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The systematics of some Italian populations of Wild boar (*Sus scrofa* L.): A craniometric and electrophoretic analysis

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Abstract

Studied craniometric and electrophoretic characters of Italian and South-Western French wild boar (*Sus scrofa* L.) populations, compared with two samples of domestic pig, to assess their taxonomic relationships and to check the actual validity of the two Italian subspecies *S. s. majori* De Beaux and Festa, 1927, and *S. s. meridionalis* Major, 1883.

Nine craniometric traits from 41 skulls (from the ancient maremma, present Maremma and Sardinia populations) were submitted to univariate and multivariate statistic analyses. 200 wild boar specimens (from the Maremma, Sardinia and South-Western France populations) and 68 pig specimens (Landrace and Sardinian native free ranging breeds) were submitted to electrophoretic analysis.

Statistic analysis of such data has shown the Sardinian wild boar well separated from the overlapping clusters of the Italian populations. Multivariate analyses of the adjusted, Log- and Ratio-transformed data show a general overlapping of these groups. The main morphometric differences among the Italian, as well as the Western Palearctic wild boar populations, may be explained by a body size factor, probably linked to an environmental cline.

Electrophoresis has proven genetic characteristics of the studied wild boar samples at the loci LAP-Rbc and 6PGD. A dendrogram computed from the Nei's Ds shows a cluster, inclusive of the Italian Maremma and the French populations, well set apart from a second cluster with the closely linked Sardinian wild boar and Sardinian free ranging pigs. The Landrace breed and a wild population recently crossed with domestic pigs, appears very well separated.

As the Italian Maremma populations seem to belong to the same environmental cline of the Western Palearctic *Sus scrofa* populations, the opportunity to suppress the subspecies *S. s. majori* is suggested. The Sardinian wild boar is fairly well characterized morphometrically as well as genetically, so that the subspecies *S. s. meridionalis* must be maintained.

Introduction

In a monograph published in 1927, De Beaux and Festa described the new subspecies *Sus scrofa majori* dedicated to F. Major, the first who recognized its characteristics (Major 1885), to group apart the wild boar population living in the Tuscany and Latium Maremma (Italy). This population appeared to have a mean body size smaller than the nominate form *Sus scrofa scrofa* L. (Western Europe). In the same monograph the validity of the subspecies *Sus scrofa meridionalis*, proposed by *Major* in 1883, was used to describe the Sardinian wild boar population. This population, showing small body size, adapted to the poor environmental conditions of the island, posed continuous problems of identification and nomenclature (Strobel 1882; Major 1885; Miller 1912; De Beaux and Festa 1927), perhaps related to its possible origins from a breed of domestic pigs that became ferals and to the continuous interbreeding with the native free ranging domestic pigs (For a review see Apollonio et al., in press).

An immigration of wild boars from France (South-East) towards the Italian regions Liguria and Piedmont started in 1919. De Beaux and Festa (1927) pointed out these animals to belong to the nominate form *Sus scrofa scrofa* L. The distribution of wild boars in Italy around 1950 is shown in Fig. 1A. The peninsular populations filled a small portion of the potential area and were subdivided in well separated patches.

Following the decline they suffered from the XVII century to the early 1900's as a consequence of the extensive environmental modifications and of the hunting pressure man exerted, a cycle of quick expansion of the Italian wild boar populations then began.

The expansion was mainly due to the restocking for hunting purposes with animals from abroad (therefore belonging to different populations and probably to different subspecies as well). An almost continuous distribution from North-West (Piedmont) to South-West (Calabria) was achieved (Fig. 1B). The rearing of native domestic pig breeds in semi-wild conditions, as well as the purposive crossing between wild and domestic pigs, are considered to allow the diffusion of hybrid genotypes in the wild populations.

The methodological limitations of the works of Major (1883, 1885) and De Beaux and Festa (1927) as well as the demographic events the peninsular populations underwent, strongly suggest the opportunity to reconsider the systematics of the Italian wild boars.

Studying skulls from some German populations, attributed to the nominate form *Sus scrofa scrofa,* in comparison with samples from Italian and from Sardinian populations, the hypothesis of the existence of a dimensional cline linking the Western Palearctic populations, was raised (Apollonio et al., in press). The largest sizes are shown by the populations living around the North-Eastern end of the cline (Germany); the smallest sizes are shown by the populations living at the South-Western end of the cline (Central Italy and Sardinia Island, South France, South Spain). Such a dimensional trend (following the Bergman rule) may possibly by related to the diversity expressed by climatic and environmental conditions ranging from the Northern temperate decidous forest to the Southern semiarid Mediterranean scrub.

In this paper we have analyzed through univariate and multivariate statistics a sample of skulls from the Sardinian and from the Italian continental wild boar populations including some skulls (preserved in Museums) which had been partly studied by De Beaux and Festa themselves. Our aim was to check if the Sardinian population could be morphometrically set apart from the peninsular ones and if the present Maremma populations retain the characteristics they showed when were a recognized as a new subspecies.

Moreover the genetic structure of several populations whose history is well known (from continental Italy, Sardinia and South-West France) was investigated through electrophoresis of blood and tissue proteins and enzymes. These samples were compared to those from domestic pigs belonging to an improved breed (Landrace) and to a sample from native Sardinian free ranging pigs.

The hypothesis of an environmental based dimensional cline of the Western Palearctic *Sus scrofa* populations was then contrasted with the electrophoretic data, in order to detect a possible genetic cline.

Material and methods

Blood and tissue samples were taken from captured, hunted or slaughtered animals belonging to the following populations (Fig. 1C).

1. San Rossore preserve (Pisa, Tuscany) = CSR (n = 109).
   The population of this preserve was originated in 1813 from a few animals belonging to the "Maremma" stock. In 1848 the population was decimated and afterwards, to aid a fast recovery, some free ranging pigs were introduced with a consequent crossing. A new population crash occurred in 1900 followed by a recovery. From that time until now only few animals have been introduced (1967) from the Castelporziano preserve. (n = sample size).
2. Castelporziano preserve (Rome) = CCP (n = 35).
   We have no information on introductions in this area. Probably this is the only true local nucleous and, because of its former geographic range, this population could be regarded as a relict of the *majori* subspecies.
3. Nuoro district (North-East Sardinia) = CSA (n = 9).
   In this range no introduction has occurred in the past with the exception of a few animals from Corsica in the 70's. However the Corsica and the Sardinia wild boars were formerly attributed to the same subspecies *meridionalis*.

