An. Inst. Biol. Univ. Nal. Autón. Méx. 48 (1977), Ser. Biol. Exp. (1): 45-56. 30-VIII-1982

CYTOGENETIC CONTRIBUTION TO THE TAXONOMY OF THE VOLCANO MOUSE, *Neotomodon alstoni* (Cricetidae, Rodentia).*

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ABSTRACT

Comparisons between chromosome diploid numbers, relative lengths and G-banding patterns of populations belonging to the geographic races of *Neotomodon alstoni* support the postulate of the existence of one single species within the genus *Neotomodon*. The affinity of this genus with *Peromyscus* is discussed.

RESUMEN

Algunos estudios citogenéticos realizados en poblaciones pertenecientes a las razas geográficas de *Neotomodon alstoni* han permitido la comparación entre los números diploides, las longitudes relativas de los cromosomas y los patrones de bandas G. Estos datos comparativos aportan argumentos en favor de que el género *Neotomodon* es monotípico. Por otra parte, se discute la notable afinidad filogenética entre los géneros *Neotomodon* y *Peromyscus*.

INTRODUCTION

The Rodentia is a large order which includes approximately 1600 species distributed in 3 suborders, 43 families and about 354 genera (Anderson and Jones, 1967). This noticeable species diversity, together with some other factors, such as its short generation time and its wide geographical distribution, have interested students from different fields to contribute to a deeper understanding of the evolution of the order. One type of evidence most frequently applied by geneticist involved in evolutionary studies is the chromosomal analysis, since the number, structure and banding patterns of chromosomes are cytological features which are useful in the study of phylogenetic relationships amongst some groups. Such studies, originally developed in the *Drosophila genus* (Patterson and Stone, 1952), have been particularly useful in plants as well as in Diptera, and have been used to analyze several mammalian taxonomy problems.

* Contribución 180 del Centro de Ciencias del Mar y Limnología, UNAM.

Neotomodon is a rodent genus which belong to the cricetid family. It was described originally by Merriam (1898) who, considering morphological differences, assigned three species to the *Neotomodon* genus: *N. alstoni, N. orizabae* and *N. perotensis.* In 1945 Davis and Follansbee, as a result of further morphological studies (mainly of cranial and dental characteristics) and some ecological features of the genus, concluded that there was only one species with two geographical races: *N. alstoni alstoni* and *N. alstoni perotensis. N. orizabae* was considered to be synonymous with *N. alstoni perotensis.*

In 1973, Uribe *et al.,* made cytogenetic studies on *Neotomodon alstoni alstoni,* including a proposed karyotype for this species, which was throughly analyzed. Rodríguez *et al.* (1975) investigated some cytogenetic characters of *Neotomodon alstoni perotensis* and made general comparisons between the karyotypes of the two geographic races *alstoni* and *perotensis.* The purpose of this paper is to compare the results derived from the chromosomal sampling of populations belonging to the two geographic races of *Neotomodon alstoni,* including descriptions of their banding patterns, and also to discuss some relevant aspects of their evolution in the light of chromosomal data.

