Chromosomal Variation of *Osgoodomys banderanus* (Rodentia: Muridae)*

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Accepted July, 7, 1999

Summary Chromosomal material from 76 specimens of *Osgoodomys banderanus* collected in 22 different localities was studied. The revised material was obtained from femur bone marrow from individuals which were previously injected with colchicine. A minimum of 10 mitotic fields were studied for each specimen. A number $2n=48$ was found for all the studied specimens. 3 different fundamental numbers and 4 different cytotypes were found 3 of them are not registered yet. Geographical distribution for each cytotype is presented. The G banding pattern was obtained and a cladogram with the possible chromosomal changes is presented.

Key words Chromosomes, Variation, Cytotypes, Geographical distribution, *Osgoodomys*.

Osgoodomys banderanus is a Mexican endemic murid (Hall 1981). It is present from the Southeast (state of Guerrero 15°20'N, 98°30'W) throughout the Northwest (state of Nayarit 23°N, 105°40'W). It occurs at an elevation of 20–1500 m above sea level in a dry and hot climate, particularly, it inhabits rocky areas, also around the margins of streams which have rocky walls and are surrounded by various types of vegetation: tropical deciduous forest, subdeciduous forest, oak forests or secondary vegetation (Alvarez 1968, Núñez 1993).

From the taxonomical point of view, the genus has been subjected to various changes; it was described as a species within the genus *Peromyscus* by Allen (1897). Later, a subgenus of *Osgoodomys* was established (Hooper 1958, Hooper and Musser 1964). Carleton (1980) proposed a separation within the generic level of *O. banderanus* based upon phenotypic differences.

Lee and Elder (1977) and Rogers *et al.* (1984) reported a karyotype for *O. banderanus*, based only on the study of specimens from the Jalisco state which is just a small part of the total distribution area for this species. They described it as a relatively simple one, consisting of a pair of large chromosomes, two small biarmed and the rest of the karyotype made up of 20 pairs of acrocentric autosomes which exhibited various sizes from small to large. Another report does not indicate the geographic source of the material (Stangl Jr. and Baker 1984).

The studied taxon has been considered a relict species which was separated in early stages from the ancestral phyletic branch (Hooper 1968). Research by Linzey and Layne (1969) and Carleton (1989) have lead these authors to make a similar proposal.

Since *O. banderanus* is found in many diverse environments, it is possible that interpopulation variation could be detected by comparing the karyotypes for the different populations. Therefore,

* This work is part of the Doctoral Thesis in Biological Science of the first author. Universidad Autónoma Metropolitana, México.

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the study of the karyotype of individuals collected at sites located throughout the geographic distribution area for this species, was carried out to test this possibility.

Material and methods

The chromosomic material was obtained from male and female individuals of *Osgoodomys banderanus* captured in 22 different localities and it was processed in the field. A minimum of 10 mitotic fields were studied for each one of the specimens.

Chromosomic material for preparations was obtained from femur bone marrow (Dyer 1979, Baker *et al.* 1983). The specimens were injected intraperitoneally with a colchicine (Microlab) aqueous 0.004% solution at a rate of 0.1 ml/10 g of specimen weight, 45 min before samples of chromosomic material were obtained.

The conventional chromosome classes were established according to Levan *et al.* (1964). After chromosomic figures homologation, the number of autosomic arms was considered as the fundamental number (F.N.).

A comparison of the karyotypes obtained was carried out against the standard karyotype proposed for the genus by the Committee for standardization of chromosomes of *Peromyscus* (1977, 1994).

In order to obtain G bands, the technique proposed by Baker *et al.* (1987) was followed.

On the basis of the differences shown for the cytotypes found, a cladogram was integrated with the most parsimonious routes among the possible chromosome arms additions, following a similar pattern to that presented by Greenbaum and Baker (1978) and Baker *et al.* (1979).

The specimens with cytotype A, B, and D are stored in the Universidad Autónoma Metropolitana, Universidad Michoacana de San Nicolás de Hidalgo, Universidad Nacional Autónoma de México, and Texas Tech University collections and the specimenes with cytotype C are stored in the collection of Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo.