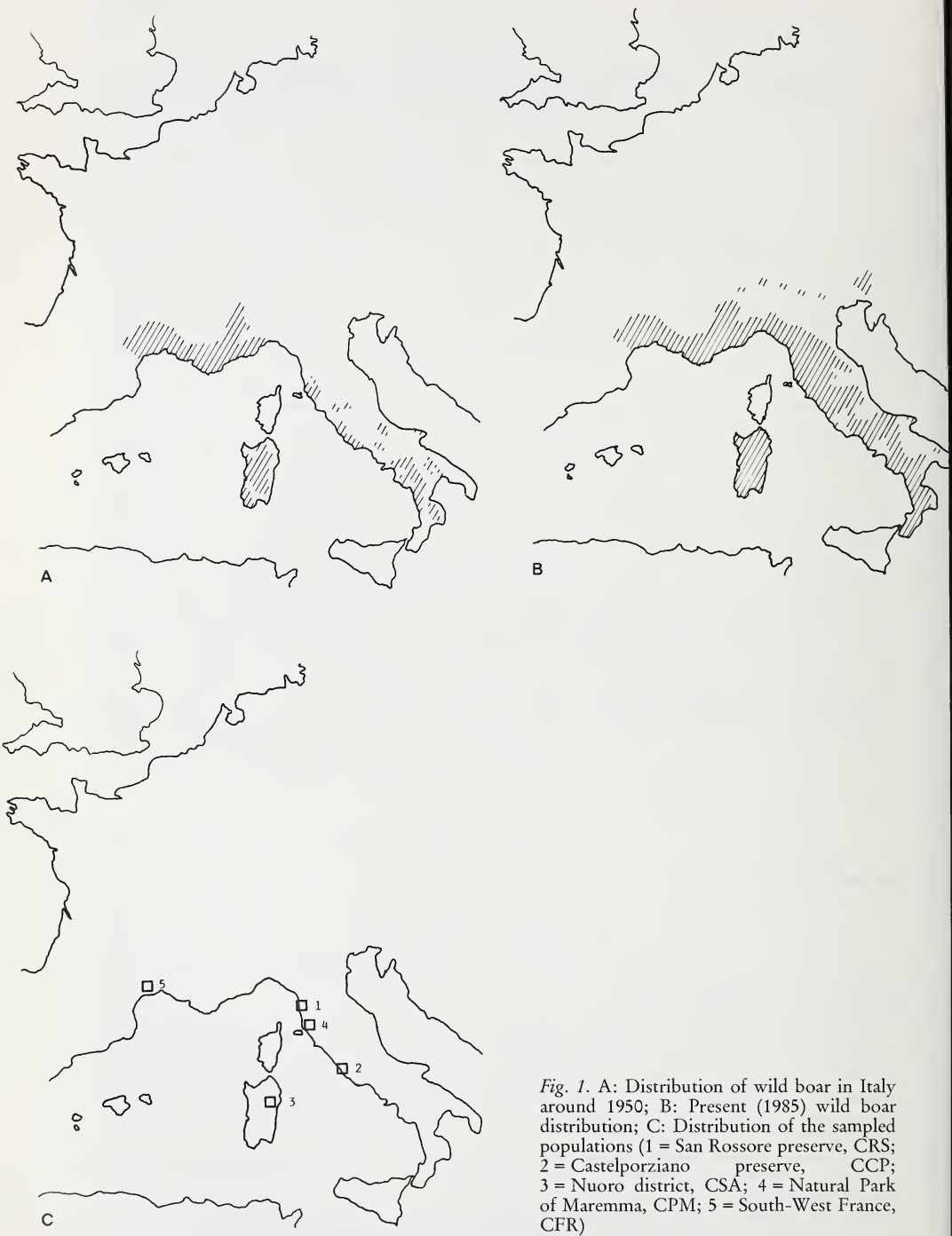

Fig. 1. A: Distribution of wild boar in Italy around 1950; B: Present (1985) wild boar distribution; C: Distribution of the sampled populations (1 = San Rossore preserve, CRS; 2 = Castelporziano preserve, CCP; 3 = Nuoro district, CSA; 4 = Natural Park of Maremma, CPM; 5 = South-West France, CFR)

4. Natural Park of Maremma (Grosseto, Tuscany) = CPM (n = 32).
   Until 1975 this area was a private hunting preserve. As before the World War II the local wild boars population was decimated by hunting and poaching, the owners planned to restock it with farmed wild boars crossed with domestic pigs (Boschi 1984). The present population shows phenotypic heterogeneity as a consequence of the past hybridization and a plan of selective cull is now carried out by the Park wardens.
5. France (South-West) = CFR (n = 15).
   Blood samples from hunted animals were obtained through the kind collaboration of Dr. G. Valet and Dr. F. Spitz (INRA, Laboratoire de la Faune Sauvage, Castanet Tolosan). They belong to populations living in several places of the Herault and Aude regions.
6. Landrace breed = MBO (n = 47).
   This sample of domestic pig was obtained from a local abbatoir (Bologna).
7. Sardinia native breed = MSA (n = 21).
   This is a sample of domestic pigs belonging to the native and free ranging Sardinian population.

Nine craniometric measurements (Tab. 1) were made on a total of 41 skulls of Italian wild boars partly belonging to the Sardinian population (CSA, n = 14♂♂; 3♀♀) and partly to the peninsular Maremma population. This sample consists of three groups:

1. Skulls (n = 13 ♂♂) belonging to the original Maremma population (CMAJ) studied by De Beaux and Festa (1927) for the identification of the subspecies *S. s. majori*. These skulls were obtained from the Natural History Museums in Milan, Florence, Turin, Genua and the Museum of the National Institute of Wildlife Biology and they include some of the specimens measured by Major (1885) and De Beaux and Festa (1927). They are therefore a sample of the Maremma population before any restocking was made.
2. Skulls (n = 8 ♂♂; 6 ♀♀) belonging to the San Rossore (CSR) preserve population, collected from hunted animals.
3. Skulls (n = 6 ♂♂; 8 ♀♀) belonging to the Castelporziano (CCP) preserve population, collected from hunted animals.

All skulls are from animals more than three years old as judged through the third molar tooth complete eruption and partial abrasion. Males and females were analyzed separately.

Size heterogeneity among the four groups was tested by analysis of variance of the observed means. An analysis of covariance (i. e. an analysis of variance applied to a linear regression model), was computed to check the presumed relationships between the length of the skull (expressed through its median superior length; character n. 3 in Tab. 1), and all the remaining craniometric variables. A set of regression coefficients, averaged over all the groups, is computed and used to adjust the group's means and the individual scores. A Snedecor's "F" test among the adjusted means was computed (Snedecor and Cockran 1967). The adjusted scores were used as input for multivariate analysis.

Multivariate relationships among groups were evaluated using two models (Morrison 1967). Principal Component Analysis (PCA) allows the description of the multivariate spatial distribution of the observed values within a cartesian system of vectors (PC) oriented along the successive maximum variability directions. The samples were analyzed as a single group in order to discriminate among group a-posteriori. The plot of the eigenvectors (loadings of the single variables) is a representation of the association pattern of the craniometric variables.

Canonical Analysis (CA) allows the visualization of the discrimination among a-priori determined groups, maximizing the between-groups versus the within-group variance. Within an orthogonal system of canonical variates (CV), the distances among groups are shown by the respective multivariate means (centroids) or by the distribution of the individual scores around their centroids.

Table 1. **List of the skull characters and acronyms of the studied samples**

1 = Condylobasal length.
2 = Overall length projected on the basal plane.
3 = Median superior length.
4 = Supraorbital width.
5 = Interorbital distance
6 = Width of nasal bones at maxillary-premaxillary suture.
7 = Height of skull with jaws clenched.
8 = Width of mandibula between condylear processes.
9 = Length of chin suture.

