GENETIC DIVERSITY ANALYSIS OF TWO DESERT BIGHORN SHEEP (*Ovis canadensis mexicana*) POPULATION IN MEXICO

[ANALISIS DE LA DIVERSIDAD GENETICA DE DOS POBLACIONES DE BORREGO CIMARRON (*Ovis canadensis mexicana*) EN MEXICO]

J. Abad-Zavaleta[1*], A.M. Sifuentes-Rincón[2], A. Lafón Terrazas[3], J. Gutiérrez Alderete[3], E. González Rodríguez[3], J.A. Ortega Gutiérrez[3], S. Del Moral[1], C.A Meza Herrera[4]

*[1]Universidad del Papaloapan. Av. Ferrocarril s/n, Col. Ciudad Universitaria, C.P. 68400, Loma Bonita Oaxaca, México. Tel. (281) 872-22-37, ext. 223, *E-mail: joeabad@hotmail.com, jabad@unpa.edu.mx; [2]Centro de Biotecnología Genómica, Instituto Politécnico Nacional. Blvd. del Maestro S/N, Col. Narciso Mendoza C.P. 88710, Reynosa, Tamaulipas, México. Tel. +52 (899) 924- 36-27. E-mail: asifuentes@ipn.mx; [3]Facultad de Zootecnia, Universidad Autónoma de Chihuahua. Periférico R. Almada Km. 1.5. C.P. 31031, Chihuahua, Chih. México. (614) 434 03 45. E-mails: alafon@uach.mx, jagual2208@hotmail.com, evegonzal@uach.mx, jaortega@uach.mx.; [4]Universidad Autónoma Chapingo. Unidad Regional Universitaria de Zonas Áridas. Bermejillo, Durango, México. (872) 77-6-01-60, cmeza2020@hotmail.com*

Corresponding author

SUMMARY

The genetic diversity of two populations of desert bighorn sheep from the Tiburon Island and the Sonora State (Sirios Mountain, El Viejo Mountain and El Encierro Mountain) was analyzed. DNA was extracted from blood samples in order to amplify a six panel of loci microsatellites. The number of average alleles found for the loci studied was 8 for the population of Tiburon Island (TI) and 7 for the Sonora State´s (SS). The expected heterozygosity and the index of polymorphic information (PIC) expected were very similar in the two populations with values of 0.716 and 0.732, 0.66 y 0.69, for the population of SS and the TI, respectively. Because of a heterozygotes deficit, both populations are not in Hardy-Weinberg equilibrium. The molecular analysis of variance indicates a moderate genetic differentiation (F_{ST} = 0.059; P =0.0001). The results of this work show that it is necessary to establish strategies to monitor and maintain the genetic diversity and purity of the population of the TI. In the case of the population of the SS, it is necessary to monitor broadening the number of samples in order to evaluate their genetic variability and to determine the reproductive but suitable handling, for the mating among pairs.

Keywords: Desert bighorn sheep; genetic variability; microsatellites; Tiburon Island; Sonora State

RESUMEN

Se analizó la diversidad genética de dos poblaciones de borrego cimarrón, provenientes de la Isla Tiburón y del estado de Sonora (Sierra Cirios, Sierra el Viejo y Sierra el Encierro). A partir de muestras de sangre se extrajo ADN que se utilizó para amplificar un panel de seis loci microsatélites. El número de alelos promedio encontrados para los loci estudiados fue 8 para la población de la Isla y 7 para el estado Sonora. La heterocigosidad media esperada y los índices de información polimórfica (PIC) fueron muy similares en las dos poblaciones, con valores de 0.716 y 0.732, 0.66 y 0.69, para la población de Sonora y la de Isla Tiburón, respectivamente. Ambas poblaciones se encuentran en desequilibrio Hardy-Weinberg debido a un exceso de homocigotos. El análisis molecular de varianza (AMOVA) indica una baja diferenciación genética (F_{ST} = 0.059; P =0.0001). Los resultados de este trabajo muestran que es necesario establecer estrategias para monitorear y mantener la diversidad genética y pureza de la población de la Isla Tiburón. En el caso de las poblaciones del estado de Sonora es necesario monitorearlas ampliando el número de muestra a fin de evaluar su variabilidad genética y determinar el manejo reproductivo más adecuado, para el apareamiento entre ejemplares.

Palabras clave: borrego cimarrón; variabilidad genética; microsatélites; Isla Tiburón; Estado de Sonora.

INTRODUCTION

Mexico occupies one of the first places in biodiversity, unfortunately, there is a great amount of vertebrate species that are threatened. In the last decades, the environmental criteria has focused in implementing conservation strategies that allow us to reduce the impact on the diversity and through the protection, conservation, recovery, reintroduction, handling and sustainable advantage of species in danger of extinction (Soberon and Llorente, 1993).

