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

Mariasilvia D’Andrea,¹ Lorraine Pariset,² Donato Matassino,³ Alessio Valentini,² Johannes A. Lenstra,⁴ Giuseppe Maiorano,¹ Fabio Pilla¹
¹Dipartimento Scienze Animali Vegetali e dell’Ambiente, Università del Molise, Campobasso, Italy
²Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy
³Consorzio per la Sperimentazione, Divulgazione e Applicazione di Biotecnica Innovative, Benevento, Italy
⁴Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Abstract

The Italian Podolian (TPPOD) 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 the 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 high-lands, 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 outliers among neutral loci can greatly affect the estimate of population index (Liu et al., 2003). Outlier loci can be efficiently identified by estimating single loci FST values (Beaumont et al., 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; Nduku et al., 2008) and to unravel the genetic distance and relationship between breeds (McHugh et al., 1994; Moadami-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 (Moioi 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.

Corresponding author: Dr. Mariasilvia D’Andrea, Dipartimento Scienze Animali e dell’Ambiente, Università del Molise, via De Sanctis snc, 86100 Campobasso, Italy. Tel. +39.0874.404818 - Fax: +39.0874.404855. E-mail:dandrea@unimol.it

Key words: Podolian cattle, Population structure, Microsatellite loci.

Received for publication: 16 June 2011. Revision received: 13 September 2011. Accepted for publication: 16 September 2011.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright M. D’Andrea et al., 2011 License PAGEPress, Italy Italian Journal of Animal Science 2011; 10:e54 doi:10.4081/ijas.2011.e54
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 (BM1824, BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH152, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILST005, ILST006, INRA065, INRA067, INRA069, INRA037, INRA039, 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 30 out of the 134 individuals balanced for the region of origin was selected and the obtained genotypes were standardized using three reference samples on was selected and the obtained genotypes were balanced for the region of origin (ISAG/FAO, 2004).

Amplification was performed as suggested by ISAG/FAO (ISAG-FAO, 2004) for cattle biodiversity investigations. The ISAG/FAO Standing Committee Recommendations (ISAG/FAO, 2004) were followed.

European and cosmopolitan breeds were also investigated in the Hungarian Podolian, in which 11 loci were investigated in the Hungarian Podolian, in which 11 loci were 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 (Manatrtinnov 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

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).

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).

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).

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).

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.

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|
| Cabanina| ITCAB       | 26  | 0.66| 0.64| 0.62| 0.05|
| Grigio Alpina | ITORA | 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), UMMJ31, 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, Istrisan, 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 Istrisan 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 Istrisan 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 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 (Hienleder 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.

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.

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. Zuchtingssb. 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., Lalouea-Fox, C., Vernesi, C., Ferrand, N., Casoli, A., Goyache, F., Raya, L.J., Conti, S., Lari, M., Martini, A., Ouragh, L., Magid, A., Atash, A., Zsinos, A, Bosco, P., Tiantephydilis, C., Ploumi, K., Sineo, L., Mallelli, 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,
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.

F AO, 2011. FA0 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 beef 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.

Hienleder, 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. Cyto.genet. Genome Res. 125:150-156.

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., 2006. Genetic characterization among some Austrian and Hungarian cattle breeds. Anim. Genet. 31:140-146.

McHugh, D.E., Lofthus, R.T., Bradley, D.G., Sharp, M., Cunningham, E.P., 1994. Microsatellite DNA variation within and among European beef cattle breeds. J. Anim. Sci. 73:3259-3268.

Moazami-Goudarzi, K., Daloe, D., Furet, J.P., Grosclaude, F., 1997. Analysis of genetic relationship between 10 cattle breeds with 17 microsatellites. Anim. Genet. 28: 338-345.

Mooli, 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., Jainil, 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., 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., Maròt-Agôts, A., Bodo, 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. DISTRACT – a pro-

[Ital J Anim Sci vol.10:e54, 2011] [page 241]
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   | 0.746                   | 0.795                   | 0.075      | 0.002   |
| INRA43 | 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                   | 0.085      | 0.001   |
| ILST5  | 0.377                   | 0.400                   | 0.813      | 0.003   |
| MM12   | 0.817                   | 0.790                   | 0.625      | 0.002   |
| INRA32 | 0.856                   | 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  |
| ILST5  | 0.717                   | 0.777                   | 0.688      | 0.002   |

SD, standard deviation.

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

**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.