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Effect of consanguinity on Argentinean Angus beef DNA traceability

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ABSTRACT

Since the 1990s several authors have envisaged the use of DNA to certify meat origin. Two major parameters must be assessed before a DNA based traceability protocol can be implemented in the food chain: (i) the information content of a DNA marker set in a specific livestock breed or group of breeds; (ii) the minimum number of DNA markers needed to obtain a statistically acceptable match probability. The objective of the present work was to establish the effect of different levels of inbreeding in the matching efficiency, and the minimum number of microsatellite markers needed, in a DNA based meat traceability program, starting from an 11-microsatellite marker panel. Samples were obtained from beef production farms in South America, where animals are typically bred under pasture-based extensive conditions. Three groups of animals with different consanguinity rates were sampled. Exclusion power (Q) was higher than 0.999998 and match probability lower than 3.01E−08, for the whole set of markers within each group. Both values were affected by consanguinity. To reach a two mismatch criteria exclusion power (Q_2) of 99.99, six markers were needed in unrelated animals whereas seven markers were needed in related animals. To reach $Q_2 = 99.9999$, 8 and 10 microsatellite markers, respectively, were needed. In general, one or two more microsatellite markers were needed to identify consanguineous animals. This study proved the DNA marker set used to be suitable for the identification of the meat from all slaughtered animals in Argentina, per week, month, and year.

1. Introduction

Consumers concern about meat origin, quality, and safety has been increasing over the last two decades. Producers have responded by developing traceability systems in order to guarantee all production and health aspects of the animals, from birth to slaughter. South America is the major beef exporter in the world. Its typical production system is extensive, with big farms and natural pasture fed animals. Due to environmental variety, several different production and commercial systems can be found, e.g. dams can be either artificially inseminated (AI) or naturally mated, and animals can be sold at any age (Rearte, 2007). Moreover, different cattle producers will have different consanguinities in their herds.

Different researchers have envisaged the use of DNA markers to certify meat origin (Arana, Soret, Lasa, & Alfonso, 2002; Lenstra, 2005, chap. 8). DNA can be used to trace meat throughout the whole food chain, from live animals to their derived beef products. Since the early 1990s, microsatellites (STR) have been the most commonly used markers for identification in forensic and animal/crop sciences (Lirón, Ripoli, Peral-García, & Giovambattista, 2007; Rogberg-Muñoz, Prando, Baldo, Peral-García, & Giovambattista, 2008).

Several researchers studied sample matching using different number and combinations of markers (AFLP, microsatellites, Single Nucleotide Polymorphisms (SNPs)) to trace meat (Dalvit, De Marchi, & Cassandro, 2007; Lenstra, 2005). These studies mainly focused on the evaluation of the information content of a marker set in a particular livestock breed or group of breeds (Negrini et al., 2007, 2009; Sancristobal-Gaudy et al., 2000), and/or the minimum number of markers needed for statistically acceptable

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exclusion power degree (Arana et al., 2002; Dalvit, De Marchi, Targhetta, Gervaso, & Cassandro, 2008; Herráez, Schäfer, Mosner, Fries, & Wink, 2005; Orrù, Napolitano, Catillo, & Moioli, 2006; Vázquez et al., 2004). The present work used commercial beef cattle herds and focused on how different consanguinity rates can affect the matching efficiency, and the minimum number of DNA markers needed for individual meat traceability.

2. Materials and methods

2.1. Biological samples and DNA extraction

This study was performed in a whole Angus beef supply chain in the Buenos Aires Province, Argentine. A 187 Angus individuals, from different farms in three typical South American production systems, were tagged with TypiFix™ ear tags (IDnostics, Prionics AG, Zurich, Switzerland). Blood samples were collected in Vacutainer® containing EDTA (BD Argentina srl, Buenos Aires, Argentina), and ear cartilage samples retrieved from the TypiFix™ ear tags. Samples included:

- 123 unrelated animals (URG), from a feeder farm that buys calves from different breeders.
- 27 related animals (RG), from a farm that fattens its own calves, and uses a natural multi sire mating system (this kind of herds typically mates 300 up to 400 dams with 9–12 sires).
- 37 half-sib animals (HSF), from a farm that fattens its own calves, and usually uses one or two bulls in an AI program; this would be the commercial herd with the highest consanguinity rate.

