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ORIGINAL RESEARCH

# Genetic evidence for past hybridisation between domestic pigs and English wild boars

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**Abstract** While governments normally take action to eradicate or control feral populations of introduced species, management becomes problematic in the rare event of an inadvertent reintroduction of a locally extinct species to its former range. Free-living wild boars became extinct in Britain around 700 years ago, but animals have recently escaped from farms and recolonised parts of England. There has been much debate about the potential ecological and economic impacts of the presence of feral populations of wild boar in England. Predicted negative impacts include disease transmission to domestic pigs, crop damage and problems caused by the species' rooting behaviour. Perhaps the strongest argument in favour of the wild boars in England is the restoration of a formerly native species. However, for the re-established populations to have an intrinsic value as an addition to English biodiversity, it would be preferable if the animals were genetically pure wild boars. We used four genetic marker systems to assess the genetic status of a wild boar population in the Forest of Dean, western England. We found high frequencies of alleles of domestic origin at the mitochondrial control region and a nuclear pseudo-gene. Microsatellite-based analyses also suggested that English wild boars had a mixed wild boar/domestic pig ancestry. Therefore, it is debatable whether the wild boar in the Forest of Dean can be regarded as a restored native species.

**Keywords** Admixture analysis · Introduction · Introgression · Invasive species · *Sus scrofa*

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## Introduction

Introduced species can be a major threat to biodiversity, especially if they become invasive. Governments and local authorities normally take action to eradicate, or at least control, feral populations of introduced species (e.g. Manchester and Bullock 2000). The issue of how best to deal with introduced species becomes problematic in the rare event of an inadvertent reintroduction of a locally extinct species to its former range. While such reintroductions can have negative consequences as their potential environmental impact has not been assessed beforehand, the re-establishment of an extinct native species can have an intrinsic conservation value.

Managed reintroduction programmes that consider genetic issues frequently favour the maintenance of a natural gene pool—genetic integrity—in the re-established populations (Vergeer et al. 2008). For example, the World Conservation Union guidelines on reintroductions recommend that a source population for reintroductions should ideally be genetically closely related to the original native stock (IUCN 1998). Furthermore, restoration programmes should also aim to re-establish populations with high

genetic functionality, i.e. population with high genetic diversity and with individuals predicted to maximise reproduction, survival, viability and persistence under wild conditions (Frankham et al. 2009; Vergeer et al. 2008). Conversely, high genetic diversity is generally considered to contribute to an introduced species becoming invasive (e.g., Lindholm et al. 2005; Roman and Darling 2007).

In contrast to most European countries, free-living wild boars (*Sus scrofa*) became extinct in Britain around 700 years ago (Rackham 1993), but have been farmed for their meat since the mid-1980s (Booth 1995). Over the last decade, some animals have escaped from wild boar farms and established breeding populations in southern and south-western England (Wilson 2005). There has been much debate about the potential ecological and economic impacts of the presence of wild boars in England (Moore and Wilson 2005; Wilson 2005). While wild boar hunting and wild boar meat could be an important source of income, perhaps the strongest argument in favour of the wild boars in England is the restoration of a formerly native species that was driven to extinction (Moore and Wilson 2005; Wilson 2005).

On the other hand, wild boars carry many diseases that are shared with livestock (Gortázar et al. 2007), and the risk of disease transmission to domestic pigs is expected to increase as wild boars and feral pigs (escaped or released domestic pigs that have established wild populations; Lowe et al. 2000) increase in number and range (Massei et al. 2011). In many European countries, the wild boar is associated with crop damage (Schley et al. 2008), which can be expected to become more widespread and frequent. Furthermore, the rooting behaviour of wild boar may destroy woodland vegetation and cause damage to species-rich grasslands (Massei and Genov 2004). Areas of remaining ancient/semi-natural woodland are scattered and relatively small, and may therefore not withstand wild boar rooting (Wilson 2005).

For the re-established populations to have an intrinsic value as an addition to native English biodiversity, the feral animals need to be true wild boars, rather than feral pigs (Wilson 2005). While the first wild boar farms sourced their initial stock from zoos, from the late 1980s onwards, animals of German origin were imported from estates in Denmark, and animals of East European origin from estates in Sweden (Booth 1995). However, it has been reported that farmers have crossed wild boars with domestic sows from rare breeds, such as the Tamworth, to increase litter size and growth rates of progeny (Booth 1995). Gongora et al. (2003) found evidence for some genetic admixture between wild boars and domestic pigs on a wild boar farm in Finland. Furthermore, as the different introduced populations go back to a small number of founders, they possibly also have reduced genetic variation, which would be undesirable in terms of species restoration.

The mitochondrial control region, the nuclear glucose-phosphate isomerase pseudogene (*GPIP*) and the melanocortin-1 receptor (*MC1R*) coat colour gene have all been used to make inferences about past or present hybridisation between domestic pigs and wild boars (e.g., Giuffra et al. 2000; Gongora et al. 2003; Koutsogiannouli et al. 2010; Lattuada et al. 2009). Studies based on the control region of mitochondrial DNA (mtDNA) support a clear phylogeographic distinction between European and Asian wild boars and provide evidence for separate domestication events in both parts of the world (Larson et al. 2005). Asian mtDNA haplotypes do occur in European pig breeds as a result of attempts at improving European with Asian breeds in the eighteenth and early ninteenth centuries (Giuffra et al. 2000). The frequency of Asian mtDNA haplotypes in European breeds has been reported to vary between 0 and 100 % (Fang and Andersson 2006). However, the presence of Asian haplotypes in European wild boars, which are expected to carry only European ones, is usually taken conclusively as evidence of the occurrence of a domestic pig ancestor in the maternal line (Giuffra et al. 2000; Scandura et al. 2011b). For example, only six European wild boars (originating from Italy, Germany and France) out of a total of 368 individuals analysed by Alves et al. (2010) and Scandura et al. (2008) carried an Asian mtDNA haplotype.

