Genetic Population Structure of Sus scrofa in Lithuania before the African Swine Fever Outbreak

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Abstract

Background: Wild boar (*Sus scrofa*) is a widely distributed ungulate whose success can be attributed to a variety of ecological features. The genetic variation and population structure of wild boar population in Lithuania before the spread of African swine fever has not yet been thoroughly studied. To characterize the amount of genetic variation and population structure of wild boar in Lithuania before the African swine fever outbreak, we genotyped and analyzed microsatellite loci for a total of 96 wild boar specimens from nine locations.

Results: In the present study, individuals were genetically typed at fifteen microsatellite loci using multiplex PCR amplification. Our data showed that that 85% of the genetic variation originated from individuals, indicating a high gene exchange between the nine subpopulations of wild boar in Lithuania. Bayesian-based clustering analysis in STRUCTURE identified two inferred genetic clusters and each of the 9 subpopulations had more than 1 cluster. A factorial correspondence analysis confirmed homogeneity and no genetic differentiation between subpopulations of *S.scrofa* in Lithuania.

Conclusions: Our results reveal that wild boar subpopulations of Lithuania before the African swine fever outbreak were still not distinguished and admixed. This study highlights the potential contribution for the future study understanding the detailed structure of wild boar population in Lithuania after African swine fever outbreak.

Background

The wild boar (*Sus scrofa*) is among the most widespread large mammals, as its natural range extends from western Europe and the Mediterranean Basin to eastern Russian Federation and Japan, throughout southeast Asia [1, 2]. For this species remarkable adaptability, wild boar populations expand their geographic range and can be found in a variety of habitats and climates [3, 4]. Successful range expansion and increasing abundance of the wild boar populations is influenced by several factors like a high ecological plasticity, high reproductive capacity, their adaptability to changing food diverse [2], lack of natural predators [5] and supplementary feeding [6]. In light of these factors, the main regulatory mechanism for the rapid increase in size of the wild boar populations is wildlife management [7, 8].

Central European wild boar subspecies also abundantly distributed in Lithuania [9]. The abundance of wild boar population poses a threat to the agriculture or present a risk for livestock health. The emergence in 2014 and persistence of African Swine Fever (ASF) in Lithuania has been linked to a relatively rapid decline of the wild boar population [10, 11]. The regulation of high wild boar populations levels through hunting partially solves the threat of ASF spreading to new regions, but it is still unknown how the intensive reduction of population numbers will affect the genetic structure of wild boars in the future. Molecular techniques can be applicable as valuable tools for improving the understanding of the genetic changes in populations, population structuring, and genetic differentiation [12]. Information about the state of wild boar population structure before the spread of ASF would allow us to determine and to compare how the spread of the virus may have influenced its population genetic structure.

The main goal of this present study was to asses the genetic diversity and genetic structure of the wild boar population in Lithuania before ASF outbreak using a set of microsatellite markers.

Results

Genetic diversity analysis of wild boars in Lithuania

Using the 15 microsatellite markers, 147 alleles were observed in the 96 wild boar samples from nine districts, ranging from 103 alleles in Vilnius to 52 alleles in Alytus (Table 1). The number of alleles for each locus (N_A) ranged from 2 to 13 with average over all loci and all sample sites of 5.02 (Table 1).

Private alleles, distinctive to a specific population, were present in all subpopulations varying from a single in Alytus to a maximum of 8 in Kaunas subpopulation (Table 1). Overall observed heterozygosity values across all loci ranged from 0.567 to 0.650, the expected heterozygosity values ranged from 0.534 to 0.678 (Table 1). Significant deviation from HWE was observed in 5 out of 15 loci at $P < 0.05$ (Table 1). In five subpopulations (Utena, Vilnius, Alytus, Marijampolė, Kaunas) observed heterozygosity differed significantly from expected heterozygosity under Hardy-Weinberg equilibrium toward heterozygosity deficiency (Table 1).