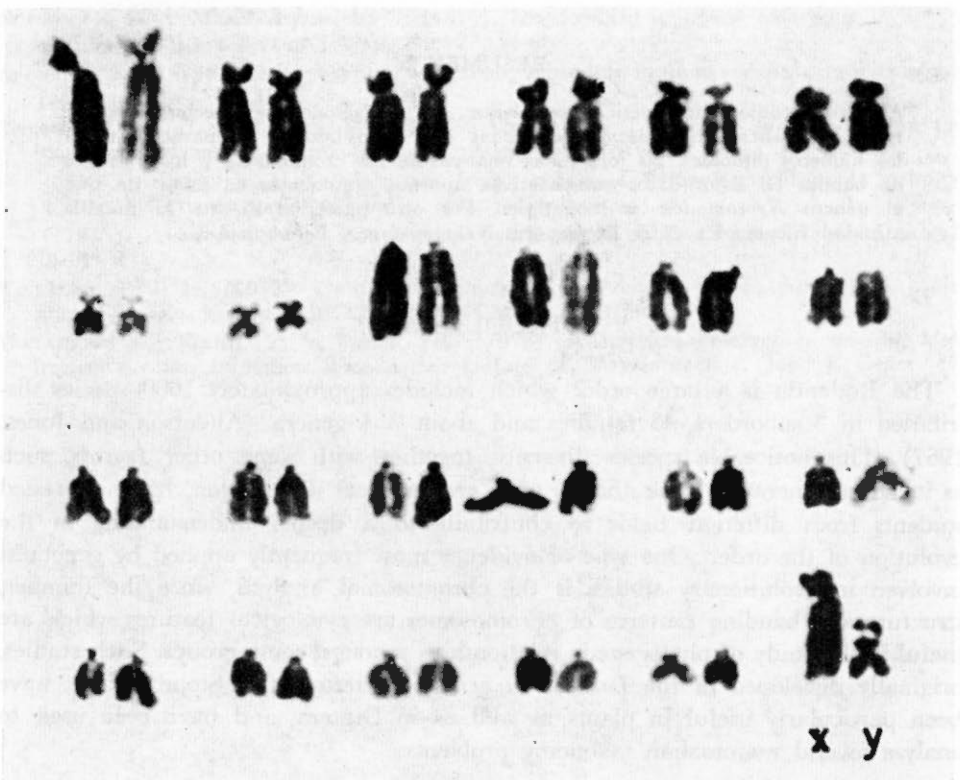

Fig. 1. Karyotype of *Neotomodon alstoni.* The populations sampled belonging to *N. a. alstoni* and to *N. a. perotensis show and identical karyotype.*

MATERIAL AND METHODS

G-banding was obtained by immersing the slides in a Giemsa-trypsin mixture, following Seabright (1971). Karyotypic data of *Neotomodon alstoni alstoni* and *Neotomodon alstoni perotensis*, from Uribe-Alcocer *et al.* (1973), and from Rodríguez-Romero *et al.* (1974) were used for statistical analysis.

RESULTS

In table 1 some of the basic karyological characters found for both geographic races are given. The proposed karyotypes are shown in figure 1. Table 2 shows the relative lengths, the centromeric index and the classification of the chromosome pairs of the karyotypes of such geographic races.

TABLE I. RELATIVE LENGTHS OF THE CHROMOSOMES OF *NEOTOMODON ALSTONI ALSTONI* AND *N. A.* PEROTENSIS AND CLASSIFICATION OF THE BIARMED CHROMOSOMES ACCORDING TO THE POSITION OF THE CENTROMERE, AS SHOWN BY THE CENTROME INDEX

Chromosome pairs	*Neotomodon alstoni alstoni*				*Neotomodon alstoni perotensis*			
	$\bar{x} \pm$ *S.E.*		*C.I.*	*Classification*	$x \pm$ *S.E.*		*C.I.*	*Classification*
1	86.40	1.27	21.55	st	85.26	2.65	21.73	st
2	71.30	1.19	22.66	st	68.89	1.32	22.24	st
3	66.30	0.95	20.57	st	64.60	1.04	22.44	st
4	60.70	0.99	21.85	st	59.10	1.46	26.49	sm
5	49.00	0.68	28.12	sm	50.97	0.99	29.03	sm
6	46.00	0.62	28.25	sm	45.13	1.07	30.42	sm
7	28.10	1.00	34.24	sm	34.00	1.79	34.32	sm
8	23.50	0.81	39.01	m	26.87	0.82	40.64	m
9	61.60	1.27			60.73	1.40		
10	54.60	0.69			54.50	1.32		
11	45.60	0.93			44.99	1.03		
12	38.20	0.47			39.27	0.71		
13	36.20	0.41			36.23	0.61		
14	33.80	0.53			34.07	0.62		
15	32.20	0.30			32.49	0.42		
16	30.80	0.39			30.56	0.54		
17	29.70	0.35			29.31	0.49		
18	28.90	0.44			28.51	0.54		
19	27.40	0.31			27.48	0.69		
20	26.10	0.48			26.41	0.68		
21	25.00	0.43			25.07	0.53		
22	23.20	0.60			23.13	0.53		
23	20.30	0.57			20.76	0.57		
X	55.10	2.02	26.94	sm	52.11	1.05	28.71	sm
Y	31.6	1.30	35.5	sm	28.3	1.10	29.7	sm

R.L. = relative length.
C.I. = Centromeric index.
st = subtelocentric.
sm = submetacentric.
m = metacentric.

With the use of conventional techniques, the uniarmed chromosomes of the karyotypes formed a series of decreasing sized elements with no possibility of sorting out the homologous pairs. The G bands supplied suitable markers to identify properly the homologous chromosomes. The centromere of biarmed chromosomes appeared darkly stained, while in the uniarmed elements it was mostly lightly stained or even unstained.

