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INVITED REVIEW

Genetic diversity in the European wild boar *Sus scrofa*: phylogeography, population structure and wild x domestic hybridization

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ABSTRACT

1. Despite the vast literature on genetic variation in the domestic pig *Sus scrofa*, little is known about genetic differentiation in wild boar populations.
2. Here we present an up-to-date review of published data on the past and recent history of the European wild boar, its genetic diversity and the spatial distribution of genetic variation throughout the continent.
3. The phylogeography of the species seems to be shaped mostly by past large-scale events (like postglacial recolonization) rather than by more recent human manipulation. Genetic differentiation is observed both on a continental and a regional scale, and non-intuitive barriers to gene flow occur.
4. From an indirect estimate, hybridization between wild boar and domestic pigs is seemingly a minor source of genetic variation for wild boar populations, yet risks are still linked to the release of captive hybrids in some areas.
5. Finally, we present future perspectives concerning the development of powerful molecular tools and their possible application to the study and management of this species.

Keywords: genetic differentiation, introgression, pig, population genetics, variation

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INTRODUCTION

The wild boar *Sus scrofa* has the widest geographical range of all ungulates and one of the widest of all terrestrial mammals. It is native to Europe, Asia and North Africa, and was introduced as a game species in all other continents, with the exception of Antarctica.

Human attitudes towards this species are contradictory: on one hand, it is considered a resource, as valuable game, and populations are sustained by artificial restocking; on the other hand, it represents an ecological pest, and control activities are invoked to eradicate or limit populations (Bieber & Ruf 2005). The future

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management of this species should be based on a full understanding of the factors determining its adaptation to a wide spectrum of environmental conditions. It is therefore of paramount importance to get further insights into overall genetic variation in the wild boar, and in particular, to understand the role of anthropogenic factors in shaping its geographical patterns.

On the basis of the phenotypic differentiation in *S. scrofa*, as many as 16 different subspecies have been described in the past (Groves 1981). Groves and Grubb (1993) classified the European wild boar into a group of 'western races', including the subspecies *S. s. scrofa* (Central-Western Europe), *S. s. meridionalis* (Sardinia and Corsica), *S. s. attila* (Eastern Europe) and *S. s. lybicus* (Southern Balkans). Nonetheless, multivariate morphometrics of skull measurements suggested the existence of a single subspecies (Genov 1999) with a possible north-east to south-west dimensional cline due to environmental variation and its effect on food availability and growth rate (Randi et al. 1989). In addition, the wild boar is the ancestor of the domestic pig, with which it shares a close genetic affinity and can hybridize. Thus, genetic introgression from the domestic pig may represent a further source of differentiation throughout the natural range of the wild boar, and may represent a major threat to its genetic integrity.

Although several researchers have sampled European wild boars, their studies have had various purposes, and so, most of the data are scattered. No molecular survey has yet been carried out to evaluate the overall genetic variation throughout Europe, on the basis of wide and comprehensive sampling. Our goal is to review the up-to-date published studies on genetic diversity in the European wild boar, with a special emphasis on phylogeographic patterns that can be attributed either to ancient demographic events or to anthropogenic factors. To this end, we also conduct a meta-analysis of the number of mitochondrial sequences deposited in GenBank. In addition, we will focus for the first time on the controversial relationship between wild boars and domestic pigs, by evaluating the results obtained with different classes of molecular markers.

ORIGIN, HISTORY AND PRESENT STATUS OF THE EUROPEAN POPULATION

The roots of *S. scrofa* are in South-East Asia (Groves 1981). Here, the genus *Sus* originated between 5.0 and 1.2 Ma (million years ago; Randi et al. 1996, Mona et al. 2007) and differentiated into several lineages during the Late Pliocene and Early Pleistocene (Lucchini et al. 2005). The spread of *S. scrofa* in the Asian continent was followed by its colonization of Europe and North Africa. The oldest records of *S. scrofa* in Europe date back to the Late Villafranchian period in the Early Pleistocene, around 1.5–1.0 Ma, and were found at the archaeological sites of Untermassfeld in Germany and Atapuerca in Spain (Rook & Martínez-Navarro 2010). However, the estimation of the time since the most recent common ancestor (TMRCA) in the Eurasian pig based on mitochondrial DNA (mtDNA) sequences contrasts with this dating, and supports a more recent differentiation within the species (0.8–0.4 Ma, Randi et al. 1996, Mona et al. 2007).