Table 1
Genetic diversity at 15 microsatellite loci in 9 wild boar (*Sus scrofa*) subpopulations

	sw2494–116 bp	s010166–206 bp	sw353140–162 bp	s0386167–181 bp	s0355241–261 bp	sw7298–112 bp	Tnfb158–193 bp	s0070270–285	s002693–105 bp	s0155145–161 bp	s0005212–264 bp	sw241098–116 bp	sw830170–182 bp	sw632156–196 bp	swr1207-223
Utena (N = 18)															
N_A	8	8	5	6	2	4	8	7	4	6	12	6	5	6	9
A_P	-	1	-	1	-	-	-	-	-	1	1	-	-	-	2
H_O	0.556	0.706	0.611	0.667	0.000	0.778	0.722	0.778	0.611	0.667	0.882	0.833	0.444	0.667	0.62
H_E	0.725	0.725	0.670	0.733	0.111	0.708	0.753	0.696	0.542	0.716	0.862	0.622	0.548	0.674	0.79
P	0.014*	0.412	0.390	0.417	0.030*	0.306	0.141	0.736	0.485	0.430	0.903	0.005*	0.205	0.316	0.01
Vilnius (N = 24)															
N_A	8	7	6	5	2	5	11	7	5	5	13	9	5	8	7
A_P	-	1	-	-	-	-	3	-	-	-	1	1	-	1	-
H_O	0.625	0.542	0.708	0.667	0.000	0.500	0.917	0.714	0.417	0.417	0.909	0.708	0.542	0.696	0.81
H_E	0.746	0.560	0.753	0.719	0.172	0.671	0.823	0.732	0.597	0.687	0.870	0.726	0.523	0.771	0.70
P	0.048	0.507	0.323	0.083	0.002*	0.018*	0.083	0.238	0.002*	0.010*	0.881	0.053	0.609	0.332	0.24
Alytus (N = 4)															
N_A	3	2	3	4	2	4	4	4	3	3	4	5	3	5	3
A_P	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
H_O	0.750	0.500	0.500	0.500	0.000	0.750	1.000	0.500	0.000	0.250	1.000	1.000	0.667	0.500	1.00
H_E	0.656	0.375	0.406	0.656	0.375	0.656	0.719	0.563	0.625	0.656	0.722	0.750	0.500	0.750	0.61
P	0.680	0.859	0.856	0.141	0.147	0.820	0.457	0.427	0.027*	0.090	0.660	0.526	0.805	0.087	0.40
Marijampolė (N = 10)															
N_A	5	7	5	4	3	5	9	5	3	6	12	7	3	7	5
A_P	1	1	-	-	1	1	1	-	-	-	1	-	-	-	-
H_O	0.500	0.600	0.700	0.400	0.100	0.900	0.900	0.600	0.667	0.500	0.900	0.800	0.333	1.000	0.77
H_E	0.480	0.730	0.705	0.685	0.485	0.665	0.855	0.695	0.623	0.690	0.890	0.740	0.438	0.809	0.67
P	0.744	0.016*	0.488	0.027*	1.000	0.104	0.699	0.058	0.533	0.075	0.837	0.989	0.060	0.243	0.48
Kaunas (N = 19)															
N_A	6	7	6	6	3	5	8	10	5	6	11	7	4	7	6
A_P	-	1	-	-	1	-	-	4	1	-	-	-	-	1	-
H_O	0.556	0.556	0.706	0.611	0.056	0.611	0.889	0.647	0.722	0.333	0.941	0.667	0.333	0.765	0.75
H_E	0.633	0.648	0.732	0.738	0.245	0.715	0.827	0.704	0.640	0.599	0.848	0.563	0.557	0.708	0.80
P	0.011*	0.184	0.293	0.130	1.000	0.021*	0.219	0.041*	0.402	0.047*	0.296	0.201	0.003*	0.341	0.38
Tauragė (N = 4)															
N_A	4	3	5	4	1	4	3	4	2	2	6	3	3	5	3
A_P	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
H_O	0.750	0.750	1.000	0.750	0.000	0.500	0.750	0.750	0.250	0.250	1.000	0.500	0.500	0.750	0.00
H_E	0.656	0.531	0.781	0.656	0.000	0.563	0.531	0.656	0.219	0.219	0.781	0.406	0.656	0.688	0.66
P	0.800	0.578	0.639	0.797	-	0.408	0.567	0.808	-	-	0.571	0.858	0.255	0.912	0.07

