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Traceability of 9 Portuguese cattle breeds with PDO products in the market using microsatellites

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ABSTRACT

Assignment tests based on multilocus genotypes are becoming increasingly important to certify the origin of livestock products and assure food safety and authenticity. The potential of microsatellites for determining the origin of beef products among cattle breeds present in the Portuguese market with the Protected Denomination of Origin (PDO) was studied. Methodologies were used to establish the number of populations under study and to allocate individuals to their original population. The STRUCTURE program was used to perform the strictly Bayesian method and the GENECLASS 2 program was used to accomplish two types of assignment tests. The STRUCTURE program converged to 9 populations, precisely the number of populations under study. Regarding the individual allocation, the strictly Bayesian method implemented by the STRUCTURE program allowed 96% of correct allocations when running the program without the knowledge of the source populations and 98% when the STRUCTURE program was run knowing the source populations of the animals. In the assignment test performed by the GENECLASS 2, 95% and 97% of individuals were correctly allocated by the frequency and the Bayesian methods, respectively. These results show the potential feasibility for traceability scheme based on microsatellites.

1. Introduction

Traceability is defined as the ability 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 EC regulation 178/2002 as “the ability to trace and follow a food, feed, food producing animal or ingredients, through all stages of production and distribution”. The consumers' lack of confidence, in particular towards food of animal origin, is due to several reasons, including food safety and socio-economical changes. The B.S.E. (Bovine Spongiform Encephalopathy) has certainly been the most serious food scandal of the last years causing a drastic reduction of beef consumption in all Europe; it was followed by the dioxin crisis and avian influenza in the poultry sector, and more recently by the crisis of horse meat (Ciampolini, Leveziel, Mozzanti, Grohs, & Cianci, 2000; Goffaux, China, Dams, Clinquart, & Daube, 2005; Stoyke, Hamann, Radeck, & Gowik, 2013). This last crisis proved that only genetic tests could ensure with absolute certainty the contamination with horse meat. In addition, the incidence of food borne diseases, due to microbial contamination of processed food, has also increased in the last decades (Opara & Mazaud, 2001).

Nowadays, consumers are much more aware of ecological and environmental matters and the demand for organic food and for products obtained in an eco-sustainable way has increased. All of these reasons contribute to the need of finding a system to trace food products. Traceability is the answer to the consumers' demand for transparency and it is becoming synonymous with safe and quality food. To answer these demands, we have witnessed the emergence of products with seals of the European Union in the markets. These labels guarantee the quality, with quality being understood as the characteristics of the products, their production methods, as well as the geographical origin and traditions. The seal of Biological Agriculture (BA) ensures that products are free of chemicals and are produced in the most natural way possible. Geographical Indications guarantee the quality of their products and ensure their geographic origin. The same happens with Protected Denomination of Origin (PDO) and Protected Geographical Indications (PGI). Traditional Specialties Guaranteed (TSG) assures that the products with this seal are produced in the most traditional way possible. In Portugal, we have 10 PDO products in the market

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associated with specific cattle breeds. Alentejana, Arouquesa, Barrosã, Cachena, Brava de Lide, Marinhoa, Maronesa, Mertolenga, Mirandesa and Preta are all breeds that have their meat certified with the warranty seal PDO of the European Union in the Portuguese market. This has had a positive impact on the conservation policies of these genetic resources because the certified product can be associated with more sustainable production systems and because it brings economic gains to rural areas, usually more marginalized. Traceability of certified products in relation to the breeds from which they originate is very important if we want to give credibility to this specialized production.

The outlined objectives for this study were to research the extent to which the differences in allele frequencies among 9 populations of Portuguese cattle breeds with PDO products in the market are sufficient or not for the definition of reference populations with reference markers and ascertain whether multilocus genotypes can be used in the allocation of individuals in these populations so that they can be used in the future in the traceability of these breeds. With this purpose, we performed a comparison of three methods of allocating individuals based on molecular information provided by 30 microsatellites, using the probabilistic approach performed by the STRUCTURE and GENECLASS 2 programs.

2. Material and methods

2.1. Animals

The animals sampled are representative of 9 Portuguese breeds with PDO products in the market and include fifty ($N = 50$) animals of Alentejana (ALT), Arouquesa (ARQ), Barrosã (BRS), Marinhoa (MRH), Maronesa (MRN), Mertolenga (MRT), Mirandesa (MRD), and Cachena (CCH) breeds and forty ($N = 40$) animals of Brava de Lide (BRV) breed. All animals used were registered in the herd book of the respective breed.

2.2. DNA extraction

DNA was extracted from whole blood samples collected by jugular venipuncture into sterile VACUETTE vacuum tubes of 9 ml containing K3-EDTA as the anticoagulant. DNA was isolated by the saline method proposed by Miller, Dykes, and Polesky (1988).

