Genetic differentiation among geographically isolated populations of Criollo cattle and their divergence from other Bos taurus breeds

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Genetic differentiation among geographically isolated populations of Criollo cattle and their divergence from other *Bos taurus* breeds[1]

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ABSTRACT: The microsatellites HEL5, HEL9, INRA063, and BM2113 were used to analyze genetic similarities and differences of geographically isolated Criollo cattle herds in Mexico. Criollo cattle from five counties within the state of Chihuahua and one county from the state of Tamaulipas (n = 60) were sampled. The five counties in Chihuahua included Cerocahui (n = 14), Chinipas (n = 10), Guachochi (n = 15), Morelos (n = 30), and Temoris (n = 9). Samples of DNA were amplified by PCR and separated on a 7% polyacrylamide gel. Microsatellite size was established by comparison to M13mp18 DNA ladder and a documented set of four bovine controls. Allele frequencies and genotypic deviations from Hardy-Weinberg equilibrium were tested using the GENEPOP program. Eleven alleles were generated at HEL5 for the populations sampled (149 to 169 bp). Allele frequencies were greatest for the 163-bp allele in Criollo cattle from Cerocahui, Chinipas, Moralos, and Tamaulipas (0.23 to 0.5). Cattle from Guachochi had an allele frequency of 0.38 for the 151-bp allele, and cattle from Temoris had an allele frequency of 0.25 for the 149- and 167-bp alleles, with no 163-bp allele. Amplification with HEL9 produced 12 alleles (145, 149 to 169 bp) and showed common high-frequency alleles at 149, 157, and 159 bp for animals from all regions. The Chinipas population showed a moderate allele frequency at 145 bp; no other regions contained this allele. For INRA063 there were five alleles with 182 and 184 bp in low frequency. For BM2113 there were 10 alleles in the Criollo cattle (125 to 143 bp), with an equal distribution of frequencies for all alleles. In two regions, Guachochi and Morelos, genotypic frequencies deviated from Hardy-Weinberg equilibrium. Cattle from the Temoris region were genetically most distant from Criollo cattle of the other five regions.

Key Words: Genetic Analysis, Genetic Variation, Microsatellites

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Introduction

Criollo cattle, also known as Corriente, have experienced a unique genetic evolution due to limited management, natural selection, and adaptation. These cattle are reputed to be heat-resistant and have inherent longevity from living in the region of Mexico known as the Sierra Madre, which contains steep canyons and limited forage. Many of the Criollo remaining in Mexico are exported for use in rodeo; the annual demand is 40,000 steers for rodeo alone (Fierro and Torres, 1996). Pure Criollo, which exist in small, isolated populations, have dwindled rapidly in numbers during the last 30 yr. Production of these cattle in the Sierra Madre may be very efficient, because they live and reproduce in areas where other cattle may not survive. They also are raised and sold by the native people of the Sierra Madre.

Microsatellites have been proven to be useful markers for a variety of purposes, such as genome mapping, parentage determination, disease research, cancer research, and determination of genetic variation (Caskey et al., 1992; Glowatzki-Mullis, et al., 1995; Garcia-Moreno et al., 1996). The high variability of microsatellites and their distribution give them advantages over other markers. Arranz et al. (1996a) showed that microsatellite loci were much more useful than protein markers in determining heterozygosity and genetic distances between Brown Swiss and three breeds of Spanish cattle.

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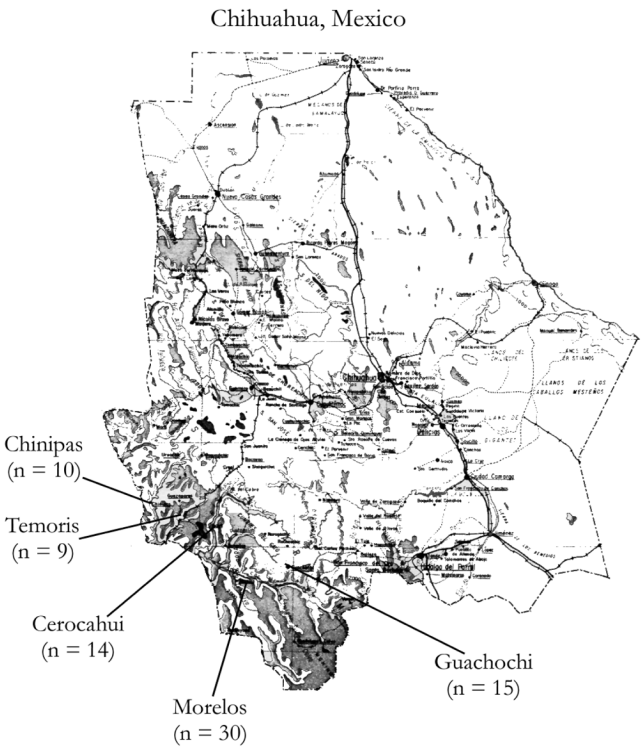

Figure 1. Map of Chihuahua, Mexico, showing five regions containing Criollo cattle that were sampled.