CSR = San Rossore preserve, Pisa, Tuscany, (n. 1 in Fig. 1c).
CCP = Castelporziano preserve, Roma, Latium, (n. 2).
CSA = Nuoro district, Sardinia, (n. 3).
CPM = Natural Park of Maremma, Grosseto, Tuscany, (n. 4).
CFR = South-West France (n. 5).
MBO = Domestic pig sample, Landrace breed.
MSA = Sardinia native domestic pig breed.
CMAJ = Sample of skulls belonging to the ancient Maremma population.

Multivariate analysis of the observed values was contrasted with multivariate analysis of the following data transformations:

1. Base-10 logarithms (Log), to correct for unequal character variances among groups linked to different sample size;
2. Ratios between each variable and the presumed general skull size (median superior length), to remove the influence of size variation among groups from the observed values;
3. Adjusted scores computed from the analysis of covariance, to remove the influence of size from the observed values.

Plasma, red blood cells (Rbc) and tissue homogenates (liver, heart) were submitted to electrophoresis following three techniques:

1. Polyacrylamide gel in horizontal slabs (PAGE) using an LKB (Bromma, Sweden) equipment;
2. Cellulose acetate membranes (CAM) using a Sartorius (Göttingen, W. Germany) equipment;
3. Vertical polyacrylamide gel in a discontinuous system (Davis 1964).

Buffer solutions and staining followed standard recipes (Tab. 2). A total of 33 loci were usefully resolved.

From the gels the allele frequencies were computed. Expected single locus heterozygosities, based on Hardy-Weinberg equilibrium, were tested against the observed heterozygosities (H_L), using a X^2 test. The mean heterozygosity over all the scored loci (H) was computed for each population, as well as the percent of polymorphic loci (P). A matrix of genetic distances among groups was computed

Table 2. **List of the studied loci and electrophoretic methods**

Locus[a]	Alleles[b]	Electro-phoresis[c]	Buffer	pH electro-de buffer	Method[d]	Tissues
1. G6PD	a	CAM	Tris-borate-EDTA	pH 8.7	A	Liver
2. LDH-A	a	PAGE	Tris-maleate	pH 7.4	B	Liver/Rbc
3. LDH-B	a	PAGE	Tris-maleate	pH 7.4	B	Liver/Rbc
4. AK	a	CAM	Phosphate	pH 6.25	A	Liver
5. GOT-S	a	PAGE	Tris-citrate II	pH 8.0	C	Liver
6. GOT-M	a	PAGE	Tris-citrate II	pH 8.0	C	Liver
7. MDH-S	a	PAGE	Tris-citrate II	pH 8.0	C	Liver/Heart
8. MDH-M	a	PAGE	Tris-citrate II	pH 8.0	C	Liver/Heart
9. ME-M	a	PAGE	Tris-citrate II	pH 8.0	C	Liver/Heart
10. ME-S	a	PAGE	Tris-citrate II	pH 8.0	C	Liver/Heart
11. SOD	a	PAGE	Phosphate-citrate	pH 5.9	B	Liver
12. PGM-1	a	CAM	Tris-maleate	pH 7.4	A	Liver/Rbc
13. EST-Rbc	a	PAGE	LiOH	pH 8.6	D	Rbc
14. IDH-S	a	PAGE	Phosphate-citrate	pH 5.9	B	Liver
15. IDH-M	a	PAGE	Phosphate-citrate	pH 5.9	B	Liver
16. ACP-1	a	PAGE	Tris-citrate	pH 8.0	B	Liver
17. ACP-2	a	PAGE	Tris-citrate	pH 8.0	B	Liver
18. α GPDH	a	CAM	Tris-borate-EDTA	pH 8.7	A	Liver
19. LAP-plasma	a	PAGE	LiOH	pH 8.6	D	Plasma
20. Pt-plasma 1	a	VERT	Disc. Davis	pH 8.3	E	Plasma
21. Pt-plasma 2	a	VERT	Disc. Davis	pH 8.3	E	Plasma
22. Pt-plasma 3	a	VERT	Disc. Davis	pH 8.3	E	Plasma
23. Pt-Rbc 1	a	VERT	Disc. Davis	pH 8.3	E	Rbc
24. Pt-Rbc 2	a	VERT	Disc. Davis	pH 8.3	E	Rbc
25. Pt-Rbc 9	a	VERT	Disc. Davis	pH 8.3	E	Rbc
26. Alb	a	VERT	Disc. Davis	pH 8.3	E	Plasma
27. Hb-A	a	VERT	Disc. Davis	pH 8.3	E	Rbc
28. Hb-B	a	VERT	Disc. Davis	pH 8.3	E	Rbc
29. PGI	a, b	CAM	Tris-borate-EDTA	pH 8.7	A	Liver
30. PGM-2	a, b	CAM	Tris-maleate	pH 7.4	A	Liver
31. 6PGD	a, b	PAGE	Phosphate	pH 7.0	B	Liver/Rbc
32. LAP-Rbc	a, b	PAGE	LiOH	pH 8.6	D	Rbc
33. Tf	a, b	VERT	Disc. Davis	pH 8.3	E	Plasma

[a] Loci nomenclature follows Harris and Hopkinson (1976). – [b] a = fast allele, b = slow allele. – [c] PAGE = polyacrylamide gel electrophoresis; CAM = cellulose acetate membrane electrophoresis; VERT = vertical polyacrylamide gel electrophoresis. – [d] A = Grunbaum (1981); B = Harris and Hopkinson (1976); C = Selander (1971); D = Ferguson (1980); E = Davis (1964)

Table 3. Mean values and adjusted means of the studied skull characters (males only)

Character[a]	CSA (n = 14)[b]		CMAJ (n = 13)		CSR (n = 8)		CCP (n = 6)		"F"-test	
	Obs[c] (± 1 s.e.)	Adj[d]	Obs (± 1 s.e.)	Adj	Obs (± 1 s.e.)	Adj	Obs (± 1 s.e.)	Adj	Obs	Adj
3	302.36 ± 5.27		361.38 ± 5.29		344.00 ± 8.25		366.66 ± 4.71			
1	275.36 ± 4.68	300.80	324.85 ± 4.08	306.50	304.50 ± 5.55	306.47	337.00 ± 3.49	314.74	**	*
2	304.14 ± 5.10	335.02	358.00 ± 4.89	335.75	329.37 ± 7.26	331.77	361.83 ± 4.40	334.82	**	n.s.
4	92.21 ± 1.53	100.47	103.08 ± 1.91	97.13	96.75 ± 1.81	97.39	105.50 ± 2.09	98.28	**	n.s.
5	66.14 ± 1.08	72.07	75.23 ± 2.02	70.96	71.87 ± 1.43	72.33	77.50 ± 0.76	72.31	**	n.s.
6	23.07 ± 0.79	26.14	29.93 ± 0.85	27.71	26.62 ± 0.82	26.86	30.00 ± 0.96	27.31	**	n.s.
7	165.57 ± 4.58	181.39	196.54 ± 4.21	185.14	186.25 ± 2.72	187.48	208.67 ± 4.52	194.83	**	n.s.
8	107.21 ± 1.87	115.38	119.61 ± 1.77	113.73	115.37 ± 1.75	116.01	119.17 ± 1.35	112.02	**	n.s.
9	74.86 ± 2.28	84.01	87.23 ± 1.94	80.63	82.12 ± 3.76	82.83	93.83 ± 3.02	85.83	**	n.s.