Total DNA was extracted from whole blood samples using DNAzol® (Invitrogen, Carlsbad, CA) following the supplier's instructions. Ear cartilage were retrieved from the TypiFixTM ear tags using a pneumatic extractor, and DNA extraction was performed using Nettexc Clean Columns kit (IDnostics, Prionics AG, Zurich, Switzerland; http://www.nexttec.biz).

2.2. Genetic markers, PCR amplification and genetic analysis

DNA genotyping was performed using 11-microsatellite markers standardized and recommended by the International Society for Animal Genetics (ISAG, http://www.isag.org.uk/): *ETH3*, *ETH10*, *ETH225*, *INRA023*, *BM1824*, *BM2113*, *SPS115*, *TGLA53*, *TGLA122*, *TGLA126*, and *TGLA227*.

The same DNA samples were independently genotyped at the Functional Genomics Laboratory (Parco Tecnologico Padano, Lodi, Italy), using the StockMarks® kit (AppliedByosystems, USA), and at IGEVET laboratory (Facultad de Ciencias Veterinarias, UNLP-CONICET, La Plata, Argentina), using a self developed kit. Fragments were resolved in an automatic DNA sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and a MEGABACE 1000 (GE Healthcare, USA), respectively (detailed protocols available upon request from the corresponding author). Allele sizes were standardized between laboratories using genotyping data from DNA reference samples and the ISAG nomenclature.

2.2.1. Statistical analysis

Allele frequencies were assessed by direct counting. The level of genetic variability was estimated by allelic diversity (Na; total number of alleles, average number of alleles, and number of alleles per locus), and the observed (Ho) and unbiased expected (He) heterozygosity using GENEPOP package (Raymond & Rousset, 1997). Hardy–Weinberg equilibrium (HWE) for each locus within population was estimated by F_{IS}, using the test included in the same package.

Mismatch distribution among pairs of samples in Complete Sample – CS (RG and URG combined samples), RG, URG, and HSF were calculated. Mismatch exclusion power (Q) and match probability (MP) were estimated for each marker and for the whole set, according to Weir (1996). All the parameters were calculated using algorithms programmed with Visual Basic, and implemented into Excel software (available upon request from the corresponding author).

3. Results and discussion

The total and average Na, average Ho and He for the whole marker set within CS, URG, RG, and HSF, are summarized in Table 1 (gene and genotype frequencies and single locus data are available upon request from the corresponding author). It needs to be underlined the low percentage of putative alleles (4.54%) compared to values previously reported (e.g. 23% in Dalvit et al., 2008), this discordance could be explained by the sampling as all animals came from the same (Angus) breed. Furthermore, Na was more affected by the consanguinity rate increment than He (Table 1); and the degree of Na and He observed in RG and URG were comparable to those previously reported for STR in other Angus populations (Lirón et al., 2007; Machugh, Shriver, Loftus, Cunningham, & Bradley, 1997). HWE tests over all loci within each sample group were also performed: three of them exhibited significant deviations from theoretical proportions (Table 1). The observed rate of disequilibrium was higher than expected by chance and could be a consequence of the population structure (Whalund effect) in CS and URG, or consanguinity in RG and HSF as most of the disequilibrium was due to homozygous excess.

Q for match between pairs of samples was estimated for each microsatellite within each group (Table 2). In addition, Q was estimated for the whole set of markers within each group, considering one and two mismatch criteria, resulting in exclusion powers higher than 0.99999997 and 0.999998 for Q_1 and Q_2, respectively (Table 1). Furthermore, MP was estimated, for each STR and for the whole set in each group (Tables 1 and 3), ranging from 2.41E−11 to 3.01E−08. These data showed that in the worst case-scenario (HSF) using 11 microsatellites, it is expected to find three individuals out of 100 million with the same genotype.

