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Short communication

# Genetic diversity of Greek sheep breeds and transhumant populations utilizing microsatellite markers

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ABSTRACT

Genetic variation in 13 local sheep breeds, raised in different regions of Greece, was assessed using 10 polymorphic microsatellite markers and was compared with that of three transhumant populations from the Greek Prefectures of Epirus, Sterea Ellada and Thessaly. The total number of alleles per marker ranged from 8 to 32 alleles, and transhumant samples exhibited higher values for allelic number, observed/expected heterozygosity and allelic richness than the local ones. Estimates of inbreeding coefficient ($F_{IS}$) were significant only in the transhumant population of Epirus (*$P < 0.05$). The genetic differentiation of the native breeds was low ($F_{ST} = 4.9\%$), indicating admixture, though all analyses provided clear evidence for the isolation of Thraki and Sarakatsaniko breeds. Differentiation of the three transhumant populations was much lower ($F_{ST} = 0.2\%$), indicating a high rate of gene flow between them. The admixture analysis, using Bayesian methods, suggested that many different local breeds have been used for breeding purposes in each of the three transhumant populations. Among the breeds with high proportion of membership to transhumant farming was Karagouniko which, as already known from literature, is used for the upgrading of numerous sheep populations in Greece.



## 1. Introduction

Sheep (*Ovis aries*, L.) were among the first domesticated animals (about) for a variety of reasons, such as their relatively inexact food requirements, ease of handling, versatility of products (milk, meat and wool) and their important role as sacrificial animals in many religions (Bokonyi, 1976). In Greece, sheep farming plays an important role in its rural economy and together with goat breeding, they represent approximately the 43% of the animal production and 13% of the total agricultural production. The Greek sheep population presents high morphological and productive variation, while showing great adaptability to local environmental conditions (Hatziminaoglou, 2001).

Characterization of animal genetic resources is a prerequisite for successful management programmes and formation of directives in national livestock development. Data on genetic variation within and among breeds provide information for management at breed level (i.e., control of inbreeding) and help, as well, to identify divergent breeds that may harbour distinct genotypes due to local adaptation and are, therefore, worthy of conservation efforts. Sheep genetic resources in Greece were formed under the impact of different processes, such as geographical isolation, genetic drift, selection, crossbreeding and transhumant farming practices (Ligda et al., 2009). All of these factors contributed to the current picture of sheep breeds and varieties in Greece, where the main feature is the presence of high percentage of crossbred animals, resulting from uncontrolled crossbreeding within the local population as well as with non-native breeds (Hatziminaoglou, 2001).

Microsatellite markers are characterized as codominant and highly polymorphic systems and have quickly become one of the best choices of molecular markers for estimating genetic diversity in livestock (Baumung et al., 2004). In recent years, these markers have been successfully applied in studies regarding the genetic diversity between and within Greek and other European sheep breeds (Bizelis et al., 2007; Koutsouli et al., 2007; Lawson Handley et al., 2007; Peter et al., 2007; Ligda et al., 2009; Mastranestasis et al., 2015). However, these assays considered only populations that are characterized as pure-bred. The aim of the present study was to obtain information about the levels and patterns of genetic diversity in Greek sheep breeds as well as to examine the extent of admixture in transhumant farming, as a precursor toward the

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implementation of future management and conservation programmes.

## 2. Materials and methods

### 2.1. Sampling and DNA extraction

A total of 464 blood samples were collected from 13 local sheep breeds and three transhumant sheep populations. Unrelated animals were sampled, while keeping a minimum of five separate flocks per breed. Regarding the transhumant populations, samples from at least 10 different flocks contributed to each of the three populations. The 13 native breeds used in the present study were the following: Agrinio, Argos, Orino, Boutsiko, Chios, Kalarritiko, Karagouniko, Katsika, Kymi, Lesvos, Pilioritiko, Sarakatsaniko and Thraki. Demographic details for these breeds can be found in Bizelis et al. (2007) and Ligda et al. (2009). The three transhumant populations represent the respective Prefectures of Epirus, Sterea Ellada and Thessaly. These populations came from a number of sheep farms that were widely distributed in each of the above Greek Prefectures.

Genomic DNA was isolated from fresh or frozen whole blood, using the PureLink Genomic DNA Mini Kit (Invitrogen by Life Technologies). The quality and quantity of DNA were checked using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). The DNA concentration of each sample was adjusted to 20 ng/μl through dilution with distilled water and arrayed into 96-well PCR plates.

### 2.2. Genotyping

Multiplex PCR reaction method amplifying simultaneously 10 microsatellite loci (MAF214, OARAE129, OARCP49, OARFCB304, ILSTS005, ILSTS011, INRA063, OARFCB128, OARFCB20 and SRCRSP001) was carried out. Microsatellite markers were chosen from the panels recommended by the Food and Agriculture Organization (FAO, 2011) and the International Society for Animal Genetics (ISAG, 2010). All multiplex PCRs were performed in 10 μl volume containing 5–6 μl of 1X KAPA2 G Fast Multiplex PCR Kit (KAPABIOSYSTEMS), 3–4 μl of primer mix (0.3 μM for each primer) and 1 μl (~20 ng) of template DNA. Cycling conditions for the multiplex amplification consisted of an initial 95 °C denaturation step for 3 min followed by 35 cycles of 30 s at 94 °C, 90 s at 60 °C and 60 s at 72 °C, with a final extension at 72 °C for 10 min. Fluorescently labelled PCR products were separated on an ABI 3500 Genetic Analyzer (Applied Biosystems). Alleles were sized and individuals genotyped using the STRand 2.4.59 software (Toonen and Hughes, 2001).

