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GENETIC DIVERSITY OF ALENTEJANO AND IBERIAN BREEDS ASSESED BY POLYMORPHISMS OF MAJOR GENES

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Abstract. Alentejano and Iberian breeds are biogeographically located in the southwest of the Iberian Peninsula. These breeds shared a set of common characteristics. Both are phenotypically very similar showing a low growth rate and a high intramuscular fat content and are reared under extensive systems in open-range fields. The aim of the current study, carried out within the framework of the TREASURE project, intends to characterize of the genetic diversity of Alentejano and Iberian breeds using the genotyping data of 32 polymorphisms located on 26 major genes. These polymorphisms were genotyped in a total of 950 animals belonging to Alentejano, Iberian and 18 additional European breeds. The low mean values of observed (Ho) and expected (Hs) heterozygosity and F_{IS} point out a low genetic diversity in the analyzed breeds. The genetic distances estimated using Ds and F_{ST} revealed values were close to 0 (0.005 and 0.030, respectively), suggesting that these breeds are genetically similar. The population structure analyzed with multivariate methods such as Discriminant Analysis of Principal Component and admixture analyses showed that both breeds were grouped within the same cluster. These results are supported by other authors based on mitochondrial sequences who reported short genetic distances between these breeds. Although complementary analyses using a larger number of markers should be performed, the results of the current analyses support the hypothesis of Alentejano and Iberian could be different strains of the same breed.

Keywords: Alentejano, Iberian, breed, polymorphisms, genetic diversity, multivariate analyses

Introduction

Alentejano and Iberian are Mediterranean pig breeds with a common origin located in the southwest of the Iberian Peninsula. While the population of Alentejano pig is located in Portugal, the population of Iberian pig is located in Spain. Although there have been interchange of individuals, mainly dams and sires, it has been limited due to a border effect. Both breeds have a lower growth rate compared to other commercial breeds like Duroc or Landrace, a higher fat deposition, and high-quality meats due to a higher intramuscular fat content. In addition, the optimal rearing conditions for Alentejano and Iberian animals are under extensive systems taking advantage of open range fields where the animals walk freely and are fed with acorns and grass. Both breeds are very appreciated for their high quality meat and are the basis for the production of local products *(Silió, 2000; Ramos et al., 2003).*

Although the breeding strategy of Alentejano has been substantially different to other autochthonous breeds, some crosses with Berkshire and Landrace breeds during the 1950s have taken place *(Fraçao, 1984)*. On the other hand, Iberian breed is not introgressed with lean European nor Chinese breeds *(Alves et al., 2003)*, but is actively crossbred with Duroc since this cross provides a higher daily gain and lean percentage in comparison to purebred animals *(Perez-Serrano, 2008; Sánchez-Esquilache, 2011).*

The TREASURE project is a multidisciplinary European project focused on the study of the diversity of local breeds and productions systems for high quality traditional products and sustainable products. Within the scope of TREASURE the objective of the current study was the characterization of the genetic diversity of Alentejano and Iberian pig breeds using the genotyping data of variants located in 26 major genes. These variants were genotyped both in Alentejano and Iberian and in 18 other autochthonous pig breeds.

Material and Methods

A total of 48 animals from Alentejano and Iberian breeds and 854 animals from 18 other European pig breeds were included in the study. Genomic DNA was extracted from blood samples with a standard phenol:chloroform protocol

(Sambrook et al., 1989). 32 variants located on 26 major genes (*MC1R*, *TYRP1*, *NR6A*, *PCK1*, *RYR1*, *IGF2*, *MC4R*, *LEPR, PHKG1*, *SCD*, *GBP5*, *TAS2R39*, *TAS2R4*, *TAS2R38*, *MUC4*, *ESR1*, *CYP2E1*, *LEP*, *CAST*, *MTTP*, *CYB5A*, *FTO*, *PPARGC1A*, *CAPN1*, *PPARD*, and *CTSL*) were genotyped using TaqMan® OpenArray® technology.

Genetic variability at the different loci in each population was measured with observed (Ho) and expected (He) heterozygosity *(Nei, 1987)*. Population structure was evaluated using the F_{IS} statistic *(Nei, 1987)*. Ds and F_{ST} distance measures were estimated according to *Takezaki and Nei (1996).* All the estimates were computed using the library hierfstat in R environment *(Goudet, 2005)*. In addition, in order to represent geometric relationships among the pig breeds, principal component analyses were carried out using the library FactoMineR in R environment *(Lê et al., 2008)*.

Population genetic structure was analyzed through Discriminant Analysis of Principal Component (DAPC) and the optimal number of clusters was identified through the Bayesian Information Criterion (BIC). Derivation of group membership probabilities has also been calculated using both α-score optimization and cross-validation (1000 replications). Both analyses were performed using the library Adegenet v.2.0.2 in R environment *(Jombart, 2008)*.

