PAPER

Genetic characterization and structure of the Italian Podolian cattle breed and its relationship with some major European breeds

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Abstract

The Italian Podolian is an autochthonous breed belonging to the grey steppe cattle group. In the past, the Podolian was present throughout all of the Adriatic and the southern part of Italy, but now it is restricted to four regions and displays morphological differences according to the region. It is thought that the Podolian contributed heavily to the genetic make-up of the Chianina and Romagnola breeds, while contrasting opinions exist regarding the relationship between the Podolian and the Piemontese. This paper aimed at characterizing genetic variability and structure by means of microsatellite *loci* to assess the relationship between the Podolian and the most relevant European breeds, and to detect evidence of selection in the considered breeds by investigating the existence of outlier *loci*. The DNA of 134 Italian Podolian individuals from 14 different herds in 4 breeding areas were collected, genotyped at 30 microsatellite *loci* and compared to 13 Italian and European breeds, including the grey type ones and using N'dama as the outgroup. The Podolian showed the highest values of observed heterozygosity and polymorphism content. Moreover in a subset of 50 samples (chosen according to geographic origin), polymorphism data analysis evidenced the absence of a geographically determined genetic structure. The hierarchical population clustering evidenced a Podolian cluster at K=5 and the phylogenetic tree based on Reynold's distances confirmed the genetic links between the Podolian breeds. Two *loci* presented significantly low FST values, suggesting that they could be under the effect of balancing selection. It can be concluded that Italian Podolian has no genetic subdivision, contains a notable amount of genetic variability and is closely related to the other Podolian type breeds.

Introduction

The Italian Podolian (ITPOD) is one of the grey cattle breeds reared in Italy (including the Romagnola and Chianina) and is part of a larger European family (i.e. the Hungarian Grey, Istrian).

The presence of Podolian in Italy has been shown since the ancient times (Baker and Manwell, 1980; Ciani and Matassino, 2001; 2007). In the past, the breed was reared throughout all of the Adriatic and the southern part of the country, but currently the breeding areas are restricted to the regions of Southern Italy. The Italian Podolian originated in the Podolian region of Eastern Europe, as indicated by its name, and it is thought that the breed related to Chianina and Romagnola breeds (Astolfi *et al.*, 1983) and also to Istrian Grey (FAO, 2011). The breed is well adapted for exploiting the Mediterranean scrub and highlands, so it can be considered as a genetic resource for future breeding options aimed at enhancing animal production in marginal areas by using the environment in a sustainable way. In the eighties, a selection scheme aimed at improving meat production was implemented, but the use of artificial insemination is still limited and the selection scheme is not fully applied. For this reason, a genetic structure that differentiates Podolian populations reared in different areas may be hypothesized due to the genetic isolation between population and could justify the slight morphological differences observed between the regions. Over the last few decades, this breed has experienced a drastic reduction in numbers and now about 24,000 animals are registered in the herd book (ANABIC, 2011), while the total population is estimated to consist of more than 100,000 heads.

In order to ascertain genetic variability and population structure at the DNA level, different types of markers can be investigated. A fundamental requisite is the absence of selection pressure on the considered *loci*, since it was demonstrated that the inclusion of a few out-

liers among neutral *loci* can greatly affect the estimate of population indexes (Luikart *et al.*, 2003). Outlier *loci* can be efficiently identified by estimating single *loci* FST values (Beaumont and Nichols, 2003). Microsatellites have been extensively used to characterize the population genetics of local and cosmopolitan cattle breeds (Brenneman *et al.*, 2007; Canon *et al.*, 2008; Dadi *et al.*, 2008; Dalvit *et al.*, 2008; Ndumu *et al.*, 2008) and to unravel the genetic distance and relationship between breeds (McHugh *et al.*, 1994; Moazami-Goudarzi *et al.*, 1997; Ciampolini *et al.*, 1995; Canon *et al.*, 2001; Del Bo *et al.*, 2001; Wiener *et al.*, 2004; European Cattle Genetic Diversity Consortium, 2006; Martin-Burriel *et al.*, 2007; Sun *et al.*, 2008). Using the same molecular markers, the genetic variability of other Podolian breeds of Eastern Europe has been investigated Georgescu *et al.*, 2009; Manatrinon *et al.*, 2008), as well as that of the Italian Podolian (Moioli *et al.*, 2004; D'Angelo *et al.*, 2006). However, in the previous studies the existence of genetic structure in the Italian Podolian as well as its relationship with the main European breeds have never been investigated. Moreover, the existence of outlier *loci* has never been tested. The aim of this paper was to characterize the genetic variability and structure of the Italian Podolian and to ascertain the genetic relationships between this breed and the Italian breeds included in the RESGEN project and the most relevant European breeds. The genomes were analyzed by means of microsatellite markers and the existence of outlier *loci* and their effect on genetic distance and structure were also considered.

