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Genetic characterization and inventory of the Bísaro pig through visible effect genes. Their utilization in the genotypic comparison between populations and in the establishing of a nucleus for *in vivo* genetic conservation[†]

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SUMMARY - Observation in certain traditional pig farms of interior northern Portugal made it possible to identify animals which had visible variants (colour, shape and position of ears, head, size, dorsal line) that characterized the ancient Bísaro pig. Due to the importance of these observations in the field of genetic resource, they conducted the development of actions in the conservation, characterization and evaluation of that type of pig and had the objective of determining production and processing potentials. This study deals with the first phase of that project which includes the identification and characterization of the Bísaro pig: a quantitative and genetic inventory was carried out on the herd (Bísaro); a first selection of animals was made (based on visible traits). The aim of these measures was to create two *in vivo* genetic conservation nuclei (in Minho and Trás-os-Montes) where animals could be studied. In these nuclei, genotypes resulting from reproduction and pairing were used to make a list of existing genes and genotypes (coat colour and patterns). In the same nuclei, the breed was morphologically characterized by comparing existing genotypes and these with the Bísaro pig population described 150 years ago.

Key words: Breed, Bísaro, inventory, visible variants, nuclei, genotypes.

RESUME - *"Caractérisation génétique et inventaire des porcins de race Bísaro à travers des gènes à effets visibles. Utilisation dans la comparaison génotypique entre populations et dans l'établissement d'un noyau pour la conservation génétique* in vivo*". L'analyse des fréquences phénotypiques ségréguées (des gènes à effets visibles, type et couleur du pelage), obtenues par accouplement à l'intérieur de la race Bísara et par croisement avec le sanglier, a permis d'identifier les allèles (appartenant aux locus A, I, E, He, Be) de la population Bísara ainsi que les combinaisons génotypiques les plus probables pour chaque phénotype. L'inventaire a prouvé la quasi-extinction de la race et deux noyaux (région Minho and Trás-os-Montes) de conservation* in vivo *en lignée paternelle et en lignée maternelle ont été constitués, ce qui permet d'assurer la conservation de 2n chromosomes purs ainsi que leur étude en station à partir de généalogies connues. L'inventaire génétique a également permis de quantifier les fréquences génotypiques de la population qui présente une plus grande variabilité dans la région de Minho, ce qui est en rapport direct avec la plus grande variabilité génotypique des verrats dans cette région. L'étude morphologique réalisée sur les noyaux a permis de caractériser et comparer la population entre régions et avec l'ancien porc Bísaro.*

Mots-clés : *Race, Bísaro, inventaire, polymorphismes visibles, noyaux, génotypes.*

Introduction

After R. Bakewell (1725-1795) carried out the first work in animal selection in sheep, studies began in the phenotypic characterization of domestic species (Martels, 1854; Sanson, 1867; Cornevin, 1898; Diffloth, 1909). This is how the XIX and XX centuries witnessed the birth of the first genealogical books (England) and at the same time the "creation" of breeds defined by the concept of standard or ideal animal. This concept includes visible characteristics, such as skin colour or type of hair, and morphological characteristics of the body or parts of it. In general terms, this concept implied the selection and reduction of visible polymorphisms in each population, thus suppressing genotypes and

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corresponding genes. This phenomenon contributed to the homogenization of populations during the XIX century, and partially to the decrease of genetic variability observed at the end of the same century.

Pigs of Celtic descent (Sanson), referred to by Bernardo Lima (1865) as pigs of the Bísaro type, were found throughout western Europe during the XIX century, including Portugal from the Tagus river to Galiza. To quote this author, these pigs were characterized by "large body; big, sometimes flabby, drooping ears; long snouts; convex or arched back; long legs; several varieties of hair colour: variable, black, spotted or white". These varieties took on several designations according to colour, size and region. Pigs from the Beira region were smaller, black or spotted and those from Galiza were bigger, white or white with spots. They were also called "cerdões" if they had abundant hair or "molarinhos" if they had lack of it. These pigs had slow growth, little backfat and a lot of skin and bone.

In the same publication, B. Lima (1865) states that these types of pig (Bísaro; Celtic) are of the same type as those found throughout western Europe (France, Switzerland, Germany, Denmark) and England. Such breeds were described by Sanson (1867) and some of them by Cornevin (1889): Podolienne, Bohême, Dano-scandinave, Bavaroise, Bakonyer, Flamande, Bretonne, Craonaise, Augeronne, Normande, Irlandaise, Lorraine, Beira do Litoral (Bísaro).

