

# Genetic Traceability of Cinta Senese Pig (*Sus scrofa domesticus* L.): A Study of the Meat and Processed Products by Microsatellite Markers

Monica Scali<sup>1</sup>, Rita Vignani<sup>2</sup>, Marco Quintetti<sup>1</sup>, Jacopo Bigliuzzi<sup>1</sup>, Elisa Paolucci<sup>2</sup>, Mauro Cresti<sup>1</sup>

<sup>1</sup>Department of Life Science, University of Siena, Siena, Italy

<sup>2</sup>Serge-Genomics S.r.L., Siena, Italy

Email: [monica.scali@unisi.it](mailto:monica.scali@unisi.it)

Received 9 January 2015; accepted 21 May 2015; published 26 May 2015

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## Abstract

Traceability based on DNA analysis is attracting increasing interest due to the crisis of confidence that consumers show towards the products of animal origin. The present work discusses a genetic traceability system of meat and processed products from an historical Tuscan native pig breed, the Cinta Senese. The study is based on a panel of 8 ISAG (International Society for Animal Genetics) DNA microsatellite markers usage done both on pigs and derived products. The SSRs panel allowed us to obtain a unique fingerprint of the individuals to be used as a tracer “downstream” in the processed products. The molecular method used proved that the hams, analyzed just before commercialization, were obtained from Cinta Senese pigs and that the analyzed meat products derived from the Cinta Senese were produced at least with 95% of Cinta Senese meat. In perspective, the molecular testing could be introduced as a voluntarily adopted method for proving intrinsic quality of many regional food products.

## Keywords

*Sus scrofa domesticus* L., Genotyping, SSRs, Meat Molecular Traceability

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## 1. Introduction

The Cinta (meaning belt) Senese (of Siena)—(CS) is an autochthonous pig (*Sus scrofa domesticus* L.) breed

**How to cite this paper:** Scali, M., Vignani, R., Quintetti, M., Bigliuzzi, J., Paolucci, E. and Cresti, M. (2015) Genetic Traceability of Cinta Senese Pig (*Sus scrofa domesticus* L.): A Study of the Meat and Processed Products by Microsatellite Markers. *Food and Nutrition Sciences*, 6, 712-726. <http://dx.doi.org/10.4236/fns.2015.68074>

widespread in Tuscany, that had almost been lost during the '60s. Originating in Tuscany from around the areas of Siena, it is believed that this ancient hardy breed is more than likely bred in Roman times, although the earliest acknowledgements found are dating from the Middle Ages. Within Siena's Town Hall, an historical image of the CS can be found in painting entitled "Effects of Good Government in the Country" by artist Ambrogio Lorenzetti (1338). Free-range pig farming is of great significance, both for animal health and for low-impacting farming to the environment. Up and into the 1950's many people owned one or more CS for their meat, but a period of decline of the CS was attributed to a massive cross breeding with white pig breeds, which needed to be kept for a much shorter period than the CS before butchering. Among those, Large White (LW), that was introduced to Italy from the UK and Landrace (L) that was introduced mainly from several Scandinavian Countries.

However, the white breeds could not adapt to the same wild or semi-wild farming and terrain that the CS was so familiar with. This led to a successful crossbreeding between the CS and the white breeds that allowed avoiding extinction of the CS breed due to an excessive inbreeding. On the other hand, the interbreeding crossings arouse doubts concerning the genetic identity of the original CS breed.

In the year 2000, a voluntary Consortium was established in Siena, today known as "Consorzio di Tutela della Cinta Senese" (Consortium for the Defence of Cinta Senese). Products are given a tag confirming their origin, that is, a Protected Designation of Origin (PDO), which means the animal from which the produce is derived was born raised and slaughtered in Tuscany.

Several CS products (Salami, lard, pancetta, bacon and other cured meats, sausages, capocollo, and shoulder) can be purchased from delicatessens within Tuscany's provinces of Siena, Grosseto and Florence and that can be considered a typical Italian Regional food that characterizes the Mediterranean diet. The authentic products will have a label "Cinta Senese D.O.P" attached, but at the moment no molecular testing has been associated to this label with the exception of research studies, including the present. The CS meat is rich of high quality nutraceuticals, such as a favorable unsaturated/saturated fatty acids ratio, a high content in branched aminoacids and a quite low atherogenic and thrombogenic indexes [1].

The rediscovery of the CS pig is due mainly to the increasing demand for typical Italian Regional quality food by consumers. These pigs are characterized, in fact, for rich and tasty meat, whose characteristics are appreciated for processing into traditional salami.

Traceability of food products has become an important field of study for proving the intrinsic quality of typical food and is fundamental to ensure that consumers are supplied with genuine products. DNA testing of food proves its composition and maintains a high level of consumer confidence in relation to food quality and safety [2] [3].

There are several analytical methods available to the scientific community to assess the composition and geographical origin of the main ingredients of food. Many different techniques have been increasingly introduced in the framework of documental traceability programmes in compliance with National and International regulations, or by producers that voluntarily wish to ensure their products have an extra guarantee of quality.

