ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

project terra lemnia

GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE SHEEP OF LEMNOS, LESVOS AND AGIOS EFSTRATIOS ISLANDS

terra lemnia project / Strategy 1.1 / Activity 1.1.3

August 2020

Editors:
EIRINI TARSANI **PhD, Agricultural University of Athens**
ANTONIS KOMINAKIS **Ass. Professor, Agricultural University of Athens**
IOANNIS HADJIGEORGIOU **Ass. Professor, Agricultural University of Athens**

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

project terra lemnia

GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE SHEEP OF LEMNOS, LESVOS AND AGIOS EFSTRATIOS ISLANDS

terra lemnia project / Strategy 1.1 / Activity 1.1.3

August 2020

EDITORS:

EIRINI TARSANI
PhD, Agricultural University of Athens

ANTONIS KOMINAKIS
Ass. Professor, Agricultural University of Athens

IOANNIS HADJIGEORGIOU
Ass. Professor, Agricultural University of Athens

Coordinated by:

MedINA
MEDITERRANEAN INSTITUTE FOR NATURE AND ANTHROPOS

terra lemnia project | www.terra-lemnia.net

Funded by:

MAVA 2017-2022 Mediterranean Strategy Outcome M6
Loss of biodiversity by abandonment of cultural practices

MAVA
FONDATION POUR LA NATURE

Contents

1. Framework of the research in Lemnos 2

2. Genetic diversity and population structure of the sheep of Lemnos 4

2.1. Data and genetic analysis 4

2.2. Results 5

2.2.1. Genetic diversity 5

2.2.2. Genetic differentiation (FST) and genetic distance 5

2.2.3. Population structure 6

2.3. Conclusions 11

1. Framework of the research in Lemnos

Lemnos' livestock farming is dominated by dairy sheep amounting up to 75% (10,580 LU) of the island's total LU. Goat and bovine farming follow, contributing to 12% (1,730 LU) and 10% (1,400 LU) of the island's total LU, respectively. Sheep and goats kept on the island show a remarkably high phenotypic heterogeneity (Picture 1).

Picture 1. A flock of ewes and rams on Lemnos island demonstrating high phenotypic heterogeneity.

According to local reports and historical data, an extensive crossing of the local sheep population with the Lesvos sheep took place in the 1960s, with the aim to upgrade milk performance. This crossed population was gradually expanded on the island and maintained for about thirty years when the introduction of other sheep breeds began resulting in new crossings. In a recent survey on 40 farms, performed under the "Terra Lemnia" project, the majority of farmers characterized their animals as crossings between the local and the Lesvos sheep breed. Nevertheless, also purebred animals belonging to the so-called ***local Lemnos sheep*** (Pictures 2, 3, 4) were also reported as well as crossings with the Chios (Picture 5) and/or other foreign breeds (e.g. Lacaune) (Picture 6).

Picture 2. Sheep of the so-called *local Lemnos* population ranging the rough terrain of Vigla mountain.

Numerous visits of sheep farms, performed by the AUA team (Ass. Prof. A. Kominakis and Ass. Prof. I. Hadjigeorgiou) confirmed the high phenotypic variability both within and between sheep flocks. This variability was associated with local adaptation to the individual environmental conditions under which the various flocks are maintained with most distinctive that of the Vigla mountain (Lemnos island). Driven from these observations, an exhaustive sampling and genetic analysis were undertaken aiming, first to explore the genetic differences between the Lemnos, Lesvos and Agios Efstratios islands and second, to detect population structure of the sheep of the Lemnos island.

2. Genetic diversity and population structure of the sheep of Lemnos

2.1. Data and genetic analysis

A total number of n=445 animals were genotyped with the ovine 50K SNP array (n=54,227 SNPs). The animals were dispersed in three North Aegean islands of Lemnos (n=307 ewes distributed in 18 herds), Lesvos (n=117 ewes distributed in 6 herds) and Agios Efstratios (n=21 ewes in 1 herd). Quality control (QC) was performed at the marker (SNP) level and SNPs were excluded due to: (i) call rate lower than 0.95, (ii) minor allele frequency (MAF) lower than 0.05, (iii) deviation from Hardy–Weinberg genetic equilibrium based on the Fisher exact test p-value<10^{-4} and (iv) linkage disequilibrium r^2 values greater than 0.50 (within windows of 50 SNPs and increment size of 5 SNPs). Application of QC at the marker level resulted in a total number of 39,885 autosomal SNPs retained for further genetic analyses (GA). Subsequent GA included: (i) estimation of genetic diversity levels at island level, (ii) estimation of levels of genetic differentiation between island populations and (iii) detection of population structure via Principal Components Analysis (PCA).

