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Further Occurrence of a Karyotype of $2N = 14$ Chromosomes in two Species of Chilean Didelphoid Marsupials

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All the species of marsupials of the American superfamily Didelphoidea known in their chromosome make-up have been found to fall into one of three groups of taxa, each one having a basically similar karyotype. These three different karyotypes are characterized by particular chromosome numbers in the diploid set (14, 18 or 22) and by particular chromosome morphologies (REIG and BIANCHI 1969, REIG and LÖBIG 1970).

Among the didelphids, a $2N=14$ chromosomes karyotype was first described in *Caluromys derbianus* and observed in *Marmosa mexicana* (BIGGERS, FRITZ, HARE and McFEELY 1965). Afterwards, this kind of karyotype was found in *Marmosa robinsoni* (REIG 1968) and it was later verified in *Caluromys philander*, *Metachirus nudicaudatus*, *Marmosa murina* and *Marmosa cinerea* (REIG and LÖBIG 1970). In all these taxa, the chromosome complements are almost identical, the only significant difference lying in the tiny X chromosome, which may be either acrocentric or sub-

metacentric. Inferring from the condition found in these species, we can speak of a standard $2n=14$ didelphid karyotype, composed of (A) three pairs of large, submetacentric autosomes; (B) one pair of medium sized, metacentric autosomes; (C) two pairs of small, acrocentric autosomes, and a sexual XY-XX system formed by a minute, submetacentric or acrocentric X chromosome, and an even smaller, telocentric Y chromosome.

A slight departure from this standard karyotype was found in *Marmosa fuscata*, where the autosomes of group C and the X chromosome are metacentric (REIG and SONNENSCHNEIN 1970). In fact karyotypes of 14 chromosomes closely alike to the above described didelphid standard, and also differing from it only in minor variations in the position of the centromere in some of the autosome pairs, are widely distributed among the marsupials. They occur in many Australasian species, including all the studied species of the superfamilies Dasyurioidea and Perameloidea as well as several species of the Phalangerioidea (HAYMAN and MARTIN 1969). Furthermore, they have been recently described in *Caenolestes obscurus*, *Caenolestes fuliginosus* and *Lestoros inca* of the South American superfamily Caenolestioidea (HAYMAN, KIRSCH, MARTIN and WALLERS 1971).

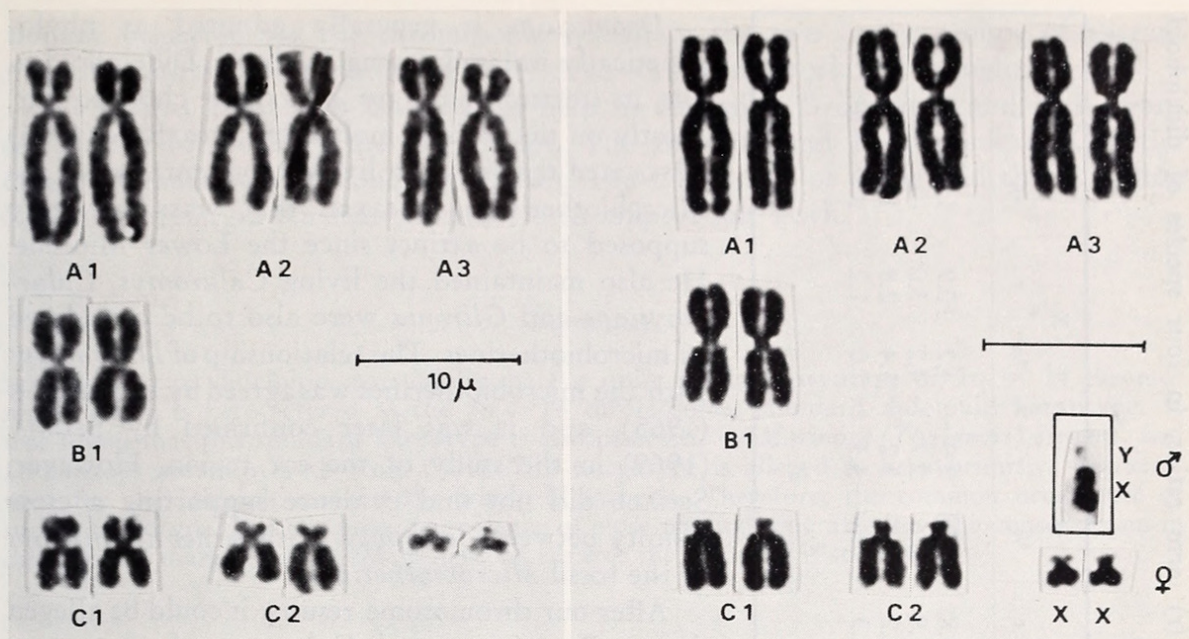
The variations in gross chromosome morphology as regards the described standard found in this large array of taxa, are actually small, and they may be accounted for a relatively little number of chromosome repatternings, mainly due to pericentric inversions. The same holds for the didelphid karyotypes of 22 or 18 chromosomes, which reinforces the pattern of chromosome stability found in the Methatheria altogether. Such a wide-spread occurrence of similar chromosome sets is a remarkable fact which opposes the general picture of chromosome diversity found in the different groups of the Eutheria, in which chromosome multiformity at the Family and generic level is the rule (see the various reports in BENIRSCHKE 1969, and also REIG and KIBLISKY 1969). It is, therefore, of interest to check the extent of the occurrence of this karyotype in other taxa of marsupials the chromosomes of which have not yet been described.