	sw2494–116 bp	s010166–206 bp	sw353140–162 bp	s0386167–181 bp	s0355241–261 bp	sw7298–112 bp	Tnfb158–193 bp	s0070270–285	s002693–105 bp	s0155145–161 bp	s0005212–264 bp	sw241098–116 bp	sw830170–182 bp	sw632156–196 bp	swr1207-223
Klaipėda (N = 4)															
N_A	2	4	4	3	3	4	4	3	5	2	5	3	3	4	5
A_P	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-
H_O	0.500	0.500	0.750	0.250	0.250	0.750	0.750	0.750	0.750	0.500	1.000	0.750	0.750	1.000	0.50
H_E	0.375	0.563	0.688	0.594	0.594	0.719	0.719	0.531	0.688	0.375	0.781	0.531	0.531	0.688	0.75
P	0.858	0.439	0.749	0.084	0.084	0.857	0.736	0.574	0.915	0.858	0.656	0.572	0.563	0.403	0.08
Šiauliai (N = 7)															
N_A	6	6	5	6	1	4	5	4	4	4	7	5	2	7	4
A_P	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
H_O	0.714	0.714	0.429	0.714	0.000	0.857	0.857	0.667	0.571	0.286	0.714	1.000	0.571	0.714	0.83
H_E	0.745	0.796	0.745	0.786	0.000	0.704	0.735	0.597	0.459	0.653	0.786	0.704	0.408	0.704	0.69
P	0.212	0.256	0.028*	0.115	-	0.424	0.542	0.731	0.562	0.019*	0.940	0.114	0.555	0.876	0.56
Pnevėžys (N = 6)															
N_A	4	4	3	5	4	4	5	5	3	3	6	5	4	5	7
A_P	-	1	-	-	2	-	-	-	-	-	-	-	-	-	1
H_O	1.000	0.333	0.667	0.667	0.167	0.667	1.000	0.667	0.333	0.333	1.000	0.833	0.667	0.667	0.66
H_E	0.694	0.694	0.569	0.792	0.514	0.681	0.792	0.611	0.292	0.500	0.778	0.722	0.514	0.667	0.83
P	0.172	0.039*	0.604	0.132	0.013*	0.484	0.386	0.788	0.907	0.173	0.361	0.631	0.489	0.825	0.09
Total (N = 96)															
N_A	10	13	9	7	7	6	14	11	7	7	21	9	5	11	10
A_P	1	6	2	1	5	1	4	4	1	1	5	1	0	2	3
H_O	0.661	0.578	0.675	0.581	0.064	0.701	0.865	0.675	0.480	0.393	0.927	0.788	0.534	0.751	0.66
H_E	0.635	0.625	0.672	0.706	0.277	0.676	0.750	0.643	0.521	0.566	0.813	0.641	0.520	0.718	0.72
P	0.999	0.028*	0.875	0.002*	0.000*	0.955	0.212	0.974	0.007*	0.000*	0.879	0.622	0.977	0.548	0.04

N_A: number of alleles; A_P: private alleles; H_0: observed heterozygosity; H_E: expected heterozygosity under HWE; P:the probability of Hardy-Weinberg equilibrium (p* < 0.05: significant departure from Hardy-Weinberg equilibrium);

Genetic differentiation and population structure analysis

Pairwise FST and Nei's genetic distances (D_{Nei}) among subpopulations are shown in Table 2. Nei's genetic distances and FST analysis indicated a low or no genetic differentiation between all pairs of subpopulations (Table 2). All wild boar subpopulation pairs were not significantly differentiated from one another.