Picture 3. Lambs of the local sheep population at Vigla mountain.

2.2. Results

2.2.1. Genetic diversity

Average expected heterozygosity (He) of across Lemnos, Lesvos and Agios Efstratios islands was as high as 0.388 (SD: 0.11), 0.386 (SD: 0.11) and 0.374 (SD: 0.14), respectively. The respective average observed heterozygosity (Ho) was 0.376 (SD: 0.11), 0.384 (SD: 0.12) and 0.372 (SD: 0.16) for Lemnos, Lesvos and Agios Efstratios islands. As, for biallelic alleles (SNPs) such as those used here, Ho values can range from 0.2 (low diversity) to 0.5 (highest diversity) the aforementioned values are indicative of relatively high genetic diversity of sheep raised on all the three islands.

Picture 4. Ewes of the indigenous sheep population at Vigla mountain.

2.2.2. Genetic differentiation (FST) and genetic distance

Table 1 presents estimates of genetic differentiation (F_{ST}) for pairs of island populations. Note, that the value of genetic differentiation (F_{ST}) can theoretically range from 0.0 (no differentiation) to 1.0 (complete differentiation). Present F_{ST} estimates ranged from 0.009 (Lemnos - Lesvos) to 0.028 (Lesvos - Agios Efstratios) and are indicative of little genetic differentiation between the three island populations. This finding fully complies with the scenario of past inter-breeding events in form of extensive gene flow from Lesvos (Pictures 7, 8) to Lemnos and possibly from Lemnos to Agios Efstratios.

Table 1. **Pairwise genetic differentiation (F_{ST}) among the three North Aegean islands' sheep populations**

Population	***Lemnos***	***Lesvos***
Lesvos	0.009	-
Agios Efstratios	0.022	0.028

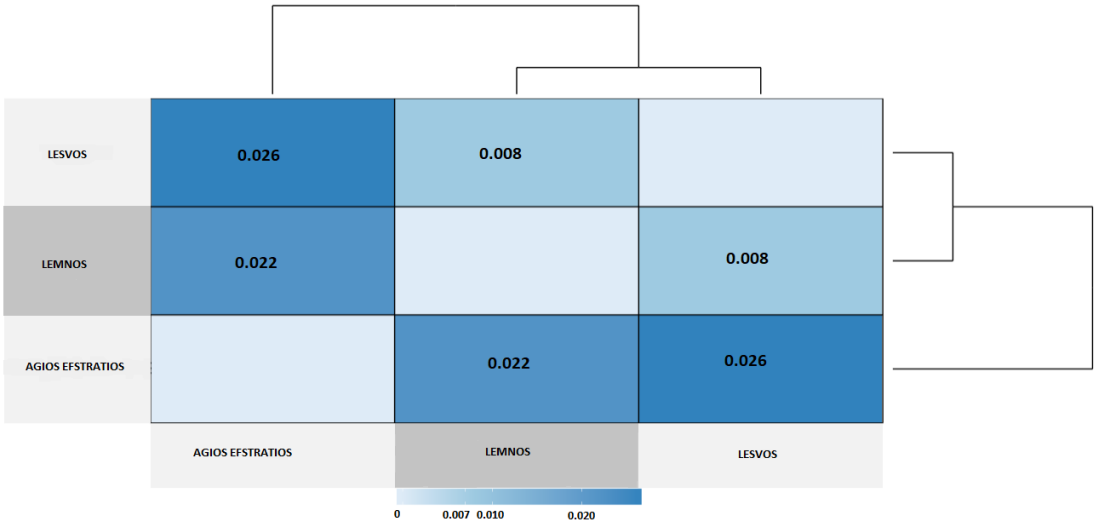
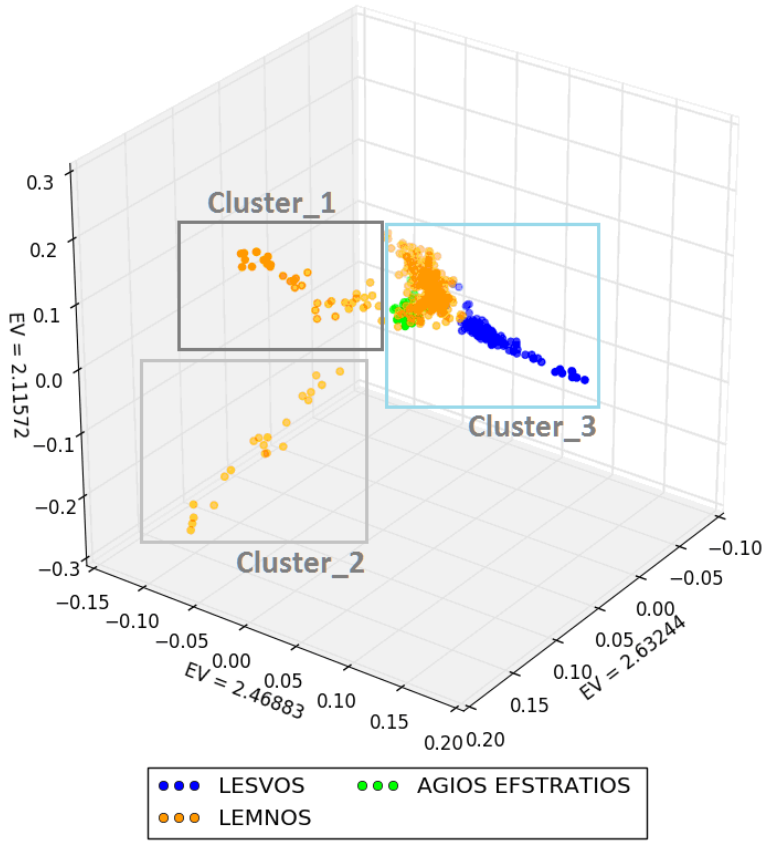


