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Identification of mtDNA Lineages of *Sus scrofa* by Multiplex Single Base Extension for the Authentication of Processed Food Products

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Supporting Information

ABSTRACT: A genetic method to identify the breed of origin could serve as a useful tool for inspecting the authenticity of the increasing number of monobreed foodstuffs, such as those derived from small local European pig breeds. Mitochondrial DNA (mtDNA) is practically the only reliable genomic target for PCR in processed products, and its haploid nature and strict maternal inheritance greatly facilitate genetic analysis. As a result of strategies that sought to improve the production traits of European pigs, most industrial breeds presently show a high frequency of Asian alleles, while the absence or low frequency of such Asian alleles has been observed in small rustic breeds from which highly prized dry-cured and other traditional products are derived. Therefore, the detection of Asian ancestry would indicate nonconformity in Protected Denomination of Origin products. This study presents a single base extension assay based on 15 diagnostic mtDNA single nucleotide polymorphisms to discriminate between Asian and European *Sus scrofa* lineages. The test was robust, sensitive and accurate in a wide range of processed foodstuffs and allowed accurate detection of pig genetic material and identification of maternal ancestry. A market survey suggested that nonconformity of products derived from Portuguese breeds is an unusual event at present, but regular surveys both in the local populations and in commercial products would be advisible. Taking into consideration the limitations presented by other methodologies, this mtDNA-based test probably attains the highest resolution for the direct genetic test for population of origin in *Sus scrofa* food products.

KEYWORDS: *Sus scrofa*, food products, mtDNA, authentication, primer extension assay

INTRODUCTION

Increasing awareness of the composition of food products and the need to support correct labeling have triggered the development of molecular methods to ascertain the authenticity of animal and plant derived products. Earlier approaches have relied on the analysis of species-specific proteins, making use of a multiplicity of immunological and electrophoretic methods.[1] These analyses were plagued with complications related to the denaturation of proteins during the processing of products, false positives due to cross-reactivity and the tissue dependency of protein expression.

The use of DNA sequence information obtained by PCR presents several advantages over protein-based methods. DNA is present in all types of tissue, is relatively stable under extreme conditions and processing technologies, requires low amounts of test samples, and can potentially provide information that allows for species, breed and individual identification. In recent years, DNA-based methods have become promising tools for the scientific description of food composition, with a variety of methodologies allowing for the detection or confirmation of species identity in food products: DNA hybridization, restriction fragment length polymorphisms (RFLPs), real-time PCR, DNA sequencing or microarrays.[2]

The ability to trace farm animals back to their source breed is a particularly important element of quality control systems that aim to detect fraud and to validate particular productions. Many typical food products are protected by the European labels PDO (Protected Denomination of Origin) or PGI (Protected Geographical Indication) and are only allowed to be prepared from one breed. Mediterranean countries such as Italy, France, Spain and Portugal lead the number of PDO and PGI products of economic value of which the prestigious dry-cured pork products derived from local breeds, generally produced in rearing systems located in less competitive areas, represent typical added values like tradition and high quality. An example for this is the Alentejano pig, a Portuguese breed produced by extensive farming in the southern ecosystem of pastureland interspersed with Mediterranean oaks. The premium dry-cured goods derived from the Alentejano pig constitute an important aspect of the Portuguese cultural heritage and a commercially interesting niche recently rediscovered that is marketed at significantly higher prices than nondifferentiated products derived from other breeds such as Large White, Landrace, Pietrain and Duroc. Therefore, it is likely that pressure for rapid market expansion will stimulate the proliferation of lesser products under consumer-attractive denominations. Genetic certification of the breed of origin would be a valuable tool for the protection of the original products and could contribute to increased breed profitability.

