Genetic characteristic among Austrian and Hungarian cattle breeds

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Introduction

Carinthian blond (CB) and Waldviertel blond (WV) cattle used to be widespread in and around the Alpine region around 1900. During the following century the size of both populations was reduced drastically whereas Austrian Simmental and Brown Swiss became popular as high-yielding dual purpose breeds. According to ÖNGENE (2007) CB aroused from a mixture between Slavonian and Franconian cattle from Germany while WV was composed of many breeds; the three major breeds were Celtic, Hungarian Grey (HU, a pure breed originated from Hungary with grey coat color and known to be quite disease resistant) and Franconian cattle. At the present time, CB and WV populations are considered as highly endangered in Austria (ÖNGENE, 2007). Conservation breeding programs for those breeds were established 1995, where phenotypic conservation criteria are not considered to be sufficient to maintain original breeds' characteristics. Additional sources of information like pedigree and molecular genetic information might be used. Therefore the goal of this study was to investigate differences and similarities of closely related populations based on microsatellite marker information, to assess the genetic diversity, breed relationships, population structure and possible bottlenecks of CB, WV and HU populations.

Material and Methods

Animals and microsatellite markers

Blood from 60 animals per breed was sampled. All animals were genotyped for 29 microsatellite markers (Table 1) recommended for genetic diversity studies by the FAO (http://www.projects.roslin.ac.uk/cdiv/markers.html).

Statistical analysis

Each population was tested for Hardy-Weinberg equilibrium using GENEPOP v3.4 (http://genepop.curtin.edu.au/; Raymond and Rousset 1995). A sequential Bonferroni correction (α = 0.05) was used to correct for multiple comparisons (Rice 1989).

Observed and expected heterozygosity across breeds were estimated by GENETIX v4.05 (http://www.genetix.univ-montp2.fr/genetix/intro.htm). Additionally observed and expected heterozygosity and mean number of alleles across loci were estimated by EXEL MICROSATELLITE TOOLKIT (S.D.E.Park, http://animalgenomics.ucd.ie/sdepark/ms-toolkit/). Numbers of private alleles were derived by the software package CONVERT (http://www.agriculture.purdue.edu/fnr/html/faculty/Rhodes/Students%20and%20Staff/glaubitz/software.htm). Fixation coefficients (F_{IS}, F_{IT} and F_{ST}) were calculated using POPGEN v1.32 (http://www.ualberta.ca/~fyeh/download.htm). The number of effective migrants per generation (*Nm*) was based on F_{ST} estimates (Weir&Cockerham 1984) and was calculated as $Nm = (1-F_{ST})/4*F_{ST}$ (Wright 1969) by GENETIX.

Further, genetic relationships among breeds and individuals were derived using D_A (Nei et al. 1983). Distance matrices were calculated using POPULATION v1.2.30beta2 (Olivier Langella; http://ftp.bioinformatics.org/pub/populations/). Robustness of the UPGMA of population tree was tested by 10 000 bootstraps on loci and the cladogram was drawn with PHYLODRAW (http://pearl.cs.pusan.ac.kr/phylodraw/). Assignment of individual cattle to their most likely breed was performed by GENECLASS2 (Cornuet et al. 1999; http://www.montpellier.inra.fr/URLB/) using a frequencies based method (Paetkau et al. 1995) with 1 000 simulated individuals. We assessed bottlenecks using one method provided by the software package BOTTLENECK v1.2.02 (http://www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html). This method, the heterozygosity excess method, was described by Cornuet and Luikart (1996). It exploits the fact that allelic diversity is reduced faster than heterozygosity during a bottleneck, because rare alleles are lost rapidly and have little effect on heterozygosity, thus producing a transient excess in heterozygosity relative to that expected in a population of constant size with the same number of alleles. Two statistical tests have

been proposed to evaluate such differences. We applied standardized differences test (T2 values) and Wilcoxon sign-rank test (probabilities-one tail for H excess) to estimate the probability of heterozygosity excess.

