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Genetic diversity and maternal origins of indigenous sheep populations in north Ethiopia

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ABSTRACT

This study investigated maternal origin and haplotype variants in four sheep populations in the northern Ethiopian Tigray and Afar regions: Abergelle, Elle, Begait, and Tigray Highland. The research involved amplifying a 1088-bp mtDNA control region and sequencing 24 samples and examined haplotype diversity and maternal origins. The maternal origins were determined by using 517 bp of the mtDNA d-loop region, which was matched to globally defined reference sequences. The AMOVA analysis reveals 29.78 % variation in four populations with 13 mutational sites dividing haplogroups A and B. The presence of diverse maternal origins in those indigenous sheep populations is very crucial for future conservation and breeding efforts. To properly grasp their genetic potential, it will therefore be necessary to decipher the entire genome landscape of the indigenous sheep resources in Tigray regional state, the north Ethiopia.

1. Introduction

With 42.9 million sheep and 52.5 million goats, Ethiopia has one of the world's largest ruminant populations, accounting for around 10 % of Africa's total population and 4 % of the global population (CSA, 2021; FAOSTAT, 2019). Sheep play important roles for smallholder farmers in Ethiopia. They are used to support the livelihoods, contribute to asset, social, cultural, and environmental values, ensure food security, and provide income for farmers (Edea et al., 2017). Formulation of appropriate strategies for long-term maintenance and use of the genetic variation within livestock species requires characterization of animal genetic resources to identify the variation and appropriate germplasm that are optimal for each system. However, the effort towards identification and characterization of the sheep genetic resources of north Ethiopia is not sufficient. Except few studies on phenotypic characterization of the four indigenous sheep populations (Abergelle, Tigray Highland, Elle and Begait) mainly found in Tigray Regional State of north Ethiopia (Solomon et al., 2011; Mulata et al., 2014) there is no detailed study at genetic and molecular levels. Therefore, this study was aimed to investigate the haplotype diversity and maternal origins of the four indigenous sheep populations in Tigray Regional State of north Ethiopia.

2. Materials and methods

2.1. Description of sampling sites and study sheep populations

Samples were collected from Tigray and Afar regions in Ethiopia, with the ecology ranging from 1204.5 masl to 1076 masl (Solomon et al., 2011). The study sheep populations, Begait, Tigray Highland, Abergelle, and Elle, exhibit varying phenotypic appearances as shown in (Fig. 1).

2.2. Sampling strategy and DNA extraction

Nasal swab samples were collected using Performagene Livestock's nasal swab DNA collection kit (DNA Genotek, Kanata, ON, Canada) from a total of 48 indigenous sheep populations that include Abergelle (n=10), Tigray Highland (n=24), Elle (n=5) and Begait (n=9) in Tigray and Afar Regional States in Ethiopia. As shown in (Figure S2) 24 out of 48 sheep individuals were successfully sequenced (accession number:

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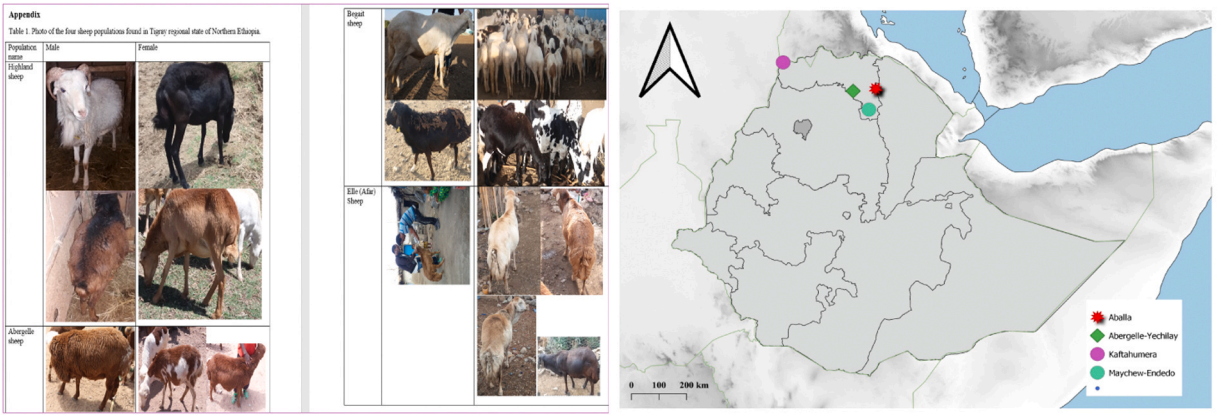

Fig. 1. The study depicts sheep populations in Ethiopia, including regions: Tigray and Afar, and districts: Kaftahumera, Abergelle-Yechilay, Mychew-Endedo, and Aballa.

