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Genetic Diversity Within and Among Four South European Native Horse Breeds Based on Microsatellite DNA Analysis: Implications for Conservation

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Abstract

In the present study, genetic analyses of diversity and differentiation were performed on four Basque-Navarrese semiferal native horse breeds. In total, 417 animals were genotyped for 12 microsatellite markers. Mean heterozygosity was higher than in other horse breeds, surely as a consequence of management. Although the population size of some of these breeds has declined appreciably in the past century, no genetic bottleneck was detected in any of the breeds, possibly because it was not narrow enough to be detectable. In the phylogenetic tree, the Jaca Navarra breed was very similar to the Pottoka, but appeared to stand in an intermediate position between this and the meat breeds. Assuming that Pottoka is the breed less affected by admixture, the others gradually distanced themselves from it through varying influences from outside breeds, among other factors. In a comparative study with other breeds, the French breeds Ardanais, Comtois, and Breton were the closest to the four native breeds. Three different approaches for evaluating the distribution of genetic diversity were applied. The high intrabreed variability of Euskal Herriko Mendiko Zaldia (EHMZ) was pointed out in these analyses. In our opinion, cultural, economic, and scientific factors should also be considered in the management of these horse breeds.

For centuries, horses were raised in what are now the autonomous communities of the Basque Country and Nafarroa by the ancient method of keeping herds of mares roaming free for most of the year and rounding them up from time to time for branding and selection of animals for sale (Donezar 1952). From the 18th century onward, advances in farming, the sale of communal land, and the deforestation of large areas for wood to be burned as fuel reduced the area available for livestock in Bizkaia and Gipuzkoa, and herd sizes declined by as much as two-thirds. These changes were not felt so strongly in Araba and Nafarroa, and herd sizes were maintained (Pérez 1999). In the 20th century, horses in general were affected by the mechanization of agriculture and the abandonment of shepherding. The largest population declines were in the light and semilight breeds, including all members of the Cantabrian pony group. Heavier breeds raised for meat were able to maintain or even increase their numbers (Garcia-Dory 1987).

At present, there are four recognized autochthonous breeds of horse in the Basque autonomous community (BAC) and Nafarroa (Figure 1), all of which have special protected status. Information on these breeds can be found in the international databanks of the Food and Agricultural Organization (FAO) at www.fao.org/dad-is. The two light breeds, Pottoka (POT) and Jaca Navarra (JN), are members of the generic group called Cantabrian-Pyrenean ponies. The Euskal Herriko Mendiko Zaldia (EHMZ) and Burguete (BU) breeds may be considered as heavy breeds, and as such fit into a series of groupings arising from crossbreeding between autochthonous or local mares and imported stallions, mainly from French breeds such as Ardanais and Comtois (Pérez de Muniain 1999). Both EHMZ and BU are draft/meat animals and are raised mainly for consumption. The BAC and Nafarroa are the only areas in the European Union where there is fully extensive horse breeding for meat (Legarra et al. 2000). Breed associations are working with provincial

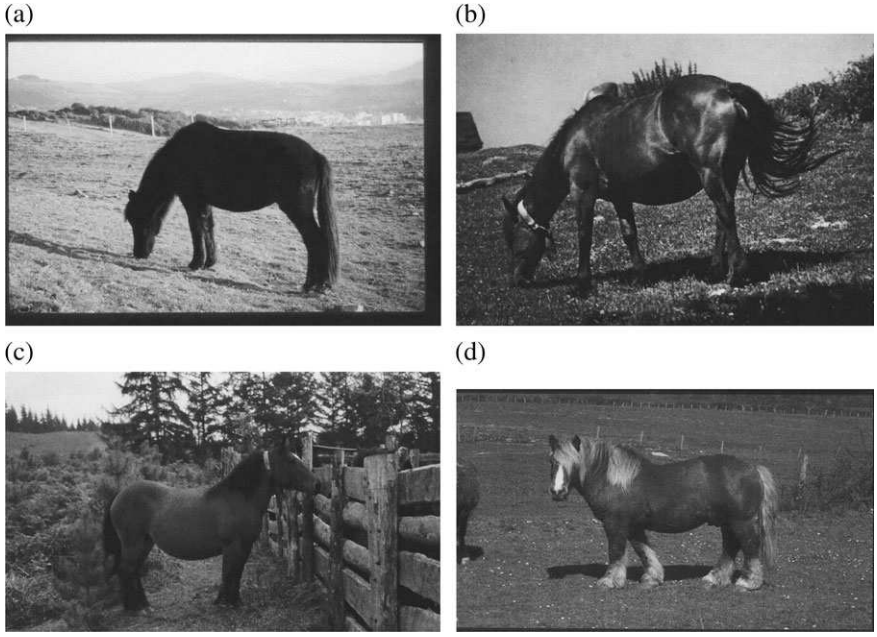

Figure 1. The four native breeds analyzed: (a) Pottoka and (b) Jaca Navarra ponies, and (c) Euskal Herriko Mendiko Zaldia and (d) Burguete medium-size horses.