The desert bighorn sheep (Ovis canadensis) is a much appreciated species due to its ecological value and economic potential; nevertheless, it is threatened (CESPEDES, 2003). It lives in arid and semiarid mountainous lands of the Occident of America; in Mexico it is currently found in unsteady lands with rocky hillsides in the states of Sonora, Baja California Norte and Baja California Sur, where three subspecies are distributed: O. c. mexicana, O. c. cremnobates y O. c. weemsi. It is a opportunist fodder, it adapts to the available vegetation in the season. Copulation occurs between July and December. A female has an average of one offspring per year and they are born between January and June (SEMARNAT, 2001). Adult males get to reach 1.95 m in total length and the cross up to 1.01 in height in average. Whereas females exhibit reach up to 1.88 in length in total and they have a lower size. Male horns can reach up to 27 cm in length; however, female horns are not bigger than 38 cm. As mentioned before, it is not surprising that the weight of the adult animal varies from a range from 79 up to 158 kilograms.

Because of their cynegetic interest, in some farms of the northwest of Mexico, sheep breeders have been established for the conservation and management of the species; however, the populations are frequently small and closed. These conditions can generate a reduction of the allelic diversity and genetic variability in the lineage (Fitzsimmons *et al.* 1997; Conde, 2000). In turn, in the future, this could lead to health hazards and to adaptation to environmental, reproductive and genetic changes (Stangel *et al*, 1992).

Nowadays, the analysis of the genetic diversity relies on the use of molecular markers. From these markers, the use of microsatellites stands out. These molecular markers are highly polymorphic. They also allow us to evaluate the degree of genetic variation among species, subspecies and populations (Gotelli et al. 1994; Taylor et al. 1994).

In Mexico, there are only a few studies on the desert bighorn sheep and specifically in genetic analyses they are almost null, despite the importance of hunting to save these specie, because since 1995, the Tiburon Island (TI) population has been used as a source for establishing other bighorn sheep populations in mainland Sonora, and further re-introductions to Chihuahua, Coahuila and Nuevo Leon. However a new allelic variety has not been supplemented to compensate the low genetic diversity, which is the result of the original limited stock of 20 desert bighorn sheep. Therefore, the objective of the present study was to evaluate the degree of genetic diversity of two populations of desert bighorn sheep; one originally coming from the Tiburon Island and another one coming from some ranches in the Sonora State, with the intention of promoting the design and implementation of reproductive strategies aimed at preventing some of the problems provoked by inbreeding.

MATERIALS AND METHODS

Sampling and DNA extraction

Five to 10 ml of blood from the jugular vein of 105 stored desert bighorn sheep were collected In the Tiburon Island (TI; n=77) and the Sirios Mountain, El Viejo Mountain and El Encierro Mountain of the Sonora State (SS; n=28). We isolated DNA from whole blood sample by means of the kit GFX Genomic Blood DNA Purification (Amersham).

Genotyping

The genetic diversity of the two populations was evaluated using six microsatellites loci previously reported as equipment to analyze the genetic diversity in ovine (Ovis aries and Ovis canadensis) BM143, MAF36, MAF70 and OarFCB11 (Forbes et al., 1995; Ellegren et al., 1997; Gutierrez et al., 2000; Hedrick et al., 2001; Lopez, 2004); additionally two microsatellites loci (INRA40, TGLA53) widely used in cattle because of their high polymorphism were used (Peelman et al. 1998, Salazar et al. 2004; Zamora et al. 2004). The conditions of PCR for each loci were

settled varying the concentrations of MgCl2, the concentrations (50 ng) of DNA, dNTP' s 0,4 mm (Promega, Cat. U1240) and Taq polymerase 0,125 U/(l. (Promega, Cat. A351H) in a final volume of 10 (l. The PCR products were analyzed within the secuenciator (LI-COR, 1999) and the initiators were marked with IRDye 800. The products of PCR became denaturalized to 95 °C for 5 minutes. The product was visualized by electrophoresis in a poliacrilamide-bisacrilamide gel of to 6.5 % during 1:30 hr.

Data analysis

Allele size data for each locus were obtained using SAGA GTTM software. 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). Heterogeneity in allele frequencies among pair wise comparisons and exact tests for conformances to Hardy-Weinberg equilibrium (HW) at each locus within samples were performed using the program GENEPOP 3.1, with the Markov chain method (Raymond & Rousset, 1995). The genetic structure of populations was determined by an Analysis of Molecular Variance (AMOVA) using the Arlequin software (Schneider et al. 2000), under a hierarchical design that considered variations among populations and within individuals from each population.

