

Genetic variability in the endangered Asturcón pony assessed using genealogical and molecular information

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Abstract

The aim of this analysis is to assess the genetic diversity in the black-coated Asturcón pony population using genealogical and molecular methods in order to ascertain the possible impact of the inclusion of the bay Asturcón individuals on the breed's recovery programme. Pedigree information registered in the studbook of the black-coated Asturcón (including a total of 1080 individuals) was analysed. Additionally, 261 blood samples from the black-coated Asturcón individuals were obtained and genotyped for 15 microsatellites. Furthermore, 58 blood samples were obtained and genotyped from bay Asturcón individuals in order to place the results within the context of an unselected population with no known genealogies. The results indicated high losses of genetic representation of founders in the present population of black-coated Asturcón ponies. Roughly 60% and 30% of the founder stallion and dam lines, respectively, are lost in the present population. Average inbreeding was 4.7%. The equivalent number of founders and equivalent number of ancestors were 18.1 and 13. The most relevant founders and ancestors identified belonged to the three major studs involved in the recovery of the breed. However, the results highlight the dependence of the breed on the management of the government-run Cayón stud, which has increased its genetic contribution to the breed over time from 35.6% to 50.1%. At a molecular level, genetic variability assessed in the black-coated Asturcón was lower than that observed in the bay Asturcón. Expected heterozygosity, $F_{IS(m)}$ and rarefacted average number of alleles per locus were 0.755 and 0.828, 4.1% and 1.3%, and 9.5 and 9.2, respectively, for the black-coated and bay Asturcón. The expected molecular coancestry in the black-coated Asturcón base population ($E(f_0)$) took a value of 0.229, which was near to the molecular coancestry computed in the bay Asturcón (0.231). Implications of the reported results in the recovery programme of the Asturcón pony breed are discussed.

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1. Introduction

The Asturcón is a 'Celtic type' native Iberian horse population (Aparicio, 1944) that is morphologically and genetically related to other breeds settled in the Iberian North-western Atlantic areas, such as the Garrano in

Portugal or the Faco galego, Losino and Pottoka in Spain (Jordana et al., 1995; Royo et al., 2005a). The Asturcón pony breed is considered one of the oldest representatives (García-Dory, 1980) of native Iberian horse populations. The Asturcón individuals coincide basically with the horses described by the Roman chroniclers during the Cantabrian Wars 80 B.C. (Álvarez Sevilla, 1995).

The Asturcón population decreased in size dramatically and the breed became highly endangered after the

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Spanish Civil War (Álvarez Llana, 1995; Álvarez Sevilla, 1995). The recovery of the Asturcón pony breed commenced in the 1970s with 21 black-coated reproductive individuals managed in semi-feral conditions on the Sueve Range in Eastern Asturias. The process is well documented (García-Dory, 1980; Álvarez Llana, 1995; Royo et al., 2005b) and led to the foundation of the Asturcón pony studbook in 1981 and the breeders association (ACPRA) in 1987 (Álvarez Llana, 1995; Álvarez Sevilla, 1995).

In spite of the fact that black-coated Asturcón individuals were used for the recovery of the breed, most historical evidence indicates that the majority of Asturcón individuals were bred in Western Asturias and were bay in colour (Álvarez Llana, 1995). Available veterinary morphological descriptions and photographs made during the first third of 20th century do not allow differentiating between the black- and the bay-coated Asturcón individuals (Álvarez Llana, 1995). Moreover, the presence of black animals in the Western Asturcón population and bay individuals in the Asturcón population of Sueve has been widely reported (García-Dory, 1980; Álvarez Llana, 1995). During the late 1990s, some breeders grouped together in the ‘García-Dory’ Association initiated a conservation programme for the Western (bay) Asturcón, also known as Caballo de Corro (Royo et al., 2005a,b). Preliminary studies carried out involving both Asturcón populations failed to find any genetic or morphological reason to consider the two Asturcón strains as separate populations (Royo et al., 2004). In late 2005, the bay Asturcón, in which the recessive black allele is present at a frequency of 42% (Royo et al., 2005c), has been included in the ACPRA studbook and breeding programme (ACPRA, 2006, personal communication).