However, the determination of the origin and parentage of these breeds is not a simple matter because it is not possible to say with certainty that the Bísaro pig or any other breed is solely of Celtic descent. According to several bibliographic records, during the period of ocean discovery, pigs circulated throughout the world, especially pigs of Chinese, Asian and African descent. Besides this fact, the Bísaro type pig was not the only one in Portugal (M. Pinto, 1878); the Alentejana breed from the south of Portugal, as well as Chinese and English breeds (Yorkshire, Berkshire, Chesterwhite, Leichester) could also be found. These breeds were improved for meat production and resulted from the crossing of Celtic, Chinese and Napolitan pigs. It was during this time that the genetic erosion process began to increase, leading to the extinction of hundreds of breeds throughout Europe and almost to the extinction of our present-day Bísaro pig.

Recently, Legault (1997) reviewed and updated studies on hereditability of coat colour in pigs [Ollivier and Sellier (1982); Johansson *et al*. (1992, 1996); Kijas *et al*. (1996); Mariani *et al*. (1996)], having shown that visible effect genetic polymorphisms, along with biochemical and molecular markers of the DNA chain may be useful in the identification of some crossbreeding schemes, in the determination of the genetic origin of populations, in the evaluation of the genetic constitution of populations that are not very well known, as well as contributing to the evaluation of variability. Genetic variants of hair colour may also contribute to the image of high quality regional products and to the study of the genetic evolution of populations. For example, that author suggests that the presence of a white spot (He gene) on the snout of some European breeds (such as Berkshire) may be explained by the importation of pigs in the XIX century from southern China where that polymorphism is common. In New Zealand, Clarke and Dzieciolowski (1991) studied the genetic contribution of original breeds (ancient) to today's breeds through morphological comparison and hair colour variants. Works in this field will be impeled by recent advances in molecular genetics, namely the identification of locus KIT [dominant white, chromosome 8 (Johansson Moller *et al.,* 1996)] and the discovery of locus E, chromosome 6 (Kijas *et al.,* 1996; Mariani *et al.,* 1996) which are responsible for the extension of colouring, as well as by advances expected in upcoming years for the genetic mapping of the pig.

In fact, the genetic interpretation of visible effect gene phenotypes (colour and patterns) through their genotypic and genic frequencies in certain loci, has been used as a method for genetic patrimony inventory and characterization (Audiot, 1995). In the northern interior of Portugal (Santos Silva, 1996), some works in traditional pig farms identified a number of animals with visible genetic variants (coat colour, shape and position of ears, size, dorsal line) that characterized the ancient Bísaro pig. These observations proved to be of interest in the field of genetic resources and therefore lead to the development of a series of actions* in the areas of conservation, characterization and evaluation of that type of pig with the objective of determining production and processing potentials. This study deals with the first phase of this project which includes the identification and characterization of the Bísaro pig. During this phase, the following were carried out: a quantitative and genotypic inventory (coat colour and patterns) of the Bísaro pig herd; a first selection (individual/visible traits) of females and males with the objective of setting up two *in vivo* genetic conservation nuclei (Minho and Trás-os-Montes); in these nuclei and based on the type of pairing carried out, it was possible to suggest a list of genes found in the herd (genes responsible for the colour and patterns), as well as genotypes, their frequencies and geographic distribution.

In these nuclei (genetic material previously characterized and identified genealogically), studies were carried out on reproduction, production and meat quality performances. At the same time, a blood bank was set up in order to support genetic studies based on biochemical polymorphisms and sequences of nucleotide bases of the DNA molecule.

Materials and methods

General methodology

A questionnaire was distributed in the regions of Minho and Montalegre and the number of pigs (males and females) was recorded when these were over one year of age and had Bísaro standards (Lima, 1865). Each of these animals were characterized in terms of coat colour and distribution on the body. In this questionnaire, a survey was made regarding the number of pig farms, their structure and production methods used. According to the morphological evaluation carried out, ten females and three males (*in vivo* conservation nuclei) were chosen to represent different variants of colour (spotted and white). Random pairing was also carried out in order to establish genotypes and their respective alleles based on segregated phenotypic frequencies. The morphometric characterization was later carried out in the same animals and was used to compare both conservation nuclei (Minho and Trás-os-Montes).

List of genotypes and genes

18 pairings were carried out between 13 females and 6 males previously evaluated and recorded in the Book of genealogy. A phenotypic record of the coat (colour and patterns) was made for all piglets resulting from these pairings. After this, and considering observed and expected frequencies in descendants, genotypic and allelic interpretations were carried out according to tables elaborated by Legault (1997).

Geographical distribution of colour variants

Comparison (2, homogeneity test and two to two) of phenotypic frequencies of coat colour observed in the studied regions; Pearson correlation tests.