As in most temperate mammal species, present-day patterns of genetic variation in the wild boar population may be the consequence of Quaternary contraction or expansion events due to climatic fluctuations (Hewitt 2004). In particular, the transition between the Würmian glaciation and the subsequent interglacial period led to a range contraction of temperate species into refugial areas, mostly located in

southern peninsulas, and a subsequent spread of relict populations to occupy the re-established favourable habitats.

Sommer and Nadachowski (2006), reviewing the faunal assemblages at a number of European archaeological sites, provided an overview of wild boar distribution in Europe during the last glacial maximum (23000–16000 years before present; BP). At approximately 20000 years BP, *S. scrofa* inhabited the Iberian peninsula, southwestern France, the Italian peninsula and the Balkans, from Greece northwards to Slovenia and Croatia. When the climate became milder, the species recolonized the rest of the continent. In the absence of comprehensive genetic data, it is impossible to establish the spatial and temporal patterns of this large-scale recolonization.

The recent demographic history of the wild boar in Europe was greatly affected by humans. The species was once widespread and common throughout the continent. Direct human exploitation and habitat transformation caused a strong decline during the 19th and 20th centuries (see Apollonio et al. 2010). The species was already extinct in the British Isles (before the 17th century), and during that period, it disappeared also in other countries, such as the Netherlands, Denmark, Czech Republic, Switzerland, Slovenia and Baltic Countries. Its recolonization of Central Europe started from relict populations surviving in France, Germany, Poland, Slovakia, Hungary and Romania. A contraction of the distribution range due to local extinction was observed in the Italian, Iberian and Balkan peninsulas.

A sudden growth in wild boar populations started more or less simultaneously in many countries after World War II (Saez-Royuela & Telleria 1986). In Germany, the population increased from 50000 in the 1960s to the present 500000 individuals (Wotschikowsky 2010). A similar picture emerged from hunting statistics in France (Maillard et al. 2010).

OVERALL GENETIC VARIATION AND DIVERGENCE BETWEEN EUROPEAN AND NON-EUROPEAN POPULATIONS

Chromosome number

European and Asian wild boars differ in their karyotype or chromosome number. While Asian *S. scrofa* have 2n = 38, karyotypes with 2n = 36 are found in Europe. This reduction in the number of chromosomes is due to a Robertsonian translocation involving chromosomes 15 and 17 (McFee et al. 1966). European populations happen to have a numerical polymorphism with 36, 37 and 38 chromosomes, as observed in many European countries (see Table S1 and references therein). Karyotypes with 2n = 37 arise from crossings between individuals with 2n = 36 and 2n = 38. The ancestral condition for the species is probably 2n = 38; this is now the most common in Asian wild and domestic pigs. A chromosomal number of 2n = 36 is a derived status that is now very common in Europe. At least in some areas, the observed polymorphism might be the result of artificial translocations and hybridization with domestic pigs.

In the absence of a survey on a continental scale, it is impossible to define any geographical border between the 'western' (2n = 36) and the 'eastern' (2n = 38) karyotypes. Nevertheless, no correspondence was found between chromosome number and genetic variation in European wild boars (Fang et al. 2006), and this is not surprising, given that animals carrying different karyotypes can breed successfully and produce fertile hybrid offspring (Arroyo Nombela et al. 1990).

