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[1]*Unitat de Genètica I Millora Animal, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Venezuela; and* [3]*Servicio de Ganadería, Diputación Foral de Bizkaia, Avda. Lehendakari Aguirre, 9, Bilbao, Spain*

Genetic conservation of five endangered Spanish donkey breeds

By J. Aranguren-Mendez[1,2], J. Jordana[1] and M. Gomez[3]

Summary

The genetic diversity of most livestock species is being reduced, and it is not possible to preserve all of these livestock breeds. In order to preserve as much of the genetic diversity as possible, we must first have a robust method of measuring genetic diversity among breeds. Three different methods of study that graphically represent relationships among breeds are presented; Weitzman's method, principal component analysis (PCA) and a neighbour-joining tree with allele sharing. Diversity was evaluated on the basis of 15 microsatellite markers typed over a total of 513 DNA samples collected from five Spanish donkey breeds. Breed differentiation was confirmed by the clustering based on the genetic distances between individuals, which essentially grouped all individuals in discrete clusters. The genetic distance among breeds was used to measure the global diversity of the set in breeds considered, and to evaluate the marginal loss of diversity attached to each breed. The Catalana breed appeared to be the most 'unique' in the set considered. In addition to this, the usefulness of global evaluations of diversity using molecular markers to choose breeds is worthy of conservation.

Zusammenfassung

Genetische konservierung fünf bedrohter spanischer Eselrassen

Die genetische Vielfalt der meisten Haustierarten ist bedroht, und es ist nicht möglich alle Rassen zu erhalten. Um möglichst viel der genetischen Diversität erhalten zu können, müssen zuerst robuste Methoden zur Schätzung der genetischen Variabilität zwischen Rassen entwickelt werden. Es werden drei Methoden zur graphischen Darstellung der genetischen Beziehungen zwischen Rassen vorgestellt; Weitzman's Methode, Hauptkomponentenanalyse (PCA) und ein 'allele-sharing neighbour-joining tree'. Die genetische Diversität wurde auf der Basis von 15 Mikrosatellitenmarkern, typisiert an 513 DNA-Proben von fünf spanischen Eselrassen, bewertet. Die Rassendifferenzierung wurde durch eine Clusteranalyse der genetischen Distanz zwischen den Einzeltieren, die alle Individuen in diskrete Cluster einordnet, bestätigt. Die genetische Distanz zwischen Rassen wurde genutzt, um die Gesamtdiversität zu messen und um den marginalen Variabilitätsverlust für jede Rasse zu bewerten. Der katalanische Esel wurde als die am weitest außenstehende Rasse identifiziert. Zusätzlich werden umfassende Bewertungen der Diversität mittels molekularer Marker als sehr nützlich für die Auswahl erhaltenswürdiger Rassen erachtet.

Introduction

In genetic conservation, the main objective consists of preserving variability within populations under the hypothesis of correlation between genetic variation and the population's viability. When economic resources are scarce, it is important to prioritise populations for preservation, decisions of where to allocate resources or to establish preservation plans should be based on information that ensures, to the greatest degree possible, the future viability and success of the preserved populations and of the species (Falk 1991).

Consequently, one of the first stages in the conservation programme of species consists of the evaluation of their genetic variability, their distribution among their populations,

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and the possible detection of rare alleles, as an indicator of populations with unique genetic variants (GONZÁLEZ-CANDELAS and MONTOLÍO 2000).

The Food and Agriculture Organization (FAO) of the United Nations has been mandated by its member nations to manage global animal genetics resources, and much important progress has been made in the last few years (http://www.fao.org/dad-is/). However, resources are limited, and priorities will have to be set for breed conservation, for breed development programmes and for evaluation studies (BARKER 1999).

This article presents the results of the analysis of microsatellite variability in five endangered Spanish donkey breeds (Andaluza, Catalana, Mallorquina, Encartaciones and Zamorano-Leonesa breeds). Our main goals consist of studying the genetic diversity of the five populations of donkeys, through the analysis of three methods which study genetic variability, based on microsatellite data: Weitzman's diversity, the principal components analysis (PCA) and the allele-shared analysis.