Mitochondrial DNA (mtDNA) is practically the only reliable target for genetic analysis in low quality or low quantity DNA samples such as processed foodstuffs because it is several-fold more abundant than nuclear DNA. Strict maternal inheritance results in an individual normally possessing only one allele for each gene, and sequence ambiguities that result from heterozygous genotypes are avoided. The relatively high mitochondrial

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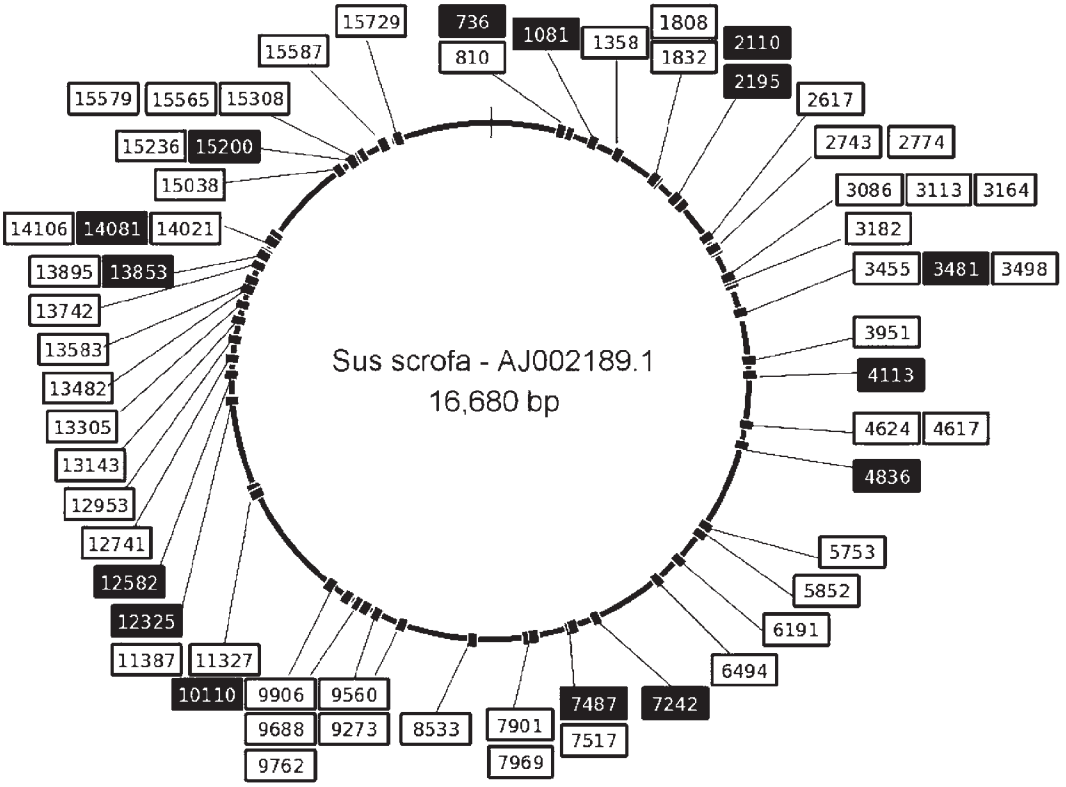

Figure 1. Schematic representation of the *Sus scrofa* mitochondrial genome, and the approximate location of the 63 polymorphic positions that allow for discrimination between the Asian and the European phylogenetic clades, numbered according to the reference sequence Genbank AJ002189.[8] Polymorphic positions surveyed in the multiplex single base extension assay are shown in black squares.

mutation rate allows for the accumulation of nucleotide differences in closely related lineages. Because of these properties, many applications in the field of genetic identification of processed animal products make use of mitochondrial DNA polymorphisms.[3]

