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Use of single nucleotide polymorphisms to study variability in local and cosmopolitan pig breeds reared in Italy

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SUMMARY – The genetic characterisation of endangered local breeds is an important step to preserve them from extinction. Moreover, these breeds could be useful to explain the phenotypic differences of productive and reproductive traits and to maintain sustainable agriculture in the regions were they are bred. With this research we characterised four Italian local breeds (Casertana, Calabrese, Cinta Senese, Nero Siciliano) and six widespread breeds reared in Italy (Italian Large White, Italian Landrace, Duroc, Belgian Landrace, Hampshire, Piétrain) for single nucleotide polymorphisms (SNPs) detected in 15 loci.

Keywords: Pig, local breeds, SNP, genetic diversity.

***RESUME –** "Utilisation de SNP pour étudier la variabilité chez des races porcines locales et cosmopolites élevées en Italie". La caractérisation génétique des races porcines locales est importante pour prévenir leur extinction. Il faut envisager leur sauvetage pour contribuer à expliquer les différences de le caractéristiques productives et reproductives et aussi pour rendre possible un élevage durable dans leur région d'origine. L'objectif de cette étude est de caractériser quatre races locales italiennes (Casertana, Calabrese, Cinta Senese, Nero Siciliano) et six races cosmopolites élevées en Italie (Large White Italien, Landrace Italien, Duroc, Landrace Belge, Hampshire, Piétrain) pour les single nucleotide polymorphisms (SNPs) détectés dans 15 loci.*

***Mots-clés :** Porc, races locales, SNP, diversité génétique.*

Introduction

Calabrese (CA), Casertana (CE), Cinta Senese (CS), Mora Romagnola (MR), and Nero Siciliano (NS) are the Italian local breeds classified as endangered by EAAP recommendations (Simon and Buchenauer, 1993) and included in the pig pedigree registry of ANAS (National Association of Pig Breeders). They represent the porcine genetic resources still alive in Italy after the lost of several breeds during the 20th Century when the cosmopolitan breeds were introduced. These breeds can provide potential sources of novel gene variants useful to elucidate the molecular basis contributing to phenotypic differences for productive, reproductive and disease resistance traits. Moreover, the maintenance of these traditional breeds can allow a better utilisation of the specific environment where they are bred and the preserving of typical products. A prerequisite of a biodiversity conservation programme is a description of genetic variability among breeds that can be obtained using anomymous DNA markers like microsatellites (San Cristobal *et al*., 2005) that can be complemented with information of polymorphisms in genes coding for proteins with known function (Ciobanu *et al*., 2001).

To this aim we studied genetic variability in four porcine Italian local breeds and six international pig breeds reared in Italy using single nucleotide polymorphisms (SNPs) in major and candidate genes for performance traits.

Materials and methods

DNA samples of 10 pig breeds were used, some of them already included in the EU PigBioDiv project (San Cristobal *et al*., 2005). For each breed the minimum and the maximum numbers of samples analysed were as follows: CA (8-16), CE (8-17), CS (13-15), NS (27-119), Italian Large White (LW;25-257), Italian Landrace (IL;21-150), Duroc (DU;25-154), Belgian Landrace (BL;16-44), Hampshire (HA;10-37), and Piétrain (PI;10-48). The animals were tested for SNPs in 15 loci mapping on 11 autosomes (Table 1).

Table 1. List of the analysed loci used for this research and their chromosome localisation (Chr)

Locus	Symbol	Chr	References
2,4-dienoyl CoA reductase 1, mitochondrial	DECR1	4	Davoli *et al.* (2002)
Cathepsin B	CTSB	14	Russo *et al.* (2002)
Cathepsin F	CTSF	2	Russo *et al.* (2004a)
Cystatin B	CSTB	13	Russo *et al.* (2002)
Estrogen receptor	ESR	1	Short *et al.* (1997)
Glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)	GAA	12	Fontanesi et al. (2003)
Myopalladin	MYOP	14	Davoli et al. (2003a)
Myosin light chain 2 (Clone PWHLC2-24)	HUMMLC2B	3	Davoli *et al.* (2003b)
Myosin, heavy polypeptide 4, skeletal muscle	MYH4	12	Davoli *et al.* (2003b)
Na+, K(+)-ATPase subunit alpha 2	ATP1A2	4	Blazkova *et al.* (2000)
Phosphoglycerate mutase 2	PGAM2	18	Fontanesi *et al*. (2003)
Protein kinase, AMP-activated, beta 1 non-catalytic subunit	PRKAB1	14	Fontanesi *et al.* (2003)
Protein kinase, AMP-activated, gamma 3 non-catalytic subunit	PRKAG3 (I199V)	15	Milan et al. (2000), Fontanesi *et al.* (2003)
Pyruvate kinase, muscle	PKM2	7	Fontanesi et al. (2003)
Ryanodine receptor 1 (Calcium release channel)	RYR1 (CRC)	6	Fuji *et al.* (1991), Russo *et al.* (1993)

Genotyping was carried out by PCR-RFLP and PCR-SSCP techniques as previously reported. For a complete description of the methods see the references included in Table 1. DISPAN program (Ota, 1993) was used to estimate allele frequencies, average heterozygosity (H) within breed and Nei's genetic distances (D) between breeds (Nei, 1972).

Results and discussion

A total of 34 distinct alleles were observed analysing 15 loci. Italian Large White, Italian Landrace, and Nero Siciliano were polymorphic for all loci whereas Casertana showed the lowest number of polymorphic loci (Table 2). A high level of genetic variability in Nero Siciliano breed, was already reported in the PigBiodiv project, where microsatellites were used (San Cristobal *et al*., 2005).

