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Genetic diversity of Nigerian Indigenous Sheep breeds at the β-Lactoglobulin gene locus

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Abstract

The study assessed genetic diversity of four Nigerian sheep populations namely; Balami, Yankasa, Ouda and West African Dwarf (WAD) making use of blood sample. Extracted DNAs were used to study polymorphism at the β-lactoglobulin gene locus using RLFP-PCR process. Results revealed the percentage polymorphic locus was 100% while Shannon's information index, observed homozygosity, expected heterozygosity, unbiased expected heterozygosity and fixation index were 0.656, 0.516, 0.464, 0.477 and -0.075, respectively. The gene flow (Nm) for all the population was estimated to be 7.65. The pairwise Fst was low and within the range of 0.0004(between Balami and WAD) to 0.0520 (between Balami and Yankasa). Variation within and between the populations of sheep shows that a large proportion of the observed variance (98% at $p < 0.01$) occurred within the breeds while only 2 % of the variance ($p < 0.01$) was contributed due to differences among the breeds. The nearest genetic distance was between the Balami and WAD (0.001) and between Ouda and the WAD (0.001). Phylogenetic analysis revealed three clusters. The present study showed that effort should be made to prevent the wearing away of the genetic make-up of the sheep populations considering the negative fixation index.

Keywords: Cluster analysis; fixation index; RLFP; PCR; sheep; indigenous

Introduction

Milk and milk products from ruminants like sheep and goat could play vital roles in the agricultural sector of most countries (Selvaggi et al., 2015) particularly the developing ones if given more attention. The milk of sheep is mainly used in cheese making (Selvaggi et al., 2014a). This may not be unconnected with its peculiar composition and properties which makes it suitable for specific productive purposes (Selvaggi et al., 2014b). β-lactoglobulin, representing 60 to 65% of the total protein in milk, is the major whey protein in ruminant milk (Selvaggi et al., 2015). It was the first protein in which polymorphism was first reported and it is made up of 162 amino acids forming stable dimers in milk (Kontopidis et al., 2004). β-lactoglobulin gene is located on ovine chromosome 3 and it is one of the particular genes affecting milk traits in sheep (Selvaggi et al., 2015). The effect of the gene is felt in the mammary gland in pregnant animals and during lactation in a tissue-specific manner (Selvaggi et al., 2015). The gene is reported to be highly polymorphic in cattle; 12 polymorphic types are found in cattle and the A and B types are the most frequent. The difference in the polymorphic forms is commonly linked to differences in milk protein yield and value (Selvaggi et al., 2015).

In sheep, three alleles A, B and C have been observed, their difference brought about by one or more amino acid changes. For example, the variant A differs from the variant B only at position 20 (Tyr→ His) in the amino acid sequence (Selvaggi et al., 2015). A new and rare variant (C) was discovered by Erhardt (1989); the author opined that this type is a kind of variant A but with an exchange at location

148 of a single amino acid (Arg→Gln). However, the most common types are the A and B and these have been detected in Turkish Karacabey Merino sheep (Elmaci et al., 2007), Teleorman Black sheep of Romania (Gras et al., 2016), Harry, Noami, Sawakni and Nagdi sheep of Saudi Arabia (El-Shazly et al., 2012) and, in Kangal and Tuj sheep of Turkey (Shahin et al., 2011). A high preponderance of the AB type was reported in Bergshaf, Friesian, Polish Merino and Polish mountain sheep (Kawecka and Radko, 2011); Awassi, Daglic, Akkaramen, Karakas and Norduz sheep of Turkey (Shahin et al., 2011); Awassi sheep of Jordan (Jawasreh et al., 2019); Karagouniko and Chios sheep of Greece (Triantaphyllopoulos et al., 2017); Bulgarian dairy synthetic sheep population (Gencheva, 2019); Hungarian, Romanian, Slovakian, Croatian, Bulgarian, Serbian and Bosnian sheep (Kusza et al., 2015) and, local and improved local Awassi sheep of Palestine (Rasheydeh et al., 2020). However, Kevorkian et al. (2008) and Gencheva (2019) did not observe the presence of either the B or A genotype in Karakul sheep, Stara Zagora and Pleven Blackhead sheep of Bulgaria. A review of sheep breeds and the different polymorphic forms of β-lactoglobulin found was undertaken by Salvaggi et al. (2015). Most sheep breed of Indian origin were reported to have a preponderance of the B allele prompting Arora et al. (2010) to suggest that the B allele is possibly the most inherited type of β-lactoglobulin in Indian sheep.