Table 2
Pairwise F_{ST} (above diagonal) and Nei's genetic distance D_{Nei} (below diagonal) between Lithuanian wild boar subpopulations

	1	2	3	4	5	6	7	8	9
1.Utena		0.004 NS	0.025 NS	0.014 NS	-0.006 NS	0.048 NS	0.032 NS	0.019 NS	-0.032 NS
2.Vilnius	0.084		0.007 NS	-0.011 NS	-0.007 NS	0.005 NS	-0.007 NS	0.012 NS	-0.003 NS
3.Alytus	0.194	0.135		-0.032 NS	-0.004 NS	0.069 NS	-0.016 NS	0.039 NS	0.006 NS
4.Marijampolė	0.132	0.071	0.176		-0.019 NS	0.029 NS	-0.033 NS	0.013 NS	0.023 NS
5.Kaunas	0.067	0.052	0.151	0.071		0.005 NS	-0.010 NS	0.002 NS	-0.001 NS
6.Tauragė	0.239	0.166	0.268	0.257	0.160		0.027 NS	0.046 NS	0.072 NS
7.Klaipėda	0.255	0.161	0.267	0.159	0.152	0.293		0.032 NS	0.038 NS
8.Šiauliai	0.090	0.095	0.215	0.141	0.085	0.233	0.231		0.047 NS
9.Panevėžys	0.096	0.148	0.201	0.206	0.126	0.321	0.294	0.152	
NS-non-significant population differentiation									

Additionally, three-dimensional factorial correspondence analysis (3D-FCA) was also conducted in order to determine the degree of structuring of wild boar subpopulations (Fig. 1). FCA results indicated admixture between individuals from different districts. These results suggested that intensive hunting pressure, widespread distribution, no presence of geographical barriers can influence genetic composition and population structure of the wild boar subpopulations in Lithuania.

The result of analysis of molecular variance (AMOVA) showed that 85% of the total genetic variation originated from individuals, while 15% came from differences among individuals within the populations, and 0% was observed among populations (Table 3). Statistical analysis of fixation index (F_{ST}=0.000) and analysis of molecular variance revealed no significant genetic differentiation between the wild boar subpopulations (Table 3). Other F-statistics revealed significant values for F_{IS} = 0.150 ($p < 0.001$) and F_{IT} = 0.150 ($p < 0.001$). These data indicate that higher genetic variability of *S. scrofa* is mainly distributed within individuals (Table 3).

Table 3
Analysis of molecular variance (AMOVA) of wild boar subpopulations based on various genetic groupings

Source of variation	df	SS	MS	Est. Var.	%	F-statistics	Value	P-value
Among populations	8	48.766	6.096	0.000	0%	F_{ST}	0.000	0.507
Among individuals within population	87	531.270	6.107	0.795	15%	F_{IS}	0.150	0.001
Within individuals	96	433.500	4.516	4.516	85%	F_{IT}	0.150	0.001
Total	191	1013.536		5.311	100%			

The population structure analysis showed the optimum number of subpopulations K which explained that wild boar subpopulations could be divided into two clusters (K = 2) using the Evanno method (Fig. 2). Through the graphic visualization of the population structure, there was no separation of genetic groups, and each of the 9 subpopulations had more than 1 cluster (Fig. 2). Increasing the number of structure groups beyond K = 2 did not influence changes in population structure.

Discussion

The genetic structure of wild boar population from Lithuania has not yet been thoroughly studied. The microsatellite analysis could lead to new and important inferences about the structure and differentiation of *S. scrofa* population before and after African swine fever.

Genetic diversity and variation

Most of the loci used in this work had been analyzed in previous studies with wild boar in Europe. We have detected similar genetic variation patterns in the investigated loci of wild boars as compared with other genetic studies. The analysis of microsatellite polymorphisms revealed that the level of genetic diversity obtained in Lithuanian wild boar population (H_O=0.622) was in the same range of that reported in Bulgarian wild boar (H_O=0.63) [13], Italian

populations (Ho = 0.63), Hungarian wild boar populations (Ho = 0.75) [14], in wild boar populations from Portugal (Ho = 0.627) [15] and Poland (H_0 = 0.51) [16]. A similar level of heterozygosity (Ho = 0.63) was reported in wild boar populations inhabiting East Asia [17].

Analysis of molecular variance (AMOVA) revealed high intra-population genetic variation in wild boar population in Lithuania (Table 3). A similar trend involving genetic variation that mainly distributed within population has also been reported for Bulgarian populations [13]. The high intra-population variability and genetic homogeneity are influenced by gene flow, which is impacted by distribution and connectivity of populations [18, 19].