Materials and methods

Population samples

Currently, the five Spanish donkey breed figures fit in the category of endangered breeds, proposed by the FAO Expert Consultation (FAO, DAD-IS http://fao.org/dad-is), as well as general information on those breeds, which may be found here. The number of individuals sampled was as follows: 87 Andaluza (AND), 140 Catalana (CAT), 104 Mallorquina (MALL), 74 Encartaciones (ENC) and 108 Zamorano-Leonesa (ZAM). In addition, nine Moroccan asses (MOR) were included as a reference population. Donkey DNA was prepared from whole blood according to standard methods involving lysates of the washed white cells and phenol–chloroform–isoamyl alcohol (25:24:1) extraction (AUSUBEL et al. 1987).

Microsatellite markers

Out of the 15 equine microsatellite markers used, 12 (ASB2, AHT4, AHT5, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, and VHL20) were included in a kit (Stock-Marks® for horses, Equine Paternity PCR Typing kit, PE Applied Biosystems Division; Foster City, CA), which was used with slight modifications in some polymerase chain reaction (PCR) conditions. The other three markers (HMS1, HMS5 and HTG15) were obtained from the literature of equine markers (BREEN et al. 1994). Primer sequences, reaction conditions and data collections have been described previously (ARANGUREN-MÉNDEZ et al. 2001).

Statistical analyses

Genetic distances can also be used to measure diversity, as proposed by WEITZMAN (1992, 1993). This approach has been implemented here to provide a further upward hierarchical representation of the breeds and to evaluate marginal losses of diversity due to various patterns of breed extinction, as advocated by THAON D'ARNOLDI et al. (1998).

Genetic distances between breeds were calculated based on allelic frequencies in each breed (available from the authors on request). Two measures of distances were used, namely the Reynolds' (REYNOLDS et al. 1983) and the standard Nei distances (NEI 1972), taking into account the corrections needed for a small sample size (NEI, 1978).

PCA was performed using the SAS procedure PRINCOMP (SAS 1990) according to the recommendation of CAVALLI-SFORZA et al. (1994). A neighbour-joining (NJ) tree (SAITOU and NEI 1987), using individual animals as operational taxonomic units (OTUs), was constructed with a distance matrix derived from the simple allele-sharing statistic (Bowcock et al. 1994). The distance was obtained using the MICROSAT computer program (Minch et al. 1995). The PHYLIP package (Felsenstein 1995) was then used to construct the tree from the distance matrix.

Results

Table 1 gives the Reynolds and Nei standard genetic distances. The smallest values were obtained for the AND-ZAM and MALL-ENC pairs by Reynolds and Nei distances, respectively. The largest were between AND-CAT for both distances.

Weitzman's representation, based on Nei's standard distance, is shown in Figure 1, where the branch length for each breed can be read, approximately, measuring its relative contribution to the corresponding diversity function. A clear discrimination is observed between two groups, i.e., (i) a first group constituted only of the Andaluza breed (south of Spain), and (ii) another group involving all breeds of black coat of the north of Spain (Catalana, Encartaciones, Mallorquina and Zamorano-Leonesa breeds).

The marginal losses of diversity attached to each breed, which may be taken as a measure of their 'uniqueness', are shown in Table 2, based on the two distances considered. On

Table 1. **Reynolds' distance estimate (below the diagonal) and standard Nei genetic distance (above the diagonal) among five Spanish donkey breeds**

	Andaluza	Catalana	Mallorquina	Encartaciones	Zam-Leonesa
Andaluza	–	**0.1324**	0.1074	0.0797	0.0737
Catalana	**0.0618**	–	0.0801	0.0767	0.1140
Mallorquina	0.0561	0.0441	–	*0.0597*	0.0702
Encartaciones	0.0452	0.0413	0.0379	–	0.0723
Zam-Leonesa	*0.0372*	0.0537	0.0393	0.0407	–

The greatest distances are given in bold and the smallest distances in italics.