Materials and methods

A total of 134 unrelated individuals belonging to 14 different herds in 4 Italian regions (2 from Molise, 1 from Puglia, 10 from Basilicata and 1 from Campania) were sampled by collecting peripheral blood samples. The DNA was isolated from lymphocytes by classical phenol-chloroform extraction methods (Sambrook *et al.*, 1989). The individual genotypes were determined using the panel of 30 microsatellites suggested by FAO (ISAG-FAO, 2004) for cattle biodiversity investigations (*BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH152, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILST005, ILST006, INRA005, INRA023, INRA0032, INRA0035, INRA037, INRA063, MM12, SPS115, TGLA53, TGLA122, TGLA126, TGLA227*). Amplification was performed as suggested by the ISAG/FAO Standing Committee Recommendations (ISAG/FAO, 2004).

Furthermore, a subset of 50 out of the 134 individuals balanced for the region of origin was selected and the obtained genotypes werw standardized using three reference samples on order to make them comparable to the genotypes obtained by the European Cattle Genetic Diversity Consortium in the RESGEN project N. CT98-118 of the European Union 5th Framework Programme. The breeds considered for comparison were the Italian Grey Podolian type breeds: Romagnola (ITROM), Chianina (ITCHI), and Piemontese (ITPIM), other Italian breeds: Rendena (ITREN), Cabannina (ITCAB), Pezzata Rossa Italiana (ITPRI) and Grigio Alpina (ITGRI), a grey coated breed apparently not related to the Podolian breed, and Istrian (CRIST), a Podolian breed from Croatia. Some of the most representative European and cosmopolitan breeds were also considered: Swiss Brown (CHSWB), Simmental (CHSIM), Holstein Friesian (NLFRH), and Limousine (FRLIM). N'Dama (NDA) breed was selected as outgroup. An average of 40 genotyped individuals per breed was analyzed. Genotypes from the Podolian dataset were elaborated with the GENEPOP ver. 3.1d computer package (Raymond and Rousset, 1995) to obtain allelic frequencies and the number of alleles for each *locus*, and the observed and expected heterozygosities were obtained with the ARLEQUIN ver. 2.000 (Schneider *et al.*, 2000) computer package by applying the method of Guo and Thompson (1992). The computations required to detect outlier *loci* were performed using the approach proposed by Beaumont & Nichols (1996). The detection of outlier *loci* was performed following the FST outlier approach developed by Beaumont and Nichols (1996) further developed by Beaumont and Balding (2004), and implemented in the FDIST2 software (*http://www.rubic.rdg.ac.uk/~mab/software. html*). This method evaluates the relationship between FST and He (expected heterozygosity) by calculating the expected distribution of Wright's coefficient FST vs He under an island model of migration with neutral markers. This distribution is then used to identify outlier *loci* that have excessively high or low FST values compared to neutral expectations. Such outlier *loci* are candidates for being under directional selection.

Structure software 2.3 (Pritchard *et al.*, 2000) was used to reveal the existence of genetic variability partitioning. In order to analyze the relationships between the European breeds, ten independent runs of each K, testing K ranging from 2 to 15, with 100,000 Markov chain Monte Carlo (MCMC) iterations and a burn-in period of 100,000, were performed using the admixture model considering allele frequencies between populations as independent with no prior information about individual membership. The method by Evanno for testing K (Evanno *et al.*, 2005) was applied to detect the strength of the genetic structure detected by the software. DISTRUCT (Rosenberg, 2004) software was used to visualize the estimated membership coefficients. PowerMarker software (Liu and Muse, 2005) was applied in order to compute Reynold's genetic distances between breed pairs and to construct and display the phylogenetic tree using the neighbour-joining (NJ) algorithm, which was visualized using Treeview software (Page, 1996).

