Review

Genetic traceability of livestock products: A review

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Abstract

Traceability is the ability to maintain the identification of animal, or animal products, all along the production chain. It represents an essential tool to safeguard public and animal health and to valorize typical production systems. European food legislation is particularly strict and traceability systems, based on product labeling, have become mandatory in all European countries. However, the implementation of this system does not ensure consumers against fraud. Paper documents can be counterfeit so researchers have focused on the study of genetic traceability systems based on products identification through DNA analysis. In fact DNA is inalterable, detectable in every cell, resistant to heat treatments, and allows for individual, breed or species identification. Even if results are promising, these techniques are too expensive to be converted in routine tests but they could be a trusted tool for verification of suspected fraud. The present review proposes a synthesis of the major advances made in individual, breed, and species genetic identification in the last years, focusing on advantages and disadvantages and on their real future applications for animal productions.

Keywords: Traceability; Molecular markers; DNA; Livestock products; Meat

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1. Traceability: what and why

Traceability is defined as a system able to maintain a credible custody of identification for animals or animal products through various steps within the food chain, from the farm to the retailer (McKean, 2001). In particular, this
term was defined by the European Regulation (ER) 178/2002 as “the ability to trace and follow a food, feed, food producing animal or ingredients, through all stages of production and distribution”. While, following the ISO 8402 standard norms, traceability is defined as “the capacity of establishing a product’s origin process history, use and provenance by reference to written records” (ISO, 1994). However, like other traceability definitions, ISO 8402 does not define which parameters have to be measured or how history or origin should be determined. As proposed by Golan, Krissof, Kuchler, Nelson, and Price (2004) a traceability system might be characterized by: its breadth, depth, and precision. The breadth depends on the amount of information recorded (e.g. feed regime, pedigree information or details of animal’s veterinary care), the depth consists on how far, back or forward, the system tracks (to a grain elevator, farm or field), in many cases, the depth is determined by the breadth or attributes of interest. Finally, the precision is the degree of assurance with which the system can pinpoint the movement of a particular product, and is described with reference to an acceptable error rate.

In the last few years traceability issues have grown in importance due to the consumers’ increasing attention to food quality matters. The consumers’ lack of confidence, in particular towards food of animal origin, is due to several reasons including both food safety and socio-economic changes. Bovine spongiform encephalopathy (BSE) has certainly been the most serious food safety problem of the last years, causing a drastic reduction of beef consumption in all Europe. It was then followed by the dioxin crisis and the avian influenza in the poultry sector (Ciampolini, Leveziel, Mozzanti, Grohs, & Cianci, 2000; Goffaux, China, Dams, Clinquart, & Daube, 2005). Furthermore, the incidence of food borne diseases due to microbial contamination of processed food, has increased in the last decade leading to additional food scares in the buyers (Opara & Mazaud, 2001).

Besides these “food scandals” socio-economical reasons have also contributed to increase people’s interest in what they eat and in how and where it is produced. For example, it is worth mentioning that the main reasons for the negative trend on meat consumption, are not due only to the negative impact of the food scandals involving meat products, but also to the new food habits of the younger generation and the progressive decline of the organoleptic meat properties (Cozzi & Ragno, 2003). In fact, for red meat, a loss of taste and flavor has been observed, probably related to the reduced marbling (Kerry & Ledward, 2002). In the same way, the reduction of intramuscular fat deposition seems to have a negative effect on meat tenderness (Seideman, Koohmaraie, & Crouse, 1987). At present, consumers are more aware than years ago of ecological and environmental matters, and the demand for organic food and for products obtained in eco-sustainable systems has increased (Opara & Mazaud, 2001), nevertheless the industrialization processes, as well as the market globalization, have made difficult for people to keep a check on food processing methods (Ajmone-Marsan, Milanesi, & Negrini, 2004).

All these reasons have contributed to the need of finding a system to trace food products. Traceability is the answer to the consumers’ demand of transparency and it is becoming synonymous with safe and high quality food. Authorities and scientists are still debating on how the perfect trace back system should work and several authors have compared, in their publications, the efficacy of different traceability methods (Barcos, 2001; Marchant, 2002; Meuwissen, Velthuis, Hogeveen, & Huirne, 2003; Stanford, Stitt, Kellar, & McAllister, 2001) focusing on animal identification. They stated that a good system should be convenient, easy to use and read, durable, respecting animal and public health, and able to avoid fraud. Several identification methods have been studied including different kinds of tags, ruminal bolus or retinal analysis. At present policymakers have implemented mandatory methods based on tags or labels as will be described in the next chapter, such methodologies are easy to use but often cannot prevent fraud (Barcos, 2001; Stanford et al., 2001). Debates on food safety issues and on traceability matters involve not only policymakers and scientists but also economists as, implementation of traceability systems, is strongly related to cost. Realization of any kind of system results in costs and benefits for both industries and consumers; in particular for food companies it is a tool to counterattack liability claims and to improve recall efficacy but, on the other one hand, consumers willingness to pay for this service must be studied (Meuwissen et al., 2003).

Though, traceability issues concern many different aspects tied not only to food safety and policymakers decisions but involving economic aspects and consumers’ decision making behavior, implementation of such systems must necessarily cover all these aspects.

