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## Pedigree analysis in the Andalusian horse: population structure, genetic variability and influence of the Carthusian strain

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### Abstract

The studbook of the Andalusian horse comprising a total of 75,389 individuals (6318 of them classified as Carthusians) was analysed in order to ascertain the genetic history of the breed and, specially, to evaluate its genetic variability and the influence of the Carthusian strain in the breed. Although there is no possible way to identify studs acting as Nucleus in the breed, there is a high concentration of genes and individuals' origin. The effective number of studs producing grandfathers was of 10.6. The equivalent number of founders was 1948.5 individuals (370.5 Carthusians). The effective number of founders was 39.6 and the effective number of ancestors 27. Only 6 ancestors were necessary to explain 50% of the genetic variability of the breed. The average values of inbreeding and average relatedness for the whole Andalusian horse population were, respectively, 8.48% and 12.25%. The same values for the Carthusian strain were higher (9.08% and 13.01%) even though the generation interval for the strain was larger than that for the whole population (12.43 versus 10.11 years). In any case, inbreeding in Andalusian horse breed seems to have a remote origin and linked to the Carthusian individuals. The total contribution of the Carthusian founders to the populations accounted for up to 87.64% of the population. In turn the Carthusian ancestors explained 80.46% of the genetic variability of the breed. No differentiation was found between Carthusian and non-Carthusian reproductive individuals using genealogical  $F_{ST}$  (0.000026). It can be concluded that the distinction between Carthusian and non-Carthusian individuals within the Andalusian horse breed does not have genetic support. The unbalanced use of the Carthusian individuals with lower average relatedness values for reproduction is proposed to preserve the genetic variability of the breed.

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### 1. Introduction

The assessment of the within-population genetic variability and gene flow is necessary before the

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implementation of selection programs to establish appropriate management of the genetic stock. Some simple demographic parameters, largely dependent on the management and mating policy, have a large impact on the genetic variability. Additionally, the study of the population structure and demography can highlight important circumstances affecting the genetic history of the population.

The Andalusian (Spanish Purebred) horse is considered the most ancient horse breed in the Iberian Peninsula (Aparicio, 1944). Its importance is more than of national interest, as it is involved in the formation of some other horse breeds such as Lusitano, Lippizan and present native horse American strains. Within the Andalusian studbook, the only recognised strain is the Carthusian. This strain is formed by a small number of individuals descendant from those bred by religious orders since the XVth century (Valera et al., 1998). Breeders preferentially use Carthusian individuals for reproduction thus probably limiting the available genetic variability in the breed.

Andalusian horse breeders are interested in the implementation of a selection program including type (Molina et al., 1999), functional (Koenen et al., 2004) and fertility traits. The aim of this work is to analyse the information of the Andalusian horse studbook to contribute to the knowledge of the structure of the population and to evaluate its genetic variability in terms of inbreeding and genetic representation. The analysis will pay a great deal of attention to the influence of the Carthusian strain in the breed. This analysis will suggest appropriate strategies to monitor matings and manage genetic variability to enlarge the selection basis useful for a selection program.

## 2. Material and methods

Information from all the individuals registered in the Andalusian horse studbook from its foundation in the XIXth century to 31st December of 1998 was available, comprising a total of 75,389 (39,453 females) animals. The Andalusian horse studbook organisation did not register individuals with unknown genealogies after 1913. Andalusian horse breeders associations classify as Carthusian: (a) founder indi-

viduals with particular historical origin; and (b) individuals resulting from matings between Carthusian parents. In consequence, up to 6318 individuals (2869 females) were registered as Carthusian. Up to 22,884 (2947 Carthusians) of the registered animals (5870 stallions, 412 of them being Carthusians) were used for reproduction.

To characterise the structure of the population, the following parameters were analysed:

- Studs contributing with stallions to the population. We followed the criteria proposed by Vassallo et al. (1986) classifying the studs as: (a) nucleus studs, if breeders use only their own stallions, never purchase stallions but sell them; (b) multiplier studs, when breeders use purchased stallions and also sell stallions; and (c) commercial studs that never sell stallions. The methodology we use here is suitable for analysing populations of an unknown structure and enabled us to analyse the data without predefining a nucleus in our population.
- Genetically important studs. This parameter was analysed by the 3 methods proposed by Barker (1957): (1) appearances of the males from a stud as fathers; (2) appearances of each stud in the father-of-stallion line; and (3) total appearances of the reproductive males from a stud in all the possible pathways. An appearance in the parental generation was scored as 4, an appearance in the grandparental generation was scored as 2, and an appearance in the great-grandparental generation was scored as 1.

