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Review: Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs

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Abstract. *Sheriff O, Alemayehu K. 2017. Review: Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs. Asian J Agric 1: 46-51.* Microsatellites have been widely accepted and employed as useful molecular markers for measuring genetic diversity and divergence within and among populations. The various parameters developed so far to measure genetic diversity within and among populations are observed and expected heterozygosity (Ho and He), the mean number of alleles per locus (MNA), polymorphic information content (PIC), genetic distance and phylogenetic or tree building approach. The objective of this review was therefore to quantify the genetic diversity studies of domestic sheep populations using microsatellite markers and their contribution in supporting sustainable sheep breeding programs. From the review, it is possible to see that there were high population genetic variations in all the studied sheep populations, poor levels of population differentiation and high levels of inbreeding. On the other hand, low estimates of heterozygosity and mean number of alleles and employing only few and weak markers were observed in some of the studies. The gaps observed in the previous genetic diversity studies of the sheep populations may demand further works to reveal more information on the population structures and to start appropriate and sustainable breeding programs.

Keywords: Genetic diversity, microsatellites, sheep, sustainable breeding

Abbreviations: DA: Cavalli-Sforza genetic distance, DS: Nei's standard genetic distance, FAO: Food and agricultural organization of the united nations, F*IS*: Level of inbreeding, F*ST:* Genetic differentiation between subpopulations, He: Expected heterozygosity, Ho: Observed heterozygosity, HWE: Hardy-Weinberg equilibrium, MNA: Mean number of alleles per locus, PIC: Polymorphic information content

INTRODUCTION

Domestic farm animals are crucial for food and agriculture, providing 30 to 40 percent of the agricultural sector's global economic value (FAO 2000). Despite their invaluable contribution to the global economy, there is a rapid loss of genetic resources in farm animals and the world loses two breeds of its valuable domestic diversity every week (FAO 2000). Hence, there should be an urgent mechanism to maintain and document the diversity of livestock genetic resources and design appropriate strategies for conservation and sustainable use, particularly in developing countries (Hanotte and Jialin 2005).

Maintenance of livestock genetic diversity is a key to the long-term survival of most species and should be done based on comprehensive information regarding the structure of the populations, including sources of genetic variability within and among populations. It also requires adequate implementation of conservation priorities and sustainable management programs (Mahmoudi et al. 2011) to be widely used to categorize livestock species in the world (Cardellino and Boyazoglu 2009).

Genetic diversity (the variation of alleles and genotypes present in a population) provides a basis for adaptive and evolutionary processes (Frankham et al. 2002). The current pool of diversity in livestock has been created by the forces of both natural and artificial selection (Groeneveld et al. 2010). These forces encompass processes such as mutations, adaptations, segregation, selective breeding, and genetic drift (Groeneveld et al. 2010). Future generations of domesticated species are wholly dependent on genetic variation which will be observed from genetic differences between breeds, between populations within a breed and between individuals within a population (Groeneveld et al. 2010).

Globally, sheep have the highest number of recorded breeds, contributing 25% to the total mammalian breeds adapted to a broad range of environments (Gizaw et al. 2008). The adaptation of different breeds to a broad range of agroecology provides the necessary variability that offers opportunities to meet the increased future demands for food and provide flexibility to respond to changing markets and needs (Wollny 2003). To date, more than 1078.2 million sheep populations are kept in different parts of the world with the following share in million: Asia (452.3), Africa (287.6), Northern America (6.9), Central

America (8.1), Caribbean (3.1), South America (73.1), Europe (133.9) and Oceania (113.1) (Mahmoud, 2010).