TABLE II. COMPARISON OF SOME KARYOLOGICAL CHARACTERS FOUND IN *Neotomodon alstoni alstoni* AND *Neotomodon alstoni perotensis*

	Neotomodon alstoni alstoni	*Neotomodon alstoni perotensis*
Diploid number	48	48
Fundamental number	62	62
Sexual differenciation system		
Females	XX	XX
Males	XY	XY
Biarmed chromosomes pairs	8 + Sexual	8 + Sexual
One-armed chromosomes Pairs	15	15

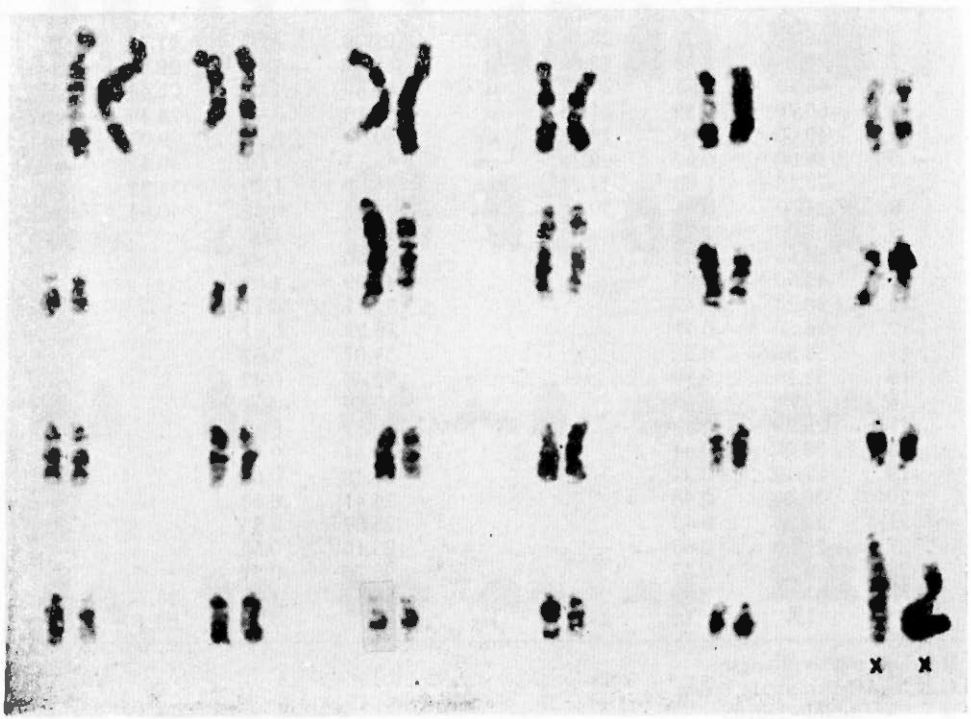

Fig. 2. G-Banding pattern of the chromosomes of *Neotomodon alstoni.*

A female G-banded karyotype of *Neotomodon alstoni alstoni* is shown in figure 2. The 22 pairs of autosomal homologous chromosomes were matched according to the displayed banding patterns. No systematic variations were discerned between any pair of homologous, nor between the autosomes from a female and those from a male, although some mismatchings eventually occurred, which were considered as being due to technical procedures, because they seemed to appear randomly, affecting different pairs of chromosomes in different cells.

It was not possible to find any difference between the G-banding pattern displayed by the chromosomes of the sampled populations of *Neotomodon alstoni alstoni* and *N. alstoni perotensis.*

In figure 3 an idiogram of the common G-banding pattern of the geographic races of *Neotomodon alstoni*, is shown. Fine bands which appeared as subdivisions of larger bands on elongated chromosomes are not represented in the idiogram as their occurrence is infrequent in comparison with the most conspicous and constantly appearing bands which should be a more useful diagnostic feature of the genus.

The proposed G-banding pattern of both geographic races of *Neotomodon* follows closely, if not identically, with the patterns of the long arms and some short arms of *Peromyscus eremicus* as shown by Pathak *et al.* (1973).

The mean length values of every pair of chromosomes and the centroameric index of biarmed chromosomes of *Neotomodon alstoni alstoni* and *Neotomodon alstoni perotensis* were compared by means of Students' T Test and showed no significant differences at a 0.95 confidence level.

Identification of the Y-chromosome has been reassessed. In earlier publications (Uribe-Alcocer *et al.*, 1973; Rodríguez-Romero, 1973, 1974) it was assumed that the Y-chromosome was a larger subtelocentric chromosome different from the one we now propose: i.e. the smaller element of the uneven pair in the male complement, a medium sized (R. L. 31.6 28.3%) submetacentric, with a secondary constriction in the short arm. In some cells the block distal to this secondary constriction is not visible, and the chromosome shows only a short arm fragment. This lead us to consider it as member of the series of uniarmed chromosomes. Further studies are required in order to know whether the eventual appearence of this block was produced by differential staining affinities of this region and the rest of the chromosome, or by a deletion of this chromosome section in some cells.