The pedigree completeness level was characterised by computing:

- Generation lengths. This is the average age of parents at the birth of their useful offspring. We computed this for the 4 pathways (father–son, father–daughter, mother–son and mother–daughter) using birth dates of registered animals together with those of their fathers and mothers.
- Number of traced generations. We computed this as those generations separating the offspring of its furthest known ancestor in each path. Ancestors with no known parent were considered as founders (generation 0).

- Number of equivalent generations. This was computed as the sum of  $(1/2)^n$  where  $n$  is the number of generations separating the individual to each known ancestor.

To characterise the genetic variability of the population we analysed the following parameters:

- Effective number of founders ( $f_e$ ) (Lacy, 1989), defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population under study. This is computed as:  $f_e = 1 / \sum_{k=1}^f q_k^2$  where  $q_k$  is the probability of gene origin of the  $k$ th founder and  $f_e$  the real number of founders.
- Effective number of ancestors ( $f_a$ ). This is the minimum number of ancestors, not necessarily founders, explaining the complete genetic diversity of a population (Boichard et al., 1997). Parameter  $f_a$  does not fully account for gene loss by drift from the ancestors to a reference population but complements the information offered by the effective number of founders accounting for the losses of genetic variability produced for the unbalanced use of reproductive individuals producing bottlenecks. To compute  $f_a$  one must consider only the marginal contribution of an ancestor, which is the contribution made by an ancestor that is not explained by other ancestors chosen before.
- Individual inbreeding coefficient ( $F$ ). This is defined as the probability that an individual has two genes identical by descent (Wright, 1931).
- The average relatedness coefficient (AR) of each individual (Gutiérrez et al., 2003; Goyache et al., 2003). The average relatedness (AR) coefficient of each individual is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal. AR can then be interpreted as the representation of the animal in the whole pedigree regardless of the knowledge of its own pedigree and, numerically, is twice the probability that two random alleles, one from the animal and the other from the population in the pedigree (including the animal), were identical by descent. The AR coefficient for each individual in the pedigree is computed as the average of the coefficients in the row correspondent to the individual in the numerator relationship

matrix **A**. Notice that both alleles from the individual are included in the population. Thus, the AR coefficient accounts simultaneously for the coancestry and inbreeding coefficients. We can calculate AR for the founder animals by assigning to each individual a value of 1 for its belonging to the population, 1/2 for each son the animal has in this population, 1/4 for each grandson and so on, and weighting by the size of the population. Obviously, the addition of the scores across founder animals is the total size of the population.

Structure of population was assessed from genealogical information by means of  $F$  statistics (Wright, 1931) for each known generation and each defined subpopulation. Wright's  $F$  statistics have been computed following Caballero and Toro (2000). These authors have formalised the pedigree tools necessary for the analysis of genetic differentiation in subdivided populations starting on the average pairwise coancestry coefficient ( $f_{ij}$ ) between individuals of two subpopulations,  $i$  and  $j$ , of a given metapopulation including all  $N_i \times N_j$  pairs. For a given subpopulation  $i$ , the average coancestry, the average self-coancestry of the  $N_i$  individuals and the average coefficient of inbreeding would be, respectively,  $f_{ii}$ ,  $s_i$ ,  $F_i = 2s_i - 1$ . Finally, the Wright's (1931)  $F$ -statistics are obtained as  $F_{IS} = \frac{\bar{F} - \bar{f}}{1 - \bar{f}}$ ,  $F_{ST} = \frac{\bar{f} - \bar{f}}{1 - \bar{f}}$ , and  $F_{IT} = \frac{\bar{F} - \bar{f}}{1 - \bar{f}}$ , where  $\bar{f}$  and  $\bar{F}$  are, respectively, the mean coancestry and the inbreeding coefficient for the entire metapopulation, and,  $\bar{f}$  the average coancestry for the subpopulation so that  $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ .

