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Genetic structure of the wild boar population in Portugal: Evidence of a recent bottleneck

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Abstract

The present study assesses the degree of genetic structure and the presence of recent genetic bottlenecks in the wild boar population in Portugal. One hundred and ten individuals were sampled after capture during organised legal drive hunts, conducted in 58 municipalities across the continental territory, during the game seasons of 2002/2003 and 2003/2004. Individuals were genetically typed at six microsatellite loci using multiplex PCR amplification. Significant deviations from Hardy–Weinberg equilibrium were found for the total population of wild boar in Portugal. Wild boar population genetic structure was assessed using Bayesian methods, suggesting the existence of three subpopulations (**North**, **Centre** and **South**). Tests were conducted to detect the presence of potential migrants and hybrids between subpopulations. After exclusion of these individuals, three sets of wild boars representative of respective subpopulations were distinguished and tested for the effects of recent bottlenecks. Genetic distances between pairs of subpopulations were quantified using F_{ST} and R_{ST} estimators, revealing a variation of 0.138–0.178 and 0.107–0.198, respectively. On the basis of genetic and distribution data for Portuguese wild boar from the beginning of the 20th century, a model of strong demographic decline and contraction to isolated refuge areas at the national level, followed by a recovery and expansion towards former distribution limits is suggested. Some evidence points to present admixture among subpopulations in contact areas.

Keywords: *Sus scrofa* L.; Genetic structure; Bottleneck; Microsatellites; Bayesian statistics

Introduction

Wild boar (*Sus scrofa*, Linnaeus, 1758) is a widely distributed ungulate whose success can be attributed to a variety of ecological features such as opportunistic omnivorous behaviour (Massei et al. 1996; Fonseca 2004), high prolificness and adaptive plasticity (Fonseca et al. 2004). It is described as a philopatric species depending on age class (Spitz and Janeau 1990; Lemel et al. 2003), and presenting differences on annual and seasonal space use (Keuling et al. 2007). However, evidence indicates low genetic flow among local populations (Okumura et al. 1996).

According to Fonseca (2004), wild boars were once very abundant in Portugal. However, at the beginning of the 20th century, the species was confined to mountain areas near the national border with Spain and to some royal hunting areas, due to strong hunting pressure (Fonseca 2004). By the 1960s, wild boar populations were extremely reduced due to both continued over-hunting and an outbreak of classic swine fever. In 1967, wild boar hunting was banned in Portugal (Serôdio,

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1985 **in** Fonseca 2004), and by the end of the 1970s the species began to recover. However, at that time the distribution of wild boar remained fragmented. Nowadays, wild boars are found throughout the country, with the exception of major urban areas and some parts of the coastline (Fonseca 2004), and they are of "low concern" in terms of their national conservation status (Cabral et al. 2005). Similarly in Spain, the wild boar is distributed throughout most of the continental territory (Rosell and Herrero 2002).

Wild boar populations are widely exploited and managed throughout Europe. However, knowledge of the genetic, demographic and ecological impacts of these management practices remains incomplete (Randi 1995). The genetic impact of wild boar relocations was considered by Vernesi et al. (2003). Animal health can be impacted by relocating wild boars (Fernandez-de- Mera et al. 2003), particularly when these animals come in contact with farmed domestic pig (Vidal et al. 2006; Melzer et al. 2006).

Wild boar and domestic pig belong to the same species, *S. scrofa*, Linnaeus, 1758. There is some evidence that pig domestication occurred independently in Europe and Asia (Giuffra et al. 2000; Larson et al. 2005). The divergence between ancestral forms of wild boar has been estimated to have occurred much earlier than that estimated for its domestication (Giuffra et al. 2000).

The species genome has been widely deciphered. Large contributions were made by the "PigMap" (Archibald et al. 1995) and "USDA Pig Genome" (Rothschild 2003) programs in Europe and the USA, respectively. Full sequencing of *S. scrofa* mitochondrial DNA was achieved by Ursing and Arnason (1998). Microsatellite mutation rates were estimated for this species by Yue et al. (2002) with 7.52×10^{-5} per **locus** and generation. Lowden et al. (2002) tested several microsatellite markers developed for domestic pig in wild suiforms, finding a high level of conservation in the studied loci. Several studies on domestic pig breeds' biodiversity have been performed using these kinds of markers (e.g. Harcet et al. 2006; San Cristobal et al. 2006). Microsatellites were also applied in studies of feral pig populations in Australia (Hampton et al. 2004; Spencer et al. 2006). However, only a few studies have applied pig microsatellites to wild boar populations in Eurasia (Vernesi et al. 2003; Lorenzini 2005; Fickel and Hohmann 2006).

The main goal of this study was to assess the genetic variability and the degree of genetic structure of the wild boar (*S. scrofa*) population in Portugal, using a set of microsatellite markers. We also aimed to identify the main factors determining the degree of genetic structure and variability. Finally, we compared the results of this study with the available documented data on the demographic history of wild boars in Portugal.

Material and methods

Sampling

Wild boar samples were collected during the hunting seasons in 2002/03 and 2003/04, in 58 municipalities across continental Portugal (Fig. 1). Blood was collected in K_3EDTA tubes, from 110 wild boars (shot during drive hunts) and stored at 4 °C. Each animal was described according to weight, sex and estimated age. In the lab, a portion of each sample was transferred to FTA® individual cards (Whatman) and stored at room temperature. The remaining blood sample was stored at −20 °C for subsequent use.

DNA extraction

DNA was extracted from blood dried on FTA® cards, using the standard *Chelex*® procedure described by Walsh et al. (1991). Extractions were performed in a total volume of 200 µl, using small areas of the blood card (1–2 mm^2). Samples were used immediately for amplification or stored at −20 °C for later use. In the latter case, samples were stirred and centrifuged prior to amplification.

Amplification and genotyping

Six markers were chosen (from an original set of 91 pairs of primers) based on their known polymorphism, chromosome location, annealing temperature, size range, fluorescence dye applicability and performance under constant amplification conditions. Marker selection was performed with the aim of multiplex amplification of several markers, and a level of compromise among primer specific criteria was established. The chosen markers (chromosome location between brackets) were: *S0008* (I), *SW986* (V), *SW1129* (VI), *SW1701* (VII), *SW1517* (II) and *SW828* (III)—(GenBank access numbers: M97235, AF235422, AF235199, AF235485, AF253650, AF253852). Relevant information about the markers can be accessed through the websites of the NAGRP Pig Genome Coordination Program (http://www.animalgenome.org/resources/fprimerset9.html) and the US Meat Animal Research Centre (http://www.marc.usda.gov/genome/genome.html). The markers were divided in two triplex amplification sets (*S0008*, *SW986*, *SW1129* and *SW1701*, *SW1517*, *SW828*). Both sets were amplified in an Eppendorf Mastercycler® device, using a Qiagen Multiplex PCR Kit®, adding 2 µl of Qsolution® to the reaction mix, following manufacturer's instructions. An annealing temperature of 58 °C was applied to all markers. The final concentration of each primer was 0.2 µM, and 2.5–5 µl of *Chelex* extract were used, in a final reaction volume of 25 µl. For both triplex sets, the amplification program consisted of 15 min at 95 °C; 30 cycles of 30 s at 94 °C, 3 min at 58 °C, 60 s at 72 °C, and a final extension of 30 min at 60 °C. Amplified products were analysed by capillary electrophoresis in an automated sequencer ABI PRISM™ 310 and allele sizing was performed with GENESCAN® (v.3.1.2, Applied Biosystems).

Data analysis

After genotyping all individuals, allele frequencies for the six loci and potential deviation from Hardy–Weinberg