hétérozygotes Ho varie de 0,55 à 0,61 et le nombre moyen d'allèles (NMA) de 5,47 à 6,47. Les indices de différenciation génétique (F_{st}) sont différents entre les cinq sous populations ($P<0,01$). Des allèles spécifiques de population sont identifiés aux loci ILSTS033 (allèle 254) et ILSTS005 (allèle 182) dans la sous population du Togo avec des fréquences respectives de 5,41 et 12,82%. L'allèle 226 du locus ILSTS103 est totalement (100%) fixé dans la sous population togolaise et presque (98,75%) dans la sous population de la vallée au Bénin. L'évaluation du marqueur spécifique du chromosome Y révèle l'introgression de gènes de zébu dans les cinq sous populations allant de 37,5% dans la vallée du Bénin et sur le Plateau Est à 100% sur le Plateau Ouest. L'analyse de mélange utilisant le programme STRUCTURE (k = 2) confirme les observations phénotypiques concluant à l'introgression de gènes de zébu au sein des populations. Des variations de mélange sont observées entre troupeaux au sein d'une même sous population. Alors que les animaux dans la vallée au Bénin présentent un niveau faible d'introgression de gènes de zébu, c'est la sous population du Plateau Est qui paraît être la plus pure.

Resumen

La diversidad genética y la introgresión de genes de zebú en la población de bovinos a lo largo de la costa del golfo de Guinea que va desde Togo hasta Nigeria han sido evaluados a partir de 15 marcadores microsatelitares autosomos y un marcador específico del cromosoma Y. La media de heterocigosis Ho varía de 0,55 a 0,61 y el número medio de alelos (NMA) de 5,47 a 6,47. Los índices de diferenciación genética (F_{st}) son diferentes entre

las cinco sub-poblaciones (P<0,01). Han sido identificados alelos específicos de poblaciones en los loci ILSTS033 (alelo 254) e ILSTS005 (alelo 182) en la sub-población del Togo con frecuencias respectivas de 5,41 y 12,82%. El alelo 226 del locus ILSTS103 está totalmente fijado (100%) en la sub-población togolesa y casi (98,75%) en la sub-población del valle del Benín. La evaluación del marcador específico del cromosoma Y revela la introgresión de genes de zebú en las cinco sub-poblaciones que van de 37,5% en el valle del Benín y en la Meseta Este, hasta 100% en la Meseta Oeste. El análisis de mezcla utilizando el programa STRUCTURE (k=2) confirma las observaciones fenotípicas que indican la introgresión de genes de zebú en el interior de las poblaciones. Variaciones de mezclas han sido observadas entre rebaños al interior de una misma población. Mientras que los animales del valle del Benín presentan un nivel de introgresión de genes de zebú bajo, la sub-población de la Meseta Este parece ser la más pura.

Key words*: Cattle, Microsatellites, Genetic diversity, Zebu genes introgression.*

Introduction

West African taurine cattle (*B. taurus*) breeds are under threat of extinction due to uncontrolled breeding practices by farmers aiming to enhance the size and the productivity of their animals. However, it is recognised that animals of these breeds are well adapted to their local environments and remain productive in areas where Zebu or crossbreeds are often unable to survive (Agyemang *et al.* 1991; Uza, 1997). The phenotypic traits of these animals are the result of hundreds of years of natural selection in relation to the local environments under permanent tsetse, ticks and helminth challenge. It is clear that uncontrolled crossbreeding with exotic breeds poses a threat to the adaptive traits of this unique animal genetic resource. This uncontrolled crossbreeding is the result of poor agricultural policies with no long term breeding goals, each farmer developing his own objective with emphasis on short-term results.

In the early eighties, the Food and Agriculture Organisation of the United Nations started alerting public opinion on the subject of animal genetic resources conservation and management (FAO, 1981). At the Earth Summit in 1992 in Rio de Janeiro, 157 countries signed the United Nations convention on biological diversity and subsequently FAO (1992) launched a special program for the global management of farm animal genetic resources. The aim of this program is to maintain in each species a maximum genetic diversity of the gene pool to allow for future unforeseen needs in the development of sustainable animal production systems. In addition the program aims at prompt actions to preserve animal breeds at risk of extinction.

Studies in the last decade have shown that African cattle were most likely domesticated within the African continent, separately from the other centres of domestication in the Fertile Crescent and in the Indian sub-continent, from the wild African auroch *B. primigenius* (Bradley *et al.*, 1996). Their separate origin indicates that they represent a unique set of genetic characteristics. Today, the remaining pure African taurine cattle are only found within the West African taurine cattle living in tsetse-infected areas. These populations, given their origins and their adaptation to local environmental conditions (e.g. disease resistance), represent a unique genetic resource.