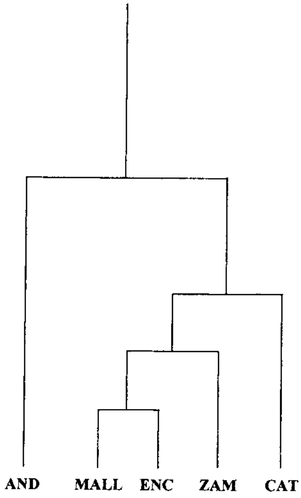

Fig. 1. Dendrogram of relationships established by Weitzman's method using standard Nei distance among five Spanish donkey breeds

Table 2. **Marginal losses of Weitzman's diversity in Spanish donkey breeds**

	Reynolds distance			Nei standard distance			
Breed loss (Q)	Diversity $V(S\backslash Q)$	Absolute loss ΔV	Relative loss $\Delta V/V$ (%)	Diversity $V(S\backslash Q)$	Absolute loss ΔV	Relative loss $\Delta V/V$ (%)	Average $\Delta V/V$ (%)
None (0)	1810	0	0.00	3424	0	0.00	0.00
Andaluza (1)	1323	487	26.91	2460	934	28.15	27.53
Catalana (2)	1312	498	27.51	2373	1051	30.70	29.11
Mallorquina (3)	1403	407	22.49	2814	610	17.82	20.16
Encartaciones (4)	1431	379	20.94	2827	597	17.44	19.19
Zamorano-Leonesa (5)	1438	372	20.55	2722	702	20.50	20.53
(1) + (2)	786	1024	56.57	1320	2104	61.45	59.01
(2) + (4)	824	986	54.48	1520	1904	55.61	55.05
(1) + (4)	944	866	47.85	1863	1561	45.59	46.72
(1) + (2) + (4)	407	1403	77.51	723	2701	78.88	78.20
(2) + (3) + (5)	452	1358	75.03	797	2627	76.72	75.88

$V(S\backslash Q)$ = diversity after dropping Q from S; $\Delta V = V(S) - V(S\backslash Q)$ = absolute value; $\Delta V/V$ = relative value (distance multiplied by 10 000).

average, the highest and lowest losses of diversity are incurred with the extinction of the Catalana and the Encartaciones breeds, respectively. It can also be seen, in Table 2, that the loss of the Andaluza + Catalana + Encartaciones breeds induces a markedly higher loss than the sum of the corresponding individual breeds losses.

The first three principal factors in the principal components (PCs) analysis are plotted in Figure 2. The first PC accounts for 52.0% of the underlying variation, the second PC condenses 15.0% and the third PC accounts for 13.8% of the variation. The first component, which explain more than the half of the existent variation, clearly separates the Spanish donkey breeds from the Moroccan ass population, and within the Spaniards, grouping closely the Catalana and Mallorquina breeds, in a similar way to the obtained in other studies (Aranguren-Méndez et al. 2001).

Figure 3 shows a NJ phylogenetic tree, constructed from the simple allele-sharing distance between 109 individuals, 20 animals taken at random from each population of Spanish donkeys, except for the Moroccan ass population in which all nine individuals were used. Of the 100 Spanish donkeys represented in the tree, only 11 were not clustered with animals from the same population, and some of the breeds are very closely clustered. For example, all AND and ENC animals are found in discrete clusters. The other breeds showed a comparable level of clustering, as do the CAT and MALL breeds. The ZAM and Moroccan animals show a more fragmented pattern of clustering, with animals from these two populations split into a number of distinct groups spread over the tree.

Discussion

Three different methods of study and its graphic representation of breed genetic diversity are presented: Weitzman's method, PCA and the neighbour-joining tree with allele sharing.