The aim of the present analysis is to assess the remaining genetic diversity in the black-coated Asturcón pony population using genealogical and molecular methodologies in order to ascertain the possible impact of the inclusion of the bay Asturcón individuals on the ACPRA breeding programme.

2. Materials and methods

2.1. Data and sampling

Genealogical information recorded in the black-coated Asturcón studbook from its foundation to December 2002 was obtained from ACPRA, summing 1080 individuals. Studbook information was analysed in order to identify stallion and dam lines, defined respectively as unbroken descent through male or female animals only from an ancestor to a descendant (Cunningham et al., 2001).

ACPRA also provided a total of 261 blood samples from the individuals born during the following foaling seasons: 1998 (89), 2000 (85) and 2002 (87). The whole available samples were used as reference population (RP) for further analyses. In order to illustrate possible losses of genetic variability over time, the corresponding yearly cohorts (C1998, C2000 and C2002) were also used as reference populations when needed. Additionally, 58 blood samples from bay Asturcón individuals (which constitute basically all the adult individuals at the sampling period during the winter of 2004) were obtained in order to place the results within the context of an unselected, closely related population. Notice that pure breed matings had just started in bay Asturcón at the sampling period and genealogical information was not available (Goyache et al., 2005).

2.2. Genealogical analyses

Genealogical analyses were carried out using the ENDOG v3.0 program (Gutiérrez and Goyache, 2005). Available pedigree information for each individual in the dataset was assessed by computing the equivalent complete generations (g_e). The parameter g_e is computed as the sum over all known ancestors of the term $(1/2)^n$, where n is the number of generations separating the individual from each known ancestor (Maignel et al., 1996). Notice that, on average for a given reference population, g_e is equivalent to the ‘discrete generation equivalents’ (T) proposed by Woolliams and Mäntysaari (1995), thus characterizing the amount of pedigree information in datasets with overlapping generations.

Genealogical information was analyzed in order to obtain the full coancestry (f ; Malécot, 1948) matrix of the black-coated Asturcón pedigree, the individual coefficient of inbreeding (F) (Wright, 1969), defined as the probability that an individual has two identical alleles by descent, and the individual average relatedness coefficient (AR) (Goyache et al., 2003; Gutiérrez et al., 2003), defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to the animal. The three parameters were computed using RP and each sampled cohort as the reference population and further averaged.

To characterise possible unbalanced genetic contributions to the present population, the following parameters were analysed: a) equivalent number of founders computed weighting the unknown parent as half a founder; b) 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: $f_e = 1/\sum_{k=1}^f q_k^2$, q_k being the probability of gene origin of the k th founder and f the real number of founders; and c) the effective number of ancestors (f_a) (Boichard et al., 1997), which accounts for the losses of genetic variability produced by the unbalanced use of reproductive individuals, also taking into account bottlenecks in the pedigree, and defined as the number of equally contributing ancestors that would be expected to produce the same genetic diversity as in the population under study. The parameter f_a was computed as $f_a = 1/\sum_{j=1}^a q_j^2$, where q_j is the marginal contribution of an ancestor j ; in other words, the genetic contribution made by an ancestor that is not explained by other ancestors chosen previously.

From the coancestry information, Wright's (1969) F_{IS} statistic was obtained as $F_{IS} = \frac{\bar{F} - \bar{f}}{1 - \bar{f}}$, where \bar{F} is the mean inbreeding coefficient for the entire population, and \bar{f} the average coancestry for the subpopulation (Caballero and Toro, 2000, 2002).