MtDNA sequences are particularly well studied in the genus *Sus*. A worldwide survey based on the analysis of the mitochondrial control region identified two major phylogenetic clades representing two important and independent pig domestication events, one in Europe and another in Asia.[4] Thus, Asian and European *Sus* populations constituted distinct phylogenetic branches until the advent of long distance cultural and commercial exchanges from the 17th century onward, when European pig populations started to be crossbred with Asian animals, resulting in improved prolificacy, growth and desired meat characteristics. As a result of this effort, most present industrial breeds (e.g., Duroc) are known European−Asian hybrids, while local rustic European breeds have low frequencies of Asian haplotypes.[5] An extensive study of mtDNA lineages in the autochthonous Portuguese breeds and wild boars has revealed very low frequencies of Asian alleles, suggesting such admixtures occurred rarely until now, which stands in contrast to the high prevalence of Asian lineages in industrial breeds.[6] Therefore, the detection of Asian lineages in traditional food products derived from breeds without a known record of Asian lineages in the population would indicate the use of other animals and nonconformity with denomination of a local of origin.

In this work, we present the development and application of a multiplex single base extension (SBE, also known as minisequencing or SNaPshot) assay making use of 15 diagnostic single nucleotide polymorphisms (SNPs) for the identification of Asian and European lineages in fresh and highly processed pig and wild boar food products. The test proved to be robust, easy to perform, and potentially useful for the rapid screening of a large number of samples.

MATERIALS AND METHODS

Identification of Mitochondrial SNPs for Discrimination of Asian and European *Sus scrofa* Lineages. Complete mitochondrial sequences from *Sus scrofa* were retrieved from the NCBI database using Geneious software (www.biomatters.com) and aligned with MUSCLE version 3.6[7] against a reference sequence (Genbank AJ002189).[8] We used all Asian and European complete mtDNA sequences from previously published articles,[8−12] including the only complete mtDNA sequence from Italy,[13] for a total of 48 haplotypes. Parsimony-informative sites with two variants were identified using the DnaSP 5.10.00 software.[14] Geographic assignment of each position was obtained using a phylogenetic tree of complete sequences estimated in BEAST[15] with the GTR+I+G model as determined in JmodelTest and two runs of 20^6 generations.

A total of 63 polymorphic positions along the whole mitochondrial genome were identified among the 241 parsimony-informative sites as diagnostic for the purpose of the discrimination between the Asian (A) and the European E1 lineages (Supplementary Figure 1 in the Supporting Information), of which 15 were included in the SBE assay (Figure 1).

A phylogenetic tree with partial mtDNA sequences (control region positions 15435 to 16046 in the reference sequence) was built using additional sequences from Italy[16,17] and Turkey[16,18] to demonstrate that Near Eastern (NE) lineages could also be discriminated (Supplementary Figure 2 in the Supporting Information). The final tree was generated by maximum likelihood calculations using Phyml with HKY85+I+G, determined in JmodelTest[19] and approximate likelihood ratio test for branches' support (aLRT).[20]

Table 1. Sequences (5′−3′) of the mtDNA Fragments Containing the 15 Diagnostic SNPs, Designated by the Number of the Surveyed Nucleotide Position in a Reference Mitochondrial Genome (Genbank AJ002189)[8a]