Genetic diversity evaluates the total number of genetic characteristics in the genetic makeup of a species (NBII, 2011) and this diversity varies by different degrees between and within species tested at both the protein and DNA levels (Nevo, 2001). The genetic diversity of livestock species allows animal husbandry to be practiced in a wide range of environments and with differing objectives. It provides the raw material for selective breeding programmes and allows livestock populations to adapt favorably to condition changes in the environmental (Reed and Richard, 2013). Those individuals that can adapt to changing environmental conditions are more likely to survive and produce offspring bearing those alleles (Pullin, 2002). High genetic diversity is therefore essential for a specie to evolve. Species that have less genetic variation are at a greater risk as they are more exposed to unhealthy reproduction, and their offspring are less likely to deal with problems such as inbreeding (Willis et al., 2006).

A careful search of literature revealed a dearth of reported works on the genetic diversity of Nigerian sheep breeds at the *β*-lactoglobulin gene locus and this prompted the present work which aim is to evaluate genetic diversity at the *β*-lactoglobulin gene locus in four Nigerian indigenous sheep breeds.

Materials and methods

Animals

Blood samples were collected from 76 sheep belonging to four different breeds (16 from Balami, 20 from Ouda, 20 from Yankasa, and 20 from West African Dwarf). The animals were sampled at two locations in Nigeria namely; Ibadan, where the West African Dwarf sheep were sampled and the National Animal Production Research Institute (NAPRI), Shika, Zaria where the other three breeds were sampled.

DNA extraction and RFLP-polymerase chain reaction

DNA was extracted from whole blood using a ZYMOBeadTM Genomic DNA kit (ZYMO Research Corporation). *β*-Lactoglobulin genotypes were identified as described by Feligini et al. (1998) and Anton et al. (1999). In the first step, the 120bp fragment of the sheep *β*-lactoglobulin gene was amplified using forward primer 5-CAACTCAAGGTCCCTCTCCA-3 and reverse primer 5-CTTCAGCTCC TCCAGGTACA-3. PCR amplifications was performed in reaction mixtures of 25μL containing 12.5μL of 2×PCR Master Mix (ZymoBIOMICS™ PCR PreMix), 0.5μM of each primer, and 25-75ng genomic DNA. Amplification was performed in a Biologix thermal cycler (TC1000-G), programmed for an initial denaturation at 95^{o}C for 10 minutes, followed by 35 cycles each with denaturing at 93^{o}C for 15 seconds, annealing at 60^{o}C for 30 seconds, extension at 72^{o}C for 30 seconds,

and a final extension at 72ºC for 10 minutes. In the second step, the 105bp fragment of the sheep *β*-lactoglobulin gene was amplified using forward primer 5-TCAGGACCCCGGAGGTGGACAAC-3 and reverse primer 5-CCTCCAGCTGGGTCGGGTTGAAG-3. The cycling programme began with an initial denaturation step (1 minute at 94ºC) followed by 30 cycles consisting of 15 seconds at 94ºC, 1 minute at 60ºC, 10 seconds at 72ºC, and final elongation for 10 minutes at 72ºC. The same PCR reaction mixtures used in the first step was used for amplification. In both cases, PCR products (12*μ*L) were digested with 8U of *Ras*a and 10U of *Mspl* restriction enzyme in a 20*μ*L total reaction volume for 2 hours at 37ºC. The restriction fragments were directly analyzed by electrophoresis using 3% agarose gel in 1×TAE buffer, stained with ethidium bromide, and visualized under Ultra Violet (UV) light to detect amplification.

Statistical analysis

The measurement of genetic diversity including number of alleles (Na), effective number of allele (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), Shannon's information index (I), % polymorphic locus, fixation index (F), F-st statistics, Analysis of Molecular Variance (AMOVA) and Nei's unbiased genetic distance (Nei, 1978) was estimated using GenAIEx 6.2 software (Peakall and Smouse, 2012). A dendrogram based on Nei's unbiased genetic distances, using the unweighted pair group method with arithmetic mean (UPGMA), was generated to show the genetic distances of the populations or subpopulations investigated in this study using PowerMarker V.3.25 to estimate Nei's genetic distances between pairs of sheep.

