ASSESSMENT OF GENETIC VARIABILITY AND RELATIONSHIPS AMONG THE MAIN FOUNDER LINES OF THE SPANISH MENORCA HORSE BREED USING MOLECULAR COANCESTRY INFORMATION

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Abstract

The Menorca Horse is an Spanish endangered horse breed, mainly located in the Balearic Island. Although there are some important populations in other European countries, as France, Italy, Holland… It is characterized by its black coat colour and its relationship with the popular activities in the Island and its environment. A study of the actual genetic variability has been assessed in order to quantify the genetic diversity of the population within its breeding and conservation programme. The genotypes of 753 registered horses were analyzed for 16 microsatellite markers in the Central Laboratory of Veterinary (Algete), belonging to the Spanish Ministry of Environment and Rural and Marine Affairs. The average number of alleles per *locus* was 7.69, and the average expected and observed heterocigosities were 0.710 and 0.723, respectively. The average molecular coancestry value (*fij*) for the whole population was 0.291 and the mean self-coancestry value was 0.638. Genetic relationships among three groups of horses by their founder-lines (same line via father and mother) were also estimated using molecular coancestry information. A total of 134 horses were included in the analysis. All of them belongs to the main founder-lines registered in this breed: Mudaino (M), Torretrençada (T) and Son Quart (S). For these three groups, the average molecular coancestry was 0.312 and the mean self-cancestry was 0.661. Mean kinship distance between groups was 0.322. The between groups molecular coancestry values (*fij*) varied from 0.263 (for the ST groups pair) to 0.288 (for the M-T pair). Molecular coancestry values within groups varied from 0.318 (for S group) to 0.399 (for the T group). F-statistics, F_{IS}, F_{ST} and F_{IT} for the groups analysed were, respectively -0.026, 0.038 and 0.013.

Introduction

This study estimates the genetic variability and the relationships between the main founder lines of the Spanish Menorca horse breed using molecular coancestry information. The importance of the assessment of the genetic relationships among the main founder lines of the Spanish Menorca horse breed is based in their recuperation. Most published studies on this task have been carried out using microsatellites (Azor *et al.*, 2006, 2007; Cañón *et al.*, 2000; Morais *et al.*, 2005).

Caballero and Toro (2002) have formalised the way in which it is possible to obtain coancestry coefficients from molecular information. Eding and Meuwissen (2001), using simulated data, showed that molecular coancestry has some interesting properties, namely that average kinship between populations become constant very quickly after population fission, causing between-population diversity to remain constant. However, the number of studies using molecular coancestry to assess the genetic relationships between breeds is scarce (Álvarez *et al.*, 2005).

Material and Methods

We have use 753 genotypes analyzed for 16 microsatellites (table 1) markers in the Central Laboratory of Veterinary (Algete), belonging to the Spanish Ministry of Environment and Rural and Marine Affairs.

Parameters characterising genetic variability (such as observed and expected heterozygosity and average number of alleles per locus), and within and between-populations molecular coancestry coefficients (*fij*) and kinship distance (*Dk*) were computed using the program MolKin v3.0 (Gutiérrez *et al.*, 2005) which is freely available at http://www.ucm.es/info/prodanim/html/JP_Web.htm. The molecular coancestry between two individuals *i* and *j* can be computed at a given locus using the following scoring rules (Caballero and Toro, 2002; Eding and Meuwissen, 2001): $f_{ij,l} = \frac{1}{4}\left[I_{11} + I_{12} + I_{21} + I_{22}\right]$

where *Ixy* is 1 when allele *x* on locus *l* in individual *i* and allele *y* in the same locus in individual *j* are identical, and zero otherwise. Notice that this value can only have four values: 0, ¼, ½ and 1. The molecular coancestry between two individuals *i* and *j* (*fij*) can be obtained by simply averaging over *L* analyzed loci

$$f_{ij} = \frac{\sum_{l=1}^{L} f_{ij,l}}{L}$$

The kinship distance between two individuals *i* and *j* is *Dk* = [(*si* + *sj*)/2] – *fij*, where *si* is self-coancestry for the individual *i*. The within and between-populations molecular coancestry and *Dk* are computed by simply averaging the corresponding values for all the within- or between-population pairs of individuals. Using also the program Molkin the *F*-statistics, F_{IS}, F_{ST}, and F_{IT}, and Polymorphic Informative Content (PIC) were computed (Gutiérrez *et al.*, 2005).

