

## GENETIC AND PRODUCTIVE CHARACTERIZATION OF THE BURLINA CATTLE BREED

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Original scientific paper

### SUMMARY

*The Burlina (BUR) is a local Italian cattle breed, its main feature is to be a small sized animal well adapted to live in difficult environment such as mountain areas. Nowadays only 350 cows are enrolled in the Italian Herd Book due to its substitution by more productive breeds. This study proposes a phenotypic and genetic characterization of the BUR breed in comparison with Holstein Friesian (HFR) and Brown Swiss (BSW). The comparison of productive traits showed lower production than HFR and BSW as well as longer productive life and more favourable reproductive traits in BUR animals. The genetic analyses, performed by microsatellites markers, showed the high level of heterozygosity and the genetic distinctiveness of BUR. These findings approve the feasibility of a conservation scheme and suggest the profitability of breeding BUR, especially in difficult environment where the low production can be compensated by longevity and the economic loss by the added value of its typical dairy production.*

*Key-words: Burlina cattle breed, genetic characterization, conservation.*

### INTRODUCTION

The Burlina (BUR) is local dairy cattle breed reared in North-East Italy; it is a small sized animal with black spotted coat, well adapted to live in difficult environment as marginal mountain areas thank to its good grazing characteristics. BUR is mainly found in the mountain area of Veneto region (Del Bo et al., 2001) where it has always been reared and appreciated by local farmers. Despite of that, the BUR population was almost destroyed during the First World War and later, during the 1930's - 40's. Several actions were carried out to replace it with the more productive and widespread Brown Swiss (BSW) and Holstein Friesian (HFR). The number of animals drastically decreased since 1980 it has been enrolled in the Italian Herd Book of local breeds. Nowadays, about 350 cows are enrolled, most of them located in the Treviso and Vicenza provinces of Italy (AIA, 2006). Recently, interest in BUR has increased because it can exploit mountain pasture better than BSW and HFR, and thus is better able to safeguard pastures in the unstable and fragile mountain environment (Cozzi et al., 2004). In less productive farms, BUR can achieve higher production than HFR which requires higher inputs to perform optimal results (Bittante et al., 1992).

In addition, typical cheese called Morlacco traditionally from BUR milk has been produced. It is a raw whole milk cheese, preferably obtained from cows at pasture to achieve a special herbs flavor. The strong link among product, breed and environment could be a starting point for implementing a conservation program for BUR in its native area where it can perform better and its breeding could become profitable and competitive. Aim of this study was the productive and genetic characterization of the BUR breed in comparison with the cosmopolitan HFR and BSW. The productive characterization was carried out by collecting data on milk yield and quality. The genetic characterization was performed using 12 microsatellite (STR) markers in order to study the genetic variability of the breed and its genetic distinctness. The collected information represented the first step for establishing the feasibility of a conservation scheme for the safeguard of the BUR breed.

### MATERIAL AND METHODS

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### Productive and reproductive traits characterization

Data from the Italian National Breeders Association were collected for the characterization of productive and reproductive traits from 1986 to 2002. Data, referred to herds located in the Treviso Italian province where BUR is mainly reared, and included information on the three studied breeds: BUR, HFR and BSW. The collected data were: number of controlled cows, number of closed lactations, production (milk yield, fat and protein percentage), age at first calving, average age at calving, days open, lactation period and number of inseminations. Descriptive statistics were calculated for each trait per year in order to characterize BUR in comparison to HFR and BSW.

