Assessing losses of genetic variability in the endangered Mallorquí horse

I. Álvarez[1], L.J. Royo[1], L. Pérez-Pardal[1], I. Fernández[1], L. Payeras[2], F. Goyache[1]

[1]Área de Genética y Reproducción Animal, SERIDA-Deva, Gijón (Asturias), Spain
[2]AECABMA, Inca (Mallorca), Spain

ABSTRACT: Information contained in the studbook of the endangered Mallorquí horse was analysed to assess the genetic variability of the breed. Also, the effect of selection for coat colour variation was assessed using a set of 15 microsatellites genotyped on 68 Mallorquí reproductive individuals previously diagnosed for the presence of the chestnut allele on the melanocortin-1 receptor gene. Mean inbreeding for the whole pedigree (310 individuals) was 2.5% while that for the individuals born during the last three years of recording (reference population) was 4.7%. Only 70% of all the founders have genetic representation in the reference population. A comparison of the parameters such as effective number of founders, effective number of ancestors and founder genome equivalents documented that the losses of founder genes occurred very soon after the implementation of conservation strategies. The parameter F_{IS} computed from genealogical information was positive (0.029) for the whole population and negative (–0.024) for the reference population, thus illustrating the effort of the Mallorquí horse breeders to avoid matings between relatives in later generations. A total of 14 individuals were heterozygotes for the chestnut allele (allele frequency of 10.6%). The rejection for reproduction of the chestnut heterozygote individuals would not affect the overall gene diversity of the population. However, the total allelic richness would decrease both at the within-subpopulation (1.2%) and total contribution level (0.4%). The chestnut heterozygote individuals are a within-breed reservoir of rare alleles that should be preserved to avoid risks for the future viability of the breed.

Keywords: conservation; genealogies; selective decisions; Mc1r; gene diversity; allelic richness

The Mallorquí breed is an extremely endangered horse population bred in the Mallorca Island (Balearic Islands, Spain). Probably related with the also endangered Menorquí horse and the extinct Catalonian horse, the Mallorquí population suffered a dramatic bottleneck during the second half of the 20th century due to the import into the island of Spanish Arab, Spanish Purebred and, particularly, Trotter horses (Llamas et al., 1992; Azor et al., 2007). A recovery programme started in the late 1980's using 5 stallions and 18 mares. The first official studbook was published in 1993. The limited size of the founder population quickly made it impossible to plan matings between unrelated individuals thus affecting the genetic variability of the breed.

Inbred matings caused an increase in the frequency of occurrence of individuals showing recessive chestnut coat colour (see Royo et al., 2008 and Thiruvenkadan et al., 2008 for reviews on the coat colour inheritance). Since the only coat colour allowed in the Mallorquí breed studbook is

Supported by Grant No. MICIN-INIA RZ2008-00010.

solid black, breeders implemented mating policies avoiding the use for reproduction of individuals suspicious to be carriers of the chestnut allele. This actually means a reduction in the number of the individuals available for reproduction, therefore affecting the viability of the population.

There is an increasing interest in the assessment of the genetic diversity in livestock populations (see as recent examples Li et al., 2008 and Kusza et al., 2009). This is particularly true in small horse populations (Royo et al., 2007; Avdi and Banos, 2008). In this research the genealogical information recorded in the Mallorquí horse studbook is analysed to ascertain the genetic variability of the breed. Also, a representative sample of reproductive individuals was genotyped for a set of microsatellites and diagnosed for the presence of the recessive chestnut allele. Losses of genetic variability due to the rejection for reproduction of the individuals carrying the chestnut allele were quantified. Consequences for the conservation programme of the breed are discussed.

MATERIAL AND METHODS

Genealogical analyses

Genealogical information recorded in the Mallorquí horse studbook from its foundation to December 2007 was obtained from the breeders association (AECABMA), comprising 310 individuals (154 males). In order to characterise the present genetic variability of the breed, individuals born in the period 2005–2007 (66) were used as reference population. Those individuals with no known parents were considered as founders.

The following parameters were computed for the whole population and for the reference population: equivalent complete generations (g), number of founders (individuals with no known parents), effective number of founders (f_e), effective number of ancestors (f_a), founder genome equivalents (f_g), computed from the average coancestry of the populations (Caballero and Toro, 2002), F_{IS}, the individual inbreeding coefficient (F), the individual average relatedness coefficient (AR). Generation length (and standard error) was computed for the whole dataset. These parameters are described elsewhere (Goyache et al., 2003; Gutiérrez et al., 2005a; Royo et al., 2007) and were computed using the ENDOG v.4.6 programme (Gutiérrez and Goyache, 2005). See the ENDOG User's Guide for a detailed description of the procedures used.

