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Population genetic structure and milk production traits in Girgentana goat breed

Salvatore Mastrangelo[A,B], *Marco Tolone*[A], *Maria Montalbano*[A], *Lina Tortorici*[A], *Rosalia Di Gerlando*[A], *Maria Teresa Sardina*[A] *and Baldassare Portolano*[A]

[A]Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Edificio 4, Ingresso G, 90128 Palermo, Italy.
[B]Corresponding author. Email: salvatore.mastrangelo@unipa.it

Abstract. The aim of this work was to evaluate the genetic status of the Girgentana goat, an endangered breed from Sicily (Italy), using microsatellite markers. Furthermore, as the main purpose of the Girgentana breed is milk production, quantitative milk traits were investigated, including fatty acid profile. Molecular data from *CSN1S1*, *CSN2*, *CSN1S2*, and *CSN3* casein genes were also used to infer haplotypes. A total of 264 individuals were collected. Samples of Maltese ($n = 41$) and Derivata di Siria ($n = 33$) goat breeds were also used to understand the genetic relationship among breeds. Test-day records for milk production were collected to determine daily milk yield, fat, protein, casein, lactose, and somatic cell count. Individual milk samples were also collected for fatty acid extraction. Wright's statistics, gene flow, Nei genetic distance, factorial correspondence analysis, and Bayesian assignment test showed the existence of genetic variability and differentiation among breeds. The AMOVA results indicated that 89.96% of the total variance was partitioned within populations. The Girgentana breed appears to have a subdivided population, and has not experienced a recent bottleneck. A high variability in milk yield was observed. Mean morning milk yield was 1448 ± 404 g, with 4.30 ± 0.87% and 3.72 ± 0.44% of fat and protein percentages, respectively. The average somatic cell count found in Girgentana goat milk was higher than the threshold of 1 500 000 cells/mL advised in Europe for fresh milk. Gross milk and fatty acid composition were similar to that reported in the literature for other local goat breeds.

Additional keywords: casein genes, microsatellite markers, milk yield and gross composition.

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Introduction

In recent years, there has been great interest in recovering and preserving local livestock breeds and populations (e.g. Cornale *et al*. 2014; Vahidi *et al*. 2014; Parejo *et al*. 2015), considering that the existence of a large gene pool is important for preservation of potential future breeding and development of sustainable animal production systems.

Effective management of farm animal resources requires comprehensive knowledge of the breeds' characteristics (Groeneveld *et al*. 2010). Such exhaustive overview represents the starting point for the development of a preservation program. Molecular tools allow the characterisation of genetic resources at DNA level. Because of favourable characteristics, such as abundant number, high polymorphism and codominant inheritance, microsatellite DNA markers have been extensively used for several applications in livestock genetics (e.g. Tolone *et al*. 2012; Rosa *et al*. 2013; Vahidi *et al*. 2014; Parejo *et al*. 2015), and remain a choice for animal biodiversity studies of local breeds (Pons *et al*. 2015).

Many autochthonous breeds have an endangered status, or they even face extinction, because of their replacement by highly productive foreign breeds and progressive abandoning of low income rural activities. An interesting situation is represented by the Girgentana goat, an ancient local breed reared in Sicily. Over recent years, this breed has become almost extinct, in part as a consequence of the marked decrease in fresh goat milk consumption. In fact, it is listed by the Food and Agriculture Organisation with an endangered risk status (Canón *et al*. 2006).

The aim of this work was to evaluate the genetic status of the Girgentana goat breed using molecular information generated from microsatellite markers. Furthermore, as the main purpose of this breed is milk production, quantitative milk traits were investigated, including fatty acid (FA) profile. Molecular data from casein genes were also used to infer haplotypes.

Materials and methods

Biological samples

A total of 264 samples of the Girgentana goat breed enrolled in the Herd Book were randomly collected in several flocks located in four different areas of Sicily (Area 1 = Palermo, Area 2 = Agrigento, Area 3 = Enna, and Area 4 = Caltanissetta). In addition, samples of Maltese (n = 41) and Derivata di Siria

(n = 33) goat breeds were used to understand the genetic relationship among breeds. From each animal ~10 mL of blood were collected from the jugular vein, using vacuum tubes containing EDTA as anticoagulant. Genomic DNA was extracted from buffy coats of nucleated cells using a salting out method (Miller *et al*. 1988). After checking the quantity and quality of DNA using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), samples were diluted to a final concentration of 50 ng/μL in ultrapure water and stored at 4°C until use.

Microsatellite amplifications

A total of 20 microsatellite markers were chosen according to the International Society for Animal Genetics and Food and Agriculture Organisation guidelines, in order to be polymorphic and to map all over the genome. Genotypes were determined by means of five multiplex fluorescent polymerase chain reactions (PCR) and fragment lengths determined in a single semi-automated electrophoresis run by using an ABI PRISM 3130*xl* Genetic Analyser and GeneMapper 4.0 with recommended protocols (Applied Biosystems, Foster City, CA, USA). Each PCR reaction was performed in a total volume of 10 μL containing 100 ng of genomic DNA, 1X PCR buffer with $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, primers mix and 1 U of *Taq* DNA polymerase (Fermentas, Hanover, MD, USA). The thermal cycling conditions were an initial denaturation at 95°C for 10 min, 35 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 2 min, followed by final extension at 60°C for 30 min.

To evaluate data quality and repeatability, a total of five samples per breed were genotyped in duplicate. Moreover, to ensure correct genotype scoring, visual inspection was carried out independently by two experienced operators.

Genetic variability of microsatellite markers in Girgentana breed

Number of alleles per locus (k), observed and expected heterozygosity (H_o and H_e, respectively), polymorphic information content, Hardy–Weinberg equilibrium (HWE) and null allele frequencies for Girgentana breed were estimated using Cervus version 3.0.3 (Kalinowski *et al*. 2007).