Several researchers have investigated the probability of matching two samples by chance, using a different number, and type of markers (microsatellite or SNP), in the context of individual

Table 1
Summary statistics of genetic variability and Hardy–Weinberg disequilibrium. Total (Na), observed (Ho) and expected (He) heterozygocities, p value of Hardy–Weinberg disequilibrium (HWE p-value), number of loci deviating from HWE, and whole set exclusion power for one (Q_1) and two (Q_2) mismatch criteria, and minimum number of markers (MNM): MNM_1 to reach $Q_2 = 0.9999$ and MNM_2 to reach $Q_2 = 0.999999$. CS, combined sample; URG, unrelated animals group; RG, related animals group; HSF, half-sib family.

Breed code	Na	Ho	He	HWE p-value	HWE deviations	MP	Q_1	Q_2	MNM_1	MNM_2
CS	8.00	0.680	0.734	0.0009	3	2.41E−11	0.99999999998	0.999999998	6	8
URG	7.91	0.671	0.734	0.0063	2	1.61E−11	0.99999999998	0.999999998	6	8
RG	5.73	0.707	0.706	0.0733	3	7.76E−10	0.99999999922	0.999999947	7	10
HSF	4.55	0.571	0.608	0.0000	4	3.01E−08	0.99999996993	0.999998466	7	>11

Table 2
Exclusion power for match samples (Q) estimated for 1–11 microsatellites in combined sample, unrelated animals group, related animals group, half-sib family. Genetic markers were given from higher to lower gene diversity.

No. of loci	Combined sample		Unrelated animals group		Related animals group		Half-sib family	
	Q_1	Q_2	Q_1	Q_2	Q_1	Q_2	Q_1	Q_2
1	0.95204468543	–	0.95137119824	–	0.91685144421	–	0.912829041	–
2	0.99723729120	0.897197329	0.99657990914	0.884460729	0.99159914535	0.824218017	0.991816566	0.827134487
3	0.99980354670	0.990123555	0.99959221746	0.983211772	0.99839815751	0.959683559	0.998794813	0.96756358
4	0.99997530107	0.998586538	0.99993996397	0.997180597	0.99960936857	0.988957068	0.9997905	0.993365819
5	0.99999706504	0.999810275	0.99999295656	0.999616234	0.99993837118	0.997928785	0.999959767	0.998556686
6	0.99999963276	0.999973693	0.99999902382	0.999940745	0.99999255991	0.999695766	0.999992185	0.999687226
7	0.99999994896	0.999996027	0.99999985463	0.999990345	0.99999888560	0.999948105	0.99999843	0.999930918
8	0.99999999251	0.999999373	0.99999999159	0.999999305	0.99999987615	0.999993242	0.999999581	0.999980418
9	0.99999999895	0.999999906	0.99999999892	0.999999903	0.99999997616	0.999998599	0.999999829	0.999991774
10	0.99999999985	0.999999986	0.99999999986	0.999999987	0.99999999538	0.999999709	0.999999938	0.999996902
11	0.99999999998	0.999999998	0.99999999998	0.999999998	0.99999999922	0.999999947	0.99999997	0.999998466

Q_1, one mismatch criteria; Q_2, two mismatch criteria.

Table 3
Match probability estimated for 1–11 microsatellites in combined sample (CS), unrelated animals group (URG), related animals group (RG), half-sib family (HSF). Genetic markers were given from higher to lower gene diversity.

No. of loci	CS	URG	RG	HSF
1	4.80E−02	4.86E−02	8.31E−02	8.72E−02
2	2.76E−03	3.42E−03	8.40E−03	8.18E−03
3	1.96E−04	4.08E−04	1.60E−03	1.21E−03
4	2.47E−05	6.00E−05	3.91E−04	2.10E−04
5	2.93E−06	7.04E−06	6.16E−05	4.02E−05
6	3.67E−07	9.76E−07	7.44E−06	7.82E−06
7	5.10E−08	1.45E−07	1.11E−06	1.57E−06
8	7.49E−09	8.41E−09	1.24E−07	4.19E−07
9	1.05E−09	1.08E−09	2.38E−08	1.71E−07
10	1.49E−10	1.38E−10	4.62E−09	6.20E−08
11	2.41E−11	1.61E−11	7.76E−10	3.01E−08

