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# Demographic genetics of the endangered Amiata donkey breed

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### ABSTRACT

The demogenetic structure of the Amiata donkey, an endangered breed from Central Italy, was investigated using information from pedigrees. Genealogical data of 602 donkeys reared in Tuscany were recorded in a database and analysed by the computer package ENDOG. Population size increased from 89 subjects in 1995 to 503 (129 males and 374 females) in 2005. Animals were distributed among 152 herds, but the effective number of herds was 21, suggesting that a small number of herds provided stallions for the entire breed. The maximum number of traced generation was 4, the mean maximum generation was 1.14, the mean complete generation was 0.53, and the mean equivalent generation was 0.78. The average relatedness coefficient (AR) in the 503 alive animals was 0.94% while the mean F was 0.29% so the effective population size was 172.41. Among 24 animals with a 4-generation history, 3 (12.5%) were 25% inbred. Although the incompleteness of genealogical information did not permit accurate inference of the current values of population genetic parameters, the present work represents a first step towards an efficient management of the breed.

*Key Words:* Amiata donkey, Local breed, Pedigree analysis, Inbreeding.

### RIASSUNTO

#### DEFINIZIONE DEI PARAMETRI DEMOGRAFICI E GENETICI DELL'ASINO DELL'AMIATA MEDIANTE ANALISI GENEALOGICA.

*L'asino dell'Amiata è una razza autoctona toscana in pericolo di estinzione; attraverso l'analisi delle informazioni derivanti dalle schede di registrazione anagrafica è stata analizzata la struttura demografica della razza. I dati genealogici di 602 asini allevati nel comprensorio della Toscana sono stati archiviati in un database informatizzato ed analizzati mediante il programma ENDOG. Gli effettivi numerici della popolazione sono aumentati da 89 soggetti nel 1995 a 503 (129 maschi e 374 femmine) nel 2005. I capi erano distribuiti in 152 allevamenti, sebbene il numero di allevamenti effettivi fosse pari a 21, ovvero solo un ristretto numero di allevamenti ha fornito gli stalloni impiegati per l'intera popolazione. Il numero massimo di generazioni era pari a 4, il numero mediato tra tutti gli individui era 1,14, il numero medio di generazioni complete era 0,53 ed il numero medio di generazioni equivalenti era 0,71. La parentela media tra i 503 animali vivi è risultata di 0,94% mentre la consanguineità media è risultata pari a 0,29% con un numero effettivo di popolazione*

pari a 172,21. Dei 24 animali con informazioni disponibili su quattro generazioni, 3 (12,5%) presentavano un coefficiente di inbreeding pari al 25%. Sebbene l'incompletezza delle informazioni genealogiche non abbia permesso una stima accurata dei valori attuali per i principali parametri genetici, il presente lavoro costituisce un primo passo nella direzione di una gestione più efficiente e consapevole della razza.

Parole chiave: Asino dell'Amiata, Razza autoctona, Analisi del Pedigree, Consanguineità.

## Introduction

Domestic animals have had profound effects on food production for humankind and on human societies. Today there are roughly 3,000 breeds and breed varieties of the seven major livestock mammalian species (cattle, pig, sheep, goat, horse, donkey and buffalo; Hall and Ruane, 1993). However, a large proportion of them are endangered, i.e., they include < 1,000 breeding females or < 20 breeding males (Scherf, 1995, 2000). In particular, most local breeds of Europe have suffered a critical decrease in numbers, mainly as a consequence of the agriculture industrialization. The donkey was mainly used as a work animal in the Mediterranean area of Europe. Although it has now lost its principal reason for breeding, donkey breeds represent irreplaceable resources as a genetic reservoir of gene pools, and the few remaining endangered populations should be actively preserved.