Table 2. Average heterozygosity (H) ± standard error (SE) and polymorphic loci for each breed

Breed	H ± SE	No. of polymorphic loci	Monomorphic loci
LW	0.378 ± 0.037	15	–
IL	0.367 ± 0.045	15	–
DU	0.279 ± 0.053	13	ESR, MYH4
HA	0.297 ± 0.051	13	ESR, RYR1
PI	0.329 ± 0.046	13	ESR, PRKAB1
BL	0.355 ± 0.047	14	PRKAB1
CS	0.287 ± 0.052	13	HUMMLC2B, PRKAB1
CA	0.348 ± 0.043	14	CTSF
CE	0.330 ± 0.053	12	MYOP, PGAM2, RYR1
NS	0.304 ± 0.032	15	–

Three loci (*ESR*, *PRKAB1* and *RYR1*) were monomorphic in at least 2 breeds (Table 2). For two loci *RYR1* and *ESR* it is worth to report on the results obtained. At the *RYR1* locus, allele 1843T was found in 3 local breeds, Cinta Senese, Casertana and Nero Siciliano with frequencies of 0.07, 0.16 and 0.08, respectively. This allele was found in heterozygous state with the exception of a homozygous TT Nero Siciliano pig. The presence of allele 1843T in Nero Siciliano was already reported by Russo *et al*. (2004b) with a lower frequency. For the three local breeds, these results may indicate crossing with other breeds in which this *RYR1* allele is present. So far, no report indicates poor meat quality or stress susceptibility in Italian local breeds, thus it will be interesting to evaluate if their genetic background may dilute or eliminate the negative effect of allele 1843T.

At the *ESR* locus Casertana and Calabrese breeds showed a frequency of allele B of 0.21 and 0.39, respectively, a level close to 0.35 found in Italian Large White. In Meishan and Large White breeds, allele B has been associated with an increase in litter size (Rothschild *et al*., 1997; Short *et al*., 1997). The level of variability at the *ESR* locus makes it possible to study the influence of this gene on reproductive performances in the local breeds. The study of genes affecting these traits is important because reproductive parameters like the number of piglet born alive per parity, low in local breeds, need to be improved (Franci *et al*., 2001).

Average heterozygosity (Table 2) ranged from 0.279 for Duroc to 0.378 for Italian Large White. Local breeds compared with the cosmopolitan ones, maintain a quite high level of heterozygosity. Calabrese, Casertana and Nero Siciliano presented higher values than Duroc and Hampshire although a low level of genetic variability in endangered local breeds could be expected owing to their reduced population size. Among the local breeds, Cinta Senese showed the lowest average heterozigosity (0.287) and Duroc among the cosmopolitan ones presented the minimum value (0.279). The low level of heterozygosity described here for Duroc is in agreement to what was observed by Davoli *et al*. (1996) who calculated an average heterozygosity of 0.2927 analysing 25 RFLPs by Southern blot analysis.

Table 3. Nei's genetic distances ± standard error between breeds. The grey box outlines the comparisons between the local and the cosmopolitan breeds

	LW	IL	DU	HA	PI	BL	CS	CA	CE
IL	0.0636 ±0.0243								
DU	0.1296 ±0.0640	0.0128 ±0.0745							
HA	0.0769 ±0.0234	0.1041 ±0.0423	0.1426 ±0.0714						
PI	0.1347 ±0.0979	0.1069 ±0.0824	0.1890 ±0.1010	0.1596 ±0.1036					
BL	0.1827 ±0.1031	0.1067 ±0.0865	0.2742 ±0.1158	0.2464 ±0.1114	0.0395 ±0.0224				
CS	0.1291 ±0.0649	0.0861 ±0.0293	0.1515 ±0.0854	0.0813 ±0.0420	0.1547 ±0.0990	0.2634 ±0.1188			
CA	0.1032 ±0.0479	0.1092 ±0.0457	0.1776 ±0.0840	0.1590 ±0.0855	0.2247 ±0.0937	0.2447 ±0.0944	0.2490 ±0.1109		
CE	0.0843 ±0.0764	0.1452 ±0.0905	0.0753 ±0.0296	0.1021 ±0.0399	0.1827 ±0.1124	0.2648 ±0.1362	0.1991 ±0.1046	0.1733 ±0.0772	
NS	0.0326 ±0.0112	0.0862 ±0.0241	0.1004 ±0.0616	0.1091 ±0.0540	0.1361 ±0.0857	0.2131 ±0.0983	0.1559 ±0.0892	0.1000 ±0.0467	0.1067 ±0.0750

The Nei's standard genetic distances calculated are reported in Table 3.

Genetic distances calculated between the Italian local and some cosmopolitan breeds (Italian Large White, Italian Landrace, Duroc and Hampshire) are unexpectedly reduced and in some cases lower than those calculated for the comparisons of local breeds one to each other. The minimum value of distance (0.0326) was obtained between Nero Siciliano and Italian Large White.

Cinta Senese is, on average, less similar to the other local breeds. This result could be partially explained by the geographic distance of Tuscany in the central part of Italy (the main rearing area of this breed) from Southern regions were the other local breeds are bred and the consequent isolation with unlikely crossbreeding.

Conclusions

This study is a first contribution to the knowledge of genetic variability in four Italian local breeds using SNPs. In the studied local breeds for the analysed loci, we found an unexpectedly high value of heterozigosity comparable to that was observed in cosmopolitan breeds. The presence of a quite high level of genetic variability, mainly in genes that have been associated to or could affect performance traits, is an interesting element to evaluate for the genetic improvement of local breeds by plans of marker assisted selection. Additional studies including more SNPs and larger samples are needed for a more detailed genetic analysis of these populations.

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