The algorithm implemented in STRUCTURE *(Pritchard et al., 2000)* was also used to determine the most likely number of partition in the dataset, irrespective of breed of origin. The assignment of the individuals to populations considered an ancestry model with no admixture and correlated allele frequencies. The burning period was set to 50,000 and the number of MCMC replications after burning was 200,000 for $2 \leq k \leq 25$ (k number of clusters).

Results and Discussion

A total of 26SNPs were successfully genotyped. The overall Ho and Hs values per locus ranged from 0.024 to 0.414 and from 0.025 to 0.415. The F_{ST} value from all loci was 0.27, pointing out that 27% of differences are due to breed and 73% caused by differences among individuals.

A total of 17 and 19 SNPs segregated in Alentejano an Iberian, respectively. Table 1 shows that both Alentejano and Iberian breeds have low mean values for Ho and Hs and also low values for F_{IS}, which suggests a low genetic diversity at the studied loci. In addition, the genetic distances estimated, Ds and $F_{ST,}$ were very low (0.005 and 0.030, respectively), pointing out that these breeds are genetically similar.

Table 1. Obseved (Ho) and expected (He) heterozygosity, F_{IS} statistic for each breed, and Nei's genetic distances (Ds and F_{ST}) between Alentejano and Iberian breeds

	Ho	Hs	F_{IS}	Ds	F_{ST}
Alentejano	0.121	0.129	0.052	0.005	0.030
Iberian	0.131	0.149	0.078		

Figure 1 shows the plots of the first versus the second component (Figure 1a) and the second versus the third component (Figure 1b) of the principal component analysis. In the Figure 1a) two clusters can be discerned being Alentejano and Iberian the main representatives of one of the clusters, while the rest of the breeds are on the other one. In addition, in the Figure 1b, Alentejano and Iberian are grouped again together in the same cluster.

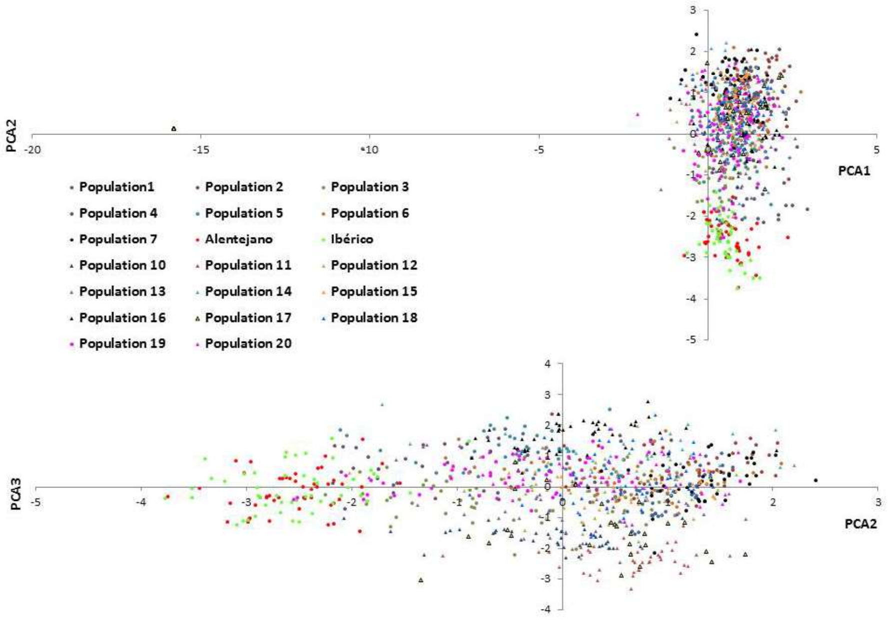

Figure 1. Diagram showing the relative position of the genotyped individuals defined by principal component factor scores.

DAPC analysis confirmed an original shared signal between Alentejano and Iberico which grouped in the same cluster (Figure 2a) and this result was supported by STRUCTURE where the across-run average of estimated ln probability of data (ln Pr(X|K)) reached a plateau at K=14 maintaining the two mentioned breeds in the same cluster (Figure 2b).

