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Allozyme variability in Bulgarian wild boar populations

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Genetic variability in 42 wild boars *Sus scrofa* Linnaeus, 1758 sampled from two Bulgarian populations was studied using multilocus allozyme electrophoresis. A sample of 37 wild boars from central Italy was used for comparison. Percent polymorphism (over 40 studied loci) and heterozygosity were higher in southern Bulgaria (P = 12.5; H = 0.028) than in northern Bulgaria (P = 5.0; H = 0.025) wild boar populations. Nei's genetic distance (D = 0.002) and Wright's F_{ST} (0.05) indicated the existence of significant ($p < 0.01$) genetic divergence among the two populations. Italian wild boars showed higher variability (P = 20; H = 0.049), and their average genetic distance from the Bulgarian samples was D = 0.013. Genetic variability of Bulgarian wild boar populations might be determined by their demographic histories (bottlenecks, isolation and expansion), and by possible cross-breeding with domestic pigs. Genetic divergence between populations is correlated with morphometric variation between the northern plain and the southern mountain Bulgarian wild boars (Genov *et al.* 1991). These findings suggest the possibility of natural selection and adaptation to different habitats.

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Introduction

A simultaneous increase in number and distribution range of wild boar *Sus scrofa* Linnaeus, 1758 populations occurred in recent years over the whole of Europe. This trend have been favoured by the action of several factors such as the improvement of habitat conditions following farmers' abandonment of some rural areas, variations in type and extension of dominant plantation crops, introduction and restocking of wild boar populations, limitations to hunting and better management practices (Geptner *et al.* 1961, Pavlov *et al.* 1974, Fadeev 1975 and 1989, Genov 1981, Erkinaro *et al.* 1982, Saez-Royuela and Telleria 1986, Apollonio *et al.* 1988).

Wild boars disappeared from many regions in Bulgaria during local wars and World War I (1912 – 1918) due to increasing hunting pressure. The formerly widespread and continuous population (Ivanov 1906) was fragmented in small isolates in the Rhodope mountains, Sredna Gora mountains, and in Rila – Pirin

Massiv, in southern Bulgaria; in the eastern part of the Balkan Range; and in Dobrudja and Ludogorie forests in north-eastern Bulgaria (Genov *et al.* 1991) (Fig 1). Wild boar populations increased and expanded their ranges in Bulgaria starting from the end of the 1950s. In north-eastern Bulgaria reintroduction and restocking were carried out using captive-reared local animals. The isolated southern mountain populations expanded during the 60s and recently came again in contact. Genov *et al.* (1991) indicate the possibility of cross-breeding of domestic pigs, reared in semi-wild conditions, with wild boars in the norheastern part of the country (Balkan range, Ludogorie and Dobrudja plains). Notwithstanding the recent expansion of population ranges, northern and southern Bulgaria wild boars seem to be actually isolated, because the Balkan Range and many human-inhabited areas constitute effective barriers to dispersal.

Genov *et al.* (1991) studied skull characters of wild boars from Bulgaria using multivariate methods, and described the exsistence of two morphological ecotypes: a larger type occurring in the northern plains, and a smaller type occurring in the southern mountains. In this paper we analyse genetic variability of wild boars sampled from the two Bulgarian populations using multilocus protein electrophoresis. Aim of the study was to describe genetic polymorphism within populations, and to estimate the extent of genetic divergence between south and north Bulgaria populations. A sample of wild boars belonging to a population from central Italy has been used for comparison.

Materials and methods

Tissue samples (liver and heart) were obtained from 42 Bulgarian and 37 Italian wild boars. Bulgarian wild boars were collected from the following areas (Fig. 1): northern Bulgaria (Eastern Balkan range and Ludogorie plains, n = 16); southern Bulgaria (Rhodope, Sredna Gora, Ograzden, Pirin and Rila Mountains, n = 26). Those two populations belong to the north and south Bulgarian wild boar phenotypes, which have been previously described by means of multivariate craniometrical analysis (Genov *et al.* 1991).