Morphology and morphometry

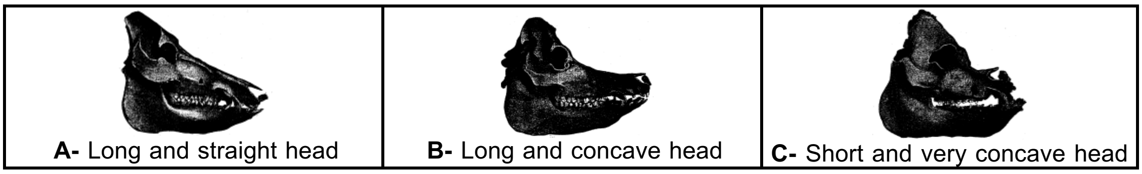
Comparison and variance analysis of the characterization of both conservation nuclei (Minho and Trás-os-Montes). Biometric measurements and morphological evaluations were carried out in all animals (adults with at least one farrowing) as follows: *Heights*: wither (alc), croup (alg), half back (almd); *Depth of chest*: *Lengths*: body (cc), barrel (ct), head (ccb), paw (cp); *Widths*: shoulders (le), breast (lp), hindquarters (lps); *Perimeters*: torax (pt), abdomen (pab), shin (pc).

The morphological evaluation followed the parameters bellow:

(i) Shape of the head. (Fig. 1)

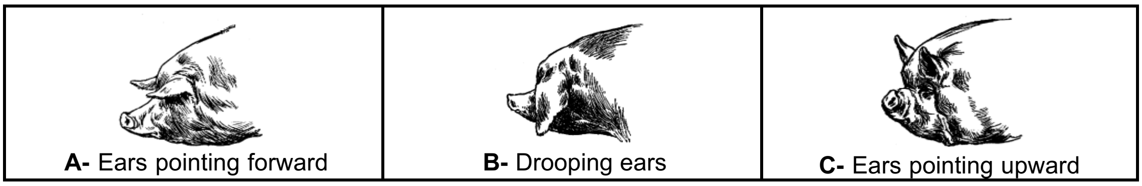
(ii) *Dorsal line*: (saddle, arched or straight); *Profile of croup*: (straight or concave).

(iii) Size and shape of ears. (Fig. 2)



A- Long and straight head
B- Long and concave head
C- Short and very concave head

Fig. 1. Morphological classification according to Cornevin (1898).



A- Ears pointing forward
B- Drooping ears
C- Ears pointing upward

Fig. 2. Morphological classification according to Sanson (1867) and Cornevin (1898).

Results and discussion

Phenotypic frequencies obtained for each type of pairing

Table 1 shows phenotypes obtained from pairing. This table shows frequencies of segregated phenotypic variants, summarizes frequencies obtained in each litter and groups them according to type of pairing. A total of 18 litters and 160 piglets were used.

Inventory of the main colour types and presumable alleles in the population

Agouti (A) locus

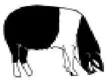
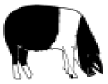
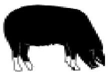
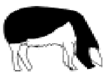
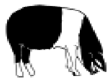
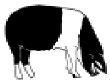
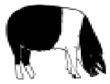
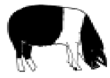
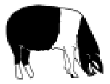
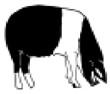
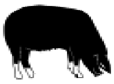
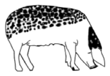
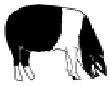
Some pairings between grey Bísaro pigs (mixture of white and black hairs) result in grey piglets that have light horizontal stripes (not quantified). These stripes alternate between light grey and dark grey and like the wild boar, this particular hair characteristic disappears with age. The cross between grey Bísaro pigs and wild boar originated 100% grey pigs, some of which have white snouts and others a black stripe. These observations lead us to believe that A gene (dominant) is present in the Bísaro population, although in low frequency.

Extension (E) locus

This locus, responsible for the extension of colour, was located (Kijas *et al.,* 1996; Mariani *et al.,* 1996) near the S0035 marker in chromosome 6. Due to its epistasis relation (hipostatic locus) with locus **I** (white or colour inhibitor, epistatic locus, see ahead), nuances in coat colour are observed only in the absence of dominant **I** allele. On the other hand, locus E has a modifying effect on the size of the white belt which is determined by the Be^w dominant gene of the White Belt locus.

Several variants were observed for the extension of colour: entirely grey pigs (greyish when they are "cerdões") except on the extremities (snout and members) and edges of ears; the same as the one mentioned above, except they have a white belt (Be^w gene, Locus Belt; see ahead); the same as the one mentioned above, except for a black snout; totally white; white with a coloured spot of variable size located on the hind flanks and dorso (a white spot may or may not exist on the head); the same as the one mentioned above (white), except they have many small spots – "malhinhas" – instead of a large spot.