DISCUSSION

Our results show that the sampled populations have a very similar karyotype, whose measurements are statistically equal. This point is cleary shown in table 2 where some of the various karyological characteristics compared are found to be identical. There does exist, however, differences which could be important, for example chromosome pair 4 of *Neotomodon alstoni alstoni* has been classified by Uribe-Alcocer *et al.* (1973) as subtelocentric while the same pair of *Neotomodon*

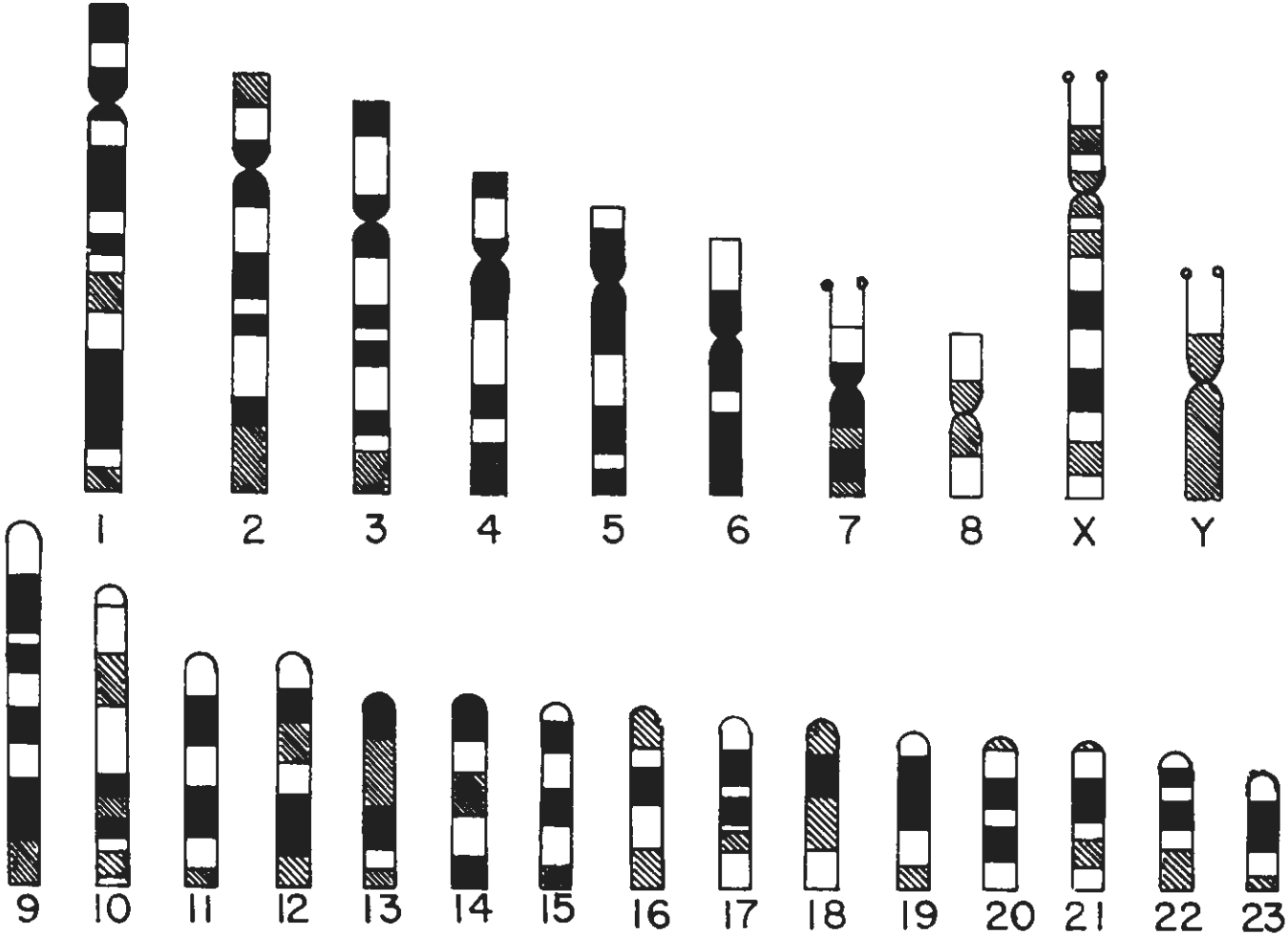

Fig. 3. Idiogram of the common G-Banding patterns found in the subspecies of *Neotomodon alstoni*. These bands are the most constantly found, although they do not appear in every banded karyotype. Some bands show further subdivisions in some karyotypes.