Weitzman's diversity defines the diversity expected after a given period of time based on the extinction probability of each element of the set considered. If *n* elements are endangered, second survival–extinction patterns may occur with given probabilities, and for each pattern the resulting diversity may be calculated (Thaon d'Arnoldi et al. 1998). Knowing the pairwise genetic distances and the risk status of a given set endangered breeds, as expressed through their respective probabilities of extinction, an order of priority for a cryo-preservation programme could thus be established.

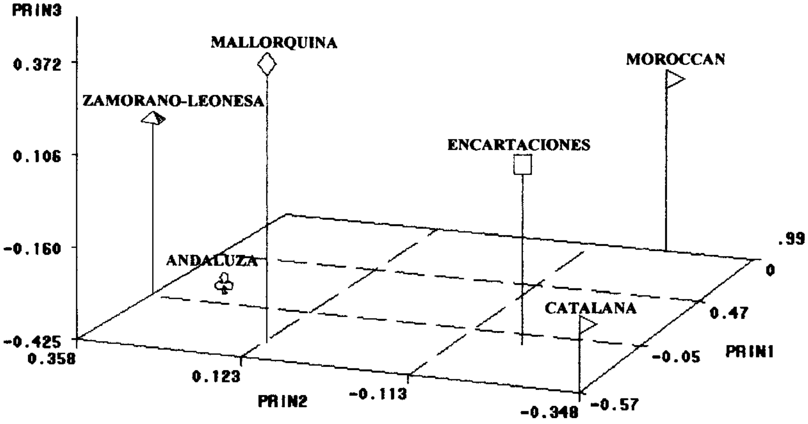

Fig. 2. PCA of allele frequencies from 13 microsatellite loci typed in six populations from five Spanish donkey breeds, and one Moroccan ass breed. The first PC accounts for 52.0%, second PC condenses 15.0% and the third PC condenses 13.8% of the variation

In this study, an opportunity is given for evaluating the global diversity in the set of breeds considered, using Weitzman's (1992, 1993)approach. Table 2 clearly shows the wide range of contributions of each breed to overall diversity, ranging from about 19.19% (ENC breed) to 29.11% (CAT breed). Based on these distances (Reynolds and Nei distances), the AND, CAT and ENC breeds altogether account for 80% of the total diversity, which is an indicator for the potential value to preserve these local endangered breeds in the maintenance of biodiversity. The loss of diversity caused by extinction in a set of breeds can be estimated by the sum of the ordinates in the nodes, which would disappear from the tree if the extinct breeds were to be removed, without any other change. Thus, just by looking at Table 2, it is obvious that the loss of the CAT breed would cause the decrease of diversity three or four times greater than the loss of the MALL or ENC breeds. Although the microsatellite-based phylogeny and the Weitzman diversity tree (Fig. 1) showed some identical clusters, it should not be expected that they will give the same information (Barker et al. 2001).

However, the results and the conservation decisions that one could take of the Weitzman's diversity analysis based on genetic distances should be taken with caution, as recent studies seem to indicate that these methods could be inappropriate for within-species breed conservation, because they ignore within-breed variation (Caballero and Toro 2002).

Multivariate analysis of microsatellite allele frequencies (PCA) has previously been shown to be a powerful tool to reveal the underlying evolutionary history and admixture among distantly related populations (Schmid et al. 1999; Stahlberger-Saitbekova et al. 2001). However, its use as a technique to discern relationships among closely related populations is questionable (MacHugh et al. 1998).

The used markers did not show any breed-specific allele allowing simple identification of the breed and allocation of each one of the individuals as to its breed origin. However, the NJ tree of individuals is in very good agreement with its population's structure (Fig. 3). Of the 100 spanish donkeys examined, 89 (89%) form discrete clusters that coincide with the breed of origin of the sample. The position of 11 individuals (11%) is not clearly defined in the tree, and the Moroccan ass shows a more fragmented pattern of clustering. Within-breed, these samples tend to form sub-clusters and they correspond to their source subpopulations to a very high degree (data not shown). Previous studies, which have used a