Similarly, the nuclear pseudo-gene *GPIP* has Asian and European alleles with a fairly large sequence divergence and the absence of intermediate forms. Giuffra et al. (2000) found allele *GPIP*1* only in Japanese wild boars. Allele *GPIP*3* was found at a high frequency in Asian domestic pigs, at low-to-intermediate frequencies in European domestic pigs and at low frequencies in European wild boars. Conversely, allele *GPIP*4* was found to occur at high frequencies in both European wild boars and domestic pigs, and at low frequencies in Asian domestic pigs. While the possibility that the alleles represent an ancestral polymorphisms showing significant allele frequency differences between the continents cannot be excluded, the presence of *GPIP*3* in a European wild boar population is usually taken to be indicative of a domestic ancestor (Giuffra et al. 2000, 2003).

Sequence diversity at the *MC1R* coat colour gene is also relevant in this context. Altogether 13 different alleles, associated with five different colour phenotypes, have been detected in pigs. The wild-type allele (historically referred to as $E^{+}$) has been identified exclusively in wild boar, while all domestic breeds, with the exception of Mangalica from Hungary, carry mutant *MC1R* alleles (Fang et al. 2009). The *MC1R* coat colour gene has been used to differentiate between meat from wild boars and domestic pigs (Fajardo et al. 2008) and to detect wild/domestic hybrids in

Greece (Koutsogiannouli et al. 2010). Finally, Scandura et al. (2011a) used ten microsatellite loci to obtain evidence for past hybridisation between Sardinian wild boars and pigs from different sources (Italian mainland, central Europe, domestic pigs).

In the present article, we used these four different marker systems (mtDNA, *GPIP*, *MC1R* and microsatellites) to test whether wild boar populations in the Forest of Dean, western England, were pure-bred or had a mixed wild boar–domestic pig ancestry. We also used the microsatellite loci to assess the level of genetic diversity of the same population.

## Methods

We collected tissue samples from 20 wild boars culled in the Forest of Dean, in western England near Bristol. The wild boar breeding population in the Forest of Dean probably originated from escapees from a farm in the late 1990s. In 2004, a boar, or possible a hybrid, was reported to have escaped from a local abattoir and a further 25–30 animals were illegally released in the area (Wilson 2005). Given its history, it is questionable whether the wild boar population in the Forest of Dean consists of pure-bred wild boar closely related to populations from continental Europe.

We also obtained tissue or DNA samples from five domestic pig breeds (Berkshire, $N = 26$; Tamworth, $N = 11$; Hampshire, $N = 9$; Landrace, $N = 6$; and Large White, $N = 7$), from 36 commercial domestic pigs cross-bred from different breeds (which of the breeds precisely was unknown to us) and from European wild boars from five different localities (Western Belgium, $N = 27$; Croatia, $N = 20$; north-western France, $N = 15$; eastern Germany, $N = 18$; and Luxembourg, $N = 23$). We also included 19 samples collected in two hunting areas in southern Luxembourg where wild boars had been illegally released at the end of the 1990s. Previous genetic analyses have shown these 19 individuals to be strongly differentiated from the local population (Frantz et al. 2009), suggesting that they were related, if not identical, to the individuals that had been illegally released. Finally, we obtained one sample from a farm, which, according to the owner, was breeding animals of mixed wild boar–domestic pig ancestry. Tissue samples were stored in absolute ethanol, and DNA was extracted using a ammonium acetate-based salting-out procedure (Miller et al. 1988).

We obtained sequence information on the mtDNA control region, the *GPIP* pseudo-gene and the *MC1R* coat colour gene from the 20 boars from the Forest of Dean. In addition, given the dearth of information on the background frequency of the Asian alleles in European wild boar populations, we also determined the genotype at the *GPIP* locus in the 50 wild boars from Belgium and Luxembourg. Using primers pigCTR22L and pigCTR515G (Fickel and Hohmann 2006), we amplified a 493-bp fragment of the mitochondrial control region that included a diagnostic region that allowed Asian and European haplotypes to be distinguished (Fang and Andersson 2006). We used primers GPIP2 and GPIP3 (Giuffra et al. 2000) to amplify a 308-bp fragment of the *GPIP* pseudo-gene, which allows Asian and European alleles to be differentiated. We designed two primers (Forward: 5′-TCGCCCATGTACTACTTCGT-3′; Reverse: 5′-GTGGTGGTAGTAGGCGAT-GA-3′) to amplify a 345-bp fragment of the *MC1R* gene that included the single-nucleotide polymorphisms between codon positions 95 and 166 (following Fang et al. 2009). This allowed us to differentiate the European wild type ($E^{+}$) from all other alleles, as well as most alleles from each other.

We amplified fragments using 10-μl polymerase chain reactions (PCRs) that contained approximately 10 ng of DNA and 0.5 units of Biotaq DNA Polymerase (Bioline, London, UK) in the manufacturer's buffer with a concentration of 1.0 μM of each primer, 1.5 mM $MgCl_2$ and 0.1 mM of each dNTP. The PCRs were performed in a thermal cycler (MJ Research) using the following procedure: one cycle of 3 min at 95 °C, followed by 36 cycles of 94 °C for 30 s, specific annealing temperature (mtDNA: 59 °C, *GPIP* 63 °C, *MC1R*: 60 °C) for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. Successful PCR products were precipitated with ethanol and forward and reverse strands sequenced using Big Dye Terminator chemistry (Applied Biosystems, Carlsbad, CA, USA) on an ABI 3730 capillary DNA automated sequencer (Applied Biosystems, Carlsbad, CA, USA).