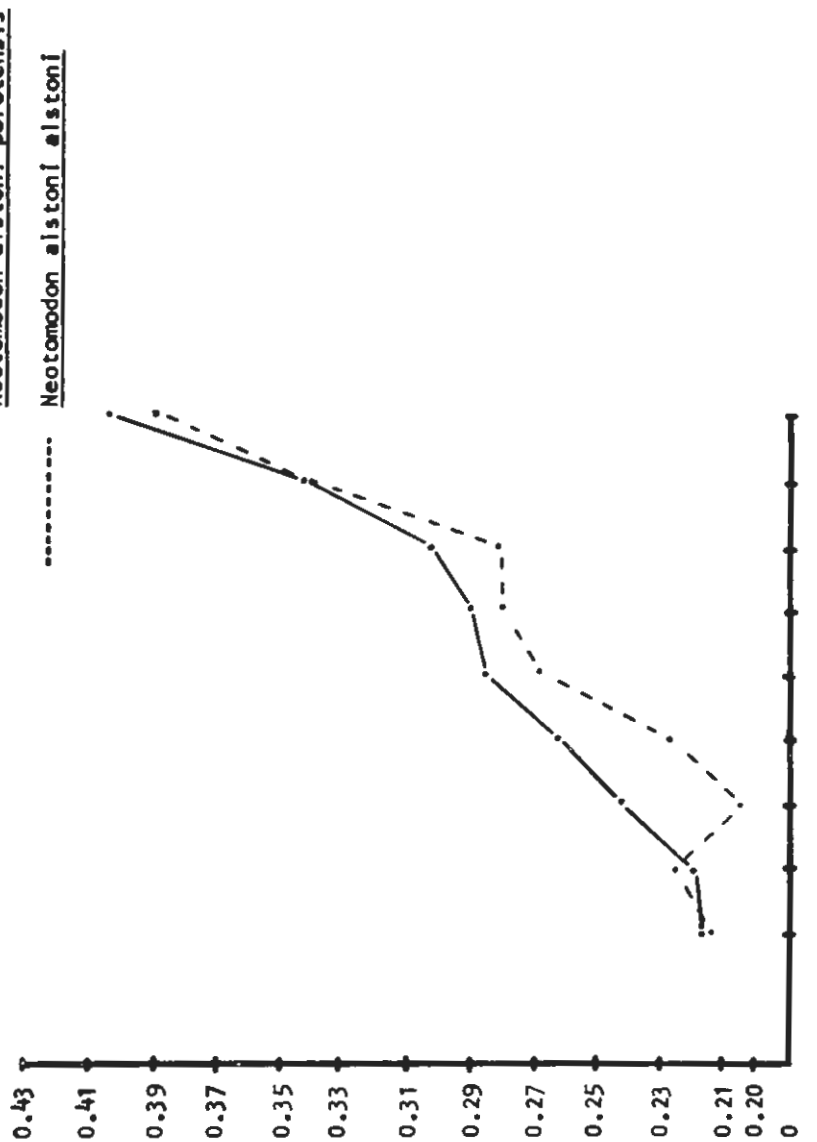

Fig. 4. Comparison of the centromeric index of the biarmed elements of the karyotype of *Neotomodon alstoni alstoni* y *N. a. perotensis*.

alstoni perotensis was classified as submetacentric by Rodríguez-Romero *et al.* (1975), as is shown in table 2. This seemingly important difference can be explained by the criteria followed to assign chromosomes to different groups with regard to the position of the centromere. In the former case, the centromeric index is 21.85, while in the latter it corresponds to 26.49. Statistical analysis does not indicate ignificative differences between the lenght nor the centromeric index of these two pairs of chromosomes. However, the arbitrary limit for submetacentric chromosomes lies in the centromeri index value of 25, above which chromosomes are submetacentric, and below which they are subtelocentric. This limit lies among the centromeric indexes found for both geographic races. Hence, pair 4 of *Neotomodon alstoni* alstoni has been assigned to the subtelocentric group, while the same pair of *Neotomodon alstoni perotensis* has been assigned to the submetacentric group, being both pairs in fact, very similar. A centromeric displacement of 1/20 of the length of the chromosome, or an equivalent error in measurement, would account for the slightly different arm ratio values.

The genus *Neotomodon* has morphological similarities with the genera *Peromyscus* and *Neotoma* (Merriam, 1898; Goldman, 1910; Davis and Follansbee, 1945). Karyologically, the populations studied are closer to the genus *Peromyscus,* as both have 48 chromosomes, which is the diploid number found for all species of *Peromyscus.* Also in both species the X-chromosome comprises about 5% of the total length of the haploid set (Ohno, 1967). The fundamental number 62 (Matthey, 1945) is found within the range displayed by species of the genus *Peromyscus.* There are even some species of the genus *Peromyscus* with a karyotype which shows eight biarmed autosomes and the same diploid number, e.g. *Peromyscus gossypinus* and *Peromyscus truei.* From a comparison between chromosome numbers, *Neotomodon* it not as close to *Neotoma mexicana,* a sympatric species, with a diploid number of 52 and with a fundamental number of 52 (Baker and Mascarello, 1969), or 54 (Paulete Vanrell *et al.*, 1971).