2. The European legislation on traceability

The European Union (EU) has always paid great attention to food safety, first of all because the agro-alimentary sector on the whole is very important for the European economy. The EU is the biggest producer of food products and beverages in the world (European Commission, 2000) with food and beverage industries producing 15% of the total EU manufacturing output, corresponding to 600 billion Euro. The second reason can be found in the Treaty of Rome (1957) instituting the EU, and stating that one of its aims is the “achievement of a high level of health protection” and “the strengthening of consumers’ protection”. So, food safety measures have always been present in EU legislation but, in the last years, in particular after the BSE outbreak in the early 1960s, the legislation had been implemented in order to be faithful to its aims regarding health protection and to gain consumers’ trust.
The three most important EU documents regarding food safety are the Green Paper on the general principles of food law in the European Union (1997), The White Paper on Food Safety (2000) and the ER 178/2002 (applied from 1st January 2005). In particular with the latter a traceability system has been introduced in the food sector, even if for the beef industry such system already existed thanks to the ER 1760/2000 and 1825/2000 issued soon after the BSE crisis.

The Green Paper on the principles of EU legislation about food products (1997), is made up of six parts regarding different aspects of food safety such as: the actual legislation of member countries, the need to simplify EU legislation and, above all, the need to implement it for better protecting consumers’ health, a must after the BSE outbreaks.

The White Paper on Food Safety (2000), followed few years later, containing strategies for updating the actual legislation; among the given suggestions there were: the institution of an independent European Alimentary Authority, the risk analysis as main instrument for food safety, the application of the precaution principle, the need of controls on food products and the consumers’ information. In addition, for the first time, it introduced the concept of traceability for feed and animal products “from farm to fork” and transparency was the leitmotiv of the entire document.

The White Paper was the base for ER 178/2002 applied from the 1st January 2005. This regulation has stressed the importance of a traceability system declaring that “the experience demonstrated how the impossibility to reconstruct the trail of a food could be a danger for the market of such product” while, a traceability system, able to keep all the information regarding food production, can help to proceed to its recall in case of danger without damaging the entire sector. So, since 2005, the regulation has become mandatory for all member countries which must define a traceability system for the whole food sector; in addition it permits to achieve an agreement among the different member countries legislations in which several differences were present leading to problems regarding the free exchange of food among them. Though ER 178/2002 is the fundamental law regarding food safety, it has been followed by several other regulations; for animal products the most important are 852/2004, 853/2004, 854/2004 and 882/2004, all of them corroborate the importance of a traceability system and the need to control them by authorities.

Not only EU has such a strict legislation on food products traceability; in fact traceability systems based on animal identification have been implemented in several countries. In Canada, Australia and New Zealand a trace back system based on tagging was established in 2001, in Japan as well strict rules have been established in the same year, and in Brazil and Argentina traceability systems are in use, even if with different depth. In the United States (USA) a trace back system was proposed even if it would not be mandatory or provide comprehensive information (Marchant, 2002; Smith et al., 2005). Moreover, in the last few years the discussion on the identification and registration of genetically modified organisms (GMOs), between the EU and the USA, contributed to an increase in the traceability requirements and transparency in food chains. Labeling of GMOs is obligatory in the USA only if the product differs essentially from the “original”, e.g. if the nutritional value differs, or if the product contains an allergen that it is not present in the original. The EU demands that all GMO products, with a GMO contamination of >0.9%, must be labeled as such.

3. Conventional and geographical traceability

Traceability systems are mandatory in all EU member countries and, as described before, they are particularly important for livestock and animal products. Anyway there are several types of traceability depending on how it is obtained and on what information it furnishes.

The so-called conventional traceability consists of the labeling system, such as in the beef sector, and of the management of processed food by batches. It is extremely useful for keeping individual information for each animal and is less expensive and easier to achieve than other methods. For example, in the beef industry, legislation requires the identification of each animal through ear tags with a specific code given by the Veterinary Services, a passport accompanying the animal in all its movements, and a central database collecting all the information. The identification code must be maintained also after slaughter on the carcass and on every meat cut. As mentioned this method presents several advantages, ear tags are quite durable, easy to apply and to read allowing for fast data transmission. They are also easy to remove even if they cannot be easily used again (Barcos, 2001). Furthermore, being based on paper documents, they could be counterfeit (Cunningham & Meghen, 2001). The General Food Law refers not only to meat but to every food and feed products, this has several implications for producers; in fact the source of all ingredients must be traced and processors must be able to prove that their suppliers can provide food traceability as well. Conventional traceability applies to everything contributing to food safety, including packaging, closures, seals, jars, etc. and covers everything that happens to the products before, during and after the manufacturing, packaging, and distribution; all this information must be stored, resulting in an enormous collection of data that must be accurate, easy accessible and maintained for an extended period of time (Schwägele, 2005).

Geographic traceability instead does not aim to identify an individual or a batch but the geographic origin of a product through the study of “track elements” such as volatile compounds, microbial flora, stable isotopes and infrared spectroscopy (Franke, Gremaud, Hadorn, & Kreuzer, 2005; Mauriello, Moio, Genovesi, & Ercolini, 2003;
Genetic traceability, according to its name, is based on the identification of both animals and their products through the study of DNA. In fact, DNA molecule has the feature to be enormously variable among individuals (expect for monozygotic twins and clones) allowing to distinguish among them (Mackie et al., 1999; Cunningham & Meghen, 2001). Other important features are: a-DNA is inalterable during animal life; b-DNA is stable to the different treatments of processed food; c-DNA is present in every cell of the organism.