FST analyses revealed no genetic structure among subpopulations of wild boar in Lithuania suggesting high rates of gene flow or little separation in time between populations. Conversely, the obtained higher genetic differentiation (FST = 0.0816) among Bulgarian populations occurs due to such geographical barriers as mountain ridges and human impact [13]. One possible explanation for the low FST values we observed is that the wild boar is migratory species and has a relatively large home range [16].

Population structure

In this study, we analyzed the population structure of *S.scrofa* population in Lithuania. At the individual level, FCA and STRUCTURE cluster analysis were performed. STRUCTURE analysis provided evidence for two genetic groups. The phenomen of two genetically distinct clusters can be occurred as a result of demographic history. Nikolov et al. 2009 [13] has identified two subgroups in their study of Bulgarian wild boar populations and detected that Balkan Mountain Range acts as a natural migration barrier. Our results differed from those of Ferreira et al. 2009 [15], where Portuguese wild boar formed three subpopulations (North, Centre and South) for effects of a recent genetic bottleneck. A factorial correspondence analysis confirmed homogeneity and no genetic differentiation between subpopulations of *S.scrofa*.

Conclusions

Microsatellite loci analyses revealed that wild boar subpopulations of Lithuania before the African swine fever outbreak were still not distinguished and admixed. Future studies with extensive sampling will be helpful for understanding the detailed structure of wild boar population in Lithuania after African swine fever outbreak.

Methods

Sampling

Tissue samples of wild boar were collected during a 5-year period (2009–2013) from 9 districts in Lithuania (Fig. 3). A total of 96 *S.scrofa* individuals legally harvested by the licensed hunters in different parts of Lithuania were investigated. We chose to focus on a single population of Lithuania and sample size that would be sufficient to characterize population-level genetic diversity when using microsatellites. To compare and reveal genetic diversity among major regions, we grouped the Lithuanian wild boar population into nine groups based on their regional origins: Utena = 18, Vilnius = 21, Alytus = 4, Marijampolė = 10, Kaunas = 19, Tauragė = 4, Klaipėda = 4, Šiauliai = 7, Panevėžys = 6. Fresh muscle, spleen and blood were sampled from unprotected wild boars and either stored in plastic tubes (5–30 ml) filled with 96% alcohol or kept frozen at the temperature of − 20 °C. All samples were legally collected and deposited into the State Food and Veterinary Service Republic of Lithuania (SFVS). The study did not involve collection of samples from live animals. Ethics statement was not required. Hunters collected samples in accordance with their national regulations on wild boar management.

In this research, samples were extracted with "DNeasy Blood and Tissue Kit" (Qiagen, Catalog. No. 69506) following the manufacturer's. The concentration and the purity of isolated DNA were determined with Nanodrop 2000 Spectrophotometer (Thermo Scientific, DE, USA). Samples were used immediately for amplification or stored at -20ºC for later use.

Amplification and genotyping

A set of 15 microsatellite markers were selected from the list of microsatellite markers recommended by the International Society of Animal Genetics (ISAG)-Food and Agriculture Organisation (FAO) [20]. The markers were grouped into two multiplex (SW24, S0386, S0355, SW353, SW936, SW72, S0070, S0107 and S0026, S0155, S0005, SW2410, SW830, SW632, SWR1941) reactions based on their size and annealing temperature. The PCR reactions were carried out in a total volume of 25 µL, containing 1 µL of DNA template, fluorescent forward primer (2 µM) and non-fluorescent reverse primer (2 µM), and 2x QuantiTect Multiplex PCR NoROX Master Mix (Ref. 204743, QIAGEN GmbH). PCR reactions were carried out in the following steps: 10 min an initial denaturation at 95 °C, 30 or 35 cycles at 95 °C for 30 s depending on the primer set used, annealing at an optimal temperature ranging from 57–58 °C, extension at 72 °C for 1 min, then a final extension at 72 °C for 30 min. The ABI 3100 (Applied Biosystems, USA) DNA Analyzer was used for genotyping alleles with a GeneScanTM-500 ROX size standard (Applied Biosystems). Gene Mapper 3.7 (Applied Biosystems) software was used to estimate the size of the alleles.