Table 1. Summary of phenotypic frequencies obtained in each litter

Pairing			Resulting phenotypes (%)						
Phenotype		N							
		8	71	21	4			4	
		1		80	20				
		1	22				11		67
		2	80	10		10			
		1	40	20		10	20	10	
		1						50	50
		1						30	70
Wild boar		3	44		56				

These genotypes, with the exception of "malhinhas", are similar to those obtained by Legault (1997) in his experiments with French autochthon breeds, European and Chinese breeds (Limousine, Basca, Jinhua, Meishan, Piétrain and Hampshire). This leads us to conclude that the same E and E^P genes (respectively, black spot of variable size in the posterior area of the body and head or only in the posterior area/black body with white extremities) exist in the Bísaro breed. In relation to the "malhinhas" phenotype, we did not find any parallel with other works we are familiar with. This fact may suggest the existence of a still unknown mutation of series E (e^m) which is recessive to E and E^P. The extension of small spot on the body – "malhinhas" – varies from animal to animal (high variability). Similarly to big black spots of homologous location, the extension of small spots seems to have high heritability and may be determined by selection. This variant is the most rare and should be recessive to all loci. From now on, we will call this variant "Malhadinhos".

After having analysed the questionnaire distributed at farms, as well as the reproduction at the nucleus, we observed that no red, rusty, brown or orange coloured pigs were found. This fact leads us to consider that the Bísaro breed suffers no influence from the e recessive gene (red, rusty coloured; Duroc breed) and from others which may originate those types of colour.

White (I) locus

Locus I is epistatic for locus E, responsible for the white colour or inhibition of colour (I -), identified in chromosome 8, linked to ALB and PDGFRA genes (Johansson *et al.,* 1992). This locus is still being studied today and is presently the best known. The white and white/spotted phenotype was described by Lima (1865) and Pinto (1878) as being part of the Galega variety. However, the quantity and type of spots was never described by these or any other authors.

When coloured, all progenitors and descendants are grey (greyish colour) and no black animal was found. According to Legault (1997), greyish hair is a particular genotype (specific genetic combination involving both locus I and E (I^di EE). Its aspect is due to the mixture of black and white hair. This explanation coincides with our observations. However, observations of the skin revealed that it is greyish both in animals with a lot of hair ("cerdões") and in those with none ("molarinhos"). This fact may be due to a dilution factor of black pigmentation.

The crossing of white Bísaros among themselves resulted in white and spotted ("malhadinhos") piglets in a 7:3 proportion, thus revealing the heterozigotic state of progenitors for locus I. The backcross between White and "Malhadinhos" resulted in a greater number of the latter (5:5). This suggests two things: one is based on the explanation given before which admits the existence of a new e^m recessive gene in locus E, thus forming "malhadinhos" when the animal is recessive in locus I (ii); another explanation may be related to the existence of an i^{ml} recessive gene for all genes which belong to series I, except for i. This gene may be dominant or may have incomplete penetration in relation to E/E^P, thus resulting in the "malhadinho" phenotype (i^{ml} i^{ml} E -). The verification of this hypothesis may be possible by pairing "malhadinhos" with each other; confirmation will be possible if the segregation of this same genotype is 100%.

On the other hand, when at least one progenitor is grey, the segregation of mainly grey animals (mixture of white and black hair) leads us to admit the high frequency of the I^d allele in the population, as suggested by Legault (1997). Allele I^d is responsible for that phenotype when E is present, i.e., the I^di EE genotypic combination already mentioned. The cross between the wild boar and grey Bísaro females resulted in 100% grey piglets, which leads us to admit that females were I^d I^d homozigotic.

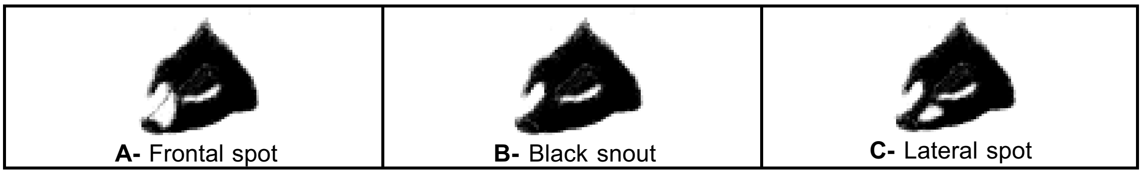
The low percentage (4%) of "malhadinhos" piglets which resulted from crossing grey animals could thus be explained through the I^d i^{ml} heterozigotic genotypic formula of progenitors. However, the continuation of works and the advances in molecular genetics in the future, namely in relation to chromosomes 8 and 6 (locus I and E, respectively), may explain these and other hypotheses.

White head or Hereford (He) locus

As already mentioned, in coloured phenotypes there is a high percentage of animals with a white spot on the snout (Fig. 3). According to Legault (1997), this characteristic is a result of a dominant gene (allele He, Locus He) which is possibly due to the importation of Chinese pigs during the XIX century. This white spot is characteristic in Berkshire or Poland-China European breeds and in Dahua bei and Moncai Chinese breeds.