Results

Genetic diversity of β-lactoglobulin gene locus across Ouda, Balami, Yankasa and West African Dwarf sheep

The various parameters of genetic differentiation at the *β*-lactoglobulin gene locus of Nigerian sheep breeds are presented in Table 1. The Ne varied from 1.600 alleles per locus in the Yankasa to 1.992 in Balami sheep. Shannon's information index varied from 0.562 in the Yankasa to 0.691 in the Balami. Generally, Yankasa sheep had lower values of Ho (0.20), He (0.375) and uHe (0.385) while Balami had higher values of Ho (0.813), He (0.498), and uHe (0.515). The entire locus was found to be polymorphic. In terms of fixation index, the lowest value (-0.631) was observed in the Balami while the highest value (0.467) was observed in the Yankasa sheep.

Table 1. Genetic differentiation at the *β*-lactoglobulin gene locus of Nigerian sheep breeds

Population	N	Na	Ne	I	Ho	He	uHe	F	%P	Nm
Balami	16	2	1.992	0.691	0.813	0.498	0.514	-0.631	100	
WAD	20	2	1.980	0.688	0.500	0.495	0.508	-0.010	100	
Yankasa	20	2	1.600	0.562	0.200	0.375	0.385	-0.467	100	
Ouda	20	2	1.956	0.682	0.550	0.489	0.501	-0.125	100	
Mean	19	2	1.882	0.656	0.516	0.464	0.477	-0.075	100	7.65
SE	1.000	0.000	0.094	0.031	0.126	0.030	0.031	0.225	0.000	

SE = standard error, Na = number of alleles, Ne = number of effective alleles, I= Shannon's information index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, %P = percentage of polymorphic locus, Nm = gene flow, WAD = West African Dwarf.

The pairwise F-statistics (Fst) values measured for the four breeds of sheep are presented in Table 2. The Fst value showed very low genetic differentiation among the breeds. It was 0.0004 between WAD and Balami, 0.052 between Balami and Yankasa and, 0.002 between Ouda and Balami. Genetic differentiation between WAD and Yankasa was 0.044, and 0.001 between WAD and Ouda. It was observed to be 0.034 between Yankasa and Ouda. The widest difference was between the Balami and

WAD, while the least was between the Balami and Yankasa sheep, closely followed by that between WAD and Yankasa and Ouda sheep.

Table 2. Pairwise Fst values presented for Nigerian sheep breeds

	Balami	WAD	Yankasa	Ouda
Balami	0.000			
WAD	0.0004	0.000		
Yankasa	0.052	0.044	0.000	
Ouda	0.002	0.001	0.034	0.000

WAD = West African Dwarf

Variation within and between populations of sheep breeds was estimated using AMOVA (Table 3). The results revealed that a large proportion (98%) of the observed variance (0.98 at $p < 0.01$) occurred within the breeds (the individual difference of animals) and only 2% of the variance (0.02 at $p < 0.01$) was contributed due to differences between the breeds.

Table 3. Summary of AMOVA showing variation within and between sheep population

Source of variation	df	SS	MS	Estimate of variance component	% variation	P-value
Among population	3	1.172	0.391	0.004	2	0.001
Among individual	72	16.144	0.224	0.000	0	
Within animal	76	19.000	0.250	0.250	98	0.001
Total	151	36.316		0.254	100	

Genetic distance and identity of the four sheep populations studied

The result of the Nei genetic distance of the four sheep breeds is shown in Table 4. The nearest genetic distance was between the Balami and WAD (0.001) and, between Ouda and WAD (0.001) while the farthest distance was between Balami and Yankasa (0.083).

Cluster analysis based on Nei's standard distance

A cluster analysis generated based on Nei's standard distance matrix is presented in Figure 1. In the first cluster, it was observed that Balami and Yankasa are more similar to each other than they are to Ouda and WAD. The Ouda is also genetically closer to Balami and Yankasa than it is to WAD as seen in the second cluster.

Table 4. Pairwise population matrix of Nei genetic identity and Nei genetic distance of Nigerian sheep breeds

	Balami	WAD	Yankasa	Ouda
Balami	**1**	0.999	0.921	0.996
WAD	0.001	**1**	0.934	0.999
Yankasa	0.083	0.068	**1**	0.951
Ouda	0.004	0.001	0.050	**1**

WAD = West African Dwarf; genetic identity above the diagonal and genetic distance below the diagonal.