### 2.3. Statistical analysis

Number of alleles (A), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$) and allelic richness ($R_S$) were calculated using GENETIX 4.05.2 (Belkhir et al., 1996) and FSTAT 2.9.3.2 (Goudet, 1995) statistical packages. FSTAT was also used to calculate population pairwise $F_{ST}$ values (multilocus) and F-statistics fixation indexes ($F_{IT}$, $F_{IS}$, and $F_{ST}$) per locus (Weir and Cockerham, 1984). Estimates and confidence intervals based on resampling schemes were provided for all F-statistics: the Jackknife procedure was applied over loci and a confidence interval of 95% was computed with 1000 bootstraps. Moreover, the significance of deviation from Hardy-Weinberg equilibrium (HWE) for each population (based on $F_{IS}$ values) was assessed with randomisation procedures, implemented also in FSTAT, followed by strict Bonferroni correction (Rice, 1989).

In order to define the degree of differentiation among the studied populations, we used factorial correspondence analysis of individual multilocus genotypes through GENETIX 4.05.2 software. Sample relationships were also explored by estimating the genetic distances according to Reynolds et al. (1983), using the GENDIST program in PHYLIP 3.6 statistical package (Felsentein, 2004). A tree topology based on the genetic distances was obtained according to UPGMA algorithms using a NEIGHBOR subroutine, implemented also in PHYLIP 3.6. Assessment of support for clusters on the tree (through 1000 bootstrap resampling of loci) was performed with SEQBOOT subroutine. Finally, we examined the membership of the local sheep breeds to each individual of the three transhumant populations using GeneClass 2.0 software (Piry et al., 2004). The Bayesian methods of Rannala and Mountain (1997) and Baudouin and Lebrun (2001) were chosen as criteria for computation, setting the assignment threshold of scores at $^*P < 0.05$ level of significance and using Monte-Carlo resampling method for calculating the probability of each computation (1000 simulated individuals). These methods assign an individual to the population in which the individual's genotype is most likely to occur.

## 3. Results and discussion

### 3.1. Genetic variation

In total, 157 different alleles were found in the 464 samples analyzed with 10 microsatellite loci. Polymorphism of the markers was medium to high, ranging from 8 (OARAE129, ILSTS011) to 32 (OARCP49) alleles. Summary statistics for genetic diversity are presented in Table 1. The transhumant population Sterea Ellada exhibited the highest mean number of alleles (12.6) and allelic richness (7.339), while the lowest respective values were found in Sarakatsaniko local breed (5 and 4.591). The average number of alleles in the three transhumant populations ranged from 9.4 to 12.6, whereas the respective range for the 13 native breeds was lower (5 to 8.4). Additionally, $R_S$ mean ranged from 6.699 to 7.339 in the transhumant samples, being higher than that in the local breeds (4.591 to 6.550).

The $H_o$ values in the transhumant populations ranged from 0.6768 to 0.7426 with a mean of 0.7198, while the respective range in the local breeds was lower (0.5836 to 0.7398) and averaged 0.6886. The $H_e$ mean was also higher in the transhumant samples compared to that in the native breeds (0.7611 and 0.7042, respectively) (Table 1). Regarding the local sheep breeds, the results for $H_o$ and $H_e$ were similar to those reported by Ligda et al. (2009) (0.696 and 0.739, respectively), where 310 animals of 10 different Greek breeds were analyzed with 28 microsatellite markers.

Average $F_{IS}$ values over all loci ranged from −0.048 to 0.138, being positive in 15 out of 16 studied populations. However, testing for deviation from HWE, based on 3200 randomizations and Bonferroni correction, revealed a significant deficit of heterozygotes only in Epirus population ($^*P < 0.05$, Table 1), indicating the absence of noticeable inbreeding phenomena in the overall populations' panel.

### 3.2. Genetic differentiation and admixture analysis

Average $F_{ST}$ over loci and samples was generally low ($0.036 \pm 0.003$), showing that most of the genetic diversity is explained within populations. According to pairwise multilocus $F_{ST}$ estimates, most genetic differentiation was distributed among local breeds (0.0084-0.1525), whereas differentiation between the three transhumant populations was much lower (0.0002-0.0037) (Table 2), indicating a high rate of gene flow between them. In accordance with this result, the transhumant samples exhibited higher values for A, $H_o$, $H_e$ and $R_S$ compared to the local ones. Regarding the 13 native breeds, the average $F_{ST}$ value of 0.049 was similar to that found by Ligda et al. (2009) from the analysis of 10