F_1 from crossing the wild boar with white snout Bísaro females resulted in 50% descendants with that same characteristic. This result confirms the existence of a dominant gene which is responsible for the white snout. On the other hand, pairing between black snout animals resulted in 100% black snout piglets (hehe) and the crossing of white snout animals (done 8 times) resulted in an 8:2 proportion (white: black snout). Therefore, heterozigotic and homozigotic Bísaro pigs exist for this locus.

The white spot on the edge of the ears (Fig. 3) has a specific pattern which is found in 100% of pigs with coloured heads. This mark is specific to this breed and can also be seen in Bísaro pigs with grey hair only. Since we are not able to relate this unusual characteristic with any other types of spots or their size, we are lead to believe that there is a specific recessive mutation of unknown chromosomatic location which may be considered "exclusive" to this breed. These may be use as an image of marketing of products. Although it is not the only one.



A- Frontal spot **B-** Black snout **C-** Lateral spot

Fig. 3. White colour polymorphism on the snout and ear.

White Belt (Be) locus

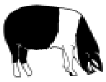
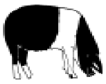
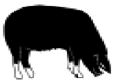
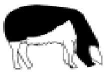
This locus is responsible for the white belt in the wither and shoulder areas through the Be^w dominant genetic polymorphism. This characteristic is observed in Wessex Saddleback and Hampshire pig breeds, as well as in cattle (Lankenvelder) and other mammals.

The F_1 from the cross between the Wild Boar and three Bísaro white belted females resulted in 50% piglets with the same characteristic in all litters. This result shows that the white belt of the Bísaro pig is due to the Be^w dominant gene in locus White Belt and that females are heterozigotic (Be^w be). For the same reason (heterozigotic), pairing between belted pigs resulted in a mean of 9:1 proportion.

Genotypic interpretation

The following Table 2 shows genotypic interpretation and varieties based on former results and the genic and genotypic tables of Legault (1997). Send locus C, D and R (dilution factors of colour and eye colour, respectively), allelisms very complex, they were not used.

Table 2. List of phenotypes found and their probable genotypic composition

Phenotype	Genotype	Phenotypic description	Common phenotypic description	Name of variety
	A^wa I^di EE hehe Be^wbe	Grey/spotted/ white snout/white belt	Spotted	Galega
	A^wa I^di EE hehe Be^wbe	Grey/spotted/ black snout/white belt	Spotted	Galega
	A^wa I^di EE hehe bebe aa ii EE hehe bebe	Grey or black/ white paws	Black	Transmontana/ Beirã
	A^wa I^di EE aa ii EE	Grey or black/ greatly spotted	Spotted	Transmontana/ Beirã
	aa ii EE	Grey or black/ not very spotted	White with spots	Transmontana/ Beirã
	aa $i^{ml}i^{ml}$ EE or aa ii e^me^m	Malhadinho	White with spots	Galega
	aa Ii EE	White	White	Galega
Wild boar	A^wA^w ii EE hehe bebe	Wild		

Quantitative inventory and geographical distribution of the herd

Breeder herd and its geographical distribution

The quantitative results of the questionnaire are shown in annex 1. Questionnaires were answered in 52 farms that had 133 Bísaro pigs [52 males (only 27 were breeding) and 81 females] considered as being pure. The following animals were chosen per region and sex (Table 3).

These farms are small (2.6 breeders/farm) and follow the traditional production methods used in Minho and Trás-os-Montes (Santos Silva, 1996). Since there is a low number of females on these farms, males do not fully use their reproductive potential, which means an additional cost in herd maintenance is needed. For this reason, farmers often decide to "share" males with other farms normally known as "mating posts". From the 52 farms which answered the questionnaire, 13 had a Bísaro male they shared with others. This system is the most widely used in the Minho region.

Table 3. Summary of the adult breeder herd (male and female) in each region

Category	Entre Douro e Minho	Montalegre	Total
Boars	19	33	52
Sows (>1 year)	42	39	81
Total males and females	61	72	133
Total number of farms	29	23	52
Breedes farms (collective boar)	10	3	13
Female/male ratio	2.21	1.18	1.56
Stocking rate	2.1	3.1	2.6

Geographical distribution of the different phenotypic groups (visible polymorphism of coat colour)

From the 124 pigs phenotypically inventoried (55 in Minho and 69 in Montalegre), 78% are grey, spotted and belted. 15% are white; 4% are spotted and 3% are totally black or grey. Although the number of animals is low, there is some variability in the Bísaro population. Variability is higher in the Minho region than in Montalegre. Phenotypic frequencies (colour variants) differ significantly according to region (2 = 60.2; *P*<0.001) in the following proportions: spotted with belt (65% in Minho and 88% in Trás-os-Montes); white pigs (20% and 11.4%); totally black or grey (7.2% and 1.5%). In relation to spotted pigs without belts, in Minho 9% were inventoried, while in Montalegre no animals of this type were found. These results suggest a more diversified genomic pool of the Bísaro pig in Minho (Figs 4 and 5).