Traditionally, the species or variety identification of food ingredients can be performed through the chemical analysis of proteins or secondary metabolites with NIR spectroscopy or mass spectrometry. Chemical-based methods are currently also used to obtain information on the safety requirements in regard to undesired chemical molecules, such as pesticides or other synthesis molecules that may have been used during growth or storage of the ingredients ([4] [5]) or for studying the nutraceutical content [6]. Examples of such studies have recently improved the content of international publications databases and include different case studies concerning different food categories. Among others, there are studies on livestock products and meat [7]-[9], wine and spirits [10]-[13], several plant species like cocoa beans, chicory, wheat and garlic [14]-[18], olive oil [19]-[26], on eggs [27].

The presence of DNA traces in different food matrixes, together with the extraordinary chemical stability of this molecule, renders DNA an ideal candidate for tracing the composition and origin of food. Molecular traceability is nowadays becoming a common methodology for different areas of food industry.

Molecular traceability methods have been boosted by GMO traceability law requirements, especially in European Countries where GMO planting and use is strictly regulated by law or even forbidden in particular food categories, like food for babies [28]. Examples of GMO tracking that concerns the food industry include corn [29], and soybean [30].

DNA tracing is used for proving the genuinity of milk and cheese products [31], meat [32]-[37], fish-

containing derivatives [38] [39], olives [40], olive oil [41]-[47], and others [48]. The detection and reading of DNA residues, even in conditions of highly degraded traces of DNA, is made possible through the use of a classical molecular technique, the Polymerase Chain Reaction (PCR) both in the classical and more recent version, the Real-Time PCR (RT-PCR). While the classical PCR provides a qualitative assay, the RT-PCR, which is commonly used also in medicine as a diagnostic tool, can estimate the quantity of DNA initially present in a sample. Both could be considered the key techniques for nucleic acid amplification. PCR is in fact also widely used in forensic science for the genotyping of LCN (Low Copy Number) DNA traces found in crime or disaster scenes [49].

Thanks to next-generation sequencing technologies, there is a wide range of choice of DNA molecular markers that are currently available to the scientific community for different focused studies, such as genetic population analysis, for monitoring genetic identity and variability in a natural population, for parentage testing or for tracing meat through the entire food chain.

SNPs markers are considered the newest type of molecular markers that are supposed to offer great opportunities in mammals since they are abundant in the genome, are genetically stable and can be detected in a high throughput system [50]. Unfortunately, allele frequencies for SNP markers in Cinta Senese pig populations are not available and their use for Cinta Senese authentication has never been explored.

The most commonly used molecular markers for genetic identification and traceability in forensic and animal/crop sciences are the SSRs or microsatellite [51]-[53]. These molecular markers are highly polymorphic, informative and interspersed throughout the entire genome and for their characteristics have been proposed for tracking food, including meat and wine [54].

Recent works showed as SSR markers are suitable for distinguish genotypically the Cinta Senese breed from the main white breeds spread in Italy and that the use of a general, neutral SSRs genotyping approach, seems appropriate in determining the pig breed identity [53]. Therefore, the aim of this work was to verify the utility of the SSR markers for the implementation of a molecular traceability system through the whole Cinta Senese production chain, from meat to processed products.

## 2. Materials and Methods

### 2.1. Sampling and Genomic DNA Extraction

The meats coming from six different CS pigs post-mortem were numbered consecutively (from CSmeat1 or CScarne1 to CSmeat6 or CScarne6) and used for the extraction of genomic DNA by the GenElute Mammalian Genomic DNA Kit (Sigma-Aldrich, MO, USA). All the samples were analyzed in triplicate.

The same commercial kit was used for the extraction of genomic DNA from two CS salami, each analyzed in triplicate. In particular, 250 mg of salami meat, including fat, were homogenized using the Tissue Lyser Disruption System (Qiagen) and 25 mg of the homogenized meat were used for DNA extraction.

Likewise, the GenElute Mammalian Genomic DNA Kit was used to extract genomic DNA from each of the following samples always analyzed in triplicate: fresh CS ham belonging to a butchery batch that included, among others, the six individuals used for the meat analysis (Ham1 or Prosciutto1); pigskin coming from the same fresh CS ham (Pigskin1 or Cotenna1); aged CS ham not belonging to the butchery batch of the six individuals used for the meat analysis (Ham2 or Prosciutto2).

All the DNAs were analyzed on agarose gel and quantified by biophotometer (Eppendorf) using the ratio 260/280 nm to assess the purity of DNA and the ratio 260/230 to exclude the presence of contaminants which absorb at 230 nm. The amount of DNA extracted from all kinds of samples varies from 15 to 25 µg.

### 2.2. SSRs Genotyping

The genomic DNA of all samples (meats, salami and hams) were amplified at 8 SSRs loci (S0026, SW240, S0068, SW122, SW632, SW1828, SW1067, S0226) (**Table 1**) recommended by ISAG/FAO and selected as most suitable for genetic identification of CS breed according to polymorphic information and allele frequency reported by [53].