We also genotyped all samples at 14 unlinked microsatellite markers (Hampton et al. 2004). Microsatellite genotyping was performed in three multiplexed PCRs (Frantz et al. 2009). Multiplex 1 contained loci *S0002 & SW911*, multiplex 2 loci *S0026*, *S0097*, *Sw122*, *Sw857*, and *Sw951* and multiplex 3 loci contained *S0005*, *S0090*, *S0155*, *S0226*, *Sw240*, *Sw632*, and *Sw936*. In the case of multiplex 1, PCR reactions were performed in 10-μl volumes, each containing approximately 10 ng of DNA. The final reaction concentrations consisted of 0.5 units of Biotaq DNA Polymerase (Bioline) in the manufacturer's buffer with a concentration of 1.0 μM of each primer, 1.5 mM $MgCl_2$ and 0.1 mM of each dNTP. PCRs cycles were followed as described above, with an annealing temperature of 60 °C. Multiplexes 2 and 3 were performed using the Qiagen Multiplex PCR Kit. Each reaction contained 1× QIAGEN Multiplex Master Mix and 0.2 μM of each primer. In addition, 0.5× Q-solution was added to Multiplex 3. After drying 1 μl of DNA (10 ng/μl) overnight in a 384-well PCR plate (Greiner Bio-One, Stonehouse, UK), multiplex reactions were performed in a total

volume of 2 µl. Multiplex reactions started with a 15-min denaturation at 95 °C, followed by denaturation at 94 °C for 30 s, annealing at 55 °C for 90 s and extension at 72 °C for 1 min. The final incubation was at 60 °C for 30 min. Individuals genotyped at fewer than ten microsatellite loci were excluded from the downstream analyses.

Microsatellite genetic diversities of the different pig breeds and the wild boar populations were estimated as the mean number of alleles per locus ($A$), observed ($H_O$) and unbiased expected ($uH_E$) heterozygosities using GENETIX 4.05.2 (Belkhir 2004). Allelic richness ($A_R$) was estimated using the programme FSTAT (Goudet 1995). We excluded the nine Hampshire, six Landrace and seven Large White pigs, as well as loci S0090 and SW911 (genotyping difficulties in two different populations), to allow meaningful estimation of $A_R$. We used GENETIX v.4.05.2 to perform a factorial correspondence analysis (FCA) to visualise the genetic distance between the English boars and the reference samples. The programme POPULATIONS 1.2.30 (Langella 1999) was used to calculate Nei's standard genetic distance ($D_s$) between the breeds and wild boar populations and, together with TREEVIEW 1.5 (Page 1996), to construct an unrooted neighbour-joining tree from the $D_s$ distance matrix. Bootstrap support was obtained by 1,000 bootstraps on locus.

The programme STRUCTURE (Pritchard et al. 2000) was used to determine the degree of genetic admixture between our reference populations and the English boar. We estimated $K$, the number of subpopulations or clusters, by performing ten independent runs of $K = 1$–14 with $10^6$ iterations following a burn-in period of $10^5$ iterations, using the model with correlated allele frequencies and assuming admixture. Assignment results for the most-supported number of subpopulations—ignoring the two runs of $K = 10$ with the lowest log-likelihood values; see "Results" section—were averaged manually across the ten independent runs and summarised using programme DISTRUCT 1.1 (Rosenberg 2004). We also performed STRUCTURE admixture analyses of ten datasets consisting only of the English wild boar and each reference population/breed in turn. We performed ten independent runs of $K = 2$ for each dataset, using the same parameters as above, and manually averaged assignment values across these runs.

## Results

Sequence analysis of a 493-bp-fragment of the mtDNA control region from 20 wild boars from the Forest of Dean

**Table 1** Variable sites in the porcine mtDNA control region among the two haplotypes observed in the introduced English wild boars (in bold) and similar or identical published sequences

| Haplotypes | Nucleotide position | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15543 | 15565 | 15571 | 15579 | 15587 | 15592 | 15615 | 15675 | 15728 | 15740 | 15824 | 15839 | 15886 |
| **Euro**[a] | **T** | **G** | **C** | **C** | **C** | **A** | **T** | **T** | **A** | **T** | **C** | **T** | **C** |
| EH6[b] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| EH8[b] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| EH15[b] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| H8[c] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| H14[c] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| H20[c] | . | . | . | . | . | . | . | . | . | C | . | . | . |
| **Asia**[d] | **C** | **A** | – | **T** | **T** | **G** | **C** | **C** | **G** | . | **T** | **C** | **T** |
| AH19[b] | C | A | – | T | T | G | C | C | G | . | T | C | T |
| EA18[e] | C | A | – | T | T | G | C | C | G | . | T | C | T |
| EA163[e] | C | A | – | T | T | G | C | C | G | . | T | C | T |

*Dots* (.) and *dashes* (-) indicate matches and gaps, respectively, with the master sequence (Euro). Nucleotide positions are initially numbered according to the complete pig mtDNA reference sequence (Ursing and Arnason 1998), but change later due to the insertion

[a] Genbank accession number JF304430.1

[b] From Fang et al. (2006)

[c] From Alves et al. (2010)

[d] Genbank accession number JF304431.1

[e] From Larson et al. (2010)