The proportion of genotypes in males and females does not differ significantly (2 = 5.99; *P*>0.05), i.e., sub-populations of males and females have the same genotypic constitution. When compared to the geographical distribution of varieties 100 years ago, today both regions tend to show a decrease in white varieties (genotype I -, locus I, 14.5% genotypic frequency) and totally black ones (3%) in relation to grey varieties (carriers of the I^d mutation, locus I) which have a white belt (Be^w- genotype, locus Be, 78% genotypic frequency). Similarly to that period, a greater proportion of white and spotted white varieties (Galega) are found in Minho (23%) than in Montalegre (15%). This difference is observed because white males (5 in Minho and 1 in Montalegre) and totally black ones are not frequently used. The majority of males in both regions are belted: 97% in Montalegre and 50% in Entre Douro e Minho. The number of females from each genotype and region is directly correlated (r = 0.94; *P*<0.05) to the number of males from each genotype (Fig. 6).

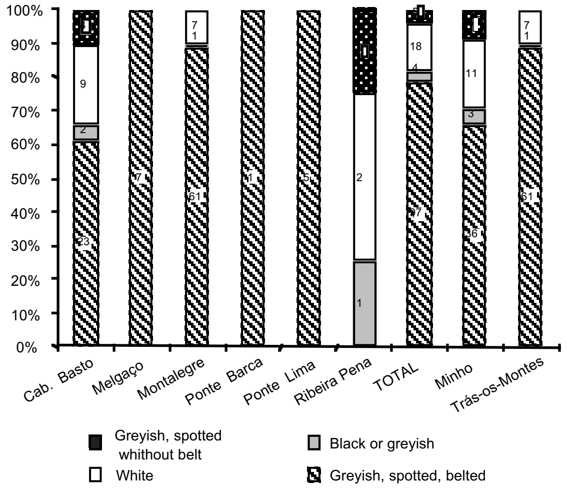


Fig. 4. Phenotypic frequencies of the different Bísaro types per region.

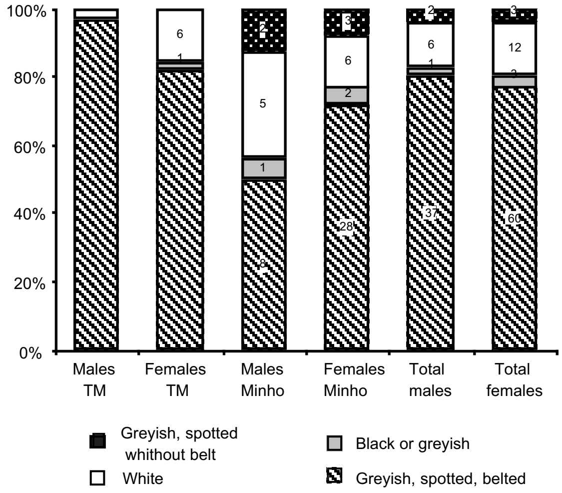


Fig. 5. Proportion of different phenotypes according to sex and region.

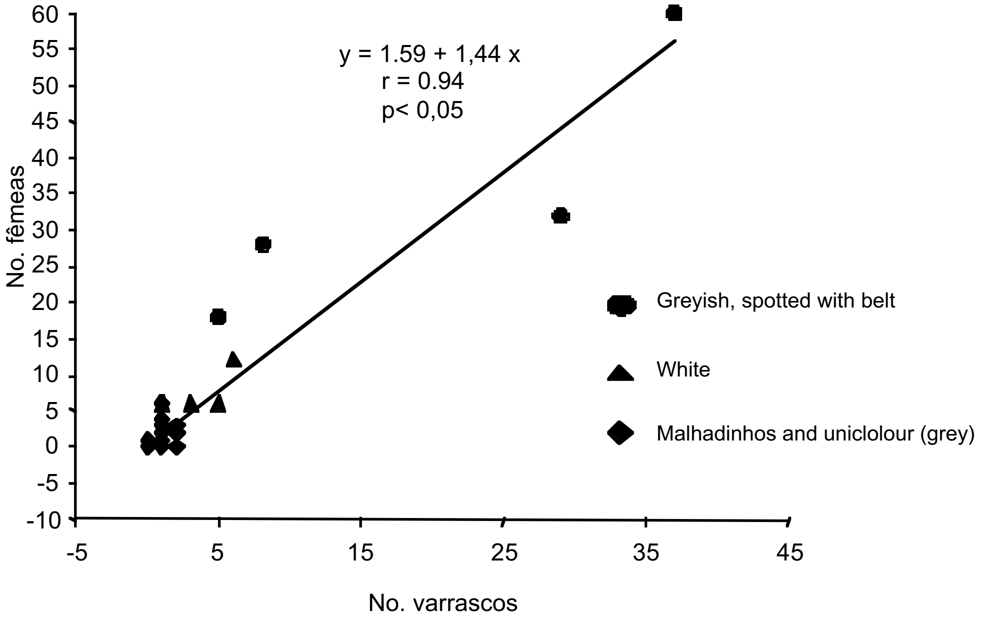


Fig. 6. Correlation between the number of males and females according to genetic type.

