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Allozyme variation of Cottontail rabbits (*Sylvilagus*) from Mexico

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Abstract

We examined the allozyme variation of cottontail rabbits of the genus *Sylvilagus* from Mexico, and described their genic relationships. Samples of kidney and heart were run in horizontal starch-gel electrophoresis to assess the variation of 23 presumptive loci, and the BIOSYS-1 software was used to compute estimates of genic variation. Results showed that 60.8 % of the loci were polymorphic. *S. floridanus* was the most genically variable rabbit as revealed by mean number of alleles per locus and percentage of polymorphic loci. The fixation index showed genetic differentiation among species. The smallest genetic distance was between *S. floridanus* and *S. brasiliensis* whereas the largest one was between *S. mansuetus* and *S. audubonii*. A phenogram showed *S. mansuetus* branching out first, *S. audubonii* next, and finally *S. floridanus* and *S. brasiliensis* together. In conclusion, *S. floridanus* showed the largest genic variation, *S. mansuetus* was the most distinctive rabbit, and *S. audubonii* and *S. brasiliensis* were the most closely related species.

Key words: *Sylvilagus*, cottontail rabbits, allozymes, electrophoresis, Mexico

Introduction

Cottontail rabbits of the genus *Sylvilagus* are a speciose group that occurs in the New World (Hoffmann 1993). Unfortunately, genetic variation and species relationships within the genus have been barely examined (Scribner and Warren 1986). Reports in the literature on these topics are limited to scarce and scattered references. This is remarkable if we consider the high species richness and population abundance of this genus in North America (Chapman and Ceballos 1990).) Just the Mexican territory, for instance, hosts eight species of *Silvilagus*, four being endemics (Cervantes et al. 1994). The genetic variation of Mexican cottontail rabbits still remains unexplored.

Genetic distance estimates are available for few lagomorph species (Glover et al. 1977; Grillitsch et al. 1992). Only recently, Halanych and Robinson (1997) provided useful information to gain insight into the evolutionary history of some cottontail rabbit species using sequence data from mtDNA.

Sylvilagus floridanus, a cottontail species occurring in Mexico, is genetically variable in Maryland, U.S.A., due to its intensive introduction to that state (Chapman and Morgan 1973; Morgan and Chapman 1981). Isolated populations of the same species in Texas, U. S. A., are genetically differentiated and characterized by periodical high dispersion rates and low genetic flow due to agricultural land use (Van Den Bussche et al. 1987). *S. audubonii* from Texas, another cottontail rabbit occurring in Mexico, displays a

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high genetic similarity to Texan populations of *S. floridanus*, although no gene flow between them is expected to take place (SCRIBNER and WARREN 1986).

These data thus suggest the presence of genic variation within and among species of *Sylvilagus* and may provide an estimation of relationship between species. Therefore, the purpose of this study is to examine the allozyme variation of selected species of Mexican *Sylvilagus*.

Material and methods

Rabbits were collected with a shotgun. Specimens of black-tailed jackrabbit (*Lepus californicus*) from a Mexican locality were included as outgroup. Samples of heart, kidney, and liver were removed and immediately frozen in liquid nitrogen. All specimens were preserved as standard museum vouchers and deposited in the mammalian collection (Coleccion Nacional de Mamiferos, CNMA, formerly IBUNAM) of Instituto de Biologia, Universidad Nacional Autonoma de Mexico, in Mexico City, Mexico.

Localities are listed by species and sample sizes are indicated in parenthesis as follows. *Sylvilagus floridanus*: 14 km W Villa de Arista, Municipio Moctezuma, San Luis Potosi, Mexico, 1620 m (2); 2 km W Santa Maria del Mar, Municipio Juchitan, Oaxaca, 5 m (3); 10 km NW + 2 km E La Rosa Amarilla, Municipio La Manzanilla, Jalisco, Mexico, 2050 m (3); 11 km E + 1.5 km N San Jose de Gracia, Municipio Marcos Castellanos; Michoacan, Mexico, 2100 m (3). *S. audubonii*: 140 km NE Gomez Palacio, Municipio Mapimi, Durango, Mexico, 1189 m (3). *S. mansuetus*: Isla San Jose, Municipio La Paz, Baja California Sur, Mexico, 5 m (3). *S. brasiliensis*: El Chajul, Municipio Ocosingo, Chiapas, Mexico (1); km 35 road Catemaco-Balzapote, Municipio Catemaco, Veracruz (1). *L. californicus*: same locality as that for *S. audubonii* (2).