**Table 2** Summary of the alleles present at one mitochondrial and two nuclear genetic markers in the 20 introduced wild boars from the Forest of Dean

| Individual | mtDNA | *GPIP* | | *MC1R* | |
|---|---|---|---|---|---|
| | Haplotypes | Allele 1 | Allele 2 | Allele 1 | Allele 2 |
| Boar1 | **Asia** | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar2 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar3 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar4 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar5 | **Asia** | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar6 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar7 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar8 | Europe | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar9 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar10 | **Asia** | ***GPIP*3*** | ***GPIP*3*** | $E^+$ | $E^+$ |
| Boar11 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar12 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar13 | **Asia** | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar14 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar15 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar16 | **Asia** | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar17 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar18 | Europe | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |
| Boar19 | Europe | ***GPIP*3*** | *GPIP*4* | $E^+$ | $E^+$ |
| Boar20 | **Asia** | *GPIP*4* | *GPIP*4* | $E^+$ | $E^+$ |

Haplotypes/alleles inherited from a domestic pig ancestor are highlighted in bold. Please refer to Table 1 for further information on the mtDNA haplotypes, and to main text for further information on the alleles of the nuclear genes

revealed only two haplotypes (Table 1). Both haplotypes were characterised by 13 variable sites, consisting of 12 transitions and one insertion/deletion. One haplotype, found in nine individuals, was closely related to control region sequences belonging to the main European phylogenetic group (Alves et al. 2010; Fang et al. 2006). The other haplotype, found in 11 individuals, was identical to control region sequences first identified in Asian domestic pigs and belonging to the Asian haplogroup (Larson et al. 2010). Our Asian haplotype also matched haplotype AH19 that Fang et al. (2006) identified in a Belgian wild boar (Table 1). We found sequences corresponding to the Asian allele *GPIP*3* in nine of the 20 English boar, including two individuals that had the European mtDNA haplotype (Table 2). *GPIP*3* was also found in four out of the 50 Luxembourg and Belgian wild boars that were tested. These four individuals, originating from the Belgian population, consisted of one homozygote and 3 heterozygotes individuals. Finally, all 20 English individuals were homozygous for the wild-type $E^+$ allele at the *MC1R* coat-colour gene.

The wild boar population in the Forest of Dean had a lower genetic diversity at the microsatellite loci than the continental wild boars and domestic pigs (Table 3), both in terms of number of alleles, allelic richness and unbiased expected heterozygosity. Only the individuals from the Tamworth breed were genetically less diverse than the English wild boars. The FCA positioned the English wild boars between the domestic pigs and the western European wild boar populations (Fig. 1). One Belgian wild boar clustered with the English boar in the FCA. The individuals introduced to Luxembourg clustered with the wild boar populations and the one farmed wild boar with the domestic pigs. An unrooted neighbour-joining tree based on $D_s$ confirmed that the English wild boars were distinct both from domestic pigs and wild boar populations (Fig. 2).

The genetic admixture analysis using programme STRUCTURE clustered the English wild boars with the domestic pigs—with some admixed individuals—when running the programme assuming that the dataset consisted of two genetic units ($K = 2$; Fig. 3). Overall, the STRUCTURE algorithm suggested the dataset to consist of ten different genetic units, as the highest log-likelihood values with good convergence were obtained for $K = 10$ (Fig. S1). At $K = 10$, the English wild boars formed a distinct cluster with very little evidence of admixture either with domestic pigs or wild boars (Fig. 3). One Berkshire pig appeared to be admixed

**Table 3** Genetic diversity measures of wild boar and domestic pig populations analysed in this study

| Population | $N^a$ | $A^b$ | $A_R^c$ | $uH_E^d$ | $H_O^e$ |
|---|---|---|---|---|---|
| Wild boar | | | | | |
| Croatia | 20 | 4.4 | 3.8 | 0.557 | 0.424 |
| France | 14 | 4.5 | 4.3 | 0.566 | 0.564 |
| Belgium | 27 | 5.8 | 4.9 | 0.643 | 0.632 |
| Germany | 18 | 4.5 | 4.1 | 0.594 | 0.590 |
| Luxembourg | 23 | 5.1 | 4.3 | 0.595 | 0.581 |
| Introduced Luxembourg | 19 | 3.5 | 3.3 | 0.552 | 0.658 |
| Domestic pigs | | | | | |
| Berkshire | 26 | 4.9 | 3.9 | 0.569 | 0.594 |
| Tamworth | 11 | 3.3 | 3.2 | 0.502 | 0.524 |
| Hampshire | 7 | 4.0 | – | 0.625 | 0.545 |
| Commercial crossbreeds | 36 | 7.1 | 5.8 | 0.754 | 0.789 |
| Landrace | 6 | 3.6 | – | 0.647 | 0.607 |
| Large White | 7 | 4.2 | – | 0.681 | 0.643 |
| English wild boar | 20 | 3.4 | 3.2 | 0.523 | 0.578 |

[a] Number of individuals genotyped

[b] Average number of alleles per locus

[c] Average allelic richness per locus: populations Hampshire and Landrace/Large White as well as loci SW911 and S0090 were excluded to permit calculation of $A_R$ with a meaningful sample size of ten diploid individuals