2.3. Molecular analyses

Total DNA was isolated from blood samples following standard procedures (Sambrook et al., 1989). A set of 15 microsatellites (AHT4, AHT5, ASB17, ASB2, CA425, HMS1, HMS2, HMS3, HMS7, HTG4, VHL20, ASB23, HMS6, HTG10 and HTG7) was analyzed in all the sampled individuals. The PCR products were electrophoretically separated using an ABI PRISM™ 3100 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).

Analyses on molecular information were carried out using the MolKin program (Gutiérrez et al., 2005a). At the reference population level, the following parameters were computed: expected heterozygosity (H_e ; Nei, 1987); average number of alleles per locus (A), corrected in order to account for sample size using Hurlbert's rarefaction method (1971) as $A[g] = \sum_i \left[1 - \prod_{k=0}^{g-1} \frac{N - N_i - k}{N - k} \right]$, where g is the specified sampled size, N the number of gene copies examined at a given locus ($N > g$), and N_i the number of occurrences in the i^{th} allele among the N sampled gene copies.

The full molecular coancestry matrix among the genotyped individuals was computed. The molecular coancestry (M) between two individuals i and j is the probability that two randomly sampled alleles from the same locus in two individuals are identical by state (Caballero and Toro, 2002). Molecular coancestry between two individuals i and j at any given locus can be computed using the following scoring rules (Caballero and Toro,

2002; Eding and Meuwissen, 2001): $M_{ij,l} = 1/4[I_{11} + I_{12} + I_{21} + I_{22}]$, where I_{xy} is 1 when allele x at locus l in individual i and allele y at the same locus in individual j are identical, and zero otherwise. Notice that this value can only have four values: 0, 1/4, 1/2 and 1. The molecular coancestry between two individuals i and j (M_{ij}) can be obtained by simply averaging over L analyzed loci as $M_{ij} = \frac{\sum_{l=1}^L M_{ij,l}}{L}$. Additionally, molecular mean kinship (Mk; Gutiérrez et al., 2005a) was computed as the average molecular coancestry of each individual with the rest of the population, and further averaged for each cohort under study.

The parameter $F_{IS(m)}$ was computed from molecular information as $F_{IS} = \frac{(1 - H_0) - \bar{M}}{1 - \bar{M}}$, where H_0 is the observed homozygosity for the entire population, and \bar{M} the average molecular coancestry for the subpopulation (Caballero and Toro, 2002).

The expected molecular coancestry value in the base population ($Ef_{(m)0}$) was computed using RP and each cohort as the reference population, following Toro et al. (2003), as $Ef_{(m)0} = (f_{(m)t} - f_t)/(1 - f_t)$, where $f_{(m)t}$ and f_t are respectively the molecular and the genealogical coancestry for the generation t th cohort.

3. Results

A total of 7 stallion lines and 50 dam lines were identified in the black-coated Asturcón studbook. Only 3 stallion lines were represented in the reference population: *Lotu* (55.6%), *Yoplait* (26.9%) and *Esllabayu* (17.5%). Some 38 dam lines were identified in the reference population; the most represented are those from *Eslavaya* (22.4%), *Romana* (7.7%), *Polvorina* (6.3%) and *Rápida II* (6.3%), the remainder being represented below 4%. Up to 22 dam lines are represented in the RP at a frequency of 1% or lower. The frequencies found in the RP for the stallion and dam lines were basically the same within cohorts.

Table 1 details the 6 ancestors and the 6 founders contributing the most. Except for *Moro* which is the stallion descendant of the founder *Yoplait* selected as ancestor, the listed individuals were born during the 1970s and belonged to the three studs that were initially involved in the recovery of the Asturcón pony: the local Suevo studs grouped together in ACAS, the government-run stud owned by the public administration to contribute to the recovery of the breed (Cayón) and the private stud of Antón Álvarez Sevilla. Four of the listed individuals were selected both as major ancestors and founders (*Eslavaya*, *Esllabayu*, *Rápida II* and *Polvorina*). The ancestor contributing the most (*Lotu*) explained 22.9% of the genetic variability, while the

