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RESEARCH ARTICLE

Assessment of genetic structure in Mexican charolais herds using microsatellite markers

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Abbreviations: HW: Hardy-Weinberg
PCR: polymerase chain reaction
PIC: polymorphic information content

Knowledge of livestock genetic diversity is an essential step to respond to commercial demands and reach production objectives in different environments and production systems. The evaluation of animal genetic diversity is achieved by using molecular markers. Microsatellites are the most used markers for studies of this type. Eleven microsatellites were used to evaluate the genetic variation from three populations of Charolais cattle located in northeast Mexico. The studied populations exhibited a high allelic variability with a mean heterozygosity of 0.5. A moderate genetic differentiation between the Charolais populations (F_{ST} = 0.079; P < 0.001) was observed. This suggests subdivisions in Charolais breed established in Mexico, due to genetic material origin, reproductive and selective management and local isolation.

Breeding and conservation programs can be determined by characterizing the genetic variation of livestock (Notter, 1999). Genetic improvement may reduce significantly the genetic variation within populations (Vasconcellos et al. 2003). To date, there are an increasing number of specialized breeds spread mainly for the development of reproduction systems (*i.e.* artificial insemination, embryo transfer, etc.) conducting to a reduction on their population effective size and complicating sustained genetic improvement (Mapletoft and Hasler, 2005). The Holstein breed is a clear example of widespread germplasm for milk production improvement. An evident consequence is a large Holstein population maintained only for a few sires. In such cases genetic diversity evaluation, is primordial in order to avoid the deleterious consequences (Notter, 1999; Williams, 2005).

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Microsatellites are an important tool in population genetic studies. They are highly polymorphic, presents simple mendelian inheritance and codominance (segregation of homozygotes and heterozygotes (Boichard et al. 1998)).

Versatility for adaptation to a wide range of management conditions has made the Charolais a highly used beef cattle breed in the world. In was imported to Mexico in the 1930's. The best animals have been selected for performance and phenotypic characteristics. Because the breed is so adaptable to varying conditions, it has been used in almost all states of the country as purebred or crossbred cattle.

Genetic diversity of the Charolais developed in Mexico can be a consequence of several factors. Some of these factors are the differences in management and in selective decisions, and the use of imported genetic material. The study of genetic variation in different populations will eventually allow the understanding of the effect of germplasm introduction, gene flow and/or selection movements. The objective of the present study was to evaluate the genetic molecular variation at three founder Charolais populations from different environments in Mexico.

MATERIALS AND METHODS

Whole blood was collected from one hundred-ninety four non-related purebred Charolais cattle in three ranches. Two ranches are located in Nuevo Leon, and one in Veracruz, Mexico. The selection criterion at each ranch is breed pattern, conformation, and weight gain. These ranches are national distributors of the Charolais genetics. Each ranch was identified as a subpopulation, according with the origin of genetic material used for improvement. Ranch 1, imported genetic material only from France; ranch 2, utilizes its own and local genetic material; and ranch 3 imports genetic material from France, UK and Ireland.

Isolation of DNA was done as is reported by Peelman et al. (1998). Genetic diversity was determined by using eleven microsatellites loci; Table 1 describes loci sequences and polymerase chain reaction (PCR) conditions. Amplification profiles consisted of one cycle at 95ºC during 10 min; five three-step cycles of 45 sec at 95ºC, 45 sec at 62ºC (temperature was reduced 2ºC each cycle), 45 sec at 72ºC; 25 cycles with the following steps each one of 45 sec at 95ºC, 55ºC 45 sec (this temperature was changed for each loci according with Table 1), 45 sec at 72ºC; and finally one cycle of 10 min at 72ºC. PCR products were denaturized at 95ºC for 5 min, and then electrophoresed on a 6.5% denaturing polyacrylamide-bisacrilamide gel during 2 hrs on a LI-COR sequencer (LI-COR, Inc. Lincoln, Nebraska USA) model 42001G.

Allele size for each locus was obtained using SAGA GT^{TM} software (LI-COR, Inc. Lincoln, Nebraska USA). Numbers of alleles (k) observed (HO) and expected (HE) heterozygosity, allelic frequencies, and the polymorphic information content (PIC) for each locus and in combinations of loci were estimated using CERVUS 2.0 software (Marshall et al. 1998). Diversity analysis was performed using the same software. Population structure was established by an Analysis of Molecular Variance (AMOVA), using Arlequin (Excoffier et al. 2005). Effective number of alleles was estimated according to Li et al. (2004).