Genetic differentiation among breeds

Wright's fixation indices among breeds (F_{is}, F_{it} and F_{st}) were estimated with FSTAT version 2.9.3 (Goudet 1995) using the Weir and Cockerham method (Weir and Cockerham 1984). The average number of effective migrants exchanged per generation (gene flow, Nm) was calculated with the following formula: $Nm = (1 - F_{st})/(4\ F_{st})$, as applied in Genetix version 4.03 (Belkhir *et al*. 1996). Moreover, the same software was used to perform factorial correspondence analysis (FCA) based on the individual multilocus and genetic distances (Nei minimum distance). Analyses on Nm and FCA were applied among breeds (Girgentana, Maltese, and Derivata di Siria) and among four different groups of Girgentana goats corresponding to the four different sampling areas. The significance of the fixation indices was tested using Arlequin version 3.11 (Excoffier *et al*. 2005) through the locus-by-locus analysis of molecular variance (AMOVA) procedure among breeds. Bayesian model-based clustering method implemented in Structure version 2.3.1 (Pritchard *et al*. 2000) was used to analyse the genetic structure, identify the true number of populations and assign the individuals to each cluster (K). The number of assumed clusters ranged from 1 to 7. The model used was based on an assumption of admixture and correlated allele frequencies. For each K, 50 independent runs were performed. All runs consisted of a burn-in period of 10 000 steps, followed by 100 000 Markov Chain Monte Carlo iterations. The most likely number of K clusters fitting the observed data was established by plotting LnPr(G|K) values obtained in the 50 independent runs for each K, as suggested by Pritchard *et al*. (2000). Following Evanno *et al*. (2005), we also calculated Delta K (ΔK), an *ad hoc* statistic based on the second order rate of change of the likelihood function, $L''(K)$, with respect to K. Graphic representation of these statistics were obtained with the web-based Structure Harvester program (Earl and vonHoldt 2012). Finally, the bottleneck hypothesis on Girgentana breed was investigated using Bottleneck version 1.2.2 (Cornuet and Luikart 1996). Two-phase mutation model and the Wilcoxon signed-rank test were used to detect the possible presence of a recent bottleneck. This approach is considered the most appropriate for microsatellite markers (Piry *et al*. 1999). The qualitative descriptor of the allele frequency distribution, 'mode-shift' indicator, was also used for assessing distortion in allele frequency, indicative of possible bottleneck.

Milk yield and gross composition

Test-day (TD) records for milk production on Girgentana goat kidding from 2011 to 2013 were collected in four different flocks, following an A4 recording scheme (ICAR 2007). Samples were taken both from animals with known and unknown genotypes at microsatellite markers. The first TD record was performed 31 days after kidding. Milk from the first 30 days of lactation was suckled by the kids. All goats were milked manually twice a day and milk from morning and evening milking was collected to determine daily milk yield (MY), fat (FAT), protein (PRT), casein (CAS), lactose (LCT) and somatic cell count (SCC). Milk samples were analysed using a Combifoss 6000 (FOSS Electric, Hillerod, Denmark) instrument. The original information was edited to guarantee the quality of the data to be analysed. Records were removed from the dataset when information of TD for MY, FAT, PRT, CAS, LCT and SCC were missed. SCC was transformed as log value (SCC ($\log_{10}$)). After editing, the dataset consisted of 870 TD records of 302 goats. Statistical analysis was performed using the PROC UNIVARIATE procedure of SAS statistical package (SAS Institute Inc., Cary, NC, USA).

Fatty acids analyses

Individual milk samples were collected during lactation from 100 goats, homogeneous for milk production, days of lactation, bodyweight and diet. The milk sample of each goat was collected during the morning milking, immediately stored at 4°C in a portable refrigerator, and transported to the laboratory of Dipartimento Scienze Agrarie e Forestali, University of Palermo, where it was lyophilised and frozen at −20°C until analysis. Before analysis, the lyophilised milk sample was

solubilised by adding a corresponding volume of ultrapure water. Milk samples were prepared following the Rose-Gottlieb's method (AOAC 1995) for FA extraction. For trans-esterification of lipids, 2 N KOH in methanol was used; the FA methyl esters were analysed with a chromatographic method (Sağdıç *et al*. 2004). The determination of the FA profile was performed by gas chromatography SHIMADZU GC-2010 with flame ionisation detector. The FA methyl esters were injected into capillary column (Zebron ZB-WAX Plus 30 m × 0:32-mm id, 0.2-mM film), identified according to the retention times and quantified by calibration curves. The results of FA were expressed as g/100 g FAT.

Casein haplotypes

Molecular data of casein (CAS) gene polymorphisms for Girgentana breed from our previous studies on *CSN1S1* (Mastrangelo *et al*. 2013*a*), *CSN2* (Tortorici *et al*. 2014), *CSN1S2* (Palmeri *et al*. 2014), and *CSN3* (Di Gerlando *et al*. 2015), were used to infer haplotypes. Haplotypes distributions were analysed using HAPLOTYPE procedures of SAS (SAS Institute Inc.). Haplotype frequencies were calculated under the null hypothesis of no linkage disequilibrium and under the alternative hypothesis of association between CAS genes.

Results and discussion

Genetic variability of microsatellite markers in Girgentana goat breed

Microsatellite markers with their chromosome number, primer sequences, allele size range, and multiplex set are reported in Table S1, available as supplementary material for this paper.