Most parameters have been computed using the program ENDOG v 3.0 (Gutiérrez and Goyache, 2005). When needed statistical analysis were carried out using the SAS program (SAS/STAT™, 1999).

### 3. Results

#### 3.1. Demographic analysis

Fig. 1 shows the distribution of records during the history of the studbook of the breed. Most individuals (78.9%) were registered from 1980. During the first 5 years of the 1990s the number of registrations represented 28% of the total and from 1995 to 1998

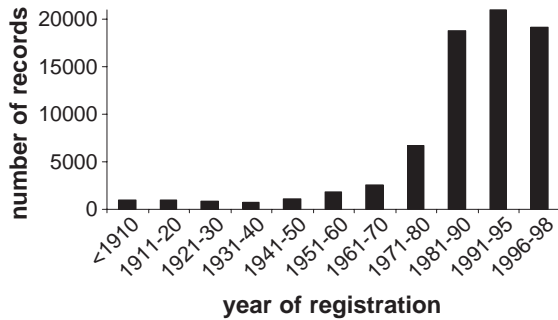


Fig. 1. Number of registrations in the Andalusian horse studbook by periods of 10 years in the analysed dataset.

they reached 25.6%. Individuals were distributed in a total of 7469 studs. From these, roughly 25% had more than 5 individuals registered. Up to 605 registered studs had between 21 and 80 records and only 90 studs had 81 or more records.

Fig. 2 characterises the completeness level of the Andalusian horse studbook by both the percent of

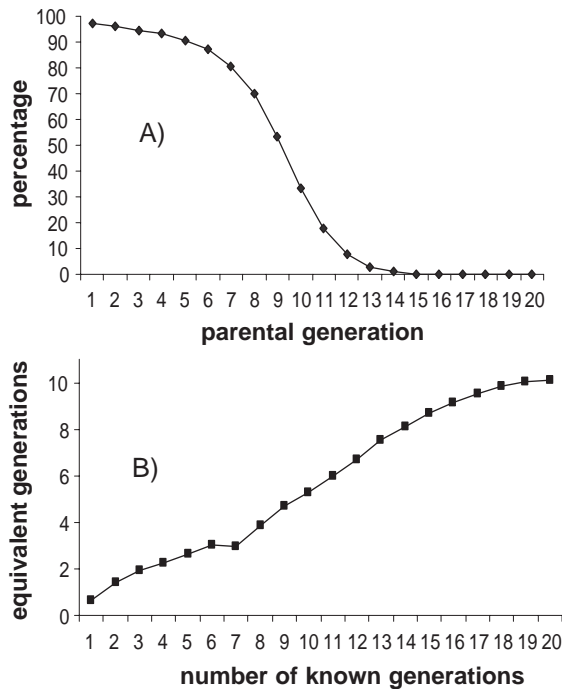


Fig. 2. The completeness level of the Andalusian horse studbook assessed by means of: (A) percent of ancestors known per parental generation, being parental generation 1 that corresponds to parents, 2 that corresponds to grandparents, etc.; and (B) average number of equivalent generations per known generations.

ancestors known per parental generation and the average number of equivalent generations per generation. For the first five generations of ancestors, the pedigree knowledge was higher than 90%. However, it drops dramatically to 80% in the parental generation 7th and to 33% in 10th. After the 11th parental generation the percent of known ancestors is less than 10%. The average number of equivalent generations per generation known shows a gap in the 7th generation. After that, this parameter increases gradually to reach average values around 10 for the 18th generation or later. The combined information of Fig. 2 characterises the difficulties of obtaining genealogies derived from the Spanish Civil War period.

The classification of the Andalusian horse studs according to the origin and use of the stud's stallions (Vassallo et al., 1986) is given in Table 1. No stud can be considered as nucleus. Up to 42.6% of the studs were classified at the multiplier level using their own stallions and both purchasing and selling stallions. The other studs were classified as commercial studs that never sell stallions. No disconnected studs were found.

Average generation interval for the whole pedigree was 10.11 years while this was substantially higher for Carthusians (12.43 years). Table 2 shows generation lengths for the four pathways parent–offspring. All the generation interval pathways for the Carthusian individuals were higher than those computed for the whole Andalusian horse populations. In any case the stallion pathways were very similar but slightly longer than those involving mares.