RESULTS AND DISCUSSION

All loci showed high polymorphism in all ranches (Table 2). Twelve alleles per loci was the average. Allele number per locus ranged from 7 alleles in ETH10 locus, to 18 alleles in TGLA53. The allele diversity was similar among the ranches. Ranch 2 showed a unique allelic series for marker ETH10 (from 203 bp to 217 bp). ETH10 locus also showed the most different allele number between ranches. Ranches had unique alleles. Ranch 2 had seven at five loci, meanwhile the ranches 1 and 3, presented six and five, respectively.

Expected heterozygocity was higher than the observed for all loci in the three ranches. The overall mean heterozygocity was 0.500. Observed heterozygosity ranged from 0.388 to 0.780, from 0.235 to 0.816, and from 0.230 to 0.732 for ranches 1, 2, and 3, respectively. Heterozygosity is a reliable estimator of genetic diversity (MacHugh et al. 1998). MacHugh et al. (1998) reported a value of heterozygosity for Charolais of 0.525. Maudet et al. (2002) found a heterozygosity of 0.640 in Charolais in France. These heterozygocity values are similar than those estimated in the present study, suggesting the genetic diversity of Charolais in Mexico is comparable to that reported in Europe.

An overall departure from Hardy-Weinberg expectation (HW) was ascertained in the present study for all markers with the exception of BMS1886 ($P < 0.01$; Table 3). Deviation of genetic equilibrium, might suggest a subdivision in the whole population due to a Wahlund effect (Hartl and Clark, 1997).

Based on the analysis of molecular variance (Table 4), there was a moderate differentiation ($F_{ST} = 0.079$; $P < 0.001$) among ranches (Hartl and Clark, 1997). Variation within and between the ranches was of 92.03 and 7.97%, respectively. Jordana et al. 2003, reported an analysis in eight Southwest European beef cattle breeds, their results showed a genetic differentiation between breeds of 6.8%, authors pointing out that those values are similar to those founded in humans, dogs, sheep, goats, rabbits and pigs. Similarly, another study in European beef cattle, reported, values of differentiation between breeds of 7.0%; this degree of differentiation was related to a relatively low genetic flow and high reproductive isolation (Cañón et al. 2001). Moioli et al. (2004), reported between isolated Italian breeds an estimate of $F_{ST} = 0.06$, even lower than the

one found for our studied subpopulations. Considering the similarity of our results with those previously found between breeds even species, they could be an evidence of subdivisions within the Mexican Charolais breed, suggesting a direct consequence of genetic material origin and reproductive or selective management practices.

Fernández et al. (2006) suggested that subdivision of an animal population may have negative effects conducting to a reduced population size and, therefore, it could generate higher inbreeding levels and a depression at fitness-related traits. One way to avoid that side-effect is to allow certain degree of gene flow between subpopulations. However, Notter (1999), indicated that the maintenance of allelic diversity is achieved in subdivided rather than panmictic populations so long these are large enough to pass through the inbreeding effects, or managed for an occasional crossing to renew heterozygosity. Application of these concepts to evaluated populations, could avoid the inbreeding effects, a recommendation is to continue with the current reproduction practices, which are based on using imported semen.

These results show the relationships at the evaluated populations, not the complete history of the breed in Mexico. Further studies must be done in order to confirm the subdivisions within Charolais breed in Mexico and its implications in the design of crossbreeding strategies focused to exploit the productive advantages of each subdivided breed-population.

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APPENDIX

TABLES

Table 1. Chromosomal location, primer sequences and PCR conditions for microsatellite loci used in this study.