Once the DNA is extracted from the chosen matrix (it can either be animal tissue, blood, muscle, hair, sperm, faeces or even a processed food such as cheese or canned meat) it is analyzed by molecular markers to obtain a fingerprint or specific allelic frequencies allowing for individual, breed or species identification. Since the introduction of the polymerase chain reaction (PCR) in 1989, many different markers have been discovered and studied, at present the most widely used are microsatellites also known as short tandem repeats (STR) and single nucleotide polymorphism (SNP) (Mariani et al., 2005). As already mentioned DNA analysis furnishes different levels of identification: the individual one is of great interest for the verification of a meat cut and it is strictly linked to food safety, while breed and species discrimination are interesting to detect fraud and to protect and valorize typical productions. The use of these technologies in animals and their products is just an extension of techniques already in use for human testing and routinely applied in forensic casework (Cunningham & Meghen, 2001).

4.1. Individual genetic traceability

Animal individual identification is useful for safeguarding public and animal health and providing safe products for both domestic and export consumption. In addition also national diseases monitoring and eradication programs depend heavily on correct animal identification (Cunningham & Meghen, 2001); though, after the BSE outbreaks in the EU and the foot and mouth disease in the United Kingdom, trace back systems have become an issue of international concern (Barcos, 2001; Stanford et al., 2001). As already mentioned, the beef sector suffered a serious crisis after BSE outbreaks, and, since then, consumers are worried about meat quality, its origin and integrity all through the food chain until consumption. As a consequence, the EU has regulated the beef labeling system with ER 1825/2000 (Arana, Soret, Lasa, & Alfonso, 2002) that is substantially based on paper documents and tags. Typing of DNA has been proposed for future implementation as an individual identification method due to its precision, durability and possibility to overcome limits of conventional traceability systems. Studies have been conducted on many different cattle breeds. Their aim was to assess a panel of molecular markers able to discriminate one individual from another. To test the panel efficacy the so-called match probability (MP) is calculated, it is defined as the probability to find, by chance, two individuals sharing the same genotypic profile at the studied loci (Weir, 1996). For example, if the frequency of all alleles detected at all analyzed loci is the same and equal to 0.25 the cumulative probability (%) of a chance match is 0.125\(^n\) × 100, where \(n\) is the number of loci.

The most widely used markers are microsatellites (Peelman et al., 1998; San cristobil-Gaudy et al., 2000; Arana et al., 2002; Vázquez et al., 2004; Herraeza, Schafer, Morsener, Fries, & Wink, 2005; Dalvit et al., 2006; Orrù, Napolitano, Catillo, & Moioli, 2006) and the most recent SNP (Heaton et al., 2002; Heaton et al., 2005; Herraeza et al., 2005). In Table 1 are shown the results of these researches, the type of markers utilized and the breeds studied. They reveal the efficacy of both markers for individual traceability with different results depending on the type, number and level of polymorphism of chosen markers. No author obtained MP values higher than one over one million evidencing a good power of discrimination of the method. However, to choose which is the best MP discrimination threshold the population size has to be considered; for a population of four thousand animals MP values in the order of 10\(^{-6}\) are adequate but if several million animals are bred such a value does not ensure a good level of discrimination.

It is worth mentioning an important aspect when choosing the markers and the breeds to analyze; Orrù et al. (2006) in their study on four cattle breeds observed that the informative content of each microsatellite varied from one breed to another depending on the typical breed allelic frequencies and on the presence of private alleles (alleles always present in one breed and always absent in the others); though when implementing a genetic trace back system it would be interesting to choose different panels for each breed or, to contain costs, to choose a panel permitting the achievement of good efficacy in all breeds. In both
cases preliminary analyses on all breeds are needed to
determine the genetic structure of each population.

The mentioned studies concern the identification of a
single meat cut but also tracing of individual animal’s
products in mixtures should be ensured considering that
ground meat, sausages and potted meat present a greater
health risk than carcasses and meat cuts (Barcos, 2001).
For this purpose Shackell, Mathias, Cave, and Dodds
(2005) recently published an article on the possibility to
use microsatellites for tracing ground beef mixtures. Micro-
satellite markers were used to analyze samples containing a
mixture from several individuals. In this case, it was impos-
sible, looking at the electropherograms obtained after the
analysis of fragments, to distinguish among true alleles,
“stutters” (amplified fragments that does not correspond
to a true allele) and their interaction; so instead of assign-
ing alleles, a DNA “signal” profile was created for each
marker and sample including the area of every observed
peak. They achieved a good success rate distinguishing
individuals among mixtures containing meat from up to
five different animals, although when considering more
individuals results were not satisfactory. Thus such tech-
nique could be the appropriate tool to verify for example
that the correct batch has been recalled, as suggested by
the authors.

Even if the beef sector is obviously the most involved
Goffaux et al. (2005) highlighted that such a system could
be applied in Belgium to pigs where traceability stops at
the slaughter-house making it impossible to link a piece
of meat to an animal. They proposed the use of 21 SNP
markers giving a MP of $7 \times 10^{-6}$, such test was considered
sufficiently significant as the total Belgian pig population is
of about $7 \times 10^{-6}$.

In conclusion, effective genetic meat traceability could be
possible but it faces two problems: the high costs of
analyses and the management of the collected individual
samples. The second one is particularly tricky. If research
could define the most appropriate markers, reducing the
number and consequently the costs, a new organization of
the beef chain is needed. The all national herd has to
be sampled, possibly by the Veterinary Services when
applying ear tags, and samples must be conserved to be
analyzed in case of need. This will necessarily lead to the
creation of “banks” in which samples, like hairs, could
be easily stored. As suggested by Cunningham and Meghen
(2001) DNA analyses will be required only in same cases,
for particular investigations and on a random basis, in this
way the integrity of the ear tag could be guaranteed; such
system has already been implemented by an Irish super-
market chain. In addition as suggested by Barcos (2001)
a harmonization and standardization of individual trace
back systems in all countries would be advisable as world
trade of animals and animal products has grown and public
health has to be ensured, it is worth mentioning that some
European governments are actually considering this possi-
bility (Cunningham & Meghen, 2001).