authorities and the Basque government to determine and analyze the characteristics of all four breeds of horses in the BAC and Nafarroa, and to evaluate the degree of similarity between animals on different farms and between these populations as a whole and neighboring populations.

Genetic characterization is the first step in breed conservation and may have implications for future breeding strategies. To achieve the genetic characterization of these populations we have chosen microsatellite markers. Ease and accuracy of typing, together with high levels of polymorphism and spread distribution in the genome, make microsatellite loci an attractive potential source of information about population histories and evolutionary processes (Goldstein and Schlötterer 1999). Microsatellites have been successfully applied to parentage and relatedness testing in horses (Bowling et al. 1997; Marklund et al. 1994) and their usefulness for estimating genetic distances among closely related populations has been documented (Takezaki and Nei 1996). Horse breeds around the world have been analyzed by microsatellites, including the Przewalski horse (Breen et al. 1994), Spanish Celtic breeds (Cañon et al. 2000), Norwegian breeds (Bjørnstad et al. 2000; Bjørnstad and Røed 2001), and various European and Asian breeds (Tozaki et al. 2003; Vilá et al. 2001).

In the present study, we estimated the genetic diversity of the four native breeds, investigated the effect of genetic bottlenecks, and estimated the amount of genetic differentiation between these breeds. We applied three different approaches to evaluate the distribution of genetic diversity and corresponding conservation priorities. This information can be helpful for the correct management of the four native horse populations located in the Cantabrian-Pyrenean area.

Materials and Methods

Breeds and Sampling

A total of 417 animals representing four native breeds were analyzed (Figure 2). In sampling, we attempted to avoid closely related animals and to take samples only from animals that met the standards for each breed. POT (Figure 1a) is classified into three different types according to coat color and morphological characteristics: A, B, or C (Bizkaiko Foru Aldundia 1997). Type A is for animals that meet all breed standard requirements; type B is for animals that fail to meet one or more such requirements (height, coat color); and type C is for animals with one parent belonging to a different breed and animals far removed from the standard. Animals sampled in Bizkaia were all from type A, animals sampled in Araba were type B, and, after comparison of their allelic frequencies, animals from types A and B were grouped in Gipuzkoa. We maintained type C animals in another group (POTC). For EHMZ (Figure 1c), we selected animals from all over the distribution area of the breed and distributed in four main areas. A preliminary statistical comparison was done and one of the main areas (Gorbea) was significantly different from the others, so we differentiated it from the other areas (EHMZG). Moreover, animals known to have any admixture with other breeds were included in a different group, which we called mixed EHMZ (EHMZX). Twenty thoroughbred horses were also included for comparison purposes.

Microsatellite Typing

Twelve microsatellites were genotyped in the horse breeds, including nine markers recommended for parentage testing