Table 1

Classification of the studs registered in the studbook of Andalusian horse according to the origin and use of the stud's stallions

Classification	Using purchased stallions	Using own stallions	Selling stallions	Number of herds	Percentage of purchased stallions
Nucleus studs	No	Yes	Yes	0	0
Multiplier studs	Yes	Yes	Yes	421	69.8
Commercial studs	Yes	Yes	No	71	87.6
Disconnected studs	Yes	No	No	996	100
Disconnected studs	No	Yes	No	0	0

Only those herds with more than five records in the Andalusian horse studbook (1859) have been included in this analysis.

Table 2

Generation intervals and standard deviations (SD) for the four pathways parent–offspring for the whole pedigree of the Andalusian horse breed and for the Carthusian strain

Pathway	Whole pedigree		Carthusians	
	N	Years	N	Years
Stallion–son	5474	10.39	640	11.99
Stallion–daughter	15,862	10.36	1457	11.24
Mare–son	5477	9.99	634	11.35
Mare–daughter	15,830	9.80	1470	11.62
Average		10.11		11.50

### 3.2. Concentration of gene and individuals origin

Table 3 shows major information on the concentration of gene and studs origin in the Andalusian horse. Base population was formed by 1465 individuals with unknown parents (280 Carthusian) and 967 additional animals (181 Carthusian) from which only one parent was known. Thus, weighting the unknown parent as half a founder, the equivalent number of founders for the Andalusian horse breed was 1948.5 individuals (370.5 for the Carthusian strain). Total number of studs producing stallions was 954 (12.8% of the total). However, the effective number of studs producing stallions was of 32.9. This parameter is reduced to 10.6 and 7.6 when effective number of studs is computed, respectively, for those producing grandfathers and great-grandfathers, showing a limited concentration of the studs of origin of the animals. The stud contributing the most to the

Table 3

Parameters characterising the concentration of gene and studs origin in the Andalusian horse

Total number of animals	75,389
Animals with unknown parents	1465
Equivalent number of founder animals	1948.5
Effective number of founder animals	39.6
Number of ancestors explaining 100%	331
Number of ancestors explaining 70%	13
Number of ancestors explaining 50%	6
Effective number of ancestors	16.5
Actual and effective (in brackets) number of studs supplying fathers	954 (32.9)
Actual and effective (in brackets) number of studs supplying grandfathers	337 (10.6)
Actual and effective (in brackets) number of studs supplying great-grandfathers	139 (7.6)
Stud contributing the most to the genetic stock	17.0%

population (*Domínguez Hermanos*) had 17% and is a well reputed ‘Carthusian stud’. Seven additional studs contributed between 1.4% and 4% to the genetic stock while the others contributed less than 1%.

The effective number of founders was 39.6. The number of ancestors (founders or not) explaining 100% of the genetic variability of the breed was 331. However, only 6 individuals were necessary to explain 50% of the genetic variability of the breed and 13 to explain 70%. The effective number of ancestors was 27. Reliability of the results obtained using Boichard et al.’s (1997) methodology was tested by re-running the analysis after definition of sequentially bigger reference population. Results were the same across re-analysis.

Table 4 details the 10 ancestors and the 10 founders contributing the most. Most selected founders were born before 1901 while most selected ancestors were born in the first 10 years or the 1920s of the XXth century. The ancestor contributing the most (*Ameri-*

Table 4

Description of 10 ancestors and 10 founders contributing the most to the genetic variability of the Andalusian horse

	Sex	Year of birth	Carthusian	Explained variability	AR
<i>Founder</i>					
Coronel	Male	1850	Yes	1.23	8.19
Peligrosa	Female	1850	Yes		5.64
Ramillete I	Female	1850	Yes		4.29
Principe I	Male	1900	Yes		3.99
Sola.	Female	1900	Yes		3.99
Odiosa	Female	1913	Yes		3.99
Mantequera.	Female	1850	Yes		3.74
Inspirado II	Male	1850	Yes		3.68
Inspirada	Female	1908	Yes	1.37	2.96
Coronel II	Male	1900	Yes		2.81
<i>Ancestor</i>					
Americano	Male	1927	Yes	15.77	
Destinada	Female	1923	Yes	12.62	
Celoso III	Male	1933	Yes	6.27	
Oficiala 1913	Female	1913	Yes	5.94	
Maluso	Male	1949	No	5.37	
Presumido	Male	1921	Yes	4.62	
Zurrona	Female	1919	Yes	3.62	
Rurita 1919	Female	1919	Yes	3.33	
Receloso 1907	Male	1907	Yes	2.85	
Hechicero 1914	Male	1914	Yes	2.77	