All PCR reactions were carried out in an Eppendorf Mastercycler Gradient PCR in a total volume of 12.5 µl, containing 10 ng of genomic DNA, 0.25 mM dNTPs (Promega), 0.25 µM each primer (one of them being fluorescein labeled), 1X Green GoTaq<sup>®</sup> Reaction Buffer containing 1.5 mM MgCl<sub>2</sub> (Promega), 0.1 U Go Taq<sup>®</sup>

**Table 1.** Microsatellite primer sequences.

Locus	Forward Primer	Reverse Primer
S0026	AACCTTCCCTTCCAATCAC	CACAGACTGCTTTTACTCC
SW240	AGAAATTAGTGCTCAAATTGG	AAACCATTAAGTCCTAGCAAA
S0068	AGTGGTCTCTCCCTCTTGCT	CCTTCAACCTTTGAGCAAGAAC
SW122	TGTCTTTTTATTTTGCTTTTGG	CAAAAAAGGCCAAAAGATTGACA
SW632	TGGGTTGAAAGATTTCCCAA	GGAGTCAGTACTTTGGCTTGA
SW1828	AATGCATTGTCTTCATTCAACC	TTAACCGGGGGCACTTGTG
SW1067	TGCTGGCCAGTGACTCTG	CCGGGGGATTAACAAAAAAG
S0226	GGTAAACTTTTNCCTCAATACA	GCACCTTTAACTTTCATGATGCTCC

DNA Polymerase (Promega). PCR conditions included an initial denaturation step of 5' at 95°C, 39 cycles of 30' at 95°C, 30' at 55°C, 1' at 72°C and a final extension of 10' at 72°C.

The PCR products were separated on 2% agarose gel stained with ethidium bromide to identify possible imperfections and to decrease the rate of failure in capillary electrophoresis.

2 µl of PCR product and 12.5 µl of an internal size standards (Et-Rox-400, GE) were denatured at 95°C for 2' and kept on ice. The allele sizing was done by capillary electrophoresis, based on laser scanning of fluorescence-marked DNA fragments. Genotyping was done on MegaBACE 500 DNA Analysis System and evaluated by software Fragment Profiler version 1.2 (GE Healthcare, USA). In **Figure 1** two typical electropherogram images are shown.

### 2.3. Dendrogram of Similarity

The similarity dendrograms have been obtained using NTSyS 2.1 software, the clustering algorithm is SAHN and among the different options, UPGMA was selected as hierarchical method for the tree construction.

## 3. Results

### 3.1. CS Meat Genotyping

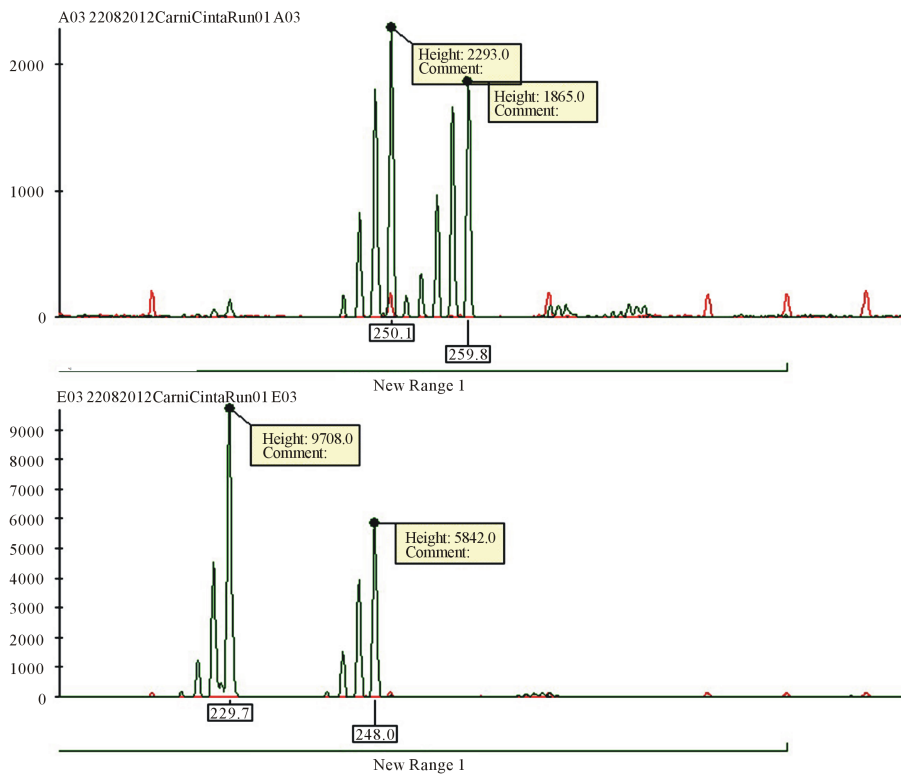
The present study focused to the development of a genetic traceability plan along the CS supply chain, from meat to the processed products. The tracking is based on the comparison between the DNA profiles of six CS meat samples, coming from different CS individuals, with the DNA profiles obtained from the processed products. The SSRs markers are frequently used in breeding program for their discrimination power. The effectiveness of a SSRs panel is usually tested by the probability of identity (PI) that is the probability of finding, by chance, two individuals with the same profile. Therefore, to ensure that each individual is univocally characterized it is essential to use a set of sufficiently polymorphic loci.

