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EFFECTS OF DIFFERENT HUNTING MANAGEMENT ON THE GENETIC STRUCTURE OF WILD BOAR POPULATION IN SOUTH ITALY

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Summary: We focus on the effect of different hunting management (high protected reserves, National Park and managed hunting area) on wild boar (Sus scrofa) population in Italy, using genetic markers. Genetic analyses were carried out on a 300-bp of the mtDNA in 140 samples and 800 GenBank sequences. We analysed levels of molecular diversity and the mismatch distributions for each population from management hunting areas. Sixteen haplotypes were identified in the wild boar data set, belonging to the Italian, European and Asiatic clades. Intriguingly, the high protected reserves show a single Italian haplotype, while the other two areas show a similar genetic pattern with high genetic diversity and variability. In these latter the signature of past reinforcement was evident. In the National Park poaching may be responsible for the impoverishment of autochthonous (Italian) haplotypes.

Key words: wild boar; hunting management; mtDNA; protected and unprotected areas; Italy

Introduction

The wild boar, *Sus scrofa*, is an important game species. In the eighteenth and nineteenth centuries, for indiscriminate hunting, the species experienced demographic reduction and some local extinction. Since 1980, changes in agricultural practices, placement of artificial feeding sites, progressive decreasing of its predator favoured the increase in wild boar populations over the entire European range [1, 2]. In this scenario the extensive reintroduction for hunting probably played a critical role [3, 4, 5, 6, 7, 8, 9, 10, 11]. A large number of wild boar from Eastern Europe restocked in Central Europe [12], including Italy, with no particular attention paid to the possible consequences for the autochthonous groups. Actually, wild boar populations are widely exploited throughout Europe. In some cases it is considered a resource, as game species, with populations hunted and sustained by artificial restocking, but in the other cases, it represents a pest [12, 13].

In Italy, wild boar, like other game species, is managed differently in the same regions. In particular, we could have *High Protected Reserves (HPR)*, in which hunting and reinforcement of wild boar populations is forbidden, *National Park* (NP) in which these activities were developed up to 1995 and *Managed Hunting Areas* (MHA) (Italian *AT , Ambiti Territoriali di caccia)* in which the hunt and reinforcement are allowed (Table 1). In all those three different types of managed areas wild boar populations have home. Inferences about population structure and genetic traits allow us to get information on the optimum strategies of management for this resource, the wild boar.

In this paper we tried to evaluate the effect of management on wild boar population genetic structure using molecular genetics tools.

Material and Method

Hair, skin, skeletal muscle, and ear tissue from 140 wild boars were collected in 3 different sampling areas across Italy: a HPR, a NP and a MHA (see Table 1). Total genomic DNA was extracted using standard phenol–chloroform method [14]. To amplify the mtDNA partial control region (CR) we used two primers developed by Okumura et al. [15] (mitL76, 5'-AATATGCGACCCCAAAAATTTAACCATT-3' and mitH62, 5'-CCTGCCAAGCGGGTTGCTGG-3'). All amplified fragments were purified using GFX PCR DNA and gel band Purification Kit (GE Heathcare, UK) to be used as sequencing templates. Nucleotide sequences of both

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strands were determined by using an Applied Biosystems 3100 DNA sequencer with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems).

Table 1 - Areas with different hunting management. N= number of wild boar examined in each typology areas

	Wild boar reinforcement	Harvest activity	Harvest period	Natural predation	N
High Protected Reserve	Never	No	Never	No	13
National Park	Up to 1995	Poaching	Always	Some wolf	93
Managed Hunting Area	Currently	Hunt	From Sept to Dec	Some wolf	34

We performed the set of sequence alignment including wild boar from our study areas and 800 GeneBank sequences, from modern wild boar and pig breeds, chosen as representative of the current genetic diversity of Western Eurasia wild boar [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. Haplotypes were collapsed from the entire data set using collapse version 1.2 software (Posada, available at http://darwin.uvigo.es), setting deletions as fifth state.

We produced intraspecific mtDNA phylogenies. Phylogenetic analysis was performed by using MrBayes software v.8 [25] and model parameters identified by ModelTest [26]. Under the HKY85+G+I model, parameter estimates (including posterior probabilities) and consensus trees, resulting from five MrBayes runs of at least 1 million generations each, were recorded and contrasted. The posterior probabilities listed on both trees represent the lowest recorded values amongst all runs.

Levels of molecular diversity such as Number of polymorphic sites, haplotype (Hd) and nucleotide (πn) diversities, sequence conservation (C), homozigosity, mean number of pairwise nucleotide differences (k) and respective variance were calculated with Arlequin version 3.01 [27] for our three wild boar populations.

Mismatch distributions were performed for these populations, according to the sudden expansion model, by DNAsp [28]. Confidence intervals were obtained using a parametric bootstrap approach based on 10,000 simulated samples [29]. Populations that experienced a demographic expansion are expected to present a unimodal mismatch distribution. Conversely, populations experiencing demographic equilibrium should generate multimodal distribution [30, 31].

Results and Discussion

Mitochondrial sequence data (300 bp) were obtained from 3 populations, of which 13 samples from the HPR (High Protected Reserve), 93 from the NP (National Park) and 34 from MHA (Managed Hunting Area). Bayesian phylogenetic tree built by all sequences, with other obtained from GenBank, allowed us to assign our samples to different wild boar clades. In fact, previous genetic studies [22, 32, 33] have identified in Italy the presence of the three major *S. scrofa* mtDNA lineages: Asian, European, and Italian clades. The first two clades are widely distributed, while Italian haplotypes are not observed outside Italy.