[a] See Table 1 for the list of characters. – [b] See 'Material and Methods' for the description of the populations. – [c] Observed means. – [d] The adjusted means were computed following the formula; $\bar{y}i = \bar{y}i - b_j (\bar{X}_i - \bar{X})$, where: $\bar{y}i$ is the adjusted mean; $\bar{y}i$ is the correspondent observed mean; b_j is the regression coefficient obtained through analysis of covariance and correspondent to the j character; $\bar{X}i$ is the mean of the character 3 for the i group; $\bar{X}$ is the general mean of the character 3 (from: Snedecor and Cockran 1967). – ** $p \leqslant 0.01$; * $p \leqslant 0.05$; n.s. = not significant

following Nei (1972) and Rogers (1972). These distances were clustered through an UPGMA procedure (Sneath and Sokal 1973) in order to obtain a phenogram depicting the probable genetic relationships among the groups.

Results

Analysis of covariance

Mean values of the 9 skull characters (males only) and the corresponding adjusted means are shown in Tab. 3. The analysis of variance of the observed means shows highly significant differences for all the characters, among the 4 wild boar groups ($p \leqslant 0.01$). The Sardinian population is particularly well separated from the Italian CCP one. Its dimensions appear larger than the present (CSR) as well as the old (CMAJ) Maremma populations. After analysis of covariance between the median superior length (considered as independent variable and as a good extimator of the size of the skull) and all the other variables (supposed depending from size) a set of adjusted means is obtained. The mean differences among the 4 groups appear to be very small and not statistically significant, character n. 1 excluded ($P \leqslant 0.05$), when the size factor is removed.

The "F"-test of the adjusted means is a test of parallelism among the regression lines of each variable on the size of the skull. We can observe that, size factor excluded, no significant dimensional difference among groups remains to suggest possible allometric variations. The difference between the Sardinia and the CCP groups is explained by the significance of the character n. 1 mean difference between the 4 groups.

PCA and CA computed with the observed values

About the 90 % of the total variability is explained by the two principal components PC-I and PC-II. The plotting of the male sample scores shows clear elliptic and elongated clusters (Fig. 2A) suggesting a high correlation among characters due to the effect of size variation (Sneath and Sokal

1973). All the characters show similar loadings on PC-I, behaving as they were the expression of a single size factor. Groups overlap each other along a linear sequence following size variation. Shape differences, expressed through some inverse correlations between lengths (characters n. 1, n. 2 and n. 3) and widths (characters n. 4 and n. 5), come out from the eigenvectors plot on PC-II. This principal component explains only the 4,4 % of the total variability and therefore it cannot produce any discrete cluster on PC-II (Fig. 2A). Similar results came out from CA, the order of the groups being the same PCA produces, while a better discrimination among them is evident. The Sardinian and the CCP groups are fairly separated at the ends, while CSR and CMAJ are near the middle of the distribution, with large overlaps (Fig. 2C). The separation between the Italian and the Sardinian populations appears rather clear.

Both PCA and CA give similar results by computing the male as well as the female values (Fig. 2B and 2D). (Measures on CMAJ females not available). The Sardinia females appear very clearly separated from the CSR and CCP ones, completely overlapping between them. The PC-I eigenvector shows a similar loading structure both in males and in females.

PCA and CA of the Logs and Ratios

PCA computed using the Log-transformed data produce distributions similar to those obtained from the observed values (Fig. 3A; Fig. 3B). The percent of explained variance and the structure of the eigenvectors remain also unchanged, both in males and in females, showing the noninfluent effect of sampling variance on multivariate outputs. CA of these data cannot produce any output being the determinant of the within groups matrix too small to give a possibility of discrimination.

PCA computed from the Ratio-transformed data show the effect of the dramatic reduction of variability, being the groups almost totally overlapping (Fig. 3C; 3D). While in males the Sardinian group separates a little from the Italian samples, in females the wide separation observed using the observed and the Log-transformed data, disappears completely. Moreover, the total phenotypic variability is distributed among several principal components. Using the observed and the Log-transformed data, PC-I explains about the 85 % of the variability and PC-I and PC-II together explain more than the 90 %. Working with the Ratio-transformed data we obtain a PC-I explaining only the 37 % of the total variability and we must cumulate the first six PCs to exceed the 90 %.

The eigenvectors structure shows possible allometric differences among the samples, but the residual variability after the data transformation is so small not to allow any discrimination.

CA computed from the Ratio-transformed data does not produce any output because of the great reduction of variability. The removal of the variation linked to different skull size, make impossible any multivariate discrimination among groups.

PCA and CA of the adjusted values

Adjusting the data in order to remove the effect of the size of the skull, we obtain clusters, computed through PCA, showing a spherical shape within the first two PC (Fig. 4A). the elliptic shape of the clusters is lost because of the data transformation: the correlation of each character with the size of the skull has been removed. The 4 groups now clearly overlap, especially if projected on PC-I. PCA produces PC-I and PC-II explaining the 50 % of the total phenotypic variability. These first two components are roughly equivalents (PC-I = 33 %, PC-II = 21 %). Without size effect the residual phenotypic variability is largely distributed among the principal components. The eigenvectors structure is similar both on PC-I and on PC-II and it expresses shape differences within the samples.

CA computed on the adjusted values is once again more efficient in discriminating among groups (Fig. 4B). Some possible elements of allometric variation allow a small