Protein polymorphism

Several researchers investigated biochemical variation in European wild boar populations in the 1980s and 1990s, based on electrophoretic protein polymorphisms. Hartl and Csaikl (1987), Randi et al. (1989, 1992), Hartl et al. (1993) and Ernhaft and Csányi (1995) screened allozyme loci in populations from Austria, Italy, Bulgaria, Germany, France and Hungary, and in reference samples from domestic pigs (see Supporting Information). Altogether, they identified 19 polymorphic loci (95% criterion) in European wild boars. Average heterozygosity ranged between 0.015 and 0.050 in Austrian populations, between 0.012 and 0.030 in Italian populations, and between 0.021 and 0.040 in Bulgarian samples. These levels of biochemical variation were considered to be medium compared to other large mammals (Baccus et al. 1983), although they were reported to be lower than in European red deer *Cervus elaphus* and roe deer *Capreolus capreolus* populations (Hartl et al. 1993).

Mitochondrial DNA

European wild boars have different mtDNA from those inhabiting Eastern Asia (Giuffra et al. 2000, Okumura et al. 2001, Kim et al. 2002, Larson et al. 2005). Haplotypes observed at mitochondrial coding and non-coding regions (cytochrome B gene, cytB and control region, CR) by Giuffra et al. (2000), and confirmed by sequencing of the near-complete mtDNA genome (Kijas & Andersson 2001), clustered into three phylogenetic groups. European wild boars showed the E1 and E2 haplogroups, while Japanese wild boars carried the A haplogroup. On the basis of a molecular clock, the TMRCA between A and E1 was dated back to 900000 years BP. Noticeably, this partition is reflected by domestic pig breeds from Europe and Asia, thus indicating an independent domestication of *S. scrofa* in the two continents.

Investigating the CR-mtDNA sequence variation in a number of domestic pig breeds and wild boar populations worldwide, Larson et al. (2005) found a plethora of mitochondrial clades in south-eastern Asia, where *S. scrofa* originated, while only the two lineages E1 and E2 were confirmed in Europe. In this continent, Scandura et al. (2008) surveyed the CR-mtDNA diversity of a total of 646 sequences of both wild boars and domestic pigs. The Italian peninsula represented a hotspot of genetic diversity, hosting both European mtDNA clades, of which E2 was endemic to Italy. MtDNA allelic richness (i.e. allelic diversity corrected for sample size) was almost twofold higher in Italian wild boars than cumulatively in five other non-Italian populations (10.9 vs. 6.0). This result agrees with the high level of allelic richness observed at 10 microsatellite loci in the Italian peninsula (Italy = 7.96, rest of Europe = 7.54; Scandura et al. 2008). The E1 lineage is also present in the Near East and in North Africa (Figs S1 and S2), as revealed by Ramirez et al. (2009) and Hajji and Zachos (in press).

Y-chromosome

Ramirez et al. (2009) also quantified worldwide Y-chromosome variation in pigs, by partial sequencing of six separate regions. Seven single nucleotide polymorphisms (SNPs) were grouped into three different haplotypes (H1, H2 and H3), representing two divergent lineages (H1/H2 vs. H3). Unlike the even partition of Asian wild boars into the two lineages, a sharp dominance (94%) of the H1/H2 lineage was found in the European wild boar population.

Overall genetic variation

A noticeable genetic divergence exists between European and East Asian *S. scrofa*, in both wild populations and domestic breeds (Giuffra et al. 2000, Kijas & Andersson 2001, Larson et al. 2005, Megens et al. 2008). All studies that have been carried out so far, concerning karyological, biochemical and genetic diversity, concordantly prove a sharp differentiation between eastern Asian and European populations. An east-to-west gradient of genetic variation seemingly exists. East-Asian wild boars have higher mitochondrial, Y-chromosome and microsatellite diversity than their European conspecifics (Larson et al. 2005, Ramirez et al. 2009, Luetkemeier et al. 2010). This pattern of genetic variation may reflect past expansion dynamics from the species' area of origin (south-eastern Asia). This large-scale pattern of variation may have arisen from (i) one (or more) ancient long-distance colonization events, followed by divergence of isolated lineages, (ii) geographical isolation due to local extinction in a previously continuous distribution range, or (iii) isolation by distance due to restricted gene flow in Eurasian wild boar populations. However, insufficient sampling (with a large gap in western Asia) prevents us from identifying possible 'suture zones', and thus from giving support to any of the aforementioned hypotheses.