Homogenates of kidney and heart were analyzed for electrophoretically detectable protein variation and prepared according to the methods of SELANDER et al. (1971). Procedures for horizontal starch gel electrophoresis also followed those of SELANDER et al. (1971).

A total of 23 presumptive loci were examined as follows (abbreviations and IEC numbers follow HARRIS and HOPKINSON 1976): buffer system tris-citrate I (pH 6.7–6.3) was used for malate dehydrogenase (MDH-1, MDH-2, 1.1.1.37), lactate dehydrogenase (LDH-1, LDH-2, LDH-3, 1.1.1.27), acid phosphatase (ACP, 3.1.3.2), glucose-phosphate isomerase (GPI, 5.3.1.9), 6-phosphosphogluconate dehydrogenase (PGD, 1.1.1.44), glucose dehydrogenase (GDH-1, GDH-2, 1.1.1.47), purine nucleoside phosphorylase (NP, 2.4.2.1), and general proteins (GP); buffer system tris-citrate II (pH 8) for malic enzyme (ME-1, ME-2, 1.1.1.40), L-glutamate dehydrogenase (GLUD, 1.4.1.3); buffer system PGI-potassium phosphate (ph 6.7) for isocitrate dehydrogenase (ICD, 1.1.1.42), aldolase (ALD, 4.1.2.13), superoxide dismutase (SOD, 1.15.1.1), xantine dehydrogenase (XDH, 1.2.3.2); buffer system tris-malate EDTA (pH 7.4) for sorbitol dehydrogenase (SDH, 1.1.1.14), hexokinase (HK, 2.7.1.1); and buffer system litium hydroxide (A = 10 %, B = 90 %) for alcohol dehydrogenase (ADH, 1.1.1.1), and esterase (EST, 3.1.1.1).

Alleles at each locus were designated by mobility relative to the most common allele at that locus. Results were summarized in the form of individual genotypes by locus for each individual.

Estimates of allelic frequencies, polymorphism, heterozygosity, WRIGHT's (1965) F-statistics, coeffcients of genetic distance (D) of ROGERS (1972) and of unbiased distance (D) of NEI (1978) were computed using the BIOSYS-1 program of SWOFFORD and SELANDER (1981). The coeffcients of genetic distance were calculated with the inclusion of monomorphic loci. Clustering of distance matrices was performed using the unweighted pair-group method with arithmetic averages procedure (UPGMA; SNEATH and SOKAL 1973; SWOFFORD and SELANDER 1981).

Results and discussion

Of the 23 loci examined electrophoretically, 14 (60.8 %) were polymorphic (Tab. 1), whereas GP, ME, GDH-1, ADH, MDH-1, LDH-2, GPI, SOD, and ACP were monomorphic. Rare variants (frequency of the most common allele as greater than 0.95) were

Table 1. Alleles (a–d), allele frequencies (in parenthesis), sample size (n), average number of alleles per locus (AVER), percent of polymorphic loci (POLY), and expected average individual heterozygosity (HETE) for leporids (*Sylvilagus mansuetus, S. floridanus, S. audubonii, S. brasiliensis* and *Lepus californicus*) from Mexico. Only polymorphic loci (14 out of 23) are listed. Estimate of POLY includes only those loci for which dominant allele has a frequency less than 0.95. See text for loci abbreviations.