[d] Average unbiased expected heterozygosity

[e] Average observed heterozygosity

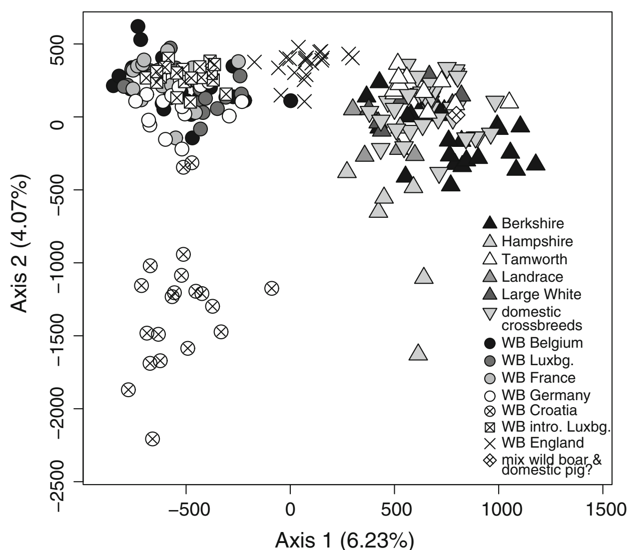


**Fig. 1** Factorial correspondence analysis of swine belonging to different breeds and wild boar populations, based on 14 microsatellite loci. Percentage of the total variation explained by each of the two axes is given

with the English wild boars. With the exception of the Large Whites and the domestic crossbreds, the different domestic breeds formed separate genetic clusters. The wild boars from Belgium, France and Luxembourg clustered together, suggesting relatively little genetic differentiation between these populations, and the other wild boar populations were each genetically distinct. Two Belgian wild boars were shown to be admixed with the Berkshire and Landrace breeds, respectively. The Berkshire-admixed wild boar was the same individual that clustered with the English wild boars in the FCA. We also found evidence of admixture of the introduced individuals from Luxembourg with the local population, suggesting that some of the sampled individuals were in fact descendants of the introduced boar. The analysis of the datasets consisting of only the English wild boars, and each reference population/breed in turn did not identify any further hybrids (Fig. S2). Two Berkshire pigs appeared to be admixed with the English wild boars. However, the STRUCTURE analysis with the complete dataset suggested these two individuals to be a genetic mixture of different breeds (Fig. 3).

## Discussion

After 700 years of extinction in the wild, feral populations of wild boar have been established in southern England following accidental releases of farmed wild boars. Similar to feral pigs, which are considered to be one of the world's 100 worst invading species (Lowe et al. 2000), introduced wild boars can damage crops, stock and property, and transmit diseases to domestic pigs and humans (Wilson

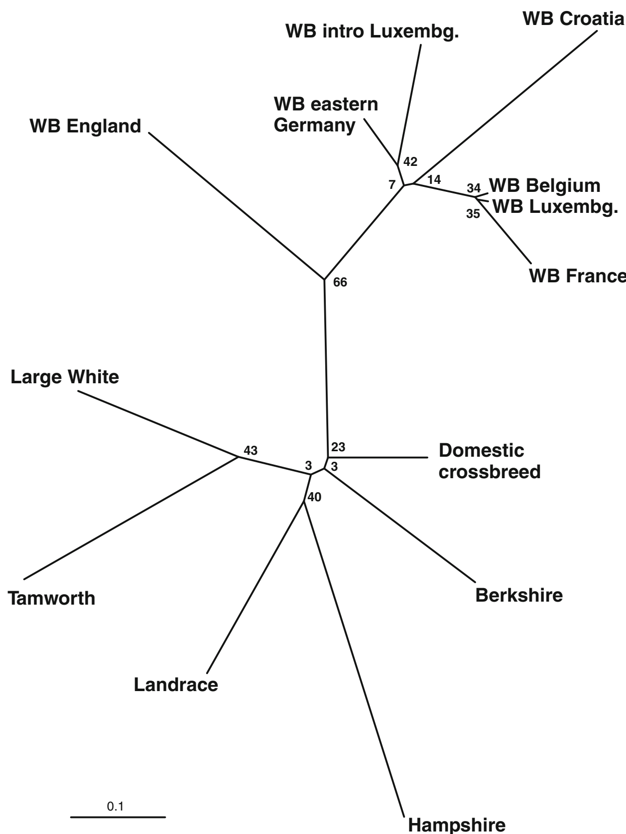


**Fig. 2** Neighbour-joining tree based on Nei's standard genetic distance ($D_S$), estimated using 14 microsatellite loci, for all pig breeds and wild boar populations. Bootstrap support was obtained by 1,000 bootstraps on locus

2005). While the restoration of a natural component of woodland ecosystems might be desirable, wild boars can cause serious ecological damage by their rooting behaviour (Wilson 2005). Consequently, there has been much debate about how to best manage these newly established populations, and, given uncertainty about the long-term impact of the species, eradication of all the established populations has been one of the considered management options (Moore and Wilson 2005).

Perhaps the best argument in favour of the presence of wild boars in England is the restoration of a formerly native species. However, the sequences obtained from a fragment of the mitochondrial control region suggested that more than half the analysed wild boars from the Forest of Dean had a mixed wild boar–domestic pig ancestry. This contrasts with phylogeographic studies that found between 1 and 3 % of the control region sequences obtained from wild boars to be of Asian origin (Alves et al. 2010; Fang et al. 2006; Scandura et al. 2008). Conversely, Lattuada et al. (2009) found a high incidence of Asian haplotypes in a recently reintroduced population of wild boars in northern Italy, confirming that high frequencies of Asian mtDNA can be reached locally, as a result of releases of wild boar genetic status of which had not been verified beforehand.

Similar to the mitochondrial DNA, an Asian allele of the nuclear *GPIP* pseudo-gene was found in just under half of the 20 English wild boars analysed here (frequency of *GPIP*3*: 0.25). Gongora et al. (2003) found the same Asian allele in six out of 20 wild boars in a farm in Finland, which was taken as evidence of crossbreeding with domestics. Giuffra et al. (2000) found *GPIP*3* in two out of 13 (frequency of *GPIP*3*: 0.12) and in one out of 20 (frequency of *GPIP*3*: 0.04) wild boars from Poland and Italy, respectively. In the present study, we detected an Asian allele in four out of 50 individuals from Belgium and Luxembourg (frequency of *GPIP*3*: 0.05). Given the history of the English population, the higher-than-background-level frequencies of the *GPIP*3* allele in the English wild boars probably resulted from relatively recent introgression with domestic pigs. Nevertheless, further information on the frequencies of this allele in natural populations is needed to corroborate this conclusion and assess how informative the *GPIP*3* allele is in terms of detection of recent hybridisation between domestic pigs and wild boars.