Molecular analyses

Blood samples from a total of 68 Mallorquí horse reproductive individuals were obtained. Total DNA was isolated by standard procedures (Sambrook et al., 1989). Following previous analyses (Royo et al., 2007) a set of 15 microsatellites (AHT4, AHT5, ASB17, ASB2, CA425, HMS1, HMS2, HMS3, HMS7, HTG4, VHL20, ASB23, HMS6, HTG10 and HTG7) was analysed in all the sampled individuals. These markers are among those selected for the national official parentage test in Spanish horse breeds (Bouzada et al., 2008). The PCR products were electrophoretically separated using an ABI PRISM™ 310 DNA sequencer (Perkin Elmer). Allele sizes were scored against the genScan-500 LIZ size standard (Perkin Elmer) using the GeneMapper™ Software v3.7 (Applied Biosystem).

The presence of two chestnut alleles reported in the literature (Marklund et al., 1996; Wagner and Reissmann, 2000) on the melanocortin-1 receptor gene (MC1r; locus Extension) was tested using the RT-PCR protocol described in Royo et al. (2008). The PCR assays were performed by means of the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using optically clear PCR plates and sealing films (Bio-Rad Laboratories). Primers and dual-labelled allele-specific oligonucleotide probes were designed by Beacon Designer software version 2.0 (Bio-Rad, Barcelona, Spain) and synthesized by Sigma-Genosys (Haverhill, UK) and Eurogentec (Liege, Belgium).

The microsatellites used were described computing the number of alleles per locus, expected heterozygosity (H_e) and polymorphic informative content (PIC) using the MolKin programme (current version v3.0; Gutiérrez et al., 2005b). Also, the parameter F_{IS} was computed at the marker level according to Weir and Cockerham (1984), using the GENEPOP v1.2 programme (Raymond and Rousset, 1995).

For descriptive purposes, the following parameters characterising genetic diversity were computed at the whole population level using also the programme MolKin: observed heterozygosity, Nei's (1987) gene diversity (GD, i.e. expected heterozygosity) and F_{IS} (Nei, 1987). Additionally, molecular

coancestry (f; Caballero and Toro, 2002) and molecular mean kinship (M_k; i.e. the average molecular coancestry of each individual with the rest of the population; Gutiérrez et al., 2005b) were computed at the individual level and, when necessary averaged for the whole genotyped population or for each carrier subpopulation.

Losses of diversity arising when Mallorquí individuals carrying the chestnut allele were rejected for reproduction were assessed according to Caballero and Toro (2002) and Petit et al. (1998) using also the MolKin programme. The Caballero and Toro (2002) methodology uses as criterion the maintenance of the maximum overall Nei's (1987) gene diversity in a population (see formula (5) of Caballero and Toro, 2002, and the MolKin's Users' Guide). Briefly, average GD of a population depends on the within-subpopulation coancestry and the between-subpopulations Nei's minimum distance (1987). This allows to separate the contributions to the total GD due to the within-subpopulation diversity (f_{ii}) and the between-subpopulations genetic distance, being:

$$GD_T = GD_W + GD_B$$

where:

GD_T = total contribution to GD

GD_W = contribution to the within-subpopulation diversity

GD_B = contribution to the between-subpopulations diversity

Petit et al. (1998) used Hurlbert's (1971) rarefacted (i.e. corrected for sampling size) number of alleles per locus (k) to asses the contribution of the i^{th} subpopulation to the total allelic richness as:

$$C_t^g(i) = \frac{k_T^g - k_{T\backslash 1}^g}{k_T^g - 1}$$

where:

k_T^g = Hurlbert's (1971) estimator of the total allelic richness in the whole analysed population

$k_{T\backslash 1}^g$ = estimator of the total allelic richness when the i^{th} subpopulation is excluded

The contribution to total allelic richness of a subpopulation due to its divergence can be obtained as:

$$C_t^g(i) = \frac{1}{n}\left(\frac{k_i^g - k_{S\backslash i}^g}{k_T^g - 1}\right)$$

and the contribution of this subpopulation due to its own allelic richness ($C_D^g(i)$)simply by the difference

$$C_D^g(i) = C_T^g(i) - C_S^g(i)$$

Table 1. Genealogical parameters characterising genetic variability in the whole Mallorquí horse population and in the reference population defined