Introgression with Zebu cattle in West African taurine cattle populations, however, is common (MacHugh *et al*., 1997; Hanotte *et al*., 2000) and is diluting progressively the African taurine genetic background of these breeds. Ultimately, this introgression will result in the loss of the unique genetic adaptation of these breeds. Currently, the extent and pattern of Zebu introgression into the indigenous taurine populations is well described by Hanotte *et al*. (2002). This study, however, did not include the Lagune breed. The current paper is therefore a complementary study to that of Hanotte *et al*. (2002), targeting the Lagune breed in the Bight of Benin. Results of this study can be used to identify suitable pure West African taurine populations for their inclusion in breeding programs that aim at conserving and utilising these unique indigenous genetic resources. These results could also be the starting point for the implementation of a sustainable breeding programme for livestock production in that region.

Material and Methods

Sampling

Blood samples were collected from different cattle subpopulations in Benin, Nigeria and Togo. Forty animals, of which eight were males, were sampled for each identified subpopulation. Three subpopulations have been sampled in Benin, which are Benin Plateau West (BPW), Benin Valley (BV)

and Benin Plateau East (BPE). Two neighbouring regions of the target area in Benin have been considered (Figure 1) in Nigeria (NG) and Togo (TG) resulting in a maximum of 200 sampled cattle.

Appropriate materials such as Wattman filter paper, evacuated blood collection tubes (5 ml) and Eppendorf tubes for stocking buffy coats were used for handling samples. Blood samples were collected from each animal in two evacuated blood collection tubes containing 3.2% EDTA and the buffy coat (white cells) was taken after spinning the tubes at 2400 rpm at ambient temperature. Collected buffy coats were added with 8M urea solution and kept at ambient temperature. To be on the safe side, blood samples were also collected on Wattman paper and dried at 40°C in oven, then stored at ambient temperature. Sampling included as many animals from different herds as possible to avoid direct relationship between sampled individuals. In other terms, one male and four females were sampled per herd and a total of eight herds were visited for each identified subpopulation. All samples were conveyed to the laboratory of genetics at ILRI campus in Nairobi (Kenya) for processing.

Laboratory procedures

DNA was extracted from the buffy coats with standard procedures as described by Sambrook *et al.* (1999). Sixteen markers, of which one was on the Y chromosome, were used for genotyping. They were *TGLA126*, *TGLA122*, *ILSTS033*, *ILSTS013*, *ILSTS008*, *ILSTS005*, *ILSTS006*, *ILSTS036*, *ILSTS028*, *ILSTS023*, *ILSTS103*, *ETH152*, *BM2113*, *BM1824*, *AGLA293* and *INRA124*. Polymerase Chain Reaction (PCR) for amplification of microsatellite loci were performed as described in Hirano *et al.* (1996) and Kemp *et al.* (1995).

Genotyping and gel analysis were performed using automatic DNA sequencer ABI 377 and associated analysis software Genescan™ (version 3.1) and Genotyper™ version 2.0).

Data analysis

Marker allele frequencies have been analysed using the Microsatellite toolkit program (Park, 2001). The genetic diversity of each population was evaluated by computing the mean number of alleles per locus (MNA), the observed heterozygosity (H_{obs}), and the expected heterozygosity (H_{exp}) using the microsatellite toolkit (Park, 2001) as well as GENEPOP, version 3.3 (Raymond and Rousset, 1995). Subpopulations have been checked for HWE using the exact probability test with GENEPOP. The level of genetic differentiation among populations was examined using *F*-statistics (Weir and Cockerham, 1984). Genetic distances between populations D_A (Nei *et al.*, 1983) were calculated and used to construct a phylogenetic tree