Results and discussion

All *loci* were successfully amplified and found to be polymorphic in the 134 Italian Podolian samples. The average number of alleles per *locus* was 9.9. A deviation from the Hardy-Weinberg equilibrium (P<0.05) was detected in 11 out of the 30 *loci* (Table S1, Appendix). The results of the basic population parameters in all of the examined European breeds using all the 30 *loci* are shown in Table 1. The Italian Podolian breed showed the highest values of expected (0.73) and observed (0.71) heterozygosities, while the Simmental displayed the lowest values (0.59) for both measures. The difference between expected and observed heterozygosity in the Italian Podolian resulted in a slightly positive inbreeding coefficient (0.05). The observed heterozygosities were higher than those reported for the British breeds investigated using the same microsatellite set (Wiener *et al.*, 2004), whose values ranged from 0.56 (Highland) to 0.67 (Ayrshire), and for local Spanish, French and Portuguese breeds analyzed at 16 microsatellite *loci* of the FAO list (values ranging from 0.58 to 0.71 in Salers and Barrosa, respectively) (Canon *et al.*, 2001). The same microsatellites were also investigated in the Hungarian Podolian, where a lower value of observed heterozygosity (0.66) was obtained (Manatrinon *et al.*, 2008), whereas in the Romanian Podolian, in which 11 *loci* were analyzed (Georgescu *et al.*, 2009), the value was comparable (0.76) to that of the Italian population. Genetic variability in the Italian Podolian breed was investigated in a previous study (D'Angelo *et al.*, 2006), which found a lower value of observed heterozygosity (0.44). This

Table 1. Diversity measurements for each of the cattle populations analysed in the current study.

	Code	Sample size	He	Ho	PIC	f
Podolian	ITPOD	50	0.73	0.71	0.70	0.05
Piemontese	ITPIM	48	0.72	0.71	0.68	0.03
Istrian	CRIST	45	0.71	0.70	0.67	0.03
Rendena	ITREN	34	0.67	0.65	0.62	0.05
Limousin	FRLIM	50	0.67	0.65	0.62	0.03
Swiss Brown	CHSWB	50	0.66	0.67	0.62	0.00
Cabannina	ITCAB	26	0.66	0.64	0.62	0.05
Grigio Alpina	ITGRA	28	0.66	0.64	0.62	0.06
Chianina	ITCHI	36	0.66	0.65	0.61	0.02
Holstein Friesian	NLFRH	34	0.64	0.62	0.59	0.05
Pezzata Rossa Italiana	ITPRI	49	0.64	0.65	0.59	-0.01
Romagnola	ITROM	32	0.63	0.63	0.59	0.02
Simmental	CHSIM	50	0.59	0.59	0.55	0.02

He, expected heterozygosity; Ho, observed heterozygosity; PIC, polymorphism informative content; f, inbreeding coefficient.

discrepancy can be justified by the fact that the investigation was conducted on different microsatellites and (more relevantly) in a restricted geographic area. On the other hand, in agreement with our findings, an investigation into the STAT5A *locus* evidenced a larger genetic variability in the Italian Podolian compared to the cosmopolitan breeds (Dario *et al*., 2009). A higher variability in Podolian breeds compared to Italian meat breeds was also recently reported using SNP analysis (Pariset *et al*., 2010). This high variability could be explained considering that selection scheme is not fully adopted and the population is more consistent than other local breeds. The search for outliers evidenced significant values for two microsatellites (BM1818 and HAUT24), suggesting that these *loci* may be under the effect of balancing selection, in fact as showed in Figure S1 (Appendix), these two *loci* are placed under the lower confidence limit of 95% (Beaumont and Nichols, 1996; Beaumont and Balding, 2004). These two microsatellites were never reported to be associated with phenotypic traits, but, interestingly, BM1818 maps on bovine 23 very closely (from 140 to 210 kb) to four coding *loci* (KIF13A (kinesin family member 13A), UMJM31, NUP153 (nucleoporin), FAM8A1). However, the function of the coded proteins is still unknown. The data were then analyzed both including and excluding the two outliers. While no significant variations were observed in the clustering results, some differences were identified in the NJ trees, as discussed below. In order to estimate the number of genetic clusters within the 50 Italian Podolian individuals, a parametric genetic mixture analysis in the Structure 2.3 software (Pritchard *et al*., 2000) was performed. In Figure 1, a graphical presentation of the estimated membership coefficients to the clusters of the structure analysis obtained with K=4 is displayed. A K value of 4 was chosen because this corresponds to the number of Italian regions where the animals originated from. Clusters are presented as different colors and individuals are depicted as bars partitioned into colored segments whose lengths correspond to the membership coefficients in each subgroup. The analysis did not show a partitioning of genetic variability according to geographical origin since all of the animals showed a membership that was equally distributed between the four clusters, with the notable exception of some animals that belonged to the same single herd. This finding could be explained by the transhumance. Further analysis using the same software was performed in order to estimate the number of genetic clusters among all of the examined