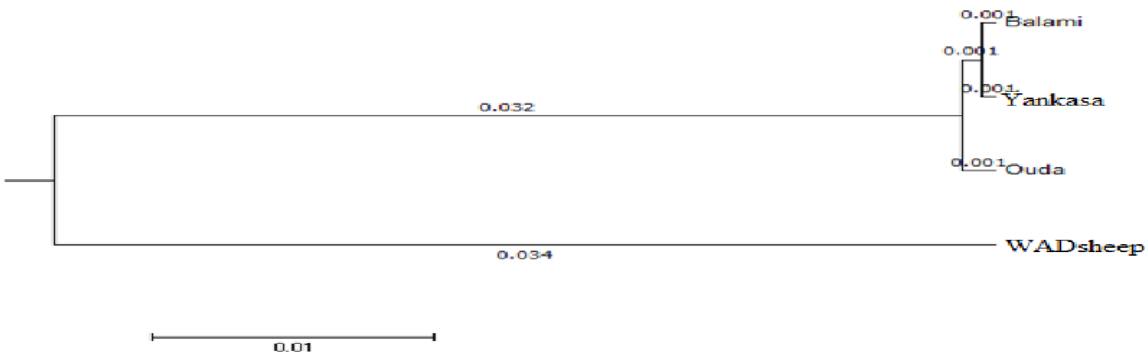

Figure 1. Dendrogram of genetic relationships at the *β*-lactoglobulin gene locus

Discussion

According to Takezaki and Nei (1996), for a genetic marker to be effective and useful in the determination of gene diversity or variation, it ought to be between 0.3-0.8. The average genetic diversity (He=0.464) for all populations of sheep in the present study confirms that restriction fragment length polymorphism is an appropriate marker for measuring genetic variation at the *β*-lactoglobulin locus. This is also supported by the 100% polymorphism recorded at the *β*-lactoglobulin gene locus. Several authors have studied polymorphism at the *β*-lactoglobulin gene locus of sheep using restriction fragment length polymorphism methods with excellent results: in Karacabey Merino sheep of Turkey (Elmaci et al., 2007); in 15 native sheep breeds of India namely Changthangi, Rampur, Bushair, Chokla, Magra, Kheri, Marwari, Sonadi, Jalauni, Muzzafarnagri, Jaissalmeri, Deccani, Mandya, Chhtanagpuri, Ganjam and Garole (Arora et al., 2010); in Turkish Awassi, Daglic, Tuj, Akkaraman, Karakas, Norduz, Guney Karaman and Kanga sheep (Shahin et al., 2011); in Polish Mountain, East Friesian, Polish Merino and Austrian Bergschaf sheep raised for milk production (Kawecka and Radko, 2011); in Noami, Sawakni, Harry and Nagdi sheep of the Kingdom of Saudi Arabia (El-Shazly et al., 2012); in Bulgarian dairy synthetic population sheep, Copper-red Shumen sheep, Stara Zagora sheep and Pleven Blackhead sheep (Gencheva, 2019); in Awassi sheep of Jordan (Jawasreh et al., 2019); and, in indigenous Awassi and improved Awassi sheep of Palestine (Rasheydeh et al., 2020). The 100% polymorphic information content is an indication of abundant genetic diversity in the sheep population.

The mean number of observed alleles per locus (2.00±0.000) observed in the study was low. This means that, even though there is diversity in the Nigerian sheep breeds, they are less genetically diverse when compared to their ancestors (Quaresma et al., 2014). Such populations with low effective number of alleles (1.882±0.094) are not only exposed to inbreeding (Willis et al., 2006), but are threatened by extinction (Leroy et al., 2013). Sheep of Indian and Tibetan origin have been reported to be high in genetic diversity (Kumarasamy et al., 2009; Sharma et al., 2016). The differences observed in the genetic diversity could be attributed to environmental factors and genetics.

Shannon information index revealed moderate genetic diversity across the populations. Shannon index value of 0.562 to 0.691 obtained for the Nigerian sheep breeds is lower than the mark of 3.5 set for high species evenness and richness (Krebs, 1999) and is an indication of low species richness and evenness. This low species richness may not be unconnected with the level of heterozygote deficiency observed among the sheep sampled. The mean Shannon's index (I) of 0.656 observed in the present study is an indication that equitability in the genetic distribution of the Nigerian indigenous sheep breeds has been largely disturbed negatively hence, the exhibited low genetic diversity. Low amount

of genetic diversity could increase susceptibility of animal populations to disease outbreaks and the appearance/fixation of detrimental alleles.