The meats coming from six different CS pigs post-mortem were genotyped at 8 SSRs loci (S0026, SW240, S0068, SW122, SW632, SW1828, SW1067, S0226) [53]. The allele sizes characterizing the genotype of each CS pig post-mortem were used for the production of a dendrogram of similarity by NTSYS 2.0, including the different pig breeds belonging to our private databank listed in **Table 2** [53].

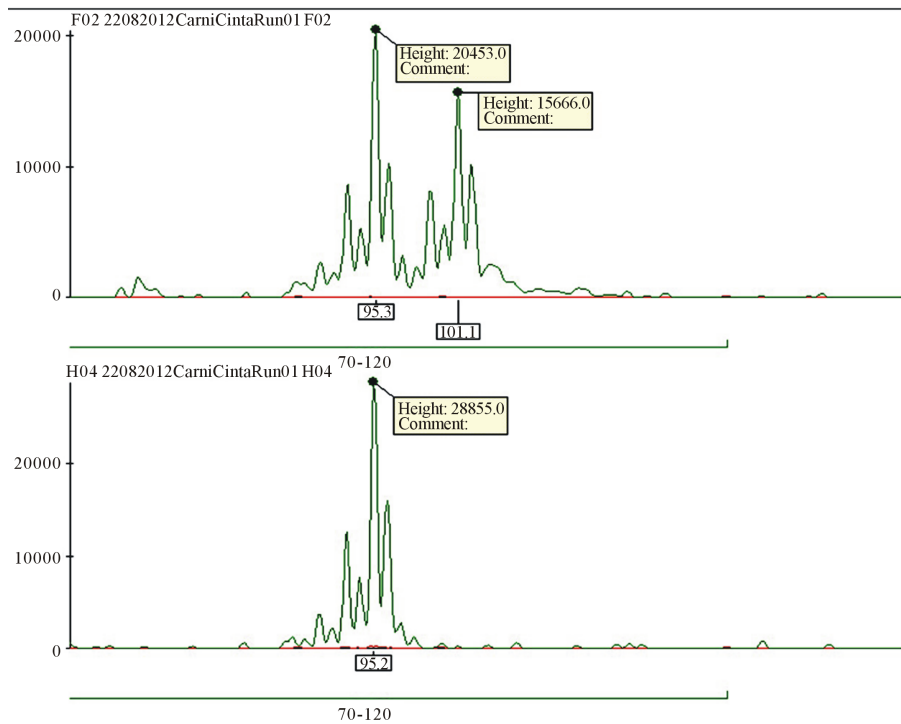
The analysis of the dendrogram of similarity reveals that the six CS meat samples share the “consensus genotype” typical of the CS pig breed and differ from other breeds and crossbreeds included as controls (**Figure 2**), verifying their belonging to CS breed.

### 3.2. Sensitivity Test of White Pig Meat in Blended CS-White Pig Meat Samples

For this aim, two white pig meat samples (White pig meat 1 and White pig meat 2), coming from two different white pigs (post-mortem) which had no relationship with the CS breed, were genotyped at the 8 SSRs loci previously listed and the allele sizes were compared with the allele sizes of the six CS meat samples above described (data not shown). The comparison reveals that the CS meat 1 shows the highest number of discriminating alleles respect to the two white meat samples (**Table 3**) and therefore it was identified as the most suitable to



(a)



(b)