4.2. Breed genetic traceability

Breed genetic traceability allows the assignment or exclusion of the breed of origin to a product. Such ability has become more and more important as today many typical products, some protected by the European labels PDO or PGI, are prepared from one breed only or cannot be made with some breeds. Examples are the Italian PDO cheese Parmigiano Reggiano “Vacche Rosse” produced only with milk obtained from the Reggiana dairy cows (Gandini & Oldenbroek, 1999) while for the meat industry both Italy and Spain obtained the PGI label for beef from several native breeds: Chianina, Marchigiana, Romagnola, Podolica, and Maremma for Italy and Pirenaica for Spain (Arana et al., 2002). Not only cattle breeds are involved in such production, the Spanish PDO Jamon Iberico made with Iberian pig breeds only (Garcia et al., 2006) is a good example. The list could be long and it is essentially made up of products typical of the Mediterranean countries such as France, Italy and Spain (Pancaldi et al., 2005) and most of the studies are performed in such nations. It is important to underline that these products are usually very ancient and their preservation consist also

Table 1

<table>
<thead>
<tr>
<th>Type and number of markers</th>
<th>Match probability</th>
<th>Breeds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR – 12</td>
<td>$1.9 \times 10^{-11}$</td>
<td>Piemontese, Chianina, Marchigiana, Romagnola</td>
<td>Dalvit et al. (2006)</td>
</tr>
<tr>
<td>STR – 10</td>
<td>$2.4 \times 10^{-8}$</td>
<td>Galloway</td>
<td>Herraeza et al. (2005)</td>
</tr>
<tr>
<td>STR – 14</td>
<td>$2.3 \times 10^{-11}$</td>
<td>Galloway</td>
<td>Herraeza et al. (2005)</td>
</tr>
<tr>
<td>STR – 17</td>
<td>$1.4 \times 10^{-13}$</td>
<td>Galloway</td>
<td>Herraeza et al. (2005)</td>
</tr>
<tr>
<td>SNP – 43</td>
<td>$5.3 \times 10^{-11}$</td>
<td>Galloway</td>
<td>Herraeza et al. (2005)</td>
</tr>
<tr>
<td>SNP – 20</td>
<td>$4.3 \times 10^{-9}$</td>
<td>Holstein Friesian and others</td>
<td>Heaton et al. (2005)</td>
</tr>
<tr>
<td>SNP – 32</td>
<td>$2.0 \times 10^{-13}$</td>
<td>American Angus</td>
<td>Heaton et al. (2002)</td>
</tr>
<tr>
<td>STR – 10</td>
<td>$&gt;10^{-7}$</td>
<td>Pirenaica</td>
<td>Arana et al. (2002)</td>
</tr>
<tr>
<td>STR – 13</td>
<td>$&gt;10^{-15}$</td>
<td>Piemontese, Chianina, Holstein Friesian, Italian Simmental</td>
<td>Orù et al. (2006)</td>
</tr>
<tr>
<td>STR – 11</td>
<td>$5 \times 10^{-12}$</td>
<td>Charolaise</td>
<td>Saneristob-Gaudy et al. (2000)</td>
</tr>
<tr>
<td>STR – 10</td>
<td>$1 \times 10^{-10}$</td>
<td>Belgium beef cattle</td>
<td>Peelman et al. (1998)</td>
</tr>
</tbody>
</table>

a STR: short tandem repeats.
b SNP: single nucleotide polymorphism.
in the protection of old traditions and cultures. The herds
of the utilized breeds are often small and endangered,
and their only chance of survival is their use for the pro-
duction of typical and high quality products. Though,
research regarding breed genetic traceability is often linked
with studies on breed characterization (Carrión et al., 2003;
Ciampolini et al., 2000; De Marchi, Targhetta, Contiero, &
Cassandro, 2003; Maudet, Luikart, & Taberlet, 2002;
Ovílo, Cervera, Castellanos, & Martínez-Zapater, 2000) and,
sometimes, also conservation through the use of
molecular markers methods (Alderson & Plastow, 2004;
De Marchi, Dalvit, Targhetta, & Cassandro, 2006). If indi-
vidual traceability is an instrument to ensure food safety,
breed traceability is a means to defend and valorize partic-
ular food products.

To assign an individual or a product to a breed two
approaches are possible, as reported by Ajmone-Marsan
et al. (2004): a-deterministic: consisting on finding molecu-
lar markers with different allelic variants fixed in different
breeds, though it will be possible to develop simple analysis
protocols without the need of statistical inference; b-prob-
abilistic: consisting on utilizing a set of markers with typi-
cal allelic frequencies in different breeds. Breed assign-
ment is obtained by statistical methods based on maximum like-
lihood functions (Paetkau, Clvert, Stirling, & Strobeck,
1995). Bayesian methods (Rannala & Mountain, 1997)
and genetic distances methods (Cornuet, Pyr, Luikart,
Estoup, & Solignac, 1999).