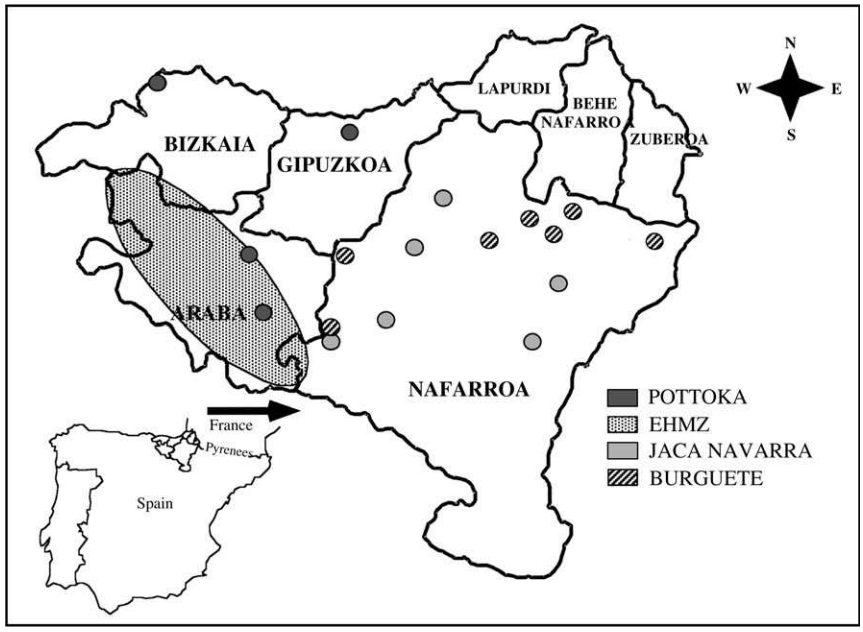

Figure 2. The Basque autonomous community with its three provinces—Bizkaia, Gipuzkoa, and Araba—and Nafarroa. Sampling points are indicated. EHMZ was sampled over the entire distribution area.

by the International Society of Animal Genetics (ISAG) Equine Genetics Standing Committee. The markers were VHL20 (van Haeringen et al. 1994), HTG4, HTG6, HTG7, and HTG10 (Ellegren et al. 1992), AHT4 and AHT5 (Binns et al. 1995), HMS2, HMS3, HMS6, and HMS7 (Guerin et al. 1994), and ASB2 (Breen et al. 1997). DNA was extracted from blood using organic solvents (Ausubel et al. 1987) and polymerase chain reactions (PCRs) was performed in a 9700 GeneAmp PCR system (Applied Biosystems, Foster City, CA). The PCR conditions were those described in the StockMarks for Horses equine genotyping kit (Applied Biosystems). PCR products were detected by capillary electrophoresis using an ABI PRISM 310 DNA genetic analyzer with GeneScan analysis software (Applied Biosystems). The Rox 350 bp internal standard (Applied Biosystems) was used for sizing alleles. Samples of the ISAG 1999 comparison test were genotyped in order to standardize our nomenclature with that of other research groups.

Statistical Analysis

The allelic frequencies, observed and expected heterozygosities, and average exclusion probabilities were estimated using the Cervus 2.0 program (Marshall et al. 1998). The test for deviation from Hardy-Weinberg equilibrium was performed with version 1.2 of the GENEPOP software package (Raymond and Rousset 1995). Based on the Bottleneck computer program (version 1.2.02) (Cornuet and Luikart 1996), two tests were used to check for bottlenecks: the sign test (Cornuet and Luikart 1996) and the Wilcoxon signed-rank test (Luikart and Cornuet 1998). As recommended by Piry et al. (1999), we used the two-phase mutation model (TPM) with 95% stepwise mutation model and 5% multistep mutations. The computer program FSTAT (version 1.2) (Goudet 1995) was used to obtain estimates of inbreeding coefficients and population subdivision based on unbiased F statistics according to Weir and Cockerham (1984).

Correspondence analysis was performed using the NTSYS software (Rohlf 1988). Genetic distances, bootstrap procedures, and neighbor-joining trees were obtained using the PHYLIP 3.5 statistical package (Felsenstein 1989). The Reynolds' distance (Reynolds et al. 1983) is based on the drift model whereby drift is the only force operating, and it seems to be the best method for very closely related breeds (Laval et al. 2002). Moreover, we compared our four native breeds with available data for seven others: the Spanish pure breed (Vega-Plá and Rodriguez-Gallardo 1998), the Uruguayan Creole horse, a descendent of Spanish horses (Kelly et al. 2002), three French draught breeds—Ardanais, Comtois, and Breton (Mériaux JC, personal communication), and two widely distributed breeds—thoroughbred (our data) and quarter horse (Bowling et al. 1997).

Three different approaches for evaluating the partition of the genetic diversity were applied:

(1) Following the Weitzman approach (Weitzman 1992, 1993), Reynolds' genetic distances were used to compute marginal losses of genetic diversity.
(2) The contribution of different populations to total diversity and allelic richness was calculated according to Petit et al. (1998) using CONTRIB (available at http://www.pierroton.intr.fr/genetics/labo/Software/Contrib). In their method, the contributions of each

Table 1. Genetic variability of 12 microsatellites in the Basque-Navarrese and thoroughbred horses