SNP	reference sequence
736	TACCGCCATCTTCAGCAAACcctaaaaaggaacaatagtaagcacaatcataGcacataaaaaCGTTAGGTCAAGGTGTAGCT
1081	GTGTGCTTGGATTACCAAAGcatagcttaaactaaagcacctagtttacacctagaagatcccaCaatgtatGGGTACTTTGAACCAAAGCT
2110	TGTATGAATGGCCACACGAGggtttttactgtctcttacttccaatcagtgaaattAaccttccCGTGAAGAGGCGGGAATAAA
2195	AAGACGAGAAGACCCTATGGagctttaattaactattccaaaagttaaacaaCtcaaccacaaagggataaaacataacttaacatGGACTAGCAATTTCGGTTGG
3481	CGCCATATTCTTCATAGCAGaatatgccaacatcatcataataaatgcatttacagcaattctcttcctaggagcatCccacgaCCCACACACACCAGAACTAT
4113	TTCCTAACACAAGCCACAGCctccatAatactaataatagccatcatcatcaacctCCTATATTCTGGCCAATGG
4836	ATTTCTACATACGACTAGCCTACTCCTCctcactgactatgttcccatccaccaaCaacataaaaataaaATGACAATTCGAACACACAAAAC
7242	CACAATGGATGCCCAAGAAGtagaaacaatttgaacaatcctacccgctattattCtaattcttattGCCCTTCCATCATTACGAAT
7487	GACAATCGAGTTGTTCTACCaatagaaataacaatccgaatattagtAtcctctgaagacgtactgcactcatgagccGTCCCATCCCTCGGTTTAAAA
10110	ACTAGTGTTTGCAGCCTGCGaagctgcactAggcctgtcactactagtaatagtatccaaCACATACGGTACCGATTACG
12325	TATACAACCGCATCGGAGACattggatttgtcctatccatagcatgattcCtaacccactcaaaCGCATGAGATCTTCAACAAA
12582	CCGCTTCTACCCCTTAATAGaaactaacaaactagttcaaacTataacactatgcctaggagctatCACCACCTTATTTACAGCAC
13853	AAACCAGAAACAAATGCTCCaaaaacagtcttattagaaactcaaacctcaggAtacatctCAGTAGCCATAGCAGTAGTA
14081	GAGGGCTTAGAAGAAAAACCaacaaacccaataacaaaaatagtacttaaaataaatgcaatataCattgtcattattctcacatggaatttaaCCACGACCAATGACATGAAA
15200	GCAGACCTCATTACACTAACatgaattggaggacaacccgtagaacacccGttcatcatcatcgGCCAACTAGCCTCCATCTTA

[a] PCR primers and diagnostic SNPs are in uppercase and bold, and SBE primers (tails not represented) are underlined.

Primer Design. Two separate sets of primers (PCR amplification primers and extension primers) are necessary for performing a SBE assay. The first are used to obtain the amplification of fragments containing the surveyed SNPs, and the second are used in the subsequent SBE reaction. PCR primers were designed for coamplification of 15 DNA fragments, each containing one diagnostic SNP. To optimize the amplification of DNA in highly processed products, these primers were designed to amplify less than 120 bp long fragments using Primer3 software.[21] Potential primer−dimer interactions were assessed with AutoDimer software.[22] Extension primers for the SBE reaction were also designed using the Primer3 software, with tails added manually and sizes adjusted after preliminary tests. Under the assumption that all SNPs had the same diagnostic value, the highest possible number of SNPs was included in the final assay based on the suitability of primers for multiplex PCR amplification and multiplex SBE.

Samples and DNA Extraction. Samples for analysis were collected from a variety of both fresh and processed commercial foods derived from pig and wild boar acquired in the retail market. The selection of these products aimed at a broad analysis of commercially available pig and wild boar food products that displayed a local breed and/or local origin in the labeling. Anonymous products (not indicating a local origin and/or breed in the label) were also analyzed. DNA was extracted from approximately 3 mm^3 pieces of sample material using a standard phenol−chloroform method.

Single Locus PCR and Sequencing. A set of 6 reference samples, for which Asian or European ancestry was previously determined by mtDNA control region analysis,[6] were used to test each PCR primer pair independently. Amplifications were performed in 5 μL reactions using 5−10 ng of DNA, 1× QIAGEN PCR Mix (QIAGEN, Germany) and 0.2 μM primers, as follows: 15 min at 95 °C; 30 cycles at 94 °C for 1 min, 60 °C for 1 min 30 s, 72 °C for 1 min; 72 °C for 10 min. DNA amplification was confirmed by silver-stained acrylamide gel electrophoresis. Each fragment was then sequenced by in both directions to confirm the presence of the diagnostic SNP at the expected position (Table 1). Presequencing purification was performed combining 1.5 μL of each PCR product and 1 μL of Exo-SAP (USB Corporation, USA) for 15 min at 37 °C followed by 15 min at 85 °C. Sequencing reactions were performed by combining 1× Big Dye Sequencing Kit v3 (Applied Biosystems, USA), 0.2 μM primer and 2.5 μL of purified PCR product, as follows: 96 °C for 2 min and 35 cycles at 96 °C for 15 s, 50 °C for 9 s, 60 °C for 2 min. Sequencing reaction products were purified with Sephadex G-50 columns (GE Healthcare, U.K.) and run on a 3130xl ABI Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions. Electrophoretic data were analyzed with Sequencing Analysis v5.2 software (Applied Biosystems, USA) and manually compared to a reference sequence.[23]