In contrast to the mtDNA and *GPIP*, all the 20 introduced animals were homozygous for the wild-type $E^+$ allele at the *MC1R* coat colour gene. Gongora et al. (2003) found that all wild boars cross-bred on a farm in Finland were homozygous for the wild-type allele as well, with the exception of one individual with an Asian wild type. Conversely, Koutsogiannouli et al. (2010) found domestic coat colour alleles in 17 % of captive and 5 % of free-ranging wild boars in Greece. The fact that the coat colour gene was not a useful marker to detect domestic pig ancestry in the English wild boar population is perhaps not surprising. Wild boar farmers that have cross-bred are likely to remove individuals from their wild boar stock that do not look like wild boar. In other words, the *MC1R* coat colour gene might only be a useful marker for detecting natural hybridisation between free-ranging domestic pigs and wild boars.

The wild boar population in the Forest of Dean had a lower genetic diversity than the other wild boar populations and most domestic pig breeds analysed in this study. This result suggests that the English boar might have reduced genetic diversity, possibly as the result of the population having a small number of founders. Comparisons with other wild boar studies are difficult as different microsatellite loci have been used to assess genetic diversity (Ferreira et al. 2006; Scandura et al. 2011a; Vernesi et al. 2003). Our study population might therefore not fulfil the restoration ideal of high genetic functionality. However, since high genetic diversity is likely to contribute to the introduced species becoming invasive, the reduced genetic diversity might be

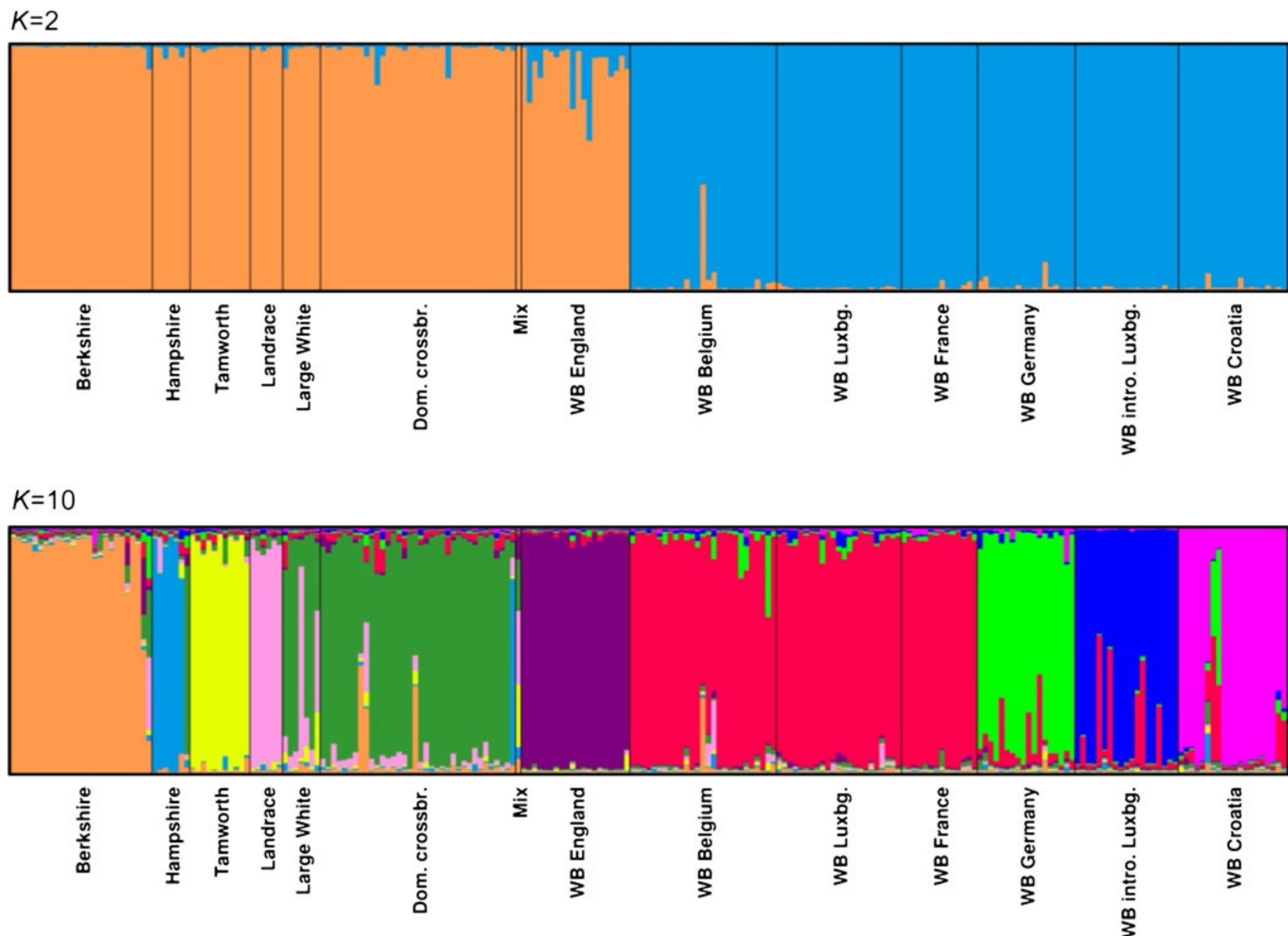


**Fig. 3** Results of STRUCTURE analysis: bar plots for $K = 2$, $K = 10$, illustrating the level of genetic admixture between European wild boar populations, introduced wild boars in Luxembourg and England, as well as different domestic pig breeds. Each individual is represented by a thin horizontal line divided into segments showing its proportions of membership of the genetic populations

desirable from management point of view. Despite the high reproductive potential of the species, there has been no dramatic increase in numbers in any of the established breeding populations, although the most likely reason for this is the culling pressure, rather than the adverse effects due to inbreeding (Moore and Wilson 2005).