Microsatellites have been used previously in human evolutionary studies and recently in bovine evolutionary studies (Ciampolini et al., 1995; Arranz et al., 1996b; Moazami-Goudarzi et al., 1997). Microsatellites have been effective in evaluating differences within a breed of cattle and determining population substructure (MacHugh et al., 1994, 1998; Ciampolini et al., 1995). Objectives of this study were to determine genetic distance between isolated Criollo cattle populations and the genetic distance of Criollo from some common beef *Bos taurus* breeds.

Materials and Methods

Sampling Procedure

Blood samples were collected from Criollo cattle in remote areas of Mexico, ensuring the populations were geographically isolated. Samples were collected from four to five herds within each region. The areas within Mexico consisted of locations within the states of Chihuahua and Tamaulipas. Chihuahua lies directly south of New Mexico and Tamaulipas borders the southernmost area of Texas. Criollo cattle within five areas of southwestern Chihuahua were sampled (Figure 1), and one area within the state of Tamaulipas was sampled. The five regions in Chihuahua that were sampled include Cerocahui (n = 14), Chinipas (n = 10), Guachochi (n = 50), Morelos (n = 30), and Temoris (n = 9). Within the state of Tamaulipas one region was sampled (n = 60). The Criollo herds within the area of Chihuahua sampled are small (less than 30 animals) and belong primarily to the indigenous people known as the Tarahumara. Diverse environments exist within the Chihuahua study site, including a mountainous area with elevations of 1,500 to 3,250 m above sea level and a subtropical region with elevations of 500 to 1,480 m above sea level. Facilities to work cattle are nonexistent and all blood samples were collected by roping and restraining animals. Multiple herds (four to five) within each region were sampled and individuals were randomly selected.

Microsatellite Analysis. Genomic DNA was extracted from whole blood using a saturated salt procedure described by Miller et al. (1988). Concentrations of DNA, reconstituted in water, were quantified using a spectrophotometer.

Amplification of genomic segments followed the procedure described by Bishop et al. (1994) but we altered the amount of template from 80 to 50 ng. Briefly, PCR consisted of 30 cycles at 94°C for 1 min, annealing temperature for 30 s, and 72°C for 1 min. Concentrations were as follows: 0.4 μ*M* primers, 1.5 m*M* $MgCl_2$, and 30 μ*M* dNTP. The final volume of each reaction tube was approximately 12 μL. The PCR products were radiolabeled by incorporation of 0.1 μCi [α^{32}P]dATP (NEN Life Science Products, Boston, MA). Forward and reverse primers were obtained from Life Technologies (Gaithersburg, MD. Electrophoresis and scoring were performed according to Bishop et al. (1994); genotypes were independently scored twice. Gels were vacuum-dried for 2 h onto filter paper (Hoefer Pharmacia Biotech, San Francisco, CA) and exposed on Biomax film (VWR Scientific Products, Willard, OH) overnight at room temperature or for 1 h at –80°C. Allele sizes were approximated by comparing to an M13mp18 ssDNA ladder (Amersham Life Science Products, Arlington Heights, IL) and also to previously genotyped bulls from the USDA Meat Animal Research Center (MARC).

The microsatellite markers used in this study were selected based on high heterozygosity measures, low ease of scoring values, high numbers of possible alleles, and location on different chromosomes. Two of the microsatellite primers used in this study were published by Kaukinen and Varvio (1993) (HEL5 and HEL9). HEL5 is located on chromosome 21 and has an annealing temperature of 52°C. HEL9 is located on chromo-

Table 1. Primer information for the microsatellites BM2113, HEL5, HEL9, and INRA063

Microsatellite	Chromosome	No. of possible alleles	Heterozygosity
BM2113	2	11	72%
HEL5	21	7	69%
HEL9	8	13	83%
INRA063	18	6	66%