Multiplex PCR and SBE Reaction. Multiplex PCR amplification of the 15 fragments containing the diagnostic SNPs was performed in 5 μL reactions using 5−10 ng of DNA, 1× QIAGEN PCR Mix (QIAGEN), and 0.2 μM primers under the single locus PCR conditions described above. Multiplex PCR products were also purified as described in the previous section.

SBE reactions were performed in a final volume of 5 μL using 1× SNaPshot Ready Reaction Mix (Applied Biosystems), 2.5 μL of purified PCR product and adjusted concentrations of each SBE primer (Supplementary Table 1 in the Supporting Information) with a thermocycler program of 25 cycles at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. A final purification step was performed using 1 μL of SAP (USB Corporation) for each 5 μL of SBE reaction product, as follows: 37 °C for 60 min, and 75 °C for 15 min. Purified SBE reaction products were run on an ABI Genetic Analyzer 3130xl (Applied Biosystems) using GeneScan Liz-120 (Applied Biosystems) as an internal size standard. The results were analyzed with GeneMapper v4.0 software (Applied Biosystems).

RESULTS

The SBE Multiplex. The processing of food products (e.g., dehydration, smoking, salting, and presence of other species and flavoring components) did not limit the ability to diagnose Asian or European maternal lineage. Fresh or frozen and diverse smoked, dry-cured and complex mixtures such as pâtés equally allowed for amplification and SBE analysis of all expected SNPs and a straightforward interpretation of the results (Figure 2). The success of PCR and SBE reactions in the wide range of dry-cured, salted and complex foodstuffs can be attributed to three crucial factors. First, the use of mtDNA as a target for PCR enabled us to obtain results in deeply processed products from which nuclear DNA is more than often unavailable for reliable and reproducible analysis. Second, the phenol−chloroform DNA extraction methodology, although somewhat laborious compared to commercial extraction kits, resulted in