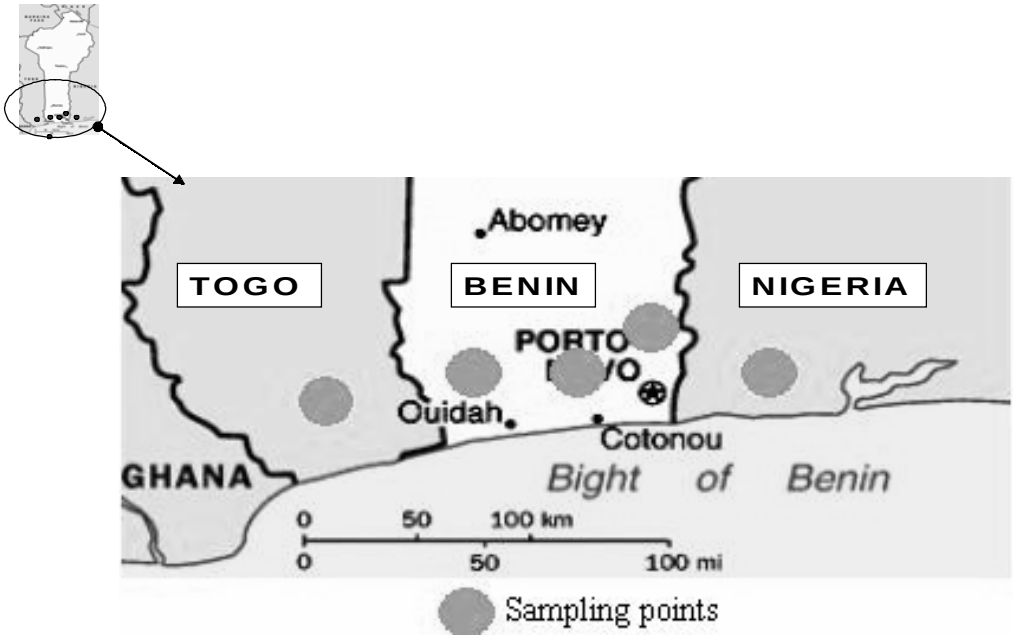

Figure 1.Benin map showing the sampled populations locations.

with the neighbour joining algorithm (Saitou and Nei, 1987). The level of Zebu introgression was estimated with the programme STRUCTURE using the ADMIX 1.0 software (Bertorelle and Excoffier, 1998). Correlations between phenotype and populations structure results were performed using Microsoft Excel.

Results

Genetic variation

From the five subpopulations under study here, 130 alleles were found for all 15 autosomal marker loci that have been genotyped over the 200 sampled animals (Table 1). The total number of alleles detected per locus ranged from 4 to 16 resulting in a mean number of alleles (MNA) per locus of 8.67. The number of alleles summed over the 15 loci per subpopulation ranged from 82 in BPE to 97 in TG (Table 1) giving a MNA per locus for each subpopulation varying from 5.47 to 6.47 (Table 2). These variations are not strong enough ($P>0.05$) to differentiate current subpopulations. However, allele 254 of the locus *ILSTS_033* and allele 182 of *ILSTS_005* are found solely in TG with a frequency of 5.41% and 12.82% respectively. Alleles 171 and 175 of *ILSTS_023* are equally distributed (50%) within TG and BV subpopulations. Further, allele 226 of locus *ILSTS_103* is fixed in TG (100%) whereas it is almost (98.75%) fixed in BV.

The expected heterozygosity computed over the 15 loci for each of the five subpopulations ranged from 0.593 (BV) to 0.673 (BPW) whereas the observed heterozygosity varied from 0.55 in BPE to 0.61 in BPW (Table 2).

None of the subpopulations deviated from HWE through all loci at 5 p. cent level. Out of the 15 loci under study, only loci *ILSTS006* ($P<0.006$), *ILSTS023* ($P<0.005$) and *ILSTS103* ($P<0.05$) deviated consistently from HWE for all five subpopulations (Table 3). Except those three loci, TG deviated ($P<0.05$) from equilibrium at two loci (*BM1824*, *BM2113*), BPW at four loci (*TGLA126*, *ILSTS033*, *ILSTS008*, *BM2113*), BV at two loci (*ILSTS008*, *ILSTS033*), BPE at three loci (*ILSTS033*, *ILSTS036*, *BM2113*), and NG at two loci (*ILSTS013*, *ILSTS036*).

Genetic distances and breed relationships

Table 4 summarizes genetic distances and gene differentiation indices between all pairs of subpopulations. The largest genetic distance (0.1183) was obtained between TG and BPE, whereas the smallest (0.0666) was obtained between

Table 1. Total number of alleles per locus and per subpopulation.

Locus	Togo	Benin			Nigeria	Total
		BPW	BV	BPE		
TGLA126	7	7	6	6	6	7
TGLA122	8	8	9	7	8	11
ILSTS033	7	5	4	7	6	9
ILSTS013	4	4	4	4	4	4
ILSTS008	4	5	4	3	3	5
ILSTS005	5	4	3	4	3	5
ILSTS006	8	5	8	5	5	9
ILSTS036	12	8	9	8	9	16
ILSTS028	6	8	6	6	7	10
ILSTS023	2	5	2	4	5	6
ILSTS103	1	5	2	4	5	7
ETH152	6	5	7	5	5	8
BM2113	12	10	7	6	9	12
BM1824	6	4	4	4	4	6
AGLA293	9	8	12	9	8	15
Total	97	91	87	82	87	130

Table 2. Comparison of genetic variability of five populations of cattle along the coastal region of Togo, Benin and Nigeria.