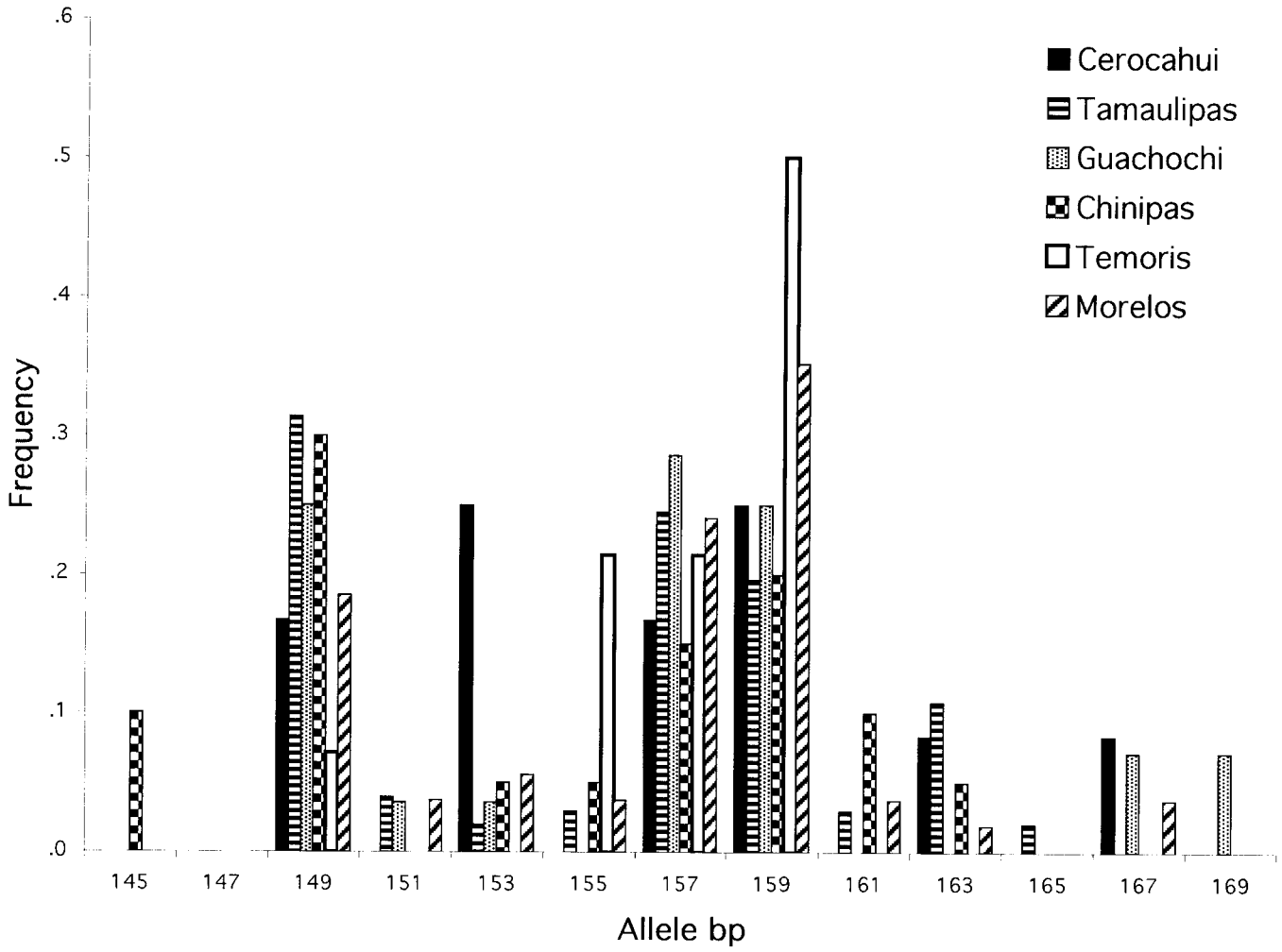

Figure 2. Allele frequencies for six Criollo populations as determined by the microsatellite HEL9. Global tests across all populations were performed by Fisher's method (Manly, 1985) and yielded the values $\chi^2 = 13.1$, $P = 0.3598$.

some 8 and has an annealing temperature of 52°C. INRA063, which was published by Vaiman et al. (1994), has an annealing temperature of 58°C and is located on chromosome 18. The microsatellite BM2113 was described by Sunden et al. (1993) and is located on chromosome 2 with an annealing temperature of 58°C. The linkage analysis for these markers that assigned positions on the bovine map was performed by Bishop et al. (1994) and Kappes et al. (1997). Information on heterozygosity, chromosomal location, and number of alleles previously detected for each of the four microsatellites used in this study is presented in Table 1.

Statistical Analysis

Allele frequencies were calculated using the computer program GENEPOP (Raymond and Rousset, 1995). The data were tested across and within geographical regions for conformity with Hardy-Weinberg expectations by GENEPOP using the methods of Guo and Thompson (1992). In order for a population to be in Hardy-Weinberg equilibrium, it must have stable allele frequencies and be a randomly mating population. There are several reasons populations may deviate from this equilibrium. Selection, either natural or artificial, will change a population's gene frequencies. Inbreeding can also be a cause of genotypic deviation from equilibrium. Two other causes are meiotic drive, the unequal selection of gametes, and migration, a large influence from an outside population with different genotypic frequencies. Additionally, global Hardy-Weinberg tests were performed using Fisher's method to combine independent test results (Manly, 1985). Fisher's method was used to combine the six populations and to combine the four microsatellite markers. Briefly, this method treats each individual test result as a random variable and is a chi-squared variate with $2n$ degrees of freedom.

The Ds method described by Nei (1972) for determining genetic distances was used. Genetic distance measures the time that has elapsed since populations were genetically equivalent. The calculations for genetic distance measures were performed by the method of

Paetkau et al. (1997). These methods of statistical analyses have been used in similar studies (Ciampolini et al., 1995; Garcia-Moreno et al., 1996; Goodman, 1998).