Table 1 shows the number of alleles (k), observed and expected heterozygosities (H_o and H_e, respectively), polymorphic information content (PIC), the significance of HWE and null allele frequency. All microsatellites were polymorphic, except *STAT5B**, which was monomorphic in Girgentana goat. A total of 129 alleles were detected across the 19 loci studied. The number of alleles per locus ranged from 2 (*ETH225*) to 11 (*BRN*) with an overall mean number of alleles per locus of 6.79. The average H_o across loci was 0.576 ± 0.204, with estimates per locus ranging from 0.137 (*ETH225*) to 0.977 (*CSRD247*). The average H_e value was 0.604 ± 0.203 with a range between 0.141 (*ETH225*) and 0.865 (*BRN*). Most markers had high levels of polymorphism with PIC ranging from 0.131 (*ETH225*) to 0.849 (*BRN*), and among all microsatellites, 14 were highly informative (PIC >0.50). Five microsatellites showed significant deviation from HWE ($P < 0.01$) (Table 1). In general, there may be several reasons for the deviations from HWE, such as the largest differences between H_e and H_o (Sechi *et al*. 2007; Rosa *et al*. 2013), the high Fis values (Nei 1987), and the high null allele frequencies.

The relatively low mean number of alleles per locus could be the consequence of past bottlenecks, which are known to affect more allelic richness than the level of genetic variability (Luikart and Cornuet 1998). The *ETH225* marker was not informative and was not further considered because it only presented two alleles. The same result for this marker was reported by Gour *et al*. (2006) in Jamunapari goats. In this study, the number of alleles was indicative of polymorphism and the final number of chosen loci (18) were sufficient for assessing genetic diversity and useful for the planned objectives. Based on gene diversity (H_e), the Girgentana breed showed a moderate genetic variation, but the average H_e showed in this

Table 1. Number of alleles (k), observed (H_o) and expected (H_e) heterozygosities, polymorphic information content (PIC), Hardy–Weinberg equilibrium (HWE) and null allele frequency (F(Null)) of each locus for Girgentana goat breed

ND, not detected; n.s., not significant; ***, $P < 0.001$. A significant *P*-value indicates deviation from equilibrium

Locus	k	H_o	H_e	PIC	HWE	F(Null)
FCB48	7	0.758	0.755	0.716	n.s.	–0.0008
FCB20	6	0.769	0.773	0.738	n.s.	–0.0899
BRN	11	0.727	0.865	0.849	***	0.0973
CSRD247	6	0.977	0.614	0.542	***	0.1583
SRCRSP0005	8	0.720	0.711	0.660	n.s.	–0.0099
OLADRB	10	0.738	0.803	0.779	n.s.	0.0416
SRCRSP0008	5	0.563	0.586	0.506	n.s.	0.0206
INRA104	3	0.455	0.444	0.347	n.s.	–0.0132
OARAE54	8	0.413	0.402	0.386	n.s.	–0.0278
MB099	3	0.183	0.203	0.184	n.s.	0.0316
MCM73	6	0.514	0.576	0.523	***	0.0998
FCB11	6	0.606	0.821	0.794	***	0.1536
BM1329	9	0.633	0.644	0.613	n.s.	0.0070
ETH225	2	0.137	0.141	0.131	ND	0.0131
INRA023	8	0.624	0.687	0.631	n.s.	0.0490
SRCRSP0024	8	0.488	0.506	0.477	n.s.	0.0253
TCRGC4	5	0.709	0.689	0.632	n.s.	–0.0120
TGLA122	8	0.431	0.461	0.429	n.s.	0.0370
MCM64	10	0.504	0.789	0.763	***	0.2213
Mean	6.79	0.576 ± 0.204	0.604 ± 0.203	0.563 ± 0.202	–	–

study was lower than those reported for Florida (0.688), Guadarrama (0.678) (Canón *et al*. 2006), Retinta Extremeña (0.709) (Parejo *et al*. 2015), Sarda (0.700), Argentata dell'Etna (0.740) (Negrini *et al*. 2012), Mascaruna (0.703) (Mastrangelo *et al*. 2013*b*), Sempione (0.690) and Vallesana (0.700) (Cornale *et al*. 2014) breeds. Several factors can contribute to decrease gene diversity, as high selection pressure, the use of artificial insemination and uncontrolled mating of related individuals. In local breeds such as the Girgentana one, selection programs are absent, but natural mating is the common practice, and the exchange of males among herds is quite unusual (Mastrangelo *et al*. 2012). This leads to an increase of inbreeding within the population due to mating among relatives and could generate population subdivision as a consequence of genetic drift.

Genetic differentiation among breeds

Wright's statistics, gene flow (Nm), Nei standard genetic distance, FCA, and Bayesian model-based clustering algorithm were used to visualise and explore the genetic relationships among breeds using genotypic data from Maltese and Derivata di Siria goat breeds.

Wright's statistics among populations were: F_{is} = 0.052 (within-population inbreeding estimate), F_{it} = 0.160 (total inbreeding estimate) and F_{st} = 0.112 (measurement of population differentiation). The F_{st} indicated the existence of genetic differentiation among populations, as reported in a previous study on phylogenetic analysis of Sicilian goats using mitochondrial hypervariable region 1 (Sardina *et al*. 2006). The separation of Girgentana from the other goat breeds was also reported by Siwek *et al*. (2011) (F_{st} = 0.10) probably due to the differences in breeding system and origin. Moreover, the reduction in the numbers of reared animals could increase the genetic differentiation due to genetic drift. The lowest genetic distance (Nei) was between Maltese and Derivata di Siria due to geographic proximity, similarities in environment, breeding practices, and past gene flow among them. These two breeds showed a higher value of Nm (Table 2) than to the Girgentana breed, confirming the presence of a shared genetic background. However, in general, the results showed low gene flow among these breeds.

Wright's F_{is} coefficient was also estimated per locus and across loci per breed (Table S2). The F_{is} values per microsatellite marker in each breed ranged from –0.584 to 0.647. The lowest mean value was found in Girgentana (0.037 ± 0.188), whereas the highest one was found in Maltese (0.118 ± 0.208). Similar results for the Girgentana breed was reported by Pariset *et al*. (2009) (F_{is} = 0.041) using single nucleotide polymorphisms. In a previous study on eight Italian goat breeds, Negrini *et al*. (2012) reported the highest value of F_{is} for Girgentana, but the number of analysed animals (n = 30) was very low compared with the sample size used in our study. It is interesting that an endangered breed such as Girgentana goat showed the lowest F_{is} value. In fact, despite the numerical reductions of the herds, an appropriate level of genetic variability has been conserved, probably due to the random mating in the breed that helps to maintain the genetic variability.