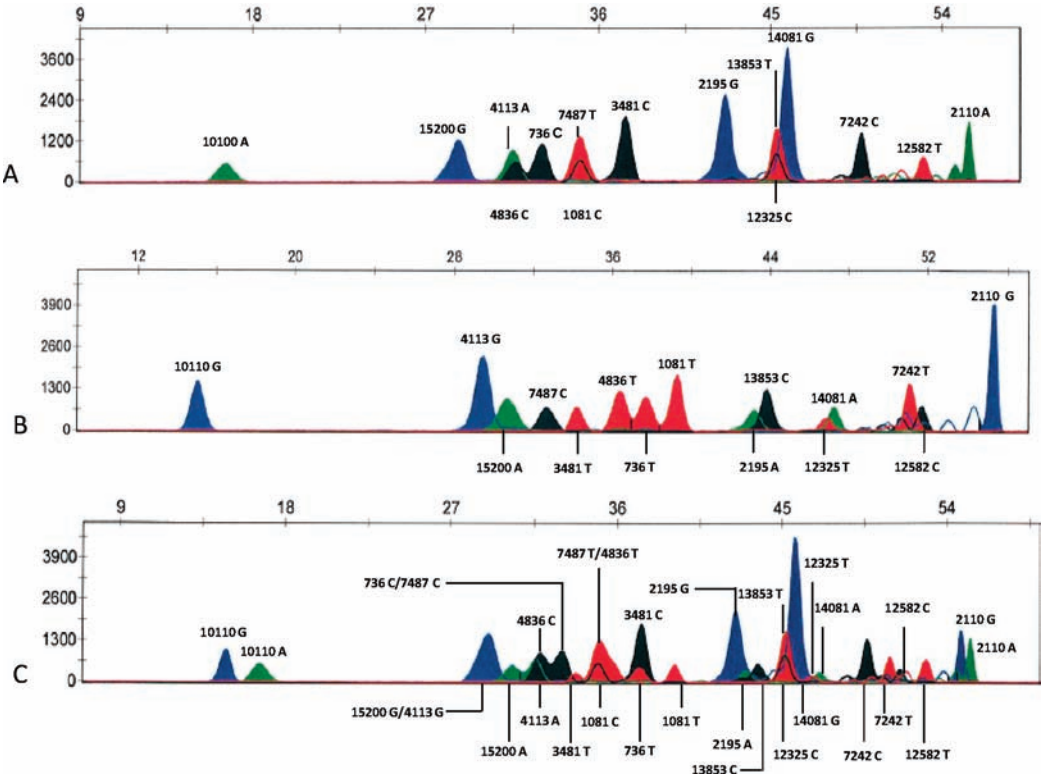

Figure 2. Example profiles obtained with the single base extension assay for determination of the European or Asian maternal ancestry in *Sus scrofa*: (A) Identification of European clade (smoked ham sample); (B) identification of Asian clade (smoked sausage sample); (C) identification of Asian and European clades in a mixture (smoked sausage). *X* axis: estimated fragment size (bp). *Y* axis: relative fluorescence intensity (RFU).

high yield and high purity of the DNA extract. Third, PCR primers were designed to obtain extremely short length fragments (75−114 bp, average = 90 bp), potentially allowing for successful PCR even in the presence of highly degraded DNA samples.

Cross-Species Reactivity. The SBE assay was tested for cross-species reactivity in one DNA sample belonging to each of the following species: *Homo sapiens* (human), *Ovis aries* (sheep), *Capra hircus* (goat), *Equus caballus* (horse), *Oryctolagus cuniculus* (rabbit), and *Mus musculus* (mouse), *Canis lupus familiaris* (dog), *Felis catus* (cat) and *Bos taurus* (cow). The results were negative for all species, thus showing that species other than *Sus scrofa* did not interfere with the results.

Mixture Tests. Whereas in products such as smoked ham it is expected that only one genetic contributor is present, such is not the case for composed products as smoked sausages and pâtés. Sensitivity tests were performed in synthetic mixtures composed of Asian (A) and European (E) DNA extracts in different proportions (1:1; 1:3; 1:5 and 1:10). Although this aspect of the methodology was not further developed and the sensitivity threshold of the test was not determined, mixed European and Asian profiles were easily interpreted (Figure 2C).

Identification of European and Asian Phylogenetic Origin in Commercial Samples. The SBE assay was used to test 64 commercial products derived from pig and wild boar (Supplementary Table 2 in the Supporting Information). Most products (81.25%) had the European mitochondrial profile. All Alentejano PDO products were consistent with a European profile and did not present nonconformities with the local purebred Alentejano breed of origin. Asian profiles were detected in three products (4.69%), of which two were smoked hams displaying an 'Iberian' or a 'Serrano' designation in the label; the other was a smoked sausage labeled with 'Traditional recipe from Alentejo'. These designations, while potentially misleading, do not imply that the products had to be manufactured from purebred Iberian pigs, and therefore the results do not indicate nonconformity but instead corroborate the sensitivity of the method. Mixed Asian/European profiles were detected in a total of nine samples (14.06%), of which four displayed the label 'Porco Preto' (two smoked sausages and two pâtés), two indicated the Portuguese 'Bísaro' breed, and one displayed the label 'Pata Negra' (smoked sausages). 'Porco Preto' and 'Pata Negra' are commercial designations for the Alentejano breed and the Iberian (Spanish) pig that do not demand pure-breeding of the source animals. 'Bísaro' is a local Portuguese breed that has shown low frequencies of Asian haplotypes.[6] Mixed profiles (Asian and European clades both present) were also detected in a pâté labeled as wild boar that also contained pork liver and fat, and in a smoked sausage labeled as containing hunted species with no reference to pork.