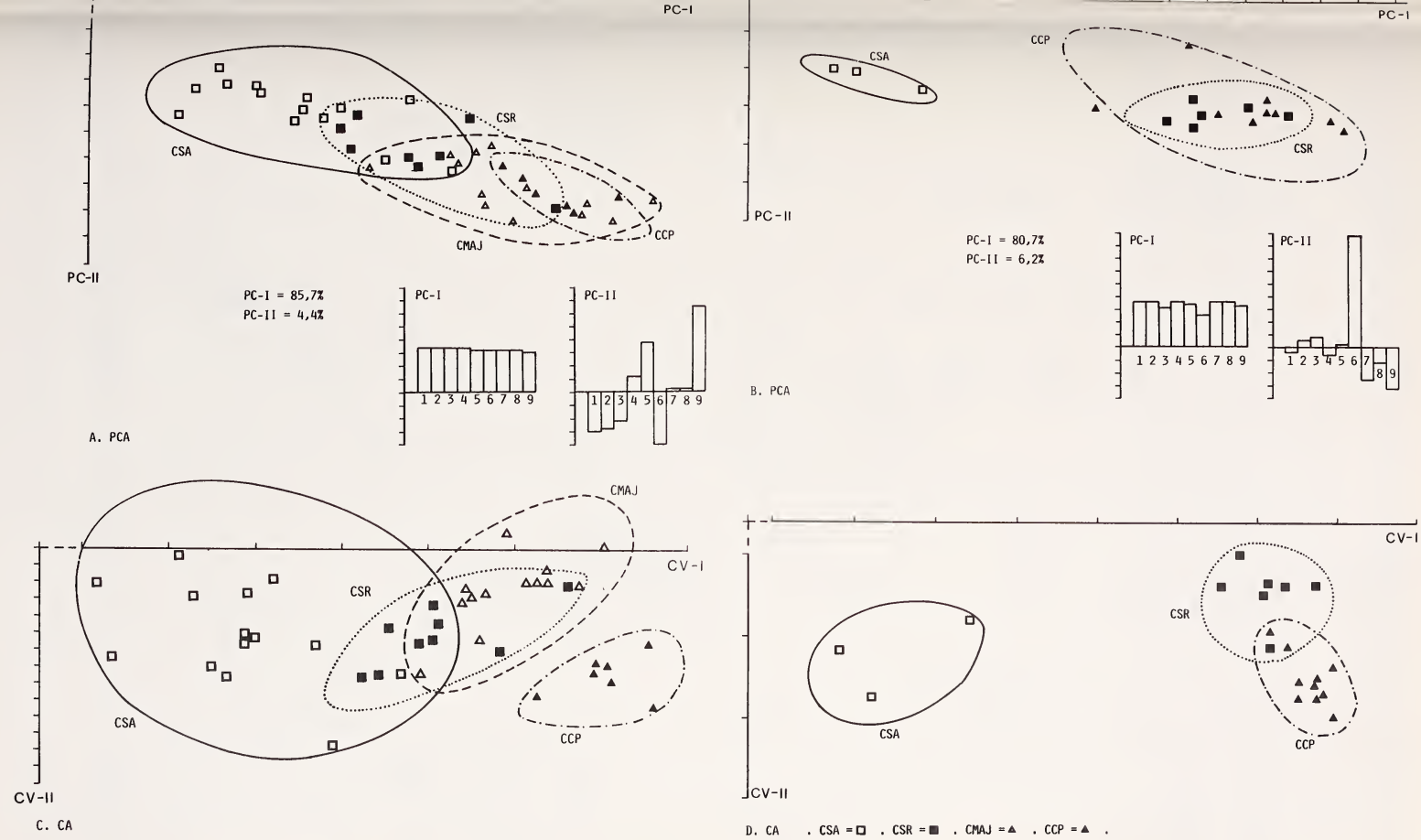

Fig. 2. Multivariate analyses of the observed values A: PCA ♂♂; B: PCA ♀♀; C: CA ♂♂; D: CA ♀♀. PC-I = first Principal Component. PC-II = second Principal Component. For the list of the sampled populations and of the craniometric characters, see "Material and methods"

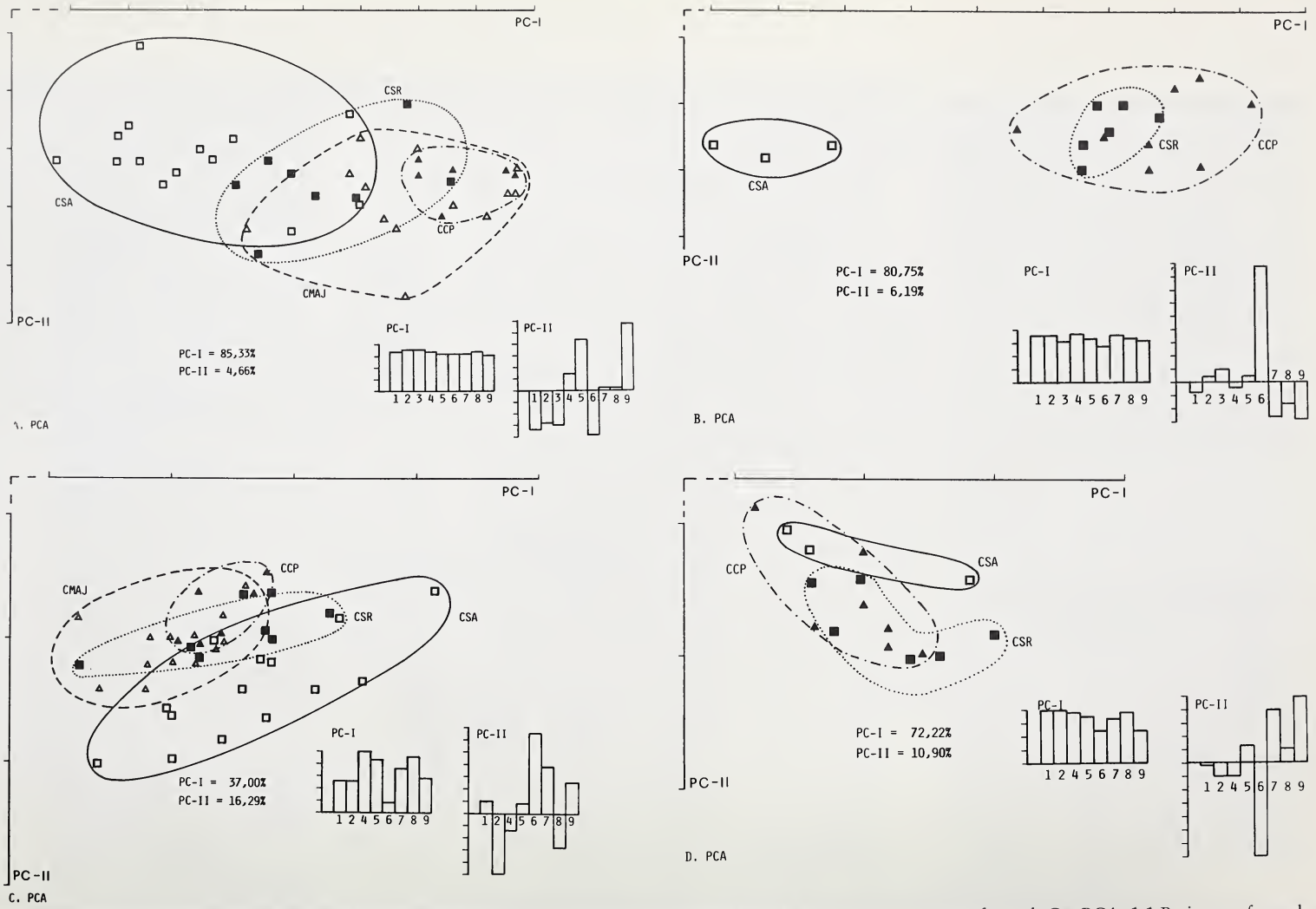

Fig. 3. Multivariate analyses of the Log- and Ratio-transformed values. A: PCA ♂♂ Log-transformed; B: PCA ♀♀ Log-transformed; C = PCA ♂♂ Ratio-transformed; D: PCA ♀♀ Ratio-transformed. PC-I, PC-II, sampled populations and characters as in Fig. 2.

separation of the Sardinia group. The portion of the total variability explained by allometry is however very small. The analysis of females produces similar results (not shown).

Electrophoresis

The listing of the polymorphic loci for each group, with the allele frequencies and their standard errors, the values of the observed heterozygosities and the χ^2 test of agreement with the expected HARDY-WEINBERG heterozygosities, is shown in Table 4.