Results

Allele Frequency Results

To determine the genetic relationship between isolated herds of Criollo cattle in Mexico, six regions within Mexico were chosen for sampling: Cerocahui, Tamaulipas, Guachochi, Chinipas, Temoris, and Morelos.

Allele frequency data for the microsatellite HEL9 are depicted in Figure 2. Twelve alleles were generated by the microsatellite HEL9 (145 and 149 to 169 bp). The frequencies of the 149-, 157-, and 159-bp fragments were similar across the populations for all regions. The 145-bp allele was present in cattle from Chinipas but absent from cattle originating in other regions. In addition, cattle from the Cerocahui region had a moderately high frequency (.25) of the 153-bp allele, but few animals from other regions had this allele. Criollo cattle from the Temoris region also displayed uniqueness by having a frequency of 0.21 at the 155-bp allele.

Allele frequency data for the microsatellite INRA063 are shown in Figure 3. Five alleles were detected by INRA063 (178 to 186 bp) in the Criollo samples, with little representation at the 182- and 184-bp alleles. Cattle from the Temoris region contained a region-specific allele at 184 bp with a frequency of 0.14; cattle from other regions did not have this allele. Another unique frequency was detected at the 186-bp allele, with a higher representation among the Chinipas samples (0.42) than among samples from the other five regions (0.06 to 0.18). In addition, this marker detected less genetic variability due to the smaller number of alleles compared to the other three markers.

Allele frequency data for the microsatellite BM2113 are depicted in Figure 4. BM2113 amplified 10 alleles