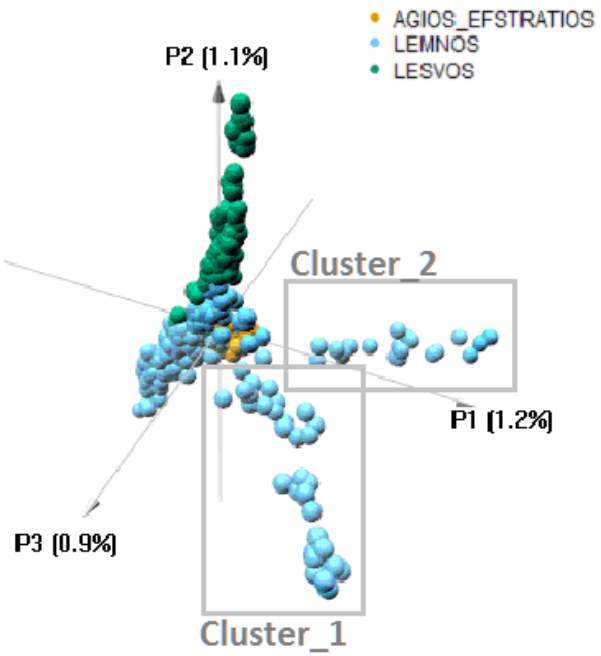
Figure 1. ***Heatmap showing Nei's genetic distances between the three islands' sheep populations***

Results of F_{ST} were also confirmed by estimates of Nei's genetic distances between the three populations (Figure 1) showing lowest genetic distances between Lemnos – Lesvos (0.008) and (relatively) higher distances between Lesvos – Agios Efstratios (0.026) and Lesvos – Lemnos (0.022) (Figure 1).