### Genetic characterization

The dataset consisted of 153 blood samples belonging to the three dairy cattle breeds: BUR (n = 80), HFR (n = 29) and BSW (n = 44). Samples were collected in different farms to obtain a representative sample from unrelated animals. Blood samples were collected from each animal in 5 ml vacutainer tubes containing sodium citrate as anticoagulant, and stored at -20°C until analysed. DNA extraction was carried out employing the “Gentra System PUREGENE DNA purification kit” starting from 300 µl of whole blood. DNA samples were then amplified by PCR in correspondence of the following 12 STR loci: BM1818, ETH185, MM12, TGLA126, BM203, TGLA122, RM12, ILST008, SPS115, BL42, ETH3 and TGLA53 (amplification protocols available on request). The studied loci were chosen in order to have high polymorphic markers spread all over the genome. Allele size was determined with a Perkin Elmer ABI Prism 3730XL Genetic Analyzer, using GeneScan 2.0 and Genotyper 3.7 software (Perkin Elmer). The obtained results were then used to perform statistical analysis as follows. Allelic frequencies, observed and expected heterozygosity, were calculated using the software Genetix 4.05.2 (Belkhir et al., 1996-2004). The Fstat 2.9.3 program (Goudet, 1995) was employed in calculations of mean number of alleles, allelic richness, and *F*-statistics estimates (Weir and Cockerham, 1984) in the total sample, and per breed. Tests for deviation from Hardy-Weinberg (H-W) equilibrium and for population differentiation were performed by the GENEPOP 3.4 software (Raymond and Rousset, 1995).

## RESULTS AND DISCUSSION

### Productive and reproductive characterization

Table 1 and 2 show the descriptive statistics of productive and reproductive traits in the three studied breeds, from 1986 to 2002, in the Treviso Italian province. First of all, it is worth observing that BUR breed suffered a drastic decrease in the number of controlled animals while HFR registered a strong increase. These data are the result of the selection goal of past years, based on improving production. In this way productive breeds such as HFR gradually substituted less productive ones such as BUR. With respect to production, BUR is the least productive breed with only half of the milk production of HFR in 2002. However, in 1986 differences among productions were not as considerable as nowadays, meaning, that no strong selection has been carried out in the BUR breed during these years. Milk quality is comparable between BUR and HFR while, as expected, BSW milk showed better characteristics.

**Table 1. Descriptive statistics of productive traits in the Burlina (BUR), Holstein Friesian (HFR) and Brown Swiss (BSW) breeds**

Years	N° of cows under control			Milk production (kg)			Fat (%)			Protein (%)		
	BUR	HFR	BSW	BUR	HFR	BSW	BUR	HFR	BSW	BUR	HFR	BSW
1986	554	5640	1759	3459	5628	4244	3.58	3.69	3.79	3.05	3.1	3.24
1990	230	9000	1615	4251	6504	5019	3.59	3.61	3.89	3.04	3.02	3.25
1994	228	10664	1514	4247	7226	5678	3.56	3.55	3.86	3.13	3.11	3.37
1998	269	22773	7189	4455	8464	6853	3.61	3.66	4.00	3.09	3.18	3.45
2002	257	15343	1543	4503	8671	7187	3.56	3.56	3.93	3.22	3.23	3.49

**Table 2. Descriptive statistics of reproductive traits in the Burlina (BUR), Holstein Friesian (HFR) and Brown Swiss (BSW) breeds**

Years	Average age at calving (m)			Age at first calving (m)			Days open (d)			N° of inseminations		
	BUR	HFR	BSW	BUR	HFR	BSW	BUR	HFR	BSW	BUR	HFR	BSW
1986	64	57	51	28	28	29	95	128	133	1.1	1.8	1.6
1990	72	49	56	28	27	29	105	129	131	1.3	2	1.8
1994	61	46	52	27	27	29	108	149	138	1.2	2.1	1.8
1998	60	47	55	28	27	28	111	150	146	1.4	2.2	1.9
2002	63	46	50	28	27	28	126	177	164	1.5	2.5	2.2

Reproductive traits showed a sharp worsening in the HFR and, in a smaller amount, also in the BSW during the past years. Even if deterioration has been observed also in BUR, its reproductive traits remain the best among these breeds. In particular, the average age at calving is much higher than in HFR and BSW. It means that BUR production's life is longer, days open as well seems to be more favourable in BUR being, in 2002, 50 days shorter than the HFR. Using data of 2002 it is possible to estimate the quantity of milk produced in the whole cow's career. Estimations of close lactations are 2.70 for BUR and 1.93 for HFR corresponding to 12158 and 16735 kg of milk respectively with a difference of 27%. These estimates suggest that, even if BUR production is very low, in the whole career it is acceptable. It is worth mentioning that these estimates did not account for costs related to maintenance and production requirement that are lower in the BUR bred due to its smaller size.