PHYLOGEOGRAPHY OF EUROPEAN WILD BOARS

Patterns of differentiation in the European wild boar and their temporal transformations can be derived from studies on mtDNA (Table S2). The two main European lineages have dissimilar distributions: haplogroup E1 is widespread in Europe (from Portugal in the west to Poland in the east), while the haplogroup E2 has been found in wild boars from the Italian peninsula and in Sardinia (Fig. 1e), and at high frequency in only two areas (Castelporziano Reserve and Maremma Regional Park; Scandura et al. 2008). In a study based on ancient DNA, Larson et al. (2007) revealed that the E2 clade had a very restricted distribution range even before the Neolithic (11000–5500 BC; Fig. 1a), having been found in the Italian peninsula and in Croatia only. In addition, from the analysis of ancient pig remains, they found evidence of the arrival of Near Eastern pigs in Europe during the Neolithic, but no genetic signature was detected in either modern European domestic breeds or wild boar populations (Larson et al. 2007).

In ancient samples, historical museum specimens (18th–20th centuries) and modern wild boars, two main E1 haplotypes are found (A and C; Larson et al. 2005). They have a central position and a star-like pattern in the network (Fig. S1), as well as a high frequency in European domestic pigs. By a meta-analysis of all modern CR-sequences available in GenBank, a geographical segregation of the corresponding groups of haplotypes (A-side vs. C-side) is evident (Fig. 1e and S1): C-side haplotypes with a central position in the network are mostly found in Iberia and eastern Europe, while A-side haplotypes are common in Central Europe and Italy. Ancient DNA gives a time perspective to the partitioning into phylogeographic groups. By sequencing a short (80 base pair) fragment, Larson et al. (2007) distinguished A-side from C-side haplotypes, tracking the distribution of the two haplogroups in Europe over time (Fig. 1a–d). The sharp spatial segregation that is observed in modern European boar populations was probably absent before pig domestication (Fig. 1a). On the contrary, the occurrence of A-side haplotypes in pigs from Eastern Europe might be the result of a post-Neolithic diffusion of pig lineages that had been previously domesticated in central Europe (Fig. 1d).