Results

Chromosomic material from 76 specimens (50 males, 26 females) was studied. Analysis of the results obtained for the karyotypes of the various populations of *O. banderanus* indicated that they could be arranged in four different cytotypes, all with a $2n=48$ number, and a different fundamental number (Table 1, Figs. 1a, 2A–D). The cytotypes found are the following:

Cytotype A (47 specimens, 26♂, 21♀). This cytotype has a chromosomic number $2n=48$ and a F.N.=52 (Table 1, Figs. 1a, 2A). The biarmed were a pair of submetacentric chromosomes of small size and two pairs of subtelocentric chromosomes., (one pair of those was the biggest and the other pair was the smallest of the group); while the monorameous were 20 pairs of acrocentric chromosomes (sizes varying from large to small). The G bands pattern is presented in Fig. 1b.

Cytotype B (10 specimens, 6♂, 4♀). This cytotype has a chromosomic number $2n=48$ and a F.N.=52 (Table 1, Figs. 1a, 2B). The biarmed chromosomes were a pair of metacentric (the smallest one) and two pairs of subtelocentric (the pairs of the largest sizes) while the monorameous were 20 pairs of acrocentric chromosomes (sizes varying from large to small). The G bands pattern is presented in Fig. 1b.

Table 1. Comparison of the found cytotypes in *O. banderanus* populations. $2n$ =diploid number, F.N. fundamental number, M=metacentric, SM=submetacentric, ST=subtelocentric, A=acrocentric, X and Y=sexual chromosomes

Cytotype	$2n$	F.N.	M	SM	ST	A	X	Y
A	48	52	—	1	2	20	ST	A
B	48	52	1	—	2	20	ST	A
C	48	62	2	1	5	15	ST	A
D	48	78	8	5	3	7	ST	A

Fig. 1. a) Found cytotypes (A, B, C, D) in *O. banderanus* populations. b) G-band pattern of the same cytotypes. A.-22=submetacentric; 1, 23, X=subtelocentric; 2-21, Y=acrocentric. B.-23=metacentric; 1, 2, X=subtelocentric; 3-22, Y=acrocentric. C.-21, Y=acrocentric, 20=submetacentric; 1, 11, 14-16, X=subtelocentric; 2-10, 12, 13, 17, 19, 23, Y=acrocentric. D.-4, 9, 12, 16-20=metacentric; 2, 7, 8, 11, 13=submetacentric; 1, 5, 6, X=subtelocentric; 3, 10, 14, 15, 21-23, Y=acrocentric.

Figs. 2A–D. Cytotypes found (A, B, C, D) in populations of *Osgoodomys banderanus*.

Cytotype C (13 specimens, 6♂, 7♀). This cytotype has a chromosomal number $2n=48$ and a F.N.=62 (Table 1, Figs. 1a, 2C). The biarmed chromosomes were: two pairs of metacentric chromosomes of small size, one pair of submetacentric chromosomes of equally small size and five pairs of subtelocentric chromosomes (the one with the largest size and the other four of a medium size). The monorameous were 15 pairs of acrocentric chromosomes (sizes varying from large to small). The G bands pattern is presented in Fig. 1b.

Cytotype D (6 specimens, 3♂, 3♀). This cytotype has a chromosomal number $2n=48$ and a F.N.=78 (Table 1, Figs. 1a, 2D). The biarmed chromosomes were 8 pairs of metacentric chromosomes (sizes varying from large to small) five pairs of submetacentric chromosomes (sizes varying from large to medium) and three pairs of subtelocentric chromosomes (large size). The monorameous were seven pairs of acrocentric chromosomes (sizes varying from large to small). The G bands pattern is presented in Fig. 1b.

Comparative analysis of the G chromosome bands of the four cytotypes was carried out (Table 2). Cytotype A, which agrees with the one published by Stangl Jr. and Baker (1984) was compared with the other three cytotypes and it was found that cytotype B exhibits a repetition or duplication in chromosome 1, and a pericentric inversion in chromosome 2, a deletion in chromosome 22, and an addition in chromosome 23. Cytotype C, exhibits changes in chromosomes 12, 14–16, and 20, 21 which were additions and also changes in chromosomes 22 and 23 which were deletions. Cytotype D exhibited changes in chromosomes 1 and 2, where a repetition of bands was observed, also in chromosomes 4–9, 11–13, and 16–20 which were identified as addition cases. For chromosomes 22 and 23, deletion was considered as a possibility.

Chromosomal rearrangements were not observed in any of the 4 cytotypes for numbers 3, 10, and the sexual chromosomes.