4.2.1. Deterministic approach

In the last years, researches have focused on both
approaches. The deterministic one is mainly based on the
study of genes coding for coat color, the principal character
allowing for breed differentiation and under human selec-
tion in European cattle breeds (Maudet & Taberlet,
2002). Table 2 shows a classification of the most important
identified loci based on their known functions coding for
color. Interest in these studies is mainly based on
the possibility to determine the breed of origin of cheese
finding molecular markers that are specific to each cow
breed and developing a technique to detect these markers
in cheese (a mixture of milk from several individuals). In
cattle, the pigmentation is determined by the distribution
of two pigments: eu- and pheomelanin, producing brown
or black and red to yellow pigmentation respectively.
Tyrosinase, the rate-limiting enzyme involved in the syn-
thesis of both melanins, is regulated by the melanocyte-
stimulating hormone (MSH). This hormone and several
other melanotropic peptides, stimulate melanin formation
in melanocytes by binding to the melanocortin-1-receptor
(MC1R), a G-protein-coupled receptor encoded by the
Extension gene (Robbins et al., 1993). In addition, the
amounts of eu- and pheomelanin in the melanocyte are
controlled by the agouti gene encoding the Agouti Signal
Protein (ASP), that acts as an antagonist of MSH signaling
through the MC1R, even if its mechanism of action is con-
troversial (Furumura et al., 1998). The MC1R gene has
been analyzed in different species (Crepaldi, Fornarelli, &
Marilli, 2005). In cattle populations many mutations have
been observed and three main alleles have been detected
(Klungland, Vage, Gomez-Rayas, Adalsteinsson, & Lien,
1995): the E+ so-called "wild type" encoding the normal
functional receptor, the dominant E3 caused by a T/C sub-
stitution changing the 99th amino acid to proline with a
consequent high level of eumelanin and the e containing
a G-deletion giving rise to a non-functional receptor result-
ning in pheomelanin production giving red color in homo-
zogotes. In addition four other alleles have been detected:
Rouzaud et al. (2000) and Maudet and Taberlet (2002) dis-
covered a new allele named E3 in the Aubrac, Gasconne
and Tarentaise breeds in a study considering different
French cow breeds while Graphodatskaya, Joerg, and
Stranzinger (2002) detected two new alleles in the Brown
Swiss breed (E11 and E12) and one in the Simmental breed

<table>
<thead>
<tr>
<th>Locus</th>
<th>Symbol</th>
<th>Function</th>
<th>Coding Molecule</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension</td>
<td>E</td>
<td>Involved in the melanogenesis regulation</td>
<td>melanocortin receptor</td>
<td>Controls the proportion of the two melanin types</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 (MC1R)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>agouti signaling protein (ASIP)</td>
<td>Controls the proportion of the two melanin types</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>KIT</td>
<td>Affects spotting extension and pigmentation intensity</td>
</tr>
<tr>
<td>Spotted o White Spotted</td>
<td>S W</td>
<td>Involved in the melanocytes development and migration during embryogenesis</td>
<td>mast cell growth factor (MGF)</td>
<td>Determines the roan color in the Shorthorn and Blue Belgian breeds</td>
</tr>
<tr>
<td>Roan</td>
<td>R</td>
<td>Involved in the melanocytes development and migration during embryogenesis</td>
<td>Tyrosinase-related protein 2 (TYRP2)</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td>Slaty</td>
<td></td>
<td>Involved in the melanin biosynthesis</td>
<td>tyrosinase (TYR)</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tyrosinase-related protein 1 (TYRP1)</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>myosin type V (MYO5A)</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td>Albino</td>
<td>C</td>
<td>Involved in the melanin biosynthesis</td>
<td>melanocortin receptor</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td>Brown</td>
<td>B</td>
<td>Involved in the melanin biosynthesis</td>
<td>melanocortin receptor</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td>Dilute</td>
<td>D</td>
<td>Involved in melanocytes morphology</td>
<td>melanocortin receptor</td>
<td>Controls dilution of coat color</td>
</tr>
<tr>
<td>Silver</td>
<td>PMEL17</td>
<td>Involved in melanosome structure and functions</td>
<td>melanocortin receptor</td>
<td>Controls dilution of coat color</td>
</tr>
</tbody>
</table>
Table 3: Polymorphisms of the extension locus in cattle breeds

<table>
<thead>
<tr>
<th>Allele</th>
<th>Breed details</th>
<th>Breed origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Aubrac, Gasconne, Tarentaise</td>
<td>France</td>
<td>Rouzaud et al. (2000) and Maudet and Taberlet (2002)</td>
</tr>
<tr>
<td>E&lt;sup&gt;1l&lt;/sup&gt;, E&lt;sup&gt;32&lt;/sup&gt;</td>
<td>Brown Swiss</td>
<td>Switzerland</td>
<td>Graphodatskaya et al. (2002)</td>
</tr>
<tr>
<td>e&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Italian and French Simmental</td>
<td>Italy, France</td>
<td>Graphodatskaya et al. (2002)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Chianina, Marchigiana, Piemontese, Maremmana, Valdostana Pezzata Rossa, Flamande</td>
<td>Italy, France</td>
<td>Maudet and Taberlet (2002) and Crepaldi et al. (2003)</td>
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(<sup>f</sup>). Table 3 shows the polymorphisms detected in different cattle breeds, studies have been performed also on beef breeds, in fact the beef sector also encounters commercial problems in recognizing and protecting meat of high quality derived from specialized breeds, resulting in economic losses for the farmers (Ciampolini et al., 2000). It is worth mentioning that in all the Italian beef cattle breeds analyzed (Chianina, Marchigiana, Piemontese and Maremmana) a new mutation has been detected consisting in a base substitution (C/T) in the 667-bp position resulting in an amino acid change (Arg to Trp) (Maudet & Taberlet, 2002), Crepaldi, Marilli, Gorni, Meggiolaro, and Cicogna (2003), in a study concerning Italian beef cattle breeds, confirmed the presence of such a mutation also in their samples.