DISCUSSION

The detection of Asian profiles was restricted to local smoked sausages and pâtés, suggesting that animals of nonlocal origin are frequently mixed with purebred autochthonous pigs in such products. Additionally, present legal specifications for traditional dry-cured goods in Portugal allow for the use of industrial pork fat in smoked sausages, forbidding only the use of non-purebred meat in PDO products. These results suggest that nonconformity with a local origin of the products is probably a rare event in premium products such as smoked ham; otherwise, a higher

frequency of Asian haplotypes would have been detected. In fact, the Duroc breed, which is the breed most commonly crossbred with Alentejano pigs and a substitute in traditional dry-cured products, has been characterized by frequencies of Asian haplotypes up to 50%.[6] As a result, this assay allows for the identification of nonconformity (Asian profile) in 50% of the cases of crossing or substitution with the industrial Duroc breed.

A balance between two aspects has to be considered when developing a discriminating method based on phylogenetic information. On one hand, mtDNA is the only reliable target for genetic analysis in processed food products; on the other, the structure of *Sus scrofa* populations, as in practically all other livestock species, has shown a weak correlation between haplotypes and geography,[24] i.e., extant breeds were recently created by human selection that has not erased the genetic heritage of a diverse founding pool of females. However, domestic pigs stand out by showing clear phylogenetic divergence in mtDNA sequences, allowing for a clear discrimination between European and Asiatic lineages, which is a unique feature in livestock species.

A survey of the male contribution to the genome via Y-chromosome (the paternal analogue to mtDNA) would be useful. However, this chromosome remains largely understudied in *Sus scrofa* because of the lack of sequence information necessary for polymorphism screening, as is generally the case for all domestic species. A recent worldwide study including *Sus scrofa* Y-chromosomal single-copy genes revealed an extremely low diversity of haplotypes.[25] As in the analysis of mtDNA lineages, no correlation between breeds and Y-chromosome haplotypes was found, except for a broad phylogeographic structure also dividing Asia and Europe. The low diversity of the Y-chromosome when compared to that of mtDNA and the known strong sex-bias in the domestication process and breeding of domestic animals, such as horse,[26] goat,[27] cattle,[28] and sheep,[29] presently discourage the use of Y-chromosome markers for population assignment.

To the best of our knowledge, genetic screening methods for direct breed identification in livestock are at present extremely scarce, and finding genetic markers with different allelic variants fixed in different breeds seems to be a difficult task. Among gene-based markers, genes coding for coat color (one of the most important traits under human selection that allows for breed differentiation) such as the melanocortin-1-receptor (*MC1R*)[30] have been favorite targets for the development of deterministic approaches for breed traceability. *MC1R* was found to have a fixed allele in European wild boars that allowed for the discrimination from domestic swine and the identification of wild−domestic hybrids.[31] In domestic pigs, one-to-one breed comparisons between the Spanish Iberian and the Duroc breeds showed that these could be discriminated based on either one of two *MC1R* diagnostic polymorphisms.[32] This gene was not found to be useful for direct breed identification in the Nero Siciliano breed (Italy).[33] In cattle, a fixed *MC1R* allele in the Reggiana breed allowed for the authentication of Parmigiano-Reggiano cheese by the detection of nonexpected DNA derived from some other breeds.[34] *MC1R* polymorphisms seem to be useful only in specific breed comparisons such as Iberian pig vs Duroc and Reggiana vs other cattle. Additionally, the difficult retrieval of autosomal DNA from highly processed products may limit the utility of this gene for distinguishing breeds.