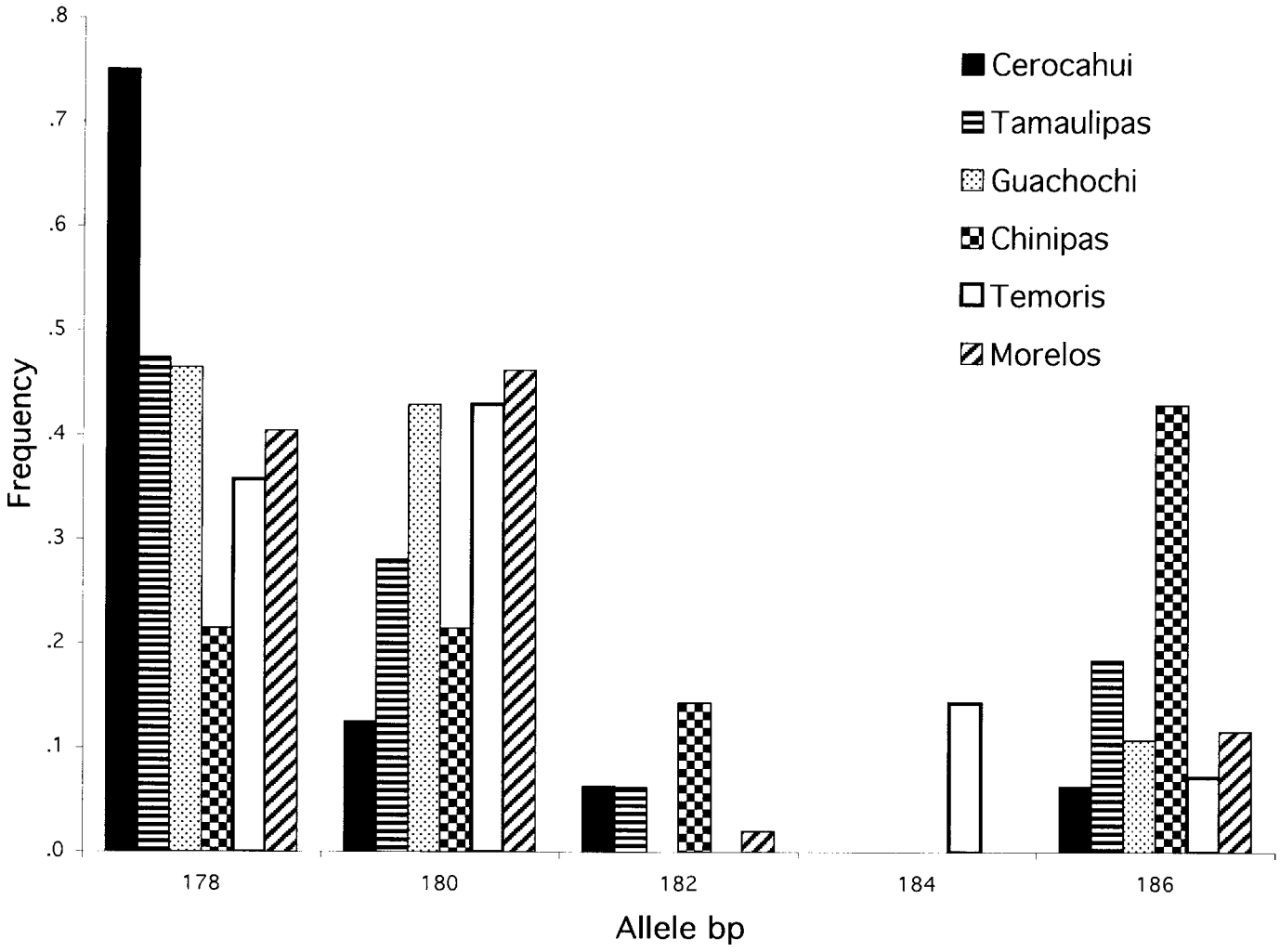

Figure 3. Allele frequencies for six Criollo populations as determined by the microsatellite INRA063. Global tests across all populations were performed by Fisher's method (Manly, 1985) and yielded the values $\chi^2 = 7.3$, $P = 0.8396$.

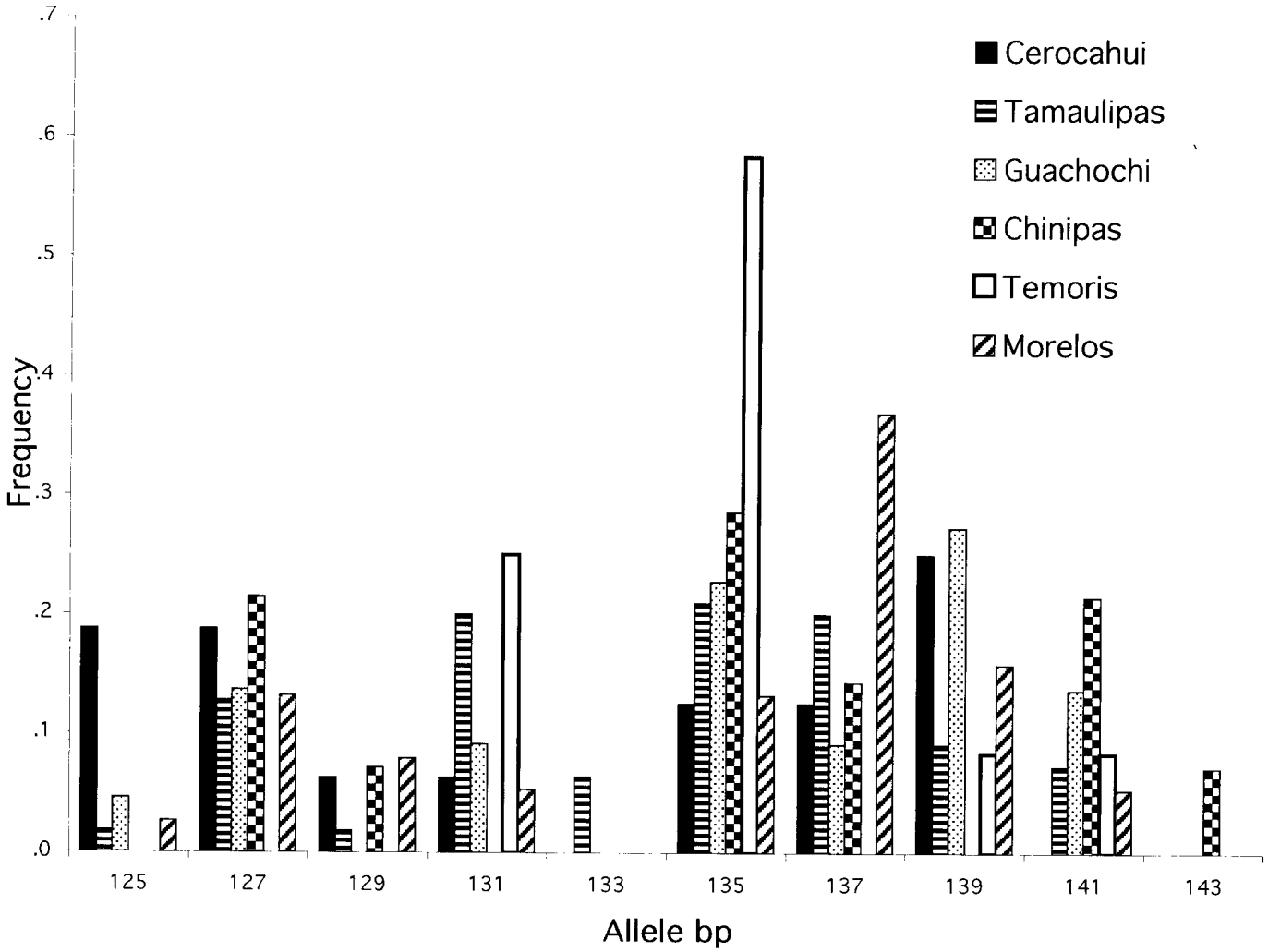

Figure 4. Allele frequencies for six Criollo populations as determined by the microsatellite BM2113. Global tests across all populations were performed by Fisher's method (Manly, 1985) and yielded the values $\chi^2 = \infty$, $P = < 0.01$.

in the Criollo samples (125 to 143 bp), yielding a fairly equal distribution of frequencies. The most common allele was the 135-bp allele, which represented over half of the Temoris region's total frequency. Cattle from the Tamaulipas region were the only ones represented at the 133-bp allele with a frequency of 0.06, and the Chinipas cattle were also the only population with the 143-bp allele, with a frequency of 0.07. In addition, the Cerocahui samples had a moderate frequency of the 125-bp allele (0.188), with little representation from other regions (0 to 0.045).