Table 1

Description of 6 ancestors and 6 founders contributing the most to the genetic variability of the black-coated Asturcón pony

Ancestor	Sex	Stallion	Mare	Year of birth	Stud	Marginal contribution	AR
Lotu	Male	–	Polvorina	1979	Cayón	22.8	22.9
Eslavaya	Female	–	–	1976	Cayón	9.1	8.5
Esllabayu	Male	–	–	1976	Álvarez-Sevilla	7.1	6.6
Rápida II	Female	–	–	1979	Cayón	5.0	4.7
Moro	Male	Yoplait	Mora	1981	ACAS	4.4	5.3
Polvorina	Female	–	–	1972	Cayón	4.4	14.5
<i>Founder</i>							
Polvorina	Female	–	–	1972	Cayón	4.4	14.5
Eslavaya	Female	–	–	1976	Cayón	9.1	8.5
Esllabayu	Male	–	–	1976	Álvarez-Sevilla	7.1	6.6
Rápida II	Female	–	–	1979	Cayón	5.0	4.7
Yoplait	Male	–	–	1973	ACAS	1.1	3.9
Sieres	Male	–	–	1978	Cayón	2.9	2.8

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

founder contributing the most to the gene pool was *Polvorina*, the mother of *Lotu*, accounting for 14.5%. Notice that *Polvorina* was also selected as an ancestor (ranking sixth in terms of contribution). This indicates that her contribution to the Asturcón pony gene pool cannot be solely explained by her son *Lotu*. The contributions of all the founders and ancestors belonging to the three major studs (ACAS, Cayón and Álvarez Sevilla) involved in the recovery of the black-coated Asturcón pony summed, respectively, 74.3% and 82.6%

of the total genetic variability of the breed. The government-owned stud (Cayón) increased its contribution to the Asturcón population after the foundation of the breed from 35.6% to reach the value of 50.1%, whilst the others decreased from levels of around 20% to 15.8% (ACAS) and 16.7% (Álvarez Sevilla).

Table 2 shows the genealogical parameters characterising genetic variability and gene origin in the black-coated Asturcón pony for the whole reference population and for each cohort. The RP had average values for

Table 2

Main genealogical parameters describing the genetic variability in the black-coated Asturcón pony and molecular parameters describing the genetic variability in both the black and the bay coated Asturcón pony populations

	Black-coated Asturcón				Bay Asturcón
	C1998	C2000	C2002	RP	
<i>Genealogical parameters</i>					
F (%)	0.052	0.041	0.047	0.047	
AR (%)	0.097	0.091	0.089	0.092	
Equivalent generations (g_e)	2.85	2.91	3.16	2.97	
F_{IS} (%)	–0.9	–1.9	–0.6	–0.7	
Equivalent number of founder animals	47.5	45.0	52.0	55.5	
Effective number of founder animals (f_e)	18.4	17.8	19.5	18.1	
Effective number of ancestors (f_a)	12	12	12	13	
Number of ancestors explaining 50%	5	5	6	6	
<i>Molecular parameters</i>					
Number of genotyped individuals	89	85	87	261	58
Expected heterozygosity (H_e)	0.739	0.775	0.788	0.755	0.828
$F_{IS(m)}$ (%)	4.1	4.5	3.9	4.1	1.3
Average number of alleles per locus (A)	7.5	7.4	7.4	9.5	9.2
Average number of alleles per locus ($A_{(78)}$)	6.9	6.9	7.0	7.3	8.6
Molecular coancestry (f)	0.266	0.278	0.268	0.264	0.231
Expected molecular coancestry (Ef_0)	0.229	0.244	0.234	0.229	–

In the black-coated Asturcón, the listed parameters have been computed for the whole reference population (RP) and for each of the available foaling season cohorts (see text).

inbreeding, average relatedness and number of equivalent complete generations of 4.7%, 9.2% and 2.97, respectively. The corresponding values for each analysed cohort were very similar to those given for the RP. Although both F and AR tend to decrease from C1998 to C2000, the average genealogical information (g_e) increases (from 2.85 to 3.16). The parameter F_{IS} computed from genealogical information showed values near 0 (–0.7% for the whole reference population), except for C2000, for which it reached a value of roughly –2%. Parameters f_e and f_a reached values of 18.1 and 13 for the RP, the number of ancestors explaining 50% of the genetic variability retained by the RP being 6. These values are basically the same for each considered cohort.