Populations	[1]MNA (±SE)	[2]H_{obs} (±SE)	3H_{exp} (±SE)
Togo	6.47 (±3.14)	0.5905 (±0.0202)	0.6410 (±0.0539)
Benin Plateau West	6.07 (±1.91)	0.6083 (±0.0200)	0.6725 (±0.0322)
Benin Valley	5.80 (±2.88)	0.5604 (±0.0204)	0.5930 (±0.0503)
Benin Plateau East	5.47 (±1.73)	0.5504 (±0.0204)	0.6194 (±0.0296)
Nigeria	5.80 (±2.01)	0.5603 (±0.0203)	0.6338 (±0.0383)

[1] Mean number of alleles per locus.
[2] Observed heterozygosity.
[3] Expected heterozygosity.

Table 3. Hardy-Weinberg exact probability test by population.

Locus	Togo	Benin			Nigeria
		BPW	BV	BPE	
TGLA126	0.1266 (0.0094)	0.0324 (0.0058)	0.9689 (0.0032)	0.3372 (0.0138)	0.1626 (0.0086)
TGLA122	0.0918 (0.0143)	0.1416 (0.0109)	0.5619 (0.0241)	0.3201 (0.0204)	0.2617 (0.0191)
ILSTS033	0.0122 (0.0035)	0.0000 (0.0000)	0.0302 (0.0026)	0.0018 (0.0009)	0.2594 (0.0157)
ILSTS013	0.3394 (0.0082)	0.2777 (0.0082)	0.9153 (0.0024)	0.5509 (0.0065)	0.0219 (0.0018)
ILSTS008	0.3769 (0.0107)	0.0052 (0.0014)	0.0024 (0.0007)	0.0842 (0.0049)	0.8024 (0.0036)
ILSTS005	0.1651 (0.0080)	0.0052 (0.0071)	0.3149 (0.0082)	0.7353 (0.0076)	0.6628 (0.0049)
ILSTS006	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0057 (0.0018)	0.0000 (0.0000)
ILSTS036	0.0797 (0.0167)	0.1021 (0.0100)	0.1006 (0.0160)	0.0294 (0.0053)	0.0001 (0.0001)
ILSTS028	0.0805 (0.0088)	0.1186 (0.0129)	0.2908 (0.0173)	0.2782 (0.0152)	0.3055 (0.0202)
ILSTS023	0.0000 (0.0000)	0.0001 (0.0001)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
ILSTS103	-	0.0003 (0.0003)	-	0.0000 (0.0000)	0.0183 (0.0035)
ETH152	0.0672 (0.0065)	0.4927 (0.0091)	0.3976 (0.0174)	0.9354 (0.0049)	0.3382 (0.0115)
BM2113	0.0396 (0.0076)	0.0110 (0.0028)	0.2903 (0.0158)	0.0089 (0.0016)	0.3507 (0.0163)
BM1824	0.0414 (0.0064)	0.9355 (0.0020)	1.0000 (0.0000)	0.8824 (0.0045)	0.0616 (0.0055)
AGLA293	0.5448 (0.0222)	0.6256 (0.0170)	0.2169 (0.0274)	0.0676 (0.0097)	0.0907 (0.0103)

NG and BPW. This result is in contrast to what would be expected given the geographic location of these subpopulations (Figure 1). The smallest coefficient of differentiation was obtained between TG and BPW, and the highest was between TG and BPE which is in agreement with the largest genetic distance obtained. The F-statistics results showed that the five populations were significantly different from each other (P=0.0001). Estimates of F_{ST} for each locus and for each population pair are summarized in Table 5.

The neighbour-joining tree based on genetic distance D_A is represented in figure 2. It shows the existing relationships among the five subpopulations according to studied loci. As it would be expected, BV and BPE are close subpopulations as shown in this figure.

Population structure (admixture)

Genetic admixture analysis shows that all five subpopulations are introgressed with Zebu genes but to different degrees (Figure 3), whereas subpopulations from TG and BPW harbour high levels of introgression at 0.753 and 0.651, respectively. BPE is the least introgressed with introgression coefficients equal to 0.188. BV and NG have intermediate coefficient, 0.309 and 0.390, respectively. On individual basis, the level of

Table 4. Genetic distance between five cattle subpopulations along the coastal region of Togo, Benin and Nigeria calculated using D_A (Nei et al., 1983) below diagonal and Weir and Cockerham (1984) F_{ST} above the diagonal.