The allele frequencies determined for the microsatellite HEL5 are shown in Figure 5. For HEL5, there were 11 alleles for the Criollo samples (149 to 169 bp). Allele frequencies were greatest for all regions for the 151- and 163-bp alleles, excluding cattle from Temoris, which had allele frequencies of 0.25 at 149 and 167 bp. In addition, the Temoris samples, unlike all others, did not contain the 163-bp common allele.

Hardy-Weinberg Equilibrium

Hardy Weinberg calculations were determined for each individual region, combining the allele frequency data for all four markers used. The results of the Hardy Weinberg test are shown in Table 2. The only regions deviating from equilibrium were Guachochi and Morelos.

Genetic Distances Determined Within Criollo Regions

Genetic distance calculations were performed combining the four markers used to analyze the six populations of Criollo cattle. Presented in Table 3 are genetic distance measures between populations from the regions sampled. Genetic distance measures for cattle from the six regions showed that Criollos from five out of the six areas have small distances, and therefore seem to be closely related. The Temoris sample, however, had large distances compared to the other five

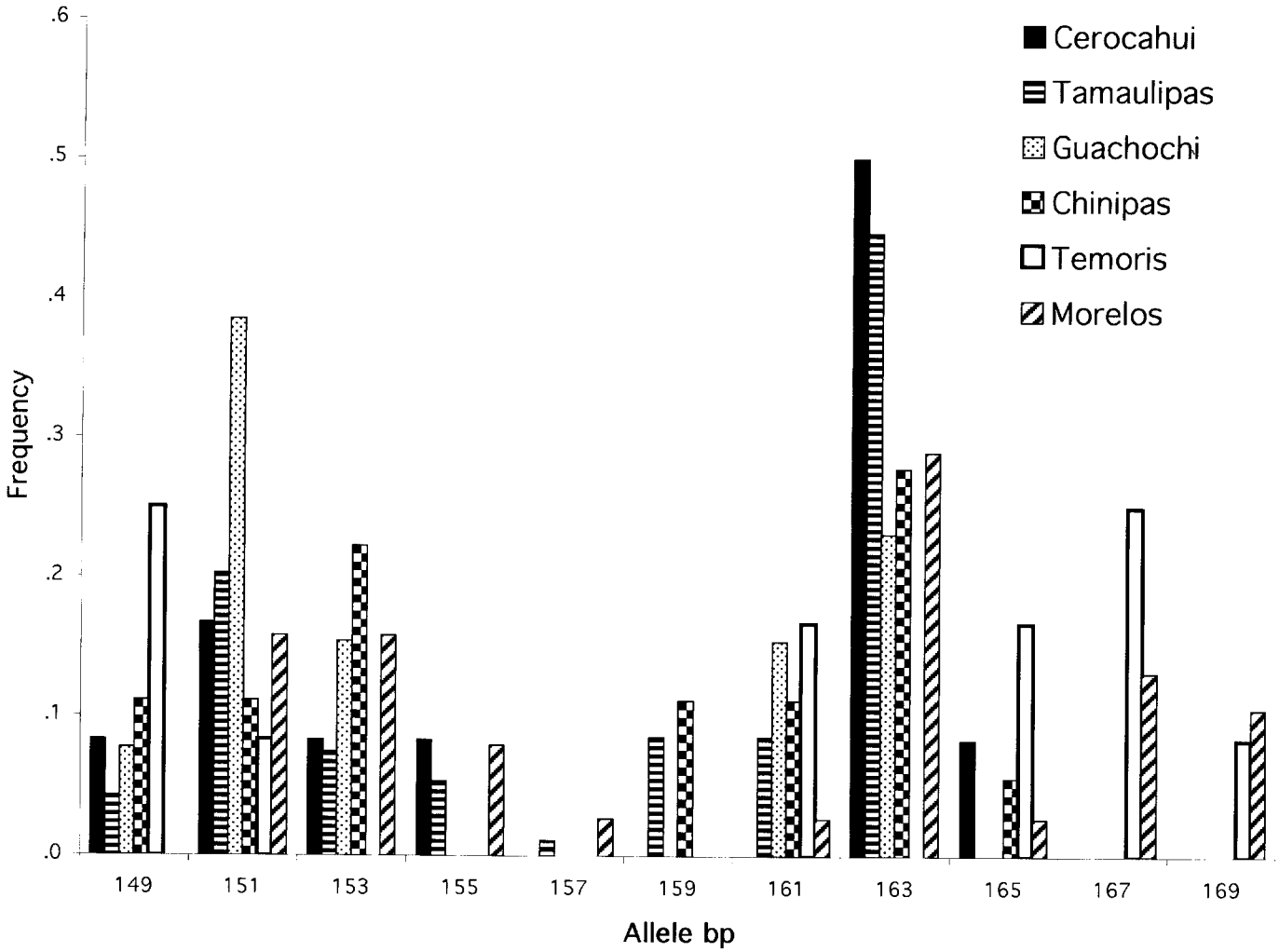

Figure 5. Allele frequencies for six Criollo populations as determined by the microsatellite HEL5. Global tests across all populations were performed by Fisher's method (Manly, 1985) and yielded the values $\chi^2 = 14.7$, $P = 0.2590$.

regions, with Ds (Nei, 1972) values ranging from 0.350 to 0.664. The five closely related regions had Ds distances that ranged from 0.120 to 0.303, excluding the Chinipas and Cerocahui cattle, which seemed to have a larger distance at 0.464.

Table 2. Hardy-Weinberg values as calculated by GENEPOP using the methods of Guo and Thompson (1992) and Fisher's Method (Manly, 1985) for six regions of Criollo cattle

Population	χ^2 value	*P*-value
Cerocahui	1.6	0.9903
Tamaulipas	12.4	0.1322
Guachochi	—[a]	<0.01
Chinipas	14.4	0.0730
Temoris	2.8	0.9471
Morelos	—[a]	<0.01

[a]χ^2 value beyond detection limits of GENEPOP.

Table 3. Genetic distances determined for six Criollo regions (Ds; Nei, 1972). Each method used the combination of four microsatellite markers (HEL9, INRA063, BM2113, and HEL5)

Population	Ds
Cerocahui and Tamaulipas	0.156
Cerocahui and Guachochi	0.228
Cerocahui and Chinipas	0.464
Cerocahui and Temoris	0.664
Cerocahui and Morelos	0.240
Tamaulipas and Guachochi	0.120
Tamaulipas and Chinipas	0.200
Tamaulipas and Temoris	0.461