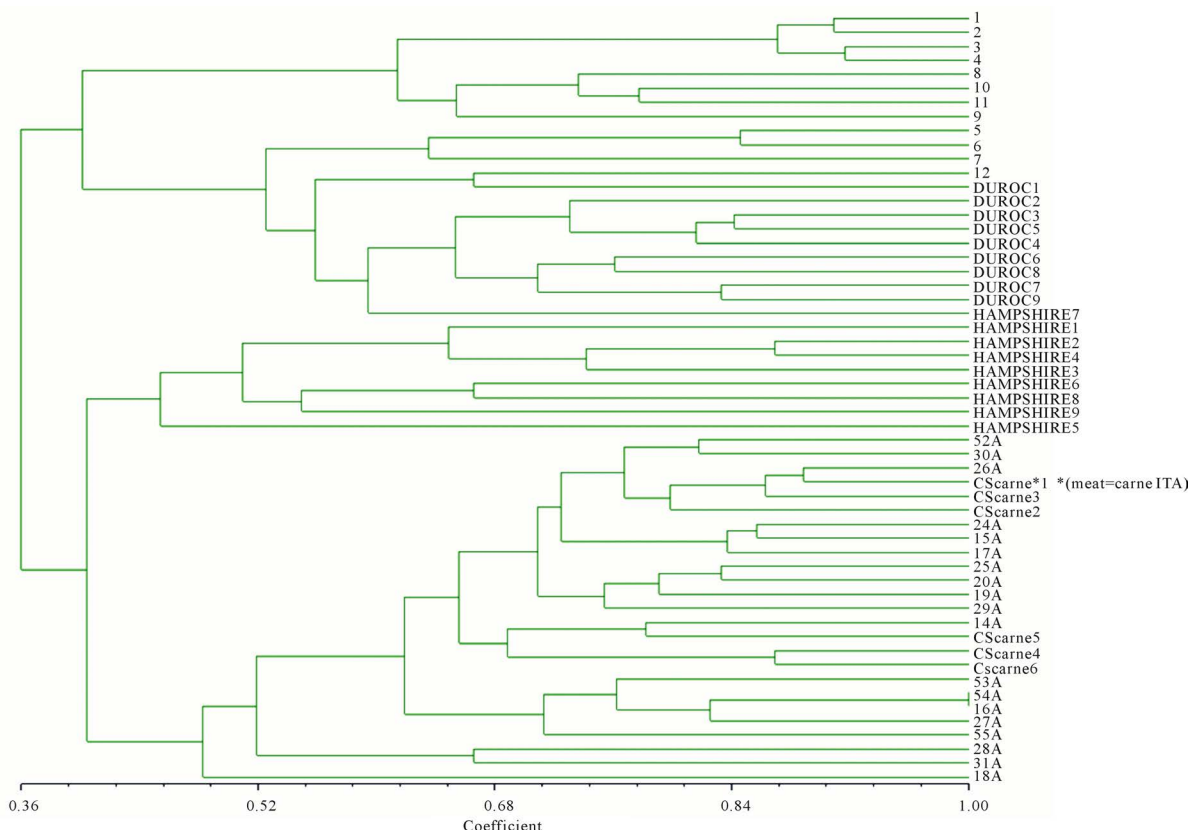
**Figure 1.** Typical electropherogram images. CS meat DNA amplification at S0068 locus (upper line, (a)) compared to the white pig meat profile (lower line, (a)). White pig meat DNA amplified at S0026 locus (upper line, (b)) compared to the CS meat profile (lower line, (b)).

**Table 2.** List of pig breeds analyzed.

Breed	Reference number
	25A
	20A
	19A
	29A
	14A
	53A
Cinta Senese	54A
	16A
	27A
	55A
	28A
	31A
	18A
	1
Landrace	2
	3
	4
	5
Large White	6
	7
	8
	9
Large White × Landrace	10
	11
Landrace × Cinta Senese	12
	Duroc1
	Duroc2
	Duroc3
	Duroc4
Duroc	Duroc5
	Duroc6
	Duroc7
	Duroc8
	Duroc9
	Hampshire1
	Hampshire2
	Hampshire3
Hampshire	Hampshire4
	Hampshire5
	Hampshire6
	Hampshire7

produce blended samples (CS-White pig meat samples) to be analysed for the lower limit of detection of white meat.

**Table 3** shows the White pig meat 1 and 2, for this set of samples, have discriminating alleles in 7 out of 8 loci. In particular, 4 SSRs loci (S0026, S0068, SW1067, S0226) have alleles that differ by more than 7 bases and therefore more easily identifiable.



**Figure 2.** Dendrogram of genetic similarity obtained by the “unweighted pair-group method with arithmetic mean (UPGMA).” The six CS meat samples (from Cscarne 1 to Cscarne 6) analyzed show a genotypic profile corresponding to the Cinta Senese breed, differing from other pig breeds listed in **Table 2**.

**Table 3.** Meat genotyping. Comparison of the allele sizes of the White pig meat 1 and 2 and the CS meat 1. The discriminant alleles shown in bold in the last right column, are univocally associated with the meat coming from the 2 different white pigs analyzed.

	White pig meat 1	White pig meat 2	CS meat 1	Discriminant alleles
S0026a	102	95	95	
S0026b	102	95	95	<b>102</b>
SW240a	97	97	97	
SW240b	97	97	108	
S0068a	230	230	250	
S0068b	256	250	260	<b>230/256</b>
SW122a	114	125	121	
SW122b	118	125	130	<b>114/118/125</b>
SW632a	160	162	162	
SW632b	173	162	169	<b>160/173</b>
SW1828a	94	94	96	
SW1828b	98	98	100	<b>94/98</b>
SW1067a	160	160	168	
SW1067b	160	170	170	<b>160</b>
S0226a	180	192	178	
S0226b	198	198	178	<b>180/192/198</b>

Therefore, White pig meat 1 and White pig meat 2 were mixed separately with the CS meat 1 according to the percentages shown in **Table 4** and the blended samples obtained were genotyped at the 4 SSRs loci above mentioned in order to establish the lower limit detection of the white pig meat.