	Togo	Benin Plateau West	Benin Valley	Benin Plateau East	Nigeria
Togo	-	0.0205	0.0341	0.0564	0.0493
Benin Plateau West	0.0795	-	0.0349	0.0310	0.0258
Benin Valley	0.0866	0.0917	-	0.0335	0.0463
Benin Plateau East	0.1183	0.0740	0.0809	-	0.0377
Nigeria	0.1103	0.0666	0.0914	0.0688	-

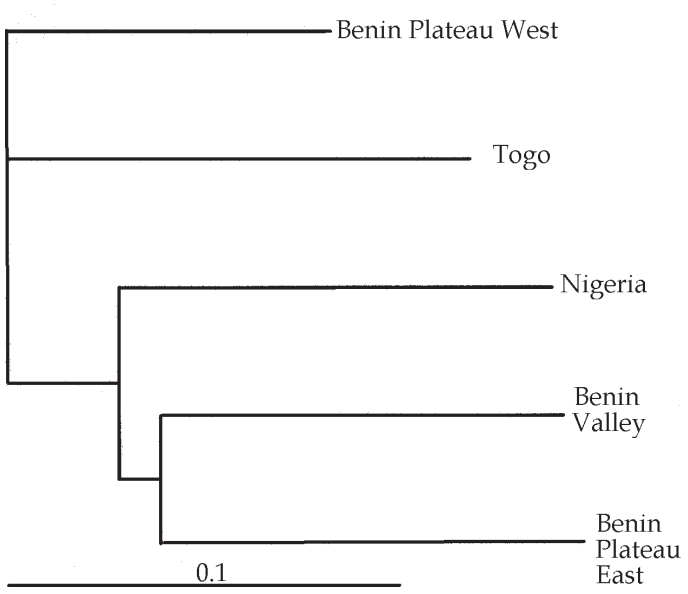

Figure 2. Neighbor joining dendogram showing the genetic relationship among the five sampled subpopulations along the coastal region from Togo to Nigeria.

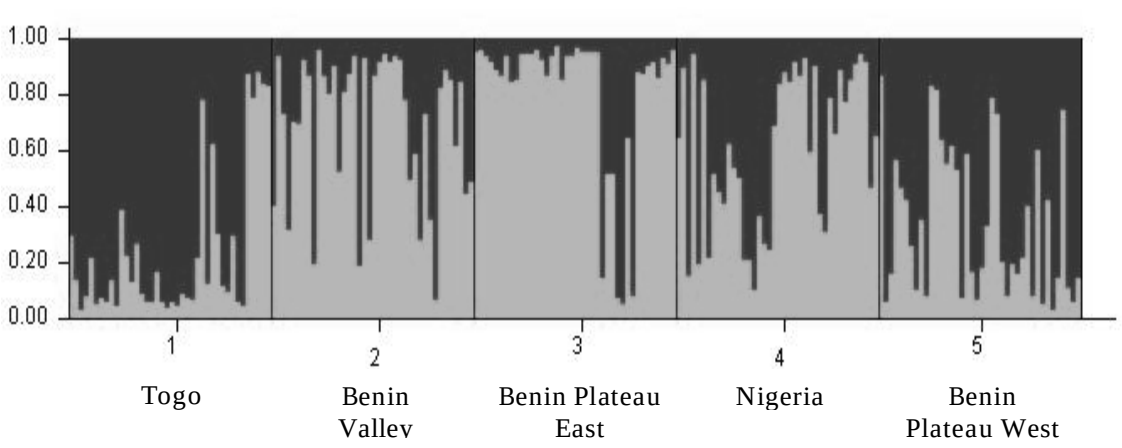

Figure 3. Admixture analysis showing zebu introgression in sampled subpopulations.

Table 5. Estimate of F_{ST} for each of the 15 loci and for each population pair.

