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Development of a Microsatellite-Based Method for the Differentiation of European Wild Boar (*Sus scrofa scrofa*) from Domestic Pig Breeds (*Sus scrofa domestica*) in Food

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ABSTRACT: Twenty microsatellites (simple sequence repeats, SSR) were used to discriminate wild boar from domestic pig and to identify mixtures of the two. Reference groups of wild boar and pig samples were collected from the UK and Europe for genetic assignment tests. Bayesian Analysis of Populations software (BAPs) gave 100% correct assignment for blind wild boar and pig samples and correctly identified mixed samples. DNA was extracted from 12 commercial food samples (11 labeled as containing wild boar) including patés, salamis, and sausage, and good SSR profiles were obtained. Eleven samples were correctly assigned as pig, and two as mixed meats. One sample sold as wild boar meat was clearly assigned as pig. A further 10 blind samples of meat cuts were analyzed, eight wild boar and two pig, and all were correctly assigned.

KEYWORDS: *wild boar, domestic pig, SSR, food authenticity, Bayesian analysis of population structure*

INTRODUCTION

The pig (*Sus scrofa domesticus* L.) was first domesticated from wild boar (*Sus scrofa* L.), approximately 9000 years ago. Currently, more than 250 breeds of domestic pig are farmed,[1] and pork has become an important global food source. In recent years, consumers have become more discerning, and the requirement for meat from named traditional breeds of pig has increased. Coupled to this has been the increase in demand for wild boar meat. This is regarded as a premium product with a stronger, more gamey flavor, which can command a higher market price. Hence, there is a danger of fraudulent substitution of pig meat for the more expensive wild boar meat. To detect substitution, there is a need to be able to discriminate between pig and wild boar meat for the enforcement of food labeling regulations, and for proof of authenticity.

Physical differences between wild boar and pig include a larger head, more coarse fur, and straight tail in the adult wild boar as compared to pig, with the major difference between the two visible in the young where wild boar piglets are striped. A distinctive coat color is often associated with certain breeds; for example, Tamworth pigs are ginger, Duroc are red, Hampshire pigs are black, and Gloucestershire Old Spot are creamy colored with dark brown spots. Methods for species identification that are sensitive and robust enough to be applied to food matrixes now tend to be based on DNA measurement.[2,3] This has led to some interest in two of the coat color genes, the melanocortin receptor 1 gene (*MC1R*) and *KIT*, which may be useful for the identification of polymorphisms associated with breed differences.[4−8] Indeed, Fernandez et al.[5] successfully differentiated Duroc from Iberian pigs using the *MC1R* gene, although the study was not extended to other breeds. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approaches of Fajardo et al.,[6] Marklund et al.,[7] and Alderson and Plastow[8] appear to be promising methods to differentiate pig breeds, but cannot be used to detect the presence of wild boar in a pig−wild boar meat mixture. Mitochondrial DNA (mtDNA) genotyping has also been used to study genetic diversity between populations of pigs,[9] and between pig and wild boar populations.[10−12] Although some private alleles were identified, (i.e., an allele only found in pig, or only in wild boar), they did not occur at a high enough frequency to be useful. For example, Alves et al.[13] detected three single nucleotide polymorphisms (SNP) in mtDNA, which occurred solely in wild boar, when compared to Iberian and Duroc pigs, but only at a frequency of 0.22, meaning that approximately 1 in 5 wild boar would carry these SNPs, whereas a frequency nearing 1.00 would be desirable for an SNP to act as a potential discriminatory marker. Fernandez et al.,[5] in addition to using an SNP unique to wild boar, supplemented their data with the use of four microsatellite markers. Microsatellite markers (simple sequence repeats, SSR) have been used to discriminate between closely related plant varieties, for example, olive oil[3] and Basmati rice,[2] and offer a means to discriminate between pig and wild boar, because over 1200 SSR markers were identified in the domestic pig in the mid 1990s.[14−21] These markers were developed to aid in breeding programs for marker-assisted selection for economically important traits, including leanness, growth rate, hardiness, fecundity, disease resistance, flavor, and meat quality. However, a number of these markers have also been used to study genetic diversity within pig breeds,[22−25] between populations of wild boar,[26] and between wild boar and pigs.[27]

The aim of this study was to develop a method for the discrimination of wild boar from domestic pig breeds and domestic pig/wild boar meat mixtures. This method could then provide a means of authenticating wild boar meat product composition, and, therefore, be a considerable aid in the enforcement of food labeling regulations.