The use of donkeys as riding and pack animals has been always important in central Italy, because of the landscape characterized by rugged hills. A particular donkey breed was present in the area of the Amiata mountain (a large inactive volcano) at the end of the 19th century, characterized by uniform grey mantle with typical striping of the limbs, slim shape, and withers height up to 140 cm. In the first three decades of the 20th century, the Amiata donkey probably received some genetic contributions from southern breeds, in an attempt to increase its size. However, breeders have generally avoided extensive breeding with outside animals, as the features of the Amiata donkey were already well adapted for use in that territory; therefore, an apparently homogenous population has been preserved, with a well identifica-

ble phenotype. After a bottleneck in the years 1945-1970 due to the advent of rural motorization, the population has started increasing again, because of specific actions carried out both by private and public organizations. More than 200 farms are active today in central Italy and they maintain a population of about 700 registered animals. Some farms are also present in more distant regions. The Anagraphic Register of the Amiata breed was established in 1993, and is maintained by the association of breeders of the Grosseto province (APAG). Being an endangered breed, subjects with an unknown pedigree have been allowed to be recorded, provided that females scored at least 60 and males at least 75 points on morphological evaluation. Recording is still open for females, whereas it has been closed since 1997 for stallions.

The Anagraphic Register is maintained on paper only. The aim of this paper was to establish an electronic database of the Amiata donkeys raised in Tuscany, and to evaluate demographic and genetic parameters of the breed by analysing the available pedigree information.

## Material and methods

The genealogical records of the Tuscan Amiata donkey were collected at APAG in 2005. For each animal, the form included: name, ID code, date of birth, breeding farm, ID codes of both parents, and microchip number. After being entered into an electronic database, genealogical information was used to determine several demographic and genetic parameters, including whole population inbreeding level, mean average relatedness coefficient (AR), effective population size ( $N_e$ ). All calculations were performed using the

Table 1. Herds, with more than six animals, classified according to the origin and use of the herd's stallions.

Herd type	Using foreign father	Own father	Selling father	Number of Herds	% foreign father
Nucleus	Not	Yes	Yes	0	0.00
Multipliers	Yes	Yes	Yes	6	72.80
Multiplier	Yes	Not	Yes	1	100.00
Commercial	Yes	Yes	Not	2	62.50
Commercial	Yes	Not	Not	12	100.00
Isolated	Not	Yes	Not	0	0.00

Table 2. Main genealogical parameters computed for the Amiata donkey pedigree.

Source	n.
Total number of animals	602
Reference population	286
Base population	316
Number of ancestors of the reference population	135
Effective number of ancestors of the reference population	42
Number of ancestors explaining 50% of genetic variability	18
Effective population size of founders	114.87

program ENDOG v3.0 (Gutiérrez and Goyache, 2005; Gutierrez *et al.*, 2005).

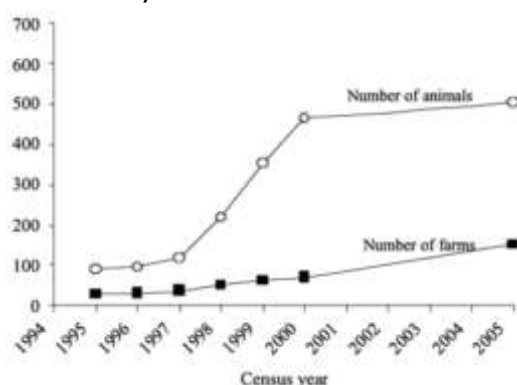
## Results and discussion

### *Demographic data and herd composition*

The total number of registered animals was 602 (174 males and 428 females). Among these, 503 (83.5%) were alive at the time of survey, 374 (74.4%) females and 129 (25.6%) males. The living animals were born between 1980 and 2004 (average births per year = 20.0 heads, peak 51 births in 2001). About 80% of the animals were born in the last ten years. Mean generation lengths were  $7.0 \pm 3.7$  in females ( $6.9 \pm 3.8$  mother-daughter,  $7.1 \pm 3.5$  mother-son), and  $5.9 \pm 2.6$  in males (practically coincident for father-daughter and father-son). The animals were distributed among 152 farms (4.0 heads per farm on

average). Previous data about year-to-year population size and number of farms were available for the period 1995-2000 (Arzilli, 2002). Since Anagraphic Register forms did not specify the death dates of registered animals (they are only classified as "dead" or "alive"), no estimates of year-to-year population size and number of farms were possible for the subsequent period (2001-2004). As seen above, at the time of survey, 503 live animals were present. As shown in Figure 1, the population size increased from 89 heads distributed among 28 farms in 1995 (mean 3.2) to 465 heads distributed among 70 farms in 2000 (mean 6.6). In 2005, most farms (129, or 85%) raised < 5 heads, 16 farms (10.5%) raised 6 to 15 heads, 6 farms (4%) raised 16 to 40 heads, and 1 farm raised 60 individuals. The effective number of farms was 21. A classification of the herds with > 6 animals in

