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AN ASSESSMENT OF THE GENETIC DIVERSITY IN THE WILD BOAR POPULATION FROM THE PODUNAVLJE-PODRAVLJE HUNTING AREA

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Over the past decade, tremendous progress has been made in mapping and characterizing the swine genome. Lowden et al. (2002) successfully applied 31 microsatellite markers developed for domestic pigs in wild suiformes, finding a high level of conservation. Vernesi et al. (2003) used microsatellites to evaluate the genetic impact of the demographic decline and translocation in Italy, stressing the importance of microsatellite markers for conservation and management strategies. Ferreira et al. (2008) found supporting evidence of a bottleneck in Portuguese of wild boar populations, followed by an expansion from refugial areas towards historical distributions, using microsatellites. Mitochondrial and nuclear markers were used to test the postglacial dispersal of European wild boars, its domestication and hybridization with pigs, overhunting and demographic decline, recent expansion and translocations (Scandura et al. 2008). The results showed that European wild boars have the signature of postglacial demographic expansion, except for the Italian populations, which seem to preserve a high proportion of preglacial diversity. They also concluded that a wider sampling in Iberia and the Balkans would be necessary to identify which refugium area contributed most to the present gene pool of the European wild boar.

Wild boar in the West Balkan region is a native and popular game species, with wide distribution. Besides the fact that the genetic diversity and structure of game species is one of the most important aspects in wildlife population management and conservation, only few data on genetic variability of wild boars in Serbia have been presented so far (Djan et al. 2008). The aim of this research was to assess the genetic diversity in the wild boar population from the Podunavlje-Podravlje hunting area using microsatellites. The description of the genetic structure of this population represents a first step towards the characterization of wild boars in the West Balkan region, which is a major point in the development of conservation and management strategies.

Muscle tissue samples from 51 wild boars were collected from the Podunavlje-Podravlje hunting grounds located in the triangle between the Danube and Drava rivers. The wild boars were hunted on the part of this hunting area called Ludoš which is isolated from the rest by the rivers, but in this region wild boars have been translocated from all parts of the Podunavlje-Podravlje hunting area. The samples were divided into two subpopulations: young (6 month – 2 year-old individuals) and adults (4 year-old and older individuals). DNA was extracted by standard Proteinase K digestion (Kocher et al., 1989). Five polymorphic microsatellites were selected: S0068 and S0005 (Fredholm et al. 1993),

SW251 and SW857 (Rohrer et al. 1994) and SW2429 (Alexander et al., 1996). Amplification reactions were performed in 20 μl, with 10 pmol of each primer, 200 μM dNTPs, 1x*Taq* buffer, 1.5U *Taq* DNA polymerase, 2.5 mM $Mg(OAc)_2$ and 25 ng of genomic DNA. The PCR program consisted of 92°C for 2 min, followed by 30 cycles of 94°C for 30s, T_a for 30s, and 72°C for 30s, and a final extension of 72°C for 5 min (Rohrer et al., 1994). Amplified PCR products were analyzed on standard silver stained 6% denaturating polyacrylamide gels.

The frequency of the major allele (F), the number of alleles per loci (A), the number of private alleles, expected heterozygosity (He), observed heterozygosity (Ho), polymorphism information content (PIC) and inbreeding coefficient were estimated using GDA (Lewis and Zaykin 2001) and Popgene (Yeh and Boyle 1997). The linkage disequilibrium test was performed using Genepop (Raymond and Rousett 1995). Allelic richness (Rs) was evaluated using FSTAT (Goudet 2002) software. The distribution of gene diversity mean was performed according to Nei (1973). The total genetic diversity mean (H_T) is partitioned into two components: gene diversity within (H_S) and among (D_{ST}) subpopulations in FSTAT. The proportion of total gene diversity among the subpopulations (G_{ST}) was calculated $G_{ST} = D_{ST}/H_T$. The Microsat software (Minch 1997) was used to produce the proportion of shared alleles distance matrix among pairs of individuals. This distance matrix was submitted to analysis of molecular variance (AMOVA; Excoffier et al., 1992) using Arlequin software (Schneider et al., 2000). The significance of ϕ statistics was obtained after 10000 random permutations.