Among the polytypic American murine opossums (genus *Marmosa*) the chromosomes of species belonging to the subgenera *Marmosa*, *Micoureus* and *Marmosops* have been reported (REIG and SONNENSCHNEIN 1970, REIG and LÖBIG 1970). Chromosomes of members of the subgenus *Thylamys* (*elegans* plus *microtarsus* groups of TATE 1933) are undescribed so far. At the same time, knowledge of the chromosomes of the peculiar *Dromiciops australis*, of the Valdivian forest of Chile and Argentina, is also of particular interest, since this animal differs morphologically from the true didelphids and shows close affinity to the fossil microbiotheres, as shown by REIG (1955) and confirmed by SEGALL (1969).

We have studied the chromosomes of three individual (two females and one male) of *Marmosa* (*Thylamys*) *elegans elegans* (WATERHOUSE), obtained from Central Chile, 3 Km S of Cachagua, in the Province of Aconcagua. They were part of a series of five individuals caught in one line of forty traps set during two nights, in a shrubby community close to the sea, described by the senior author elsewhere (REIG 1970). The same line of traps yielded *Akodon olivaceus* and *Phyllotis darwini*. *Spalacopus cyanus* was also caught in the vicinity.

Thanks to the help of Dr. Hugo Campos from the Instituto de Zoología, Universidad Austral de Chile, we obtained one female specimen of *Dromiciops australis australis* (PHILIPPI), caught near Valdivia, in Southern Chile. Unfortunately, our attempts to catch additional specimens of this taxon by ourselves resulted unsuccessful, even after extensive trapping in an area close to Mehuin where the animal was obtained before.

Chromosome spreads from the bone marrow and the spleen were studied in the



A. The chromosomes of *Dromiciops australis australis* (Philippi) from Valdivia, Chile; female. Bone marrow. Colchicine pretreatment. Giemsa stain. — B. The chromosomes of *Marmosa (Thylamys) elegans elegans* from near Cachagua, Aconcagua, Chile. Female; in the box, the gonosomes of a male individual. Bone marrow. Colchicine pretreatment. Giemsa stain

four specimens, following the colchicine-hypotonic pretreatment-air dried technique already described (FERNÁNDEZ 1968). Additionally, we obtained chromosome spreads of *Marmosa elegans* from the corneal epithelium, following the technique described by FREDGA (1964). The specimens of *Marmosa* are catalogued in the collection of mammals of the Instituto de Zoología of the Universidad Austral, in Valdivia. The single specimen of *Dromiciops* was given to the Museo de Historia Natural of Santiago.