Loci	TG / BV	TG / BPE	TG / NG	TG / BPW	BV / BPE	BV / NG	BV / BPW	BPE / NG	BPE / BPW	NG / BPW
TGLA126	0.0407	0.0369	0.0170	0.0060	0.0006	0.0006	0.0375	0.0538	0.0346	0.0118
TGLA122	0.0303	0.1558	0.0162	0.0340	0.0498	0.0256	0.0099	0.1476	0.0879	0.0096
ILSTS033	0.0302	0.0583	0.0703	0.0208	-0.0015	0.0268	0.0213	0.0659	0.0757	0.0190
ILSTS013	0.0049	0.0095	0.0432	-0.0055	-0.0007	0.0009	0.0316	0.0280	0.0346	0.0847
ILSTS008	0.0061	0.0561	0.0333	-0.0032	0.0053	-0.0101	0.0024	-0.0023	0.0607	0.0256
ILSTS005	0.0627	0.0315	0.0523	0.0442	-0.0043	-0.0120	0.0072	-0.0076	-0.0033	0.0080
ILSTS006	0.0007	0.0079	0.0923	-0.0032	0.0363	0.0924	0.0056	0.1066	-0.0056	0.1163
ILSTS036	-0.0052	0.0009	0.0036	0.0006	-0.0047	0.0050	0.0053	-0.0004	-0.0012	-0.0041
ILSTS028	0.0363	0.0478	0.0132	0.0149	-0.0120	-0.0032	0.0065	0.0086	0.0062	0.0146
ILSTS023	0.0000	0.1077	0.1950	0.1281	0.1081	0.1955	0.1285	0.0183	-0.0050	0.0041
ILSTS103	0.0000	0.2046	0.1727	0.1606	0.1813	0.1465	0.1391	-0.0009	-0.0101	0.0020
ETH152	0.0193	0.0695	0.0320	-0.0119	0.0339	0.0156	0.0125	-0.0012	0.0617	0.0248
BM2113	0.0713	0.0317	0.0107	0.0032	0.0419	0.0609	0.0383	0.0234	0.0333	0.0106
BM1824	0.0846	0.0485	0.0714	-0.0037	0.0309	-0.0113	0.0562	0.0181	0.0478	0.0494
AGLA293	0.0765	0.0618	0.0026	0.0022	0.0777	0.0644	0.0688	0.0523	0.0397	0.0162
All loci	0.0341	0.0564	0.0493	0.0205	0.0335	0.0463	0.0349	0.0377	0.0310	0.0258

TG=Togo; BPW=Benin Plateau West; BV=Benin Valley; BPE=Benin Plateau East; NG=Nigeria.

Figure 4. Typical Lagune cattle from Benin Plateau East (height is 81 cm; girth circumference is 112 cm).

Zebu introgression varies from 0.028 in TG to 0.967 in BPE.

Analysis of the *Y* chromosome reveals male Zebu introgression in males of all five subpopulations with a frequency of indicine *Y* chromosome ranging from 37.5% in BV and BPE to 100% for BPW. TG and NG have respectively 87.5 and 62.5%. The correlation coefficient between the admixture results and those of the *Y* chromosome analysis is 0.93.

Discussion

With the upgrade breeding performed by livestock producers along the coastal regions of the three countries involved in this study, the observed variation between populations was expected. Indeed, looking for taller animals, mainly for market purposes, Borgou breed and Zebu have been introduced in diverse herds (Figure 5) resulting in the higher MNA for these populations. This is the case in TG and BPW (Table 2). This result is supported by the admixture analysis which gives the higher proportion of Zebu genes to subpopulations TG and BPW. In contrast, the subpopulation BPE has the least genetic variation in terms of MNA associated with a low heterozygosity. The low genetic variation in this subpopulation could be explained by the higher level of inbreeding in this subpopulation. This explanation is supported by the management system of these animals. Farmers used to keep their animals under their own habitat during night, then they are tethered to trees and moved from time to time to access forage. Bulls are owned by few people that hire them to non-owners for breeding. This means that breeding is under control, so as to ensure these animals are kept as pure as possible for their use in certain ritual ceremonies and as a dowry. Subpopulations TG and BV, however, have in common some alleles, that is allele 171 and 175 from *ILSTS023* at 50%, allele 226 of locus *ILSTS103* at the rate of 100% for the former and 98.75% for the latter. This result is surprising given that they are separated by subpopulation BPW which has lower frequency for these alleles (results not shown).