Breed	*N*	MNA	H_o	H_e	PE_{pu}	*PE*
EHMZ	143	7.75 ± 1.36	0.724 ± 0.086	0.738 ± 0.087	0.9954	0.9999
EHMZ-G[a]	119	7.67 ± 1.43	0.729 ± 0.085	0.739 ± 0.085	0.9953	0.9999
EHMZG[a]	24	6.08 ± 1.38	0.700 ± 0.102	0.716 ± 0.126	0.9922	0.9997
EHMZX	20	5.75 ± 1.54	0.687 ± 0.170	0.669 ± 0.123	0.9768	0.9989
Burguete	45	6.25 ± 1.66	0.707 ± 0.147	0.698 ± 0.132	0.9902	0.9997
Pottoka	131	8.08 ± 1.93	0.751 ± 0.063	0.762 ± 0.073	0.9975	0.9999
Gipuzkoa	78	7.83 ± 1.64	0.736 ± 0.076	0.765 ± 0.065	0.9973	0.9999
Bizkaia	36	6.75 ± 1.76	0.777 ± 0.099	0.728 ± 0.089	0.9928	0.9998
Araba	17	5.83 ± 1.34	0.766 ± 0.172	0.706 ± 0.106	0.9865	0.9995
Pottoka C	16	6.42 ± 1.31	0.691 ± 0.139	0.735 ± 0.108	0.9929	0.9998
Jaca Navarra	62	7.25 ± 1.96	0.765 ± 0.096	0.744 ± 0.102	0.9960	0.9999
Thoroughbred	20	4.33 ± 1.56	0.633 ± 0.145	0.650 ± 0.131	—	—

Data shown corresponds to the mean number of alleles per locus (MNA), observed (H_o) and expected (H_e) heterozygosity, and probability of paternity exclusion (*PE*) and probability of parentage exclusion when both parents are unconfirmed (PE_{pu}).

[a] EHMZ-G, all subpopulations of Euskal Herriko Mendiko Zaldia, except Gorbea; EHMZG, Gorbea subpopulation only.

population to the total diversity (CT) and the total allelic richness (CTR) are divided into two components, one related to the within-population diversity (CS and CSR) and the other to the divergence from the remaining populations (CD and CDR). As allelic richness is dependent on sample size, to standardize the results of allelic richness across populations the technique of rarefaction was implemented.

(3) We applied the method of Caballero and Toro (2002) based on the molecular coancestry between all pairs of individuals, referring to identity by state instead of identity by descent.

Results

Genetic Variation

Estimates of the mean number of alleles (MNA), observed and expected heterozygosities, and probabilities of parentage exclusion are presented in Table 1. The greatest variability was found in POT and the lowest in BU, the latter presenting significant differences with the other three breeds for both MNA and heterozygosity ($P < .05$). The probability of paternity exclusion (PE) for the 12 loci ranged from .999953 in POT to .999732 in BU. These markers are therefore an effective tool for horse parentage verification in these breeds. In the Hardy-Weinberg equilibrium conformity test, HMS3 was not in equilibrium for POT ($P < .01$) and JN ($P < .05$), and HTG6 was not in equilibrium for POT ($P < .05$). Recently a base substitution in the sequence flanking the HMS3 marker has been described, the mutation being located in the M allele priming site (Achmann et al. 2001). Thus, in this study, allele nonamplification can be the reason for the detected deviation from equilibrium in this marker. The remaining populations were in equilibrium for all 12 markers tested. The allele frequencies are available from the authors upon request.

The Bottleneck computer program was developed to identify bottleneck events within the last 12 to 15 generations. Since the minimum generation interval in horses is 8 years (Ollivier 1999), 12 to 15 generations would mean 96 to 120 years before present. Analysis of microsatellite data by means of two tests (Table 2) showed no indications of population bottlenecks for the pony breeds POT and JN, with about an equal number of loci showing an excess and deficiency of heterozygotes.