A description of the chromosomes of *Dromiciops australis* (Fig. A), based in the same individuals reported here, has been informally advanced by two of the present authors (SPOTORNO and FERNÁNDEZ 1971). They are very like those of *Marmosa elegans* (Fig. B), and both species possess the standard $2n=14$ chromosomes didelphid karyotype slightly modified in the position of the centromeres of the pairs of autosomes A 1, C 1 and C 2 and of the X chromosome, as shown in the Table. *Marmosa elegans* shows a simple XY-XX sexual system, with a submetacentric X chromosome and a very minute Y chromosome of the dot-like type. Needless to say, we do not know the size and structure of the Y chromosome in *Dromiciops australis*. As regards its X chromosome, we assign to it the two components of the pair of smaller chromosomes of the complement of the single available female individual. However, this assignment is provisional and it was only based in comparisons with other taxa of the Didelphidae.

Our results show that the $2n=14$ chromosomes didelphid standard karyotype is shared by all four subgenera of living *Marmosa*. Therefore, it is quite probable that all the species of *Marmosa* have a very similar karyotype, and that only very minor chromosome changes have been involved in the evolutionary diversification of this genus, in which an authorized revision recognized 49 living species (TATE, 1933). More significant is the finding of the same type of chromosome set in *Dromiciops*, which confirms the fact that this karyotype is not only shared by members of a given genus, but also by different genera the relationships among which are not necessarily close.

Values of (0%) relative lengths of the chromosomes as a percent of the female haploid set, and (r) arm ratios, in four species of didelphoid marsupials with 2n = 14 chromosomes

	A1		A2		A3		B1		C1		C2		X		Y	
	0%	r	0%	r	0%	r	0%	r	0%	r	0%	r	0%	r	0%	r
<i>Caluromys derbianus</i> ¹	23.5	1.7	20.6	1.7	18.8	1.6	15.0	1.1	9.0	4.8	9.0	2.9	4.2	10.9	1.1	α
<i>Dromiciops australis</i> ²	22.8	2.6	20.4	1.6	19.2	2.4	15.0	1.1	9.8	1.9	8.6	2.7	4.2	3.0	—	—
<i>Marmosa elegans</i> ²	24.0	1.9	20.6	1.5	20.2	1.8	14.4	1.1	9.0	8.2	8.2	5.2	3.4	2.8	1.3	α
<i>Marmosa fuscata</i> ²	21.8	1.7	19.8	1.5	18.6	1.5	14.8	1.1	9.4	1.3	8.6	4.4	7.0	1.2	2.5	α

¹ From Legator et al., Life Sci. 5, 397 (1966). — ² Original data.

Dromiciops is generally admitted as phylogenetically isolated among the living Didelphoidea, but its relationships are not quite clear so far. Mostly on the basis of molar structure, REIG (1955) advocated that it was a living representative of the Microbiotheriinae, a taxon that was previously supposed to be extinct since the Lower Miocene. He also maintained the living *Caluromys*, *Caluromysiops* and *Glironia* were also to be considered as microbiotherines. The relationship of *Dromiciops* with the microbiotherines was agreed by PATTERSON (1965), and it was later confirmed by SEGALL (1969) in the study of the ear region. However, SEGALL did not find evidence supporting a close affinity between *Caluromys* with either *Dromiciops* or the fossil *Microbiotherium*.

After our chromosome results, it could be alleged that a *Dromiciops* and *Caluromys* share a very similar karyotype, they should be closely related. However, this conclusion would be based on a misuse of the chromosome evidence for phylogenetic inference, as the possession of 14 chromosomes is most probably a primitive character state for the Didelphidae, as it was claimed by REIG and BIANCHI (1969). In addition, HYMAN and MARTIN (1969) believe that this karyotype may be considered ancestral for the whole of the Metatheria. Sharing of a primitive character state (synplesiomorphy in HENNIG's phylogenetic vocabulary: HENNIG 1966, BRUNDIN 1968) does not indicate close phylogenetic kinship; instead, this must be inferred from the common possession of derived character states (synapomorphy). Therefore, our present results do not afford new relevant evidence to settle the conflicting views on the relationships between *Dromiciops* and *Caluromys*. In any case, it is now evident that at least one undoubted member (*Dromiciops*) of the microbiotheres, a group which may prove to deserve full family status, possesses the 2n=14 karyotype.