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■ MATERIALS AND METHODS

Samples. The reference samples, used to construct the assignment model, are shown in Table 1. Meat mixtures, commercial food, and blind samples are shown in Table 2. Wild boar tissue samples, mainly ear tips, were provided by Fera colleagues, from culled animals living wild in the Forest of Dean (Gloucestershire, UK), whereas wild boar buccal swabs were obtained from animals maintained on the Fera site. Wild boar hair samples were kindly supplied by Félix Juterczenka, from local populations based at the Château de Champoulet (Champoulet, France). Cuts of wild boar meat were purchased from local butchers or online retailers. Hair and buccal swabs from traditional and rare breed pigs were obtained from farms in the local area. Pork samples from unspecified breeds, along with named pure breed and named cross-breed cuts of meat, were purchased from a range of local supermarket chains within the UK. Commercial and/or processed wild boar and pig samples were purchased from local retailers. Binary mixtures of wild boar and pig meat were made in known proportions: six analyzed in duplicate as a reference group, and nine analyzed in duplicate submitted as "blind" samples for analysis. One sample of a putative wild boar from Australia was also included in the analysis.

Table 1. Samples of Pig and Wild Boar Used as Reference Samples for Microsatellite and BAPs (Bayesian Analysis of Populations) Analysis[a]

no.	details	sample type
Wild Boar Reference Samples		
1−18	wild boar, Forest of Dean, UK	tissue
19−25	female wild boar, France	hair
26−30	male wild boar, France	hair
31−33	blind sample	tissue
34−44	wild boar, Forest of Dean, UK	tissue
Pig Reference Samples		
45	large black pig	buccal
46	large black pig	hair
47	saddleback pig	buccal
48−52	saddleback pig	hair
53	Duroc/Gloucester Old Spot cross pig	tissue
54	Hampshire cross pig	tissue
55−58	supermarket pork − unknown breed	tissue
59	Ayreshire breed pork	tissue
60	large white breed pork	tissue
61	Saddleback/Duroc cross pork	tissue
62−73	supermarket pork − unknown breed	tissue
74	"Iron age" pig	tissue
75	Saddleback pig	tissue
76−77	Gloucester Old Spot pig	tissue

[a]Sample numbering allows cross-referencing to data points in Figure 1.

DNA Extraction. DNA was extracted from wild boar ear-tips and cuts of pork meat following a variation of the salting-out method for isolation of nucleic acids.[28] Variations to the method included the use of 10 M ammonium acetate (Sigma-Aldrich, Poole, Dorset, UK) for protein precipitation, rather than using sodium chloride, and the use of isopropanol for nucleic acid precipitation rather than using ethanol. The DNA pellet was dissolved in 500 μL of 1 × TE buffer (Sigma-Aldrich). DNA was extracted from hair follicles using a Chelex method.[29] Portions (1 cm) were cut from the root end of 50 hairs and placed in 500 μL of water (where less than 50 follicles were available, the volume of water was adjusted accordingly). Following incubation at room temperature for 30 min, 0.2 × volumes of 5% (w/v) Chelex-100 (Bio-Rad, Hemel Hempstead, UK) in 10 mM Tris buffer pH 8.0 was added and incubated at 56 °C for 45 min to 1 h. The samples were then boiled for 8 min before centrifugation for 5 min at 6600*g*. The supernatant was recovered for subsequent analysis. DNA was extracted from the buccal swab samples (Omni swabs, Whatman, Maidstone, U.K.) using a QIAamp DNA Blood Mini kit (Qiagen, Crawley, U.K.) using the buccal spin protocol. DNA was extracted from commercial meat samples following an in-house meat extraction method used routinely in this laboratory.[30] The method is a modified version of the Wizard DNA cleanup system (Promega, Southampton, U.K.).