Parameter	Population	
	whole	reference
Total number of animals	310	66
Animals with unknown parents (founders)	27	19
Mean inbreeding (F)[1]	2.5	4.7
Mean average relatedness (AR)[1]	10.1	11.2
Equivalent complete generations (g)	1.8	2.4
Effective number of founder animals (f_e)	13	11
Number of ancestors explaining 100%	26	25
Number of ancestors explaining 50%	5	4
Effective number of ancestors (f_a)	12	11
Founder genome equivalents (f_g)	10	7
F_{IS}	0.029	–0.024

[1]in percentage

RESULTS

The main genealogical parameters characterising the genetic variability in the Mallorquí horse breed are given in Table 1. The mean *F* of the reference population (4.7%) was roughly a double of that of the whole population (2.5%) as a result of cumulated generations (4.7 equivalent generations vs. 2.5 for the whole dataset). However, the mean *AR* values were very close: 11.2% for the younger individuals and 10.2% for the whole pedigree. Only 70% of all founders have genetic representation in the reference population. Although the effective numbers of founders and ancestors are roughly the same, the parameter founder genome equivalents (f_g) took a lower value in the reference population (7) than in the whole dataset (10). The mean generation interval for the breed was 11.1 (±0.4) years. The parameter F_{IS} was positive for the whole population and negative for the reference population.

Table 2 gives information on the polymorphism of the genotyped markers. Twelve out of 15 markers had 6 or more alleles per locus and 14 and 13 markers had, respectively, expected heterozygosity and PIC values above 0.6. Overall, the microsatellite set typed can be considered useful to obtain sound assessments of genetic diversity.

Observed and expected heterozygosities for the genotyped population were 0.753 and 0.723, respectively. The parameter F_{IS} computed from microsatellite information was –0.055. Across loci, 103 different alleles were identified.

A total of 14 individuals (3 males and 11 females) were heterozygotes for the chestnut allele (allele frequency of 10.6%) identified by Marklund et al. (1996). The chestnut allele identified by Wagner and Reissmann (2000) was not present in the genotyped population. Across microsatellite loci, 100 different alleles were identified in the homozygous black subpopulation while only 83 were identified in the chestnut heterozygote subpopulation. Average M_k within the heterozygote subpopulation was lower than in the non-carrier subpopulation (0.266 vs. 0.279) showing that, as a whole, the heterozygote genotypes are less represented in the Mallorquí horse genetic background.

Table 3 gives the losses of Nei's gene diversity and rarefacted allelic richness occurring after the removal of the chestnut heterozygote subpopulation. This would resemble a scenario in which heterozygous individuals were rejected for reproduction. Note that positive contributions to diversity assessed by Caballero and Toro's (2002) method mean that the remaining dataset increases the overall diversity and, consequently, the assessed population would not be preferred for conservation. On the contrary, positive contributions to diversity assessed by Petit's et al. (1998) method mean that the remaining dataset has a lower number of alleles than the original one and, therefore, the assessed population would be preferred for conservation. The removal of the chestnut heterozygote individuals increased the gene diversity of the remaining population at the between–subpopulations level (–1.4%) leading to the negative total contribution to gene diversity (–1.1%). Contributions of the chestnut heterozygote individuals to allelic richness showed a different pattern: if carrier individuals were rejected for reproduction, the allelic richness of the remaining population would decrease both at the within-subpopulation (1.2%) and total contribution level (0.4%). The removal of the homozygote black subpopulation would decrease the overall gene diversity to a large extent (–1.4% for the total contribution) but it would increase the allelic richness of the remaining population (–2.1%).