Locus	Ch[1]	PCR Conditions		Primer Sequences
		$MgCl_2$[2]	Annealing Temperature (°C)[3]	
BM2123	2	3.0	55	5'-GCTGCCTTCTACCAAATACCC-3' 5'-CTTCCTGAGAGAAGCAACACC-3'
ETH10	5	3.0	55	5'-GTTCAGGACTGGCCCTGCTAACA-3' 5'-CCTCCAGCCCACTTTCTCTTCTC-3'
INRA37	10	3.0	50	5'-GATCCTGCTTATATTTTAACCAC-3' 5'-AAAATTCCATGGAGAGAGAAAC-3'
BM1824	1	1.2	55	5'-GAGCAAGGTGTTTTTCCAATC-3' 5'-CATTCTCCAACTGCTTCCTTG-3'
BMS1886	2	3.0	60	5'-CAGGGACTGAAAAATAATGCC-3' 5'-TTCCATGTTGATTGTTTCTTCC-3'
TGLA53	16	2.0	50	5'-GCTTTCAGAAATAGTTTGCATTCA-3' 5'-ATCTTCACATGATATTACAGCAGA-3'
ILSTS005	5	0.8	50	5'-GGAAGCAATGAAATCTATAGCC-3' 5'-TGTTCTGTGAGTTTGTAAGC-3'
BMS1987	2	3.0	60	5'-TGATGCAGAGAACGTTTTAATTT-3' 5'-CTTGGGGTAGGCAGAGATTT-3'
INRA23	3	3.0	50	5'-GAGTAGAGCTACAAGATAAACTTC3' 5'-TAACTACAGGGTGTTAGATGAACTC-3'
HEL5	21	1.0	50	5'-GCAGGATCACTTGTTAGGGA-3' 5'-AGACGTTAGTGTACATTAAC-3'
TGLA44	2	3.0	65	5'-AACTGTATATTGAGAGCCTACCATG-3' 5'-CACAACTTAGCGACTAAACCACCA-3'

[1]Chromosome on which the microsatellite is located.
[2]$MgCl_2$ concentration (mM).
[3]Anneling temperature during PCR.

Table 2. Allele frequency of eleven microsatellite loci for three studied Charolais populations.

Locus/Allele size	Subpopulation/Allele frequency			Locus/Allele size	Subpopulation/Allele frequency			Locus/Allele size	Subpopulation/Allele frequency		
	1	2	3		1	2	3		1	2	3
BM2113				*BMS1886*				*INRA23*			
125	0.0900	0.0256		131	0.0612		0.0070	193			0.0390
127	0.0200	0.0513	0.0380	133	0.4694	0.4394	0.4437	195			0.0519
129	0.0600	0.0641	0.0063	135		0.0152		197	0.0204	0.0676	0.0584
131	0.3300	0.2949	0.0190	137		0.0303	0.0141	199	0.0306	0.0135	0.1948
133	0.1200	0.1282	0.3481	139	0.0102		0.0352	201	0.1122	0.0135	0.0649
135	0.1800	0.1538	0.2215	143	0.0102			203	0.1224	0.0946	0.2143
137	0.1900	0.1923	0.1646	145	0.0102	0.0606	0.0141	205	0.1224	0.1892	0.1429
139	0.0100	0.0256	0.1519	147	0.1122	0.1061	0.0423	207	0.2755	0.1081	0.0390
141		0.0641	0.0380	149	0.1327	0.1970	0.1761	209	0.0816	0.1892	0.0584
143			0.0127	151	0.0612	0.0455	0.0423	211	0.0306	0.0811	0.0779
				153	0.0102	0.0303	0.0704	213	0.0714	0.0946	0.0325
				155	0.0510	0.0606	0.0915	215	0.0408	0.0405	0.0195
ETH10				157	0.0714	0.0152	0.0634	217	0.0204	0.0811	0.0065
203		0.0113						219	0.0612	0.0135	
205		0.0113		*TGLA53*				221	0.0102		
209		0.1857	0.2000	145			0.0072	223		0.0135	
211		0.2138	0.7467	147			0.0217				
213	0.2128	0.0578	0.0267	149	0.0900		0.0072				
215	0.6809	0.0227	0.0133	151	0.1200	0.1429	0.0725	*HEL5*			
217	0.1064	0.0113	0.0133	153	0.1500		0.0580	159	0.0104		
				155	0.0900	0.0143	0.1304	161	0.0417	0.0556	0.0878
				157	0.1300	0.2143	0.2536	163	0.0521		
INRA37				159	0.0400	0.1000	0.1232	165	0.4792	0.0694	0.1757
114		0.0263		161	0.0300	0.0429	0.0725	167	0.0208	0.2083	0.0338
118			0.0065	163	0.900	0.0857	0.0725	169		0.1528	
120		0.0395	0.0260	165	0.0700	0.1571	0.0290	171		0.0278	
122			0.0260	167	0.1000	0.1143	0.0797	173		0.0139	
124			0.1364	169	0.0400	0.0429	0.0290	175	0.0417		0.0676
126	0.2857	0.2237	0.2338	171	0.0300			177	0.0313	0.0139	0.0541
128	0.3265	0.2500	0.1688	173	0.0200	0.0286		179	0.3229	0.1806	0.0541
130	0.0612	0.0526	0.1299	175		0.0286	0.0435	181		0.0972	0.0135
132	0.2245	0.2763	0.1948	177		0.0143		183		0.0694	0.2095
134	0.0612	0.0921	0.0325	183		0.0143		185		0.0139	0.2770
138			0.0130					187		0.0972	0.0270
144	0.0102	0.0263	0.0065	*ILST005*							
146	0.0306	0.0132	0.0260	179		0.0128					
				181		0.1282	0.0800	*TGLA44*			
				183	0.1735	0.4359	0.4133	141			0.0133
BM1824				185	0.4592	0.3718	0.5000	143	0.0213	0.0469	0.0600
174		0.0405		187	0.3673			145		0.0156	0.0600
176		0.1081		189		0.0256	0.0067	147			0.0133
178	0.2300	0.2568	0.0400	191		0.0128		159		0.0156	0.0067
180	0.1900	0.3784	0.3000	193		0.0128		161	0.2128	0.1250	0.2266
182	0.2800	0.0541	0.2200					163	0.0532	0.1094	0.1267
184	0.0200	0.0135	0.2600	*BMS1987*				165	0.5213	0.2500	0.3200
186		0.0811	0.0067	108			0.0063	167	0.1064	0.2344	0.1400
188	0.1600	0.0676	0.0333	110		0.0921	0.2250	169	0.0638	0.1406	0.0333
190	0.1200		0.1133	112	0.5300	0.3947	0.2750	171	0.0106	0.0469	
192			0.0267	114	0.0300	0.0263	0.0437	175	0.0106	0.0156	
				118		0.0132					
				120		0.1053	0.1937				
				122	0.4100	0.2237	0.2250				
				124		0.0263	0.0188				
				126	0.0300	0.1184	0.0125				

