



Ancient autochthonous genetic type (AAGT) ‘Casertana’ pig : genetic characterization by microsatellite analysis

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Introduction

AAGTs may represent a precious source of genetic variability able to meet the dynamic consumer needs as well as to valorize peculiar microagrieosystems. Casertana pig AAGT, known ever since antiquity and defined the ‘Italian pig pride’ by Hoesch (early half of 10th century), is currently, mostly present in Campania region (Italy). A wide safeguard programme promoted by ConSDABI is in progress for this AAGT.

The aim of this research was to contribute to knowledge of genetic variability ‘status’ of this AAGT.

Material and Methods

Microsatellite analysis, up to today, has been carried out on 30 subjects belonging to ‘Casertana’ (CT) AAGT examining 20 microsatellite loci (table I). The typification involved: (a) DNA isolation from blood samples (Sambrook *et al.*, 1989); (b) DNA amplification of 20 microsatellite loci; (c) individual genotype assignment by ABI PRISM 310 automated sequencer (Applied Biosystem); (d) use of GENEPop (Raymond e Rousset, 1995) and GENETIX (Belkhir, 2000) to estimate: (i) allele number per locus; (ii) expected and observed heterozygosity; (iii) deviation from Hardy-Weinberg equilibrium. FST (Inbreeding or Fixation index of subpopulation relative to the total population) was determined by FSTAT (Goudet, 2001) in order to estimate the degree of differences among subpopulations.

For CT AAGT, data were elaborated: (i) only on subjects typified by ConSDABI; (ii) on 58 pigs, integrating the above 30 animals with other 28 heads typified in the context of European PigBiodiversity project (Ollivier *et al.* 2005; SanCristobal *et al.*, 2006) using blood samples provided by ConSDABI experimental Farm. Furthermore, a third statistic elaboration, limitedly to 10 loci, interested a comparison among three Italian AAGTs [CT (n = 58); ‘Calabrese’ (CA, n= 19); ‘Cinta senese’ (CS, n=30) reared at ConSDABI too.



Results

CT (n=30) for 20 microsatellite loci

- More polymorphic loci :
 - ♦SW2038, SW1370, SO017, SW1823, SW1556, SW1873, each with 5 alleles, accounted for 40% of the total allele number relieved.
- Average allele number per locus: 3.8 ± 0.93 (c.v.= 24 %).
- Average expected and observed heterozygosity: 0.58 ± 0.13 (c.v.= 23 %) and 0.66 ± 0.17 (c.v.= 27 %), respectively.
- No locus diverged significantly from Hardy-Weinberg equilibrium under heterozygote deficit hypothesis.

CT (n=58) for 10 microsatellite loci

- More polymorphic loci: S0005 with 6 alleles and SW936, SW911, SW 240, each with 5 alleles, accounted for 51,3% of the total allele number relieved.
- Average allele number per locus: 4.1 ± 1.14 (c.v.= 28 %).
- Average expected and observed heterozygosity: 0.57 ± 0.10 (c.v.= 18 %) and 0.58 ± 0.09 (c.v.= 15 %), respectively.
- 3 loci diverged significantly from Hardy-Weinberg equilibrium under heterozygote deficit hypothesis.

TABLE I - Investigated microsatellite loci.

MICROSATELLITE LOCUS				
N	ACRONYM	CHROMOSOMAL LOCATION	ALLELE SIZE, bp	FORWARD AND REVERSE PRIMER SEQUENCE (5'-3')
1	SO155	1	150-166	TGTTCTCTGTTCTCTCTGTTTG AAAGTGGAAGAGTCAATGGCTAT
2	SW240	2	96-115	AGAAATAGTGCTCAATTTGG AAACCTTAAGTCCCTAGCAAA
3	SW1370	2	88-109	AGAGCAGTGGTCTGCTAAGATG GAATTCCTAAATTTACTTGCC
4	SW1695	2	171-205	ATAAGGAAATCAGGCTGAGC TCCAGGAGCTACCATGCG
5	SW72	3	100-116	ATCAGAACATGGCGCGCT TTTGAAATGGGGTGTTC
6	SW153	4	219-243	CCAGTGTCTCTTTTGAGG ATGAGTTGTGGTGTAGTGCG
7	SO005	5	205-248	TCCTTCCTCTGCTGAACCTA GCACCTCTGATCTGGGTA
8	SW1823	6	201-217	CAGGTCATGCTGTAGTGAAGG GAGCTTGGGCTACGTAGTG
9	SO228	6	222-249	GGCATAGCTGGCAGCAACA AGCCACCTCATCTTATACACT
10	SW1873	7	105-138	TATAATCTGGTGAACTACCC ATCAGATGTGCTATACCTCG
11	SW1928	7	83-107	TAGGTCAGTGATCTCTTC ACGAGAACTCGAACCTCG
12	SO101	7	197-216	GAATGCAAGAGTTCAGTGAAG GTCTCCCTCACACTTACCGCAG
13	SO017	8	155-179	CTAGAGAAAATCTGAGGTT GTTTGAATGGAGTCTGTGA
14	SW911	9	153-177	CTCAGTCTTTGGAGTGAACC CATCTGTGGAAAAAAGGCC
15	SW857	14	144-160	TGAGAGGTCAGTTACAGAGACC GATCTCTCTCAATCCCAT
16	SW1556	14	220-274	TCCAGACCTGATGATTTAG AGGTTGCTGGAGATGTGAAGC
17	SW2038	14	107-127	GCCGAGAAACCTCTCAC TAGCTGTTCAGTGCCACC
18	SO355	15	243-277	TCTGGCTCTACACTCTCTTGATG TTGGGTGGGTGCTGAAATAAGGA
19	SW936	15	80-117	TCTGGAGTCAGCATAGTGCC GTGCAAGTACACATCAGGG
20	SW1835	16	147-167	TATGGGGGCCCTAAAAGAC AAGGCCCTTAACCTCTCAG