Locus	*Sylvilagus mansuetus*	*Sylvilagus audubonii*	*Sylvilagus brasiliensis*	*Sylvilagus floridanus*	*Lepus californicus*
GLUD	b	b	b	a (0.091) b (0.633) c (0.273)	b
ICD	c	c	c	a (0.091) b (0.182) c (0.720)	c
6PGD	a (0.667) b (0.333)	a	a	a	a
GDH-2	b	b	b	b	a
MDH-2	a (0.333) b (0.667)	b	b	b	b
NP	b	b	b	b	a
ME-2	b	b	b	b	a
LDH-1	b	b	b	a (0.125) b (0.875)	b
LDH-3	b	b	b	b	a
ALD	c	b (0.677) c (0.333)	c	b (0.125) c (0.875)	a a
XDH	b	b	b	b	a
HK	a	a	a	a	b
SDH	a	a	a	a	b
EST	b	a	a	a (0.667) c (0.333)	d
n	3	3	3	8	2
AVER	1.1	1.0	1.0	1.3	1.0
POLY	8.7	4.3	0.0	21.7	00
HETE	0.046	0.023	0.000	0.084	0.000

not present. The locus with the highest number of alleles per locus (= 4) was EST, followed by ALD, ICD, and GLUD (= 3). All loci appeared as single banded homozygotes.

Among the polymorphic loci, seven were polytypic among species of *Sylvilagus* whereas the other seven (GDH-2, ME-2, NP, LDH-3, XDH, HK, and SDH) were fixed for the same allele in all species of *Sylvilagus* (Tab. 1). All loci were monomorphic in *S. brasiliensis* and *L. californicus.*

Sylvilagus floridanus showed a slightly larger average number of alleles per locus (Tab. 1). Proportions of polymorphic loci (95 % criterion) averaged 8.7 among *Sylvilagus* species and ranged from 0 to 21.7. *S. floridanus* also displayed the highest polymorphism value and alternate alleles at four loci (GLUD, ICD, LDH-1, EST). Therefore, *Sylvilagus floridanus* was the most genically variable rabbit.

This is similar to what has been found in several localities of the United States of America for the same species (MORGAN and CHAPMAN 1981; SCRIBNER and WARREN 1986; VAN DEN BUSSCHE et al. 1987). For instance, populations of *S. floridanus* and *S. audubonii* from Texas displayed 33 and 25 % of polymorphism, respectively (SCRIBNER and WARREN 1986). On the other hand, polymorphism recorded in brown hares (*Lepus europaeus*)

from Central Europe was 16.3 % (HARTL et al. 1990). In contrast, no polymorphic loci for *S. brasiliensis* was recorded herein.

In our study no rabbit species showed heterozygote individuals. Similarly, heterozygote deficiencies were noted for populations of *S. floridanus* from Texas (VAN DEN BUSSCHE et al. 1987), although previous studies of *S. floridanus* and *S. audubonii* revealed fair amounts of heterozygosity (MORGAN and CHAPMAN 1981; SCRIBNER and WARREN 1986). Selected populations of the European wild rabbit (*Oryctolagus cuniculus*) from East Anglia, England, also showed heterozygote deficiencies at most loci (SURRIDGE et al. 1998). Moreover, low levels of heterozygosity were reported in pikas (*Ochtotona princeps*) from Colorado, U.S.A. (GLOVER et al. 1977).

The expected genic heterozygosity for *S. floridanus* calculated from Hardy-Weinberg assumptions was 8.4 % (Tab. 1), whereas the mean value for vertebrate populations is 5–6 % (SELANDER and JOHNSON 1973). In contrast, expected figures for *S. mansuetus* and *S. audubonii* were lower (4.6 and 2.3 %, respectively).

Sylvilagus species occurring in Mexican territory thus show detectable protein variation. The extent of variation is, however, comparatively low.

Our estimates of genetic distance (NEI's, 1978, unbiased distance) between species turned out to be relatively low compared to those of other vertebrate populations. Our results are comparable to those found for conspecific populations whose coefficients of similarity are generally at the 0.90's level (SELANDER and JOHNSON 1973; HARTL et al. 1990). This is particularly true for genetic distances among the species pairs *S. audubonii* – *S. brasiliensis*, *S. brasiliensis* – *S. floridanus*, and *S. audubonii* – *S. floridanus* (Tab. 2). Populations of *S. audubonii* from Texas also displayed high genetic similarity (ROGER's similarity index = 0.884) to sympatric *S. floridanus* (SCRIBNER and WARREN 1986). *S. floridanus* from the same region displayed NEI's genetic distances between populations ranging from 0.20 to 0.388 (VAN DEN BUSSCHE et al. 1987).