SSR Analysis. DNA extracts from all samples were analyzed using 20 SSRs (primers shown in Table 3). The primers were initially selected from the PIGMAP program developed by the Roslin Institute Edinburgh, which utilizes highly polymorphic microsatellites to study pig breed biodiversity. The references giving details of the markers are shown in Table 3. Markers were chosen that showed high polymorphism within and between breeds (information on heterozygosity values, *H*, number of alleles, and average polymorphism information content, PIC, are shown in Table 3). Forward primers were fluorescently labeled at the 5′ end using one of three labels, FAM, HEX, or NED (Applied Biosystems, Warrington, U.K.). PCR reactions comprised 20 ng template DNA (apart from DNA extracted from hair where 100 ng was used), 0.5 μM each primer, 0.06 mM dNTPs, 1.5 mM magnesium chloride (apart from primer pairs SW911, SW936, SW1111, and SW1828 for which the optimum magnesium chloride concentration was 0.9 mM), 0.7 U Red Hot Taq DNA polymerase (Abgene, Epsom, U.K.) and 1 × buffer IV (supplied with the Red Hot Taq DNA polymerase). Total reaction volume was 15 μL. Reactions were run on a Hybaid Multiblock PCR system (Hybaid, Basingstoke, U.K.) with the following thermal cycling protocol: 94 °C for 5 min, followed by 35 cycles (40 cycles for DNA from hair) of 94 °C for 30 s, 55 °C (or 60 °C for primer pairs S0155 and SW24) for 30 s, 72 °C for 30 s, with a final step of 72 °C for 60 min. Amplification products were multiplexed prior to electrophoresis through a 36 cm capillary filled with POP-7 polymer (Applied Biosystems) mounted in an Applied Biosytems 3130xl Genetic Analyzer. SSR profiles were analyzed using GeneMapper v3.7 software. Any individual samples that had 20% or more alleles missing, due to poor DNA quality, were excluded from analysis.

Assignment Tests. To visualize the data, a principal coordinate (PCO) plot of pairwise simple allele matching distances was made using Genstat 12 (VSN Int. Ltd.). Bayesian Analysis of Populations (BAPs) software v5.2[31,32] was used to assign samples to reference groups of pig and wild boar using the "admixture based on predefined clustering" module with the probability of samples being admixtures of the reference groups calculated by a 1000 replicate bootstrap. BAPs is the only program, to the best of our knowledge, which allows samples with more than two alleles (as is the case with meat mixtures) to be analyzed for admixture from genetic data. 44 wild boar and 33 pig samples were used as reference populations for BAPs.

■ RESULTS AND DISCUSSION

SSR Analysis of Pure Wild Boar and Pig Samples. The 20 SSR markers chosen for analysis were found to generate clear profiles with DNA extracted from tissue or buccal samples. DNA extracted from hair tended to be of poor quality and quantity, and therefore, initially, for these types of samples, the number of PCR cycles was increased to 55. However, this resulted in a large number of extraneous peaks. The number of cycles was then lowered to 40, and with an increase in the amount of DNA template per reaction, to 100 ng from the original 20 ng, resulted in clear SSR profiles.

Analysis of the SSR data generated from the wild boar and pig reference samples in Table 1 indicated that samples of pure, unprocessed wild boar could be easily discriminated from pure unprocessed pig samples. This is evident in the clear separation of pig and wild boar genotypes in the PCO plot (Figure 1). Between the two reference populations, F_{st} values varied considerably for each SSR locus, reflecting the varying frequency of alleles among the two groups (F_{st} minimum = 2%, maximum = 25%). There were no fixed private alleles observed among the reference groups.

Table 2. Assignment of Pig/Wild Boar Mixtures, Commercial, and Blind Test Samples by SSR Using BAPs[a]

no.	description	expected % wild boar	BAPs mean % wild boar ($n = 2$)	BAPs P (mixture)
w/w Mixtures				
78–79	wild boar 1/pork 1 mixture	20	33	0.98
80–81	wild boar 1/pork 1 mixture	40	42.5	1
82–83	wild boar 1/pork 1 mixture	60	58.5	1
84–85	wild boar 1/pork 1 mixture	80	77.5	1
86–87	wild boar 1/pork 1 mixture	100	100	0
88–89	wild boar 1/pork 1 mixture	0	0	0
90–91	wild boar 2/pork 2 mixture	25	51	1
92–93	wild boar 2/pork 2 mixture	75	82	1
94–95	wild boar 3/pork 3 mixture	40	70	1
96–97	wild boar 3/pork 3 mixture	60	79	1
98–99	wild boar 4/pork 4 mixture	40	33	1
100–101	wild boar 4/pork 4 mixture	50	44	1
102–103	wild boar 4/pork 4 mixture	60	46	1
108	Australian wild boar	100	0	0
Commercial Samples				
110	80% wild boar salami	≥20	0	0
111	wild boar paté	≥20	0	0
112	wild boar paté	≥20	0	0
113	wild boar sausage	≥20	62	1
114	wild boar salami	≥20	0	0
115	wild boar paté	≥20	0	0
116	wild boar sausage and pork and venison casserole	≥20	0	0
117	21% wild boar, pork paté	21	25	1
118	diced wild boar, local butcher	100	0	0
119	diced wild boar, local butcher	100	0	0
120	Gloc. Old Spot pork	0	0	0
121	pork sausage, unknown breed	0	0	0
Blind Samples				
122	pork chop, unknown breed	0	0	0
123	wild boar ear tip, Forest of Dean	100	100	0
124	wild boar ear tip, Forest of Dean	100	100	0
125	wild boar ear tip, Forest of Dean	100	100	0
126	wild boar ear tip, Forest of Dean	100	100	0
127	Tamworth pork chop	0	0	0
128	wild boar ear tip, Forest of Dean	100	100	0
129	wild boar ear tip, Forest of Dean	100	100	0
130	wild boar ear tip, Forest of Dean	100	100	0
131	wild boar ear tip, Forest of Dean	100	100	0