The parameters describing the genetic variability in the Asturcón pony breed at a molecular level are also given in Table 2. In order to compare the black-coated Asturcón with a population that has not been under selection, most parameters are also given for the bay Asturcón. Expected heterozygosity was 0.755 for the RP and 0.828 for the bay Asturcón. The analysed populations showed heterozygote deficiency characterised by positive $F_{IS(m)}$ values. However, heterozygote deficiency was higher for the black-coated Asturcón (4.1% for the RP and similar values for each sampled cohort) than for the bay Asturcón (1.3%). The average number of alleles per locus was similar for both the RP and the bay Asturcón (9.5 and 9.2, respectively). However this parameter is highly influenced by sample size and should be rarefacted (Hurlbert, 1971). After rarefaction, the bay Asturcón showed higher values than the RP (8.6 vs. 7.3, respectively). The within-population molecular coancestry showed that the RP population (and similarly each sampled black-coated Asturcón cohort) had higher genetic identity than the bay Asturcón (0.264 vs. 0.231, respectively). The expected molecular coancestry in the base population ($E(f_0)$) was computed for the RP and each sampled black-coated Asturcón cohort. This parameter took a value of 0.229 for the RP and from 0.229 to 0.244 for each cohort. The computed values are close to the ‘real’ molecular coancestry computed for the bay Asturcón.

4. Discussion

The overall results presented here highlight the significant losses of founder representation that have occurred in the black-coated Asturcón population. Roughly 60% and 30% of the stallion and dam lines, respectively, are lost in the RP. The unbalanced representation of the founders is illustrated by the effective number of founder animals (f_e) and the effective number

of ancestors (f_a). The parameter f_e constitutes over a third of the equivalent number of founder animals for the RP, whilst the ratio f_a/f_e is 71.8%. This ratio is higher than that reported in horse breeds with a higher number of traced generations such as Andalusian (41.7%; Valera et al., 2005) or Lipizzan (54.4%; Zechner et al., 2002), and also higher than the figure of 38.2% recently reported for the endangered Catalonian donkey (Gutiérrez et al., 2005b). The above figures show that the losses of genetic representation of founders occurred very quickly after the implementation of the conservation programme and that the bottleneck subsequent to a mating policy allowing the abusive use of some relevant individuals in the black-coated Asturcón breed (characterised by the ratio f_a/f_e) occurred when most founders were still available for reproduction.

The abusive reproductive use of some individuals at the beginning of the conservation efforts is mainly due to the management of the Cayón stud. This is a government-run stud founded by the extinct Institute for Conservation of Nature and further managed by the Principado de Asturias Regional Government (Álvarez Llana, 1995). The Cayón stud included most founders involved in the recovery of the black-coated Asturcón pony and selected reproductive individuals in order to homogenize particular type characteristics of the breed. The importance of the Cayón stud for the breed increased gradually due to the lower reproductive success of the ACAS and Álvarez Sevilla studs. The ACAS stud decreased its activity because of the advanced age of the owners, whilst the Álvarez Sevilla stud is managed in semi-feral conditions that are highly determined by harsh environmental conditions and attacks by wolves, thus contributing only slightly to the studbook. However, the Cayón stud has had regular reproductive success and most new studs have initiated their activity using individuals from this stud.

