Mitochondrial DNA diversity in wild boar from the Primorsky Krai Region (East Russia)

Y. Ramayo*,1, I. N. Shemeret’eva†,1 and M. Pérez-Enciso*,‡

*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain. †Institute of Biology and Soil Science FEB RAS, 159, Stoletia str., Vladivostok 690022, Russia. ‡Institut de Recerca i Estudis Avançats de Catalunya, ICREA, Barcelona, Spain

Summary

We have studied the cytochrome B gene and control region DNA variability in 14 wild boars from the Primorsky Region, in the far east corner of Russia. Variability was low (χ² = 0.00248 overall) compared with the usual estimates in these loci, indicating that this is a rather closed population. Seven haplotypes were found, and one was identical to a Chinese wild boar. Phylogeographically, the sequences clustered among several Asian clades, primarily Chinese domestic pigs and Japanese and Chinese wild boars, and are positioned within the D2 clade reported by Larson et al. [Science 307, 2005; 1618]. Although North Korean pigs should be studied, our data suggest that the Primorsky mtDNA signature is absent from domestic pigs. Sequences are available through GenBank identifiers HM010461–HM010488.

Keywords mtDNA, nucleotide diversity, pig, wild boar.

The genetic characterization of the wild ancestors of domestic species, if they exist, provides important information to understand the effects of domestication and modern selection in animal genomes. The wild boar is of particular interest because of its abundant distribution and the fact that the pig has been domesticated recurrently in different areas (Larson et al. 2005). Nevertheless, and although numerous studies have been published so far, it is unlikely that the whole mtDNA variability of Sus scrofa has been completely studied, because of the widespread Eurasian distribution of this species. Despite recent works (e.g. Cho et al. 2009), Asian populations have been relatively less well studied than their European counterparts. However, it is clear by now that Asia, being the origin of the species, harbours far greater nucleotide variability than Europe, and it therefore deserves special attention.

Some interesting wild boar populations seem not have been studied at all. Here we characterize the nucleotide diversity of mtDNA, specifically cytochrome B (Cyt-B) and the control region, of wild boar from Primorsky Krai (Far East Russia). There are several relevant questions that we wish to address given that this is, to our knowledge, the first study of this population that lies at the North East border of the species distribution. Firstly, how divergent is this population from nearby populations in North China or Korea, and how variable is it? Secondly, is there any signature of its mtDNA in modern domestic pigs? Both Cyt-B and the control region have been used extensively to study the phylogeography of species, including the pig, allowing comparison with previous works.

Wild boars were sampled in five localities from Primorsky Krai (see map in Fig. S1 and Tables S1 and S2), a region located in the extreme South East corner of Russia bordering Northern Korea and the Chinese Heilongjiang province. Primorsky Krai is the north-eastern edge of the range S. scrofa subspecies ussuricus. Further progression of the species to the north is limited by low temperatures and high snow cover during winter (Bromley 1964; Bromley & Kucherenko 1983). The region of Primorsky Krai is mainly mountainous; two-thirds of the territory belongs to the mountainous system Sikhote-Alin, and only one-third of the total territory is flat, which includes Western coastal plains and a narrow strip of plains along the coast. About 70% of the territory is covered by forests, mostly deciduous and coniferous species. In terms of climate, winter monsoon winds blow from the continent (North, North–West), resulting in clear weather with a strong cooling of the
terrain: summer winds (South, South-East) cause high humidity and heavy fog on the coast. Ecological and climatological details are available elsewhere (Bromley & Kucherenco 1983; Sheremetyev & Prokopenko 2005; Sheremetyeva & Sheremetyev 2008).

We extracted DNA from tissue preserved in alcohol from 14 individuals using a standard phenol-chloroform precipitation protocol. We sequenced 1140 bp of the cytochrome B gene (Cyt-B) and 842 bp from the control region using primers and conditions described in Alves et al. (2003). Sequences were edited with SEDScape program v2.6 (Applied Biosystems). The MUSCLE program v3.6 (Edgar 2004) was used for the multiple alignment of a collection of Asian and European wild boar mtDNA sequences retrieved from GenBank (Table S3). Population genetic parameters were obtained with DNAsp-5.0 (Librado & Rozas 2009). It should be noted that although the presence of numts or mitochondrial nuclear copies is well known and we have actually found them in some Cuban pigs (Ramayo et al., unpublished), we did not encounter any sequencing artefacts in these samples. Sequences are available through GenBank identifiers HM010461–HM010474 (Cyt-B) and HM010475–HM010488 (control region).