RESULTS

Allelic frequencies

The allelic frequency is the resulting quotient when dividing the number of equal alleles in a population by the number of total alleles. And our results that the most frequent allele in the Tiburon Island population is the 95(35%), 89 (48 %), 1120 33%) and 127 (61%), 139 (34 %) y 247 (20 %) for the BM143, MAF36, MAF70, OarFCB11, TGLA53 and INRA40 loci, respectively; whereas, for the Sonora State population, the most frequent allele was the 95 (51 %), 89 (46 %), 118 (41 %), 125 (48 %), 141 (32 %) y 245 (35 %), for the BM143, MAF36, MAF70, OarFCB11, TGLA53 and INRA40, respectively (Table 1).

Genetic diversity

As shown in Table 1, 67 % of the loci evaluated in TI were polymorphic, whereas only 50 % of the loci evaluated in SS were. The number of alleles found per locus varies among the two populations. However, in both, the locus MAF70 had the highest number of alleles in TI and SS (13 and 11, respectively) and OarFCB11, the lowest (4 alleles).

The HE and HO were different among the populations, in most of the loci, HE was higher than HO (Table 2). The MAF70 locus showed the highest value (HE=0.835) and OarFCB11 locus the lowest (HE =0.523) for the TI population, whereas in the population of SS, locus INRA40 showed the highest value (HE =0.823) and locus OarFCB11 and BM143 the lowest value (HE =0.603).

The genotype frequencies of all loci reveal that the populations show an imbalance (FIS>0), except in locus BM143 (FIS<0) of the SS population. In most of the cases, these deviations are due to a deficit of heterozygotes, probably, resulting from inbreeding and preferential mating in the studied populations.

According to the analysis of molecular variance (Table 3), there is a low genetic differentiation (0.05899; P<0.0001) among the populations. Genetic variation is explained in a 5.90 % by the variation between populations and in a 94.10% by the variation of the individuals within each population.

Table 1. Allelic frequency of desert bighorn sheep (*Ovis canadensis mexicana*)

Locus	**Allele (pb)**	**Allelic frequency (Tiburon Island)**	**Allelic frequency (Sonora State)**
BM143	91		0.0179
	93	0.1948	0.1071
	95	0.3506	0.5179
	97	0.1429	0.3571
	99	0.0455	
	101	0.0714	
	103	0.1494	
	105	0.0390	
	107	0.0065	
MAF36	87		0.079
	89	0.4805	0.4643
	91	0.2987	0.2143
	103	0.0195	0.0536
	105	0.1688	0.1964
	107	0.0325	0.0357
	119		0.0179
MAF70	118		0.4107
	120	0.3312	0.2857
	122	0.1299	0.0357
	124	0.0974	0.0714
	126	0.1429	
	128	0.0325	0.0357
	130	0.0714	0.0179
	132	0.0714	0.0357
	134	0.0325	0.0357
	136	0.0360	0.0179
	138	0.0130	0.0357
	140	0.0130	
	142	0.0130	
	144	0.0260	
	146		0.0179
OarFCB11	119	0.0260	0.0714
	123	0.0455	0.0357
	125	0.3117	0.4821
	127	0.6169	0.4107
TGLA53	135	0.1233	0.0179
	137	0.1624	0.1607
	139	0.3442	0.2143
	141	0.3182	0.3214
	143	0.0519	
	151		0.0714
	153		0.2143
INRA40	137		0.0179
	239		0.0714
	241		0.1429
	243		0.0714
	245	0.0217	0.3571
	247	0.2029	0.1250
	249	0.2174	
	251	0.1594	0.0893
	253	0.1884	0.0893
	255	0.1159	

	257	0.0942	0.0357

Table 2. Values of H_E, H_O, PIC Y H-W of desert bighorn sheep (*Ovis canadensis mexicana*)

	TIBURON ISLAND						SONORA STATE					
Locus	Number of allele	**Allelic rank**	H_O	H_E	**PIC**	**HW**	Number of allele	Allelic rank	**H_O**	H_E	PIC	**HW**
BM143	**8**	93-107	0.247	0.793	**0.760**	ns	**4**	**91-107**	0.857	0.603	**0.515**	0.0339
MAF36	**5**	89-119	0.286	0.654	**0.589**	ns	**7**	**87-119**	0.536	0.708	**0.652**	ns
MAF70	**13**	120-144	0.390	0.835	**0.813**	ns	**11**	**118-146**	0.250	0.751	**0.703**	ns
OarFCB11	**4**	119-127	0.130	0.523	**0.443**	ns	**4**	**119-127**	0.536	0.603	**0.509**	ns
TGLA53	**7**	135-143	0.364	0.754	**0.711**	ns	**6**	**135-153**	0.321	0.810	**0.768**	ns
INRA40	**7**	245-257	0.319	0.834	**0.804**	ns	**9**	**237-257**	0.357	0.823	**0.789**	ns

H_O: observed heterozygosity; **H_E**: expected heterozygosity; **PIC**: polymorphic information content; **H-W (p-val)**: Values conditions for the balance of H-W, significance level of P <0.05 by Fisher's exact test; **ns**: not significant

Table 3. Hierarchical analysis of molecular variance (AMOVA) within and among desert bighorn sheep (*Ovis canadensis mexicana*) populations.