Therefore, our results reinforce the previously known picture of a wide spread occurrence of similar karyotypes in very different taxa of the Metatheria, and the relative invariance of chromosome number and structure at the generic level in species of this group of mammals. This picture suggests that in the evolution of the Metatheria there was a definite trend towards karyotype stability, whereas the evolution of the Eutheria seems to have been connected with major changes, of both number and morphology, in the chromosomes. The reasons for this difference in the evolu-

tionary behaviour of the chromosome system in the two living groups of therian mammals are not clear, and their study deserves further work and thought.

We thanks Dr. HUGO CAMPOS for help in obtaining *Dromiciops* and Mrs. Frances Musset for reading the manuscript. The International Programme in Genetics of the Organization of American States, The University of Chile and the Comisión Chilena de Energía Nuclear contributed to support this work.

Summary

Several taxa of didelphid marsupialis share a quite similar karyotype of $2n = 14$ chromosomes which is here referred as the $2n = 14$ chromosomes standard didelphid karyotype. It was found that this standard karyotype is also possessed by *Marmosa (Thylamys) elegans*, and *Dromiciops australis*, both from Chile. This karyotype is alleged to be a primitive character state for the Didelphidae and the whole Metatheria. Therefore, the common occurrence of such a karyotype does not provide evidence of close phylogenetic affinity. The conservatism in chromosome make up of the Metatheria as regards the Eutheria is stressed.

Zusammenfassung

Verschiedene Arten der Didelphidae besitzen einen sehr ähnlichen Karyotypus von $2n = 14$; es wurde festgestellt, daß dieser Standard-Karyotypus auch bei *Marmosa (Thylamys) elegans* und *Dromiciops australis* aus Chile vorkommt. Dieser Karyotypus wird als primitives, gemeinsames Merkmal für Didelphidae und die gesamte Metatheria angesehen. Das allgemeine Vorkommen eines solchen Karyotypus ist daher kein Beweis für enge phylogenetische Verwandtschaft. Der Konservatismus der Chromosomen bei den Metatheria im Vergleich zu den Eutheria wird hervorgehoben.

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Biology of the Rice Rat (*Oryzomys palustris natator*) in a Laboratory Environment

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Introduction

Within the genus *Oryzomys*, over 100 species have been described and the extent of their habitat is shown by their presence in most of South America, Central America and Mexico with extensions to East Kansas, southern Illinois und New Jersey.

The original vernacular name „rice meadow mouse“ stemmed from the work of AUDUBON and BACHMANN (1854) and was given to *Oryzomys palustris* because it was observed in the rice fields of South Carolina and Georgia. This nomenclature has inevitably resulted in the appellation „rice rat“ being applied to all the species even though their habitat covers marshy areas along the coasts to the extremes of brush in the mountains and grassy areas in the mesquite brush. This inclusion of the species with widely differing habitats and behaviour under one genus is regarded by GOLDMAN (1918) as having originated due to the similarity in ratlike form and general appearance. Thus the genus comprises of a mixed assemblage of species with wide diversity of general characters — the whole being lightly fused together by the similarities exhibited by the molar cusp pattern.

Historical confusion of nomenclature when based on limited observations is often unavoidable, and after the first species of *Oryzomys* had been described under the name of *Mus palustris* from New Jersey by HARLAN (1837), then AUDUBON and BACHMAN (1854) erroneously placed it under the genus *Arvicola*. The full generic name for the group — *Oryzomys* — was finally introduced by BAIRD (1857) with *Mus palustris* (HARLAN) as the type species. Unfortunately, the story did not end at this point because BAIRD recorded it on a later page of his publication with a



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