European breeds. Between 2 and 15 clusters (K values) were tested using the admixture model, assuming that each individual did not necessarily have a genetic background originating from one of the K populations. Consistent results across runs were obtained and a clear clustering of breeds was observed for each K tested. In order to identify the optimal K value we applied the methodology described in Evanno *et al*. (2005), and concluded that 9 was the optimal K (Figure S2, Appendix). The most interesting K values were 3, 4, 9 and 15. This latter corresponding to the

number of breeds. The corresponding graphics are displayed in Figure 2. Jersey (1) and N'Dama (15) were well differentiated; also, Chianina (5) and Romagnola (8) individuals were distinguished. At K=4, the Podolian cluster, including the Italian Podolian, Istrian, Chianina and Romagnola breeds, was easily recognized. At K=9 (the best clustering number according to Evanno's test), the cattle group of known Podolian origin was split and a weak similarity was noted between the Istrian and Romagnola breeds only. The Italian Pezzata Rossa and Simmental were very simi-

Figure 1. Summary plot of Q estimates (estimated membership coefficients for each individual in each cluster) for K=4, obtained with a 100,000 burn-in, a 100,000 MCMC, under the admixture model, for Podolian individuals belonging to 14 Italian herds. Each individual is represented by a single vertical line broken into K (4) coloured segments, with lengths proportional to each of the four inferred clusters. Each colour represents the proportion of membership (M) of each individual (represented by a vertical line) to the K clusters. The numbers correspond to four Italian regions: 1 Puglia; 2 Basilicata; 3 Molise; 4 Campania.
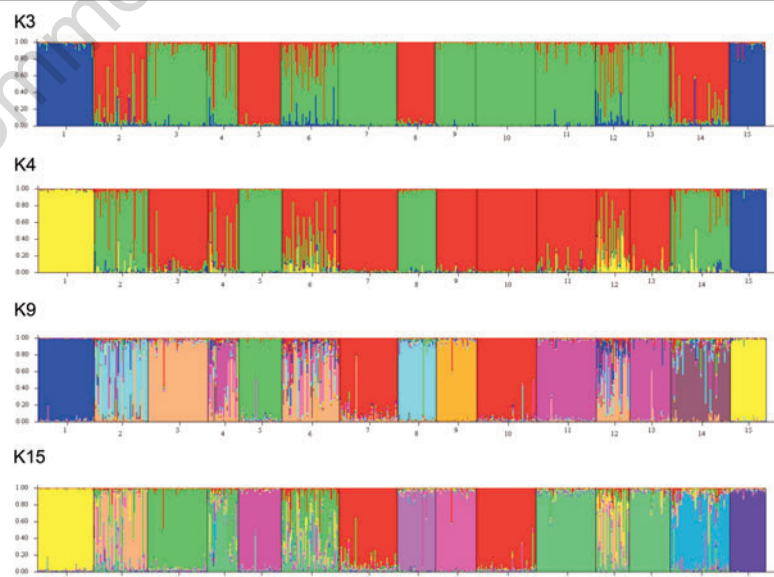

Figure 2. Summary plot of Q estimates (estimated membership coefficients for each individual, in each cluster) for K=3, 4, 9 and 15, obtained with a 100,000 burn-in, a 100,000 MCMC, under the admixture model, for the breeds analysed. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the K inferred clusters. Each colour represents the proportion of membership (M) of each individual (represented by a vertical line) to the K clusters. The numbers correspond to the following populations: 1, Jersey; 2, Istrian Podolian; 3, Limousine; 4, Cabannina; 5, Chianina; 6, Piemontese; 7, Pezzata Rossa Italiana; 8, Romagnola; 9, Holstein Friesian; 10, Simmental; 11, Swiss Brown; 12, Grigio Alpina; 13, Rendena; 14, Italian Podolian; 15, N'dama.

lar, while Jersey, N'Dama, Holstein Fresian and (to a lesser extent) Limousine were clearly noticeable. A genetic relationship was also noted between two alpine breeds (Swiss Brown and Rendena), and Piemontese, Istrian, Cabannina and Podolian displayed the higher genetic admixture. As envisaged from Evanno's test, increasing the K above 9 did not add more information, but it is worthwhile noting that at K=15, ITPRI and CHSIM were in the same cluster and the relationship between Brown and Rendena was still detectable, while the Piemontese, Grigio Alpina and Istrian breeds displayed a certain degree of genetic admixture. The tree obtained from Reynold's genetic distances (Reynolds *et al.*, 1983) between the 15 breeds is displayed in Figure S3 (Appendix). Three breed groups are clearly distinguishable in the tree: i) a cluster formed by the Piemontese, Holstein Friesian, Simmental and Pezzata Rossa Italiana breeds; ii) a cluster including the Limousine, Grigio Alpina, Rendena, Cabannina and Swiss Brown breeds; iii) a cluster including the Istrian Podolian, Italian Podolian, Romagnola and Chianina breeds. As expected, belonging all to
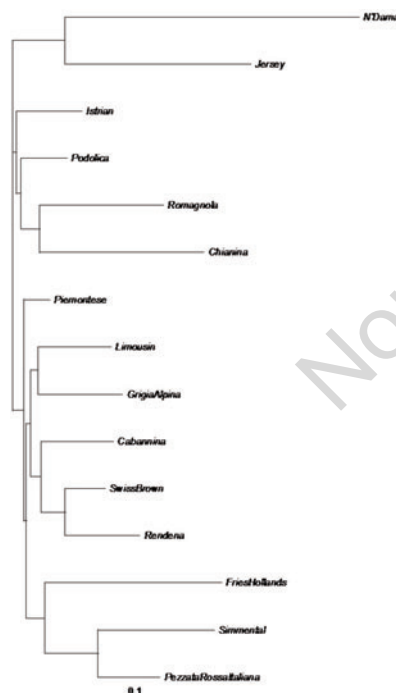

