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SHORT COMMUNICATION

Genetic study of Murgese horse from genealogical data and microsatellites

Elisa Pieragostini¹, Rita Rizzi², Grazia Bramante¹, Andrea Rosati³,
Giovanna Perrotta³, Anna Caroli⁴

¹Dipartimento di Progettazione e Gestione dei Sistemi Agro-zootecnici e forestali.
Università di Bari, Italy

²Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare.
Università di Milano, Italy

³Laboratorio di Genetica e Servizi. Cremona, Italy

⁴Dipartimento di Sanità e Benessere Animale. Università di Bari, Italy

Corresponding author: Prof. Elisa Pieragostini. PROGESA. Facoltà di Agraria, Università di Bari. Via Amendola 165/A, 70126 Bari, Italy - Tel. +39 080 5442842 - Fax: +39 080 5442504 – Email: pierelis@agr.uniba.it

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ABSTRACT

The black or rarely roan Murgese is a mesomorph horse, mainly reared in Apulia, recently selected for the saddle. The first official registry of Murgese was established in 1926. All the existing Murgese horses can be traced back to a small number of founders (46 founder mares and 9 stallions). This work aims to monitor the genetic structure of the actual population by analysing the available genealogical information from 2708 animals and a data-set containing 563 typing records of twelve microsatellites. Inbreeding coefficients were estimated for the whole sample and for the animals born from 1992 to 1999. A total of 23 generations were found. The average inbreeding coefficient was 0.0165 for the last three generations, whereas inbreeding was below 2% in animals born in the 92-99 period. The contribution of founders was unbalanced. The overall F_{IS} coefficient estimation was 0.025 and suggests that mating is generally at random in the population. However, some statistics obtained from this study, i.e. the inbreeding coefficient higher than 0.015 in the 70 animals of the 19th, 20th, and 21st generations, should induce breeders to more attention in planning mating.

Key Words: Horse, Murgese, Genealogical data, Microsatellites, Inbreeding.

RIASSUNTO

ANALISI DELLA VARIABILITÀ GENETICA E DEL LIVELLO DI CONSANGUINEITÀ NEL CAVALLO MURGESE MEDIANTE MARCATORI MOLECOLARI E DATI GENEALOGICI

Il Murgese è un cavallo mesomorfo, nero o raramente roano, allevato principalmente in Puglia e recentemente selezionato per la sella. Il primo registro ufficiale del cavallo Murgese fu stabilito nel 1926. Tutti i cavalli Murgesi attuali risalgono a un ridotto numero di fondatori (46 fattrici e 9 stalloni). In questo lavoro abbiamo analizzato la variabilità genetica della popolazione utilizzando le informazioni genealogiche disponibili e i risultati delle analisi molecolari. L'archivio anagrafico di razza relativo a 2708 animali è stato analizzato per stimare la consanguineità nell'intero campione e negli animali nati tra il 1992 e il 1999. Inoltre, sono stati analizzati i dati molecolari relativi ad un pannello di 12 loci microsatelliti tipizzati in tutti i soggetti registrati a partire dal 1980. Sono state rilevate 23 generazioni. Il coefficiente di consanguineità medio delle ultime 3 generazioni è risultato pari a 0,0165, mentre il livello medio per anno di nascita dal 1992 al 1999 si è mantenuto sotto il 2%. Il contributo degli animali fondatori non è risultato bilanciato. Il valore generale della F_{IS} , calcolato su tutti i loci, è risultato pari a 0,025 e indica che nella

popolazione l'accoppiamento è pressoché casuale. Tuttavia, alcune statistiche ottenute nel presente studio, come i valori di consanguineità superiori a 0,015 nei 70 animali della 19^{ma}, 20^{ma} e 21^{ma} generazione, consigliano una maggiore attenzione nella programmazione degli accoppiamenti.

Parole chiave: Cavallo, Murgeso, Dati genealogici, Microsatelliti, Consanguineità.

Introduction

The black or rarely roan Murgeso is a mesomorph horse, recently selected for the saddle and harness (Gandini and Rognoni, 1996; Silvestrelli, 2001). The history of the Murgeso horse is strictly connected with that of the arid and rocky hills of Apulia, Le Murge, from which it takes its name. This horse has a rustic nature (Buonavolontà and Silvestrelli, 1986) necessary for survival in such a difficult environment characterised by harsh climate (cold in the winter, hot and dry in the summer), poor pastures and the presence of enzootic pathogens.