Fig. 3 shows a proposed geographic distribution of the different cytotypes from the studied populations.

Discussion

The finding of 4 different cytotype modalities (Table 1, Fig. 1a, b) for *O. banderanus* contrasts with the findings reported by Lee and Elder (1977), Committee (1977), Greenbaum and Baker (1978), Baker *et al.* (1979) and Rogers *et al.* (1984). These authors all agree with one single karyotype for the species consisting of a group of acrocentric chromosomes except for chromosomes 1, 22, and 23 which are biarmed. This cytotype is the most common in the entire distribution area for this species (Fig. 3).

The 4 cytotypes have a number $2n=48$ but 3 different fundamental numbers were found which agrees with Lawlor (1974) who claims that there are no fusion or centric fission which could reduce or increase the chromosome number.

Table 2. Probable chromosomal rearrangements registered by G-bands in the four cytotypes in *O. banderanus*.

Cytotype A	Cytotype B	Cytotype C	Cytotype D
Chromosome			
1	Duplication		Duplication
2	pericentric Inversion		Duplication
3			
4			Addition
5			Addition
6			Addition
7			Addition
8			Addition
9			Addition
10			
11			Addition
12		Addition	Addition
13			Addition
14			Addition
15			Addition
16		Addition	Addition
17			Addition
18			Addition
19			Addition
20		Addition	Addition
21		Addition	
22	Deletion	Deletion	Deletion
23	Addition	Deletion	Deletion
x			
y

Fig. 3. Geographical distribution of the found cytotypes (A, B, C, D). Localities are shown in the map and cited in the text.

Cytotype A exhibits autosomes and sex chromosomes which have the G chromosomal band pattern reported by Stangl Jr. and Baker (1984) for *O. banderanus* (Fig. 1b) and it was detected in *O. banderanus* specimens.

Cytotype B, this modality differs slightly from the cytotype A modality. In this work it was detected in *O. b. vicinior* specimens and exhibits the following probable chromosomal rearrangements: one repetition, one pericentric inversion, one deletion and one addition. (Tables 1, 2, Fig. 1b).

Cytotype C, in this cytotype as well as in cytotype B, notable differences could be appreciated regarding the chromosome type. It exhibited an intermediate number of biarmed chromosomes (8) when compared to populations with the modalities of cytotypes A, B, and D and it presented as probable chromosomal rearrangements 6 additions and 2 deletions. (Tables 1, 2, Fig. 1b).

Cytotype D exhibits the following probable chromosomal rearrangements: 2 duplications, 14 additions and 2 deletions. The fundamental numbers indicate that the modality D karyotype is the population that presents the largest number of autosomic arms. (Tables 1, 2, Fig. 1b).

Due to the fact that the 4 karyotype modalities exhibit the same diploid number it is possible that Robertsonian changes did not occur. Rather, the probable chromosomal changes coincide with those reported in the literature (Hsu and Arrighi 1968, Arrighi *et al.* 1976, Greenbaum and Baker 1978, Yates *et al.* 1979). These authors suggest that the heterochromatin additions and pericentric inversions have played a prominent role in the Peromyscines evolution. Moreover, it has been reported that pericentric inversions have been important in the appearance of new populations within Peromyscines (Baker *et al.* 1979).

The presence of a karyotype which differs in the size of a biarmed chromosome (number 22 in cytotype A and number 2 in cytotype B) indicates the need for morphometric and electrophoretic studies for further characterization of the two registered subspecies.

The above considerations suggests the possibility of a further taxonomic subdivision for *Osgoodomys banderanus*, in a similar way as that followed by Baker *et al.* (1983) where they reported "chromosomal races" for *Peromyscus leucopus*. Lee *et al.* (1972) also contributed in a similar way to the specific recognition of *Peromyscus attwateri* through their studies on the chromosomal variation within the populations of *Peromyscus boylii*.

Cytotypes C and D suggest that *O. banderanus* populations have had the opportunity since its early origin of a radiation with an impact on their karyotype. Therefore, it would be desirable to study the reproductive aspects within these populations, as has been suggested by Zimmerman *et al.* (1978) in order to clarify taxonomic doubts.