This phenomenon shows how farmers manage this genetic resource; for reproduction, they substitute sows with others (consanguinity) that are the offspring of active males, thus privileging the selection of Be^{w} dominant genes (locus White Belt) along with the grey phenotype. The low genotypic frequency of this type of animal in the herd is explained by the fact that totally black or spotted (recessive) pigs are seldomly used. In relation to the totally white variety and whether they are male or female, these animals were seldomly used by farmers because they were after mixed up with the most used "industrial" breeds of the region (Landrace, Large White). This lead more conservative farmers to select the more genuine and characteristic hair, such as the grey genotype, striped with a white snout and white marks on the edges of the ears (aa/I^{d}i/EE/Hehe/Be^{w}be). Today, this genotype represents 78% of the Bísaro breed herd.

Morphology

Shapes of head and ears

All animals (Minho and Trás-os-Montes) had the head and ears shaped like the original breed: concave snout and long, drooping ears, respectively (Figs 1 and 2).

This result confirms the Celtic origin of the breed in evolutional terms (Sanson, 1867; Cornevin, 1988; Bernardo Lima, 1865; Macedo Pinto, 1878).

Biometry

Heights: wither (alc), croup (alg), half back (almd); *Depth of chest: Lengths*: body (cc), barrel (ct), head (ccb), paw (cp); *Widths*: shoulders (le), breast (lp), hindquarters (lps); *Perimeters*: torax (pt), abdomen (pab), shin (pc) (Table 4).

Table 4. Morphometric results of both conservation nuclei (Minho and Trás-os-Montes)

Medida (cm)	N	Minho	Trás-os-Montes	Mean	Sig.
Heights					
Wither	22	87.9	86.5	87.0	NS
Croup	22	96.1	90.5	92.5	NS
Half back	22	97.5	93.5	95.0	NS
Depth of chest	22	51.9	56.9	55.1	NS
Lengths					
Body	22	179.5	182.2	181.2	NS
Head	22	29.8	29.6	29.6	NS
Widths					
Shoulders	22	33.8	31.2	32.2	NS
Breast	22	31.8	30.0	30.7	NS
Head	22	13.8	14.4	14.2	NS
Perimeters					
Torax	22	139.8	133.5	135.8	NS
Abdomen	22	155.3	136.4	143.2	*
Shin	22	22.8	21.2	21.8	*
N teats	17	13	12	12.4	NS

*($P<0.05$); NS: non significant

Results from morphometric measurements taken in both nuclei do not show significant differences in terms of size and shape. Global means of 181 cm in length, 95 cm in height and 31 cm in mean width (shoulders and hindquarters) show the original characteristics of this Celtic descent breed: tall animals, although narrow, long and weakly conformed. In both nuclei, the height measured at the middle of the dorso (95.0 cm) was higher than at the croup (92.5 cm) and wither (87 cm), thus showing another of the breed's original characteristics which is a convex dorsal line.

Conclusions

This work showed and confirmed the utility of studying visible effect polymorphisms for the genetic evaluation and characterization of populations. In relation to initial doubts regarding the pig population considered, this study confirmed that they belonged to the Bísaro breed with its original and probable genotypes (varieties of Galiza, Trás-os-Montes and Beira regions). This inventoried population continued to develop along the Celtic line. Alleles were identified (from the

A, I, E, He, Be locus) and the most probable genotypic combinations for each phenotype (Table 2) were suggested with basis on segregated phenotypic frequency analysis obtained through pairing Bísaro pigs and crossing with the wild boar. On the other hand, this study allowed us to confirm if our opinion coincided with the genic and genotypic tables of Legault (1997) regarding the phenomenon of hair colour heredity. As a result of this study, we may suggest that there are two new specific polymorphisms in the Bísaro pig: the "Malhadinhos" phenotype which may be a result of a probable recessive mutation from series I; white spot on the ears in pigs with coloured hair on the head (unknown locus) (Fig. 3).

In relation to the demography of the population, the quantitative inventory proved that the breed is endangered. Two *in vivo* conservation nuclei (20 females and 6 males) were set up (sire line and maternal line) in order to ensure the conservation of 2n pure chromosomes and the possibility of studying their development in a station based on already known genealogies.

The genetic inventory allowed us to confirm that the population is proportionally made up of 85% spotted grey varieties (with and without belt) and 15% white, while the greatest genotypic variability is found in Minho; this variability is directly correlated with a great genotypic variability of males used. In relation to morphometric characteristics, this study proved that populations from both regions do not differ.