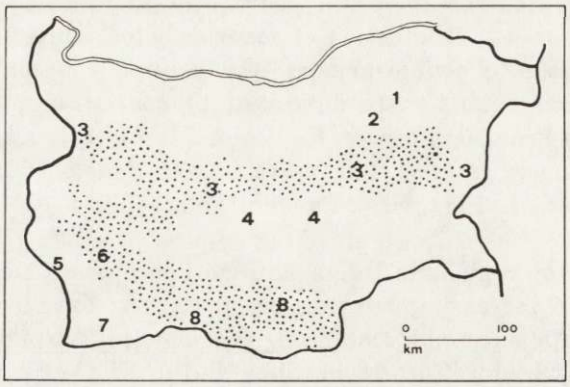

Fig. 1. Map of Bulgaria. Regions where the wild boar samples (sample size) were collected are shown: 1. Dobrudja (10); 2. Ludogorie (6); 3. Balkan Range (9); 4. Sredna Gora (4); 5. Ograzhden (2); 6. Rila Massiv (5); 7. Pirin Mountains (2); 8. Rhodopes Mountains (4).

Tissue samples were stored at –20°C during field collection, and –80°C in laboratory, until processing. About 1 gr of each tissue was separately homogenated in 1 ml of 0.01 M Tris /HCl, pH 7.5, 0.001 M Na2.EDTA, 0.001 M B-mercaptoethanol buffer, and centrifuged for 15 min at 13,000 rpm, at 4°C. Clear supernatants were diluted in 1 volume of a 40% glycerol solution, aliquoted in Microtiter plates, and frozen at –80°C until used. Vertical (VPAGE) and horizontal (HPAGE) polyacrylamide gel electrophoresis (7.5% monomers concentration in the resolving gels) were used to study allozyme variation at 40 loci, using the following buffer systems: 1 – discontinuous Tris/Glycine, pH 8.3 VPAGE (Davis 1964): AMY-1, AMY-2 (3.2.1.1); MDH-1 (1.1.1.37); FUM (4.2.1.2); non-enzyme heart proteins (HPT-1 to HPT-8); CK (2.7.3.2); LDH-1, LDH-2 (1.1.1.27); G6PDH (1.1.1.42); 2 – discontinuous Tris/Glycine, pH 8.5 VPAGE (Jolley and Allen 1965): SOD-1, SOD-2 (1.15.1.1); ME-1, ME-2 (1.1.1.40); HB-1, HB-2; PGM-2, PGM-3 (2.7.5.1); GPI (5.3.1.9); GOT-1 (2.6.1.1); GDH-1, GDH-2 (1.1.1.47); AK (2.7.4.3); 3 – Barbituric acid, pH 7.0 VPAGE (Williams and Reisfeld 1964): leucyl-alanine PEP-A (3.4.11.); leucyl-glycyl-glycine PEP-B (3.4.11.); 4 – Phosphate, pH 7.0 HPAGE (Harris and Hopkinson 1976): 6PGD (1.1.1.44); 5 – Tris/Borate pH 8.9 VPAGE (McLellan 1982): liver a-naphthylacetate esterases (EST-1, EST-2, EST-3; 3.1.1.1); 6 – Tris/Phosphate, pH 8.3 HPAGE (Harris and Hopkinson 1976): MPI (5.3.1.8); 7 – Histidine/MES, pH 6.1 VPAGE (McLellan 1982): IDH-2 (1.1.1.42); GOT-2 (2.6.1.1); ACP (3.1.3.2); ADH (1.1.1.1). Several enzymes were resolved in more than one buffer system. Staining recipes were adapted from Harris and Hopkinson (1976). Electromorphs were presumed to have a simple genetic basis, and were considered as alleles. Alleles were coded by their mobility from the starting line, with the most anodal allele coded as "a". Isozymes were coded with numbers, –1 being the most anodal.

The program BIOSYS-1 (Swofford and Selander 1989) was used to compute allele frequencies, effective allele number (*A*), percent polymorphic loci (*P*), and heterozygosity (*H*) values. Agreement with Hardy-Weinberg expectations was tested by chi-square test (Sokal and Rohlf 1981). Genetic differences among populations were estimated by *F*-statistics (Wright 1978), and tested with contingency chi-squares of heterogeneity (Workman and Niswander 1970). Nei's (1978) and Rogers' (1972) genetic distance matrices were computed, and were clusterized using UPGMA (Sneath and Sokal 1973) method.