The official denomination of the breed as well as the first 'individuals' registration were established in 1926. The existing Murgeso horses can be traced back to a founder group constituted by 46 mares and 9 stallions (Buonavolontà and Silvestrelli, 1986). In 1990 the Ministry of Agriculture and Forestry (MAF, Italy) established the Anagraphic Register of the equine populations identifiable as ethnic groups (Gandini and Rognoni, 1996), among which the Murgeso horse.

Focal points in the conservation policy and in the genetic management of the Murgeso are the Regional Institute for the Improvement of Horse Populations (IIP, Foggia), founded in 1926 as Stallion Stud, and the National Association of Murgeso horse and Martina Franca breeders (ANAMF, Martina Franca, TA, Italy), founded in 1948. The Murgeso population has now recovered to more than 1500 breeding animals, with 1080 registered males, 107 stallions, and about 350 fowls (ANAMF – IIP, 2003). Before registration, animals are typed for microsatellite markers, which are nowadays a powerful molecular tool for individual identification, parentage control, and genetic analyses as already shown in the horse species (Marklund *et al.*, 1994; Cañon *et al.*, 2000; Cunningham *et al.*, 2001; Achman *et al.*, 2004).

The knowledge of inbreeding from genealogical data is an important starting point for the

genetic improvement of a breed. Research into the equine specie has been already developed. In Italian Haflinger horse (Gandini *et al.*, 1992), average inbreeding ranged from 1.21 % of 1925-33 to 6.59 % in 1979-87. In Lipizzan horses from eight European studies, the average inbreeding coefficients varied from 8.6 to 14.4% between studies, with an overall mean of 10.8% (Zechner *et al.*, 2002).

Moreover, different studies compared genealogical and molecular information. Dunner *et al.* (1998) proposed the management of Asturcon pony by examining both the studbook and ten microsatellite markers, and assessing the reliability of molecular information in populations where no studbooks exists. Curik *et al.* (2003) carried out an investigation on the relationships between inbreeding, microsatellite heterozygosity, and morphological traits in Lipizzano horses.

This work aims to monitor the genetic variability of the Murgeso horse, by analysing both the genealogical information and the molecular data from microsatellite analysis, as a starting point for a genetic improvement program of the breed which is to be developed in the next future.

Material and methods

Genealogical analyses

Genealogical data were extracted from the Murgeso register, provided by the IIP. After removing conflicting information, the edited genealogical data set included 2708 horses (1058 mares, 157 stallions, 1493 foals), born from 1961 to 1999.

Inbreeding coefficients were estimated using Minbreed program (Gandini and De Filippi, 1998). The inbreeding trend was analysed by birth year for animals born from 1992 to 1999, estimating the average generation interval of the breed equal to 8 years from the genealogical data set. The effective number of founders was also computed.

Microsatellite analysis

A data set containing 563 typing records of a panel of twelve microsatellites used for parentage control (AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, HTG7, LEX03, VHL20) was analysed. The F_{IS} coefficients (Weir and Cockerham, 1984) were estimated for each locus and overall, together with the probability for the true F_{IS} value not to be higher than zero, using the Fstat program (Goudet, 1995).

Results and discussion*Genealogical data*

The pedigree file included 2708 animals, out of which 546 founders (62 sires and 484 mares) and 7 animals with only one parent known. Using the Minbreed program a total of 23 overlapping generations were found. The average number of foals born per year was 55.8, ranging from a minimum value of 1 in 1961 to a maximum value of 141, in 1988.

The actual and equivalent founder numbers were

553 and 55.64. The more balanced the founder contribution, the more the effective number of founders approach the actual number of founders. The difference between these two parameters implies that in the Murgesse not all the founder animals contributed equally to the population.