Ancestors were selected following Boichard et al. (1997) while founders have been selected by their individual Average Relatedness coefficient (AR).

cano) explained 15.8% of the genetic variability, while founder contributing the most to the gene pool (Coronel) accounted for 8.62%. All the most important founders were Carthusian and the total contribution of the Carthusian founders to the populations totalled 87.64%. Only two founders were selected as ancestors in the 14th (Coronel) and 126th places. Up to 103 of the total number of ancestors selected using the methodology proposed by Boichard et al. (1997) were Carthusian. These 103 animals explained 80.46% of the genetic variability of the breed (including Carthusians). The non-Carthusian ancestor contributing the most (Maluso, selected in the 5th position) accounted for 5.37%.

### 3.3. Inbreeding and genetic representation

The average values of  $F$  and AR for the whole Andalusian horse population were, respectively, 8.48% and 12.25% (Table 5). The same values for the Carthusian strain were 9.08% and 13.01%. Up to 93.4% of the registered individuals are inbred. As a consequence,  $F$  and AR values for inbred animals are only slightly higher than those computed for the whole population. The figures corresponding to males and females were very close and slightly higher for males. Despite Carthusians had shallower pedigrees than the non-Carthusian individuals (reflected in a lower number of generations traced) average  $F$  and AR for the strain are significantly higher than those for non-Carthusians (13.01% and 14.00% versus 8.06% and 12.09% for, respectively,  $F$  and AR).

Fig. 3 shows the trends of inbreeding computed by year of birth of the individuals for the whole pedigree and for Carthusians. In order to distinguish between

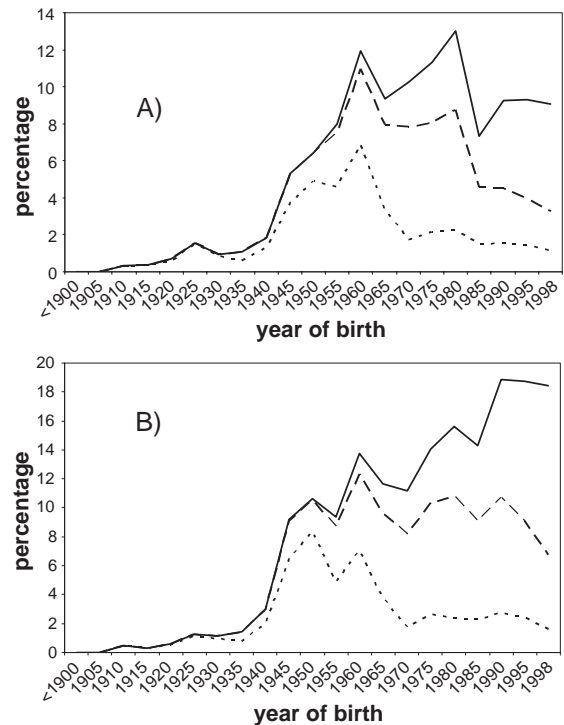


Fig. 3. Average inbreeding values in the Andalusian horse studbook by year of birth of the animals computed using all the available genealogical information (solid line) and using only the last 10 (dotted line) and the last 5 generations (dotted thin line) for (A) the whole individuals registered in the studbook and (B) the Carthusian individuals.

close and remote inbreeding the trends of the inbreeding by year of birth computed using only data from the last 10 ( $F_{10}$ ) and the last 5 ( $F_5$ ) generations were shown. Roughly speaking,  $F$  is consistently below 1% till 1938 (average  $F$  of 0.75% for the period). After this year, the average inbreeding of the

Table 5

Average values of inbreeding computed for the whole pedigree ( $F$ ) or using only the 5 ( $F_5$ ) or the 10 last generations ( $F_{10}$ ), average relatedness (AR), number of known generations and number of equivalent generations for the whole pedigree of the Andalusian horse and for the inbred animals