Figure 3. Phylogenetic relationships between the 15 breeds studied. The genetic distances were calculated from allelic frequencies by using Reynold's distances (1983) after removing the two outlier loci and using 28 microsatellites. The reconstruction was performed using the neighbour-joining clustering method.

the same Podolian Type the Italian Podolian, was found to belong to the same branch with Romagnola, Chianina and Istrian Podolian. This result confirms the common origin of these breeds and the tight linkage between the Podolian cattle of different countries, as was also observed by AFLP fingerprinting (Negrini *et al.*, 2007), although the Istrian and Italian Podolian breeds displayed a lower genetic distance than that existing between the Chianina and Romagnola breeds (Figure S3, Appendix). The Piemontese, linked to the Podolian breed, was not positioned in the same branch and this result is consistent with our structure results and with previous studies that investigated other genomic *loci* (Astolfi *et al.*, 1983; Ciampolini *et al.*,1995; Moioli *et al.*, 2004). The Piemontese breed is also not so close to Limousin as was reported using SNP markers (Negrini *et al.*, 2008). However, considering the mitochondrial DNA haplotypes, the Piemontese has the same polymorphisms as the other Italian Podolian breeds (Beja-Pereira *et al.*, 2006; Pellecchia *et al.*, 2007; Ciani and Matassino, 2001; 2007; Matassino and Ciani, 2009). For this reason, the Piemontese, Italian Podolian and the other Italian Podolian-derived breeds were assigned to the Bos primigenius taurus subspecies (Hiendleder *et al.*, 2008; Matassino and Ciani, 2009). Other breed groups can be clearly detected in the tree. Interestingly, when the tree was drawn excluding the two outlier *loci* (Figure 3), the cluster formed by the Piemontese, Holstein Friesian, Simmental and Pezzata Rossa Italiana breeds was split and Piemontese was placed alone on a different branch. This latter tree was better fitted to the breed history and illustrated how the inclusion of outlier *loci* in breed comparisons can influence the results.

Conclusions

The results obtained indicated the existence of consistent genetic variability within the Podolian breed that could be the result of a weak selective pressure for performance, and highlight the importance of this breed in the constitution of cattle biodiversity. The microsatellite polymorphisms analyzed did not show a genetic structure within the Italian Podolian populations reared in different regions. It can be concluded that the Italian Podolian breed is genetically homogenous despite its moderate phenotypic variability and the low use of artificial insemination; nonetheless, it must be pointed out that sires are exchanged between herds. However, this

data must be completely confirmed by analyzing the consistent sampling of Italian Podolian individuals from Calabria. Compared to the other European breeds, the Podolian showed the highest genetic variability in terms of expected heterozygosity and PIC values. The structure analysis as well as the genetic distance trees highlighted the close relationship between the Italian Podolian breed and the other Podolian breeds from Italy and Croatia (with the exception of the Piemontese). Moreover structure analysis also showed the relation among Alpine breeds (Swiss Brown and Rendena) while even at maximum K value Italian Pezzata Rossa and Simmental where not distinguishable.

References

ANABIC, 2001. Available from: www.anabic.it [accessed 14th April 2011].

Astolfi, P., Pagnacco, G., Guglielmino-Matessi, C.R., 1983. Phylogenetic analysis of native Italian Cattle breeds. Z. Tierz. Zuchtungsbio. 100:87-100.

Baker, C.M.A., Manwell, C., 1980. Chemical classification of cattle. 1 Breed groups. Anim. Blood Groups Bi. 11:127-150.

Beaumont, M.A., Balding, D.J., 2004. Identifying adaptive genetic divergence among populations from genome scans. Mol. Ecol. 13:969-980.

Beaumont, M.A., Nichols, R.A., 1996. Evaluating loci for use in the genetic analysis of population structure. P. R. Soc. B. 263:1619-1626.