Table 1
Haplotype diversity (Hd) and nucleotide diversity (π) of the studied populations.

Population	N	S	h	Hd±SD	π (Pi)±SD	K
Tigray highland	12	74	22	0.99±0.014	0.01884 ±0.00352	12.319
Abergelle	5	32	9	0.978 ±0.054	0.01402 ±0.00274	10.222
Begait	4	42	9	1±0.052	0.01890 ±0.00327	13.778
Elle	3	23	5	1±0.126	0.01481 ±0.00316	10.800
Total	24	137	45	0.997 ±0.005	0.02009 ±0.00364	14.566

Key: N = number of animals per population; S = segregating site; h = haplotype; Hd = haplotype diversity; π = nucleotide diversity; SD = standard deviation; K = average number of nucleotide differences.

50PP238422-PP238445). Genomic DNA was extracted from 0.5 mL of PerformageneTM sample at Holeta National Biotechnology Research Centre in Ethiopia, and the mtDNA d-loop region was sequenced using Sanger sequencing at the Department of Animal Breeding and Genetics Laboratory (SLU, Uppsala, Sweden).

2.3. Polymerase chain reaction (PCR) and purification

This study used Liu et al.'s (2016) primers for PCR, performed at the Department of Animal Breeding and Genetics Laboratory in Sweden. The PCR was performed in a 9700-thermal cycler, with initial denaturation, 35 cycles, annealing, and extension. The PCR amplification products were stored at 4°C. The study used a 9700-thermal cycler for cycle sequencing, using a mix of BigDye® Direct Sequencing Master Mi and BigDye® Direct M13 Fwd primer. The PCR conditions were 37°C, 82°C, 96°C, 50°C, and 60°C. The purified products were used for Sanger sequencing.

2.4. Data analysis

2.4.1. Population genetic diversity

The study used CodonCode aligner software to generate consensus sequences for forward and reverse primers (Richterich, 2017), while MEGA6 was used for multiple alignments (Tamura et al., 2013). Reference sequences from identified *Ovis aries* were used to define maternal origins in Ethiopian indigenous sheep populations (Table S1). DnaSP v6 was used for haplotype generation, segregating sites (S), haplotype diversity (Hd) and nucleotide diversity (π) (Rozas et al., 2017). The Arlequin ver 3.5.1.2 package was used to assess AMOVA and calculate pair-wise genetic distances among populations (Excoffier and Lischer, 2010).

Table 2
Results of AMOVA based on the analysis of the 517pb of the mtDNA d-loop in four north Ethiopian sheep populations.

Source of variations	df	Sum of squares	Variance components	Percentage of variation
Among populations	3	77.228	2.23377 Va	29.78
Within populations	40	210.658	5.26646 Vb	70.22
Total	43	287.886	7.50023	

2.4.2. Phylogenetic network analysis and population dynamics

The study used median-joining networks and neighbor-joining phylogenetic trees to investigate genetic relationships between populations, and mismatch distribution to infer historical population dynamics and expansion (Bandelt et al., 1999; Excoffier and Lischer, 2010; Tamura et al., 2013).

3. Results and discussion

3.1. Mitochondrial DNA genetic diversity and differentiation

The mtDNA genetic diversity of the four north Ethiopian sheep populations is presented in Table 1. AMOVA analysis incorporating the four populations assuming no hierarchical clusters showed that 29.78 % of the variation was due to genetic differences among populations, while 70.22 % of the total genetic variation present in north Ethiopian indigenous sheep occurred within populations (Table 2). Nigussie et al. (2019) also revealed that a high proportion (97 %) of the total genetic variation was explained by differences between individuals within populations of three eastern Ethiopian sheep breeds (Afar, Blackhead Somali, and Hararghe Highland). To examine the genetic differentiation between the four indigenous north Ethiopia sheep populations, we analyzed the average pairwise differences between and within populations using the estimated pairwise FST values. The FST values for Elle and Tigray Highland sheep, Elle and Abergelle sheep, and Elle and Begait sheep were 0.35697, 0.31289, and 0.29201, respectively, with p-value = 0.000 (Table S2).