	Whole pedigree	Inbred	Stallions	Mares	Carthusian	Non-Carthusian
$N$	75,389	70,396	35,396	38,453	6318	69,071
$F^a$	8.48	9.08	8.69	8.28	13.01	8.06
$F_5$	1.45	1.55	1.48	1.42	2.71	1.33
$F_{10}$	4.00	4.28	4.09	3.92	8.02	3.63
AR	12.25	13.01	12.55	11.97	14.00	12.09
Number of generations traced	14.59	15.37	14.92	14.28	11.89	14.86
Equivalent generations	8.26	8.72	8.46	8.08	6.59	8.41

<sup>a</sup>  $F$  and AR values are expressed in percentages.

registered individuals increases quickly to reach a maximum near to 12% in 1960. From the 1960s to 1980 average  $F$  is around 11% to finally reach levels of 13%. After this date average  $F$  values showed lower values around 9% in the 1990s. The Carthusian strain showed a similar pattern with figures always higher than those reported for the whole population. Average  $F$  for Carthusians reached 13% in 1960 and 15.6% in 1980 to consistently increase their values to levels between 18% and 19% during the 1990s. The values for  $F_5$  and  $F_{10}$  computed for the whole population showed a consistent pattern of decrease from 1960 to 1998. In the 1990s, the average values of  $F_5$  and  $F_{10}$  varied, respectively, from 1.1% to 1.4% and from 3.3% to 4.5%. The trend of decrease in the  $F_5$  and  $F_{10}$  values over year of birth of the individuals was not so clear for Carthusians. From the 1980s to the end of the available data, the values of  $F_5$  and  $F_{10}$  tended to be, respectively, around 2.5% and 10%.

Differentiation between Carthusian and non-Carthusian individuals was assessed computing the  $F_{ST}$  distance on genealogical information (Caballero and Toro, 2000). Because the size of the involved matrix was higher than the program ENDOG can manage, the analysis was limited to the reproductive individuals born before 1st January, 1993 (84% of the total). No differentiation was found between Carthusian and non-Carthusian reproductive individuals ( $F_{ST}=0.000026$ ).

#### 4. Discussion

Despite the Andalusian horse being one of the most ancient horse breeds in Europe, information obtained from the Andalusian horse studbook points towards the present population being derived from a little number of individuals that survived the introgression of Central European massive horses into the breed since the XVIth century (Aparicio, 1944; Sanz, 1992). The recovery of the breed is tributary of the individuals bred by religious orders between the XVIIIth and the XIXth centuries without the use of foreign horses. Carthusian individuals had the type characteristics used to fit the breed standard. In general, the Carthusian horses have a more 'oriental' shape always showing subconvex head profiles and grey or white coat colour. The non-

Carthusian Andalusian horses, that could have in origin some influence of foreign massive horses, are of larger size, have more convex profiles and show any true colour with a high frequency of bay coats (Sanz, 1992). The Carthusian individuals have been basically bred by a few studs (forming the so-called Carthusian studs) with usual exchanges of reproductive individuals between them (Sanz, 1992; Valera, 1997) being impossible to identify Nucleus herds for the breed.

From a demographic point of view generation intervals computed for the breed are consistent with those reported before for other horse breeds with deeper pedigrees. It is usually admitted that generation intervals in horses are long (Strom and Philipsson, 1978). Generation intervals found for Andalusian horses are consistent with those previously reported for race or riding horses. Moureaux et al. (1996) in five French horse breeds found average generation intervals from 9.7 years in Arab to 11.8 years in Trotteur Français. Other average generation intervals reported for horse breeds such as Thoroughbred or Icelandic Toelter were, respectively, 10.5 and 9.7 years (Langlois, 1982; Hugason et al., 1985). The long generation intervals usually reported in the horse are basically dependent on its recreational use (such as in the case of the analysed breed) which is not compatible with pregnancy and breeding life. In fact, this parameter is longer for the Carthusian strain. A poorer reproductive performance of the individuals of this strain due to inbreeding can not be rejected. However, the cause is more likely to be linked to management. Breeders keep Carthusian individuals for recreational use as long as possible and the beginning of their reproductive life tends to be delayed with respect to the other Andalusian horses thus influencing the time spent to select a descendant for reproduction (Gómez, 2002).