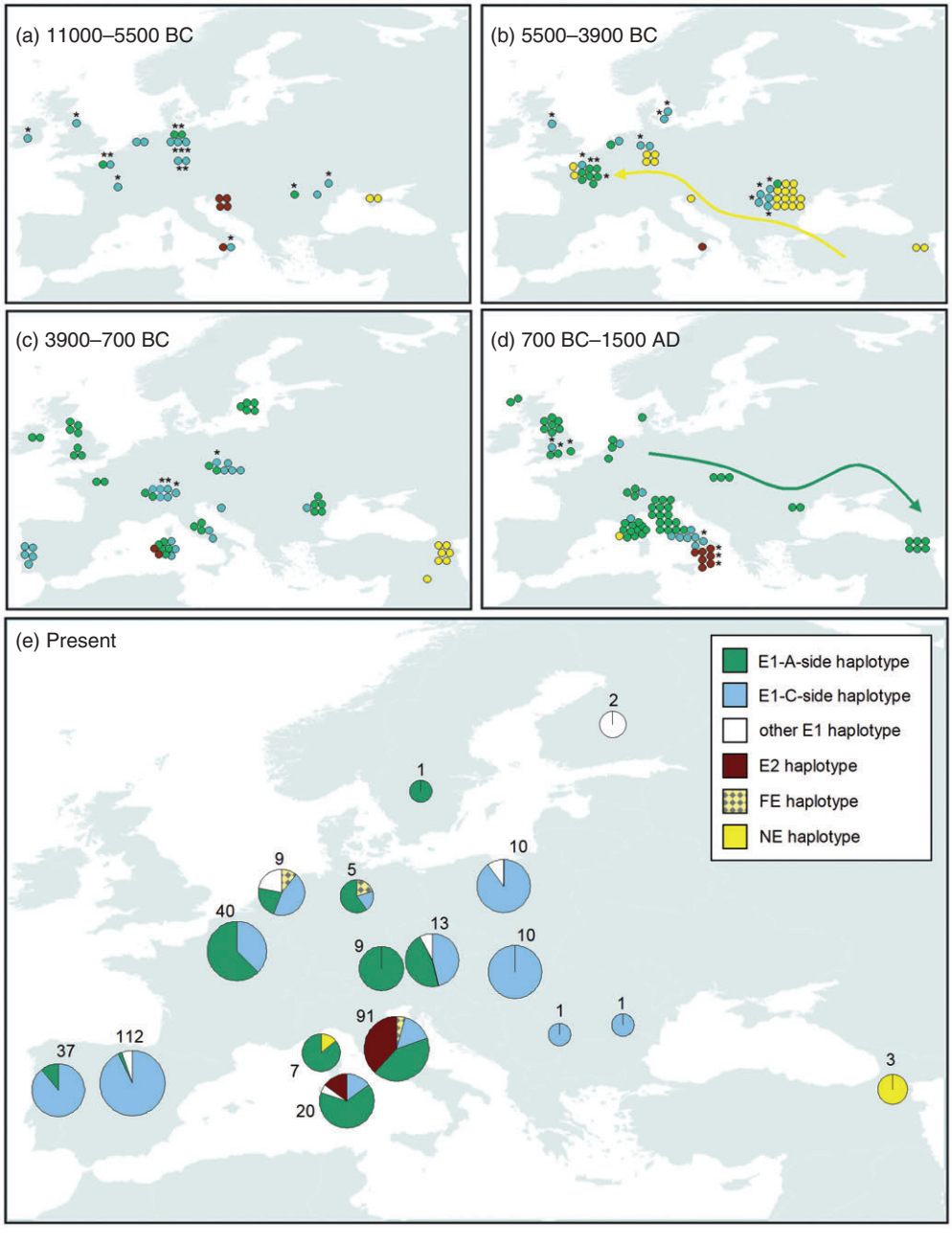

Fig. 1. Distribution of mtDNA haplogroups in ancient (a–d; Larson et al. 2007) and modern wild boars in Europe (e; Larson et al. 2005, Scandura et al. 2008, Alves et al. 2010). Haplotypes refer to the diagnostic 80 base pair fragment of the mtDNA control region that was reported by Larson et al. (2007). Circles represent sequenced individuals. Asterisks are used to mark ancient specimens classified as 'wild' by the authors. Pie charts refer to sampled populations (sample size is reported). Sampling areas are considered at a national scale (each pie chart refers to a country), apart from Sardinia, Corsica, and two discrete populations in Italy (central-southern peninsula and eastern Alps). E1 and E2 are the two European clades, FE is the main East-Asian clade (Far East) and NE is the Near Eastern clade. Arrows refer to gene flow that was probably mediated by humans. For definition of sub-clades E1-A and E1-C see Fig. S1 in Supporting Information.