AMOVA analysis was carried out to analyse the variation within and between breeds. The AMOVA results indicated that 89.96% of the total variance was partitioned among individuals of the same population (within population), whereas 10.24% of the total variance was explained by differences among individuals from different populations (among populations), respectively (Table 3).

Genetic relationship between individuals was investigated using FCA that was performed including the three breeds using the corresponding allele frequencies (Fig. 1). The first two components explained the 100% of the total inertia, 70.97% of which explained by Axis 1 and 29.03% explained by Axis 2. The results showed that the breeds formed non-overlapping clusters and are clearly separated populations (Fig. 1). Moreover, the individuals of Maltese showed a more spread cluster probably due to fewer samples and a larger genetic spectrum than the other ones.

Gene flow and FCA were also performed only for the Girgentana breed taking into account the different sampling areas. Number of migrants per generation ranged from 4.13 to 10.44 (Table 4). The distribution of the individuals described by FCA (Fig. 2) was consistent with the geographical locations of the farms in which the samples were collected, confirming at the area level that differentiation of diversity in nuclear genomes of goat breeds contains a significant portion of geographic structure, as has already been reported by other authors (Canón *et al*. 2006; Vahidi *et al*. 2014).

Population structure, degree of admixture and number of subpopulations (K) were assessed by Bayesian approach implemented by Structure. The program estimates the natural logarithm (Ln) of the probability (Pr) that a given genotype (G) is part of a given population (K). The LnPr(G|K) increased from K = 2 to K = 5, and then decreased for K = 6. For K = 5, the LnPr(G|K) was maximised and also mean variance of the LnPr(G|K) estimates was the lowest one (Fig. 3*a*). The ΔK statistics (Evanno *et al*. 2005) were also calculated and presented in Fig. 3*b*. This analysis is strongly dependent on the variability between the runs of each K. Maximal ΔK occurred at K = 2, with other peaks at K = 3 and K = 5. These results indicated that individuals were most likely separated into two genetically distinct clusters: Girgentana versus Maltese and Derivata di Siria. Other authors identified a similar

Table 2. Genetic relationship among breeds: gene flow (Nm) (above diagonal) and Nei standard genetic distance (below diagonal)

Breed	Girgentana	Maltese	Derivata di Siria
Girgentana	–	1.95	1.85
Maltese	0.087	–	2.74
Derivata di Siria	0.087	0.077	–

Table 3. Results of AMOVA for Girgentana, Maltese and Derivata di Siria goat breeds

Source of variation	Sum of squares	Variance components	Variation (%)
Among populations	2119.647	0.58229	10.04
Within populations	1741.000	5.21782	89.96

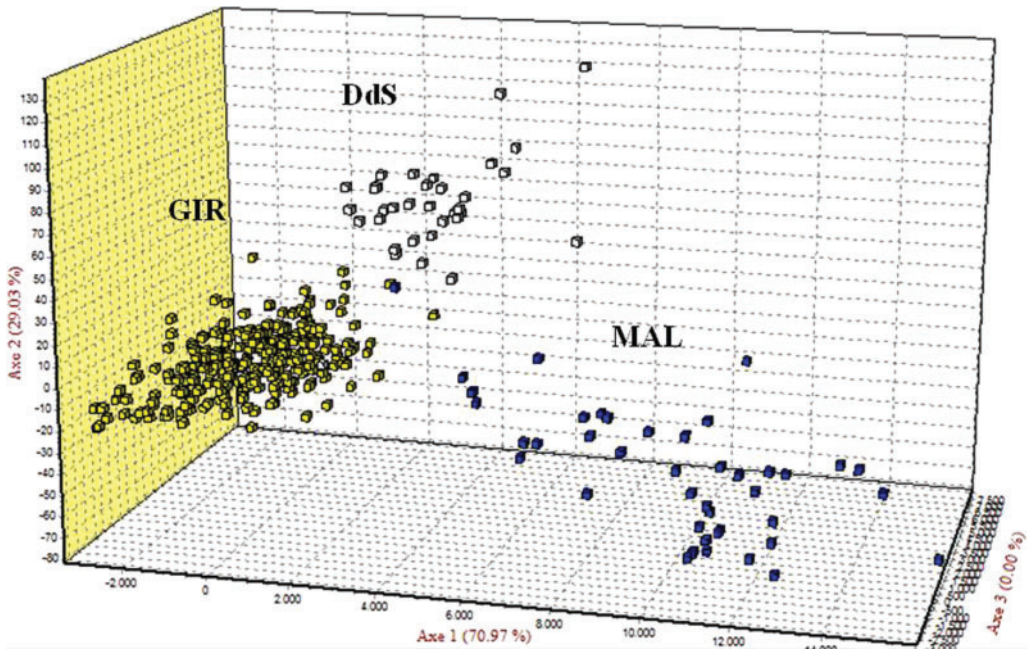

Fig. 1. Graphic representation of factorial correspondence analysis of Girgentana (GIR), Maltese (MAL) and Derivata di Siria (DdS) goat breeds. Numbers in parentheses represent the percentage of total inertia accounted by each axis.