The average inbreeding computed for the Andalusian horse (8.48%) is higher than others with high population sizes reported in the literature: 6.59% for Italian Haflinger (Gandini et al., 1992) or those reported by Moureaux et al. (1996) for five horse breeds raised in France ranging from 2.40% for Thoroughbred to 7.10% for Arab. However, inbreeding computation is very sensitive to pedigree deepness and quality and, in consequence, our figures should be only compared with those obtained from genealogical

data showing similar length. In this sense, overall inbreeding of the Andalusian horse pedigree is lower than that of 8.99% reported for North American Standardbred by MacCluer et al. (1983), that of 10.81% for Lipizzan horse reported by Zechner et al. (2002) or that of 12.5% for Thoroughbred reported by Mahon and Cunningham (1982) using pedigrees in which individuals can be traced more than 20 generations back. This level of inbreeding can be compared with that computed for the Carthusian strain (13%). However, this level of inbreeding took place, on average, in only 12 generations (see Table 5). The lengthening of the generation intervals in Carthusians is not sufficient to compensate the little number of founder individuals forming the strain. The trends of inbreeding found in the present study are highly dependent on the use of Carthusian individuals for reproduction. During the period from 1937 to 1970 the 73.4% of the stallions used for reproduction were Carthusians and the 79% of the individuals registered in the studbook during this period were sons of Carthusian stallions (Fig. 4). These figures drop substantially after 1970 to reach levels of, respectively, 7.8% and 13.3% between 1991 and 1998.

In any case, inbreeding in Andalusian horse breed seems to have a remote origin and linked to the Carthusian individuals. The values of inbreeding computed using only the last 10 ( $F_{10}$ ) and 5 ( $F_5$ ) generations (Table 5) drop dramatically and, in turn, are lower than those reported both for Lipizzan horse (Zechner et al., 2002) and, specially, for five French horse breeds (Moureaux et al., 1996). However, the same values computed for only Carthusians are higher than those computed for the Lippizan horse

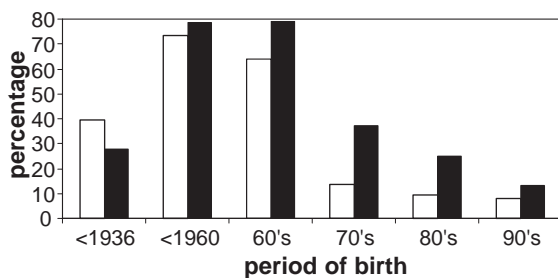


Fig. 4. Percent of stallions used for reproduction of the Carthusian strain (white bars) and percent of individuals registered in the Andalusian horse studbook which were sons of Carthusian stallions by different periods in the analysed dataset.

using 5 generations (2.7% versus 2.06%) and, especially, for those calculated using 10 generations (8% versus 5.4%) (Zechner et al., 2002). As reported for other horse populations derived from a limited number of founders (MacCluer et al., 1983; Mahon and Cunningham, 1982; Zechner et al., 2002), breeders have carried out a major effort to avoid matings with very close related individuals to limit the increase of inbreeding. During the 1960s the frequency of apparition of individuals showing a recessive disorder called 'lop neck' in the Andalusian breed induced the breeders to limit the inbred matings (Sanz, 1992). Fig. 3 illustrates that at the beginning the average level of  $F$  for the whole population dropped because matings were planned using individuals without common ancestors at least in the last 5 generations. However, the  $F_{10}$  values tended to increase during the 1970s leading to an overall increase of the  $F$  for the whole population. This was due to the scarce number of Carthusian individuals available for reproduction without common ancestors in the last 10 generations; as a consequence the effect of the program implemented by the breeders was limited in the Carthusian strain affecting the whole population. In the late 1980s and 1990s breeders associations implemented a technical program including the plan of matings by linear programming the limitation of the use of Carthusian individuals in the population and the selection of non-Carthusian lines (Valera et al., 1998). Nowadays, most Andalusian horse breeders use ad-hoc software to program matings (Gescab®, Melgarejo et al., 2000). These approaches produced a consistent decrease of the  $F_5$  and  $F_{10}$  values for the whole pedigree. However, inbreeding levels in the Carthusian strain are far from being controlled and the  $F$  for the strain tends to increase (see Fig. 3).