The results of our present study agree with the findings of the study carried out by Davis and Follansbee (1945), in which ecological and morphological characteristics shown greater affinity between *Peromyscus* and *Neotomodon,* than between *Neotomodon* and *Neotoma.*

The fact that the karyotype has 24 chromosome pairs, as does the postulated primitive cricetid karyotype (Baker and Mascarello, 1969), as wells as the close (if not identical) resemblance of the banding patterns shown by *Neotomodon* and the long arms and short arms of the chromosomes of *P. eremicus* (Pathak *et al.*, 1973) suggests that in the course of the chromosomal evolution of that group, mechanisms of addition or deletion of heterochromatic short arms were the main processes involved.

It is interesting to note the fact that populations of cricetids living at high altitudes, such as *Neotomodon* and *Peromyscus melanotis* from Mexico, and Pinaleño, Chiricahua and Santa Catalina Mountains from Southern Arizona (formerly classified as *P. maniculatus ruffinus*), have a very similar karyotype. Bowers *et al.* (1972) proposed a karyotype for the latter with the same fundamental number as *Neotomodon,* with elements which closely coincide in size as well as in centro-

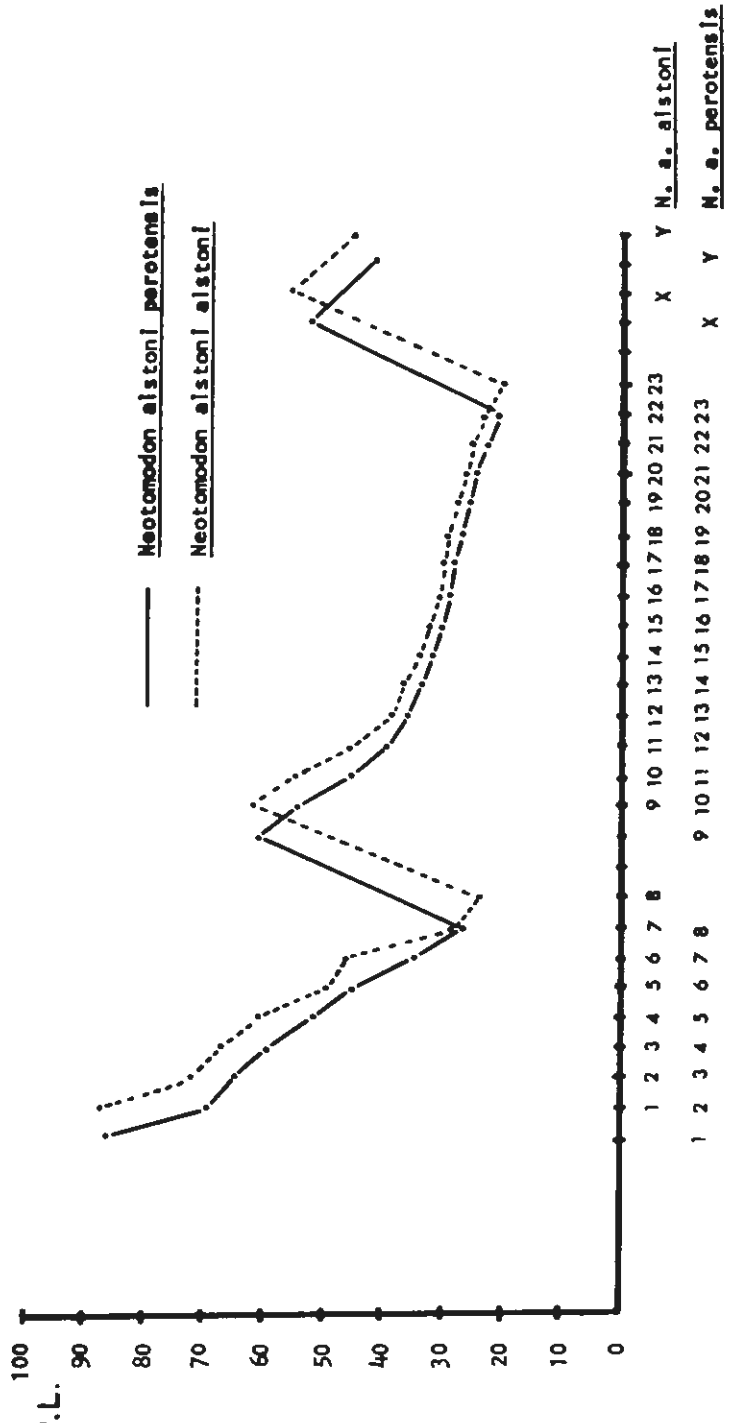

Fig. 5. Comparison of the relative lengths of the chromosomes of the subspecies of *Neotomodon alstoni*.