Tamaulipas and Morelos	0.130
Guachochi and Chinipas	0.298
Guachochi and Temoris	0.350
Guachochi and Morelos	0.134
Chinipas and Temoris	0.543
Chinipas and Morelos	0.303
Temoris and Morelos	0.387

Table 4. Allele frequencies for the marker BM2113 for Simmental, Charolais, Angus, Hereford, and Criollo cattle

Allele size	Simmental	Charolais	Angus	Hereford	Criollo[a]
123				0.1	
125		0.05	0.05	0.05	0.05
127	0.1	0.15	0.1		0.13
129					0.04
131	0.45[b]	0.15			0.11
133	0.1	0.2	0.75	0.1	0.01
135			0.1		0.26
137	0.15	0.35			0.15
139	0.2	0.05		0.65	0.14
141		0.05		0.1	0.09
143					0.01

[a]The Criollo column is an average of all six Criollo regions sampled.
[b]The underlined numbers represent the highest allele frequency for that breed.

Discussion

In this study, four microsatellite markers from four different chromosomes were shown to be useful tools to analyze the genetic structure of Criollo cattle from six regions within Mexico.

Allele Frequency Interpretation

Allele frequencies of Criollo cattle differed depending on geographic location. Three of the microsatellite markers, HEL9, HEL5, and BM2113, detected high numbers of alleles (10 to 12 alleles), which is in agreement with previous researchers who detected 7 to 13 alleles (Kappes et al., 1997). INRA063 was less informative, detecting only five alleles, but this also was consistent with previous researchers who detected six alleles (Kappes et al., 1997).

Hardy-Weinberg Equilibrium Deviations and Explanation

The Hardy-Weinberg test showed that cattle from Guachochi and Morelos regions deviated from equilibrium. By using a different alternative hypothesis (heterozygote excess or heterozygote deficiency), more information was obtained about the samples that deviated from equilibrium. Upon further testing, Guachochi cattle were found to have a deficiency in number of heterozygote individuals ($P < 0.0001$). This may imply a high inbreeding factor contributing to the large number of homozygote individuals. However, cattle in the Morelos region did not show a significant deviation due to either an excess ($P > 0.85$) or a deficiency ($P > 0.18$) of heterozygotes, although heterozygote deficiency approached significance. These tests were performed using the Score(U) test (Rousset and Raymond, 1995). The Score(U) test is a log likelihood test of the deviation from the Hardy-Weinberg Equilibrium. It differs from other tests in that it uses the maximum likelihood estimates of the allele frequencies and maximizes the likelihood of deviations with respect to other parameters.