From our data, in the NP 85% of samples belong to the European clade and 15% on autochthonous clade, of which 11% belong to Italian clade and 4% to the Asian clade. Samples in MHA belong for 82% to the European clade and 18% to the Italian one (Figure 1). In HPR instead we can find 100% of autochthonous Italian wild boars (Figure 1).

The number of polymorphic sites was very low for the latest population with only 1 polymorphic site, while we can find 8 and 7 polymorphic sites respectively in the NP and in MHA.

Relative timing of the expansion of haplotype clusters can be gleaned from a perusal of the mismatch distributions for various populations. Mismatch distribution plots were bi-modal in NP and in the MHA, although the above-mentioned population of the HPR showed a smooth, unimodal curve (Figure 1).

The mismatch distribution shows for the HPR a typical distribution of population experiencing a demographic expansion, as expected in this case. In the other two populations we can see the effect of human management throughout reinforcements, using allochthonous individuals, if we consider the result of haplotypes

clusterization. Even if in the NP the reinforcement stopped in the 1995 it is appreciable a sign of secondary contact, like in the MHA.

Substantially we observe a significant difference only in the HPR, in which the population experienced a progressive demographic expansion, probably due to the absence of both natural and human predation. The problem of wild boar in this case may emerge as a pest species in some time.

The structures shown by NP and MHA are interesting. In the first, the level of protection was not able to remove the signature of past reinforcement, and the poaching can be responsible for the impoverishment of
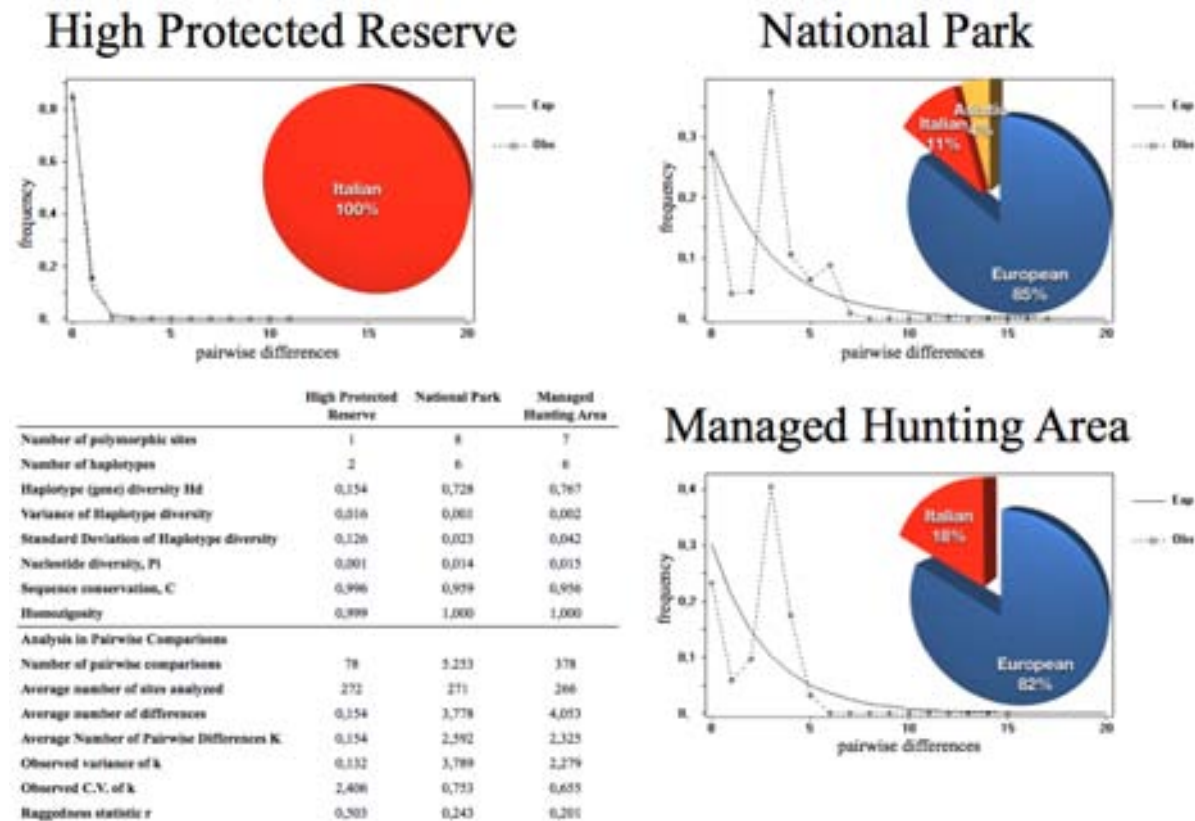

Figure 1 - Mismatch distribution of Wild boar sample. On the horizontal axis, there is the number of nucleotide site differences between pairs of individuals. Open circles show the relative frequencies of pairs with i differences. The solid line is the theoretical mismatch distribution fit.

autochthonous (Italian) haplotypes. In fact, surprisingly, in the HMA the percentage of pure Italian wild boar seems high. We hypothesised that the continuous reinforcement and the harvest in a short time, that act preferentially on the populations recently introduced in field (allocthonous), may, paradoxically, preserve autochthonous individuals that are better adapted to the Mediterranean environment and consequently harder to kill. This consideration cannot be interpreted as a favourable opinion about reinforcement with allochthonous samples that represent a bad hunting management. At the same time we want to underline, as in a NP, that the level of protection must be directed in parallel both toward the absence of introduction of allochthonous wild boars and toward the limitation of poaching. Without this joining management we may obtain the unexpected and undesirable results.

The poaching, in fact, acts in such a way as to harvest during the entire year, so that without periodic reinforcement of allochthonous, it impoverishes autochthonous Italian populations.

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