Beja-Pereira, A., Caramelli, D., Lalueza-Fox, C., Vernesi, C., Ferrand, N., Casoli, A., Goyache, F., Royo, L.J., Conti, S., Lari, M., Martini, A., Ouragh, L., Magid, A., Atash, A., Zsolnai, A., Boscato, P., Tiantaphylidis, C., Ploumi, K., Sineo, L., Mallegni, F., Taberlet, P., Erhard, G., Sampietro, L., Bertranpetit, J., Barbujani, G., Luikart, G., Bertorelle, G., 2006. The origin of European cattle: Evidence from modern and ancient DNA. P. Natl. Acad. Sci. USA 103:8113-8118.

Brenneman, R., Chase, C., Olson, T., Riley, D., Coleman, S., 2007. Genetic diversity among Angus, American Brahman, Senepol and Romosinuano cattle breeds. Anim. Genet. 38:50-53.

Bruzzone, A., Barone, C.M.A., Iamartino, D., Incoronato, C., Pilla, F., Matassino, D., 2001. Genetic characterisation of the Podolica cattle: preliminary results. Proc. 14th Nat. Congr. ASPA, Firenze, Italy,

2:58-60.

Canon, J., Alexandrino, P., Bessa, I., Carleos, C., Carretero, Y., Dunner, S., Ferran, N., Garcia, D., Jordana, J., Laloe, D., Pereira, A., Sanchez, A., Moazami-Goudarzi, K., 2001. Genetic diversity measures of local European beef cattle breeds for conservation purposes. Genet. Sel. Evol. 33:311-332.

Canon, J., Tupac-Yupanqui, I., Garcia-Atance, M.A., Cortes, O., Garcia, D., Fernandez, J., Dunner, S., 2008. Genetic variation within the Lidia bovine breed. Anim. Genet. 39:439-445.

Ciampolini, R., Moazami-Goudarzi, K., Vaiman, D., Dillman, C., Mazzanti, E., Foulley, J.L., Leveziel, H., Cianci, D., 1995. Individual multilocus genotypes using microsatellite polymorphisms to permit the analysis of the genetic variability within and between Italian beef cattle breed. J. Anim. Sci. 73:3259-3268.

Ciani, F., Matassino, D., 2001. Il bovino grigio allevato in Italia; origine. Nota 1: il bovino Macrocero. Taurus speciale 13:89-99.

Ciani, F., Matassino, D., 2007. Il bovino grigio allevato in Italia: origine ed evoluzione. Nota 2: il bovino Brachicero. Taurus speciale 18:69-76.

Dadi, H., Tibbo, M., Takahashi, Y., Nomura, K., Hanada, H., Amano, T., 2008. Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle population. Anim. Genet. 39:425-431.

Dalvit, C., De Marchi, M., Dal Zotto, R., Zanetti, E., Meuwissen, T., Cassandro, M., 2008. Genetic characterization of the Burlina cattle breed using microsatellite markers. J. Anim. Breed. Genet. 125:137-144.

D'Angelo, F., Ciani, E., Sevi, A., Albenzio, M., Ciampolini, R., Cianci, D., 2006. The genetic variability of the Podolica cattle breed from the Gargano area. Ital. J. Anim. Sci. 5:79-85.

Dario, C., Dario, M., Ciotola, F., Peretti, V., Cornicella, D., Selvaggi, M., 2009. Analysis of STAT5/A va I gene polymorhism in four Italian cattle breeds. Biochem. Genet. 47:671-679.

Del Bo, L., Polli, M., Longeri, M., Ceriotti, G., Looft, C., Barre-Dirie, A., Dolf, G., Zanotti, M., 2001. Genetic diversity among some cattle breeds in the Alpine area. J. Anim. Breed. Genet. 118:317-325.

European Cattle Genetic Diversity Consortium, 2006. Marker-assisted conservation of European cattle breeds: an evaluation. Anim. Genet. 37:475-481.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611-2620.

FAO, 2011. Fao Domestic animal information system. Available from: http://dad.fao.org/ [accessed on 16th June 2011].

Georgescu, S.E., Manea, M.A., Zaulet, M., Costache, M., 2009. Genetic diversity among Romanian cattle breeds with a special focus on the Romanian Grey Steppe Breed. Rom. Biotechnol. Lett. 14:4194-4200.

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

Hiendleder, S., Lewalski, H., Janke, A., 2008. Complete mitochondrial genomes of Bos taurus and Bos indicus provide new insights into intra-species variation, taxonomy and domestication. Cytogenet. Genome Res. 120:150-156.

ISAG/FAO, 2004. Standing Committee Recommendations. Available from: http://dad.fao.org/cgi-bin/getblob.cgi?sid=-1,50006220 [accessed 7th September 2011].

Liu, K., Muse, S.V., 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128-2129.