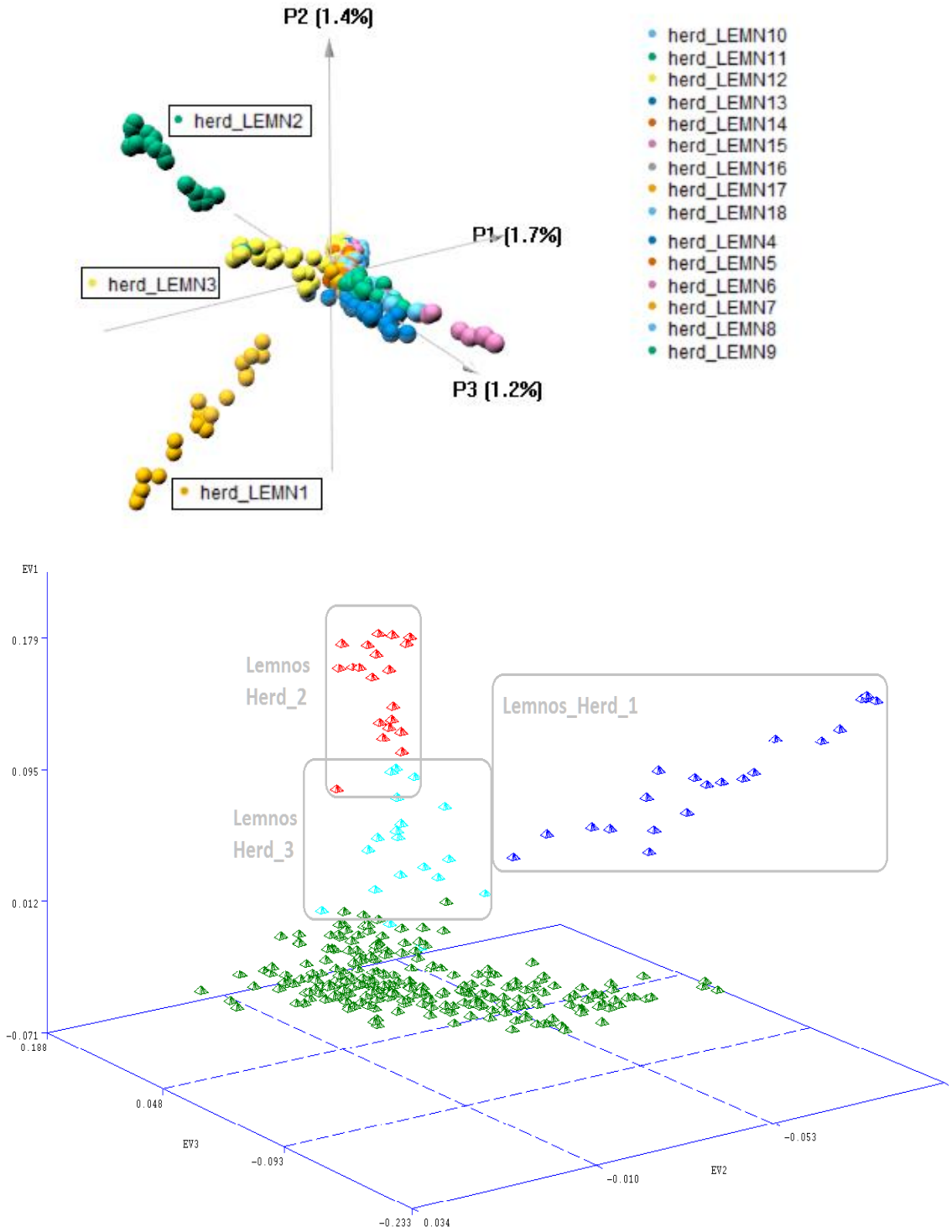
2.2.3. Population structure

Figure 2 shows results of Principal Components Analysis (PCA) in efforts to detect population structure in the three island populations. The 3D PCA plots present three distinct clusters of genotypes with two (Cluster_1 and Cluster_2) originating from the Lemnos island (Figure 2). These genotypes belong to sheep raised on three herds (coded as herd_LEMN1, herd_LEMN2, herd_LEMN3, Figure 3) that are based on the rough landscape of the Vigla mountain (Lemnos island) and are characterized by locals as the ***local Lemnos sheep***. Note that the third cluster (Cluster_3) is formed by genotypes originating from all three islands denoting a rather genetically homogenous group.





<u>Figure 2.</u> Two 3D scatterplots of the first 3 PCs of n=445 genotypes originating from three North Aegean islands (different colors denote different island origin).



<u>Figure 3.</u> 3D scatterplots of the first 3 PCs of n=307 genotypes originating from Lemnos island (different colors denote different herd).

Picture 5. Ewes and rams of a crossed population on the flat terrain of lowland Lemnos island.

Picture 6. Ewes on a semi-intensive farm on Lemnos island representing the current trend of crossing local animals with exotic breeds.

Picture 7. Ewes and lambs of Lesvos breed ranging the rough terrain of western Lesvos island.

Picture 8. Ewes of Lesvos breed ranging the rough terrain of western Lesvos island.

2.3. Conclusions

In general, the Lemnos sheep population maintains reasonably high levels of genetic diversity which should be conserved.

The low levels of genetic differentiation between the three island populations denote inter-breeding events possibly in the form of gene flow from Lesvos island.

Results of PCA revealed distinctive sheep genotypes dispersed in three flocks, raised on the highland areas of Vigla mountain (Lemnos island). These animals clearly differentiate from the rest of the animals raised either on Lemnos or Lesvos island and ***presumably belong to the local Lemnos sheep mentioned in historical reports*** and by local sheep breeders. These encouraging results could be further exploited in ***efforts to officially characterize and register them as the Lemnos sheep*** by the Greek Ministry of Rural Development and Food.