Table 2. Number of alleles per marker (*n*), chromosome location (Chr), gene diversity (*GD*, i.e. expected heterozygosity), polymorphic informative content (PIC), and F_{IS} values per marker in the analysed dataset

Marker	*n*	Chr	*GD*	PIC	F_{IS}
AHT4	8	24	0.824	0.800	–0.010
AHT5	5	8	0.753	0.709	–0.086
ASB17	6	2	0.579	0.518	0.042
ASB2	8	15	0.658	0.622	–0.051
CA425	8	28	0.768	0.731	–0.098
HMS1	6	1	0.576	0.532	0.049
HMS2	7	10	0.733	0.689	–0.0673
HMS3	7	9	0.781	0.752	–0.047
HMS7	7	1	0.745	0.704	0.040
HTG4	6	4	0.804	0.775	–0.054
VHL20	8	30	0.738	0.707	–0.029
ASB23	8	3	0.706	0.659	0.090
HMS6	4	4	0.643	0.568	–0.106
HTG10	10	21	0.826	0.804	–0.008
HTG7	5	4	0.714	0.659	–0.146

Table 3. Losses of diversity (in percentage) assessed by means of Nei's gene diversity and rarefacted allelic richness, occurring after the removal of the chestnut heterozygote subpopulation; losses arising after the removal of the homozygote black subpopulation are also given to facilitate the interpretation of the main results

Removed subpopulation	*GD*	gGD_W	gGD_B[1]	gGD_T	C_W^{28}	C_B^{28}	C_T^{28}
Chestnut heterozygote	0.716	0.3	–1.4	–1.1	1.2	–0.9	0.4
Homozygote black	0.705	–1.2	–1.4	–2.6	–1.2	–0.9	–2.1

GD = Nei's gene diversity of the remaining set after excluding the breed; gGD_W = contribution to within-population *GD*; gGD_B = contribution to between-populations *GD*; gGD_T = total contribution to *GD*; C_W^{28} = contribution to within-population allelic richness after rarefaction to 28 copies; C_B^{28} = contribution to between-populations allelic richness after rarefaction to 28 copies; C_T^{28} = total contribution to allelic richness after rarefaction to 28 copies

[1]corresponding to Nei's minimum distance

DISCUSSION

Parameters characterising the concentration of gene origins show that the viability of the analysed genetic stock is highly endangered. At the whole population level, the ratio between f_e, f_a and f_g with the actual number of founders was 48, 41 and 37%, respectively, showing that roughly 60% of the founder genes have been lost after the implementation of the purebred matings associated to the recovery programme of the breed. The close values for f_e, which accounts for the loss of genetic variability from unequal founder contributions, and f_a, which accounts for the losses of genetic variability due to bottlenecks, illustrate that losses of founder genes occurred very soon after the implementation of conservation strategies. This scenario, which was previously reported in other highly endangered livestock breeds (Royo et al., 2007; Álvarez et al., 2008), is also pointed out by the values of f_e and f_a computed for the reference population. These are basically the same, although the actual number of founders giving genes to that subpopulation is substantially lower than that for the whole population. In any case, the parameter f_g, which characterises the genetic diversity in the present population if the founders were equally represented and had lost no alleles, had the lower values, particularly for the reference population. This illustrates that a dramatic loss of variability may occur when the present-time younger individuals take the major role for reproduction.

Genealogical analyses also reflect the effort of the Mallorquí horse breeders to avoid matings between close relatives. Although the F_{IS} value computed from genealogies for the whole population is positive (0.029), that computed for the reference population is negative (–0.024). Genealogical F_{IS} characterises the departure of the random mating of a population: negative F_{IS} value means that the average *F* value within a population does not exceed the between-individuals coancestry, thus indicating that matings between relatives are avoided (Caballero and Toro, 2000; Gutiérrez et al., 2005a; Álvarez et al., 2008). Moreover, the average *AR* values computed for the whole dataset and for the reference population do not exceed twofold the value of *F*. In an ideal scenario with random matings and no population subdivision, *AR* would be approximately twofold average inbreeding of the next generation (Goyache et al., 2003; Gutiérrez et al., 2003).

The need of avoiding inbreeding has not led to an exceptional increase in the generation length in the Mallorquí horse. The generation length computed for the Mallorquí horse is within the range recently reported by Cervantes et al. (2009) for three Spanish horse breeds: 12.0 ± 0.13, 10.1 ± 0.16 and 11.4 ± 0.21 years for Spanish Anglo-Arab, Hispano-Arab and Spanish Sport horse breeds, respectively. Like the Mallorquí breed, the three listed breeds are used for saddle. The long generation intervals usually reported in the horse (see Valera et al., 2005 for a review) are basically dependent on its recreational use which is not compatible with pregnancy and breeding life.