Source of variation	**Degrees of freedom**	**Sum of Square**	**Variance component**	**Percentage of variation**
Among populations	1	10.877	0.11089	5.90
within populations	208	367.956	1.76902	94.10
Total	209	378.833	1.87991	
Fixation indices	**F_{ST} : 0.05899**	**P**=0.00000±0.00000		

DISCUSSION

Our results indicate that there are more alleles by locus in the population of the TI than in the SS. Even though, the TI population was founded from a small population. Besides, since then, 1975, genetic flow has not occurred (Hedrick et al. 2000). However, both populations showed a higher heterozygosity than the one found by Hedrick et al. (2001). In the TI, this is probably due to the number of animals evaluated, which was significantly lower (n = 14) than the one analyzed in this study (n = 77) or it might be due to the way the specimens were captured (family bias) only from one group. While in the same study, Hedrick et al. (2001) showed that Arizona's population had a higher heterozygosity (0.60, 0.54 and 0.58) than the IT. For its part, Forbes et al., (1995) found a heterozygosity of 0.57 for O. Canadensis canadensis similar result to that found in the population of the SS in this study.

The deficit of heterozygotes found in both populations can be due to the high levels of consanguinity, produced by mating among related animals (Nei, 1977; Li et al. 2000; Aranguren-Méndez et al. the 2005). In the TI population, the effective size of the first generation is estimated in 12.8 animals (Hedrick et al. 2001). Although, the population of the TI is in captivity and there is no entrance of new allelic variants, it presents suitable phenotypic characteristics.

Problems associated with inbreeding in the population have not been observed. However, they can be affected in the future, presenting the detrimental effect caused by the increment in the consanguinity putting the survival of the population at risk (Kalinowski and Hedrick, 2001; Fitzsimmons et al. 1997; Sausman, 1984).

The low genetic differentiation (F_{ST}=0.05899), in the populations in this study is due to the genetic flow that is carried out from TI to SS. Situation, that in the last decade, has occurred by means of the resettlement programs to certain regions of the SS where the desert bighorn sheep population has diminished considerably according to a population census. Gutiérrez et al., (2000), found that the calculated values of F_{ST} for 10 loci in study was 0.043 and 0.038 obtained from the Ovis aries and Bos taurus populations (O. canadensis nelsoni) from the northwest and from the populations (O. canadensis mexicana) from the Southeastern Arizona respectively. Values that are lower than the ones obtained between the populations in this study. Likewise, Worley et al., (2004) found a FST of 0.160 in 24 populations of Ovis dali, which shows a high genetic differentiation between these populations, in comparison to the populations of Ovis canadensis mexicana in discussion.

In both populations, a gene drift has occurred in a natural way. Because of a gregarious behavior of animals, preferential mating, which is conducive to the perpetuation of favorable genes carried by dominant males.

The low genetic variation in the TI is accentuated further by the genetic drift produced by the small number of animals (2 males and 11 females) from which the population was founded (bottlenecked). The effects of drift are accentuated in small size populations and they result in changes that are not necessarily adaptive (Hartl, 2000; Kalinowski y Hedrick, 2001).

Therefore, to reduce the genetic variability these stocks run the risk of adaptive problems, such as reproductive, health, and climate changes. However, the feature the populations have in common is that time has enabled them to adapt to the environment, generating a high specificity to it.

Our findings suggest that, given the status of the population that keeps TI as a supplier of genetic material for the restocking of animals to different ranches or farms in the state of Sonora and lately of Chihuahua, Coahuila and Nuevo Leon. It is desirable to maintain the degree of current genetic variability or if possible to increase it through the introduction of unrelated animals genetically distant enough to ensure a broad genetic variability, and thus the survival of the population.

On the other hand, although the population of the SS has higher values of variability in respect to TI, there is a possibility that this will decrease by an inadequate genetic reproductive management. Hence it makes sense to sample most of the subpopulations that are in the SS. And also to estimate genetic distances in order to identify those subpopulations with a lower degree of genetic variability and to implement reproductive programs aimed at increasing it.

This strategy would avoid inbreeding problems and it would increase effective reproduction and productive population. Besides, it would reduce genetic detrimental effects caused by the decline of genetic variability. When the degree of genetic variation is clarified, a genetic management program for the SS will be developed. This program will determine resettlement plans and guided crosses of individuals. In addition, policies and priority activities for the conservation, management and sustainable use of mountain sheep in Mexico will be defined.

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