Results

Eight loci (*Ck*, *Gpi*, *Got-1*, *Hpt-4*, *Hb-1*, *Pgm-1*, *Amy-1*, *Gdh-2*) out of 40 we have resolved, were polymorphic in one or more wild boar population (Table 1). All enzymes showed two alleles, except AMY-1 which showed four alleles. The three populations were quite different in average values of genetic variability. CK, HB-1 and GOT-1 were polymorphic only in the Italian population, which, therefore, showed percent polymorphism (P = 20.0) about 2 – 3 times higher than the Bulgarian populations (P = 12.5 and 5.0). Effective allele number was A = 1.0 and 1.1 in north and south Bulgaria, and it was 1.3 in the Italian sample. Average heterozygosity was about 40% higher in Italian (H_o = 0.049, H_e = 0.056) than in Bulgarian samples (H_o = 0.025 – 0.028, H_e = 0.025 – 0.035). Genotype frequencies at polymorphic loci were in Hardy-Weimberg equilibrium, with the exception of *Pgm-1* and *Amy-1* in south Bulgaria, and of *Hb-1* and *Amy-1* in Italy. These loci showed significantly ($p < 0.01$) less than expected heterozygotes. GPI, PGM-1 and AMY-1 were polymorphic in south Bulgaria, but monomorphic in north Bulgaria wild boar samples. Therefore, genetic variability estimates were, on average, higher in south Bulgaria wild boars (Table 1). Major contributions to average Tissue

Table 1. Allele frequencies at polymorphic loci and estimates of genetic variability at 40 enzyme loci in three populations of wild boar (n = sample size; P = percent polymorphic loci; A = effective allele number; H_o = observed heterozygosity; H_e = Hardy-Weinberg expected heterozygosity).

Locus	Allele n	north Bulgaria (16)	south Bulgaria (25)	Italy (37)
Ck	*a*	0.000	0.000	0.054
	b	1.000	1.000	0.946
Hpt-4	*a*	0.563	0.600	0.662
	b	0.437	0.400	0.338
Hb-1	*a*	1.000	1.000	0.865
	b	0.000	0.000	0.135
Got-1	*a*	0.000	0.000	0.041
	b	1.000	1.000	0.959
Gpi	*a*	0.000	0.020	0.162
	b	1.000	0.980	0.838
Pgm-1	*a*	1.000	0.960	0.932
	b	0.000	0.040	0.068
Amy-1	*a*	1.000	0.800	0.203
	b	0.000	0.000	0.080
	c	0.000	0.200	0.703
	d	0.000	0.000	0.014
Gdh-2	*a*	0.625	0.340	0.514
	b	0.375	0.660	0.486
P		5.0	12.5	20.0
A		1.0	1.1	1.3
Ho		0.025	0.028	0.049
He		0.025	0.035	0.056

Table 2. Genetic divergence among wild boar populations estimated as F_{ST} (Wright 1978), and assayed with contingency chi-square test (Workman and Niswander 1970). (a) n.s. = not significant; ** = $p < 0.01$; *** = $p < 0.001$.

Locus	F_{ST} estimates between Italy and Bulgaria		south and north Bulgaria	
Ck	0.037	n.s. (a)	0.000	n.s.
Hpt-4	0.007	n.s.	0.001	n.s.
Hb-1	0.094	**	0.000	n.s.
Got-1	0.027	n.s.	0.000	n.s.
Gpi	0.091	**	0.010	n.s.
Pgm-1	0.022	n.s.	0.020	n.s.
Amy-1	0.440	***	0.111	***
Gdh-2	0.055	**	0.081	**
Average	0.145	***	0.051	**

Table 3. Nei's (1978) unbiased (above diagonal), and Rogers (1972) (below diagonal) genetic distances among three wild boar populations.

Population	1	2	3
1 – south Bulgaria	–	0.002	0.009
2 – north Bulgaria	0.015	–	0.016
3 – Italy	0.030	0.036	–

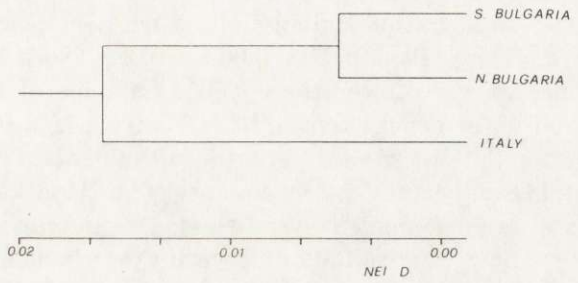

Fig. 2. UPGMA dendrogram of Nei's (1978) unbiased standard genetic distances among two Bulgarian and one Italian wild boar populations.

samples were stored at −20°C during field collection, and −80°C in laboratory, until heterozygosity were due to HPT-4 and GDH-2 (single locus H_o ranging from 0.45 to 0.49) in both populations. AMY-1 was polymorphic in south Bulgaria only, with a quite high value of heterozygosity (H_o = 0.32).