2.3. Microsatellites markers and genotyping

The microsatellites used were BM1824, BM2113, BM2613, BM1818, BM203 RM067, RM006, ETH131, ETH10, ETH225, ETH152, ETH185, ETH03, ILSTS035, ILSTS065 HEL9, HEL13, HEL11, SPS113, SPS115, TGLA345, TGLA53, TGLA227, TGLA126, TGLA122, BRRIBO, INRA023, MGTG4B, CSSM036, CYP21 and the conditions of amplification and detection of amplified products were described in Mateus, Penedo, Alves, Ramos, and Rangel-Figueiredo (2004).

2.4. Data analysis

2.4.1. Analysis with STRUCTURE

We used the STRUCTURE program version 3.0 (Pritchard, Stephens, & Donnelly, 2000) to estimate the number of population clusters (K) more likely to appear among the 9 populations studied. Data was analyzed using the Alpha and Lambda parameter defined by the default program. The definition of clusters was based on the admixture model and the assumption that allele frequencies were correlated between the breeds, as is convenient for closely related populations. To estimate the value of K (number of population clusters inferred by the data), its value was made to vary between $K = 1$ and $K = 13$ and set to run the program with a Burn-in of 50,000 and a number of MCMC repetitions after burn-in of 200,000. It was empirically determined that these values were enough for the size of the run to ensure the convergence of parameters to be estimated (Pritchard & Wen, 2003). For each value of K, 10 runs were performed, the most likely value of K was determined by the highest average of the maximum likelihood of the data (Ln P(D)) with smaller variance. The STRUCTURE program was also used to allocate individuals to their populations of origin, using the strictly Bayesian method implemented by the program. To determine the number of animals classified in each cluster, a run was made with a longer burn-in of 100,000 and a number of repetitions of MCMC after a burn-in of 1,000,000 for the most likely value of *K*. The percentage of individuals classified in each cluster was determined by considering the estimated proportion of the association of each individual genotype (Q) to each of the clusters. The percentage of subjects not included in their population of origin and misclassified in other cluster populations was also calculated.

Tests of individual allocation were also performed by the STRUCTURE program using *a priori* information about the source population of individuals, since individuals were sampled within populations with herd books established for standardized phenotypes. The run had the same characteristics as before, with K always equal to the number of populations studied.

We also analyzed the influence of the number of loci in tests of individual allocation in order to reduce the costs for each analysis. The loci were ranked in two different ways: the first, using the WICHLOCI software (Banks, Eichert, & Olsen, 2003) which allowed us to obtain a classification of the twelve most informative loci based on the differential allele frequencies and the re-sampling of 500 individuals in each population; and the second, using the GENECLASS 2 program, which allowed us to rank the twelve loci according to the percentage of subjects correctly allocated in their original populations.

2.4.2. Analysis with GENECLASS

The GENECLASS 2 program was also used to test assigning individuals to their populations of origin, based on the frequency

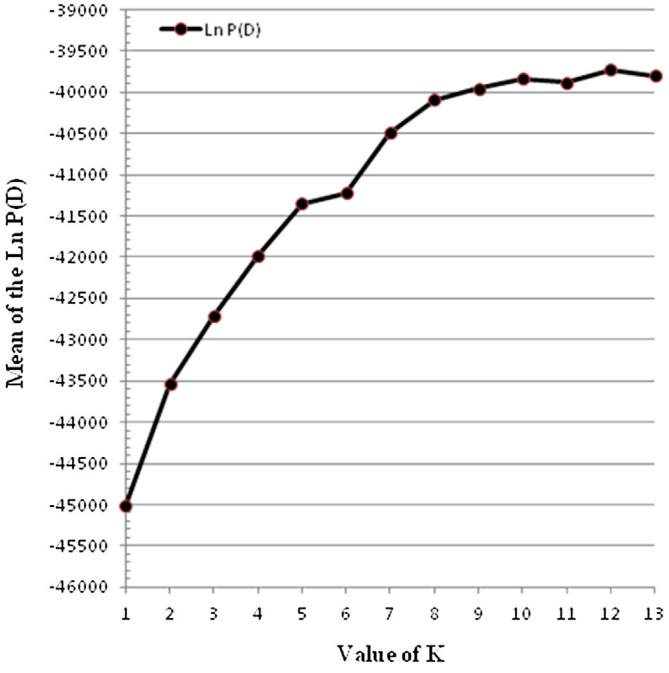

Fig. 1. Average value of the Ln P(D) for ten runs of nine Portuguese cattle breeds without information of the source populations of the animals.