From the analysis of the chromosomal material, no evidence was found that suggests inbreeding between the different cytotypes. Therefore, it is possible that there is some reproductive isolation, which supports the presence of different taxons in a similar manner to that proposed by Hinesley (1979). The idea of reproductive isolation is reinforced by the integrity of B or C karyotypes populations in spite of the fact that the areas where the specimens for these karyotypes were captured were in close geographic proximity (Sierra Coalcoman north slope, state of Michoacán).

It is worth pointing out that the studied cytotypes that do not project detectable chromosomal variation come from localities far

Fig. 4. Cladogram showing most parsimonious routes of additions of arms in chromosomes of found cytotypes in *Osgoodomys banderanus* populations.

apart within the geographic area for those karyotypes, therefore, it is possible that both cytotypes have remained invariable throughout their distribution areas.

At this time, it is not possible to answer the question of whether the existence of 3 different cytotypes to that presently recognized for the species is indicative of the possible existence of populations not recognized in the literature.

The cladogram presented in Fig. 4 shows the most parsimonious route for the additions of chromosomal arms in *O. banderanus*. This requires 20 events in order to produce cytotypes B, C, and D, starting from cytotype A which is recognized as the primitive one. (Committee 1977, Greenbaum and Baker 1978, Baker *et al.* 1979, Yates *et al.* 1979, Robbins and Baker 1981, Rogers *et al.* 1984, Stangl Jr. and Baker 1984).

Acknowledgments

Authors wish to acknowledge to: A Rocío Ortiz Muñiz and Robert D. Bradley for their comments and suggestions; Rosa María González Monroy, Edith Cortés Barberena and Patricia Pérez Vera, for their skillfull assistance in the handling of chromosomal material, to Martín Sandoval Rangel, for his assistance in the field and Alan Richards Lewis for correcting the English.

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Specimens examined (76) Specific localities for specimens of *Osgoodomys banderanus* examined in this study: Colima.—2♂: El Sauz, 8 km NE Minatitlán, 1,200 m (19°26'16"N, 104°01'W); 1♂, 2♀: 7 km SW Minatitlán, 780 (19°26'45"N, 104°06'09"W); 2♂: 9 km SW Minatitlán, 810 m (19°19'45"N, 104°06'50"W); 1♀: El Salto 4 km WSW Minatitlán, 630 m (19°22'09"N, 104°05'08"W); 6♂, 1♀: 3.4 km SW Las Canoas, 550 m (19°13'50"N, 104°07'10"W); 1♂, 1♀: 2.6 km SSE Las Canoas, 630 m (19°13'38"N, 104°08'08"W); 2♂, 1♀: 3.9 km NE Las Conchas, 73 m (18°54'25"N, 103°36'16"W); 3♂: 4.3 km NE Las Conchas, 133 m (18°54'37"N, 103°36'30"W); 3♂, 1♀: 3.3 km NW Ixtlahuacan, 60 m (18°54'09"N, 103°39'09"W); 1♂, 1♀: 1.6 km NE Las Conchas, 50 m (18°54'15"N, 103°36'W). Michoacan.—3♂, 3♀: 1 km N El Infiernillo (18°15'N, 101°54'W); 3♂, 1♀: 2 km N Caleta de Campos (18°05'N, 102°46'W); 1♂: 2 km W Cerro Colorado, 1,113 m (19°15'N, 100°21'W); 1♂, 1♀: La Yácata island, 5.12 km NNE Puerto Platanitos, 270 m (18°40'10"N, 101°52'24"W); 3♂, 1♀: Palos María, 350 m (18°48'45"N, 103°32'18"W); 5♂, 2♀: 1 km ENE Palos María, 460 m (18°48'45"N, 103°31'29"W); 3♂, 1♀: 1 km NE Palos María, 380 m (18°48'47"N, 103°32'12"W); 5♂, 5♀: Chorros del Varal, 950 m (19°35'N, 102°36'W); 1♀: 3 km E La Soledad, 9 km SE Jungapeo, 1,150 m (19°26'N, 100°29'W); 2♂, 1♀: Los Manguitos, 8.8 km ESE Dos Aguas, 1,440 m (18°46'01"N, 102°51'W). Nayarit.—1♂, 1♀: 7.6 km ENE San Blas, 60 m (21°34'28"N, 105°13'06"W); 2♂, 1♀: Arroyo La Quebrada 4.5 km W Valle de Banderas, 120 m (20°48'06"N, 105°17'12"W).