The average inbreeding computed for the black-coated Asturcón RP is lower than most of the values reported in the literature (see Valera et al., 2005, for a review), usually ranging from 6.5% to 12.5%. However, most of these inbreeding values have been computed in breeds with deep pedigrees such as Andalusian, Lipizzan or Thoroughbred. Since inbreeding computation is highly sensitive to available pedigree information, the value presented here should only be compared with those obtained from similar genealogical data. Valera et al. (2004) in the rare Losino pony breed obtained an F value of 3.4% analysing a pedigree comprising 481 individuals. Moreover, inbreeding in the black-coated Asturcón is in the upper limit of that usually reported in the literature using only five generations pedigrees (see Zechner et al., 2002, for a review). In

any case, there are no large differences between the inbreeding levels computed within each cohort and that computed for the RP. Since the late 1990s, most matings in the population (and especially in the Cayón stud) have been planned to avoid crosses between relatives, as well as to control individual contributions to the following generations in order to respectively minimize the short- and the long-term increases in inbreeding. This is reflected by the negative genealogical F_{IS} values reported in Table 2. This parameter characterises the mating policy meaning the departure from random mating as a deviation from Hardy–Weinberg proportions. Negative F_{IS} values mean 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., 2005b). Moreover, the average AR values computed for each cohort and for the RP are roughly twice the value of F . In an ideal scenario with random matings and no population subdivision, AR would be approximately twice the F value of the next generation (Goyache et al., 2003; Gutiérrez et al., 2003). However, F continues to increase in the black-coated Asturcón population. As an additional result, a significant ($p < 0.0001$) regression coefficient of the inbreeding values of the RP individuals on their g_e coefficients was computed (0.03816 ; $R^2 = 0.20$). This means that F increases with pedigree depth (a little less than 4% per discrete generation equivalent). This increase in inbreeding would give an effective number of founder animals of 13.1, which is lower than the value of 18.1 computed using founder contributions and quite close to the value of the effective number of ancestors, thus characterising the additional losses of the founder genetic background in the present population.

Molecular information was also obtained in this study using the bay Asturcón samples as a non-selected reference population. At the period of sampling, a recovery programme for the bay Asturcón population had just commenced and no significant between-individuals familial relationships were detected (average F and AR values of respectively 0% and 0.4% for a pedigree totalling 99 individuals; Goyache et al., 2005). The figures computed for H_e , average number of alleles per locus and $F_{IS(m)}$ in the bay Asturcón are typical of the base populations on which a recovery programme is based. As noted for other rare breeds (Álvarez et al., 2005), the individuals recovered in order to constitute founders of a breed are usually obtained at different genetically-isolated locations, probably leading to the fixation of different alleles (positive $F_{IS(m)}$ values). However, as a whole, these individuals capture the genetic remnant variability in the population before the bottleneck, which

is basically illustrated by the high average number of alleles per locus found.

Major molecular parameters characterising the RP and the sampled cohorts show that the present black-coated Asturcón population has lower levels of genetic variability than the bay Asturcón. Moreover, these values (especially $F_{IS(m)}$, rarefacted number of alleles per locus and f) are consistent across cohorts and do not present a clear pattern of variation over years of sampling. However, the relatively low rarefacted A and the positive and relatively large $F_{IS(m)}$ values computed for the RP and for the sampled cohorts characterise the losses of genetic variability, at a molecular level, that occurred in the black-coated Asturcón after the implementation of the recovery programme for the breed.

Toro et al. (2003) furthered the use of M in conservation programmes for the following reasons: a) there is a clear relationship between this measure in the current population and in the founder population through the genealogical coefficient; b) its value in the founder population coincides with the expected homozygosity, a classical parameter in population genetics (Nei, 1987); and c) it is the only one that is unbiased, even in the presence of inbreeding. The molecular coancestry values computed in the current black-coated Asturcón sampled populations may be compared with the expected value of this parameter in the non-sampled base population of the breed. Molecular coancestry corresponding to the bay Asturcón, as a recently recovered population, may be similar to that of the founder population of the black-coated Asturcón. If so, the Ef_0 , computed following Toro et al. (2003), can be considered a correct estimator of the existing genetic variability in a non-sampled founder population.