With respect to the distribution of the observed genetic variation within and between breeds, *F* statistics showed significant differentiation between POT subpopulations ($P < .001$). For the four EHMZ subpopulations, F_{ST} was 0.7% ($P < .01$), but the level of significance increased when we compared one of them (Gorbea) to the other three ($F_{ST} = 1.1\%$; $P < .001$). Thus EHMZ animals from the Gorbea region could be differentiated from the rest of the population. Table 3 presents F_{ST} and F_{IT} when breeds are considered in pairs. The values of genic differentiation (F_{ST}) among analyzed breeds range from 0.5% for the POTGipuzkoa-POTC pair to 8.5% for the POTAraba-EHMZX pair. Differentiation between most of them was statistically significant ($P < .01$); only three pairs of breeds or subpopulations did not show statistically significant genetic differentiation between themselves: POTC with POTGipuzkoa, JN, and EHMZ-Gorbea. It should be kept in mind that POTC is quite a heterogeneous group and the smallest one included in the

Table 2. Results of the bottleneck detection tests on the Pottoka and Jaca Navarra breeds showing the number of loci with heterozygosity excess (H_{ex}) or deficiency (H_d) and probabilities (*P*) from the sign test and the Wilcoxon signed-rank test

	Sign test		Wilcoxon signed-rank test
Breed	H_d/H_{ex}	*P*	*P*
Pottoka	6/6	.36156	.4
Gipuzkoa	6/6	.3593	.38037
Bizkaia	7/5	.17284	.33936
Araba	7/5	.17456	.30127
Jaca Navarra	5/7	.59088	.85010

Table 3. Analyses of *F* statistics of the native horse breeds and subpopulations compared in pairs (F_{ST} below the diagonal and F_{IT} above the diagonal)

	POTGip	POTBizk	POTAraba	EHMZG	EHMZ-G	JN	BU	EHMZX	POTC
POTGip	—	0.008	0.018	0.035	0.023	0.009	0.021	0.027	0.042
POTBizk	0.029	—	−0.072	−0.032	−0.006	−0.044	−0.038	−0.053	−0.028
POTAraba	0.033	0.054	—	−0.021	0.001	−0.042	−0.033	−0.054	−0.013
EHMZG	0.023	0.049	0.063	—	0.014	−0.016	−0.001	0.000	0.036
EHMZ-G	0.014	0.041	0.038	0.011	—	−0.002	0.006	0.007	0.018
JN	0.016	0.044	0.054	0.020	0.010	—	−0.024	−0.030	−0.013
BU	0.026	0.048	0.051	0.014	0.006	0.018	—	−0.017	0.005
EHMZX	0.049	0.080	0.085	0.034	0.022	0.036	0.024	—	0.013
POTC	0.005	0.038	0.038	0.009	0.009	0.015	0.017	0.034	—

analysis. The F_{ST} value over all loci between the four native breeds is 0.015 ($P < .001$), which indicates that 1.5% of the variability could be attributed to differences between them.

Breed Relationships

In the correspondence analysis (Figure 3), the first axis, which accounted for 37.5% of the total inertia, separated the light ponies—JN, POT, and POTC—from the heavier horses—BU, EHMZ, and EHMZX. The second axis, which xaccounted for 24.6% of the total inertia, separated the groups classified as admixed (POTC and EHMZX) from the well-defined breeds. In the dendrogram based on Reynolds' genetic distance (Figure 4), POT and POTC clustered on one side and the heavier breeds (EHMZ and BU) on the other, with JN in an intermediate position between them. The group of EHMZX was very distinct and divergent, as expected from the crossbreeding history of this group.

In the comparative study with other breeds, a cluster analysis based on Nei's distance matrix (Nei 1972) (Figure 5) showed the separation of the Spanish pure breed, followed by two main groups: one consisting of the four native breeds in one cluster and the French breeds in another, and the other consisting of Uruguayan Creole horse, thoroughbred, and quarter horse.

Diversity for Conservation

Table 4 summarizes the estimates of the measures of diversity at the breed level. Applying the diversity function suggested by Weitzman (1992), the biggest decreases would result from the loss of the pony breeds. The total diversity would

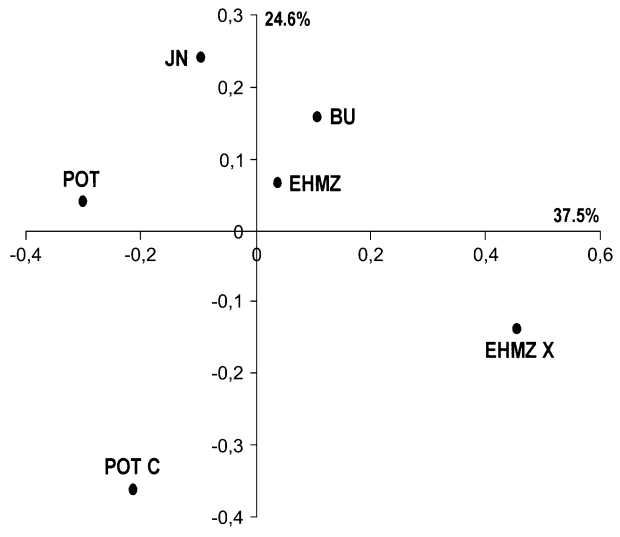

Figure 3. Correspondence analysis of allele frequencies from 12 microsatellite loci in four native horse breeds: Pottoka (POT), Jaca Navarra (JN), Burguete (BU), and Euskal Herriko Mendiko Zaldia (EHMZ), and two mixed populations Pottoka type C (POTC) and EHMZ mixed (EHMZX). The projection of breeds on axes 1 and 2 is shown.