Luikart, G., England, P.R., Tallmon, D., Jordan, S., Taberlet, P., 2003. The power and promise of population genomics: from genotyping to genome typing. Nature Rev. Genet. 4:981-994.

Manatrinon, S., Fischerleitner, F., Baumung, R., 2008. Genetic characterization among some Austrian and Hungarian cattle breeds. Arch. Anim. Breed. 51:426-437.

Martin-Burriel, I., Rodellar, C., Lenstra, J.A., Sanz Cons, C., Osta, R., Reta, M., De Argueullo, S., Sanz, A., Zaraoza, P., 2007. Genetic diversity and relationships of endangered Spanish cattle breeds. J. Hered. 98:687-691.

Matassino, D., Ciani, F., 2009. Origine e storia della "Podolica" in Italia. Proc. Int. Congr. on the tracks of Grey Podolic cattle, Matera, Italy, 3:111-124.

McHugh, D.E., Loftus, R.T., Bradley, D.G., Sharp, M., Cunningham, E.P., 1994. Microsatellite DNA variation within and among European cattle breeds. P. R. Soc. B. 256:25-31.

Moazami-Goudarzi, K., Laloe, D., Furet, J.P., Grosclaude, F., 1997. Analysis of genetic relationship between 10 cattle breeds with 17 microsatellites. Anim. Genet. 28:

338-345.

Moioli, B., Napolitano, F., Catillo, G., 2004. Genetic diversity between Piedmontese, Maremmana, and Podolica cattle breeds. J. Hered. 95:250-256.

Ndumu, D.B., Baumung, R., Hanotte, O., Wurzinger, M., Okeyo, M.A., Jailin, H., Kibogo, H., Solkener, J., 2008. Genetic and morphological characterisation of the Ankole Longhorn cattle in the African Great Lakes region. Genet. Sel. Evol. 40:467-490.

Negrini, R., Nicoloso, L., Crepaldi, P., Milanesi, E., Colli, L., Chegdani, F., Pariset, L., Dunner, S., Leveziel, H., Williams, J.L., Ajmone Marsan, P., 2008. Assessing SNP markers for assigning individuals to cattle populations. Anim. Genet. 40:18-26.

Negrini, R., Nijman, I.J., Milanesi, E., Moazami-Goudarzi, K., Williams, J.L., Erhardt, G., Dunner, S., Rodellar, C., Valentini, A., Bradley, D.G., Olsaker, I., Kantanen, J., Ajmone-Marsan, P., Lenstra, J.A., European Cattle Genetic Diversity Consortium, 2007. Differentiation of European cattle by AFLP fingerprinting. Anim. Genet. 38:60-66.

Page, R.D., 1996. TreeView: an application to display phylogenetic trees on personal computer. Comput. Appl. Biosci. 12:357-358.

Pariset, L., Mariotti, M., Nardone, A., Soysal, M.I., Ozkan, E., Williams, J.L., Dunner, S., Leveziel, H., Maróti-Agóts, A., Bodò, I., Valentini, A., 2010. Relationships between Podolic cattle breeds assessed by single nucleotide polymorphisms (SNPs) genotyping. J. Anim. Breed. Genet. 127: 481-488.

Pellecchia, M., Negrini, R., Colli, L., Patrini, M., Milanesi, E., Achilli, A., Bertorelle, G., Cavalli Sforza, L.L., Piazza, A., Toroni, A., Ajmone-Marsan, P., 2007. The mystery of Etruscan origins: novel clues from Bos taurus mitochondrial DNA. P. R. Soc. B. 274:1377-1385.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.

Reynolds, J., Weir, B.S., Cockerham, C.C., 1983. Estimation of the Coancestry coefficient, basic for a short-term genetic distance. Genetics 105:767-779.

Rosenberg, N.A., 2004. DISTRUCT – a pro-

gram for the graphical display of population structure. Mol. Ecol. Notes. 4:137-138.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, NY, USA.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin Ver 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sun, W.B., Chen, H., Lei, C.Z., Lei, X.Q., Zhang, Y.H., 2008. Genetic variation in eight Chinese cattle breeds based on the analysis of microsatellite. Genet. Sel. Evol. 40:681-692.

Wiener, P., Burton, D., Williams, J.L., 2004. Breed relationships and definition in British cattle: a genetic analysis. Heredity 93:597-602.

APPENDIX

Table S1. Diversity measurements for each analysed microsatellites in the Podolian cattle population.