[a]The % wild boar value is the proportion of the genome estimated to be represented in the wild boar reference group (% pig = 100 − % wild boar). P = probability of admixture. Sample numbering allows cross-referencing to data points in Figure 1.

Assignment Reference Groups. All reference samples were assigned to their respective subspecies (pig or wild boar) with zero admixture probability and 100% of alleles assigned to their own group. In constructing reference groups, it was difficult to obtain samples with known genetic histories; in particular, for wild boar samples, there was always the possibility of recent introgression of pig genotypes. However, the clear separation of the groups in Figure 1 was encouraging evidence that the reference samples were from distinct lineages of pig and wild boar. Initially, an Australian wild boar sample was to be included in the reference group, but it was clearly assigned as pig by BAPs and appeared intermediate in the PCO (Figure 1). This sample could be feral pig, pig highly introgressed with wild boar, or an Asian wild boar genotype, which was not encompassed in this study. It was therefore excluded from the reference group.

SSR Analysis of Mixtures, Commercial, and Blind Samples. Table 2 shows the results of SSR analysis and BAPs assignments for commercial and blind samples. For the blind samples, all whole meat pork cuts and wild boar ear samples were correctly assigned (samples 122–131) with 100% scores. For the commercial samples, Gloucestershire Old Spot chops and pork sausage were assigned as pig, as expected (samples 120 and 121).

Mixed meat samples (nos. 78–103, Table 2) were assigned by BAPs to their expected groups. All wild boar/pig w/w mixtures in the 20–80% range were assigned as mixtures with a probability of at least 0.98%. More marginal proportions were not analyzed so we do not know at what level the admixture assignment response occurs. The proportion of wild boar versus BAPs estimating % of wild boar alleles showed very good linearity for mixtures of two particular samples (wild boar 1 and pork 1, samples 78–89), R^2 = 0.9789 (Figure 2). However, when mixtures of other wild boar and pigs were analyzed, the correlation was not as good. Upon analysis of the allelic frequencies across the SSR panel for the pig versus the wild boar for each mixture, it was found that the proportion of alleles shared between the two animals inversely correlated with the accuracy of quantifying the proportion of wild boar in the mixture. For example, wild boar 3 and pig 3 were found to share many alleles, and the mean % wild boar calculated by BAPs was relatively inaccurate. However, wild boar 1 and pig 1 were found to have relatively few alleles in common, and, therefore, the BAPs calculated wild boar content of the mixture was relatively good. It was concluded, therefore, that this method would be unsuitable for the accurate quantification of wild boar meat in mixtures, but would, however, be able to identify wild boar in meat mixtures.

Table 3. List of 20 SSR Loci Analyzed for Discrimination of Pure Wild Boar and Pure Pig Breeds[a]