The amplification of the four microsatellite loci was successful in 82.4% to 94.2%, and the locus of SW857 couldn't be amplified, despite protocol modifications. All four loci were included in the statistical analysis since the linkage disequilibrium test indicated an independent segregation of loci. Loci SW251 and SW2429 are successfully amplified in wild boars for the first time, showing an adequate level of polymorphism.

A total number of 59 alleles were found in the wild boar population. The highest number of alleles per locus was found at S0068 (24), while the lowest number of 9 alleles was present at locus SW251, with an average of 14.7 (Tab. 1). Ferreira et al., (2008), in their analysis of Portuguese wild boar genetic variability, detected an average number of alleles lower than in our population A=10.17, as it was in the Italian and Hungarian wild boars (Vernesi et al., 2003) A=12.11.

A separate analysis of the young and adults showed a higher number of alleles in the young subpopulation correlating with number of samples for loci S0005 and S0068, but a significantly lower number of alleles at loci SW251 and SW2429, independent from the number of individuals (Tab. 1). The significant drop (p=0.019) of A from the young individuals to the adults could be explained by a bias in the adult samples due to the collecting of more related animals.

Among all alleles, 22 (37.29%) were found in both the analyzed groups. All the others were found only in one subpopulation (were private). Among the private alleles, 33 (60%) were found in the young group, while 4 were detected in the adults (15.38%). The average frequency of all private alleles was 62.71% (Table 1). The detected number of private alleles in the young and adult subpopulations correlates with number of individuals.

The mean allelic richness value for the analyzed population was 5.314 (4.365-6.490). In European populations the average Rs was homogenous across the wild boar populations, ranging from 2.6 to 3.5 (Scandura et al., 2008), which is lower than in our population.

The average observed heterozygosity (Ho) values for population was 0.579. Similar values were found for both subpopulations: in the young Ho=0.572 and in the adult Ho=0.571. In the European wild boar populations from Portugal (Ferreira et al., 2008) a higher Ho was detected (0.627), as was the case in the Italian and Hungarian populations Ho=0.662 (Vernesi et al.,

2003). In European populations of wild boars Ho=0.57 was calculated, which is the same as in our population (Scandura et al., 2008). According to these results, the level of genetic variability in the analyzed wild boar population is at the level for all populations on the European continent.

Deviation from the Hardy-Weinberg equilibrium was found due to a heterozygote deficiency detected for the population. The average F_{IS} values were significantly higher than zero (Table 1), indicating that inbreeding acts as a main cause for detected heterozygote deficiency. Since the samples were collected on the part of hunting area which is isolated from the rest by the rivers, and in this part are the wild boars that were translocated from all parts of the Podunavlje-Podravlje hunting area, a possible Wahlund effect might explain the heterozygote deficiency. Furthermore, the occurrence of null alleles in all analyzed loci might be responsible for some of the increased inbreeding coefficients. Heterozygote deficiency was found in the Italian (Vernesi et al., 2003) and Portuguese wild boar populations (Ferreira *et al.,* 2006) as well.

The mean Nei's gene diversity (1973) for the wild boar population was 0.887. Genetic variation within the subpopulations was much higher than among them (gene diversity mean within the subpopulations Hs=0.872 and gene diversity mean between the subpopulations D_{ST}=0.014). The genetic differentiation among the subpopulations (G_{ST}) was 0.016 which indicated that 1.6% of the total genetic diversity was distributed among the subpopulations, with 98.4% of the genetic differentiation within the subpopulation. Both values of gene diversity and portioning of nuclear diversity by the AMOVA model indicate that the within-population level acts as a major source of genetic variation, whereas the partitioning of variability due to age was clearly less pronounced.

In conclusion, the assessment of the genetic diversity in the wild boar population from the Podunavlje-Podravlje hunting area was the main objective of this study. This report represents the first genetic characterization of wild boar in the West Balkan region and it is very important for the future development of conservation and management strategies for this species. However, in future research new approaches must be taken, eventually including more individuals and more markers in order to allow the assessment of genetic structure without forcing an artificial and semi-artificial grouping of the individuals.

Table 1. Allelic variation at 4 microsatellite loci in wild boar (*Sus scrofa*) population. Population is divided into two subpopulations: YO – young individuals and AD – adults.