The results of the FCA suggest that the genetic make-up of the English wild boars was a mixture of wild boars and domestic pigs. While it is possible that the English wild boars were genetically similar to an un-sampled European wild boar population, the neighbouring-joining tree suggested that, in terms of genetic distance, the English wild boar were similarly distinct from both European wild boars and from domestic pigs. Furthermore, the overlap with the admixed wild boars from Belgium—status identified using programme STRUCTURE—does support a hybrid origin of the English animals. Similarly, when fixing the number of genetic subpopulations to two, STRUCTURE suggested that the English wild boars were genetically more similar to domestic pigs than wild boars. At $K = 2$, STRUCTURE also provided evidence of some of the English wild boars being admixed. The STRUCTURE algorithm suggested that the whole dataset consisted of ten different genetic units. At $K = 10$, the English wild boars were genetically distinct, with little direct evidence of a hybrid domestic pig-wild boar origin of the population from the Forest of Dean. Only one Berkshire pig appeared to be admixed with English wild boars. This is in contrast to the population introduced to Luxembourg, which were genetically distinct from the local population, but had individuals that had an admixed local-introduced ancestry.

If the English wild boars have a hybrid origin, why did STRUCTURE not really detect this at $K = 10$? It has been shown that the majority of British pig breeds are independent genetic units with little evidence of admixture (Wilkinson et al. 2011). It is therefore possible that we did not use an appropriate reference population to find evidence of admixture in the released wild boars. While Booth (1995) specifically names the Tamworth as a breed that has been crossed with wild boar, this does not exclude the

possibility that other domestic breeds were used for crossing as well. Also, the STRUCTURE algorithm should, in principle, only detect recent hybridisation events. Rather than wild boar farmers constantly implementing interbreeding of wild boar and domestic pig, it is more likely that the majority of farmed wild boars come from a limited stock that has been interbred with domestic pigs at the beginning of wild boar farming.

Considerations of genetic integrity are frequently ignored in restoration programmes, as the main aim is to restore the species (Dowling et al. 2005; Frankham et al. 2009). Most conservation practitioners would probably agree, however, that a restored population should be genetically as close to the original as possible (Vergeer et al. 2008). This can create conflicting management interests when a formerly native species is reintroduced in an unplanned and unmanaged fashion. For example, while reintroductions of the European beaver (*Castor fiber*) have often been performed using individuals from different localities, France has aimed to preserve its autochthonous beaver by only sourcing individuals from the population on the river Rhône. The genetic purity of French beavers is, however, now threatened by immigrants from mixed stock from neighbouring countries, and management decision needs now to be taken as how best to, if at all, manage the spread of the mixed forms (Dewas et al. 2011). Furthermore, the presence of the Canadian beaver (*Castor canadensis*) has also been shown in some Western European populations. Active intervention is clearly preferable to stop this alien species competing with the native European beaver (Dewas et al. 2012).

In the case of the wild boars in England, the intrinsic value of a reintroduced former native species must be balanced against their long-term ecological impacts, which is difficult to predict. Our results provide evidence that the wild boars in the Forest of Dean were not genetically purebred. The high frequency of the alleles of Asian origin at the mtDNA control region and the *GPIP* locus clearly show the presence of domestic pigs in their ancestral line. The microsatellite-based analyses strongly suggest that English wild boars have a mixed wild boar/domestic pig genetic make-up, without providing direct evidence for recent hybridisation. This result suggests that the wild boars in the Forest of Dean should be regarded as feral rather than a restored native species. At the very least, our results clearly show that our English study population is very dissimilar to other northern European wild boar populations and therefore unlikely to be genetically closely related to the original native stock. The wild boars in the Forest of Dean therefore do not fulfil the criterion of genetic purity ideally required by managed reintroduction programmes.

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## References

Alves PC, Pinheiro I, Godinho R, Vicente J, Gortazar C, Scandura M (2010) Genetic diversity of wild boar populations and domestic pig breeds (*Sus scrofa*) in south-western Europe. Biol J Linn Soc 101:797–822

Belkhir K (2004) Genetix 4.05.2. University of Montpellier II, Laboratoire Génome et Populations, Montpellier

Booth WD (1995) Wild boar farming in the United Kingdom. IBEX J M E 3:245–248

Dewas M, Herr J, Schley L, Angst C, Manet B, Landry P, Catusse M (2012) Recovery and status of native and introduced beavers *Castor fibris* and *Castor canadensis* in France and neighbouring countries. Mamm Rev 42:144–165

Dowling TE, Marsh PC, Kelsen AT, Tibbets CA (2005) Genetic monitoring of wild and repatriated populations of endangered razorback sucker (*Xyrauchen texanus*, Catostomidae, Teleostei) in Lake Mohave, Arizona-Nevada. Mol Ecol 14:123–135

Fajardo V, Gonzalez I, Martin I, Rojas M, Hernández PE, García T, Martín R (2008) Differentiation of European wild boar (*Sus scrofa scrofa*) and domestic swine (*Sus scrofa domestica*) meats by PCR analysis targeting the mitochondrial D-loop and the nuclear melanocortin receptor 1 (MC1R) genes. Meat Sci 78:314–322

Fang M, Andersson L (2006) Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. Proc R Soc B 273:1803–1810