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Results

In total, 213 alleles were observed at the 25 loci. The number of alleles per locus ranged from 4 (INRA35) to 14 (TGLA53) with a mean of 8.52. Null allele frequencies were estimated for each locus using EM algorithm (Dempster et al. 1977) for 10 000 replications by FREENA (http://www.montpellier.inra.fr/URLB/). We excluded 4 loci HAUT27 (15.2%), HEL13 (18.4%), ILSTS005 (11.3%) and INRA35 (36.6%) from further calculations because of estimated null allele frequencies above 10%.
The number of private alleles was highest in ETH185 (6), 3 loci had no private allele. Expected heterozygosity across all breeds varied from 0.287 (ETH10) to 0.864 (TGLA53) (Table 1). The mean number of alleles per breed ranged from 6.04±1.79 in WV to 6.52±1.81in HU and 6.76±2.11 in KB. Number of private alleles varied from 9 in WV to 27 in KB (Table 2). INRA32 in HU showed a significant heterozygote deficit ($P<0.01$). Further two loci in WV, INRA23 and INRA32, showed a significant heterozygote excess ($P<0.05$). After applying the sequential Bonferroni correction, no departure from HWE was observed across samples and loci. The exact test for population differentiation based on allele frequency variation showed that all breeds investigated were significantly different from each other ($P<0.001$). F_{ST} values indicated that around 6% of the total genetic variation can be explained by breed differences, the remaining 94% corresponding to differences among individuals.

Table 1 Chromosome (Chr.), number of alleles, number of private allele, size of DNA (bp), expected heterozygosity (H_e) and observed heterozygosity (H_o) for 29 mocrosatellite loci

Loci	Chr.	No. allele	Private allele	Length	H_e	H_o
BM1818	23	7	1	256 – 270	0.631	0.600
BM1824	1	5	-	199 – 211	0.738	0.706
BM2113	2	8	2	131 – 147	0.797	0.722
CSRM60	10	9	3	90 – 110	0.725	0.667
CSSM66	14	10	1	180 – 200	0.790	0.772
ETH10	5	7	2	208 – 222	0.287	0.289
ETH225	9	5	-	138 – 148	0.665	0.667
ETH3	19	7	2	109 – 129	0.615	0.583
ILSTS006	7	9	3	284 – 302	0.732	0.706
INRA23	3	10	1	199 – 219	0.800	0.833
SPS115	15	7	1	248 – 260	0.671	0.650
TGLA122	21	13	7	137 – 183	0.701	0.644
TGLA126	20	7	3	116 – 128	0.717	0.700
TGLA227	18	9	-	104 – 122	0.843	0.809
TGLA53	16	14	1	174 – 206	0.864	0.822
HEL1	15	9	3	102 – 118	0.753	0.669
HEL9	8	10	4	142 - 168	0.654	0.618
INRA005	12	4	1	133 – 139	0.668	0.607
INRA37	10	10	5	249 – 279	0.518	0.520
INRA63	18	7	1	168 – 180	0.672	0.661
ETH152	5	6	1	287 – 299	0.680	0.570
ETH185	17	13	6	222 – 246	0.748	0.687
HEL5	21	8	1	147 - 167	0.732	0.686
INRA32	11	8	3	169 – 185	0.636	0.583
MM12	9	11	2	173 – 197	0.804	0.789
		213	53		0.698	0.662

Table 2 Expected H_e and observed heterozygosity H_o, number of alleles and number of private alleles per breed

Population	H_e	H_o	No. Alleles	No. Private alleles
CB	0.6629	0.6747	6.76	17
WV	0.6314	0.6547	6.04	9
HU	0.6787	0.6580	6.52	27
All	0.6976	0.6624	8.52	53

Genetic structure and gene flow

Population differentiation among breeds is presented by pairwise F_{ST} coefficients (Table 3). F_{ST} coefficients ranged from 0.0649 (between CB and WV) to 0.1073 (between WV and HU). Thus, from 6.49 to 10.73% of the microsatellite variability was explained by between breeds variability while the remaining variability was explained by the variation within breeds (among individuals). Gene flow between breeds was shown by estimating the number of migrants per generation (*Nm*, where *N* was the total effective number of cattle and *m* was the migration rate). The highest migration rate was estimated between CB and WV (3.60) and the lowest migration rate between WV and HU (2.08).