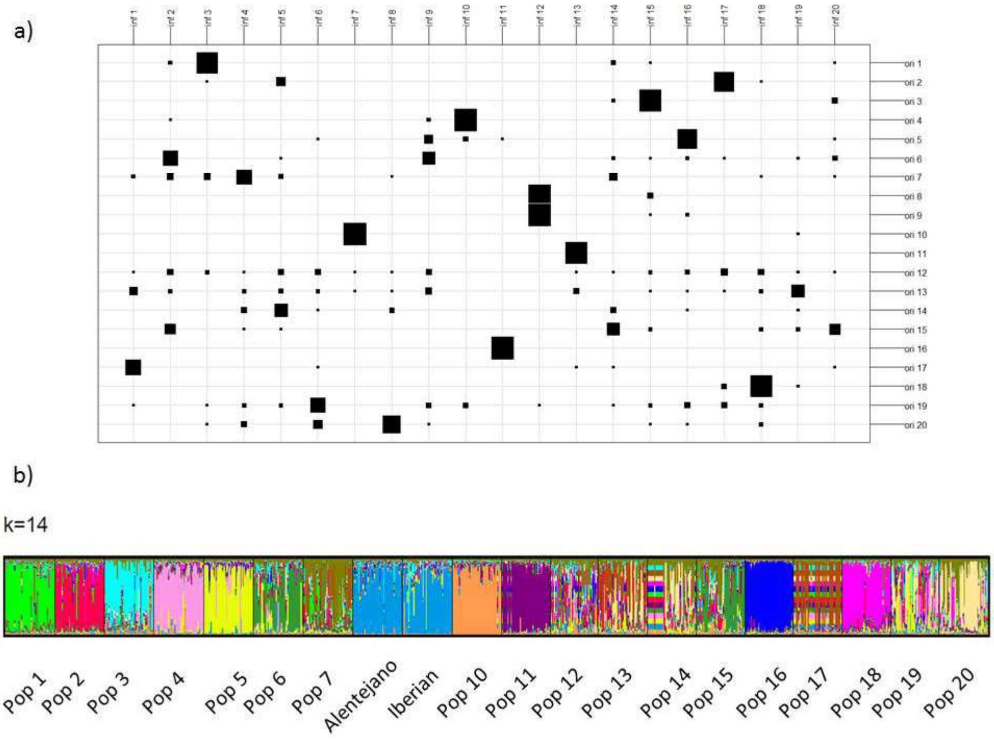

Figure 2. a) Diagram representing the number of individuals of each real population assigned to each inferred population. b) Results of admixture analyses with an optimal value of k=14

The most contributing alleles to diversity were *MC1R**3 and *MC1R**6*7 where the two investigated breeds showed intermediate and equal frequencies (MAF 0.25 and 0.10 for the two loci respectively).

Short genetic distances between Iberian and Alentejano breed were also reported by *Van Asch et al. (2012)* when they analysed the mtDNA control region (F_{ST} = 0.105). They also reported low genetic distances between Alentejano and

Iberian breeds and Iberian wild boars, suggesting an early intense gene flow between local wild populations and the domesticated herds in this region.

Although, additional analyses including a larger number of markers should be carried out to confirm these results in the whole genome, our results show a high genetic similarity between Iberian and Alentejano breeds in the analysed genes and they would be considered as different strains of the same breed.

Conclusion

Alentejano and Iberian breeds are genetically very similar for 26 SNPs located in major genes.

Genetička raznolikost alentehana i iberijske rasa ocenjena pomoću polimorfizma glavnih gena

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Rezime

Alentehano i iberijska rasa su biogeografski locirane na jugozapadu Iberijskog poluostrva. Ove rase dele skup zajedničkih karakteristika. Obe su fenotipski vrlo slične i pokazuju nisku stopu rasta i visok intramuskularni sadržaj masti i odgajaju se u ekstenzivnim sistemima, sa pripustima. Cilj ovog istraživanja, koji se sprovodi u okviru projekta TREASURE, je da se karakteriše genetička raznovrsnost alentehano i iberijske rase koristeći genotipske podatke od 32 polimorfizma koji se nalaze na 26 glavnih gena. Ti polimorfizmi su genotipirani u ukupno 950 životinja koje pripadaju alentehano, iberijskoj i 18 dodatnih evropskih rasa. Niske srednje vrednosti posmatranih (Ho) i očekivanih (Hs) heterozigotnosti i F_{IS} tačke ukazuju na nisku genetsku raznovrsnost u analiziranim rasama. Genetske udaljenosti koje su procenjene korišćenjem Ds i F_{ST} dale su vrednosti blizu 0 (0,005 i 0,030, respektivno), što ukazuje na to da su ove vrste genetički slične. Struktura populacije analizirana pomoću multivarijantnih metoda, kao što je analiza diskriminacije glavnih komponenata i analiza primesa, pokazuju da su obe rase grupisane unutar istog klastera. Ove rezultate podržavaju rezultati drugih autora, koji se zasnivaju na mitohondrijskim sekvencama koje su prijavile kratke genetičke udaljenosti između ovih rasa. Iako treba izvršiti komplementarne analize pomoću većeg broja markera, rezultati sadašnjih analiza podržavaju hipotezu da alentehano i iberijska rasa mogu u stvari biti različiti sojevi u okviru iste rase.

Ključne reči: Alentehano, iberijska, rase, polimorfizmi, genetička raznovrsnost, multivarijantne analize

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