SSR locus	primer sequence (5′−3′)	5′ fluorescent label	source reference	no. of alleles	fragment sizes (bp)	H_o	PIC
S0026	F aaccttcccttcccaatcac R cacagactgctttttactcc	FAM	16,34	5	92−104	0.38	0.41
S0070	F ggcgagcatttcattcacag R gagcaaacagcatcgtgagc	VIC	14	13	263−294	0.73	0.84
S0090	F ccaagactgccttgtaggtgaata R gctatcaagtattgtaccattagg	VIC	14,34	11	240−294	0.70	0.74
S0097	F gacctatctaatgtcattatagt R ttcctcctagagttgacaaactt	FAM	14,34	12	205−241	0.76	0.84
S0155	F tgttctctgtttctcctctgtttg R aaagtggaaagagtcaatggctat	NED	18,34	7	145−163	0.51	0.64
S0228	F ggcataggctggcagcaaca R agcccacctcatcttatctacact	VIC	20,34	10	216−239	0.59	0.70
S0355	F tctggctcctacactccttcttgatg R ttgggtgggtgctgaaaaatagga	FAM	20,34	6	242−270	0.20	0.35
SW24	F ctttgggtggagtgtgtgc R atccaaatgctgcaagcg	VIC	14,34	10	96−120	0.85	0.83
SW122	F ttgtctttttattttgcttttgg R caaaaaaggcaaaagattgaca	NED	14,34	11	113−136	0.54	0.66
SW632	F tgggttgaaagatttcccaa R ggagtcagtactttggcttga	VIC	14,34	9	159−178	0.72	0.76
SW787	F ctggagcaggagaaagtaagttc R ggacagttacagacagaagaagg	NED	14	10	138−166	0.68	0.74
SW857	F tgagaggtcagttacagaagacc R gatcctcctccaaatcccat	FAM	14,34	8	140−160	0.54	0.70
SW911	F ctcagttctttgggactgaacc R catctgtggaaaaaaaaagcc	FAM	14,34	7	154−168	0.71	0.75
SW936	F tctggagctagcataagtgcc R gtgcaagtacacatgcaggg	FAM	14,34	10	91−113	0.82	0.78
SW951	F tttcacaactctggcaccag R gatcgtgcccaaatggac	VIC	14	8	120−139	0.35	0.45
SW1067	F tgctggccagtgactctg R ccgggggattaaacaaaaag	NED	14,34	11	159−177	0.69	0.73
SW1111	F aggtcctactgtccatcacagg R gaagcagagttggcttacagtg	VIC	14	9	166−187	0.49	0.63
SW1828	F aatgcattgtcttcattcaacc R ttaaccggggcacttgtg	FAM	15,34	12	78−109	0.57	0.73
SW2008	F caggccagagtagcgtgc R cagtcctcccaaaaataacatg	FAM	15,34	10	89−120	0.64	0.81
SW2410	F atttgcccccaaggtatttc R cagggtgtggagggtagaag	FAM	15,34	9	102−128	0.55	0.45

[a]Data for the number of alleles and fragment sizes obtained, observed heterozygosity values (H_o), and average polymorphism information content (PIC)[38] are also shown.

All but two mixed pork/wild boar food product samples (110−119) were assigned as 100% pig in origin: sample 113, "wild boar sausage" was assigned as a mixture ($P = 1$) with 62% wild boar; and sample 117, "21% wild boar and pork paté", was also assigned as a mixture ($P = 1$) with 21% wild boar. Under EU regulations, mixed meat products reporting to be "wild boar" can contain as little as 20% wild boar and, therefore, in these analyses would be classified as "mixture". Given these rules, there were several anomalies in the commercial samples tested; samples 110−112 and 114−116 should at least have been categorized as wild boar/pork mixture, but no significant mixture was detected ($P = 0$). It is likely therefore that these products contained either less than 20% wild boar, or wild boar meat which contained significantly less amplifiable DNA than the pork component. In addition, two samples of diced meat from a local butcher (samples 118 and 119), purporting to be wild boar, were assigned in this analysis as pig. It is not clear if this was due to misrepresentation of the product or livestock. Given the above results of the analysis of known mixtures, it is likely that these "wild boar" diced meat samples were from a pig with less than 20% wild boar ancestry. In addition to wild boar/pig mixtures, two mixtures of different breeds of pig (Saddleback/GOS and Ayreshire/large white) and of two different individual wild boar were analyzed, and both were assigned correctly to 100% pig and 100% wild boar, respectively.