These results are promising for establishing a traceability method based on coat color markers giving the possibility to distinguish among some breeds, additional studies on other coat color genes are needed to complete the information and increase the discriminating power of such markers. Analyses on spotting gene, affecting spotting extension, for example, could in fact increase the informative power derived from extension alleles. However, studies on coat color genes are utilized also for assessing genetic diversity to maintain traditional color types for the preservation of the cultural and historical value of endangered native breeds (Kantanen et al., 2000).

Dairy and beef sectors are not the only ones involved in genetic traceability systems, more and more studies are carried out in the swine sector. In fact different pig breeds have been developed to satisfy particular market requirements; for example in the United Kingdom (UK) the Large White and the Landrace breeds were selected for bacon production while Berkshire is a pork pig. On the other one hand the Spanish market aims to valorize and protect hams obtained by Iberian pig breeds selected for outdoor rearing and production of special ham (Alderson & Plastow, 2004; Carrión et al., 2003). In both cases breed differentiation is an important tool for the protection of typical quality products. In the UK efforts have been made to discriminate Berkshire and Tamworth breeds through the use of the MC1R and KIT polymorphisms which control much of the variation of coat color in swine. Such tools might be used as part of Quality Assurance schemes for the Traditional Breeds Meat Marketing Scheme as has already happened for the British Wild Boar Association (Alderson &
Plastow, 2004; Carrión et al., 2003). In fact, in the case of wild boar, discrimination is easy due to a variant of the MC1R locus not found in commercial pigs, Tamworth and Berkshire differentiation instead requires the use of both MC1R and KIT loci analyses as reported by Alderson and Plastow (2004).

The Spanish market aims to trace Iberian pig products that have been differentiated in Spain as a component of a sustainable system supporting biodiversity and delivering outputs of the highest quality with special sensory properties. The Iberian Cured Ham has acquired an excellent reputation and can cost up to 10 times more than a normal cured ham, this led to an indiscriminate use of the term “Iberico” (Carrión et al., 2003; García et al., 2006). For the production of Iberian Cured Ham the Spanish legislation allows up to 50% Duroc origin in the animals but hams obtained from pure Iberian pigs are instead called Pure Iberian Ham. Studies on coat color genes have been performed for these breeds as well, but the variation of coat color in such breeds (from blonde to black) and the allowed use of Duroc crossbreeds, makes discrimination, through the use of these genes only, difficult. Carrión et al. (2003), collected samples from Iberian hams in some markets and analyzed them through the use of both MC1R and KIT loci and they found a selection of MC1R alleles and evidence of a new haplotype that could be the origin of the red-chestnut type but they could not identify a discrimination test. Fernández et al. (2004) solved the problem by supplementing results obtained from the analysis of MC1R with those obtained from the analysis of four microsatellite loci, of the pink-eyed dilution gene and of nine amplified fragment length polymorphism (AFLP), and were able to discriminate between pure Iberian and Duroc crossbreeds genotypes. The AFLP procedure was applied also by Alves, Castellanos, Ovíolo, Silio, and Rodríguez (2002) which highlighted the presence of nine fragments detected in the Duroc breed only while three polymorphisms were found only in Iberian pigs, use of such technology would allow the detection of crossbred animals with a whole probability of exclusion of a pig of a pure Iberian origin of 0.97 and 0.71 for the 50% and 25% Duroc crossbreeding. Further studies performed on Iberian strains only demonstrated the presence of strain-specific AFLP markers. In this case such information is important for management and conservation of highly inbred Iberian strains as differentiation among strains is not required for high quality production (Ovíolo et al., 2000). Studies on conservation and management of small populations often use AFLP markers allowing for the detection of breed specific markers, for example De Marchi et al. (2006) in a study investigating the genetic variation of four Italian indigenous chicken breeds found specific markers in every breed analyzed which could enable their differentiation on the market. However, even if the exposed results are promising and could enhance the use of AFLP markers for breed traceability methods, it must be mentioned that research should be extended to a greater number of individual samples to verify the exclusiveness of detected markers. The use of pooled samples, in fact, could be useful to highlight the presence of such markers but these differences may be due to simple differences in the allelic frequencies of the population as demonstrated by Negrini et al. (2003) in their research on some Italian cattle breeds.

In conclusion, the use of AFLP markers for breed genetic traceability is suggested by several authors (Alves et al., 2002; Negrini et al., 2003; De Marchi et al., 2006), Ovíolo et al. (2000) affirmed that using microsatellites detection of any strain-specific allele fixed in the population was impossible, evidencing a lower discrimination power than AFLP, at least for closely related individuals. However, all authors agreed with the opinion that the AFLP technique is complex, expensive, and not easy to apply to routine tests. To overcome these disadvantages it is advisable to convert AFLP markers into simpler PCR-based tests (Ovíolo et al., 2000; Alves et al., 2002), as Sasazaki et al. (2004) did in their study aimed at distinguishing Japanese Black cattle from a cross of Japanese Black and Holstein Friesian.