The lowest genetic distance recorded was between *Sylvilagus floridanus* and *S. brasiliensis* (Tab. 2), the only two cottontail rabbit species that occur as far south as the temperate and tropical habitats of South America. They mostly are allopatric species although they also may be parapatric, seldomly sympatric. In contrast, the largest genetic distance recorded herein was between *S. mansuetus* and *S. audubonii* (Tab. 2), species adapted to xeric conditions and whose ranges are very close.

Sylvilagus mansuetus, once thought to be a subspecies of *S. bachmani*, turned out to be the most distinctive rabbit of the species sample examined (Fig. 1). This cottontail is restricted to a small island of 194 km^2 in the Gulf of California, Mexico. Unfortunately, other than the original description of the species, there are no reports on the relationships of this rabbit to other cottontail rabbits. The mean genetic distance (NEI's, 1978 unbiased distance) between *S. mansuetus* and other *Sylvilagus* species was 0.057. This species dis-

Table 2. NEI's (1978) unbiased distances (above diagonal) and ROGERS' (1972) genetic distances (below diagonal) among leporids (*Sylvilagus mansuetus*, *S. floridanus*, *S. audubonii*, *S. brasiliensis*, and *Lepus californicus* from Mexico.

	Sylvilagus mansuetus	*Sylvilagus audubonii*	*Sylvilagus brasiliensis*	*Sylvilagus floridanus*	*Lepus californicus*
Sylvilagus mansuetus	–	0.071	0.051	0.050	0.599
Sylvilagus audubonii	0.101	–	0.018	0.022	0.559
Sylvilagus brasiliensis	0.072	0.029	–	0.010	0.571
Sylvilagus floridanus	0.103	0.068	0.050	–	0.567
Lepus californicus	0.464	0.430	0.435	0.446	–

played alternate alleles at two loci (6PGD, MDH-2) relative to *S. audubonii*, its nearest geographic sample examined in this study. In addition, these pair of species were fixed for one alternate allelle at the EST locus (Tab. 1).

The values of genetic differentiation (Fst) among species were relatively high (Tab. 3), except for four loci (GLUD, ICD-1, 6PGD, and MDH-2). The mean Fst was high too considering all species examined (0.851). When the outgroup (*L. californicus*) is removed from the calculations, the average is lower (about half = 0.462; Tab. 3), but still indicative of substancial species differentiation. This fits that *S. mansuetus*, *S. audubonii*, *S. floridanus*, and *S. brasiliensis* are also morphologically distinctive (CHAPMAN and CEBALLOS 1990). Similarly, populations of *S. floridanus* from Texas revealed a significant degree of genetic differentiation too (VAN DEN BUSSCHE et al. 1987).

Table 3. Fixation index (WRIGHT's Fst) for polymorphic loci calculated among leporids (*Sylvilagus mansuetus*, *S. floridanus*, *S. audubonii*, *S. brasiliensis*, and *Lepus californicus*) from Mexico, and among the same samples excluding *L. californicus*.

Locus	All samples	All samples exclusive of *L. californicus*
GLUD	0.251	0.239
ICD-1	0.177	0.168
6PGD	0.286	0.273
GDH-2	1.000	–
MDH-2	0.286	0.273
NP	1.000	–
ME-2	1.000	–
LDH-1	0.875	0.097
LDH-3	1.000	–
ALD	0.746	0.478
XDH	1.000	–
HK	1.000	–
SDH	1.000	–
EST	0.859	0.771
Mean	0.851	0.462