On the basis of mismatch distribution analysis, Scandura et al. (2008) detected a possible signature of past population expansion in the European population, which seems not to have involved the Italian peninsula. This result agrees with the presence of signs of a pre-domestication demographic expansion in modern European domestic breeds (Fang & Andersson 2006) and appears consistent with a post-glacial demographic expansion (i.e. recolonization from glacial refugia). This interpretation is supported by the difference between the networks obtained for the fast-evolving CR and the slow-evolving cytB gene (Figs S1 and S2). In the latter, the star-like pattern with a single central and very common haplotype may reflect ancient expansion that would have preceded the segregation of the aforementioned CR lineages. Therefore, the present phylogeographic structuring of CR-mtDNA in the European wild boar is probably a consequence of spatial and demographic rearrangements that occurred in more recent times.

Autosomal microsatellites cannot help detect genetic signals over a long temporal scale, but are sensitive to recent demographic events (e.g. bottlenecks). However, phylogeographic patterns emerging from the analysis of 10 microsatellites in European wild boar populations are similar (Scandura et al. 2008). As for mtDNA, an area of genetic discontinuity (the Alps) was revealed by the strong genetic differentiation of the Italian population, whereas a surprisingly high level of homogeneity was observed in the rest of Europe (from Spain to Poland). Sampling limitations, and a possible bias introduced by the set of markers used, may have affected this result. Sardinian wild boars turned out to differ from domestic pigs on the island and from mainland wild populations, thus supporting the current nomenclature, in which they are classified as *S. s. meridionalis* (Scandura et al. 2008).

GENETIC POPULATION STRUCTURE AND THE EFFECTS OF TRANSLOCATIONS

Large-scale genetic structure in the European wild boar may have been affected by humans. Modifications of local gene pools may occur by severe (or non-random) exploitation, by translocations for hunting purposes or by the release of captive animals (sometimes hybridized with domestic pigs). Vernesi et al. (2003) showed the impact these practices can have on the genetic make-up of a 'managed' boar population. The admixed nature of a target population in central Italy was proved, by a comparison with two native Italian populations and a sample of Hungarian boars. Nonetheless, patterns of genetic variation at a macro-geographic scale seem to have been only marginally influenced by recent human manipulation, as the effects of ancient demographic events are still detectable (Scandura et al. 2008). The same was noticed in the red deer (Skog et al. 2009).

At a regional scale, a general trend seems to emerge in Europe. Even in the presence of continuous habitats, wild boar populations are seldom homogenous and mostly split into genetically differentiated subpopulations. Nikolov et al. (2009) and Ferreira et al. (2009) reported on the existence of genetic structuring, respectively, in Bulgaria and Portugal. In the former, two main subpopulations were identified, one north and one south of the Thracian valley (Nikolov et al. 2009). Such north-south differentiation agrees with morphometric and allozyme data, but the boundary between the two subpopulations differs among studies (Randi et al. 1992, Hartl et al. 1993). Three genetic clusters were detected in Portugal, corresponding to one northern, one central and one southern subpopulation (Ferreira et al. 2009). In this case, the existence of topological barriers (e.g. rivers) could not sufficiently explain the

observed partition, so demographic processes and human intervention were indicated. A similar pattern was detected in a recent study on the Sardinian population (Scandura et al. in press). Here, a clear partition into three subpopulations emerges, when the effect of anthropogenic factors is removed.

Different clustering methods may happen to identify a variable number of clusters, as found in a population at the borders of the Netherlands, Luxembourg and Germany (Frantz et al. 2009). However, the authors could not exclude the possibility that deviations from panmixia due to isolation by distance had led to an overestimation of the number of clusters.