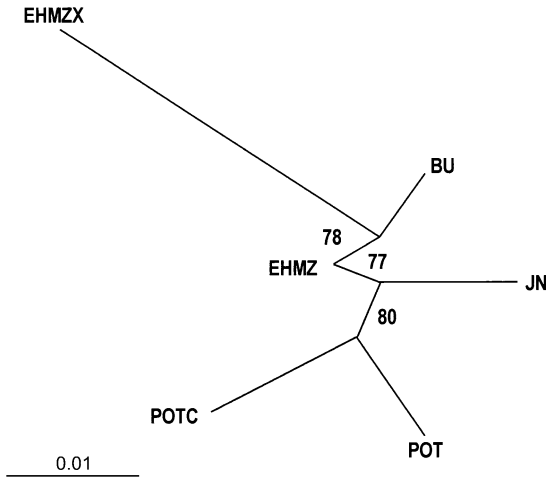

Figure 4. Genetic relationships among four Pyrenean-Cantabrian horse populations: Pottoka (POT), Jaca Navarra (JN), Burguete (BU), and Euskal Herriko Mendiko Zaldia (EHMZ). The two mixed populations, EHMZ mixed (EHMZX) and Pottoka type C (POTC), have also been included. Dendrogram was constructed by the neighbor-joining method based on Reynolds' distance. Percentages of the nodes are the bootstrap values obtained from 1000 replications.

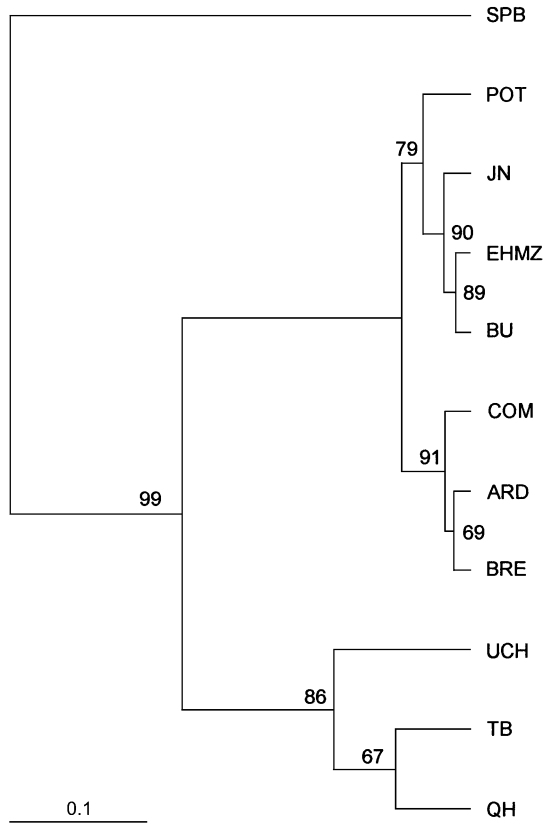

Figure 5. Dendrogram constructed from Nei's distance by the unweighted pair group method with arithmetic mean (UPGMA) shows the genetic relationships among 11 horse breeds using frequencies of six microsatellites markers. Breeds are as follows: Spanish pure breed (SPB), Uruguayan native Creole horse (UCH), Comtois (COM), Ardanais (ARD), Breton (BRE), thoroughbred (TB), quarter horse (QH), and the four Pyrenean-Cantabrian breeds: Pottoka (POT), Jaca Navarra (JN), Burguete (BU), and Euskal Herriko Mendiko Zaldia (EHMZ). Percentages of the nodes are the bootstrap values obtained from 1000 replications.

decrease by 46% if POT were lost and by 81% if POT and JN were lost. If BU were lost, the total diversity would decrease by 33%, while if the EHMZ were lost it would decrease by 17%. The contribution of different populations to total diversity and allelic richness was also calculated according to Petit et al. (1998) (Table 4). Some contributions were negative, either because the diversity of the population is lower than the mean diversity or because the population is not very divergent. As in the Weitzman approach, the breeds with the greatest contribution to total diversity were the two pony breeds, POT and JN. The main contributions in terms of total allelic richness were from POT and EHMZ. So the greatest allelic richness and diversity were not always in the same population. Lastly, we calculated the loss or gain of diversity when each breed was removed using the approach of Caballero and Toro (2002). As in the previous methods, the pony breeds represented the major loss of diversity, but after weighting for size, the EHMZ breed makes the same contribution as JN.