The effective number of founders in the Andalusian horse breed is lower than that of 48.2 reported for Lipizzan horse from a pedigree including 3867 individuals (Zechner et al., 2002) and especially than those reported by Moureaux et al. (1996) in five French horse breeds (from 70 in Trotteur Français to 333 in Selle Français) using much shorter pedigrees. However, this parameter is higher than that of 28 found for Thoroughbred horse (Cunningham et al., 2001) with a deeper pedigree. The Andalusian founder with the higher AR coefficient was genet-



ically represented in the whole Andalusian horse population in 8.2%. The contribution of the most Thoroughbred and Lipizzan founders to their breeds were, respectively of 13.5% (Cunningham et al., 2001) and 6.66% (Zechner et al., 2002). The effective number of ancestors (founders or not) should be a measure less affected by the quality of the pedigree (Boichard et al., 1997). However, in our breed this parameter is still lower than that of 26.2 reported for Lipizzan horse (Zechner et al., 2002). The contribution of the two major ancestors in the Andalusian horse breed is of 15.8% and 12.6%. Ancestor contributing the most in Lipizzan horse explained a 10.74% of the genetic variability of the breed. In addition, only 6 ancestors are needed in the Andalusian horse population to explain 50% of the genetic variability of the breed while 8 were needed in Lipizzan horses (Zechner et al., 2002). If we take into account only the contribution of founders to the gene pool we could think that they are represented in the Andalusian horse in a more balanced way than in other breeds having deep genealogies. However, information from major ancestors points towards the abusive use of some specific individuals in the Andalusian horse breed.

A major issue in the present analysis is the ascertainment of the influence of Carthusian individuals in the whole Andalusian horse breed. In the present study we estimated the contribution of Carthusian strain to the Andalusian horse population from 80.5% (using the methodology from Boichard et al., 1997) to 87.6%, (by simply summing up the AR coefficients of the Carthusian founders). Additionally,  $F_{ST}$  values computed from genealogies reflect the lack of genetic differentiation between the strain and the rest of the population. The distinction between Carthusians and non-Carthusians is not supported in genetic terms. However, the Carthusian individuals must be subject to special attention in the Andalusian horse breed because of their influence on the genetic variability of the breed. As commonly reported in other horse breeds with large or short pedigrees (Zechner et al., 2002; Cunningham et al., 2001; Glazewska and Jezierski, 2004) a small number of individuals have a large influence on the breed. The traditional techniques used to plan matings to reduce inbreeding had a moderate success within the strain. In such a situation it could be indicated to unbalance

the offspring of the parents towards those with a lower level of genetic representation in the population in an attempt to preserve the genetic variability of the breed (Goyache et al., 2003). In this sense we advocate the use of the AR coefficients as an alternative or complement to  $F$  to conserve the genetic makeup of the population. AR takes into account the percentage of the complete pedigree represented in an individual (Goyache et al., 2003; Gutiérrez et al., 2003). The preferential use of reproductive individuals with the lower AR coefficients is expected to balance the contribution the founders at a population level thus containing the increase of inbreeding. The genetic representation of the breed (assessed by means of the AR coefficients) in the Carthusian individuals is, on average, higher than that of the whole population, thus limiting the possibilities of such a program. The average AR of the 705 Carthusian stallions born after 1990 was of 18.2% and only 1% of them had AR values between 9% and 15%. The selection and unbalanced use for reproduction of these Carthusian individuals would be useful to contain the inbreeding levels in the strain and, in consequence, balance the genetic representation of the founders in the whole breed.

## 5. Conclusions

Throughout the present analysis we have analysed the pedigree information of the Andalusian horse studbook. Genealogical information characterises a population with a high concentration of gene origin. The influence of the Carthusian strain on the whole Andalusian horse population was assessed using both the contribution of the founders, the contribution of the ancestors (founders or not) and the genetic differentiation between Carthusian and non-Carthusian individuals using genealogical  $F_{ST}$ 's. The distinction between Carthusian and non-Carthusian individuals within the Andalusian horse breed does not have genetic support. The levels of  $F$  in the Andalusian horse breed have a remote origin linked to the abusive use of Carthusian stallions till the last quarter of the XXth century. The unbalanced use of the Carthusian individuals with lower AR values for reproduction is proposed to maintain the genetic variability of the breed.

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