or breed meat traceability. These works, mainly focused on the information content of a marker set within a specific breed or group of breeds (reviewed in Dalvit et al., 2007). In most of these researches biological samples were collected from different farms and performance stations in different geographical areas, or from animals that did not share ancestors, at least at grandparent level, to get as much genetic variability as possible. Table 4 summarizes the match probability estimated by different authors using microsatellite or SNPs. The present work focused on how different extensive beef farming systems affect the efficiency of tracking individual meat pieces, considering that in these systems uneven rates of consanguinity are present. As shown in Table 1, Q_1, Q_2 and MP decreased when consanguinity increased. Nevertheless the worst scenario (Q_2 in HSF) still exhibited enough exclusion power to distinguish two individual random samples within the population, and all of them reached the range of MP values previously reported (Table 3; see discussion below).

The minimum number of markers needed to reliably trace an individual or a meat product is one of the major issues, as reviewed by Cunningham and Meghen (2001). To this purpose, two different approaches were followed: (i) cumulative Q_1, Q_2 and MP (Tables 2 and 3, and Figs. 1a–c), (ii) genotype mismatch distribution between pair-wise samples (Fig. 2). These analyses clearly showed that, in the most favourable scenario (Q_1 in CS and URG), the Q value increased exponentially (whereas MP decreased exponentially), reaching 99.99 with only four markers. To obtain the same degree of Q, up to six microsatellite markers were required when consanguinity was considered (Q_2 in CS and URG, Q_1 in RG and HSF), whereas seven markers were necessary (Table 2 and Figs. 1a and b) in the worst scenario (Q_2 in RG and HSF). Similar results were observed when MP was considered, thus CS and URG MP profiles were one or two orders of magnitude below RG and HSF (Table 3 and Fig. 1c). Furthermore, the genotype mismatch distribution between pair-wise analyses showed that all sampled animals differed in at least three markers, except HSF. The mode ranged between 7 and 9 markers. In the HSF group some animals differed only in one or two markers, whereas the mode ranged between 5 and 7. Previous works investigated how population variability affects marker discrimination (Sancristobal-Gaudy et al., 2000; Arana et al., 2002; Dalvit et al., 2008). The results of

Table 4
Match probability values obtained in recent studies of individual genetic traceability in cattle using microsatellites (short tandem repeats, STR) or single nucleotide polymorphism (from Dalvit et al., 2007).

Type and number of markers	Match probability	Breeds	References
STR – 11	10^{-8}–10^{-11}	Angus	Present work
SNP – 25	10^{-8}–10^{-10}	Holstein, Simmental, Limousin, Angus, Charolais, Tux Cattle	Karniol et al. (2009)
STR – 12	$>1.4 \times 10^{-8}$	Piemontese, Chianina, Marchigiana, Romagnola, Holstein Friesian, Brown Swiss	Dalvit et al. (2008)
STR – 12	1.9×10^{-11}	Piemontese, Chianina, Marchigiana, Romagnola	Dalvit et al. (2006)
SNP – 25	5×10^{-6}	Simulated data	Weller, Seroussi, and Ron (2006)
STR – 10	2.4×10^{-8}	Galloway	Herráez et al. (2005)
STR – 17	1.4×10^{-13}	Galloway	Herráez et al. (2005)
SNP – 43	5.3×10^{-11}	Galloway	Herráez et al. (2005)
SNP – 20	4.3×10^{-8}	Holstein Friesian, and others	Heaton et al. (2005)
SNP - 37	10^{-13}	German Fleckvieh, German Braunvieh	Werner et al. (2004)
SNP – 32	2.0×10^{-13}	American Angus	Heaton et al. (2002)
SNP – 32	1.9×10^{-10}	Multi-breed composite populations	Heaton et al. (2002)
STR – 10	$>10^{-7}$	Pirenaica	Arana et al. (2002)
STR – 13	$>10^{-15}$	Piemontese, Chinina, Holstein Friesian, Italian Simmental	Orrù et al. (2006)
STR – 11	5×10^{-12}	Charolaise	Sancristobal-Gaudy et al. (2000)
STR – 10	1×10^{-10}	Belgium beef cattle	Peelman et al. (1998)