The analyses reveals that the alleles of the white meat are not detectable in cuts under than 5%, both in the blended produced with the White pig meat 1 that in the blended produced with the White pig meat 2 (**Table 5**). The lower limit of detection up to 5% recorded in this study refers to an analysis carried out by qualitative PCR.

### 3.3. CS Salami Genotyping

Two CS salami were genotyped at the 8 SSRs loci used for the six CS meat samples. By comparing the results of the salami analysis with those deriving from the analysis of CS meat, it was possible to highlight a perfect match of the allele sizes in all loci analyzed with the exception of the locus S0068 that in addition to alleles 230, 250 and 260 shows, in both salami, a 240 bp allele (**Table 6**). Comparing this result with our private databank it has been shown that the 240 bp allele is recurring for the S0068 locus in the CS breed genotype and does not occur in crossbreeds (Landrace  $\times$  Large White and Landrace  $\times$  Cinta Senese) [53] and in the other pig breeds genotyped (Landrace, Large White, Duroc and Hampshire). The results allow us to affirm that the two salami analyzed were probably produced using not only the meat coming from the six CS pig genotyped in this project, but also meat coming from other CS pigs belonging to the same butchery batch of the six CS pigs used for the meat

**Table 4.** Sensitivity test of White pig meat in blended CS-White pig meat samples.

Sample number	CS meat 1	White pig meat 1
1	99%	1%
2	97.5%	2.5%
3	95%	5%
4	90%	10%
5	85%	15%
6	80%	20%
7	75%	25%
8	70%	30%
9	65%	35%
10	60%	40%
11	55%	45%
12	50%	50%
Sample number	CS meat 1	White pig meat 2
13	99%	1%
14	97.5%	2.5%
15	95%	5%
16	90%	10%
17	85%	15%
18	80%	20%
19	75%	25%
20	70%	30%
21	65%	35%
22	60%	40%
23	55%	45%
24	50%	50%



**Table 5.** Lower limit detection of White pig meat in blended CS-White pig meat samples. The allele sizes in bold are the white meat alleles identified in the blended meat analyzed. The alleles of the white meat are not detectable in cuts under than 5%. In the CS-White1 pig meat samples, at the S0068 locus (see \* in Table 5a), the allele 256 typical of the white meat in this study, hasn't picked out, probably due to the "allele drop out" (PCR loss of one allele) phenomenon which generally occurs when the quantity of DNA is relatively low, either because the sample is limited or because the DNA it contains is degraded, and hence the test is near its threshold of sensitivity.

a—CS meat 1 - White pig meat 1												
	1	2	3	4	5	6	7	8	9	10	11	12
%	99% - 1%	97.5% - 2.5%	95% - 5%	90% - 10%	85% - 15%	80% - 20%	75% - 25%	70% - 30%	65% - 35%	60% - 40%	55% - 45%	50% - 50%
S0026		95	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>	95- <b>102</b>
S0068*	250-260	250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260
SW1067	168-170	168-170	168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170
SW226	178	178	178	178	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198	178- <b>180</b> -198
b—CS meat 1 - White pig meat 2												
	13	14	15	16	17	18	19	20	21	22	23	24
%	99% - 1%	97.5% - 2.5%	95% - 5%	90% - 10%	85% - 15%	80% - 20%	75% - 25%	70% - 30%	65% - 35%	60% - 40%	55% - 45%	50% - 50%
S0026		95	95	95	95	95	95	95	95	95	95	95
S0068	250-260	250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260	<b>230</b> -250-260
SW1067	168-170	168-170	<b>160</b> -168-170	<b>160</b> -168-170	168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170	<b>160</b> -168-170
SW226	178	178	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198	178- <b>192</b> -198

analysis. Also, the results show that in the two CS salami is not present meat coming from other pig breeds, different from Cinta Senese, and present in our databank.

### 3.4. CS Ham Genotyping

Ham1 (or Prosciutto1), Pigskin1 (or Cotenna1), Ham2 (or Prosciutto2) were genotyped at the 8 SSRs loci previously listed. The kit used for the DNA extraction was effective also for the DNA extraction from the pigskin, allowing analyzing ham that is undergoing maturation without damage or wastage for producers. The allele sizes characterizing the hams and the pigskin (Table 7) were used to produce a dendrogram of similarity including the six CS meat samples and the different pig breeds belonging to our private databank listed in Table 2 (Figure 3).

The analysis of the dendrogram of similarity reveals that the two CS hams share the genotype typical of the CS pig breed although they don't match none of the six CS pig meat samples suggesting that they belong to different pigs. This was easily predictable for Ham2, which was an aged CS ham not belonging to the butchery batch of the six CS pigs used for the meat analysis. While Ham1, since it doesn't match with anyone of the six CS meat analyzed, it's coming probably from a different CS pig belonging to the butchery batch of the six pigs used for the meat analysis.

Ham1 and Pigskin1 are exactly alike being different parts of the same sample.