HYBRIDIZATION WITH DOMESTIC PIGS: A CONTROVERSIAL ISSUE

The genetic contribution of Eurasian wild boar populations to modern domestic pig breeds is still debated (Larson et al. 2005, Luetkemeier et al. 2010), but no doubt exists about the close genetic similarity between wild boars and domestic pigs in Europe. This is confirmed by all studies using allozymes, mtDNA, microsatellites and Y-chromosome sequences. CR and cytB haplotypes largely overlap (Larson et al. 2005, Scandura et al. 2008, Ramirez et al. 2009, Luetkemeier et al. 2010). Most microsatellite alleles are shared, as well as the three Y-chromosome haplotypes (Scandura et al. 2008, Ramirez et al. 2009). F_{ST}-values (microsatellites) calculated between wild boars and domestic pigs were lower than between some different wild boar populations (Scandura et al. 2008). This result is not surprising if we consider that wild boars and domestic pigs have been sympatric for centuries (keeping pigs free in oak, *Quercus* spp., woods in autumn was common practice in Roman times and during the Middle Ages). Consequently, wild x domestic hybridization (WDH) is in the species' history, and the present genetic divergence between the two forms has mostly developed during the last two centuries (i.e. when intensive farming has progressively reduced the risk of hybridization in nature).

Very few attempts have been made to estimate the level of WDH in natural populations (see Lecis et al. 2006, Verardi et al. 2006). The first parameter that can be considered is the frequency of Asian mtDNA haplotypes. Asian haplotypes belonging to the Far East clade (A) are present at a frequency as high as 29% in European domestic pig breeds (Fang & Andersson 2006), due to a historical introgression from Chinese pigs (18th–19th century; Giuffra et al. 2000). On the contrary, European wild boars are supposed to show E1 and E2 haplotypes only. The presence of A haplotypes in wild individuals thus proves the occurrence of a domestic pig ancestor in their maternal line. Out of 368 D-loop sequences of European wild boars, included in the alignment by Alves et al. (2010), only six (1.6%) belonged to the A clade. Carrier animals were from Italy (4), Germany (1) and Belgium (1), but Asian mtDNA was also reported in boars from Luxembourg (J. Kirschning, pers. comm.). Nonetheless, a very high proportion of A haplotypes in an area of recent reintroduction in northern Italy (14/31; Lattuada et al. 2009) and in British free-ranging boars (A. Frantz, pers. comm.) that were reintroduced into England (Goulding 2001), testifies that high frequencies of Asian mtDNA can be reached locally as a result of the massive release or unintentional escape of captive-reared hybrids.

Other molecular markers have been developed that can help identify domestic genetic variants. The melanocortin-1 receptor (MC1R) locus influences animal pigmentation and shows high levels of polymorphism in domestic pigs (Kijas et al. 1998). Allelic variants, corresponding to a few mutations, can produce different colour

patterns and are responsible for the coat differences existing between domestic pigs and wild boars (Fang et al. 2009). Different alleles have been shown to have segregated in Asian and European populations (Fang et al. 2009). This polymorphism was used to detect WDH in Greece (Koutsogiannouli et al. 2010). The polymerase-chain-reaction-restriction-fragment-length-polymorphism analysis of the MC1R locus in 119 free-ranging wild boars revealed an overall frequency of 5% of domestic pig alleles, while it increased to 17% within a captive wild boar stock.

Porcine quantitative trait loci can also be useful markers to detect WDH. They are indeed associated with traits that underwent strong selection during the development of the modern pig breeds. An example of how informative these loci could be is represented by IGF2 (insulin-like growth factor 2). This gene harbours paternally expressed mutations that increase muscle growth and leanness (Van Laere et al. 2003). Ojeda et al. (2008) screened a large sample of domestic pigs and wild boars for a causative mutation (intron.3-g.3072G>A) inducing abnormal muscle development. While this SNP has reached a frequency as high as 86% in international pig breeds but only 3% in local European breeds, it was absent in 120 European wild boars.

None of the aforementioned systems can identify hybrids beyond doubt, nor can they be used to estimate the introgression rate of a given population. Nonetheless, the repeatedly low proportion (always <5%) of free-living wild boars carrying introgressed alleles suggests that the contribution of WDH to their present variation in Europe is marginal. However, the intentional or unintentional release of animals reared in captivity seems to represent the greatest threat to the genetic integrity of natural populations.

CONCLUSIONS AND PERSPECTIVES

General interest in the wild boar in Europe is growing in parallel with its diffusion and increasing abundance. However, if compared with knowledge of other large mammals, our knowledge of genetic variation in this species still appears to be fragmentary.