Statistical analysis

In order to estimate population genetic structure of wild boars in Lithuania, number of alleles per locus (N_A), the observed heterozygosity (H_O), the expected heterozygosity (H_E) under the Hardy − Weinberg assumptions were obtained in GenAlEx v6.1 [21]. Deviations from Hardy-Weinberg equilibrium (HWE) were tested with 1000 permutations across markers using Genepop v.4.0 [22]. Factorial correspondence analysis (FCA) on the microsatellite data for individual wild boars was performed using GENETIX version 4.05.2 [23]. Analysis of molecular variance (AMOVA) and F-statistics (F_{ST}, F_{IS}, and F_{IT}) were also estimated using

GenAlEx v. 6.1. To assess genetic relationships among subpopulations, we calculated pairwise Nei's genetic distances [24] between each pair of the sample sites using the software GenAlEx 6.5. We estimated FST values according to Weir and Cockerham's [25] version of Wright's F-statistic with the use of FSTAT program package [26], followed by sequential Bonferroni correction for multiple tests [27]. The genetic population structure of Lithuanian wild boars was assessed by the program STRUCTURE version 2.3.4 [28]. The probabilistic method was conducted with 200.000 replications in burn-in and 100.000 replications in the Markov Chain Monte Carlo (MCMC). Ten clustering simulations (runs) were performed for each possible value of K (K = 1 to K = 8). Then, the Structure Harvester application was used to assess the results of the probabilistic method and determine the ΔK value [29].

Abbreviations

ASF: African Swine Fever; 3D-FCA: Three-dimensional factorial correspondence analysis; AMOVA: Analysis of molecular variance; MCMC: Markov chain Monte Carlo; F_{IS}: Inbreeding coefficient; F_{ST}: Genetic differentiation between sub-populations; He: Expected heterozygosity; Ho: Observed heterozygosity; HWE: Hardy-Weinberg equilibrium; FAO: Food and Agriculture Organization of the United Nations; International Society of Animal Genetics (ISAG); NS-non-significant

Declarations

Ethics approval and consent to participate

This study did not require official or institutional ethical approval. Animals were hunted in accordance with order of the Minister of Environment of the Republic of Lithuania No. 258 (27.06.2000) concerning approval of hunting rules applicable on the territory of the Republic of Lithuania. Samples were collected as part of an infectious animal disease control program in accordance with the order of the director of the State Food and Veterinary Service of the Republic of Lithuania on the control of contagious infectious diseases in animals, 2006 March 30 No. B1-265, Vilnius, Lithuania.

Consent for publication

Not applicable

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AP, VJ and LG designed the study. AP collected samples. ŽJ, VJ and LG performed sample preparation and carried out experiments. LG, VJ and ŽJ conceived and designed the molecular genetic study and analyzed the data. LG and AP wrote the draft of the manuscript. All authors read and approved the final manuscript.