Genetic distances between pairs of subpopulations, coefficients of differentiation and

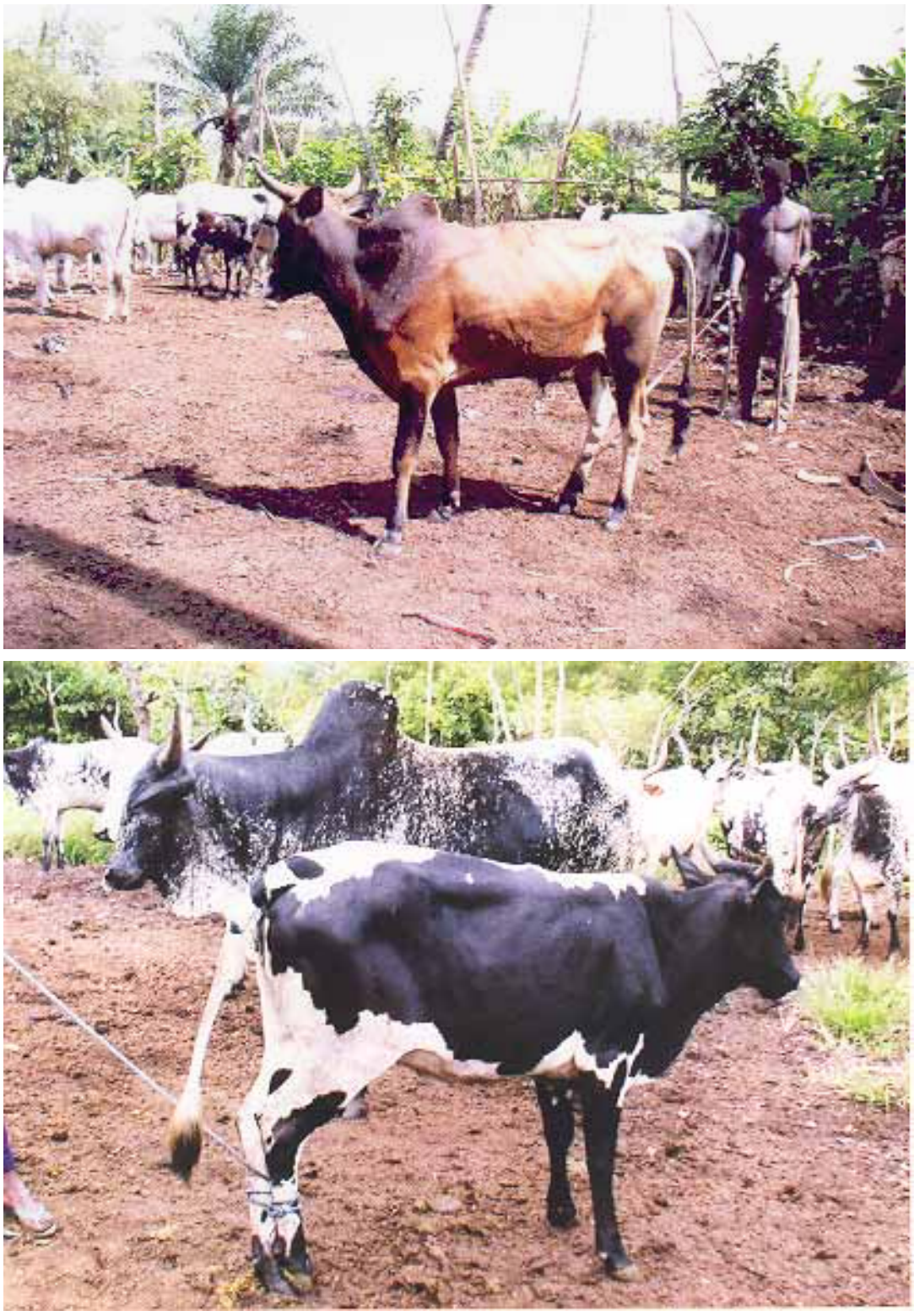

Figure 5. Zebu breeds that have been introduced in herds in the coastal regions.

F_{ST} confirm that the five subpopulations are different, although some results showed patterns that were not expected. We would expect the largest genetic distance to be between TG and BPE on one side and between NG and BPW on the other. The latter showed the least distance which is in contrast to what would be expected given the geographic location of these subpopulations (Figure 1). According to the admixture analysis, TG and NG showing the highest introgression of Zebu genes would be closer - that is not the case in current results. This could be explained by different sources of gene pools that have been used to perform the introgression. The existing relationships among subpopulations through the neighbour-joining tree clearly show that BV and BPE are close subpopulations as one could predict from figure 1.

Concurrently to blood sampling, height at wither and girth circumference were measured to relate the phenotype with genotype. There is quite an agreement between the results of the admixture model analysis and the phenotype of animals (correlation coefficient equals to 0.59), that is the higher the percent of Zebu genes, the higher is the height at wither. The availability of data on control animals (pure Zebu and pure Lagune) would have better strengthened current results.

The hypothesis that animals in the valley would be less introgressed by Zebu is true, but the population of Benin Plateau East is even purer than the one targeted in our study.