Fang M, Berg F, Ducos A, Andersson L (2006) Mitochondrial haplotypes of European wild boars with $2n = 36$ are closely related to those of European domestic pigs with $2n = 38$. Anim Genet 37:459–464

Fang MY, Larson G, Ribeiro HS, Li N, Andersson L (2009) Contrasting mode of evolution at a coat color locus in wild and domestic pigs. PLoS Genet 5:e1000341

Ferreira E, Souto L, Soares AMVM, Fonseca C (2006) Genetic structure of the wild boar (*Sus scrofa* L.) population in Portugal. Wildl Biol Pract 2:17–25

Fickel J, Hohmann U (2006) A methodological approach for non-invasive sampling for population size estimates in wild boars (*Sus scrofa*). Eur J Wildl Res 52:28–33

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to conservation genetics. Cambridge University Press, Cambridge

Frantz AC, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J Appl Ecol 46:493–505

Giuffra E, Kijas JMH, Amarger V, Carlborg O, Jeon JT, Andersson L (2000) The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154:1785–1791

Gongora J, Peltoniemi OAT, Tammen I, Raadsma H, Moran C (2003) Analyses of possible domestic pig contribution in two populations of Finnish farmed wild boar. Acta Agric Scand Sect A 53:161–165

Gortázar C, Ferroglio E, Höfle U, Fröhlich K, Vicente J (2007) Diseases shared between wildlife and livestock: a European perspective. Eur J Wildl Res 53:241–256

Goudet J (1995) FSTAT (vers 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Hampton JO, Spencer PBS, Alpers DL, Twigg LE, Woolnough AP, Doust J, Higgs T, Pluske J (2004) Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. J Appl Ecol 41:735–743

IUCN (1998) Guidelines for re-introductions. IUCN/SSC Re-introduction Specialist Group, Gland, Switzerland & Cambridge

Koutsogiannouli EA, Moutou KA, Sarafidou T, Stamatis C, Mamuris Z (2010) Detection of hybrids between wild boars (*Sus scrofa scrofa*) and domestic pigs (*Sus scrofa* f domestica) in Greece, using the PCR-RFLP method on melanocortin-1 receptor (MC1R) mutations. Mamm Biol 75:69–73

Langella O (1999) Populations 1.2.30: Population genetic software (individuals or population distances, phylogenetic trees). CNRS UPR9034, France

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L, Cooper A (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307:1618–1621

Larson G, Liu R, Zhao X, Yuan J, Fuller D, Barton L, Dobney K, Fan Q, Gu Z, Lui X-H, Luo Y, Lv P, Andersson L, Li N (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. Proc Natl Acad Sci USA 107:7686–7691

Lattuada L, Quaglia F, Iannelli F, Gissi C, Mantecca P, Bacchetta R, Polli M (2009) Mitochondrial DNA sequence variations in some Italian wild boar populations. J Anim Breed Genet 126:154–163

Lindholm AK, Breden F, Alexander HJ, Chan WK, Thakurta SG, Brooks R (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. Mol Ecol 14:3671–3682

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group, Species Survival Commission, World Conservation Union, Auckland

Manchester SJ, Bullock JM (2000) The impacts of non-native species on UK biodiversity and the effectiveness of control. J Appl Ecol 37:845–864

Massei G, Genov P (2004) The environmental impact of wild boar. Galemys 16:135–145

Massei G, Roy S, Bunting R (2011) Too many hogs? A review of methods to mitigate impact by wild boar and feral pigs. Hum Wildl Interact 5:79–99

Miller SA, Dykes DD, Polesky HF (1988) A simple salting-out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 16:1215

Moore NP, Wilson CJ (2005) Feral wild boar in England implications of future management options. Department for Environment, Food and Rural Affairs, London

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rackham O (1993) The history of the countryside. Dent, London, pp 36–37

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138

Scandura M, Iacolina L, Crestanello B, Pecchioli E, Di Benedetto MF, Russo V, Davoli R, Apollonio M, Bertorelle G (2008) Ancient vs recent processes as factors shaping the genetic variation of the European wild boar: are the effects of the last glaciation still detectable? Mol Ecol 17:1745–1762

Scandura M, Iacolina L, Apollonio M (2011a) Genetic diversity in the European wild boar *Sus scrofa*: phylogeography, population structure and wild x domestic hybridization. Mammal Rev 41:125–137

Scandura M, Iacolina L, Cossu A, Apollonio M (2011b) Effects of human perturbation on the genetic make-up of an island population: the case of the Sardinian wild boar. Heredity 106:1012–1020

Schley L, Dufrêne M, Krier A, Frantz AC (2008) Patterns of crop damage by wild boar (*Sus scrofa*) over a 10-year period. Eur J Wildl Res 54:589–599

Ursing B, Arnason U (1998) The complete mitochondrial DNA sequence of the pig (*Sus scrofa*). J Mol Evol 47:302–306

Vergeer P, Ouborg NJ, Hendry AP (2008) Genetic considerations of introduction efforts. In: Carroll SP, Fox CW (eds) Conservation biology—evolution in action. Oxford University Press, Oxford, pp 116–129

Vernesi C, Crestanello B, Pecchioli E, Tartari D, Caramelli D, Hauffe H, Bertorelle G (2003) The genetic impact of demographic decline and reintroduction in the wild boar (*Sus scrofa*): a microsatellite analysis. Mol Ecol 12:585–595

Wilkinson S, Haley C, Alderson L, Wiener P (2011) An empirical assessment of individual-based population genetic statistical techniques: application to British pig breeds. Heredity 106:261–269

Wilson CJ (2005) Feral wild boar in England status, impact and management. Department for Environment, Food and Rural Affairs, London