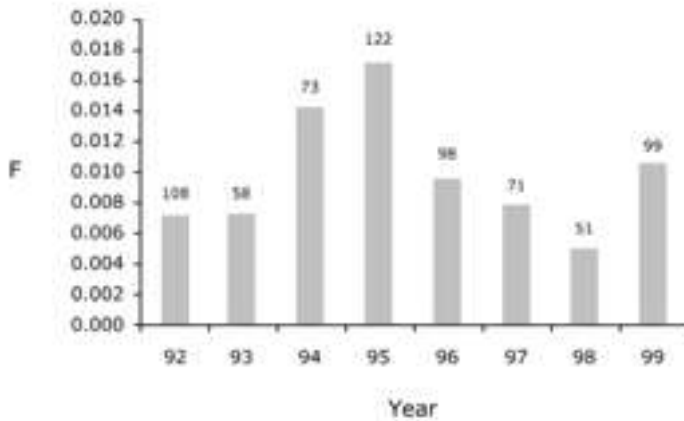
The inbreeding mean values per generation are reported in Table 1. Not considering the 22nd and 23rd generations, with high inbreeding value but involving only 3 animals, the inbreeding trend per generation is rather variable. The mean inbreeding coefficients of generations 19th, 20th, and 21st, including 70 individuals, is 0.0165.

Figure 1 shows the average inbreeding coefficients analysed for birth year in the animals born in the 1992 - 1999 period. The inbreeding was below the 2% level in each year with two peaks of 1.7% and 1% levels in 1995 and 1999, respectively. For this sample the expected contribution of founders is more balanced compared to the whole sample since the actual and effective number of founders were 358 and 58.7, respectively.

Table 1. Inbreeding coefficients (F) and number of individuals (n.) per generation.

Generation	n.	F
2	206	0
3	303	0.005
4	426	0.007
5	276	0.008
6	128	0.020
7	42	0.021
8	14	0.025
9	18	0.003
10	22	0.006
11	16	0.016
12	12	0.006
13	33	0.003
14	98	0.013
15	178	0.012
16	175	0.014
17	101	0.015
18	41	0.007
19	24	0.015
20	36	0.017
21	10	0.018
22	2	0.033
23	1	0.081

Figure 1. Average inbreeding coefficient (F) and number of animals born from 1992 to 1999.

Table 2. F_{IS} statistics calculated for each locus and over all loci and the probability value limits of the coefficients (Fstat program; Goudet, 1995).

Locus	F_{IS}	Probability (F_{IS} not >0)
HTG10	0.0276	0.075 0.070
VHL20	0.0161	0.220 0.200
HTG7	-0.0373	0.925 0.910
HTG4	0.1643	< 0.005
AHT5	-0.0267	0.905 0.885
AHT4	0.0240	0.175 0.140
HMS3	0.0852	< 0.005
HMS6	0.0504	0.020 0.020
HMS7	0.0113	0.420 0.380
LEX03	0.0271	0.075 0.060
HMS2	-0.0170	0.815 0.765
ASB2	-0.0215	0.860 0.840
All loci	0.0251	< 0.005

Microsatellites

The less polymorphic microsatellite was HTG7, with only 4 alleles, whereas the maximum number of 12 alleles was observed for three microsatellites: LEX03, HMS2 e ASB2. The average allele number for microsatellite was 9.58 (SD = 2.61). The F_{IS} statistics and the probability value limits of the coefficients are shown in Table 2. A high variability was found for the F_{IS} statistics among loci. Values ranged from -0.0373 (locus

HTG7) to 0.1643 (locus HTG4). The overall F_{IS} value, among all loci, was significantly higher than zero (0.0251). The F_{IS} values per year of birth (table 2) were close to 0 suggesting that random mating was frequent in the Murgese horse, as a confirmation of the results obtained from genealogical data. However, in 1998 and 1999 F_{IS} values (0.48 and 0.58) were higher than in most of the previous years of birth, possibly indicating a recent increasing trend of the parameter.

Table 3. F_{IS} coefficients and number of individuals (n.) per year of birth.

Year	n.	F_{IS}
1987	30	0.029
1988	32	0.008
1989	43	0.039
1990	28	0.046
1991	30	0.022
1992	48	0.021
1993	63	0.005
1994	42	0.022
1995	56	0.015
1996	70	-0.009
1997	45	0.027
1998	49	0.046
1999	30	0.058

Conclusions

The inbreeding of the Murgese population is at acceptable levels. The equivalent founder number, although unbalanced, was higher than the 50 individuals proposed by Soulé (1978) as a risk level possibly leading to genetic variation loss. In addition, the low F_{IS} values estimated on microsatellite data, indicate that random mating frequently occurs in the breed. However, some statistics obtained from this study, i.e. the inbreeding value higher than 0.015 in the 70 animals of the 19th, 20th, and 21st generations, as well as the higher F_{IS} estimates in 1998 and 1999, should induce breeders to more attention in planning mating.

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