Discussion

Genetic Variation

This study presents a genetic analysis of microsatellites markers in four autochthonous horse breeds with different morphological and production characteristics, and different degrees of admixture with other breeds. A benefit of using the chosen set of markers was that they were included in the list recommended by the FAO for diversity studies (Cothran EG, personal communication), thus our data could then be compared to those of other breeds that have influenced them the most.

The variability detected in POT and JN was very similar to that found in these breeds by Cañón et al. (2000). Overall, the levels of variability in these breeds were slightly higher than those estimated for other European or American breeds. For the four breeds, mean H_e was 0.736 ± 0.027. Although this high variability should be viewed with caution because the microsatellites were chosen for their polymorphism, the estimates can be used for comparison with other breeds. With the same set of microsatellites, the mean of 10 American breeds was $H_e = 0.694 \pm 0.032$ (Colling and Kelly 1996) and the mean of the Kladruber horse was $H_e = 0.6545 \pm 0.015$ (Horin et al. 1998). With a set of 26 microsatellites, the mean of four Norwegian breeds was $H_e = 0.641 \pm 0.060$ (Bjørnstad et al. 2000). Our results indicated a statistically

Table 4. Comparison of Weitzman's (1992) loss of diversity, contribution to total diversity and total allelic richness by Petit et al. (1998), and loss of diversity by Caballero and Toro (2002)

Breed	Weitzman	C_D	C_S	C_T	C_{DR}	C_{SR}	C_{TR}	Loss/gain (Caballero and Toro)	Loss/gain (Caballero and Toro) (w)[a]
Pottoka	−46	−0.150	1.10	0.96	10.24	3.13	13.30	−1.7	−1
Jaca Navarra	−38	0.097	3.72	0.47	−9.20	−0.21	−9.46	−0.6	−0.7
Burguete	−33	0.679	−1.60	−0.90	−11.29	−3.08	−14.36	1.3	4.5
EHMZ	−17	−0.600	0.00	−0.50	11.00	0.15	11.21	−0.2	−0.7

All values are percentages.

C_D, divergence from the remaining populations; C_S, within-population diversity; C_T, contribution of each population to total diversity; C_{DR}, divergence from the remaining populations; C_{SR}, within-population diversity; C_{TR}, contribution of each population to total allelic richness.

[a] Weighted by N_e. N_e was estimated from the population data in Rodero (2001).

significant ($P < .05$) higher level of variability in the four Basque-Navarrese semiferal native populations than in the other horse breeds that we used for comparison. In cattle, most commercial breeds present a high genetic uniformity as a consequence of management and breeding strategies (Giovambattista et al. 2001). It would appear that in horses, local breeds raised by traditional methods display higher genetic diversity than more highly selected or specialized breeds. The importance of maintaining genetic diversity in domestic species is related to the need for introducing genetic improvements into particular selected breeds and to facilitate rapid adaptation to potential changes in breeding goals (Notter 1999).

Several authors, including Garcia-Dory (1987), have suggested that population sizes of various horse breeds declined appreciably in the 19th and early 20th centuries. Although a reduction in variability might have been expected in POT and JN, no such effect was detected in our study. As discussed by Luikart et al. (1998), a bottlenecked population might not have a distorted allelic distribution for the following reasons: (1) the bottleneck was not recent or narrow enough to be detectable; (2) not enough polymorphic loci or individuals were sampled to provide sufficient power to detect a bottleneck; (3) the individuals sampled were not representative of the bottlenecked population; (4) a demographic bottleneck occurred, but not a genetic bottleneck; and (5) the bottlenecked population was not completely isolated and contained genes from immigrants that obscured the genetic effects of the bottleneck. The first scenario in POT and JN, and the last in JN are the most probable.