Table 4. Gene flow (Nm) among individuals of Girgentana breed from different sampling areas

Breed	Area 1	Area 2	Area 3	Area 4
Area 1	–	8.54	4.40	8.31
Area 2	–	–	4.13	10.44
Area 3	–	–	–	8.79
Area 4	–	–	–	–

discrepancy between methods, reporting that maximal ΔK at $K = 2$ to be an artefact (Vigouroux *et al*. 2008; Barreta *et al*. 2012). It is worth remembering that Evanno *et al*. (2005) insisted that the ΔK-based criterion should be used together with the other information provided by Structure. Therefore, based on this suggestion and the biological significance of the results, we considered that $K = 5$ reflected the most likely number of clusters that optimally represented the data structure. Results of Structure analyses are shown in Fig. 4. Maltese and Derivata di Siria breeds maintained separate clusters, whereas the Girgentana showed a more complex structure because its genome was shared among subpopulations. Table 5 showed the assignment proportion of each breed to the five most likely inferred clusters. Clusters 1 and 5 included the Maltese and the Derivata di Siria individuals with 90.2% and 91.9%, respectively. The results showed a significant proportion of assignment for these breeds and pointed out the existence of clear genetic differences between breeds, according to F_{st} value. The Girgentana showed the highest level of genetic admixture with different proportion of individuals belonging to Cluster 2 (44.5%), 3 (14.8%) and 4 (37.7%). Similar results were reported by Pariset *et al*. (2006) and Mastrangelo *et al*. (2013*b*). In fact, Sicilian goats have shown strong population admixture structure caused by geographical location of the farms, influences of natural mating and traditional breeding systems where the flock is an important breeding unit (Siwek *et al*. 2011). With the aim to know if Girgentana individuals grouped in the different clusters could respond with their sampling area, we marked the animals according to geographic area. In particular, animals from Areas 3 and 4 were assigned in Cluster 2, with some individuals from Area 2; in Cluster 4 were assigned individuals from Areas 1 and 2. Moreover, some individuals from Area 1 were assigned in Cluster 3. As expected, in general, individuals from the same area shared the same inferred cluster. Recently, Mastrangelo *et al*. (2014), in a study on local endangered cattle breeds using principal component analysis, showed that individuals that clustered together belonged to farms located in the same geographic area. The analysis with Structure software was consistent with the FCA results, confirming that the grouping of the animals in the detected clusters was correlated with their sampling area.

Bottleneck

For the conservation of genetic resources, the identification of populations that have experienced a size reduction is crucial, because a bottleneck can increase demographic stochasticity, rate of inbreeding, loss of genetic variation and fixation of deleterious alleles, thereby reducing the evolutionary potential and increasing the probability of population extinction (Negrini *et al*. 2012).

In order to test if reductions in population size have left a detectable signature in the nuclear genome, the two-phase mutation model under Wilcoxon signed-rank, sign and shift mode tests were used to investigate any recent bottleneck (heterozygosity excess) in the Girgentana goat breed. In a population at mutation-drift equilibrium, there is approximately

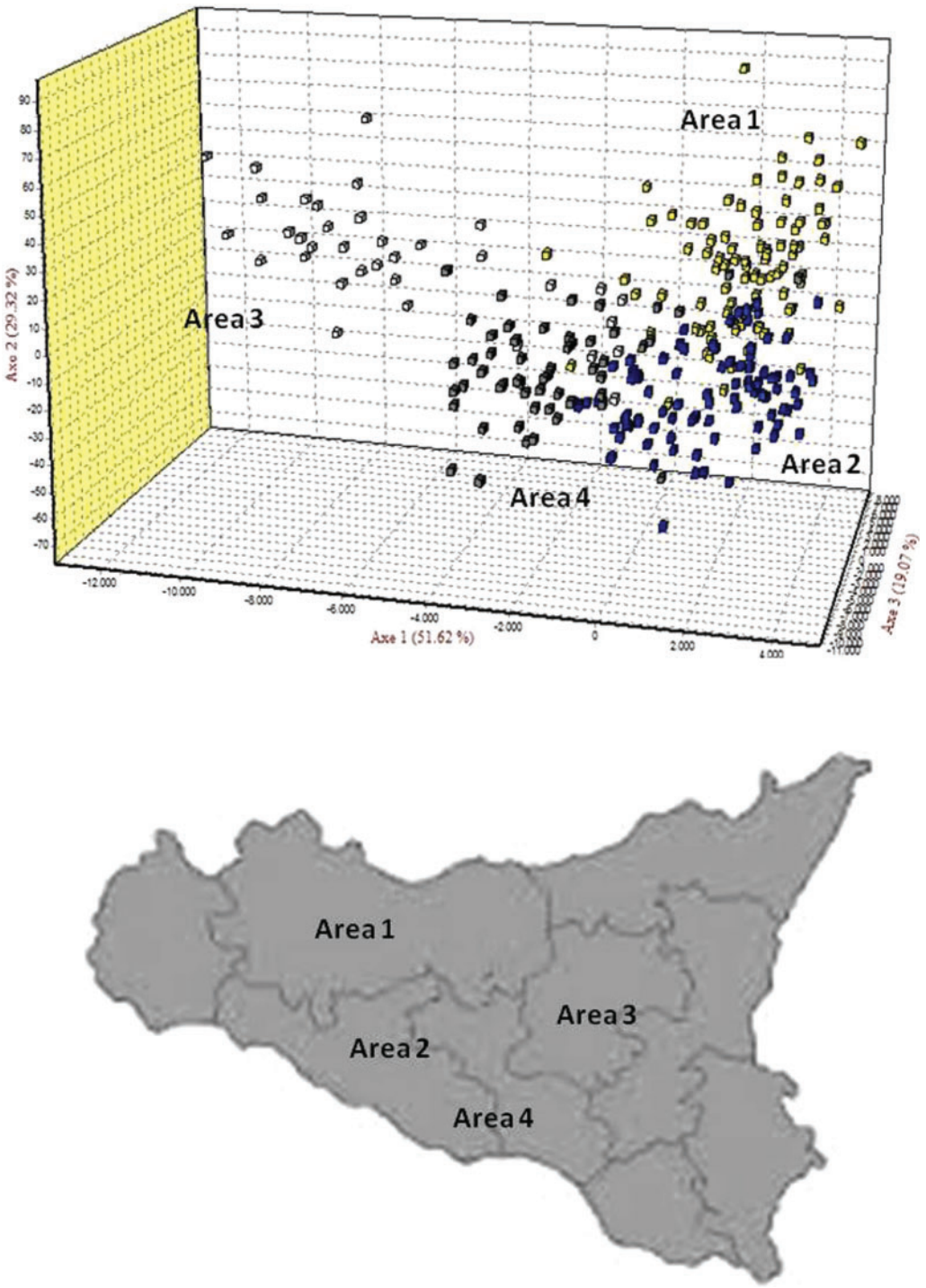

Fig. 2. Graphic representation of factorial correspondence analysis of Girgentana breed based on different areas in which the samples were collected. Numbers in parentheses represent the percentage of total inertia accounted by each axis.