The effectiveness of SSR methods for this type of analysis relies on the choice of loci used and the collection of data sets comprising the allele frequencies of all possible breeds that may be encountered to act as reference populations, weighted by the population size so that the probability that an animal assigns to one of the breeds can be determined accurately. Collection of adequate sample numbers, precluding closely related individuals, and good quality data are essential for building the reference population data set. The main drawback to the

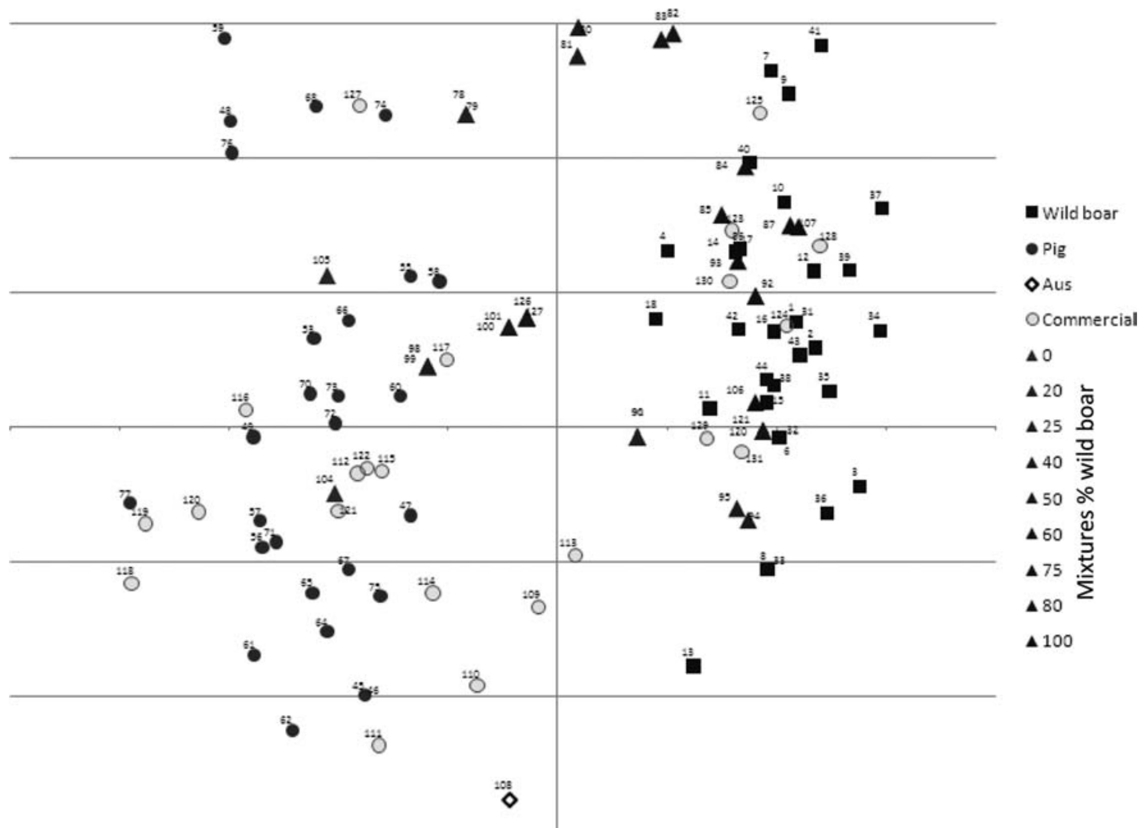

Figure 1. Principal coordinates plot (coordinates 1 and 2 = 35.86% of total variation) of pairwise simple matching distances of SSR data for pig and wild boar reference, w/w pig/wild boar mixtures, commercial meat samples, and Australian wild boar (Aus). Sample identity numbers correlate with Tables 1 and 2.

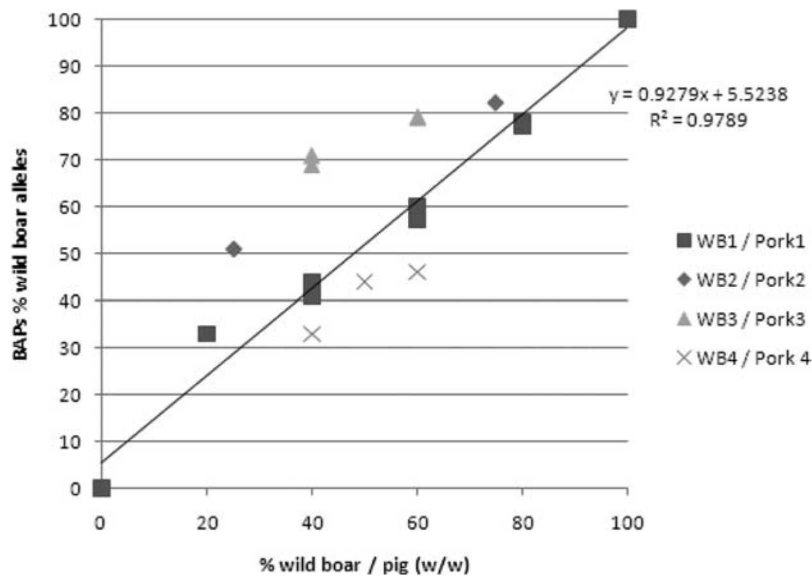

Figure 2. Plot of BAPs % wild boar alleles versus % wild boar/pork mixture. The linear regression for WB1/Pork1 mixtures is shown.