Results and Discussion

Paired average molecular self-coancestry [(*si* + *sj*)/2; below diagonal], between subpopulations molecular coancestry (*fij*; above diagonal) and, within subpopulations, molecular coancestry (*fi*), molecular self-coancestry (*si*), expected heterozygosity (He) and number of alleles per locus. The abbreviations correspond to the main founder-lines registered in this breed:
Mudaino (M), Torretrençada (T) and Son Quart (S)

Subpopulations	Between subpopulations			Within subpopulations			
	M	T	S	f_i	s_i	He	alleles
M		0.288	0.274	0.345	0.668	0.657	6.5
T	0.652		0.264	0.399	0.635	0.601	3.1
S	0.657	0.641		0.319	0.646	0.681	5.6

F_{ST} values (below diagonal) and F_{IS} values (diagonal) between Menorca horse populations

F_{IS} F_{ST}	M	S	T
M	-0.014		
S	0.034	-0.039	
T	0.009	0.023	-0.214

Number of alleles, observed and expected heterocigosity (*Ho* and *He*) and polymorphic informative content (PIC) per locus in Menorca horse breed

MARKER	ALLELES	Ho	He	PIC
AHT4	8	0.849	0.832	0.810
AHT5	7	0.775	0.763	0.730
ASB17	11	0.672	0.662	0.630
ASB2	9	0.720	0.699	0.648
ASB23	9	0.736	0.714	0.668
CA425	8	0.695	0.659	0.605
HMS1	7	0.732	0.726	0.681
HMS2	8	0.770	0.756	0.718
HMS3	7	0.781	0.747	0.706
HMS6	7	0.503	0.519	0.479
HMS7	7	0.788	0.801	0.772
HTG10	10	0.766	0.745	0.722
HTG4	7	0.720	0.722	0.682
HTG6	6	0.640	0.630	0.583
HTG7	4	0.576	0.553	0.489
VHL20	8	0.851	0.825	0.802

F- Statistics between Menorca horse populations

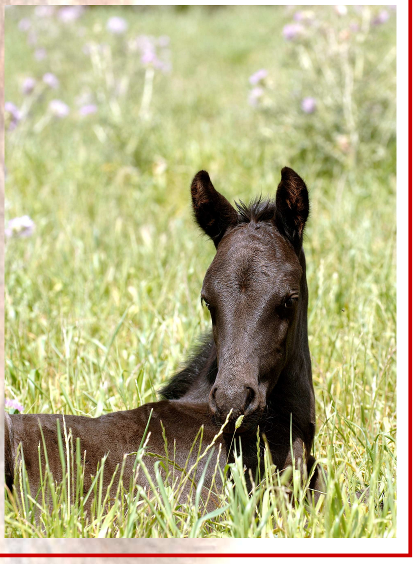
F-statistics	FIS	FIT	FST
Among subpopulations	-0.026	0.014	0.039

Most genetic distances are highly dependent on the observed allele frequencies, which are in turn highly dependent on recent evolutionary processes such as genetic drift. However, the formula used to compute the kinship distance, *Dk* = [(*si* + *sj*)/2] – *fij* has two terms that may be useful in assessing whether differentiation among populations may be recent or remote in origin; the first term [(*si* + *sj*)/2] provides information on recent differentiation whilst *fij* informs on the allele frequencies before separation of populations (Álvarez et al., 2005). *Dk* is, in consequence, a genetic distance with classical properties but corrected for the identity of the breeds before separation. In this note we show separately both terms of the between breeds *Dk*. It can be graphically shown that recent differentiation differentiates in a large extent both Asturcón populations that have a common origin (Royo et al., 2005).

Kinship distance between Menorca horse populations

Dk	M	S
S	0.383	
T	0.363	0.377

As a **CONCLUSION**, we have shown that genetic analyses based on molecular coancestry information are useful to distinguish between ancestral and recent genetic differentiation among horse breeds, thus allowing useful information on the history of the present horse populations.



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