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Figures

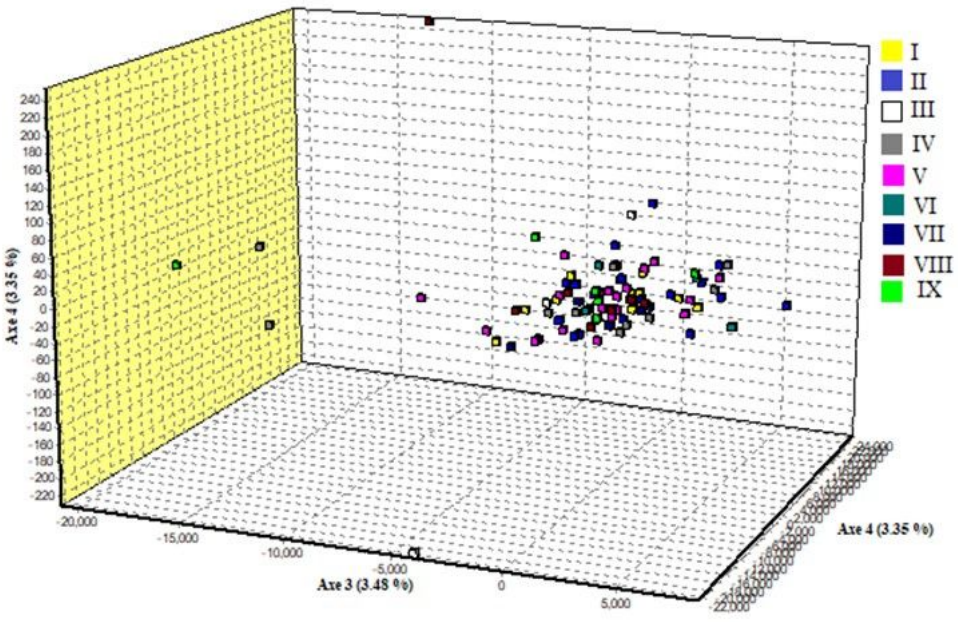

Figure 1

Three-dimensional factorial correspondence analysis (3D-FCA) showing the relationships among individuals of nine subpopulations based on fifteen microsatellite loci. The axes 1–3 explain the percentage of the variance among the individuals.

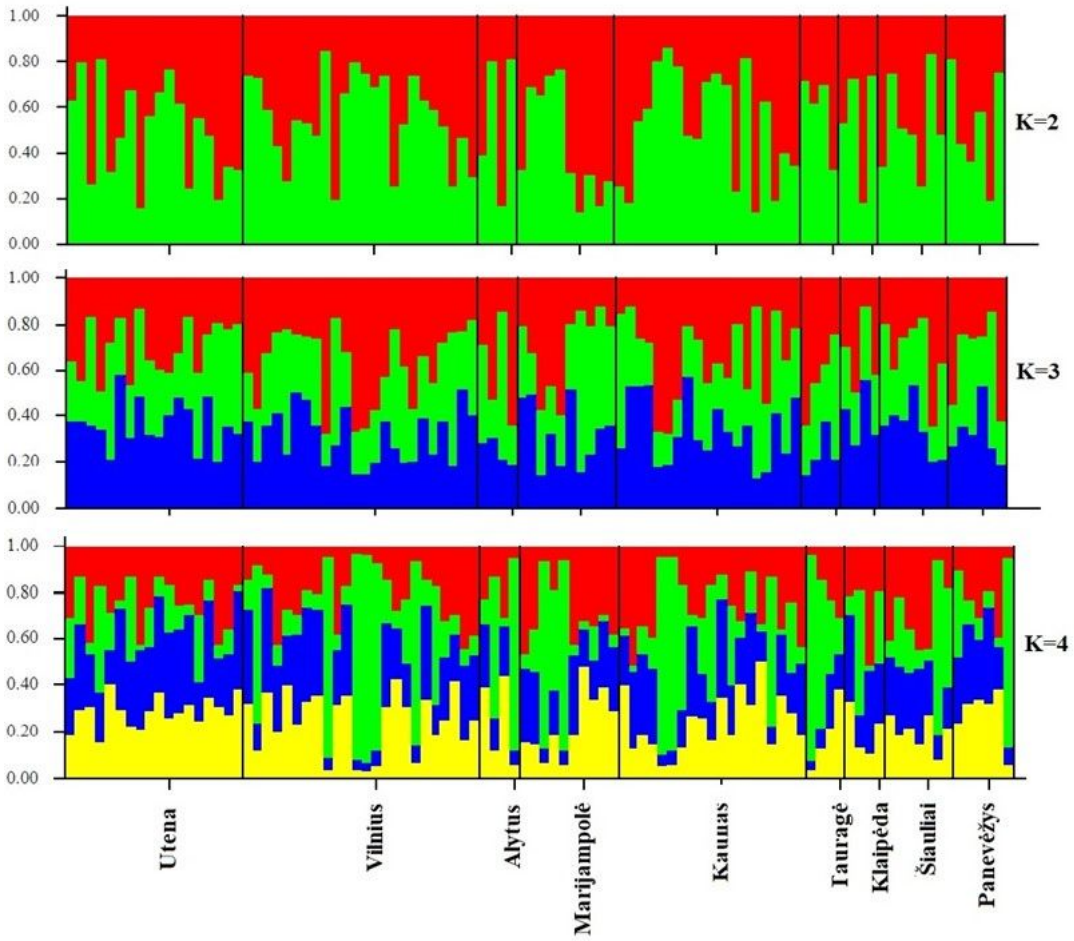

Figure 2

Cluster results from a structure analysis of 96 wild boars from 9 subpopulations and based on 15 microsatellite markers. Each individual of wild boar is represented by a single vertical bar, with colored sections indicating the likelihood of assignment to the corresponding cluster. The collection sites are separated by black lines.