Locus	Observed heterozygosity	Expected heterozygosity	P	SD
HEL1	0.789	0.807	0.282	0.003
INRA5	0.677	0.694	0.878	0.003
HAUT27	0.690	0.762	0.050	0.001
CS60	0.786	0.795	0.075	0.002
INRA63	0.620	0.679	0.065	0.002
INRA37	0.600	0.692	0.023	>0.001
CS66	0.675	0.800	>0.001	>0.001
TG227	0.833	0.867	0.071	0.001
TG126	0.715	0.771	0.025	0.001
BM2113	0.822	0.834	0.081	0.001
ETH225	0.748	0.768	0.607	0.002
ETH10	0.704	0.748	0.084	0.003
TG122	0.740	0.744	0.290	0.001
BM1824	0.679	0.688	0.819	0.003
INRA23	0.748	0.820	0.016	0.001
SP115	0.729	0.769	>0.001	>0.001
INRA35	0.464	0.463	0.005	0.001
ILSTS5	0.377	0.400	0.813	0.003
MM12	0.817	0.790	0.625	0.002
INRA32	0.656	0.649	0.042	0.001
HAUT24	0.680	0.813	0.071	0.001
ETH185	0.737	0.784	0.202	0.002
ETH152	0.644	0.729	0.121	0.002
ETH3	0.670	0.733	0.333	0.002
HEL5	0.855	0.858	>0.001	>0.001
HEL9	0.724	0.774	0.003	>0.001
TG53	0.830	0.858	0.053	>0.001
HEL13	0.746	0.729	0.253	0.004
BM1818	0.686	0.716	>0.001	>0.001
ILSTS6	0.717	0.777	0.668	0.002

SD, standard deviation.

Figure S2. Evanno's test results showing K=9 as the best value for cluster description.
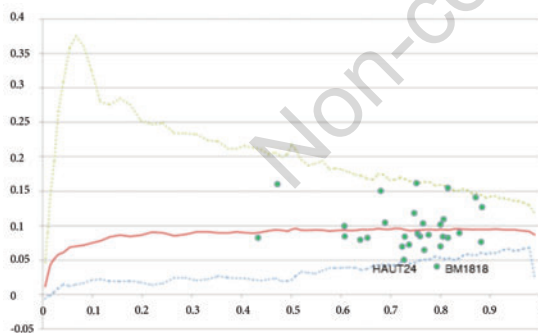

Figure S1. Upper (green) and lower (blue) confidence limits of 95% quantiles; median (red) of 50,000 replications of expected FST and heterozygosity using the coalescent model with a confidence level set to 95%, as assessed by the infinite-allele model constructed according to the method of Beaumont and Nichols (1996).
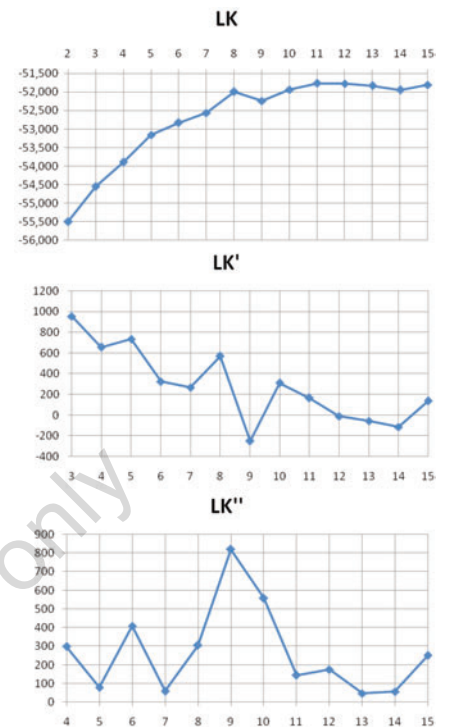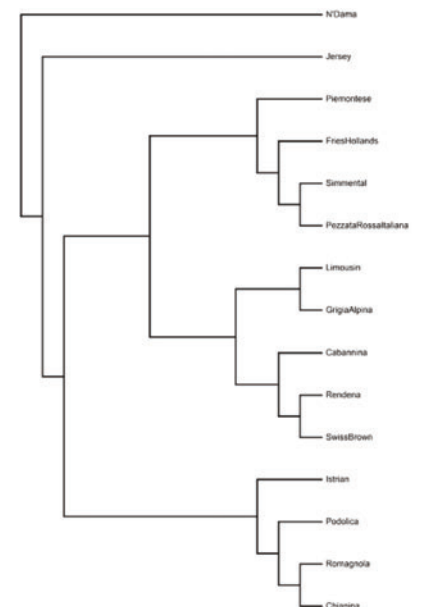

Figure S3. Phylogenetic relationships between the 15 breeds studied. The genetic distances were calculated from allelic frequencies by using Reynold's distances (1983) for all the 30 microsatellites. The reconstruction was performed using the neighbour-joining clustering method.