Selection for coat colour could increase the risk of losses of genetic variability within the Mallorquí horse breed. The two methods assayed here to quantify the contributions to total diversity of the defined subpopulations (Petit et al., 1998; Caballero and Toro, 2002) are based on variances and may

consequently yield negative contributions to total diversity (Ollivier and Foulley, 2005), regardless they could be interpreted as favourable or not. In fact, when a group of individuals is removed from a dataset, the remaining genetic variability could increase if the genetic background of the removed individuals is still represented in the conserved group. From a theoretical point of view (Caballero and Toro, 2002), the remaining genetic variability could increase as long as the removed individuals can be substituted by other more appropriate (diverse) ones. Rejection for reproduction of the chestnut heterozygote Mallorquí individuals does not affect the allelic frequencies of the remaining population to a large extent and, therefore, overall gene diversity would not be affected and even it would increase. On the contrary, the present balance of allelic frequencies is highly affected by the removal of the homozygote black Mallorquí individuals that are roughly 80% of those genotyped. However, the average number of alleles per locus would be affected by the removal of the individuals carrying the chestnut allele. Allelic richness is an important parameter in conservation genetics because it informs on the long-term evolutionary potential of a population (Petit et al., 1998). The rejection for reproduction of the chestnut heterozygote individuals would cause a loss of rare alleles while the removal of the homozygous black individuals would not (Table 3). These rare alleles are at a very low frequency at the whole population level (ranging from 0.7 to 1.5%) and, therefore, their loss would not affect the balance of allelic frequencies assessed by the method of Caballero and Toro (2002). However, Petit's et al. (1998) method assesses the uniqueness of the removed population with respect the remaining one. Alleles carried by the homozygous black individuals are also present in the chestnut heterozygous subpopulation and their removal does not have any detrimental effects on allelic richness. Overall, results highlight that the chestnut heterozygote individuals are also a within-breed reservoir of rare alleles that should be preserved. In this respect, the implementation of mating policy aimed to obtain black-coated foals from the chestnut allele carrier individuals would be advisable to avoid risks for the future viability of the breed. In summary, we have characterised here the genetic scenario in which the conservation programme of the Mallorquí horse breed is developed. Also, losses of diversity due to mating policies aimed to homogenize coat colour in the breed have been quantified. Although theoretical approaches show that the maximisation of gene diversity tends to maximise allelic richness (Caballero and Toro, 2002), methods quantifying diversity based on each of these parameters do not actually give the same but complimentary information that may be used in conservation strategies. A smart use of coat colour variation has recently been shown to be an interesting tool for the preservation of genetic variability in a genetic stock (Druml et al., 2009). The implementation of mating policies for the maintenance of the genetic background represented by the heterozygote chestnut individuals in the Mallorquí horse breed would be justified.

Acknowledgement

The authors thank to the Mallorquí horse breeders association (AECABMA) for its collaboration.

REFERENCES

Álvarez I., Royo L.J., Gutiérrez J.P., Fernández I., Arranz J.J., Goyache F. (2008): Relationship between genealogical and microsatellite information characterising losses of genetic variability: empirical evidence from the rare Xalda sheep breed. Livestock Science, 115, 80–88.

Avdi M., Banos G. (2008): Genetic diversity and inbreeding in the Greek Skyros horse. Livestock Science, 114, 362–365.

Azor P.J., Valera M., Gómez M.D., Goyache F., Molina A. (2007): Genetic characterization of the Spanish Trotter horse breed using microsatellite markers. Genetics and Molecular Biology, 30, 37–42.

Boichard D., Maignel L., Verrier E. (1997): The value of using probabilities of gene origin to measure genetic variability in a population. Genetics Selection Evolution, 29, 5–23.

Bouzada J.A., Lozano J.M., Maya M.R., Ossorio B., Trigo A., Estévez M., Mayoral T., Anadón E., Gómez-Tejedor C. (2008): Identificación genética y control genealógico equinos mediante secuencias de microsatélites de ADN. ITEA, 104, 155–249.

Caballero A., Toro M.A. (2002): Analysis of genetic diversity for the management of conserved subdivided populations. Conservation Genetics, 3, 289–299.

Cervantes I., Gutiérrez J.P., Molina A., Goyache F., Valera M. (2009): Genealogical analyses in open populations: the case of three Arab-derived Spanish

horse breeds. Journal of Animal Breeding and Genetics, 126, 335–347.