## 4. Discussion

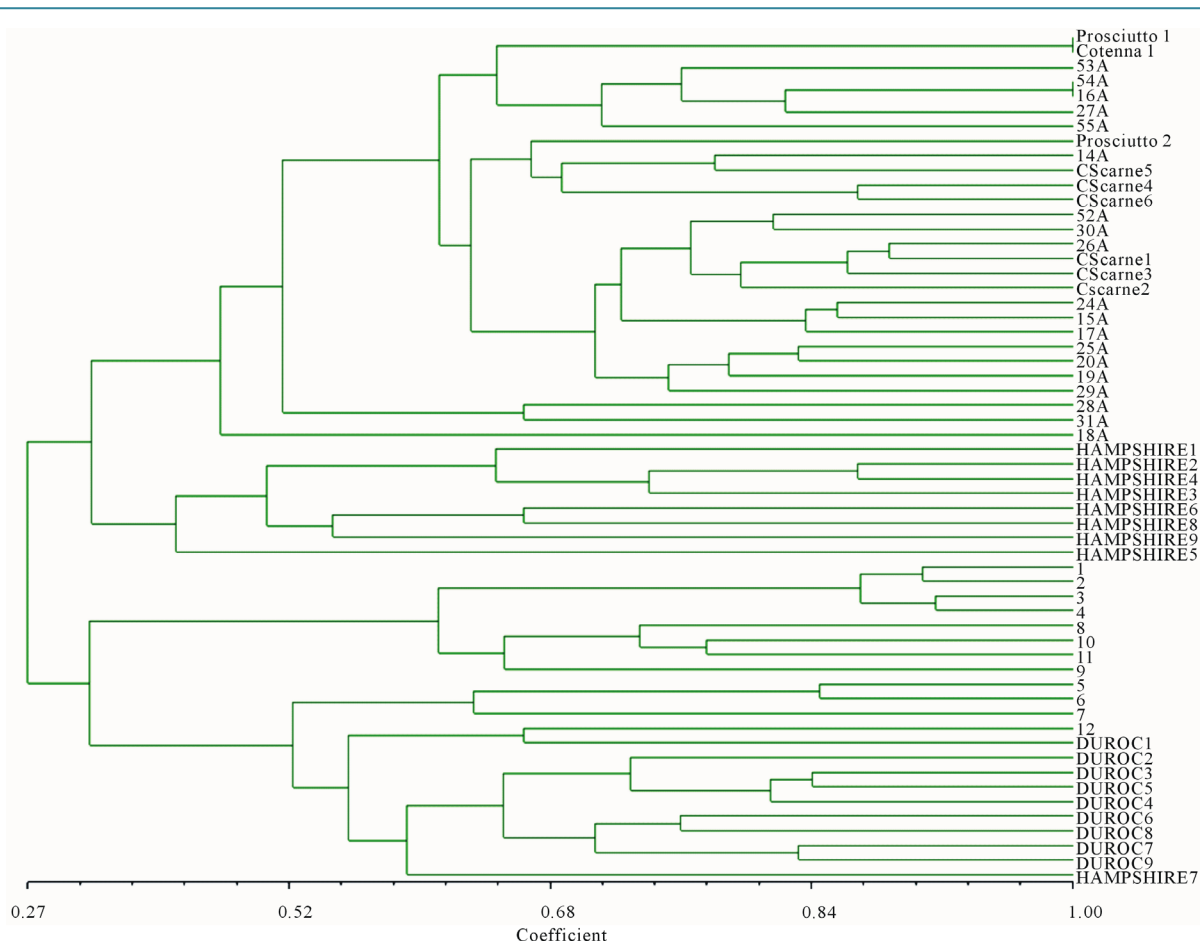
Transparency about the origin of animal products is now considered an important component of quality and safety, as perceived by consumers. It represents an important element for the competitiveness and development of local products in the national and international markets as well as for their defense against imitations and counterfeits that could harm the entire production chain. A variety of options are available for pig meat traceability, from the traditional identification method based on ear tags and tattoos, to the electronic identification and

**Table 6.** Allele sizes from the CS salami compared to the allele sizes of the six CS meat samples. The analysis reveals a perfect match of the allele sizes in all loci analyzed with the exception of the locus S0068 that shows, in both salami, a 240 bp allele.

	CSmeat 1	CSmeat 2	CSmeat 3	CSmeat 4	CSmeat 5	CSmeat 6	Salami 1	Salami 2
S0026a	95	95	95	95	95	102	95	95
S0026b	95	95	95	102	102	102	102	102
SW240a	97	97	93	97	97	97	97	97
SW240b	108	97	97	97	108	108	108	108
S0068a	250	250	250	230	230	230	230 - 240	230 - 240
S0068b	260	260	260	250	260	250	250 - 260	250 - 260
SW122a	121	114	121	114	121	114	114	114
SW122b	130	121	130	121	121	121	130 - 121	130 - 121
SW632a	162	162	162	162	162	169	162	162
SW632b	169	169	162	169	169	169	169	169
SW1828a	96	96	96	96	96	96	96	96
SW1828b	100	100	96	96	102	96	102	102
SW1067a	168	168	168	168	168	168	168	168
SW1067b	170	168	170	168	168	168	170	170
S0226a	178	178	178	178	178	178	178	178
S0226b	178	178	178	178	178	178	178	178

**Table 7.** Allele sizes of the CS pigskin and hams. Ham1 and Ham2 show four different alleles which however are recurring alleles in the CS breed genotype.