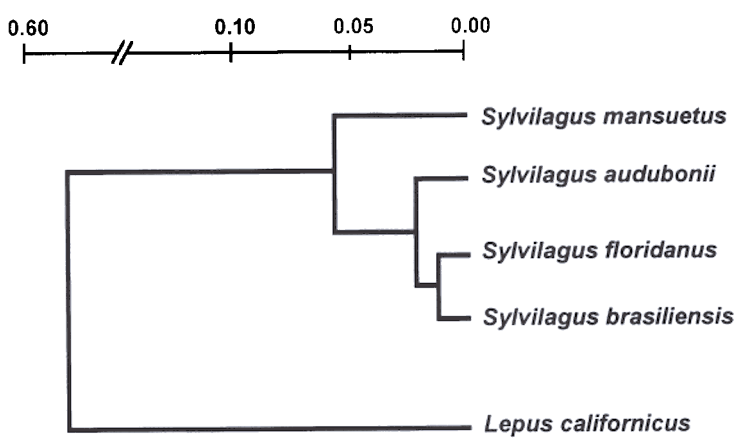

Fig 1. UPGMA tree of leporids (*Sylvilagus mansuetus*, *S. audubonii*, *S. brasiliensis*, *S. floridanus*, and *Lepus californicus*) from Mexico based on NEI's (1978) unbiased distances. The cophenetic correlation coefficient = 0.999.

The UPGMA procedure of a matrix of unbiased distances (NEI 1978) revealed three allozymic groups present within the *Sylvilagus* group (Fig. 1). The first consisted of *S. mansuetus* branching out first (fixed for one allele at the EST locus), *S. audbonii* next, and *S. floridanus* and *S. brasiliensis* together, who were relatively closely allied, and separated by a genetic distance of 0.010. None of these samples was fixed for different electromorphs relative to all of the other *Sylvilagus* samples. This arrangement may not reflect phylogenetic relationships, but allows to understand the overall genetic resemblance contained in the distance matrix computed.

Assessement of the genic relationship of *S. audubonii* to *S. floridanus* herein fit findings on the systematic relationships among ten species of *Sylvilagus* based on diploid chromosomal numbers (CHAPMAN and CEBALLOS 1990). That is, *S. audubonii* and *S. floridanus* also occur on separate branches of a dendrogram. Similarly, 12S rDNA data showed that *S. floridanus* and *S. audubonii* were not each other's closest relatives in the species set examined (HALANYCH and ROBINSON 1997).

Results presented here are the first data set that outline genic relationships among selected species of *Sylvilagus* occurring in Mexico. It is shown that samples of *Sylvilagus* species occurring in Mexico display detectable protein variation, although lower than that reported for other leporid and vertebrate species. On the other hand, *S. mansuetus* is the most genetically distinctive cottontail rabbit examined, whereas *S. floridanus* and *S. brasiliensis* are the most closely related species pair. Although the four species examined are morphologically well differentiated the genetic distances among them are smaller than expected.

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Zusammenfassung

Allozym-Variation von Wollschwanz-Kaninchen (Sylvilagus) aus Mexiko.

Wir untersuchten die Allozym-Variation von ausgewählten Arten des Wollschwanz-Kaninchens der Gattung *Sylvilagus* aus Mexiko und beschrieben deren genetische Verwandtschaften. Proben von Niere und Herz wurden horizontaler Stärkegelelektrophorese unterzogen, um die Protein-Variation von 23 vermuteten Loci zu bestimmen, und die Software BIOSYS-1 wurde benutzt, um Schätzungen der genetischen Variation zu berechnen. Die Ergebnisse zeigten, daß 60,8 % der Loci polymorph waren. *S. floridanus* war das genetisch variabelste Kaninchen, wie die mittlere Anzahl von Allelen pro Locus und der Prozentsatz von polymorphen Loci zeigten. Der „Fixations-Index" zeigte genetische Differenzierung unter den Arten. Die geringste genetische Distanz bestand zwischen *S. floridanus* und *S. brasiliensis*, die größte zwischen *S. mansuetus* und *S. audubonii*. Ein Phaenogram zeigte, daß *S. mansuetus* als erste Art abzweigt, *S. audubonii* als nächste und *S. floridanus* und *S. brasiliensis* gemeinsam. Schlußfolgernd zeigte *S. floridanus* die größte genetische Variation, *S. mansuetus* war das unterschiedlichste Kaninchen und *S. floridanus* und *S. brasiliensis* waren die am engsten verwandten Arten.

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