Microsatellites have been widely accepted as useful tools for measuring genetic diversity and divergence within and among populations (FAO 2011). So far, several genetic diversity studies on sheep have been conducted using microsatellite markers (Adamov et al. 2011). Their abundance, high level of repeat-number polymorphism, manifested as the occurrence of many alleles per locus, and co-dominant inheritance has facilitated their extensive use in genome mapping, phylogenetic inference, and population genetics in farm animals (FAO 2011). However, most of the genetic diversity studies of sheep using microsatellite markers, conducted so far, may not be as supportive as expected in revealing the required information for designing appropriate and sustainable sheep breeding programs and conservation strategies. Therefore, the objective of this review was to quantify the genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs.

GENETIC DIVERSITY AND WITHIN POPULATION VARIATION

Some of the parameters which can help to study genetic diversity within a population are the mean number of alleles per locus (MNA), the average expected and observed heterozygosity values (Halima et al. 2012b). Additionally, testing for deviations from the Hardy-Weinberg equilibrium (HWE) per population gives insight about those primary forces viz., natural selection, mutation, genetic drift, nonrandom mating, and genetic migration that derive evolutionary change (Ojango et al. 2011). On the other hand, the precision of estimated genetic diversity is a function of the number of loci analyzed, the heterozygosity of these loci and the number of animals sampled in each population (Barker 1994).

ESTIMATION OF MEAN NUMBER OF ALLELES (MNA)

The mean number of alleles is a good indicator of the genetic polymorphism within the population (Halima et al. 2012b) and it depends on sample size of the population because of the potential presence of unique alleles in a population that may occur at low frequencies (Sithembile 2011). The number of detected alleles may increase with an increase in population size. A high number of alleles imply more genetic variation (Nei 1987). Mean number of alleles that indicate the genetic polymorphism within the studied microsatellites were reported for several sheep populations (Table 1).

The mean number of alleles (MNAs) (Table 1) showed relatively lower estimates for some Ethiopian, Chinese, and South African sheep populations. For the other sheep populations, relatively encouraging estimates of MNA were reported. A high number of alleles imply more genetic variation (Nei 1987), and it is the key relevance in conservation programs. However, though those reports explain the existence of high polymorphism, the average number of alleles depends on sample size; number of observed alleles tends to increase with increasing population size (Aljumaah et al. 2012). Therefore, it is important to compare sample population sizes that are close to equal (Sithembile 2011). However, some of the studies used not only very small number of animals which is quite far from FAO recommendation for microsatellite marker analysis (FAO 2011), e.g., Hirbo et al. 2006 used only 9 animals to represent a population, but also, they used unequal sample size. This may lead to biasedness in estimating genetic parameters such as HWE and MNA, additionally, there was not any technique indicated in the papers which were employed to handle such a limitation.

It was observed that most of the sheep genetic diversity studies (Table 1) were undertaken by using few numbers of microsatellite markers. All 30 microsatellites, the maximum coverage recommended by FAO (2011), were covered only for Merino derived and Albanian sheep breeds (Ceccobelli et al. 2009; Hoda and Marsan 2012). Genetic diversity studies with a greater number of microsatellite markers, not only reveal more information on the population structures but also offer more opportunities to compare with results from previous studies undertaken with various subsets of the markers (FAO 2011).

ESTIMATION OF OBSERVED (*H*O) AND EXPECTED (*H*E) HETEROZYGOSITIES

Observed heterozygosity, the proportion of heterozygotes observed in a population, and expected heterozygosity, the percentage of loci heterozygous per individual or the number of individuals heterozygous per locus (Ojango et al. 2011) are the most widely used parameters to measure genetic diversity in a population (Toro et al. 2009). Literature suggests that heterozygosity estimates having greater than 0.5 heterozygosity values are believed to be appropriate for genetic diversity studies (Davila et al. 2009; Dorji et al. 2012). However, the heterozygosity estimates observed in some Indian, South African, Ethiopian, Chinese, Chilean, Kenyan and Nigerian sheep populations (Table 1) were below 0.5 or closer to the margin. These low heterozygosity estimates might be due to maintaining microsatellite loci that had registered values below 0.5 in the respective breeds during the analysis. On the other hand, very low heterozygosity estimates maybe because of the effect of small population size, high selection pressure in closed population, inbreeding and minimal or null immigration of new genetic materials into the population (Canon et al. 2006). The heterozygosity (both observed and expected) estimates in the remaining sheep populations are relatively high, concluding that the studied sheep populations have high amount of within-population genetic diversity.