4.2.2. Probabilistic approach

Assigning individuals to populations has a wide range of applications both in population genetics, for example for evaluating population differentiation in polar bears (Paetkau et al., 1995) or for classifying individual fish (Taylor, Beacham, & Kaeriyama, 1994) or honey bees (Cornuet, Aulagnier, Lek, Franck, & Solignac, 1996) and in forensics for verifying the authenticity of a labeled food product. The utilized methodology, based on analyses of individual multilocus genotypes, relies on the fact that individuals will have more similar genotypes when they come from the same population (Cornuet et al., 1999); these “genetic methods” are based on the likelihood that the genotype of the individual to be assigned occurs in each of two or more candidate populations (Paetkau et al., 1995; Rannala & Mountain, 1997), or on genetic distances between the individual and a population (Cornuet et al., 1999). These statistical tools could be used for the assessment of a breed traceability system. According to Cornuet et al. (1999) maximum likelihood methods, in particular the one based on a Bayesian approach, produces the best results but the population must be in Hardy–Weinberg and linkage equilibrium. Distance based methodologies can overcome this problem and could be more appropriate if these two assumptions are not fulfilled. There are other aspects affecting correct assignment such as the number of scored loci and animals, the loci variability, the population differentiation and their significance. They have been investigated by Bjørnstad and Roed (2002). According to them, both genetic differentiation and number of scored loci are highly important. For very differentiated breeds (0.200 < F< 0.259) only three loci could be sufficient to have an assignment precision of 95%. Loci having an intermediate to high variability within and across populations yield higher assignment precision, while breed sample size is not critical.
as long as more than 20 animals per breed are analyzed. Several studies on different species confirm the efficacy of the Bayesian approach if an appropriate number of markers is scored (Bjørnstad & Røed, 2001; Negrin et al., 2003; Vega-Pla, Martínez, Cabello, Rodríguez-Gallardo, & Delgado, 2003; Ciampolini et al., 2006; Dalvit et al., 2006; Filipini et al., 2006; García et al., 2006). The major problems for the effective applicability of these methods are the choice of the loci to be analyzed and the creation of a pooled database collecting the allele frequencies of all possible alternative breeds of origin, weighted by the population size in order to answer the critical question “what is the probability that this animal is actually from this breed?” (Ciampolini et al., 2000, 2006). Collection of samples from all alternative breeds is essential as maximum likelihood methods test if the analyzed sample belongs to one of the reference population and the result may be incorrect if this condition is not met (Baudouin, Piry, & Cornuet, 2004). Baudouin et al. (2004) also stressed the importance of the quality of the reference population set from which derives the quality of observed results; information about genetic diversity of the populations, their equilibrium and an adequate number of samples (collected avoiding closely related individuals) are essential.

The main criticism of the use of such methodologies lies in the difficulties of using the necessary statistical tools that often require a high familiarity, making it difficult to use them in routine tests (Baudouin et al., 2004; García et al., 2006). To try to overcome this situation some authors proposed user-friendly software, available for free on the internet, permitting computation of the necessary calculations, the most widely used are Structure (Pritchard, 2000) and GeneClass2 (Baudouin et al., 2004), both based on a Bayesian approach but the assignment procedure is different.

4.3. Species genetic traceability

Species identification in meat products has always been important for consumers because of social, religious, health and economic implications; nowadays carcasses and whole fish are rarely displayed while either fresh or frozen cuts, processed and ready to eat food are increasingly available making species identification difficult. For this reason fraudulent adulteration could take place substituting the declared meat or fish species with others of lower commercial value (Hunt, Parkes, & Lumley, 1997; Martínez & Malmheden Yman, 1998), such falsification is actually very common in game meat products resulting in great profit due to the higher prices of these species than beef or pork (Blackett & Keim, 1992; Wolf, Rentisch, & Hübner, 1999). The fish industry also is involved in species counterfeiting, especially in the case of canned fish such as tuna whose genus is made up of many different species characterized by different quality (Unseld, Beyermann, Brandt, & Hiesel, 1995). The dairy sector is the subject of frauds regarding milk and above all cheese species of origin. In fact, the greater availability and the lower cost of cow’s milk rather than goat, ewe or buffalo milk leads to fraudulent substitution in cheese manufacturing (Maudet & Taberlet, 2001). To understand the importance of these frauds it is worth mentioning than in Italy the addition of undeclared bovine milk to water buffalo milk for making cheese is the most frequent fraud reported by the Central Inspectorate for Repression of Frauds of the Italian Ministry of Agricultural and Forestry Policy in 1998 and 1999 for all foods of animal origin. In 1998 and 1999 approximately 13% of cheeses tested contained undeclared non-water buffalo milk (Rea et al., 2001).