In the wild boar samples there are 4 polymorphic loci (PGI, PGM-2, LAP-Rbc and Tf), while in the domestic pigs there are only 3 polymorphic loci (PGI, 6PGD, Tf). These polymorphisms are shared among groups, although several groups have been checked

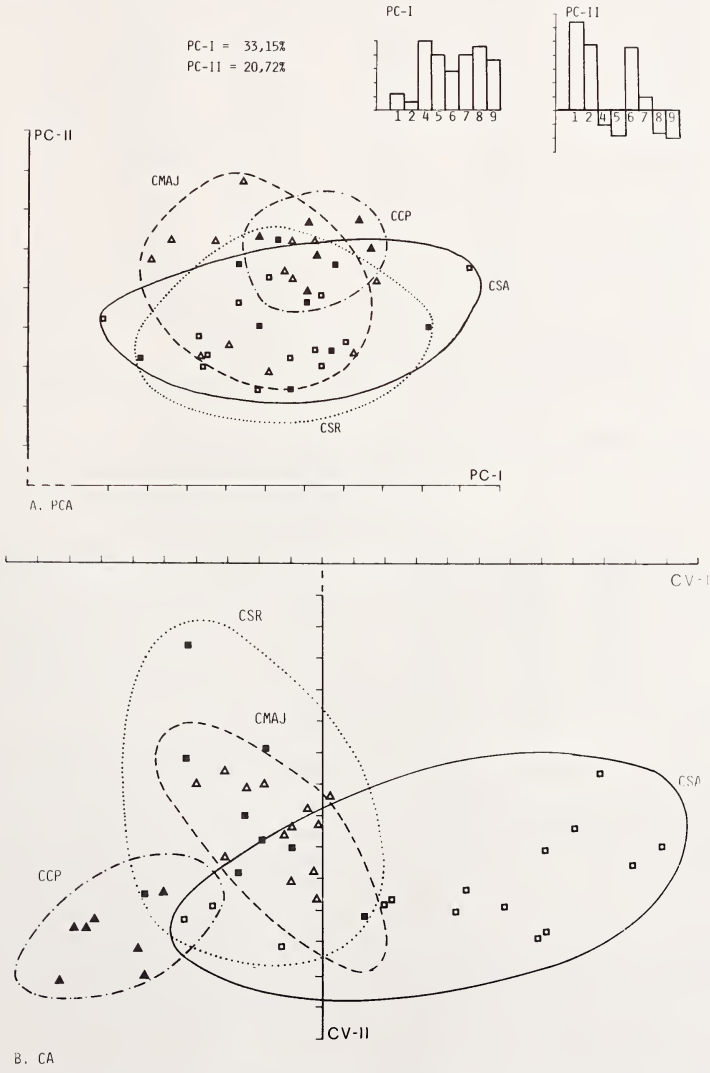

Fig. 4. Multivariate analyses of the Adjusted values. A = PCA ♂♂. B: CA ♀♀. PC-I, PC-II, sampled populations and characters as in Fig. 2

Table 4. **List of polymorphic loci, allele frequencies with their standard errors (s.e.), observed eterozygosities (H_L) and χ^2 test**

Population	Locus	Allele frequencies a	b	s.e.	H_L	χ^2	n
CSR	PGI	0.032	0.968	0.012	0.062	n.s.	109
	PGM-2	0.023	0.977	0.010	0.045	n.s.	109
	LAP-Rbc	0.014	0.986	0.010	0.028	n.s.	71
	Tf	0.250	0.750	0.056	0.375	**	30
CCP	PGI	0.171	0.829	0.045	0.284	n.s.	35
	PGM-2	0.071	0.929	0.031	0.133	n.s.	35
	LAP-Rbc	0.057	0.943	0.028	0.107	n.s.	35
	Tf	0.324	0.676	0.080	0.438	*	17
CSA	PGI	0.056	0.944	0.054	0.105	n.s.	9
	Tf	0.167	0.833	0.088	0.278	n.s.	9
CPM	PGI	0.500	0.500	0.063	0.500	n.s.	31
	Tf	0.391	0.609	0.061	0.476	n.s.	31
CFR	PGI	0.033	0.967	0.033	0.064	n.s.	15
	PGM-2	0.067	0.933	0.045	0.124	n.s.	15
	Tf	0.286	0.714	0.085	0.408	n.s.	14
MBO	PGI	0.309	0.691	0.048	0.427	*	47
	6PGD	0.596	0.404	0.051	0.482	n.s.	47
	Tf	0.063	0.938	0.027	0.117	n.s.	47
MSA	PGI	0.095	0.905	0.045	0.172	n.s.	21

* = $p \leq 0.05$; ** = $p \leq 0.01$; n.s. = not significant; n = sample size

Table 5. **Genetic variability within population**

Population	Ne	P	H
CSR	1.24	0.14	0.015
CCP	1.36	0.14	0.029
CSA	1.25	0.06	0.012
CPM	1.98	0.06	0.030
CFR	1.67	0.10	0.005
MBO	1.60	0.10	0.030
MSA	1.21	0.03	0.005
Wild boars		0.12	0.021
Domestic pigs		0.09	0.015

Ne = effective allele number at the polymorphic loci;
P = percent of polymorphism;
H = mean heterozygosity over all the studied loci

monomorphic at some of these loci, so the effective allele numbers (Ne) as well as the percent of polymorphism (P), are variable among groups (Table 5).

The Nei's genetic identities (I), and distances (D) among groups are shown in Table 6. The values of Is are very high, therefore the Ds among groups are very small (Rogers' values are highly correlated to the Nei's D values, so they are not shown here).

The Nei's Ds are clustered through an UPGMA procedure and they are shown in Fig. 5 (using the Rogers' S the same dendrogram is obtained). A first cluster includes the three continental populations: CFR, CSR and CCP, linked together by small values of D. Well separated from this cluster there is a second cluster including the Sardinia populations. The Sardinia domestic pigs (MSA) and the Sardinia wild boars (CSA) are closely linked together, but fairly away from the continental Italian groups. The crossed population living in the Natural Park of Maremma (CPM), is well separated between the wild boar cluster and the Landrace domestic (MBO) lineage.