Table 1. Estimated heterozygosity, mean number of alleles, polymorphic information content and level of inbreeding

Breed	**Country of origin**	***He***	***Ho***	**MNA**	**PIC per locus**	***F*IS**	**MS (No.)**	**Author**
Vembur sheep	India	0.73	0.52	5.88	0.69	0.29	25	Pramod et al. (2011)
Kail sheep	India	0.72	0.77	5.27	0.60	0.053	11	Ahmed et al. (2014)
sheep breeds (7)	South Africa	0.63	0.45	5-16	0.95	NA	24	Buduram (2004)
Turkish breeds (4)	Turkey	0.87	0.66	7.04	NA	0.07	17	Yilmaz et al. (2015)
Turkish native and cross sheep (11)	Turkey	0.75	0.72	5.8-11.8	NA	0.09-0.16	15	Evren (2004)
traditional sheep populations (14)	Ethiopia	0.66-0.75	NA	6.79	NA	*F*ST (0.046)	17	Gizaw (2008)
sheep breeds (3)	Ethiopia	0.50	0.33	3-23	0.69	0.236	22	Nigussie (2015)
Italian merino derived sheep (3)	Italy	0.64-0.75	0.61-0.70	5.17-8.43	NA	0.048-0.118	30	Ceccobelli et al. (2009)
Pelt sheep (3)	Iran	0.83	0.99	7.6	0.81	-0.19	15	Hatami et al. (2014)
Local Sheep (8)	China	0.54	0.59	3.8-5.4	0.49	0.404	10	Zeng et al. (2010)
Albanian Sheep (3)	Albania	0.75	0.72	8.54	0.72	0.041	31	Hoda and Marsan (2012)
Chilean sheep (4)	Chile	0.82	0.696	9-25	0.55-0.90	0.040	9	De la Barra et al. (2010)
Sheep populations (15)	Kenya	0.72	0.65	7.70	NA	0.109	15	Mukhongo et al. (2014)
Nigerian Indigenous Sheep (4)	Nigeria	0.78	0.49	8.64	0.85	0.34	15	Brilliant et al. (2012)
Sheep breeds (3)	Saudi Arabia	0.59-0.82	0.65-0.989	11.47	0.75	0.031	17	Mahmoud et al. (2017)
Trans-caucasian, Asian, European and African sheep breeds (22)	*	0.62-0.81	0.60-0.77	6.71-9.36	NA	*F*ST (0.06-0.10)	14	Hirbo et al. (2006)
Karakul sheep	Iran	0.831	0.989	8.07	0.81	-0.197	15	Nanekarani et al. (2011)
Romanian sheep breeds (4)	Romania	0.740	0.640	9.275	NA	NA	11	Emil and Marieta 2012)
NamaquaAfrikaner (3)	South Africa	0.50	0.49	3.9	0.44	0.019	22	Sithembile (2011)
Sheep breeds (10)	**	0.74	0.59	5.4-6.0	NA	0.060	10	Farid et al. (2000)

Note: MS = Microsatellite; * Azerbajan (5), Armenia (3), Georgia (2), Uzbekistan (1), Pakistan (2), Syria (1), China (1), India (1), Portugal (2), Barbados (1), UK (2) and Senegal (1); ** Canada, Iceland, USA, Denmark, UK and Kenya

Most of the observed heterozygosity values are generally closer to, but lower than, the expected heterozygosity in most of the breeds and loci indicating no overall loss in heterozygosity (allele fixation) (Araujo et al. 2006) and the populations are at Hardy-Weinberg equilibrium (HWE).