3.2. Population phylogenetic relationship

A neighbor-joining tree based on 517 bp of control region mtDNA from 48 sheep samples from four selected districts of north Ethiopia and rooted with previously studied sheep sequences showed the presence of

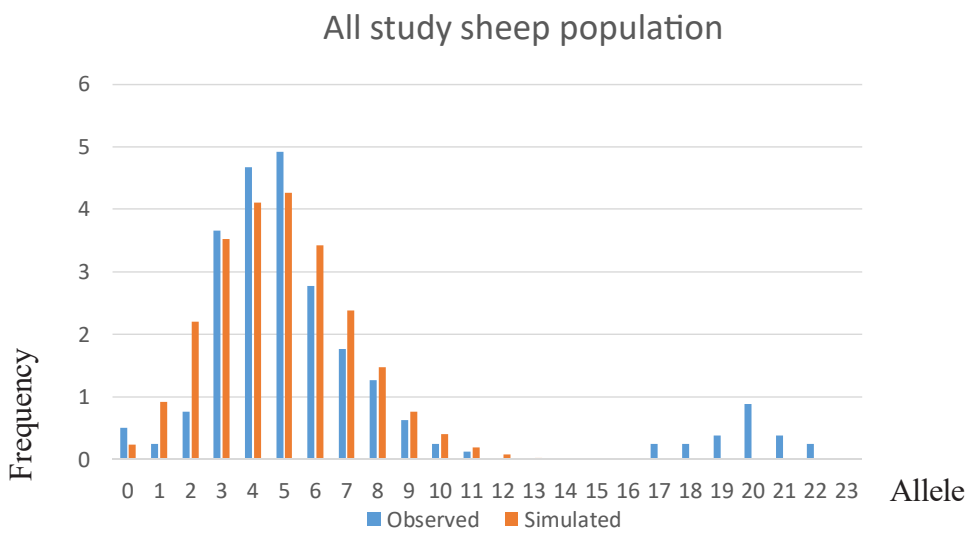

Fig. 2. Mismatch distribution pattern of all haplotypes revealed by the MJ network analysis.

two distinct haplogroups, namely, A and B, out of the five lineages reported in *Ovis aries* globally (Figure S1). As shown in Figure S1 and Figure S2, all the haplotypes except one (Tigray_HL18_ETH) were grouped under haplogroup B. In the current study, haplogroups A and B are separated by 13 mutational sites (Figure S1). The study confirms previous findings (Nigussie et al., 2019) in Ethiopia, Sudan (Gornas et al., 2011), Kenya (Resende et al., 2016), and South Africa (Ann Horsburgh and Rhines, 2010), revealing two haplogroups (B and A) in domestic sheep. In Egyptian sheep breeds, majority clustered with B haplogroup (Othman et al., 2014). African sheep share common maternal ancestry with European and Asian sheep, likely originating from the same domestication center (Muigai and Hanotte, 2013). Haplogroups C, D and E are absent in north Ethiopian sheep populations.

3.3. Population expansions

The mismatch distribution analysis of the four indigenous Ethiopian sheep populations of 517 bp mtDNA d-loop is shown in Fig. 2, and the result indicated a one-time major population expansion followed by considerable slow population expansion. The charts of the mismatch distribution for haplogroup B samples as well as the total samples of the four north Ethiopian sheep populations were multimodal (Fig. 2).

Therefore, the study found evidence of spatial and demographic growth in North Ethiopian sheep, with the first sheep likely arriving between 7500 and 7000 BP via Suez and the Sinai Peninsula (Muigai and Hanotte, 2013). Zeder (2017) also evaluated the second route, which led over Sinai before crossing the Red Sea. The fat-tailed sheep reached Africa through its northeastern region and the Horn of Africa (Muigai and Hanotte, 2013), implying that north Ethiopia may be one of the primary entrance locations for domestic sheep into the continent, notably into east Africa and Ethiopia.

4. Conclusion

The study found high mtDNA haplotype diversity in north Ethiopian sheep populations, with haplogroup B being the dominant. According to the mismatch distribution analysis, there was a onetime major and a considerable but slow sheep population expansion in Ethiopia. The presence of diverse maternal origins in those indigenous sheep populations is very crucial for future conservation and breeding efforts. Therefore, understanding the whole genome landscape of the study sheep populations may help to fully understand their genetic potential.

Author contributions

Conceptualization: MHA, AMJ and GMT; Sampling: MHA; provided guidance and supervision during sampling and laboratory analysis: AMJ and GMT; performed laboratory analysis and data management: MHA and GMT; Methodology and analyzed the data: MHA; guided data analysis and interpretation: GMT and AMJ; wrote original draft preparation: MHA and GMT; wrote, reviewed and edited the manuscript: MHA and GMT. All authors read and approved the final manuscript.

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CRediT authorship contribution statement

Anna Maria Johansson: Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **Getinet Mekuriaw Tarekegn:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Mulata Hayelom Adhena:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

We confirm that there is no conflict of interest that could have appeared to influence the work reported in this research.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.smallrumres.2024.107342.

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