Genetic Similarities and Differences Within Criollo Cattle as Determined by Genetic Distances

The most related regions were the Cerocahui and Guachochi regions, the Tamaulipas and Morelos regions, the Guachochi and Morelos regions, and the Tamaulipas and Guachochi regions. This was somewhat unexpected, because the Tamaulipas region is located in another state in Mexico, whereas the other five populations are all located in the state of Chihuahua. The calculated genetic distances revealed that the genetically most different cattle were found in Temoris, which had unique allele frequency patterns for most markers. The range of distances for all six populations using the Ds (Nei, 1972) were from 0.120 to 0.664. These values are similar to those of Paetkau et al. (1997), who detected genetic distance ranges, using the Ds method, from 0.053 to 0.431 in a comparison of six areas of Arctic brown bear with eight microsatellite markers. Arranz et al. (1996a) reported smaller genetic distances for Brown Swiss and three Spanish cattle breeds which ranged from 0.058 to 0.2011 using five microsatellite markers, analyzed by the Ds method. A study comparing European and Eastern swine breeds, performed by Paszek et al. (1998), reported larger distances ranging from 0.235 to 1.337 with the Ds method.

Genetic Similarities and Differences Across Breeds

Four unrelated breeds (Angus, Hereford, Charolais, and Simmental) were used as comparisons to better define Criollo cattle. The information for these four breeds was obtained from the Roslin Institute's Cattle Diversity Database (http://www.ri.bbsrc.ac.uk/cdiv_www/accessdb.htm). The genetic diversity of the Criollo breed compared to the other four breeds is indicated in Table 4. The Criollo breed contained 10 alleles for BM2113, whereas the other four breeds contained 4

to 7 alleles. The most prevalent allele is different for each breed, with the 135-bp allele having the highest frequency in Criollo.

Genetic distances combining the Criollo populations and comparing their average to European breeds were also calculated. In addition, genetic distances between each of the six Criollo populations and the four European breeds were determined (Table 5). The values calculated are higher than that of the within-Criollo comparisons due to the use of only one marker (BM2113). Tables 4 and 5 contain information calculated using only BM2113. The Charolais breed is the most similar or closely related to Criollo cattle, and the Angus breed is the most different (Table 5). This information is for illustrative purposes to compare the distances calculated within the Criollo samples to that of an outside breed. However, one explanation for the relatedness of the Criollo and the Charolais is geographic location. The Criollo originated from Spanish animals, and the Charolais originated in France. The Angus originated in Scotland and, therefore, was geographically distant from the Criollo. Additionally, the Charolais were originally brought to Mexico and subsequently imported into the United States.

Table 5. Genetic distances determined for six populations of Criollo and four European breeds of cattle (Angus, Hereford, Charolais, and Simmental) using the methods of Nei (1972) and the marker BM2113

Population	Ds (Nei, 1972)
Cerocahui vs Tamaulipas	0.364
Cerocahui vs Guachochi	0.180
Cerocahui vs Chinipas	0.646
Cerocahui vs Temoris	0.894
Cerocahui vs Morelos	0.303
Cerocahui vs Simmental	0.648
Cerocahui vs Charolais	0.622
Cerocahui vs Angus	2.055
Cerocahui vs Hereford	0.486
Tamaulipas vs Guachochi	0.218
Tamaulipas vs Chinipas	0.303
Tamaulipas vs Temoris	0.324
Tamaulipas vs Morelos	0.200
Tamaulipas vs Simmental	0.300
Tamaulipas vs Charolais	0.268
Tamaulipas vs Angus	1.304
Tamaulipas vs Hereford	1.299
Guachochi vs Chinipas	0.346
Guachochi vs Temoris	0.374
Guachochi vs Morelos	0.349
Guachochi vs Simmental	0.620
Guachochi vs Charolais	0.806
Guachochi vs Angus	2.133
Guachochi vs Hereford	0.395
Chinipas vs Temoris	0.456
Chinipas vs Morelos	0.420
Chinipas vs Simmental	1.727
Chinipas vs Charolais	0.813
Chinipas vs Angus	1.932
Chinipas vs Hereford	2.655
Temoris vs Morelos	1.011
Temoris vs Simmental	0.981
Temoris vs Charolais	1.876
Temoris vs Angus	2.135
Temoris vs Hereford	1.944
Morelos vs Simmental	0.678
Morelos vs Charolais	0.227
Morelos vs Angus	2.537
Morelos vs Hereford	1.038
Simmental vs Charolais	0.405
Simmental vs Angus	1.569
Simmental vs Hereford	0.944
Charolais vs Angus	0.750
Charolais vs Hereford	1.651
Angus vs Hereford	1.895

Implications

Criollo cattle from five of the six regions we sampled seem to be closely related and can be treated as homogeneous. The Temoris population, however, should be treated as a separate population for future studies. Inbreeding was detected in one of the populations, Guachochi, which may imply that this population has been more isolated than the others. The marker BM2113 detected the highest number of homozygote individuals and thus should be used in future studies to detect population substructure in the Criollo breed.

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