With the exception of Yankasa sheep, the observed heterozygosity was higher than the expected heterozygosity. Therefore, no departure from the expected Hardy-Weinberg equilibrium occurred in the sheep population studied. This is contrary to earlier reports in Tibetan sheep of India (Sharma et al., 2016). The authors reported lower mean observed heterozygosity value (0.473±0.044) compared to the 0.516±0.126 observed in this study. The high observed heterozygosity observed in the current study is reflective of lower level of inbreeding in the sheep population. Distance between the locations where the sheep were sampled probably played a part in the observed heterozygosity. If they were more closely located, the propensity for increased inter mating will be increased leading to a decrease in the level of heterozygosity. A lot of factors could have contributed to this excess of heterozygotes. It could well be that the locus is not under selection. 'Null alleles' may have been present leading to the true observation of excess heterozygotes. The null alleles are most likely to be segregating at the locus (Peter et al., 2007). Thus the difference between the observed and expected heterozygosity could be attributed to the non-random mating among the individuals of the population (Sharma et al., 2016). This was also reflected in the negative fixation index value (-0.075±0.225).

The low expected heterozygosity (He = 0.464±0.030) obtained at the *β*-lactoglobulin locus might be due to decreasing variability in the indigenous Nigerian sheep populations at the examined gene locus. This could be due to the population structure of the sheep sampled as the animals are kept in small sizes/herds. Higher expected heterozygosity has been reported for some sheep such as: the endangered Churra tensina (0.659) and Churra lebrijana (0.674) Spanish sheep breeds (Calvo et al., 2011), and in Balearic (0.62) sheep breeds (Pons and Landi, 2015). The higher the expected heterozygosity, the better the genetic diversity within that population. The low expected heterozygosity observed in this study imply a decline in the genetic diversity of the sheep studied. However, the mean expected heterozygosity observed (0.464) revealed a substantial amount of genetic diversity. This value is similar to values reported by Kucinskiene et al. (2005) and Arora et al. (2010) in Lithuanian and Indian sheep, respectively.

Unbiased heterozygosity according to Toro et al. (2009), is the most widely used parameter to measure genetic diversity within populations. The unbiased heterozygosity observed in this study was generally low to moderate with the estimates varying between 38.6 to 51.4% with an average of 47.7%. High unbiased heterozygosity has been reported for other indigenous sheep breeds such as the Red Maasia-Mutara and Maasia-Olmagogo sheep (Muigai et al., 2009), and the Ganjam sheep (Arora et al., 2010).

Gene flow refers to the successful transfer of alleles from one population of animals to another. This transfer varies among breeds, individuals and populations over time, and occurs at rates that is sufficient enough to play important evolutionary roles (Arnold, 2015). The main effect of gene flow (Nm) therefore, is the homogenization of allele frequencies between populations. The greater the gene flow between populations, the more the similarity between the population (El Hentati et al., 2012; Han et al., 2016). The estimated gene flow in the studied sheep populations (7.65) is low indicating or suggesting little migration or little cross breeding due to geographical distance. This may be a result of geographical distance between the sheep breeds (Sexton et al., 2014). Migration play key roles in modifying allele frequencies due to change in genetic diversity (Tallman et al., 2019). Okpeku *et al.* (2011) suggested that high gene flow could be due to poor breeding management such as uncontrolled mating. Substantial genetic differentiation due to genetic drift will be prevented if gene flow is diffusive and greater than unity (Udeh, 2015).

The presence of negative fixation index, an index of inbreeding as observed in the present study suggests it to be due to out-breeding (mating of individuals who are less related than the average relationship of the population) at such gene locus, and the presence of some heterozygotes. Negative fixation index in a population indicates that homozygous deficiencies may have risen from population

sub divisions owing to null alleles, genetic drift and selection against inbreeding. The negative fixation index observed in this study is in disagreement with the positive values observed in Gankam sheep (Arora et al., 2010).

The very low Fst range (0.0004-0.052) observed across all population of Nigerian sheep breeds indicate little genetic differentiation between the sampled populations, with WAD and Balami sheep depicting the highest gene flow (inter breeding potential) of 0.04%. The Fst values recorded suggest mobility and considerable exchange of genetic materials among the sheep breeds. This could be attributed to the fact that some of the animals originated from other regions and have been moved around for grazing purpose typical of the Fulani cattle rearers who move the animals all over the country seasonally in search of food.

The AMOVA results which revealed 98% genetic differentiation within the individuals than among the populations (2%) in the four sheep breeds support the Fst results. The high genetic differentiation observed within the sheep populations and the low genetic differentiation observed among the sheep population is similar to the reports of Qwabe (2011). The author reported 89.5% within population genetic differentiation, and 10.5% among population genetic differentiation in Namaqua Afrikaner sheep of South Africa.