Conservation futures

The major objective of this study was to determine the level of Zebu introgression within cattle populations along the coastal regions of the Bight of Benin. This region is known to originally be the cradle of the Lagune cattle breed (Figure 4), animals of small size with a wither height varying from 90 to 100 cm and a heart girth from 130 to 137 cm. As this study has demonstrated, there is a high tendency for Zebu or other breeds to be introgressed in this breed. Some herds and to some extent at least two populations, have been shown to be less polluted with Zebu blood. Throughout this study we placed an emphasis on naming cattle populations instead of breeds given the extent of crossbreeding, and current results support this point of view.

The hypothesis of the current study was to encounter cattle with low level of Zebu introgression that could be used for genetic resources conservation and it was expected to meet those animals in BV. Results of this study have shown that the purest taurine cattle were instead located in BPE. If such a conservation programme was to be undertaken, we would advise on the use of animals from BPE.

The status of BPE animals is the result of the breeding system in force in this area and for which objectives are clearly defined at the farmers' level. Therefore a sustainable way of handling the market for these animals remains to be identified. Indeed, there should be a strong marketing study that could identify market problems and define likely solutions. There should also be a sensitisation programme to make stakeholders be aware of different changes that might intervene. A contest and show program with good prizes could be a way of attracting farmers in that region to recognise the effort they are deploying on this breed. Any breeding programme, whatever is its goal, should integrate the indigenous knowledge and use of these animals. Throughout these actions, studies of the biochemical as well as the culinary quality of meat and milk produced from these animals should be undertaken to support the promotion of this breed.

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List of References

Agyemang, K., R.H. Dwinger, A.S. Grieve & M.L. Bah. 1991. Milk production characteristics and productivity of N'Dama cattle kept under village management in the Gambia. J. Dairy Sci. 74, 1599-1608.

Bradley, D.G., D.E. MacHugh, P. Cunningham & R.T. Loftus. 1996. Mitochondrial diversity and the origins of African and European cattle. Proc. Natl. Acad. Sci USA 93, 5131-5135.

Bertorelle, G. & L. Excoffier. 1998. Inferring admixture proportions from molecular data. Mol. Biol. Evol. 15, 1298 –1311.

FAO. 1981. Animal genetic resources conservation and management. Proceedings of the FAO / UNEP technical consultation. FAO Animal Production and Health paper 24. FAO, Rome, pp. 388.

FAO. 1992. The management of global animal genetic resources. FAO Animal Production and Health paper 104. FAO, Rome, pp. 309.

Hanotte, O., C.L. Tawah, D.G. Bradley, M. Okomo, Y. Verjee, J. Ochieng & J.E.O. Rege. 2000. Geographic distribution and frequency of a taurine *Bos taurus* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan African cattle breeds. Molecular Ecology 9, 387-396.

Hanotte, O., D.G. Bradley, J.W. Ochieng, Y. Verjee, E.W. Hill & J.E.O. Rege. 2002. African pastoralism: genetic imprints of origins and migrations. Science 296, 336-339.

Hirano, T., S. Nakane, K. Mizoshita, H. Yamakuchi, M. Inoue-Murajama, T. Watanabe, W. Barendse & Y. Sugimoto. 1996. Characterization of 42 highly polymorphic bovine microsatellite markers. Anim. Genet. 27, 365-368.

Kemp, S.J., O. Hishida, J. Wambugu, A. Rink, M.L. Longeri, R.Z. Ma, Y. Da, H.A. Lewin, W. Barendse & A.J. Teale. 1995. A panel of polymorphic bovine, ovine and caprine microsatellite markers. Anim. Genet. 26, 299-306.

MacHugh, D.E., M.D. Shriver, R.T. Loftus, P. Cunningham & D.G. Bradley. 1997. Micorsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu (*Bos taurus* and *Bos indicus*). Genetics 146, 1071-1086.

Nei, M., F. Tajima & Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evolution 19, 153-170.

Park, S.D.E. (Ed.). 2001. Trypanotolerance in West African cattle taurine and the population genetic effects of selection. PhD thesis, University of Dublin, Ireland.

Raymond, M. & F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86, 248-249.

Saitou, N. & M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406-425.

Sambrook, J., E.F. Fritsch & T. Maniatis. 1989. Molecular Cloning: A Laboratory manual. 2nd edition, Cold Spring Harbor Laboratory Press, New York.

Uza, D.V. 1997. The productivity of Muturu cattle (*Bos brachyceros*) under ranching conditions in the Southern Guinea Savanna of Benue state, Nigeria. Outlook on Agriculture 26, 19-23.

Weir, B.S. & C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358-1370.