Figure 1 Amiata donkey population size in the course of the last ten years.



terms of their genetic importance for the population was computed by ENDOG (Table 1). Among the 21 herds, more than half (12) were of the “commercial” subtype, i.e., they use only external stallions. Conversely, no herds were of the “nucleus” or “isolated” types, i.e., they do not use external stallions.

#### *Genealogical data*

Out of 602 registered animals, the number of different males being parents of registered animals was 66, and their total number of offspring was 319 (215 females, 104 males), or 4.8 mean offspring per male; the distribution was highly skewed, with ten males producing > 10 offspring (154 in total, 48.3%). On the other hand, the number of dams was 178, with 336 total offspring (1.9 mean offspring per dam); only 25 dams (14.0%) produced > 4 offspring. Table 2 summarizes the main genealogical parameters computed by ENDOG. The number of animals in the reference population (with both parents known) was 286, whereas the number of animals in the base population (one or both unknown parents) was 316. The number of ancestors contributing to the reference population was 135, whereas the effective number of ancestor was 42, or less than a third. In fact, only 18 ancestors were necessary to explain 50% of the present genetic variability in the Amiata donkey. These results point out that only a small number of males are used in reproduction. Moreover, our sample was characterized

by a relatively high number of effective founders (114.87), i.e. animals with unknown parents, which is typical of populations lacking long historical pedigree records (Gutierrez *et al.*, 2003).

Table 3 shows the distribution of the number of animals by the maximum number of traced generations. On one side, 233 animals were founders, whereas on the opposite side 24 animals had at least one of the 16 great-great-great-grandparents known. The mean maximum generation was 1.14, the mean complete generation was 0.53, and the mean equivalent generation was 0.78 (data not shown). The mean F-value of the animals with the maximum number of traced generations was 2.41%; this value resulted from 3 inbred animals with average F of 25% (Table 3). The proportion of inbred animals with genealogy traced 3 generations back was lower (1.69%), but the mean F-value was near 20%. It should be remarked that the generally low mean F and AR values could be more the effect of incomplete genealogical records than a true description of the population.

The mean F in the 503 live animals was 0.29% and the average AR was 0.94%. Using the F value estimated on the 503 live animals we obtained a value of 172.41 for  $N_e$ , and a ratio  $N_e/N$  of 0.343. As already mentioned, however, estimation of  $N_e$  in our population may be affected by the influence of the low quality of the underlying pedigree information. Despite the uncertainties, the estimated value for  $N_e$  shows that our population is in the damaged risk class ( $N_e=100-1000$ ; Scherf, 2000).

#### **Conclusions**

In the present work, a computerized database of genealogical data of the Amiata donkey was established; this represents a first step towards the safeguards and the preservation of the breed. The inbreeding level of the Amiata donkey seems to be rather acceptable, contrary to the expectation for a population

Table 3. Mean inbreeding by maximum generations.

Traced generation	N. animals	Mean F	% inbred	Average F for inbred	AR <sup>a</sup>
0	233	-	-	-	-
1	157	0	-	-	1.14%
2	129	0.48%	2.33%	20.83%	1.72%
3	59	0.42%	1.69%	19.27%	2.51%
4	24	2.41%	12.5%	25.00%	2.99%

<sup>a</sup> Average relatedness coefficient

that has suffered a severe bottleneck in recent years. However, the lack of genealogical records for a substantial number of individuals may have caused a possible underestimation of the inbreeding coefficient. In fact, a substantial increase in the average inbreeding coefficient is observed in those subjects with the maximum number of traced generations.

The relative interruption of the positive trend in the population size for the period 2001 to 2005 compared to the population expansion observed in years 1995 to 2000, together with the small number of males used for reproduction, may represent a serious risk for the conservation of the genetic background of the Amiata donkey, and suggests that further actions should be undertaken to improve the demographic and genetic management of the population.

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