The nearest genetic distance observed between the Balami and WAD and between Ouda and WAD is somewhat surprising as the sheep are so far apart geographically. Likewise, the farthest distance observed between Balami and Yankasa as they are more geographically closely located. It was expected that because the Yankasa and Balami sheep breeds are found in the arid and semi-arid region of West Africa, a higher level of gene mixing should have resulted because of inter breeding. This was however, not the case at the gene locus studied. Similar divergence from the expected was reported by Gencheva (2019) in a study of Bulgarian sheep breeds.

The cluster analysis showed the WAD to belong alone, far from the Ouda, Yankasa and Balami. This is not too surprising and might infer that to a great extent, not much interbreeding has been encouraged within the breeds possibly in an attempt to keep the genetic purity of the breeds by herders. Cluster diagram shows clearly how breeds are related or separated from each other (Wimmers et al., 2000). MacHugh et al. (1997) reported that animals cluster in a population according to their origin and geographic locations. This implies that geographically adjacent populations, are more genetically related, probably because of founder effects and interbreeding especially around bordering geographic locations. Kawecka and Radko (2011) and Gencheva (2019) obtained two distinct clusters in Bulgarian and Polish sheep breeds, respectively as against the three observed in this study. Khaldi *et al.* (2010) obtained three clusters while studying three breeds of Tunisia sheep and concluded that the differences observed between the indigenous and exotic breed studied could be because of different geographic origin, reproductive performance and morphologic appearance. The authors further opined that close relationship observed between the native breeds could be explained by a possible cross migration in time past between the breeds. Cross migration may have occurred because of short geographic distances between areas where the native breeds are distributed. Migration has a great effect on reduction of genetic differentiation between populations (Laval et al., 2000).

Conclusion

Genetic diversity study of farm animal genetic resources gives room for a breed or population to respond to selection in order to increase productivity and for better adaptability to environmental conditions which is ever changing in these seasons of climate change. The level of genetic diversity within the Nigerian indigenous sheep populations while satisfactory for now considering the negative fixation index, should be increased to avoid it wearing away. The need to retain the genetic make-up of the sheep populations in a state of purity as much as possible. This will ensure their continued survival in the various agro climes where they are presently found. Where crossbreeding becomes

unavoidable, it should be done with unrelated genetic material. The results of this study could be used as a benchmark for the four Nigerian indigenous sheep breeds.

References

Anton I. Zsolnai A. Fesus L. Kukovics S. Molnar A 1999. A survey of *β* lactoglobulin and alfa (S1) casein polymorphism in Hungarian dairy sheep breeds and crosses on DNA level. *Arch. Tierzucht. (Archives Animal Breeding).* 42: 387-392.

Arnold ML 2015. *Divergence with genetic exchange*. Oxford University Press, Oxford. 251pp.

Arora R. Bhatia S. Mishra BP. Sharma R. Pandey AK. Prakash B. Jain A 2010. Genetic polymorphism of the *β*-lactoglobulin gene in native sheep from India. *Biochemical Genetics.* 48(3-4): 304-311.

Calvo JH. Alvarez-Rodriguez J. Marcos-Carcavilla A. Serrano M. Sanz A 2011. Genetic diversity in the Churra tensina and Churra lebrijana endangered Spanish sheep breeds and relationship with other Churra group breeds and Spanish mouflon. *Small Ruminant Research.* 95: 34–39.

El Hentati H. Mohamed BH. Ali C 2012. Genetic differentiation and gene flow between the Tunisian ovine breeds Barbarine and Western thin tail using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis. *African Journal of Biotechnology.* 11(96):16291-16296.

Elmaci C. Oner Y. Balcioglu MS 2007. β-Lactoglobulin gene types in Karacabey Merino sheep breeds using PCR-RFLP. *Journal of Applied Animal Research*. 32:2: 145-148.

El-Shazly SA. Mahfouz ME. Al-Otaibi SA. Ahmed MM 2012. Genetic polymorphism in *β*-lactoglobulin gene of some sheep breeds in the Kingdom of Saudi Arabia (KSA) and its influence on milk composition. *African Journal of Biotechnology*. 11(19): 4330-4337.

Erhardt G. 1989. Evidence for a third allele at the *β*-lactoglobulin (*β*-Lg) locus of sheep milk and its occurrence in different breeds. *Animal Genetics.* 20(3): 197-204.

Feligini M. Parma P. Aleandri R. Greppi GF. Enne G. 1998. PCR-RFLP test for direct determination of *β*-lactoglobulin genotype in sheep. *Animal Genetics. 2***9**: 473.