SSR locus	CSmeat 1	CSmeat 2	CSmeat 3	CSmeat 4	CSmeat 5	CSmeat 6	Ham1 (or Prosciutto1)	Pigskin1 (or Cotenna1)
S0026a	95	95	95	95	95	102	95	95
S0026b	95	95	95	102	102	102	102	102
SW240a	97	97	93	97	97	97	97	97
SW240b	108	97	97	97	108	108	110	110
S0068a	250	250	250	230	230	230	250	250
S0068b	260	260	260	250	260	250	250	250
SW122a	121	114	121	114	121	114	121	121
SW122b	130	121	130	121	121	121	130	130
SW632a	162	162	162	162	162	169	169	169
SW632b	169	169	162	169	169	169	169	169
SW1828a	96	96	96	96	96	96	98	98
SW1828b	100	100	96	96	102	96	111	111
SW1067a	168	168	168	168	168	168	168	168
SW1067b	170	168	170	168	168	168	170	170
S0226a	178	178	178	178	178	178	178	178
S0226b	178	178	178	178	178	178	178	178



**Figure 3.** Dendrogram of genetic similarity. The hams analyzed (Prosciutto 1 and Prosciutto 2) show a genotypic profile corresponding to the CS breed detaching from other pig breeds listed in **Table 1**.

more recently, to DNA analysis. The use of DNA fingerprinting is designed as one of the more advantageous methods to track the product through the entire production chain. Indeed, each animal is genetically unique and the animal’s own DNA fingerprinting can be used to track back the origin of pig meat and derived products. In the present study, we developed a molecular traceability method, based on short DNA sequence polymorphisms analysis, to be applied along the production chain of a prestigious Tuscan pig breed, the CS, using a panel of 8 SSRs markers. It has also been shown that the genotyping technique can be used to assess the composition of CS-derived meat products (hams and salami) with a simple PCR-based amplification test.

The genetic characterization of meat from six CS pigs post-mortem has been an essential step for the realization of a complete DNA-based traceability plan, from meat to processed products. The comparison of the genetic profiles of the six meat samples against the available databanks, including the one built-up at the University of Siena [53], proved that the pig analyzed had a CS genotype. Furthermore the availability of autochthonous pig profiles belonging to the CS population, allowed to prove that the salami tested derived from other individuals, than the six taken as CS reference, but still identified as belonging to the CS breed. In fact, within the CS breed more than a fixed true-to-type genotype, there is a range of natural genetic variability that in any case, renders it possible to differentiate the CS population from the main white breeds [53].

In order to validate the method, a detection limit test was conducted in samples obtained by mixing CS and meat derived from unknown white breeds.

According to the data obtained, it was demonstrated that the meat deriving from white breeds was detectable over the CS meat above a 5% (w/w) threshold detection limit. This qualitative PCR-based approach could be used as a preliminary semi-quantitative method to assess the CS-derived products composition and the degree of purity that is foreseen by the European Union regulation for DOP or PDO (Protected Designation of Origin)

food products. This test, can be considered as subsidiary to a more accurate, but still to be developed, quantitative DNA testing, based on the use of RT-PCR. Ham is one of the best representatives of the Italian agro-alimentary production. The results method appeared to be particularly suitable for establishing CS ham origin.

The tested hams could be reconducted themselves to specific pigs within the CS population, even if not identical to the six CS references.

The knowledge of the genotypic profile of the CS pig breed is an essential starting point for the development of molecular methods useful to trace the origin of the meats used for the production of typical products.

## 5. Conclusion

Under the food traceability framework, the present work fits with the aim to provide additional techniques of study and analysis that can allow the implementation of a complete genetic traceability plan throughout the CS production chain, from meat to processed products. The opportunity to implement a plan of molecular traceability applied to meat and processed products would ensure not only an increase in the guarantees offered to consumers, but also the possibility for individual companies to differentiate themselves in the market by ensuring the authenticity of the information on the label.

## Acknowledgements

We sincerely thank Prof. Russo and Prof. Davoli (DIPROVAL, Sezione di Allevamenti Zootecnici) for having kindly provided the Duroc and Hampshire genomic DNA. The Farm “Terra di Siena” (Poggibonsi, Siena, Italy) for its collaborative help and for having provided CS meats, CS salami and hams. The present work was financed by the Amministrazione Provinciale of Siena in collaboration with ARA-Toscana (Associazione Regionale Allevatori della Toscana, Italy).

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