Druml T., Baumung R., Sölkner J. (2009): Pedigree analysis in the Austrian Noriker draught horse: genetic diversity and the impact of breeding for coat colour on population structure. Journal of Animal Breeding and Genetics, 126, 348–356.

Goyache F., Gutiérrez J.P., Fernández I., Gómez E., Álvarez I., Díez J., Royo L.J. (2003): Monitoring pedigree information to conserve the genetic variability in endangered populations: the Xalda sheep breed of Asturias as an example. Journal of Animal Breeding and Genetics, 120, 95–103.

Gutiérrez J.P., Goyache F. (2005): A note on ENDOG: a computer program for analysing pedigree information. Journal of Animal Breeding and Genetics, 122, 357–360.

Gutiérrez J.P., Altarriba J., Díaz C., Quintanilla R., Cañón J., Piedrafita J. (2003): Pedigree analysis of eight Spanish beef cattle breeds. Genetics Selection Evolution, 35, 43–64.

Gutiérrez J.P., Marmí J., Goyache F., Jordana J. (2005a): Pedigree information reveals moderate to high levels of inbreeding and a population genetic structure in the Catalonian donkey breed. Journal of Animal Breeding and Genetics, 122, 378–386.

Gutiérrez J.P., Royo L.J., Álvarez I., Goyache F. (2005b): MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. Journal of Heredity, 96, 718–721.

Hurlbert S.H. (1971): The non concept of species diversity: a critique y alternative parameters. Ecology, 52, 577–586.

Kusza S., Gyarmathy E., Dubravska J., Nagy I., Jávor A., Kukovics S. (2009): Study of genetic differences among Slovak Tsigai populations using microsatellite markers. Czech Journal of Animal Science, 54, 468–474.

Li J.Y., Chen H., Lan X.Y., Kong X.J., Min L.J. (2008): Genetic diversity of five Chinese goat breeds assessed by microsatellite markers. Czech Journal of Animal Science, 53, 315–319.

Llamas J, Castelló J.I., Antón D., García J. (1992): La Cría Caballar en Españа. ed. Darley S.A., Barcelona, Spain.

Marklund L., Johansson Moller M., Sandberg K., Anderson L. (1996): A missense mutation in the gene for melanocyte-stimulating hormone receptor (*MC1R*) is associated with the chestnut coat color in horses. Mammalian Genome, 7, 895–899.

Nei M. (1987): Molecular Evolutionary Genetics. Columbia University Press, New York, 512 pp.

Ollivier L., Foulley J.L. (2005): Aggregate diversity: New approach combining within- and between-breed genetic diversity. Livestock Production Science, 95, 247–254.

Petit R.J., El Mousadik A., Pons O. (1998): Identifying populations for conservation on the basis of genetic markers. Conservation Biological, 12, 844–855.

Raymond M., Rousset F. (1995): GENEPOP (Version 1.2): Populations genetic software for exact test and ecumenicism. Journal of Heredity, 86, 248–249.

Royo L.J., Álvarez I., Gutiérrez J.P., Fernández I., Goyache F. (2007): Genetic variability in the endangered Asturcón pony assessed using genealogical and molecular information. Livestock Science, 107, 162–169.

Royo L.J., Fernández I., Azor P.J., Álvarez I., Pérez-Pardal L., Goyache F. (2008): A novel method for routine genotyping of horse coat color gene polymorphisms. Journal of Animal Science, 86, 1291–1295.

Sambrook J., Fritsch E.F., Maniatis T. (1989): Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Thiruvenkadan A.K., Kandasamy N., Panneerselvam S. (2008): Coat colour inheritance in horses. Livestock Science, 117, 109–129.

Valera M., Molina A., Gutiérrez J.P., Gómez J., Goyache F. (2005): Pedigree analysis in the Andalusian horse: population structure, genetic variability and influence of the Carthusian strain. Livestock Production Science, 95, 57–66.

Wagner H.J., Reissmann M. (2000): New polymorphism detected in the horse *MC1R* gene. Animal Genetics, 31, 289–290.

Weir B.S., Cockerham C.C. (1984): Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Received: 2009–10–13
Accepted after corrections: 2010–05–11

Corresponding author

Félix Goyache, DVM, PhD., Área de Genética y Reproducción Animal, SERIDA-Deva, C/Camino de los Claveles 604, E-33203 Gijón (Asturias), Spain
Tel. +34 985 19 53 03, fax +34 985 19 53 10, e-mail: fgoyache@serida.org