Gras MA. Pistol G. Pelmus RS. Lazar C. Grosu H. Ghita E 2016. Relationship between gene olymorphism and milk production traits in Teleorman Black head sheep breed. *Revista MVZ Cordoba.* 21(1): 5124-5136.

Gencheva D. 2019. Single nucleotide polymorphism of the B-lactoglobulin gene in sheep breeds reared in Bulgaria. *Bulgarian Journal of Veterinary Medicine.* 1-9.

Gupta N. Gannai TAS 2007. Sheep genetic resources of India. Karanah-a finest wool breed. Monograph 50. National Bureau of Animal Genetic Resources, Karnal, India.

Han J. Yang M. Guo T. Liu J. Niu C. Yuan C. Yue Y. Yang B 2016. High gene flows promote close genetic relationship among fine-wool sheep populations (*Ovis aries*) in China. *Journal of Integrative Agriculture.* 15(4): 862-871.

Jawasreh K. Al Amareen A. Aad P 2019. Effect and interaction of β-lactoglobulin, kappa casein, and prolactin genes on milk production and composition of Awassi Sheep. *Animals* (Basel). 9(6): 382-396.

Kawecka A. Radko A 2011. Genetic polymorphism of β-lactoglobulin in sheep raised for milk production. *Journal of Applied Animal Research*. 39(1): 68-71.

Kevorkian S. Manea M. Georgescu S. Dinischiotu A. Costache M 2008. Genotyping of β-lactoglobulin gene in Karakul sheep breed. *Scientific Papers of Animal Sciences and Biotechnologies*. 41: 112– 116.

Khaldi Z. Rekik B. Haddad B. Zourgui L. Souid S 2010. Genetic characterization of three ovine breeds in Tunisia using randomly amplified polymorphic DNA markers. *Livestock Research for Rural Development. Volume 22, Article #47.* Retrieved March 16, 2020, from http://www.lrrd.org/lrrd22/3/khal22047.htm

Krebs, C 1999. *Ecological methodology*. New York. Harper Collins. 620pp.

Kontopidis G. Holt C. Sawyer L 2004. American dairy science association, invited review: *β*-Lactoglobulin: Binding properties, structure and function. *Journal of Dairy Science.* 84(4): 785-796.

Kucienskiene J. Vagonis G. Maleviciute J. Tapio I 2005. Genetic polymorphism of b-lactoglobulin in Lithuanian Blackface and Lithuanian native coarse wooled sheep. *Veterinarija IR Zootechnika*. 51(29): 90-92.

Kumarasamy P. Prema S. Ganapathi P. Karthickeyam SMK. Kanakaraj P 2009. Molecular characterization of Coimbatore breed of sheep (*Ovis aries*) in South India. *The IUP Journal of Genetics and Evolution.* 2(3): 56-65.

Kusza S. Sziszkosz N. Nagy K. Masala A. Kukovics S. András J 2015. Preliminary result of a genetic polymorphism of β-lactoglobulin gene and the phylogenetic study of ten Balkan and central European indigenous sheep breeds. *Acta Biochimica Polonica.* 62(1): 109-112.

Laval G. Iannuccelli N. Legault C. Milan D. Groenen MAM. Giuffra E. Anderson L. Nissen PE. Jorgensen CB. Beeckman P. Geldermann H. Foulley JL. Chevalet C. Ollivier L 2000. Genetic diversity of eleven European pig breeds. *Genetic selection and Evolution.* 32: 187–203.

Leroy G. Mary-Huard T. Verrier E. Danvy S. Charvolin F. Danchin-Burge C 2013. Methods to estimate effective population size using pedigree data: examples in dog, sheep, cattle and horse. *Genetics Selection Evolution* 45, 1 https://doi.org/10.1186/1297-9686-45-1

MacHugh DE. Shriver MD. Loftus RT. Cunningham P. Bradley DG 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics*. 146(3): 1071-86.

Muigai AWT. Okey AM. Kwallah AK. Mburu D. Honotte O 2009. Characterisation sheep populations of Kenya using microsatellite markers: implications for conservation and management of indigenous sheep populations. *South African Journal of Animal Science.* 39(1): 93-96.

NBII 2011. National Biological Information Infrastructure. Introduction to genetic diversity. U.S. Geological survey. Accessed from: https://psychology.wikia.org on 16/3/2020.