use of the above methodology is the necessity to use statistical tools that require a high degree of knowledge and familiarity, so that use in routine tests is difficult. This can be ameliorated to a certain extent, by the use of software available free on the Internet that is user-friendly, as used in this Article (BAPs). However, the analysis is still complex and cannot be achieved in a single, simple step, especially for cross-breeds, where classical assignment/exclusion tests using pure breeds as reference populations are inaccurate. This is illustrated by Garcia et al.[33] where the breed composition of Iberian ham was determined by the use of multilocus genotypes. Although the percentage of each breed in the genome of each sample could be determined, the statistical procedures were complex ranging from "unsupervised" methods (e.g., the software program Structure where no defined populations are used to input into the model) to

"supervised" methods (e.g., GeneClass2,[35] utilizing reference populations) to confirm the initial analysis.

Another potential problem associated with mixtures of wild boar and pork found in food products is that the two meats may not be equivalent with respect to DNA content or quality. Differences in the meat cut, water content, fat content, pre-processing, and storage conditions/freshness may all affect differentiation of wild boar in food samples. However, within predefined thresholds, for example, 20%, the detection of wild boar meat should be possible with SSR methods, provided the meats have received similar treatment.

Although the pig genome has been sequenced, leading to the availability of over 1200 SSR markers, there is only partial sequence data for the wild boar genome. Therefore, it is difficult to pinpoint differences between the two subspecies, which would lead to fixed discriminatory markers. Future work could concentrate on highly polymorphic regions such as the ribosomal ITS (intergenic transcribed spacer). There is a high degree of variation between closely related species in the ITS regions, due to their nonfunctional status, and thus low evolutionary pressure. This leads to comparison of the ITS regions being commonly used in taxonomy and molecular phylogeny of closely related taxa. However, it should be noted that SSR are neutral genetic markers. Their frequency will reflect the evolutionary history of individuals or populations, including introgressions and hybridizations between groups that phenotypically may appear very different. Where a few traits, such as meat taste and texture in the present case, are the primary concern in defining the authenticity of a meat product, then the selected polymorphisms themselves may be the preferred target. However, this would require considerable research effort to locate the genes in question, which has not yet been applied to pigs and wild boar.

A new technology, using SNP panels, has shown promising results in the identification and discrimination of pig breeds and wild boar.[36,37] Ramos et al.[36] utilized high-throughput sequencing to identify SNPs specific to 4 breeds of pig and wild boar. From an initial putative 29 416 breed-specific SNPs, 87 were found to be truly breed-specific following analysis of a large number of samples from each breed. As the costs of this emerging technology decrease in the future, SNP panels will be a cheaper approach to breed identification than SSR analysis. An SNP chip from illumina is already commercially available (PorcineSNP60 BeadChip), which features more than 62 000 SNPs spanning the porcine genome. However, as with micro-satellite analysis, analysis of the data generated using SNPs also requires a certain level of statistical knowledge. In addition, SNPs can only be used to discriminate between pure-bred animals and not cross-breeds, due to their biallelic nature. Thus, their utility in food samples, which are potentially mixed, remains to be demonstrated. Therefore, microsatellites may be more suitable for differentiation of cross-bred animals, or for predicting an animal's percentage makeup of different breeds.[37]

In summary, SSR are capable of distinguishing pure wild boar meat from pure pig breeds using 20 SSR markers, with a 100% probability according to the BAPs program. Mixtures containing 20% or greater wild boar mixed with pig (or vice versa) can be determined as mixtures, although the absolute relative quantities of wild boar and pig in these mixtures cannot accurately be determined. The method is suitable for the analysis of cuts of meat, and it can indicate the presence of wild boar and pork in processed products such as sausages, salamis, and patés.

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Notes

The authors declare no competing financial interest.

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