Locus	F	Rs	A	H_e	H_o	PIC	F_{IS}
	———————— Overall ————————						
S0068	0.177	6.490	24	0.925	0.583	0.921	0.379
S0005	0.170	5.523	15	0.876	0.553	0.864	0.378
SW251	0.321	4.365	9	0.787	0.833	0.757	–0.047
SW2429	0.304	4.878	11	0.825	0.348	0.805	0.585
Mean	0.243	5.314	14.7	0.853	0.579	0.837	0.331*
	———————— YO ————————						
S0068	0.138	6.492	22	0.926	0.575	0.921	0.390
S0005	0.179	5.544	15	0.875	0.487	0.862	0.453
SW251	0.342	4.083	9	0.763	0.868	0.727	–0.125
SW2429	0.308	4.615	9	0.807	0.359	0.782	0.564
Mean	0.242	5.183	13.8	0.842	0.572	0.823	0.332*
	———————— AD————————						
S0068	0.375	4.563	6	0.766	0.625	0.733	0.247
S0005	0.313	5.054	7	0.805	0.875	0.779	–0.021
SW251	0.250	6.000	6*	0.813	0.500	0.786	0.500
SW2429	0.286	5.091	7*	0.796	0.286	0.768	0.684
Mean	0.306	5.177	6.5	0.795	0.571	0.767	0.356*

F – frequency of the most common allele; Rs – allelic richness; A – number of alleles per locus; He – expected heterozygosity; Ho – observed heterozygosity; PIC – polymorphism information content; F_{IS} - inbreeding coefficient; *p<0.05

REFERENCES

Alexander, L.J., Rohrer, G.A., and *C.W. Beattie* (1996). *Animal Genet* **27**, 137–148.

Djan, M., Velickovic, N., Obreht, D., Gagrcin, M., Beukovic, M., and *Lj. Vapa* (2008). In: Abstracts of 7th International Symposium on Wild Boar (*Sus scrofa*) and on sub-order Suiformes (Ed, Nahlik, A.), 77. University of West Hungary.

Excoffier, L., Smouse, P.E., and *J.M. Quattro* (1992). *Genetics* **131**, 479–491.

Ferreira, E., Souto, L., Soares, A.M.V.M., and *C. Fonseca* (2006). *Wildl Biol Pract* **2**, 17-25

Ferreira, E., Souto, L., Soares, A.M.V.M., and *C. Fonseca* (2008) *Mamm Biol* **74**, 274-285.

Fredholm, M., Wintero, A.K., Christensen, K., Kristensen, B., Nielsen, P.B., Davies, W., and *A. Archibald* (1993). *Mamm Genome* **4**, 187-192.

Goudet, J. (2002). Institut d'Ecologie, Bâtiment de Biologie, Université de Lausanne, Dorigny.

Kocher, T.D., Thomas, W.K., and A. Meyer (1989). *Proc Natl Acad Sci USA* **86**, 6106-6200

Lewis, P.O., and D. Zaykin (2001). Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0.

Lowden, S., Finlayson, H.A., Macdonald, A.A., Downing, A.C., Goodman, S.J., Leus, K., Kaspe, L., Wahyuni, E., and *A.L. Archibald* (2002). *Conserv Genet* **3**, 347-350.

Minch, E., Ruiz-Linares, A., Goldstein, D., Feldman, M., and *L.L. Cavalli-Sforza* (1997). Stanford University, Stanford CA, USA.

Nei, M. (1973). *Proc Nat Acad Sci USA* Vol. **70**, No. 12, Part I, 3321-3323.

Raymond, M, and *F. Rousett* (1995). *J Hered* **86**, 248-249.

Rohrer, G.A., Alexander, L.J., Keele, J.W., Smith, T.P., and *C.W. Beattie* (1994). *Genetics* **136**, 231-245

Scandura, M., Iacolina, L., Crestanello, B., Pecchioli, E., Benedetto, M.F., Russo, V., Davoli, R., Apollonio, M., and *G. Bertorelle* (2008). *Mol Ecol* **17**, 1745-1762.

Schneider, S., Roessli, D., and *L. Excoffier* (2000). Genetics and Biometry Laboratory, University of Geneva, Geneva

Vernesi, C., Crestanello, B., Pecchioli, E., Tartari, D., Caramelli, D., Hauffe, H., and *G. Bertorelle* (2003). *Mol Ecol* **12**, 585-595.

Yeh, F.C., and *T. Boyle* (1997). *Belgian Journal of Botany* **129**, 157-163.