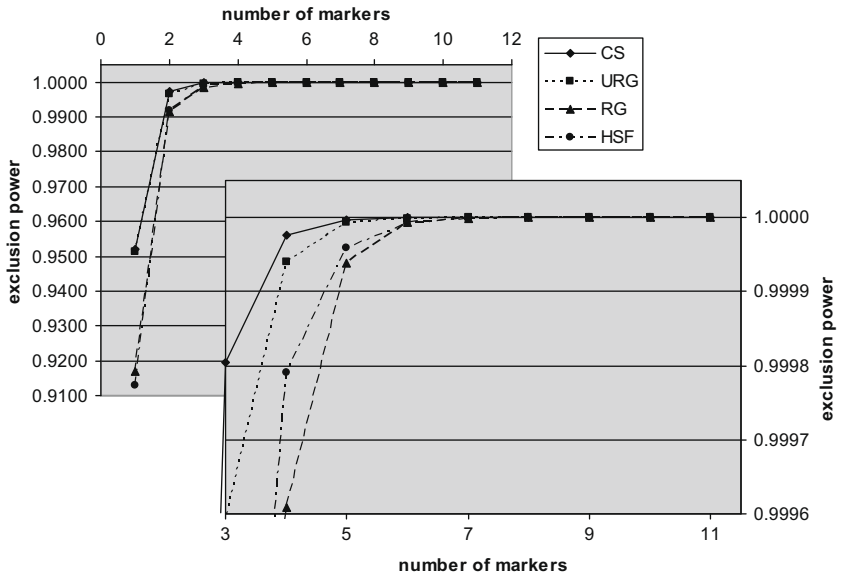

Fig. 1a. Cumulative exclusion power Q_1 calculated for combined sample (CS), unrelated (URG), related (RG) and half-sib family (HSF) groups. Genetic markers were given from higher to lower gene diversity.

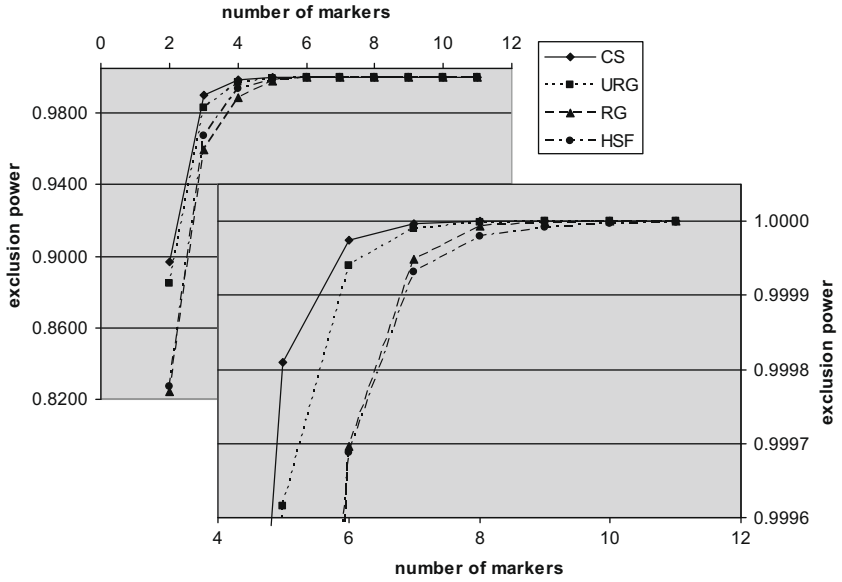

Fig. 1b. Cumulative exclusion power Q_2 calculated for combined sample (CS), unrelated (URG), related (RG) and half-sib family (HSF) groups. Genetic markers were given from higher to lower gene diversity.

the present work are in agreement with the data obtained by Arana et al. (2002), who showed, by using simulated data, that reliable individual DNA based identification was obtained using at least eight markers. MP was lower than 10^{-7} when more than seven markers were used (Table 3).