an equal probability that a locus shows genetic diversity excess or deficit. The sign test (Cornuet and Luikart 1996) determines if a significant majority of loci in a population have a heterozygosity excess, and thus if a population appears to have been recently bottlenecked. Wilcoxon test provides relatively high power when less than 20 markers are used. In Girgentana breed, the expected number of markers displaying heterozygosity excess (10.37) was equal to the observed number of loci displaying heterozygosity excess (11), and greater than the observed number of loci with heterozygosity deficiency (7) (Table 6). The mode-shift indicator showed a normal L-shaped distribution in graphical representation proportion of allele versus class of

frequency distribution (Fig. 5), which is expected for a population that has not experienced a recent bottleneck that affected genetic variability (Cornuet and Luikart 1996). Therefore, the analysis indicated that the Girgentana breed has not suffered any recent bottleneck (Table 6).

Breed conservation

The genetic diversity results presented here can be useful in outlining conservation strategies, even though it remains a subject of discussion what are the optimum weights to be given to the between- and within-breed components of genetic diversity in defining conservation properties. Following the FAO (1998) guidelines, the recommended rules for the initial stages of a conservation program are maintaining genetic diversity, defining selection within families, and creating a nucleus population. The Girgentana breed appears to have a subdivided population. The conservation management of subdivided populations requires a compromise between the control of the global genetic diversity, the avoidance of high inbreeding levels, and, sometimes, the maintenance of a certain degree of differentiation between subpopulations (Fernández *et al*. 2008). The breed showed moderate levels of gene diversity (0.604) and inbreeding (0.037). Thus, the efforts should be made to improve genetic diversity in this breed. In particular, mating decisions will play an important role in limiting the levels of inbreeding and would increase the size of this breed. Minimisation of the loss of genetic variation is equivalent to minimisation of the rate of inbreeding in the population.

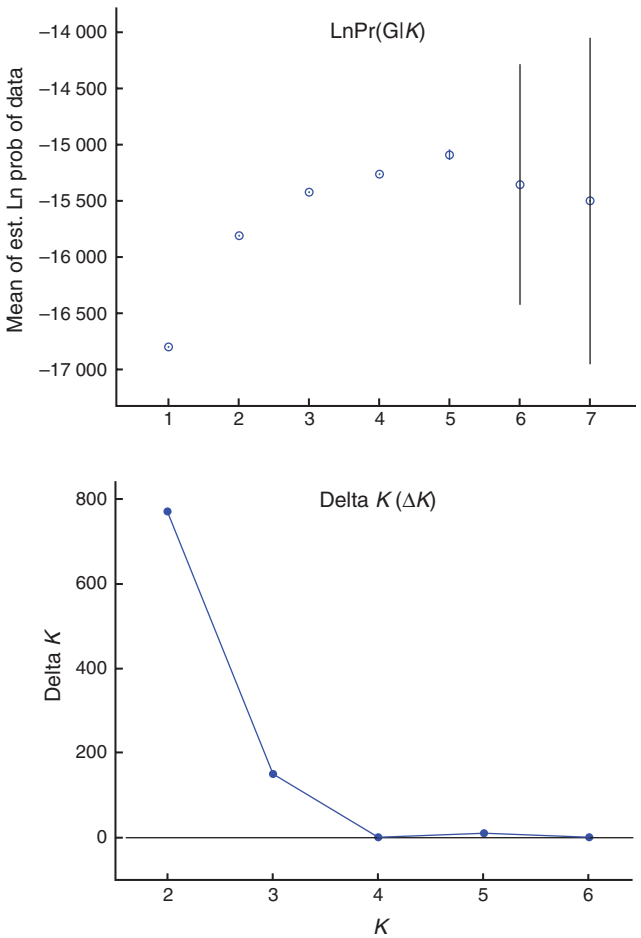

Fig. 3. Estimated posterior probabilities of LnPr(G|*K*): (*a*) LnPr(G|*K*) values are presented as a function of the number of clusters (Pritchard *et al*. 2000). Delta *K* (ΔK) values were calculated following Evanno *et al*. (2005).

Milk yield and gross composition

The descriptive statistics for milk production traits were reported in Table 7. Moreover, to better understand the variability of milk composition, a boxplot was depicted in Fig. 6. A high variability in MY was observed: the recorded levels varied from less than 1000 to ~4500 g/goat per day. The MY was higher than reported for Sarda goat breeds (Vacca *et al*. 2014), and lower than Garfagnina local goat breed (Martini *et al*. 2010). In a previous study, Todaro *et al*. (2005) reported lower MY (704 ± 323 g), FAT (3.93 ± 1.23), and PRT (3.48 ± 0.38) percentages in Girgentana breed. Similar results were reported for indigenous Greek breeds (Kondyli *et al*. 2012). Coefficient of variation (CV) for FAT was high (20%) compared with other milk production traits (Table 7) although MY had the highest CV. In fact, FAT content is the more quantitatively and qualitatively variable component of milk, depending on lactation stage, season, breed, genotype and feeding (Raynal-Ljutovac *et al*. 2008).