Table 6. **Genetic variability among populations**

Populations	CSR	CCP	CSA	CPM	CFR	MBO	MSA
CSR		0.0009	0.0006	0.0074	0.0001	0.0086	0.0020
CCP	0.999		0.0020	0.0038	0.0007	0.0081	0.0036
CSA	0.999	0.998		0.0085	0.0011	0.0071	0.0004
CPM	0.993	0.996	0.992		0.0073	0.0097	0.0097
CFR	1.000	0.999	0.999	0.993		0.0091	0.0027
MBO	0.991	0.992	0.993	0.990	0.991		0.0065
MSA	0.998	0.996	1.000	0.990	0.997	0.994	

Nei's genetic distances D (upper triangular matrix); Nei's genetic identities I (lower triangular matrix)

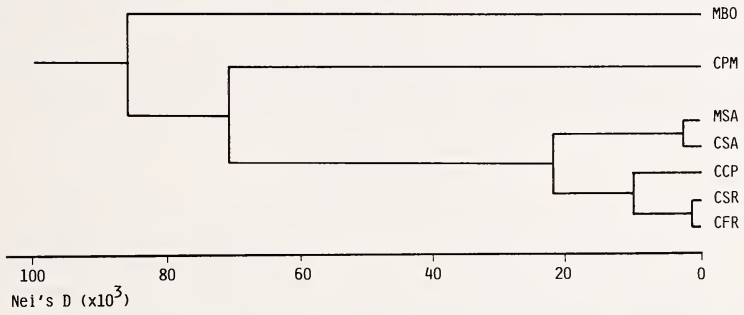

Fig. 5. UPGMA dendrogram showing possible relationships among populations computed from Nei's D values

Discussion

Morphometrics

The 4 populations we have studied were characterized through 9 skull measures the mean values of which proved significantly different. The adjusted mean values obtained after the removal of the size effect, appear not to be significantly different. So, size variation apart, these populations are not distinguishable from a univariate point of view. Multivariate analyses confirm this indication: the main factor of variation among population is a size factor. PCA computed using the observed values produces an elliptic swarm of the sample scores with a poor clustering of each populations that overlaps and dispose themselves following a linear sequence reflecting size differences.

Log-transformation of the observed values produces similar multivariate results. Ratio-transformed data produces a great reduction in variability. The population clusters show circular shape and the overlaps are wide. The prevalence of a dimensional factor in the discrimination among groups is supported by an analysis of the eigenvectors structure. PCA of the observed values gives a PC-I explaining about the 90 % of the total variability; all the characters on PC-I show similar loadings. The structure of the loadings on PC-II suggests shape differences, but the explained variability is so small not to allow any detection of clusters. PCA of the ratio transformed data distributes about the 90 % of the total phenotypic variability among the first six principal components and the loadings show shape differences on PC-I too. The main factor of variation among these 4 groups is a size factor expressed, in this case, through the median length of the skull.

Such a factor is correlated to all the skull characters we have studied, and it explains the greatest portion of the total variability. This factor is removed by adjusting the data after

the regression analysis, i. e. the variation explained through the size differences between populations is removed, so that any difference between them disappears and the residual variability is distributed on more, allometrically structured, principal components. This variability is, anyway, not great enough to discriminate among the groups.

CA model is designed to allow a maximisation of the morphometric distances among groups, so that they are discriminated better than PCA did. A better separation of the Sardinian sample on one side and of the CCP sample on the other side, is apparent.

The huge reduction of variability, within as well as between groups, following the Log- and Ratio-transformation, produces determinants of the within groups matrices so small that CA cannot give any output. No discrimination among groups is then possible.

Computing the adjusted data PCA cannot discriminate any population, while CA shows a permanent fair separation of the Sardinian group. If size differences among populations have been removed and the residual differences are due to allometric variations, we can conclude these are quantitatively very small and may fairly discriminate only a portion of the Sardinian population. The continental Italian samples overlap incompletely. The CCP sample, showing a large absolute size, plots rather apart also using adjusted data. It seems possible that small sample size and possible non-randomness in the choice of the specimens granted for study may have biased the results.

Electrophoresis

The results showed in the present work are among the few electrophoretic analyses on wild boar populations in Western Europe (Hartl and Csaikl 1987), while extensive research has been performed on several domestic pig breeds and on some wild populations in Asia and Eastern Europe (Tanaka et al. 1983; Schmid et al. 1980).

All loci we have found to be polymorphic in the wild boar, have been described as polymorphic in a wide number of domestic pig breeds (Ollivier and Sellier 1982; Franceschi and Ollivier 1981) as well as in the few studied wild boar populations (Smith et al. 1980; Hartl and Csaikl 1987).

A previously undescribed electrophoretic variation at the LAP-Rbc locus (Randi et al. 1986), was found only in the Maremma populations (CCP and CSR samples). This variant has not been detected in the Sardinian and in the French samples we have studied. This locus could be a genetic marker useful to detect differences between domestic and wild pig and could, moreover, have a narrow geographic diffusion. However, because no data was found in literature about this locus and because of the small size of some of our samples, it seems premature to draw any relevant conclusion.

The mean values of P and H we have computed for wild boars fall within the range estimated for large mammals (Nevo 1984). It is noteworthy that almost all the studied domestic pig breeds have been found polymorphic at the locus 6PGD (Rasmussen 1983; Oishi and Abe 1975; Dinklage 1969; Widar et al. 1975) while all the wild boar samples we have studied appear to be monomorphic. The polymorphism at the locus 6 PGD in the domestic pig is attributable to two alleles both present, almost in all breeds, at nearly intermediate frequencies so that even in a small sample it easily should be detectable. Moreover the presence of such a polymorphism is noteworthy in the Landrace breed we analyzed, while the Sardinian, native, unimproved domestic pigs appear to be monomorphic. A deeper discussion may start from the results published by Smith et al. (1980) on some electrophoretic analyses of samples from populations of domestic and feral pigs and of European wild boars that were introduced to the United States. The feral populations are the offspring of free ranging domestic pigs living in the Savannah River region before 1952, so they are feral since only a few generations. Another population on the Ossabaw Island is feral since about 400 years. In the Great Smoky Mountains National Park lives a population at least partly descending from a stock of introduced European wild boars.

All the domestic pig breeeds have been found polymorphic at the locus 6PGD, as well as the local domestic stock, with intermediate allele frequencies. The Savannah River feral population has been found polymorphic with practically the same allele frequencies of the domestic ones, while the Ossabaw ancient feral pigs show monomorphic the locus 6PGD. The Smoky Mountains wild boars are also monomorphic. Moreover a sample of 145 wild boars from four populations in Austria has been found monomorphic at this locus (Hartl and Csaikl 1987).

Two possible interpretations are suggested by these data:

1. Only one of the two alleles at the locus 6PGD is maintained in the wild populations. This locus is forced to be monomorphic because one allele is selected against by its own low fitness or, more probably, because of linkage with other unfavourable traits in natural environment. On the contrary this polymorphism is compatible with domestic life or, possibly, it is maintained in consequence of the artificial selection the breeders practice on the domestic breeds.
2. The domestic pig breeds intensively reared and genetically improved have an hybrid origin, deriving from European domestic breeds crossed with Asian breeds. While native European domestic breeds possibly derived from groups of European wild boars, native Asiatic breeds derived from Eastern wild populations (Epstein and Bichard 1984). Following the hypothesis that the Western wild and native domestic populations are monomorphic at the locus 6PGD, we can then suppose that some Eastern populations are polymorphic or monomorphic for the alternative allele so that the crossings performed to obtain the first improved genotypes can have produced this polymorphism at the locus 6PGD.

We must note that our CSR wild boar population, heavily interbred with domestic pigs since about 140 years, now shows an electrophoretic genetic structure similar to the other continental wild boar populations.

Native Sadinian domestic pigs appear to be monomorphic at the locus 6PGD and genetically very similar to the Sardinian wild boars. Moreover we can point out that our CPM sample is monomorphic at the locus 6PGD despite the recent heavy interbreeding with native domestic pigs belonging to the Cinta Senese breed (probably monomorphic; unpubl. observations).