Nevo E 2001. Evolution of genome-phenome diversity under environmental stress. *Proceedings of the National Academy of Sciences of the United States of America, 98(11), 6233–6240.*

Nei M 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics.* 89: 583-590.

Okpeku M. Peters SO. Ozoje MO. Adebambo OA. Agaviezor BO. O'Neill MJ. Imumorin IG 2011. Preliminary analysis of microsatellite-based genetic diversity of goats in southern Nigeria. *Journal of Genetic Resources*. 50: 105.

Peakall R. Smouse PE 2012. GenAIEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics.* 28(19): 2537-2539.

Peter C. Broford M. Perez T. Dalamitra T. Hewitt S. Erhardt G. 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Animal Genetics.* 38(1): 37-44.

Pons AL. Landi V 2015. The biodiversity and genetic structure of Balearic sheep breeds. *Journal of Animal Breeding and Genetics.* 132: 268-276.

Pillin AS 2002. *Conservation biology* (1st ed.). Cambridge University Press. 358pp.

Qwabe SO 2011. Genetic and phenotypic characterisation of the South African Namaqua Afrikaner sheep breed. MSc thesis. University of Pretoria, South Africa.

Quaresma M. Martins AMF. Rodrigues JB. Colaco J. Payan-Carreira R 2014. Pedigree and herd characterization of a donkey breed vulnerable to extinction. *Animal.* 8: 354-359.

Rasheydeh FS. Sholi N. Al-Atiyat RM 2020. Genetic polymorphisms of milk genes (β-lactoglobulin and *κ*-casein) in indigenous Awassi and improved Awassi sheep of Palestine. *Livestock Research for Rural Development. Volume 32, Article #75.* Retrieved May 11, 2020, from http://www.lrrd.org/lrrd32/5/raeda32075.html

Reed DH. Richard F 2013. Correlation between fitness and genetic diversity c*onservation. Biology*. 17(1), 230-23.

Shahin E. Karsli T. Elmaci C. Baicioglu MS 2011. Beta-lactoglobulin gene types in Turkish fat-tailed sheep breeds. *Kafkas Univ Vet Fak Derg*. 17(6): 1031-1033.

Selvaggi M. Laudadio V. Dario C. Tufarelli V 2014a. Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. *Journal of the Science of Food and Agriculture.* 94(15): 3090-3099.

Selvaggi M. Laudadio V. Dario C. Tufarelli V 2014b. Major proteins in goat milk: an updated overview on genetic variability. *Molecular Biology Reports.* 41(2): 1035-1048.

Selvaggi M. Laudadio V. Dario C. Tufarelli V 2015. *β*-lactoglobulin gene polymorphisms in sheep and effects on milk production traits: a review. *Advances in Animal and Veterinary Sciences.* 3(9): 478-484.

Sexton JP. Hangartner SB. Hoffman AA 2014. Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution.* 68: 1-15.

Sharma R. Kumar V. Arora R. Ahlawat S. Mishra AK. Tantia MS 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. *Meta Gene.* 8: 14-20.

Tallman RF. Ferguson SH. Harris LN. Hedges KJ. Howland KL. Hussey NE. Marcoux M.
Matthews CJD. Martin ZA. Moore J 2019. Migration, dispersal, and gene flow of harvested aquatic species in the Canadian artic. *In:* Bozkurt Y. Biological research in aquatic science. *IntechOpen,* DOI: 10.5772/intechopen.85902

Triantaphyllopoulos, KA. Koutsouli P. Kandris A. Papachristou D. Markopoulou KE.
Mataragka A. Massouras T. Bizelis I 2017. Effect of β-lactoglobulin gene polymorphism, lactation stage and breed on milk traits in Chios and Karagouniko sheep breeds. *Annals of Animal Science.* 17(2): 371-384.

Takezaki N. Nei M 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics.* 144: 389-399.

Toro MA. Fernandez J. Caballero A 2009. Molecular characterisation of breeds and its use in conservation. *Livestock Science.* 120: 174-194.

Udeh FU. 2015. Genetic diversity of five populations of the Nigerian local breeds of goat using Random Amplified Polymorphic DNA (RAPD) markers. MSc thesis. University of Nigeria, Nsukka, Nigeria.

Willis Y. Windig J. Hiemstra SJ 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology Systematics.* 17: 438-458.

Wimmers K. Ponsuksili S. Hardge T. Valle-Zarate A. Mathur PK. Horst P 2000. Genetic distinctness of African, Asian and South American local chickens. *Animal Genetics*. 31(3): 159-165.