Comparison among CT (n=58), CA (n= 19), CS (n= 30) for 10 microsatellite loci

TABLE II- Average allele number per locus, observed and expected heterozygosity in CT, CA and CS AAGTs.

CASERTANA		CALABRESE		CINTA SENESE	
$\bar{x} \pm \sigma$	c.v., %	$\bar{x} \pm \sigma$	c.v., %	$\bar{x} \pm \sigma$	c.v., %
4.1 ± 1.14	28	3.2 ± 0.92	29	3.9 ± 1.37	35
ALLELES, N					
OBSERVED HETEROZYGOSITY					
0.57 ± 0.10	18	0.58 ± 0.26	44	0.50 ± 0.25	50
EXPECTED HETEROZYGOSITY					
0.58 ± 0.09	15	0.50 ± 0.22	43	0.47 ± 0.22	46

- The most of alleles (65%) were common to the 3 genetic types (table III); some alleles, evidenced in different colour, were genetic type - ‘exclusive’ and their frequency ranged between a minimum value of 0.009 [allele 215, SO005 locus (CT)] to maximum of 0.447 [allele 230, SW 228 locus (CA)]; locus, the latter, monomorphic (224/224 genotype) in CS AAGT.
- F_{ST} value, equal to 0.236, indicates, according to Wright’s classification (1978), an ‘important’ diversification degree, being in the range ‘0.150÷0.250’ proper of this class.

Conclusions

In the limits of observation field, ‘Casertana’ AAGT preserves a discrete genetic variability although it derived from a low number of ‘fundators’. Furthermore, from comparison among the three AAGTs (CT, CA, CS), it emerges the presence of AAGT-‘exclusive’ alleles useful for discriminating the origin of meat product.

TABLE III - Microsatellite loci examined, alleles present and their frequency within locus in the typified AAGTs.

LOCUS			ALLELES, bp (1) AND ALLELE FREQUENCY (2)													
			(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
SW72	(2)	CT	97	0.348	108	0.223	0.429									
		CA	0.526	0.184	0.289											
		CS	0.650	0.333	0.017											
SW936	(2)	CT	90	0.088	92	0.018	0.026	0.000	0.000	0.000	0.482	0.386				
		CA	0.211	0.299	0.000	0.626	0.053	0.421								
		CS	0.000	0.000	0.138	0.000	0.431	0.397	0.034							
SW911	(2)	CT	155	0.096	159	0.535	0.175	0.035	0.158	0.000						
		CA	0.026	0.895	0.000	0.079	0.000	0.000	0.000							
		CS	0.083	0.5	0.017	0.183	0.200	0.012								
SW228	(2)	CT	220	0.000	222	0.383	0.647	0.000								
		CA	0.132	0	0.421	0.447										
		CS	0.000	0.000	1.000	0.000										
SO155	(2)	CT	146	0.063	148	0.098	0.241	0.339	0.000	0.454						
		CA	0.000	0.000	0.384	0.438	0.116	0.000								
		CS	0.250	0.050	0.000	0.033	0.517	0.150								
SO005	(2)	CT	203	0.027	215	0.009	0.000	0.670	0.018	0.000	0.205	0.000	0.000	0.000	0.000	0.071
		CA	0.000	0.000	0.000	0.289	0.000	0.458	0.342	0.000	0.231	0.000	0.000			
		CS	0.000	0.000	0.350	0.000	0.400	0.000	0.033	0.167	0.000	0.000	0.000	0.000	0.000	0.000
SW240	(2)	CT	92	0.000	94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		CA	0.000	0.000	0.500	0.447	0.000	0.000								
		CS	0.033	0.067	0.483	0.417	0.000	0.000								
SW857	(2)	CT	141	0.000	149	0.107	0.563	0.000	0.000	0.230	0.000					
		CA	0.132	0.658	0.000	0.158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		CS	0.033	0.767	0.183	0.017	0.000	0.000								
SW101	(2)	CT	206	0.000	210	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		CA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		CS	0.083	0.800	0.100	0.017										
SO355	(2)	CT	244	0.518	246	0.053	0.009	0.000	0.421							
		CA	0.974	0.000	0.000	0.000	0.026	0.000								
		CS	0.800	0.150	0.050	0.000	0.000	0.000								

*The alleles evidenced in different colour and peculiar type were detected, in the limits of observation field, exclusively in the genetic type of corresponding colour and type : CT, CA, CS.