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Annex

1. Herd's Bísaro geographique distribution

	Adress			Animals			Exploration type	
Region	Concelho	Freguesia	Lugar	Boars	Sows	Total	Colective boar	Numbers of farms
DRAEDM	Cab. Basto	Abadim	Fragata	1	1	2	1	1
DRAEDM	Cab. Basto	Abadim	Barrocas	2	1	3	1	1
DRAEDM	Cab. Basto	Abadim	Trancadas	1	3	4	1	1
DRAEDM	Cab. Basto	Alvite	Alvite		2	2		1
DRAEDM	Cab. Basto	Arco do Baúlhe	Morgado	1		1	1	1
DRAEDM	Cab. Basto	Arco do Baúlhe	Cerca Nova		1	1		1
DRAEDM	Cab. Basto	Arco do Baúlhe	Crasto		1	1		1
DRAEDM	Cab. Basto	Arco do Baúlhe	Crasto		3	3		1
DRAEDM	Cab. Basto	Arco do Baúlhe	Cerca Nova		1	1		1
DRAEDM	Cab. Basto	Pedraça	Souto do Rego	1	1	2	1	1
DRAEDM	Cab. Basto	Pedraça	Paço		1	1		1
DRAEDM	Cab. Basto	Pedraça	Souto do Rego		1	1		1
DRAEDM	Cab. Basto	Pedraça	Souto do Rego		2	2		1
DRAEDM	Cab. Basto	Pedraça	Souto do Rego					1
DRAEDM	Cab. Basto	Peinzela	Peinzela	2		2	1	1
DRAEDM	Cab. Basto	Peinzela	Peinzela		2	2		1
DRAEDM	Cab. Basto	Peinzela	Peinzela		1	1		1
DRAEDM	Cab. Basto	Peinzela	Peinzela		2	2		1
DRAEDM	Cab. Basto	Refojos de Basto	Quinta da Mata	1	2	3		1
DRAEDM	Cab. Basto	Refojos de Basto	Quinta da Mata		3	3		1
DRAEDM	Cab. Basto	Refojos de Basto	Carrezedo	2	1	3	1	1
DRAEDM	Melgaço	Fiães	Candosa	2	6	8		1
DRAEDM	Ponte da Barca	S. Martinho Crasto	Seara	1		1		1
DRAEDM	Ponte de Lima	Boalhosa	Meixieira	1	2	3	1	1
DRAEDM	Ponte de Lima	Boalhosa	Cima		1	1		1
DRAEDM	Ponte de Lima	Boalhosa	Cima		1	1		1
DRAEDM	Ribeira de Pena	Alvadia	Alvadia	1	1	2	1	1
DRAEDM	Ribeira de Pena	Alvadia	Alvadia	1	1	2		1
DRAEDM	Ribeira de Pena	Limões	Cadaval	2	1	3	1	1
DRATM	Montalegre	Pondras	Ormeche	1	2	3		1
DRATM	Montalegre	Pondras	Ormeche		1	1		1
DRATM	Montalegre	Reigoso	Currais		2	2	1	1
DRATM	Montalegre	Reigoso	Reigoso	1	4	5	1	1
DRATM	Montalegre	Reigoso	Reigoso	2	1	3		1
DRATM	Montalegre	Reigoso	Reigoso		2	2		1
DRATM	Montalegre	Reigoso	Reigoso	2	1	3		1
DRATM	Montalegre	Reigoso	Reigoso		2	2		1
DRATM	Montalegre	Reigoso	Reigoso	3	2	5		1
DRATM	Montalegre	Reigoso	Reigoso	3	1	4		1
DRATM	Montalegre	Reigoso	Reigoso	2	2	4		1
DRATM	Montalegre	Reigoso	Currais	2	2	4		1
DRATM	Montalegre	Reigoso	Currais	1	3	4		1
DRATM	Montalegre	Reigoso	Currais	2	1	3		1
DRATM	Montalegre	Reigoso	Currais		1	1		1
DRATM	Montalegre	Reigoso	Currais	2	3	5		1
DRATM	Montalegre	Reigoso	Reigoso		2	2		1
DRATM	Montalegre	Viade	Viade de Baixo	1	1	2		1
DRATM	Montalegre	Vila da Ponte	Vila da Ponte	2	2	4		1
DRATM	Montalegre	Vila da Ponte	Vila da Ponte	3	2	5		1
DRATM	Montalegre	Vila da Ponte	Vila da Ponte	2	1	3	1	1
DRATM	Montalegre	Vila da Ponte	Vila da Ponte	2	1	3		1
DRATM	Montalegre	Vila da Ponte	Choza	2		2		1
			DRAEDM	**19**	**42**	**61**	**10**	**29**
			DARTM	**33**	**39**	**72**	**3**	**23**
			TOTAL	**52**	**81**	**133**	**13**	**52**