These two hypotheses are of course not mutually exclusive: it is conceivable that the Eastern wild populations have a genetic structure dissimilar from the Western ones. Some indications are deducible from literature (Tanaka et al. 1983) but we do not know any published data on the locus 6PGD for Asiatic populations. These studies clearly state that Asian native breeds are genetically different from the improved European and American ones. If the present improved breeds have an hybrid origin their genome will bear the tracks of the parental populations. Furthermore one may think that, within the genetic background of the Western populations and within the environmental conditions the European wild boars live, the "Eastern" allele is selected against and then the locus 6PGD is driven toward monomorphism.

Anyway this locus seems to be very interesting to detect the genetic status of the European wild boar populations. Populations showing wild phenotypes but polymorphic at the locus 6PGD should be heavily suspected to be genetically polluted with improved domestic genomes. It might of course be very useful to extend the analysis and study other European native breeds and the Eastern subspecies *Sus scrofa vittatus* as well, in order to verify the genetic structure of the locus 6PGD.

In some few cases the observed heterozygosities do not agree with these expected under the equilibrium of Hardy-Weinberg (Table 4). The PGI case in the MBO sample may be the consequence of industrial rearing techniques: the breeding scheme possibly employed for the genetic improvement could have produced the observed disequilibrium. It is well known that the PGI locus is linked to the halotane locus (Rasmussen 1983). In the

domestic pig the locus PGI is polymorphic with two alleles, "a" and "b", of which "b" is always the more frequent one. The "b/b" genotype is linked with the halotane susceptibility, while the "a/a" and "a/b" genotypes are linked with the halotane resistance. In our MBO sample the frequency of "a" is clearly greater than in the wild boar populations, and the expected HARDY-WEINBERG genetic frequencies show an excess of observed alleles "a": the allele "b" could have been selected against. The Tf case in the CSR and CCP samples might be the consequence of having analyzed some hemolyzed serum samples in which the visual identification of the Tf bands may be confounded by the presence of Haptoglobin-Haemoglobin complexes. In all the other cases the agreement with the expected HARDY-WEINBERG allele frequencies is good.

The UPGMA dendrogram computed from the NEI's D matrix clearly shows a cluster including the two continental Italian and the South France populations; a second cluster including the Sardinian wild and domestic pigs and, well apart, the domestic Landrace breed and the crossed wild population living in the Parco della Maremma. In this population the recent heavy crossing with a native domestic pig breed (Cinta Senese), has clearly produced genotypes rather different from the genotypes of the wild populations living in the same range (Tuscany Maremma). The locus PGI, polymorphic in all the studied samples but generally with the "b" allele showing a high frequence near or a beyond the 90 % in the wild boar populations, seems to be a marker. In the CPM sample two alleles are present at this locus, both at the same frequency (0.50). In the domestic sample MBO the frequency of "b" is lower (0.69) than in any wild population. The Maremma Natural Park wild boars show a high phenotypic variability, and a selective program is now carried out with the aim to remove the morphologically abnormal specimens (BOSCHI 1984).

The Sardinian native domestic pigs and wild boars appear genetically very similar, suggesting a possible common origin and/or a continuous gene flow. The origin of the Sardinian wild boar is still debated (APOLLONIO et al., in press). It could be a wild continental population that became isolated or a domestic one, brought in by man in the past, which became feral. The native domestic stock could be the descendant of the original domestic population brought in by man, or it could have been tamed directly on the island starting from some wild individuals. Anyway the traditional rearing of the pigs in semi-wild conditions is still widely practised in Sardinia, so that an extensive gene flow is possible between the two populations. A strong genetic similarity is then understandable.

From the analysis of the craniometric and electrophoretic data we suggest that the Sardinian subspecies *S. s. meridionalis* should be maintained. The body size of the Sardinian wild boars is much smaller than any other Italian populations, the structure of the skull shows possible allometric differences in respect to the old and recent Maremma populations. The Sardinian lineage is fairly well electrophoretically separated from the continental cluster, including the Maremma and the French populations.

The present Maremma and France populations show a strong genetic similarity. Gene flow across these populations has probably never been interrupted for a time long enough to allow genetic divergence. The hypothesis that the size differences between the Western European populations have a prevalent environmental base is then supported. The environmental modifications produced by human activities in connection with demographic reductions European wild boars formerly have experienced certainly have originated populations reproductively isolated, but such an isolation has probably concerned populations previously not differentiated and it has not enough been prolonged to determine genetic divergence.

Craniometric and electrophoretic data suggest the opportunity to suppress the subspecies *S. s. majori* and to consider all the present Italian populations as belonging to the nominate form *Sus scrofa scrofa*.

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Zusammenfassung

Zur Systematik einiger italienischer Wildschweinpopulationen (Sus scrofa L.). Eine craniometrische und elektrophoretische Analyse

Zur Klärung taxonomischer Beziehungen und zur Überprüfung der Validität der beiden italienischen Unterarten *Sus scrofa majori* De Beaux und Festa, 1927 und *S. s. meridionalis* Major, 1883 wurden craniometrische und elektrophoretische Untersuchungen an italienischen und französischen Wildschweinpopulationen sowie an zwei verschiedenen Hausschweinrassen durchgeführt.

Neun Schädelmaße an 41 Wildschweinen (alte und rezente Populationen von Maremma und Sardinien-Populationen) wurden mittels univariater und multivariater Analyse ausgewertet. Stichproben von 200 Wildschweinen (Populationen aus Maremma, Sardinien, und dem südwestlichen Frankreich) und zusätzlich 68 Hausschweinen wurden ferner elektrophoretisch bearbeitet.

Die statistische Analyse ergab, daß die sardinische Population von den sich überlagernden Clustern der kontinentalen italienischen Populationen getrennt war. Die multivariate Analyse der bearbeiteten Daten zeigte hingegen eine allgemeine Überlappung der Gruppen. Die morphometrischen Unterschiede zwischen italienischen, wie zwischen west-paläarktischen Wildschweinpopulationen werden hauptsächlich durch den beeinflussenden Faktor Körpergröße erklärbar.

Die elektrophoretischen Untersuchungen haben genetische Besonderheiten der Loci LAB-rbc und 6 PGD ergeben. Ein Dendrogramm, erstellt an Abständen nach Nei, zeigt ein Cluster für die italienische Maremma- und die französische Population. Deutlich getrennt davon ist ein zweites Cluster für Sardinische Wild- und Hausschweine.

Abgesetzt davon wiederum erscheinen die untersuchten Hausschweine der Landrasse und auch eine Wildschweinpopulation, in die seit kurzer Zeit Hausschweine eingekreuzt sind.

Da die italienische Maremma-Population entsprechend unseren Befunden den westeuropäischen *Sus scrofa* zugeordnet werden müssen, erscheint ihr Unterarten-Status nicht valid und sollte beseitigt werden. Das sardinische Wildschwein ist demgegenüber sowohl morphologisch als auch elektrophoretisch gut characterisiert. Deshalb sollte diese Unterart weiterhin als gültig betrachtet werden.

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