Genetic divergence among populations was estimated by means of F_{ST} (Wright 1978), which measures the among population percent genetic variance (Table 2). Average F_{ST} values were 0.145 between Italy and Bulgaria, and 0.051 between south and north Bulgaria, which means that about 15% and 5%, respectively, of total genetic variance was distributed among populations. Both F_{ST} estimates were significant (contingency chi-square test; Workman and Niswander 1970). Genetic divergence between Italy and Bulgaria was extensive, and was attributable to allele frequency variability at four loci (*Hb-1*, *Gpi*, *Amy-1*, *Gdh-2*). Genetic diver- gence between south and north Bulgaria was smaller, but nevertheless significant, and attributable to only *Amy-1* and *Gdh-2* loci (Table 2).

Nei's (1978) genetic distances among wild boar populations (Table 3) were clusterized using UPGMA procedure (Fig. 2). South and north Bulgaria populations were joined at a distance 0.002, while Italy and Bulgaria populations were clustered at a distance 0.013, more than six times larger. The cophenetic correlation was 0.87.

Discussion

We have found differences in estimates of genetic variability, and significant genetic divergence between two Bulgarian wild boar populations. Percent polymorphic loci and heterozygosity was lower in the northern than in the southern population (P = 5.0 and 12.5; H_o = 0.025 and 0.028, respectively). Nei's genetic distance was 0.002, and Wright's F_{ST} was 0.051 between the two wild boar populations. A sample of wild boars from central Italy showed higher genetic variability (P = 20; H_o = 0.049) and greater genetic divergence (D = 0.013, F_{ST} = 0.14) from the Bulgarian populations.

Randi *et al.* (1989) studied some Italian and French wild boar populations and obtained values of P = 10 – 14 and H = 0.005 – 0.029. Nei's genetic distances among local populations were lower than 0.001. Samples of native Sardinian domestic pigs showed lower genetic variability (P = 0.03; H = 0.005). It is usual that local wild boar populations show different levels of genetic variability (Smith *et al.* 1980, Hartl and Csaikl 1987), as consequence of bottlenecks, small numbers, hunting pressure and management. Nevertheless, genetic variability of the two Bulgarian samples we have studies falls well within the range of P and H values estimated in other wild boar and large mammal populations (Nevo *et al.* 1984).

Several causes may have resulted in the observed genetic variability in Bulgarian wild boars. The history of northern and southern populations is different. The northern, formely widespread, wild boar population, strongly decreased during the 50s. Moreover, cross-breeding with free-ranging domestic pigs has been described in that region (Genov *et al.* 1991). Bottleneck effect (Nei *et al.* 1975) may have reduced its genetic variability. Possible gene flow between domestic and wild pigs seem to have not increased genetic variability of the northern population in comparison with southern wild boars. According to Epstein (1971) and Bökönyi (1974) in the 7th millennium B.C. domestic pigs already occurred in southeastern Europe and were locally domesticated. Rural pig breeds of ancient origins could possibly have maintained a genetic structure very similar to their parental wild populations (Randi *et al.* 1989, Mayer and Brisbin 1991, Mauget 1991). Moreover, it was documented the effectiveness of domestication and repeated bottlenecks in reducing genetic variability during human-mediated diffusion of mammalian species (Randi and Apollonio 1988).

The present southern Bulgaria wild boar population is a mixing of formely isolated mountain populations which dispersed and overlapped during the recent positive demographic trend. It is therefore possible that genetic divergence accumulated during the bottleneck and isolation phase. The recent dispersal and intermixing of previously isolated groups could have resulted in the observed higher genetic variability of the southern population. It is noteworth the low heterozygosity observed in southern wild boars (H_o = 0.028, H_e = 0.035), which was due to significant less than expected heterozygotes at PGM-1 and AMY-1 loci. These findings may indicate a Whalund effect (Hartl and Clarck 1991): we have

considered as a single panmictic populations what in reality is a recent mixing of different populations, in a state of genetic non-equilibrium. The different demographic and population genetics histories, as well as the actual isolation, act to maintain the degree of observed genetic divergence between the southern and the northern Bulgaria wild boars.

Our genetic results are in agreement with the morphometric differences discovered by Genov *et al.* (1991) between northern plain and southern mountain wild boars in Bulgaria. Different ecological conditions (i.e. food availability and winter climate; Genov 1987, Genov, in press) could explain for most of morphometric variation, although the effects of local cross-breeding with pigs, and population histories could have a role. The genetic data reported in this paper suggest that population genetic factors are probably effective in determining divergence between the studied Bulgarian wild boars. We can not exclude (but not demonstrate) that natural selection may act to determine correlate variation at the morphometric and the alloenzymatic level, so determining divergent populations adapted to plain and mountain habitats, respectively.

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