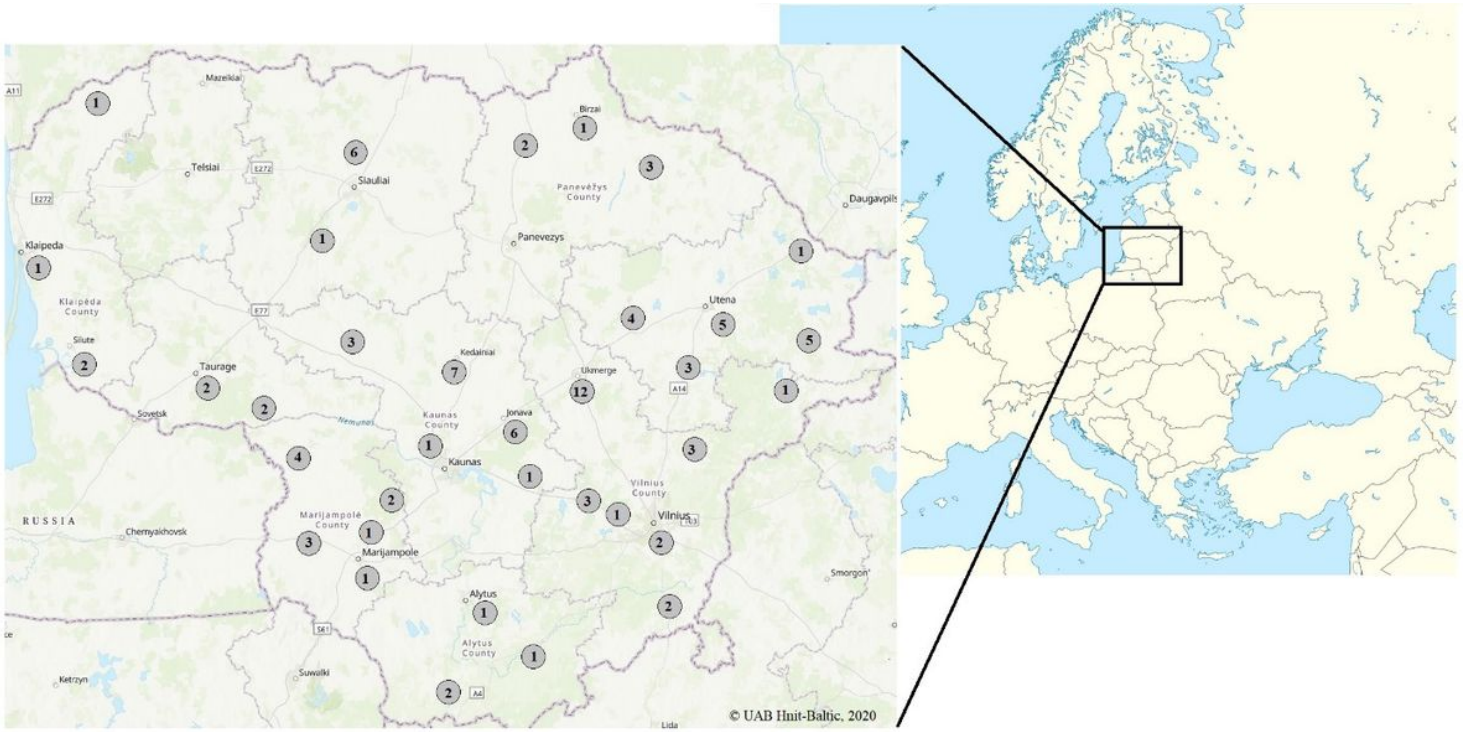

Figure 3

Geographical locations of Lithuania wild boar subpopulations in the study. The numbers indicate amount of individuals in collecting localities. (Map of Europe is downloaded from Wikimedia Commons https://commons.wikimedia.org/w/index.php?title=File:Europe_location_map.svg&oldid=352623294, map of Lithuania is downloaded from www.maps.lt)