Goat milk can display variable composition, which determines its use as drinking milk or for cheese making. This potentiality depends mainly on the milk PRT component and, more specifically, on the CAS fraction. Girgentana breed showed a proportion between CAS and PRT of 85%. This feature makes the milk of this breed very suitable for the production of dairy products.

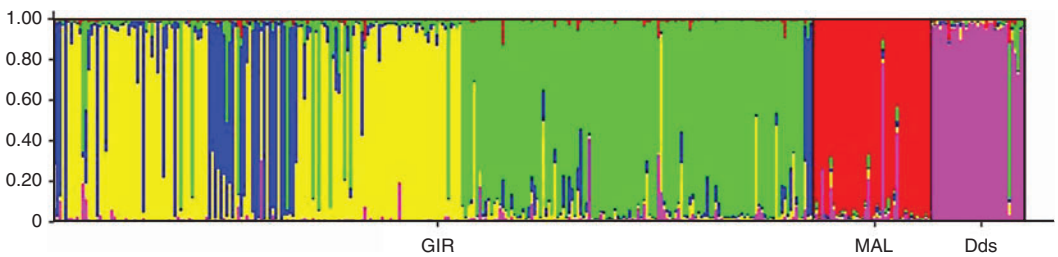

Fig. 4. Estimated population structure of the three goat breeds for *K* ranging from 2 to 7: Girgentana (GIR), Maltese (MAL) and Derivata di Siria (DdS). The most likely *K* was 5.

Table 5. Proportion of memberships of each breed in the five inferred clusters

Breed	Inferred clusters 1	2	3	4	5
Girgentana	0.010	0.445	0.148	0.377	0.019
Maltese	0.902	0.014	0.010	0.012	0.061
Derivata di Siria	0.016	0.043	0.008	0.014	0.919

Table 6. Bottleneck analysis for Girgentana goat breed using the two-phase mutation model (TPM) under Sign test and Wilcoxon rank test
Hee, heterozygosity excess expected; Hd, heterozygosity deficiency; He, heterozygosity excess

Model	Sign test	Wilcoxon test
TPM[A]	Hee = 10.37	*P* (one tail for H deficiency): 0.72456
	Hd = 7	*P* (one tail for H excess): 0.28992
	He = 11	*P* (two tails for H excess or deficiency): 0.57984
	P = 0.48085	–

[A]Parameters for TPM: variance = 30.00 proportion of Stepwise Mutation Model in TPM = 70.00%; estimation based on 1000 replications.

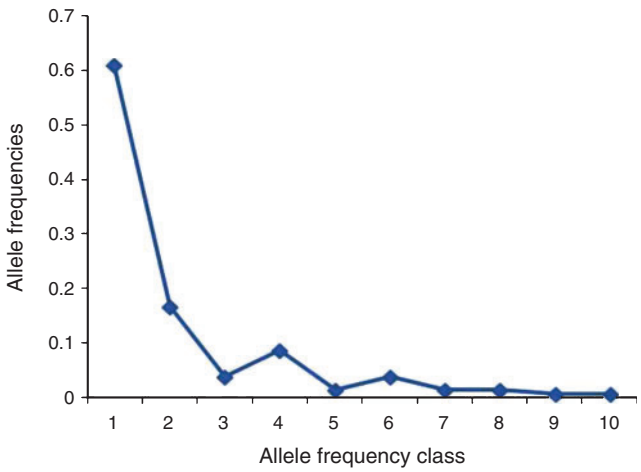

Fig. 5. L-shaped mode-shift graph of proportion of alleles and their distribution in Girgentana goat breed.

Although the threshold for SCC in goat's milk has not yet been established, the average SCC found in Girgentana goat milk, before being transformed into log value, was greater than the threshold of 1 500 000 cells/mL advised in Europe for fresh milk (Delgado-Pertiñez *et al*. 2003), with a high variability in our population (Table 7). Morgan *et al*. (2003) showed high mean levels of SCC in Greek goats (3210×10^3 cells/mL) in the middle of lactation. Todaro *et al*. (2005) reported a mean value of 426 000 cells/mL for Girgentana goat. Similar mean values of SCC ($\log_{10}$) were reported by Vacca *et al*. (2010) in local Sarda goat breed that, as Girgentana, is reared in a pastoral system, hand-milked and in the absence of modern husbandry techniques. High SCC in milk was associated with subclinical mastitis (Leitner *et al*. 2004) and it leads to modification of main milk components with negative effects on cheese manufacture. Therefore, more attention should be given to subclinical mastitis control and treatment programs for the improvement of udder health status in the Girgentana goat breed.

Table 7. Descriptive statistics of yield and composition of Girgentana goat milk

Traits	Mean ± s.d.	Max.	Min.	Variation coefficient (%)
Milk yield (g)	1448 ± 404	4544	508	28
Fat (%)	4.30 ± 0.87	7.69	1.82	20
Protein (%)	3.72 ± 0.44	6.48	2.44	12
Casein (%)	3.06 ± 0.41	5.19	1.87	13
Lactose (%)	4.69 ± 0.25	5.52	3.54	5
Somatic cell count ($\log_{10}$)	5.78 ± 0.61	7.28	4.47	11

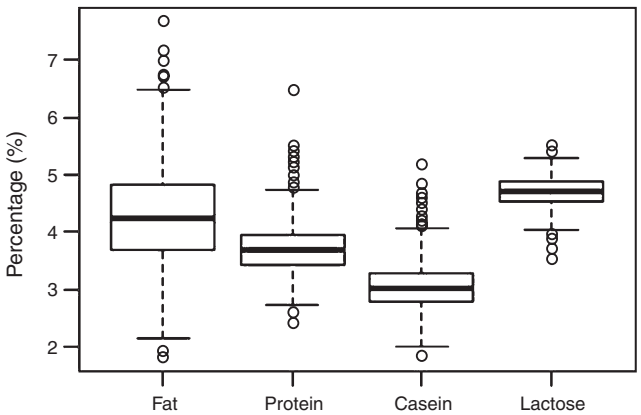

Fig. 6. Box plots with percentage (%) of fat, protein, casein and lactose in Girgentana goat milk.