### Genetic characterization

Analysis of the 12 STR permitted the detection of 96 alleles ranging from 2 (RM12 and ILST008), to 15 (TGLA122) alleles per locus with an average of 8. The allelic richness (estimate of the number of alleles per locus corrected by sample size) was calculated; BUR and HFR showed the highest number of alleles per locus (6.03 and 5.99 respectively) while BSW the lowest (5.06). Values of observed and expected heterozygosity are shown in Table 3.

**Table 3. Number of sampled animals, observed and expected heterozygosity per breed: Burlina (BUR), Holstein Friesian (HFR) and Brown Swiss (BSW), averaged overall 12 loci**

Breed	Number of samples	Average heterozygosity	
		observed $\pm$ SD	expected $\pm$ SD
BUR	80	0.677 $\pm$ 0.155	0.670 $\pm$ 0.146
HFR	29	0.639 $\pm$ 0.169	0.609 $\pm$ 0.160
BSW	44	0.563 $\pm$ 0.202	0.572 $\pm$ 0.206

Burlina was the breed showing the highest variability while BSW the lowest, differences between observed and expected values were neither significant in any of the considered breeds nor in the whole population, meaning that H-W equilibrium was respected. Heterozygosity estimates obtained for the BUR breed were moderate to high and consistent with the value reported by Del Bo et al. (2001) in a study on cattle breeds. These results are favorable for the implementation of conservation programs. High variability estimates have been detected also in other local cattle breeds (Rendo et al., 2004; Giovambattista et al., 2001) suggesting that small local populations have conserved more variability because they were not under strong selection. Estimates of Wright's  $F$ -statistics found out quite low homozygote excess in the whole population ( $F_{IT} = 8.5\%$ ), due only to differences in gene frequencies among breeds and not to a homozygote excess within them ( $F_{IS} = 0.00$ ). Such result was unexpected according to the literature. As usually, a certain amount of homozygote excess has always been detected (Jordana et al., 2003; Ciampolini et al., 2006). Our findings could be explained by the fact that in other studies deviation from H-W expectations were observed in many loci while, in our case, most of them were in equilibrium both in the whole sample and in each breed (data not shown). The estimates of Wright's  $F$ -statistics are shown in Table 4. Only BSW presented a certain excess of homozygote while BUR and HFR showed very low  $F_{IS}$  values. Homozygote excess in BUR was

remarkably low if compared with what found in other studies report on local breeds (Moioli et al., 2006), which implies that BUR has maintained most of its original variation, a good starting point for the development of a conservation program. However, these results were partially due to the presence of a significant heterozygote excess at TGLA126 locus in both BUR and HFR breeds. For this reason calculation were performed again excluding TGLA126 locus;  $F_{IS}$  estimates increased in all the studied breeds but it was still low in BUR (0.007).

**Table 4. Fixation indices. Genetic distances between breeds measured by  $F_{ST}$  (above diagonal), and  $F_{IS}$  (inbreeding within breeds) on diagonal in the studied breeds: Burlina (BUR), Holstein Friesian (HFR) and Brown Swiss (BSW)**

Breed	BUR	HFR	BSW
BUR	-0.003	0.047	0.103
HFR		-0.032	0.101
BSW			0.029

Estimates of  $F_{ST}$  showed that most of the population variance was explained by individual variability while 8.5% was explained by the presence of breeds, according to in literature findings (Schmid et al., 1999; Jordana et al., 2003). Genetic distances were measured by pair-wise  $F_{ST}$ , BSW was the most differentiated while the BUR-HFR pair was most similar. In their study on Alpine cattle breeds Del Bo et al. (2001) also found BUR to be the breed showing the lowest distance from HFR. However, test for population differentiation stated that gene frequencies among breeds were highly significant ( $P < 0.001$ ).

## CONCLUSION

In conclusion, this study demonstrated the feasibility of a conservation programme for the BUR breed. Its conservation appears important due to some very favourable features like a long productive life and good fertility. Moreover, implementation of a conservation scheme seems to be possible due to the moderate-high values of heterozygosity and the low homozygote excess.

## ACKNOWLEDGEMENT

Authors wish to thank Silvia Miotello and the Breeders Association of Treviso Province.

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(Received on 3 May 2007; accepted on 28 May 2007)