Table 3 Population differentiation among breeds based on 25 microsatellite loci was presented by F_{ST}[1] (above diagonal) and gene flow[2] (*Nm*) (below diagonal)

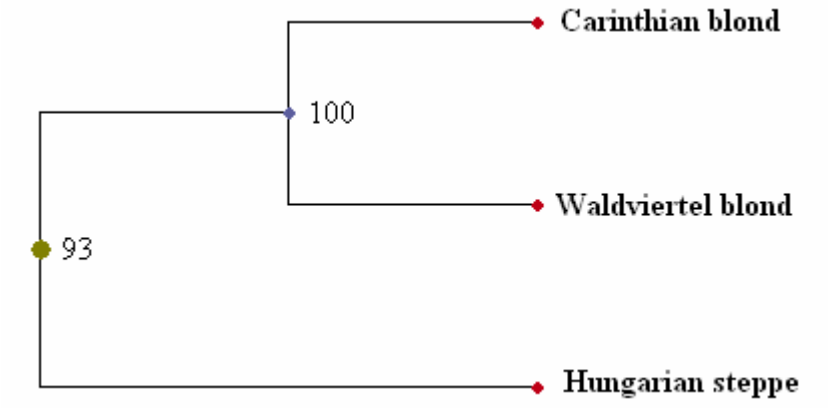
	CB	WV	HU
CB	-	0.0649	0.0685
WV	3.60	-	0.1073
HU	3.40	2.08	-

[1]F_{ST} estimates were calculated as described by Weir & Cockerham (1984).
[2]The number of effective migrants per generation (*Nm*) or gene flow were estimated using formula, $Nm = (1-F_{ST})/4F_{ST}$, derived by Wright (1969)

Breed relationships

Figure 1 presents a cladogram depicting the genetic relationships between breeds based on D_A. HU population split away from the two blond breeds. Generally the high bootstrapping values indicate a very stable phylogeny.

Figure 1 The UPGMA cladrogram of 3 breeds based on Nei et al.'s (1983) D_A, 10 000 bootstraps on loci.



Breed Assignment

The direct method of Paetkau et al. (1995) allowed the correct assignment of 97.8% of individuals to their breed of origin. However, there were 4 individuals from WV that could not be assigned to their breed and two migrants were detected ($P<0.01$).

Bottleneck Detection

We used standardized differences (T2) and Wilcoxon sign rank tests to characterize bottlenecks in three populations. The values of T2 were lower than 1.645 for all populations in both TPM and SMM (Table 4). Using the Wilcoxon rank test, the probability values were greater than 0.9 for all populations in both TPM and SMM. Results from both tests indicated that, due to mutation-drift equilibrium, a recent genetic bottleneck did not occur in CB, WV and HU. The Mode-shift indicator test was also utilized as a second method to detect potential bottlenecks, as the non bottleneck populations that were near mutation-drift equilibrium were expected to have a large proportion of alleles with low frequency. A graphical representation utilizing allelic class and proportion of alleles show a normal L-shaped distribution (data not shown here) in all populations. This distribution confirmed clearly the result that the three populations have not experienced a bottleneck recently.

Table 4 Number of loci with heterozygosity excess/deficiency and probabilities obtained from 25 microsatellites evolution models for bottleneck test (BOTTLENECK, 10 000 replications).

	Test	Pop	T2	P
1.	Standardized differences test (T2 values)			
	TPM	CB	-3.825	0.00007
		WV	-3.619	0.00015
		HU	-2.509	0.00606
	SMM	CB	-5.780	0.00000
		WV	-5.369	0.00000
		HU	-4.207	0.00001
2.	Wilcoxon test (probabilities-one tail for H excess)			
	TPM	CB		0.99518
		WV		0.98626
		HU		0.94634
	SMM	CB		0.99979
		WV		0.99875
		HU		0.99518

*parameter in TPM, 95% single-step mutation, 5% multiple-step mutation and variance among multiple steps = 12

Conclusions

As expected the differentiation between CB and WV was lowest while the migration rate was highest indicating gene flow between the Austrian blond cattle breeds. However, low levels of gene flow are most likely because of clear differentiation of the three breeds, stable trees with high bootstrap values and the high percentage of correctly assigned individuals.

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