Fatty acids analyses

Thirteen FA were separated and quantified. The FA composition of Girgentana milk was reported in Table 8. A direct comparison of our results with those from the literature is quite difficult because the contents of FA are expressed in different units, and authors do not always state the breed, lactation period, and number of analysed samples. The most abundant FA was C16:0 followed by C18:1 *c9*, C14:0, C10:0 and C18:0, which accounted for more than 75% of total FA, according to previous studies on Italian local goat breeds (Martini *et al*. 2010; Cornale *et al*. 2014). In milk of the Girgentana goat, caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids accounted on average for 1.52, 2.55, and 11.38 g/100 g FAT, respectively. These FA, are the most characteristic FA in goat milk and derived dairy products, being more abundant than in cow milk FAT, and with lauric acid (C12:0) are associated with the characteristic flavours of cheeses and can also be used to detect admixtures of milk from different species (Park *et al*. 2007). Moreover, odd- and branched-chain FA are also responsible for the typical aroma of caprine milk and cheese. The sum of short- and medium-chain FA (C4:0 to C14:0) was 34.97 g/100 g FAT.

Casein haplotypes

Knowledge of variation of CAS genes at the haplotype level has been a useful tool in biodiversity studies and in breeding

Table 8. Average fatty acid composition (g/100 g fat) in milk of Girgentana breed

Fatty acid	g 100/g fat	
	Mean	s.d.
C4:0	0.40	0.12
C6:0	1.52	0.48
C8:0	2.55	0.19
C10:0	11.38	2.71
C12:0	6.78	1.70
C14:0	12.34	0.90
∑C4-C14	34.97	3.37
C16:0	28.87	1.96
C16:1	0.18	0.09
C17:0	0.18	0.09
C18:0	8.03	1.73
C18:1 *cis-9*	13.88	2.37
C18:3	0.17	0.08
C20:0	0.26	0.04

Table 9. Haplotype frequencies in the Girgentana breed. Haplotype frequencies were calculated both under hypothesis of loci independence (H0) and taking association into account (H1)

Only haplotypes with H1 frequency higher than 0.008 were shown

CSN1S1	CSN2	CSN1S2	CSN3	H0	H1
A	C	A	A	0.122	0.368
F	C1	F	B	0.008	0.182
F	C1	A	A	0.033	0.080
A	C	A	D	0.029	0.075
A	0^1	A	B	0.008	0.052
N	C	A	B	0.008	0.040
A	C	C	D	0.002	0.037
B	C1	A	N	0.001	0.025
A	C	A	B	0.092	0.023
B	A	A	A	0.001	0.020
A	C	F	B	0.028	0.017
F	C	F	B	0.014	0.013
N	C1	F	B	0.001	0.010

strategies (Caroli *et al*. 2009). A total of 201 individuals of the Girgentana breed were used to infer haplotypes at CAS loci. Haplotype frequencies at *CSN1S1-CSN2-CSN1S2-CSN3* loci were reported in Table 9. Of the 133 inferred haplotypes from the possible combinations of CAS genes, only 13 showed association frequencies higher than 0.008. The predominant haplotype was the *A-C-A-A* (36.8%), whereas the ancestral haplotype *B-A-A-B* (Caroli *et al*. 2006) was not found in Girgentana due to the low frequency of *CSN1S1*B* allele (0.065) in this breed (Mastrangelo *et al*. 2013*a*). In a previous study Gigli *et al*. (2008) reported *A-C-A-B* as the most frequent in Girgentana goat. These results may be explained considering the different allele frequency at *CSN3*A* reported by Di Gerlando *et al*. (2015) (0.480) compared with those reported in the previous study by Gigli *et al*. (2008) (0.127). In fact, the flocks are not the same of Gigli *et al*. (2008) and number of animals within flocks has changed during these past years, and then, the individual analysed samples are not the same of previous work. Moreover, different genotyping protocols were used. In fact, the *CSN3* gene was genotyped using the Sanger sequencing protocol by Di Gerlando *et al*. (2015), whereas Gigli *et al*. (2008) used the PCR–single-strand conformation polymorphism protocol. The second most frequent haplotype was *F-C1-F-B* (18.2%) recently reported in two Italian local goat breeds but with lower frequencies (Martini *et al*. 2010; Cornale *et al*. 2014). The first five haplotypes represented more than 75% of the CAS cluster variability. The Girgentana goat was characterised by an interesting and wide variability in the CAS cluster, with haplotypes rarely found in other breeds (*N-C-A-B* and *N-C1-F-B*) containing the *N* allele at the *CSN1S1* locus, which has reported in few breeds, such as Tunisian goats (Vacca *et al*. 2009). It is well known that goat CAS are characterised by different expression levels due to strong, medium, weak or null alleles responsible for high, medium, or low CAS content in milk, depending on the CAS fraction (Caroli *et al*. 2006). Haplotype analysis of the CAS genes allows for selection of goat genetic lines for milk production with 'particular' PRT content (Sacchi *et al*. 2005). The high frequency of haplotypes containing strong alleles at each CAS gene indicates that selection for these variants should be an easy breeding objective to improve milk composition and cheese-making properties in the Girgentana breed (Martini *et al*. 2010). However, the occurrence of haplotype combinations with null and weak alleles, as *N* and *F* at *CSN1S1* and 0^1 at *CSN2* loci, encloses a high productive potential, because goats carrying these alleles may produce milk with low PRT content, which can be used for fresh milk consumption, potentially reducing the risk of developing food allergies (Bevilacqua *et al*. 2002; Martini *et al*. 2010; Ballabio *et al*. 2011).

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