Table 3. Expected and observed heterozygosity (H_E and H_O respectively), effective number of alleles (a_e), HW deviation significance, and PIC of eleven microsatellite markers used in Charolais cattle.

		Locus[1]										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Subpopulation 1												
n^2 = 1/79	H_E	0.804	0.485	0.761	0.800	0.741	0.911	0.631	0.555	0.870	0.665	0.671
	H_O	0.520	0.000	0.592	0.780	0.755	0.680	0.388	0.560	0.469	0.688	0.532
	HW[3]	***	***	**	NS	NS	NS	***	NS	***	**	NS
	a_e	4.901	1.922	4.046	4.812	3.757	10.163	2.660	2.218	7.224	2.927	2.976
	PIC	0.769	0.426	0.712	0.760	0.712	0.893	0.546	0.453	0.849	0.605	0.627
Subpopulation 2												
n = 8/45	H_E	0.834	0.651	0.807	0.774	0.757	0.885	0.663	0.769	0.891	0.880	0.843
	H_O	0.410	0.235	0.605	0.514	0.636	0.714	0.436	0.816	0.649	0.167	0.344
	HW	***	***	***	**	NS	**	**	NS	***	***	***
	a_e	5.675	2.792	4.920	4.224	3.924	7.851	2.891	4.150	8.246	7.557	5.884
	PIC	0.803	0.578	0.768	0.732	0.719	0.860	0.590	0.728	0.867	0.854	0.809
Subpopulation 3												
n = 17/63	H_E	0.781	0.404	0.846	0.783	0.755	0.880	0.577	0.788	0.877	0.834	0.813
	H_O	0.253	0.093	0.636	0.6	0.732	0.493	0.44	0.663	0.558	0.257	0.467
	HW	***	***	***	***	NS	***	***	***	***	***	***
	a_e	4.467	1.670	6.253	4.498	3.992	7.909	2.344	4.611	7.616	5.713	5.197
	PIC	0.744	0.356	0.820	0.743	0.727	0.863	0.483	0.748	0.856	0.804	0.784
Population												
n = 26/187	H_E	0.802	0.700	0.820	0.827	0.751	0.901	0.420	0.667	0.549	0.369	0.461
	H_O	0.369	0.096	0.616	0.636	0.719	0.608	0.420	0.667	0.549	0.369	0.461
	HW	**	**	**	**	NS	**	**	**	**	**	**
	a_e	4.981	3.310	5.476	5.702	3.977	9.843	2.896	3.932	9.922	7.522	4.700
	PIC	0.773	0.665	0.793	0.803	0.751	0.901	0.657	0.748	0.891	0.854	0.762

Table 4. Analysis of Molecular Variance in Charolais cattle herds in northeast Mexico.

Source of variation	Degrees of freedom	Sum Square	Variance components	Variation %
Between populations	2	76.971	0.32372	7.97
Within populations	335	1252.254	3.73807	92.03
Total	337	1329.225	4.06179	
	F_{ST} = 0.07970			