A total of 3 Cyt-B and 10 control region polymorphic sites were found (Table 1). Diversity was very low both for the Cyt-B and the control region fragments, $\pi = 0.69 \times 10^{-3}$ and $4.9 \times 10^{-3}$ respectively (Table 1). In Cyt-B, a comparison with GenBank sequences (Table S3) show that Primorsky Krai values are an order of magnitude lower than in Korean or Chinese wild boars. They are even lower than European wild boar, which is already quite uniform. There was no indication of expansion or bottlenecks, as Tajima’s D was 0.81 and Fu-Li’s D with outgroup was 0.44. Further, the mismatch distribution was multimodal, as expected under a neutral demographic model (results not presented).

All control sequences are within the D2 clade (Larson et al. 2005). This is not unexpected, as this clade comprises most of the continental Asian sequences. In relation to the Cho et al.’s (2009) work, our sequences are positioned within their K3 cluster but in a separate node. The concatenated control region and and Cyt-B sequences were arranged in seven haplotypes. None of them was previously reported in GenBank, except one, which was identical to a Chinese wild boar haplotype (GenBank accession EF545580) from the Jilin province, also close to Primorsky Krai. The low mtDNA variability of the Primorsky Krai wild boars, together with the fact that most haplotypes were unreported, suggests that this population has remained relatively isolated from the rest of Asia.

To gain further insight into the phylogeography, a Median-Joining network was constructed using the software package NETWORK 4.1 (Bandelt et al. 1999) with a collection of Asian reported sequences from China, Korea and Japan (Table S3). We built separate networks for Cyt-B and the control region (Fig. 1). As a result of the much higher mutation rate in the control region than in Cyt-B, the former network is more scattered than the latter. Overall, the Primorsky wild boars did not cluster together, and were rather interspersed among the rest of the Asian sequences, primarily Chinese and Japanese. The corresponding between-group average distances in Cyt-B synonymous positions between Primorsky wild boar and Japanese pigs were $K = 0.0045 \pm 0.0014$, Korean pigs $K = 0.0041 \pm 0.0012$, Chinese wild boar 0.0038 $\pm 0.0012$ and European wild boar 0.0138 $\pm 0.0040$, as obtained from MEGA4 (Tamura et al. 2007).

Taken together, our data show that the Primorsky krai wild boar population is a unique, isolated population that has remained stable for quite some time. Its nucleotide diversity is lower than in other wild boar populations, probably as a result of isolation. Finally, this breed seems not to have contributed to the domestic pigs. This latter conclusion is conjectural at this point, as North Korean and local Russian pigs should be studied before a final conclusion can be drawn. Nevertheless, recent work in South Korean pigs by Cho et al. (2009) did not find any Korean wild boar signature in local pigs, suggesting that Korean domestic pigs were actually introduced from China.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Cyt-B</th>
<th>Control region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$</td>
<td>$S$</td>
</tr>
<tr>
<td>Primorsky Krai</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Asian domestic</td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td>Asian wild boar</td>
<td>35</td>
<td>77</td>
</tr>
<tr>
<td>Chinese domestic</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Chinese wild boar</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Korean wild boar</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Japanese wild boar</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>European wild boar</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>

$N$, number of sequences; $S$, number of polymorphic sites; $h$, number of haplotypes; $\pi$, Tajima’s nucleotide diversity. Sequences used are listed in Supporting Tables S2 (Primorsky Krai) and S3.
Acknowledgements

We thank Olga Dolgova for logistics support and the referees and editor for their constructive comments. Y.R. was recipient of an MSc fellowship from the IAMZ (Spain) and is currently funded by the MICINN PhD programme (Spain). This work was funded by the Consolider programme and AGL2007-65563-C02-01/GAN grants (MICINN) to MPE.

References


Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Map showing sampling localities.
Table S1 Sampling localities.

Table S2 Sampling locations and GenBank accessions for each sample.
Table S3 Accession numbers for sequences used.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.