ESTIMATION OF POLYMORPHIC INFORMATION CONTENT (PIC)

Polymorphic information content (PIC) depicts the suitability of the markers and their primers used in the study for analyzing the genetic variability of a population. A marker with PIC>0.5 can be considered as highly informative and highly polymorphic, whereas 0.5>PIC>0.25 is recognized as reasonably informative and below 0.25 is measured as slightly informative (Marshall et al. 1998). In line with this, highly polymorphic markers were employed for most of the sheep populations studied (Table 1) except the local sheep breeds in China PIC=0.492. In fact, PIC is determined by heterozygosity and number of alleles (Aljumaah et al. 2012) and this makes microsatellite markers the choice for genetic characterization and diversity studies.

LEVEL OF INBREEDING (*F*IS)

*F*IS is estimated for populations that show significant deviation from the HWE and are significant for significant HWE estimation (Ojango et al. 2011). A high positive *F*IS indicates a high degree of homozygosity and vice versa, while negative values indicate low level of inbreeding (Dorji et al. 2012). Taking this background information into consideration, moderate to high inbreeding levels were reported by various scholars for different sheep populations; for instance, three sheep breeds of Ethiopia (*F*IS=0.236) (Nigussie 2015), Vembur (*F*IS=0.29) (Pramod et al. 2011), Magra (*F*IS=0.159) (Arora and Bhatia 2006) and Kheri (*F*IS=0.128) (Arora and Bhatia 2006)sheep breeds of India, some Merino derived sheep breeds of Italy (*F*IS=0.048-0.118) (Ceccobelli et al. 2009), some Turkish sheep breeds (*F*IS=0.09-0.16) (Evren 2004) eight local sheep breeds of China (*F*IS=0.404) (Zeng et al. 2010), fifteen sheep populations of Kenya (*F*IS=0.109) (Mukhongo et al. 2014) and Nigerian indigenous sheep (*F*IS=0.34) (Brilliant et al. 2012). This might be because of the small sheep population size, closed breeding system and very limited number of breeding rams used for many consecutive years. The lowest heterozygosity and MNA estimates indicated in table 1 above strengthen this justification.

However, tolerable mean values of *F*IS (0.087) for Ganjam (Arora et al. 2010), (0.0525) for Kail (Ahmed et al. 2014) and (0.0786) for Tamil Nadu (Kavitha et al. 2010) sheep breeds of India and *F*IS (0.07) for Turkish breeds (Yilmaz et al. 2015) were reported by scholars. These moderate levels of inbreeding may be a result of moderate levels of mating between closely related individuals under field conditions and maybe the uncontrolled and unplanned mating that caused high levels of inbreeding (Mekuriaw et al. 2016). On the contrary, *F*IS (-0.19) (Hatami et al. 2014) and *F*IS (-0.197) (Nanekarani et al. 2011) depict low levels of inbreeding and an excess of heterozygotes was reported for three Iranian sheep breeds and Karakul sheep breed of Iran, respectively.

GENETIC DISTANCE AND VARIATION AMONG POPULATIONS

Kalinowski (2004) had suggested that the highest genetic distance (*F*ST) be higher than 0.25, moderate to be between 0.05 and 0.25 and the lowest estimate below 0.05. In relative to many reports, the genetic distance among most of the populations obtained by many of the scholars (Farid et al. 2000 (*F*ST=0.163); Evren 2004 (*F*ST=0.002-0.146); Hirbo et al. 2006 (*F*ST=-0.001-0.183); Sithembile 2011 (*F*ST=0.105); Brilliant et al. 2012 (*F*ST=0.088); Hoda and Marsan 2012 (*F*ST=0.011); Hatami et al. 2014 (*F*ST=0.018); Mukhongo et al. 2014 (*F*ST=0.101) and Mahmoud et al. 2017 (*F*ST=0.042)) is almost negligible (<0.05) and/or moderate (0.05<*F*ST<0.25) values. Some of the authors revealed significant genetic distance estimates among populations. This implies that there is relatively low to moderate genetic sub-differentiation among the sheep populations. A fixation index (*F*ST) of about 0.15 is an indication of significant differentiation among populations (Frankham et al. 2002).