In the present black-coated Asturcón population, allele frequencies do not vary significantly with depth of pedigree, whilst genealogical kinship increases. Fig. 1 shows the variation in the AR (twice average genealogical

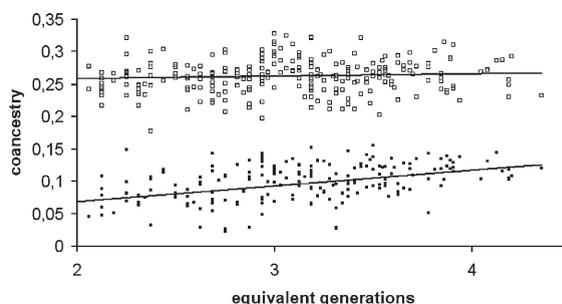


Fig. 1. Variation in the average relatedness (black squares) and molecular mean kinship (open squares) values relative to the number of equivalent generations computed for each individual in the black-coated Asturcón reference population (RP).

coancestry) and M_k (average molecular coancestry) values with respect to the individual g_e values in RP. This illustrates how individuals increase their genetic representation with pedigree depth. The regression of the AR values on g_e gives a significant ($p < 0.0001$) regression coefficient of 0.02424 ($R^2 = 0.22$), whilst the corresponding regression coefficient for M_k (0.00358; $R^2 = 0.01$) is not significant. A similar situation to that reported for the RP was assessed for Cohort 1998 and Cohort 2002, which present E_{f_0} values of around 0.23, which is the value of f in the bay Asturcón samples. However, both AR and M_k varied significantly with g_e within Cohort 2000, thus leading to ‘overestimation’ of its E_{f_0} value (0.244). Genealogical coancestry assesses the probability that an individual is identical by descent to a reference founder population in which it is assumed that all the alleles are different. The parameter E_{f_0} assumes that the present sampled population that we consider in the genealogical study has the same allele frequencies as the founder population and that these frequencies are in both Hardy–Weinberg and linkage equilibrium (Toro et al., 2003). These assumptions are not always realistic. We cannot properly expect present populations to have the same allele frequencies as founder populations. Drift or selective processes have unavoidably occurred during the formation of the breeding stock to be conserved (Toro et al., 2000, 2002; Goyache et al., 2003) and the number of allelic forms in the founder population cannot be as high as that assumed in genealogical analyses (Balloux and Lougon-Moulin, 2002). However, the present analysis leads us to accept a straightforward relationship between the value in the current population and in the founder population through genealogical coancestry (Toro et al., 2003), at least when allelic frequencies in the present population do not vary with pedigree knowledge. Moreover, the overall scenario summarised in Fig. 1 indicates that a programme such as the one implemented in the black-coated Asturcón to avoid mating between close relatives can balance allelic frequencies in spite of the fact that genealogical parameters continue to increase with depth of pedigree.

5. Conclusions

In this paper, the losses of genetic variability in the black-coated Asturcón population that occurred after the foundation of its recovery programme were assessed via genealogical and molecular information. Major losses of genetic variability occurred at the beginning of the recovery programme. The present analysis indicates that the efforts of the breeders association carried out to avoid matings between relatives have led to allelic fre-

quencies at the population level remaining fairly constant over pedigree knowledge, whilst genealogical parameters continue to increase. In this respect, relationship between genealogical and molecular coancestry is not always straightforward depending on the mating policy and pedigree depth (Fernández et al., 2005), thus justifying a combined use of pedigree and microsatellite information in some scenarios of conservation.

This paper presents molecular data computed on somatic markers for the bay Asturcón population for the first time. The inclusion of such data in the Asturcón breed studbook will allow controlling the level of inbreeding in the black Asturcón population by the allowance of some crossing between black-coated and bay Asturcón, or simply by including black-coated sons of bay individuals in the breeding scheme of the black-coated Asturcón (Royo et al., 2005c).

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