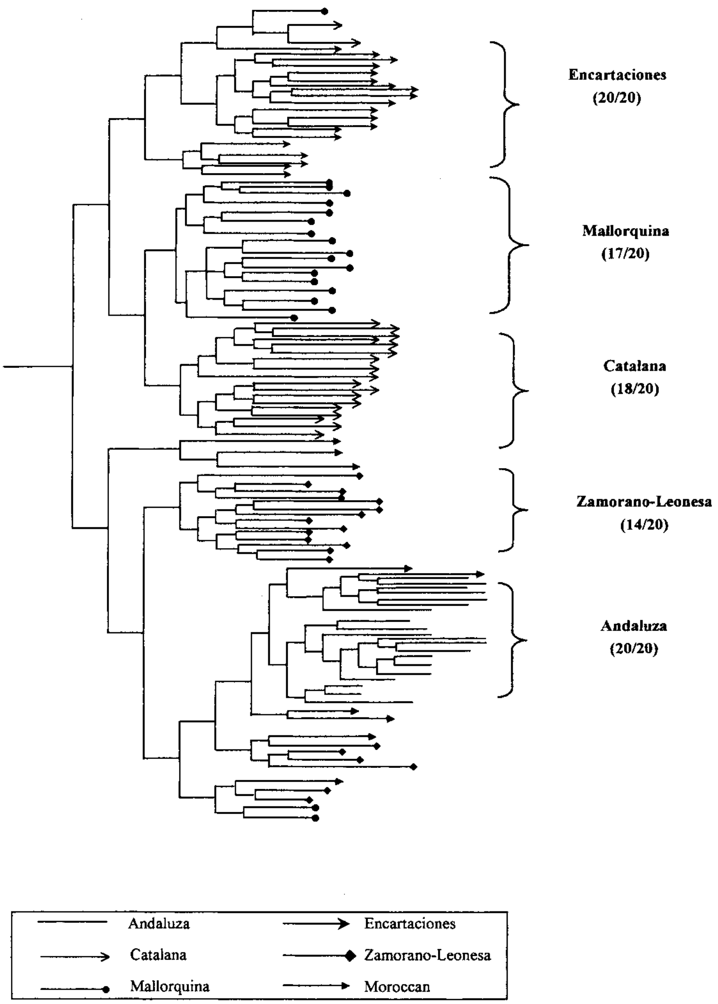

Fig. 3. Neighbour-joining dendrogram constructed from allele-sharing distance among 109 individuals from five Spanish donkey breeds, and one Moroccan ass breed. Numbers to the right indicate the fraction of individuals of the breed

microsatellite allele-sharing distance among individual organisms, have revealed a similar level of clustering within a population to that observed in Fig. 3 (MacHugh et al. 1998; Laval et al. 2000). More precisely, using breed allelic frequencies to calculate the likelihood that an animal belongs to a given breed and then assigning the animal to the breed showing the largest likelihood (using Bayesian methods as proposed by Rannala and Mountain, 1997) allowed 90.64% of the animals to be correctly assigned (data not shown).

The apparent taxonomic distinctiveness of a breed may not necessarily mean that it carries genes that are adaptively unique, as forces other than selection may have been operating. For example, random drift can affect the genetic distances among populations. When we are selecting breeds for conservation it may be important not just to consider taxonomic distinctiveness or between-population variation, but also to take measures within population diversity (Blott et al. 1998; Caballero and Toro 2002). Such measures could be included into a diversity index and population selected for conservation on the basis of this index. On the other hand, there is a need for measuring the overall diversity of a set of breeds, since prospective evaluations of diversity are required for defining appropriate conservation policies, as advocated by Weitzman (1993). In addition to this, the usefulness of global evaluations of diversity using molecular markers for the choice of breeds is worthy of conservation. We hope that this information and that reported previously (Aranguren-Méndez et al. 2001; Jordana et al. 2001) will significantly contribute to the establishment of a sensible preservation strategy for these populations.

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Author's address: J. JORDANA, Unitat de Genètica i Millora Animal, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain. E-mail: jordi.jordana@uab.es