Short tandem repeats (STRs) have been explored to obtain individual multilocus genotypes and to develop probabilistic approaches for breed assignment.[35−37] The method is based on the fact that different breeds can show differences in frequencies and numbers of fixed alleles. The complex mutation pattern of microsatellites[38] can introduce uncertainty to data analysis, and genotyping errors may arise due to stutter peaks and other artifacts (e.g., allelic dropouts, fluorescence pull-ups in capillary electrophoresis). Moreover, STRs are prone to homoplasy, i.e., the same allele can occur in unrelated lineages through convergent evolution without being identical by descent.

Biallelic markers such as SNPs and insertion and deletion polymorphisms (indels) have the advantage of being easier to genotype using automatic electrophoresis, because only two states are generally observed, and their evolution according to simple mutation models is well described. Nevertheless, a higher number of biallelic loci are needed to obtain the same resolution power compared to multiallelic markers.[39] Indels are widely spread across animal genomes and have become an abundant source of genetic markers in human studies.[40] Shared indels can confidently represent identity-by-descent because the probability of two different indels of exactly the same length occurring at the same genomic position is extremely low. Although less variable than STRs, indels overcome this limitation through the ease of multiplexing and genotyping based on electrophoretic size separation. Studies in chickens[41] and canines[42] have proposed informatics approaches to survey large-scale genomic sequence data from several individuals for the putative presence of indels and the subsequent validation of the loci's informativity as genetic markers at the population level. The lack of large-scale genome sequence information from different breeds has until now precluded the introduction of indels as genetic markers in *Sus scrofa*. New sequencing technologies and the associated cost decrease will expectedly generate massive amounts of sequence data in the near future.

Although the difficulty of finding breed markers is the common denominator in any type of genetic analysis in domestic species, autosomal STR, SNP and indel-based methods for food analysis present serious limitations compared to the mtDNA-based method we describe here. Autosomal DNA is usually difficult to retrieve from less than pristine DNA samples, as is often the case for food products, and the analysis of material that has genetic contributions from more than one individual is complex. Additionally, population assignment of samples of unknown origin requires a collection of genotypes observed in reference populations and the use of statistical tools that, in spite of user-friendly free software such as *Structure*,[43] are not practical to use in high throughput testing.

In conclusion, this single base extension methodology allows for the detection of the presence of pig-derived components in food products and the assessment of Asian or European maternal ancestry of *Sus scrofa*. The assay was robust, sensitive, and accurate in a wide range of processed foodstuffs and complex mixtures, probably due to the use of a phenol−chloroform extraction method and the use of mtDNA as a target for PCR of short fragments. Our results confirm the applicability of this method in the genetic analysis of commercial food products. The market study suggested that nonconformity with a local origin in *Sus scrofa* products derived from Portuguese breeds is still a rare event, but as market pressure increases, the situation may rapidly change. We propose that it would be advisable to perform regular surveys both in local populations and in commercial products. Moreover, surveillance could encourage animal producers to develop breeding strategies that facilitate the certification of the authenticity of their products.

As for other genetic markers described in *Sus scrofa*, none of the methodologies we have discussed are at present applicable in deterministic approaches for breed assignment. When taking these limitations into consideration, the mtDNA-based test for identification of population of origin we describe here emerges as the method most able to achieve the highest resolution in the genetic discrimination of *Sus scrofa* food products.

This assay potentially allows for the determination of the origin of *Sus scrofa* in ancient DNA samples and could be adapted to also survey diagnostic SNPs for the Near Eastern phylogenetic clade (absent from extant domestic European populations but present in archaeological specimens).[18] Thus, this assay could be extended to archaeogenetics studies focusing on the dissemination routes of the Neolithic in Europe.

■ ASSOCIATED CONTENT

Supporting Information. Additional PCR data, list of test products and phylogenetic identifications, partial overview of the alignment of 48 complete Asian and European mitochondrial *Sus scrofa* sequences, and maximum likelihood phylogenetic tree including 63 *Sus scrofa* mitochondrial control region sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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