The average *F*ST value overall microsatellite loci in the sheep populations in Ethiopia was reported to be 0.046, indicating a 4.6% of total genetic variation among populations and a 95.4% difference among individuals (Gizaw 2008). The same author reported that lack of differentiation in those phenotypically different sub-populations could be due to gene flow between the areas having close geographical distance and similar ecology. Similarly, Nigussie (2015) noted that 3% of the total variation occurred due to population subdivision, while 97% of the variation existed among individuals within the sheep populations, which might be due to migration of individuals from one sub-population to the other (Nigussie 2015). Hailu et al. (2008) and Halima et al. (2012b) also confirmed that the low genetic differentiation between sub-populations might be due to traditional uncontrolled mating practices and policies that facilitated or led to uncontrolled movement of animals through various market routes and agricultural extension systems in Ethiopia.

IDENTIFIED GAPS, THEIR IMPLICATIONS AND FUTURE PROSPECTS

One of the gaps, identified so far, is related to the expected and observed heterozygosity estimates and microsatellite loci. It is generally suggested that microsatellite loci showing *H*e and *H*o estimates of less than 0.5 were not appropriate for heterozygosity evaluation. However, microsatellite loci with

heterozygosity estimate less than 0.5 or close to that were used in some of the studies (Table 1).

Similarly, though FAO (2011) recommended the genetic diversity studies of livestock using all the 30 microsatellite markers, most of the sheep genetic diversity studies were undertaken by using a subset of the markers. For example, De la Barra et al. (2010) used only 9 microsatellites to study four Chilean sheep breeds and Farid et al. (2000) used only 10 microsatellites to study ten sheep populations in Canada, Iceland, USA, Denmark, UK, and Kenya. Hence, studying a greater number of microsatellite markers to reveal more information on the population structure is suggested in future sheep genetic diversity studies. If less than 30 microsatellites are to be used, it is important to be keen in selecting microsatellites to bring an appropriate recommendation that can support sustainable breeding strategies.

The mean number of alleles (MNA) in sheep genetic diversity studies in Ethiopia, China, and South Africa (Zeng et al. 2010; Sithembile 2011; Nigussie 2015) were below the recommended value, the microsatellite loci for genetic diversity studies should have more than four alleles (FAO 2011). This indicated that some of the microsatellite loci were not sufficiently polymorphic and were not appropriate for genetic diversity analysis.

Some of the diversity studies used not only very small number of sheep which is by far lower than the recommendation of FAO for microsatellite marker analysis (FAO 2011), e.g., Hirbo et al. 2006 used only 9 animals to represent a population, but also, they used unequal sample size. This may lead to biasedness in estimating genetic parameters such as the MNA, there was not any technique indicated in the papers which were employed to handle such a limitation.

All these gaps point out that the microsatellites which were not sufficiently polymorphic could be dropped out and it is very important to be ardent in selecting them to bring the right recommendation that can support appropriate and sustainable sheep breeding programs.

CONCLUDING REMARK

The results from this review indicated that the within population genetic diversity, in all sheep populations, is extremely higher than between population variation which might be due to the uncontrolled and random mating practiced among the breeding flocks having close geographical distance and similar ecology. There was also poor level of population differentiations, high levels of inbreeding, low estimates of heterozygosity and MNA and markers which were not sufficiently polymorphic in most of the studies. All these results demand further works to reveal more information on the sheep population structures and help to start sustainable breeding programs and policies involving the decision on pure or crossbreeding. Moreover, appropriate conservation activities on breeding farms must be taken to avoid losses of genetic diversity and thereby to support the breeding programs. It is also suggested to set up an improvement scheme for the frequent exchange of rams among farms or flocks rearing the same breed, aimed to increase genetic diversity.

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