First, approaches for species identification were based on protein analyses and immunological assay (Berger, Mageau, Schwab, & Johnston, 1988; Patterson & Jones, 1990). These methodologies present two main disadvantages: protein expression is tissue dependant, and proteins may be denatured during processing and heating (Hunt et al., 1997; Martínez & Malmheden Yman, 1998), though, the legislation still recognizes such methods as official. In fact, the reference technique for the detection of cows’ milk is based on isoelectric focusing of β-casein (European Commission, 1996). Research has been focused on the study of DNA that is present in every cell and is relatively stable to food processing, being detectable even in ripened cheese (Plath, Krause, & Einspanier, 1997). DNA based analytical approaches were investigated for the first time at the end of 1980s and beginning of 1990s, employing simple slot/blot assays using total genomic species DNA as probe, and were able to clearly identify species such pork and chicken but not among ruminant species (Bauer, Teifel-Greding, & Liebhardt, 1987; Chikuni, Ozutsumi, Kois-hikawa, & Kato, 1990; Ebbehøj & Thomsen, 1991; Wintero, Thomson, & Davies, 1990). Hunt et al. (1997) set up a method for the detection of several different species by the use of species-specific oligonucleotide probes obtaining satisfactory results until the minimum admixture level of 2.5% without the use of PCR amplification which, in that period, was still considered too sensitive and associated with many technical problems, while Janssen, Hägele, Buntjer, and Lenstra (1998) utilized PCR generated probes. Few years later, PCR-based techniques overwhelmed other methods being used. The RFLP technique was investigated by several authors on both genomic and mitochondrial DNA (Banja, Ugorski, Polanowski, & Adamczyk, 2001; Montiel-Sosa et al., 2000; Plath et al., 1997; Quinteiro et al., 1998; Ram, Ram, & Baudouin, 1996; Wolf et al., 1999) but finally the most recent techniques are based on the amplification of primers designed to give different length fragments from different species as suggested by Matsunaga et al. (1999). Mitochondrial DNA in particular, presents several advantages if compared to genomic. It is present in thousands of copies per cell improving the possibility to amplify template molecules of adequate size. The vast knowledge on its organization and the availability of reported sequences in many species, makes the design of specific primers easier and its large variability allows
reliable identification of precise species in mixtures (Mackie et al., 1999; Maudet & Taberlet, 2001; Montiel-Sosa et al., 2000). The cytochrome b gene has been widely investigated, allowing for easy and clear species differentiation both in tuna and salmon species (Bartlett & Davidson, 1991; Quinteiro et al., 1998; Rehbein, 2005; Russel et al., 2000; Unseld et al., 1995), in meat (Matsunaga et al., 1999), and in dairy products (Bania et al., 2001; Rea et al., 2001). In this last case also the study of the β-casein gene has been proposed by Plath et al. (1997) while Maudet and Taberlet (2001) suggested the use of primers in the control region also called D-loop that had already been investigated for meat differentiation by Fei et al. (1996). A recent study conducted by Bellis, Ashton, Freney, Blair, and Griffiths (2003) suggested instead the amplification of a variable intron within the highly conserved TP53 tumor suppressor gene which produces fragments of different sizes among species. The AFLP technique could also be employed for species differentiation as shown by Cassandro et al. (2005) who utilized these markers to distinguish among avian species, and such a method has already been widely used for species differentiation in plants (Cervera et al., 2000).

In conclusion, it is important to underline that most of these studies utilized commercial samples collected in supermarkets or butcheries to verify their techniques, and detected several cases of counterfeit and contamination suggesting that controls should be more strict and appropriate in order to preserve consumers against fraud.

5. Conclusions

Traceability of livestock products is an essential tool to safeguard public and animal health, and to valorize typical foods. The European Union has applied, since 2005, strict legislation on labeling systems for food products. It has been demonstrated that traceability methods based only on batch codes or paper documents can not always be trusted, being easy to be counterfeit.

At present DNA based techniques seem to be the appropriate tool for the verification of the origin of animal products and research has made enormous improvements in the last few years, moreover, these techniques are already used for human testing in forensic cases.

The major problem for their effective applicability are the high costs, unsustainable if such methods are meant to be employed as routine test, but affordable if they are needed just as verification in particular occasions (e.g. when recall of a batch is required). All types of traceability are related to increasing costs for food companies but it is essential to analyze which part of these additional costs could be translated into benefits; for example methods ensuring an efficient recall, such as DNA technologies, could prevent from recall of safe batches. Also consumers’ willingness to pay for safer food should be better studied. In general, consumers agree to pay extra for food safety issues especially in developed countries (Henson, 1996; Unnevehr, 2000), even if some authors think that actual buying decisions are mostly based on economic convenience rather than on the presence of a label or certification (Blend & van Ravenswaay, 1999). In spite of differences on consumers’ behavior, Gellynck, Januszewska, Verbeke, and Viana (2005) and Meuwissen et al. (2003) highlighted that functional attributes such as efficient products recall, the possibility to identify individual responsibility, and a complete traceability of the meat chain can be regarded as minimum requirements of a traceability systems for all consumers; keeping this aspects in mind, the potential of DNA technologies appears straightforward.

The second problem to overcome is the attainment of an agreement on markers and approaches to be utilized. As witnessed by all the studies cited in the present review, the scientific community is still debating many different approaches and guidelines are needed. The first steps have been taken by the International Society for Animal Genetics and the Food and Agriculture Organization Standing Committee which proposed sets of microsatellite markers in different species for the study of animal genetic diversity and for conservation purposes (2004). Actually more and more research is being carried out following these suggestions giving the opportunity to compare different results.

Among the three different levels of identification, the individual one regarding meat cut identification, appears the easiest to implement due the low number of markers needed, translated into low cost, and with the simpler statistical approach. The main problem is, maybe, the organization of hair sample collection from every animal at birth. Breed and species traceability is also needed but application of genetic methods is tricky. A deterministic approach seems to be simpler because statistical inference is not necessary but, at present, such techniques are not able to ensure a satisfactory level of discrimination at least for breed determination. On the other hand, probabilistic approaches are promising but the difficulties of statistical calculation have to be overcome. In conclusion, genetic traceability is a useful and trusted tool for livestock products identification and could be the solution to consumers’ lack of confidence as people rely strongly on DNA analysis. However, but to be really applicable more cooperation among researchers and people involved in the food production chain is necessary to achieve low cost and simpler organization solutions.

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