mere location with those of the karyotype of the *Neotomodon* subspecies. The karyotypic uniformity of these populations is difficult to explain by adaptative convergence alone, since the distance of at least 2000 km between the populations of Mexico and Southern Arizona rules out genetic flow. It seems more suitable to consider these separated populations as relict populations, descendants from an ancient stock that originally had a very wide area of distribution, but later in time became more reduced due probably to a combination of environmental/climatic/genotypic changes. Some populations of this ancient stock, instead of migrating back north where cooler habitas could be bound, perhaps migrated to areas with altitude and cool climate similar to the one they were adapted to. Such populations have since remained isolated, being cut off, but are nevertheless very similar karyotypically, and some populations at least, genetically (Bowers *et al.*, 1973).

The karyotype exhibited by *Peromyscus floridanus* (Hsu and Arrighi, 1968), shows a remarkable resemblance to that of *Neotomodon*. *P. floridanus*, considered a pleistocene relict (Smith *et al.*, 1973), has 48 chomosomes, 8 pairs of which are biarmed with a close correspondence in size and centromere position to the ones found in *Neotomodon*. The remaining chromosomes are acrocentrics in both groups. This genetic similarity is further strengthened by the resemblance of the structure and complexity of the phallus (Hooper and Musser, 1964). Again, it is difficult to explain the similarity of these two characters by chance alone. Furthermore, the areas of distribution of both species make improbable the existence of a direct ancestry of both groups.

Brown's hypothesis (1957) satisfactorily explains the resemblances we have noted between Mexican and Southern Arizona populations, if we assume that such similar characteristics are somewhat primitive and were common to populations that expanded to new areas some time in the past from a central population. If, due to changes in climatic conditions the distribution area of this stock was reduced, isolated populations could have been left behind becoming the nucleus of new species. Each of these species probably evolved in its own direction, keeping in common some of the characteristics found in the ancestral central populations from which the expansion occurred.

The areas of distribution of *Neotomodon*, *P. melanotis* and *P. floridanus* might correspond to peripheral areas of the general distribution of ancient stocks, where the three species remained when environmental conditions were modified after the expansion of the central group. Together with the ancentral (central) stock, the primitive populations of *Neotomodon* could have spread to areas with similar climates to the ones which they inhabit today. Through a later expansión cycle, *P. melanotis*, could have arrived once the isolating mechanisms that prevented its crossing with *Neotomodon* were established. The association of *Neotomodon* and *P. melanotis* is old. Alvarez (1966) found fossil remains of *Neotomodon* associated with *P. melanotis* and *P. maniculatus* in late Pleistocene sand at Tequesquinahua, State of Mexico.

The alternative case in which *P. melanotis* would be the new comer does not seem as likely. It is difficult to envisage that more especialized populations, like those of *Neotomodon* given the specificity of their requeriments, migrated

through zones presumably different to those to which they were adapted, and that they survived in them in spite of the existence of older, well established competing species in those areas.

Hsu and Arrighi (1968) proposed a model of karyotypic evolution of the genus *Peromyscus* (in which it is considered that *P. floridanus* is derived). Nevertheless, they stated that evolution could have been in the opposite direction and, therefore, the species considered by them as being advanced, could be in fact primitive. The generalization that primitive karyotypes are those with greater numbers of acrocentrics would therefore not hold true in this case. In studies of different populations of the genus *Peromyscus* inhabiting the islands of the Gulf of California, Lawlor (1971), taking into account karyotypic, blood and morphometric data, considered that primitive populations had a karyotype containing less acrocentric chromosomes than the descending populations. He also suggested that among those populations, the karyotypically advanced were those which included a larger number of acrocentrics. Therefore the karyotype of *P. floridanus* might well represent a primitive condition.

The structure of the phallus in *Peromyscus floridanus* and in *Neotomodon* is very similar, as mentioned before. Hooper and Musser (1964) considered that is represented a secondary reduction which took place in an ancestral stock. However, if the chromosomes and area of distribution of both species are indeed primitive, the simplicity of the phallus would be yet another primitive feature.

From the cytogenetic studies carried out in the subspecies *Neotomodon alstoni alstoni* and *Neotomodon alstoni perotensis*, it is concluded that there is not enough evidence derived from the chromosomal standpoint to affirm that the two populations studied are different. Our data show the existence of a single species as the sampled populations were karyotypically homogenous. However, the afforementioned differences in the karyotype cannot be discarded without suggesting that they might be initial stages in evolutionary pathways.