Based on the average number of slaughtered animals in Italy per year, Dalvit et al. (2008) estimated 5 loci are enough to verify the label information if the breed of origin is known. A panel of eight microsatellite markers is suitable for the reliable genetic identification of all slaughtered animals (beef and dairy breeds) in Italy per year. In Argentina approximately 1,200,000 animals are slaughtered each month, and on average one slaughterhouse processes between 500 and 800 animals a day (ONCCA, 2008; Rearte, 2007). Angus cattle represent approximately 50% of the slaughtered cattle, and 20% is represented by its crosses (Angus XXI). Data produced in the present study showed that when considering CS group: 3 (4 for HSF), 4 (5 for HSF), and 7 (9 for HSF) markers are enough to reliably identify each single individual in one slaughterhouse among all the animals slaughtered in 1 week, 1 month, and 1 year, respectively. When considering all the animals slaughtered in Argentina in 1 week, 1 month, and 1 year, 5 (7 for HSF), 6 (8 for HSF), and 7 (10 for HSF) markers are enough, respectively. Last but not least, the set of 11 markers suggested by ISAG for cattle would be suitable for DNA traceability even though all Angus slaughtered in Argentina in a year were sired by the same bull.

In conclusion, when the whole set of microsatellite was considered, Q higher than 0.999998 and MP lower than 3.01E−08 were obtained in all cases (UG, RG, CS, and HSF). However, the minimum number of markers needed for a reliable DNA based

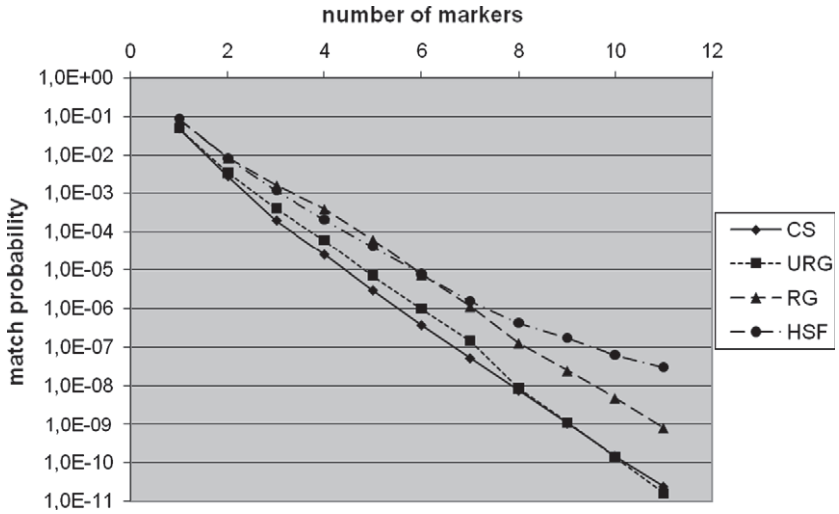

Fig. 1c. Match probability (showed in $\log_{10}$ scale) calculated for combined sample (CS), unrelated (URG), related (RG) and half-sib family (HSF) groups. Genetic markers were given from higher to lower gene diversity.

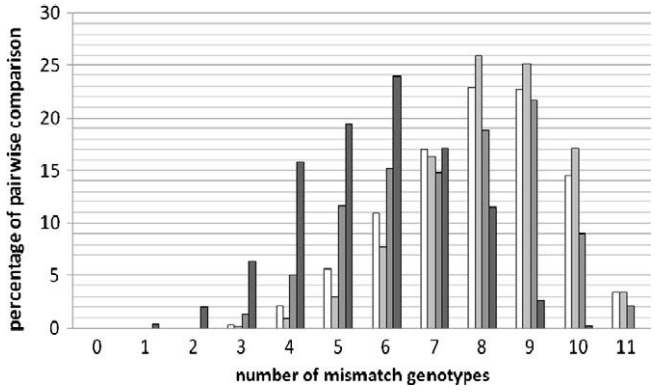

Fig. 2. Genotypes mismatch distribution between pair-wise samples. White bars, combined sample; light gray bars, unrelated animals group; dark gray bars, related animals group; black bars, half-sib family.

traceability assay increases by at least two microsatellites in the presence of consanguinity.

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