To gain further insights into the distribution of diversity, we calculated F statistics at different levels. F_{ST} estimates in POT and EHMZ showed significant differentiation between subpopulations, indicating that the within-breed diversity was high in these two breeds. The degree of genetic differentiation and the significance of the between-population F_{ST} estimates indicate low gene flow, and hence a relatively high reproductive isolation, mainly between Pottoka and the other breeds. Taking into account the four native breeds, the proportion of genetic variation attributed to the differences between them would be about 1.5% of the total variation, with the remainder corresponding to differences between individuals. Although this value was lower than the 8% obtained by Cañon et al. (2000) for Celtic horses and the 12% obtained by Bjørnstad et al. (2000) in Norwegian horses, it was still highly significant.

Breed Relationships

The neighbor-joining tree, as well as the correspondence analysis, showed a clear subdivision of the meat and the pony breeds. Interestingly, in the populational trees, JN was more closely related to POT, but appeared to stand in an intermediate position between this and the meat breeds. This could be explained by the fact that there has been some influence from other breeds in recent years, but not to the same extent as in the meat breeds. If we consider that the breed that has been least affected by admixture is POT, we might assume that this breed is the descendent of the original horse population of the area, and that the others have gradually diverged from it through various mechanisms, including introgression from outside breeds. Under this hypothesis, the closest breed to POT would be JN and the most distant would be BU. This genetic differentiation should also be appreciable in morphological terms. In the study by Jordana and Parés (1999), based on morphological data of breed prototypes, both POT and JN were placed in the Tarpan group together with other light pony breeds such as Asturcón, Gallego, Sorraia, Garrano, Jaca Soriana, and Losino. However, in terms of morphology, JN is somewhat larger than POT, though not as large as EHMZ or BU (Pérez KG, personal communication).

The results of the cluster analysis for comparison of the Basque-Navarrese horses with other breeds showed that the French breeds were closest; in fact, the draft breeds were improved by crossbreeding with French breeds in the first half of the 20th century. Even so, these formed an independent cluster and were not intermingled with the Basque-Navarrese breeds, which maintained a genetic makeup that is clearly differentiated.

Diversity for Conservation

The biological unit for conservation in domesticated animals is usually the breed. Obtaining information from molecular markers in these four breeds made it possible to create a hypothetical scenario for assessing different methods of analyzing diversity for conservation.

First, we applied a genetic distance method, such as the Weitzman function of diversity, on the four native breeds. From this analysis, it was clear that most of the diversity was present in the pony breeds. However, by summarizing breed diversity to a single genetic distance measure, the Weitzman approach fails to account adequately for the diversity and geographical structure that can be found within some breeds (Bruford et al. 2003). Taking into account the high within-breed diversity detected in meat breeds, we applied the partition of diversity of Petit et al. (1998). In this study, comparison of the Weitzman (1992) and Petit et al. (1998) approaches related to heterozygosity showed similar results, but not to allelic richness. Although the pony breeds presented the highest values for contribution of each population to the total diversity ($C_T = 0.96$ and $C_T = 0.47$ for POT and JN, respectively), the intrabreed variability of EHMZ was reflected in the highest value of divergence from the remaining populations (C_{DR}) and the second highest value for the contribution of each population to total allelic richness ($C_{TR} =$ 13.3 for POT and $C_{TR} = 11.21$ for EHMZ). From these data, it seems that further study is needed to determine the relative value of the diversity measures for defining priorities based on within-population variability. Moreover, as in previous studies in other species (Carvajal-Carmona et al. 2003; Giovambattista et al. 2001), this study emphasized the importance of considering population diversity and genetic structure within breeds as an essential component for assessing genetic resources.

Apart from their high genetic variability, we put forth three other reasons for conservation of these native breeds. The first is their cultural interest, as suggested by Ruane (1999). The cultural and historical value of the breeds may be considered, as they represent a strong link to the past in the Basque country. Second, the two meat breeds are of economic interest in that they make maximum use of the natural environment and help to maintain rural populations (Legarra et al. 2000). Third, all four breeds are of scientific interest. Even though not much is known about horse domestication in western Europe, it can be assumed that horse domestication occurred there (Bökönyi 1984). In fact, it has been suggested that horse domestication on the Iberian Peninsula occurred as early as 4500 BC (Jansen et al. 2002). Thus further analyses of these horse breeds with other molecular markers such as mitochondrial DNA or Y chromosome markers might provide relevant information regarding the origin and evolution of the domestic horse in Europe.

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