Several types of evidence showing the affinity amongst *Neotomodon* and *Peromyscus*, and the areas of distribution of both genera suggest that they probably descend from the same ancestral stock and that *Neotomodon* originated from earlier populations which also gave rise to *Peromyscus*.

LITERATURE

Al-Ais, M. (1969). Human chromosome morphology. I. Studies on normal chromosome characterization, classification and karyotyping. Can. Jour. Genet. Cytol. *11*, 307-381.

Alvarez, T. (1966). Roedores fósiles del Pleistoceno de Tequesquinahua, Estado de México. Acta Zool. Mex. *8*, 1-9.

Anderson, S. and Jones, J. K. (1967). Recent mamals of the world — A synopsis of families. Ronald Press. New York.

Baker, R. J. and Mascarello, J. F. (1969). Karyotypic analyses of the genus *Neotoma* (Cricetidae-Rodentia). Cytogenetics *8*, 187.

Bowers, J. H., Baker, R. J. and Smith, M. (1972). Chromosomal, electrophoretic and breeding studies of selected populations of dear mice (*Peromyscus maniculatus*) and black-eared mice (*P. melanotis*). Evolution *27*, 378-386.

Brown, W. L. (1957). Centrifugal speciation. Quart. Rev. Biol. *32*, 247-277.

DAVIS, W. B. AND FOLLANSBEE, L. A. (1945). The mexican volcano mouse *Neotomodon*. J. Mamm, *26*, 401-411.

HOOPER, E. T. AND MUSSER, G. G. (1964). The glans penis in neotropical Cricetines (Family Muridae) dith comments on classification of Muroid rodents. Misc. Publ. Zool., Univ. Michigan *123*, 1-57.

HSU, T. C. AND ARRIGHI, F. E. (1968) Chromosomes of *Peromyscus* (Cricetidae-Rodentia). 1. Evolutionary trends in 20 species. Cytogenetics 7, 417-446.

LAWLOR, T. E. (1971). Evolution of *Peromyscus* on northern islands in the Gulf of California, Mexico. Trans. San Diego Soc. Nat. Hist. *16*, 91-124.

MATTHEY, R. (1945). L'évolution de la formule chromosomiale chez les Vertebrés. Experientia *1*, 50-75.

MERRIAM, C. H. (1898). A new genus (*Neotomodon*) and three new species of murine Rodents from the mountains of southern Mexico. Proc. Biol. Soc. Washington 12, 127-129.

OHNO, S. (1967). Sex chomosomes and sex-linked genes. Monographs on Endocrinology. Z. Labhart, T. Mann, L. T. Samuels and J. Zander, Eds. Springer-Verlag, New York. Vol. 1.

PATHAK, S., HSU, T. C. AND ARRIGHI, F. E. (1973). Chromosomes of *Peromyscus* (Rodentia, Cricetidae). IV. The role of heterochromatin in karyotypic evolution. Cytogenet. Cell. Genet. *12*, 315-326.

PATTERSON, J. T. AND STONE, W. S. (1952). *Evolution in the Genus Drosophila*. The MacMillan Company, New York.

PAULETE, J., SCAGLIA DE PAULETE, S., LAGUARDA-FIGUERÁS, A. ÁND ROMERO, J. 1971). Cytogenetic analysis of *Neotoma mexicana torquata*. An. Inst. Biol. Univ. Nal. Autón. México *42*, Ser. Biol. Exp. 55-60.

RODRÍGUEZ-ROMERO, F. (1974). Estudios citogenéticos en *Neotomodon alstoni perotensis*. Tesis Profesional. Facultad de Ciencias, UNAM. México.

RODRÍGUEZ-ROMERO, F., URIBE- ALCOCER, M. AND LAGUARDA-FIGUERAS, A. (1975). Chromosome analysis of *Neotomodon alstoni perotensis*. Mamm. Chrom, Newsl. *16*, 117-119.

SMITH, M. H., SELANDER, R. K. AND JOHNSON, W. E. (1973). Biochemical polymorphism and systematics in the genus *Peromyscus*. III. Variations in the Florida deer mouse (*Peromyscus floridanus*), a Pleistocene relict. J. Mammal. *54*, 1-13.

URIBE-ALCOCER, M. (1972). Estudios citogenéticos en *Neotomodon alstoni alstoni*. Tesis Profesional. Facultad de Ciencias, UNAM. México.

URIBE M., LAGUARDA A., ROMERO, J., PAULETE, J. AND SCAGLIA DE PÁULETE; S. (1973). Cytogenetic analysis of *Neotomodon alstoni alstoni* Cytologia (Tokyo) *39*, 437-442.