From this review, considering all data published so far, some important indications arise:

1. Despite the dramatic changes that wild boar populations have experienced in the recent past (demographic and range fluctuations, translocations, etc.), their genetic diversity in Europe seems to have been much more affected by ancient large-scale events (e.g. postglacial recolonization) than by recent events.

2. Sharp geographic differentiations exist both at macro-scale (within the continent) and micro-scale (within local populations), and genetic discontinuities are not completely explained by the existence of physical barriers.

3. Hybridization with domestic pigs seems to occur at a very low frequency under natural conditions, but is common practice in farmed stocks, and through the release of captive animals, it represents a severe risk for wild populations.

In order to improve knowledge of the phylogeography of the wild boar, the general overview presented here needs to be supplemented by wider sampling, extended especially to north-eastern Europe and the Balkans. The priority should be to complete the screening of mtDNA diversity in European wild boar populations with two main goals: (i) to search for the presence of the E2 lineage outside the Italian peninsula, and (ii) to identify the contact zones between the Asian (Near Eastern and Far Eastern) and the European clades. A further improvement could be

achieved by the use of new statistical simulation-based frameworks (Bayesian skyline plots; Drummond et al. 2005; and Approximate Bayesian Computation; Bertorelle et al. 2010) to estimate past population dynamics by evaluating alternative scenarios. Phylogeographic studies should not be limited to a single matrilineal marker. A comprehensive survey of genetic variation should be extended to autosomal and Y-chromosome markers (microsatellites, SNPs). With respect to this, new technologies for high-throughput DNA sequencing have launched the discovery of SNPs in the pig (Amaral et al. 2009). A microarray of 60000 SNPs (Illumina Porcine 60K iSelect Beadchip, Lincoln, Nebraska, USA) has been developed to detect variation within and among pig breeds (Ramos et al. 2009). In the genomic era, it is not difficult to foresee a rapid growth in the number and resolution of analytical tools suitable for use in the wild boar.

Our knowledge of genetic variation in the wild boar, and the development of sets of polymorphic markers, can be relevant to other research fields, including those with impacts on human society, like disease control and pest management. Pioneering studies on the relationship between genetic variability and fitness have obtained encouraging results in the wild boar (Lang et al. 2000, Acevedo-Whitehouse et al. 2005), and it was claimed that even heterozygosity at a single marker can be informative of the individual's resistance to bovine tuberculosis (Amos & Acevedo-Whitehouse 2009). Similarly, improving the knowledge about reproductive biology and social behaviour is a precondition for the effective control of wild boar populations. Even in these kinds of investigations, the role of genotyping has become crucial (Delgado et al. 2008, Iacolina et al. 2009, Poteaux et al. 2009).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Median-joining network of mitochondrial control region haplotypes (411bp) in wild boars from Europe (different regions), the Near East and North Africa. Haplotype codes coincide with those used by Alves et al. 2010 (S04-S119, Fig. 4) and Hajji and Zachos in press (X and Y). Haplotypes A and C from Larson et al. 2005 (corresponding respectively to S22 and S29) are shown in the centre of the network. The related groups of haplotypes (A-side and C-side) that can be identified by sequencing an 80bp DNA fragment in ancient specimens are delimited (see Fig. 1).
Figure S2. Median-joining network of mitochondrial cytochrome B haplotypes in wild boars from Europe (different regions), the Near East and North Africa. Novel haplotype codes are used. Haplotypes were obtained by collapsing published sequences for a total length of 895bp (Randi et al. 1996, Randi et al. 2002, Ojeda et al. 2006, Mona et al. 2007 and Ramirez et al. 2009).
Table S1. Variation in chromosome number in European wild boars.
Table S2. List of relevant publications concerning genetic and biochemical variation in the European wild boar (WB) and related populations.

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