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Karyological studies of the South American rodent *Myocastor coypus* Molina 1782 (Rodentia: Myocastoridae)

Estudio cariológico del roedor sudamericano *Myocastor coypus* Molina 1782 (Rodentia: Myocastoridae)

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ABSTRACT

A cytogenetic study of "feral" and "cognac hybrid" phenotypes of Uruguayan *Myocastor coypus* rodent was performed with the purpose to investigate its evolution and systematics position. The karyotype 2n=42; FN=80 was integrated by biarmed chromosomes. The sexual X chromosome, was a metacentric one and the Y was a small acrocentric. Mitotic chromosomes using G, C and Alu I endonuclease banding were analyzed. The NOR localizations and meiotic chromosomes were examined. The comparison between "feral" and "cognac hybrid" individual showed no notorious differences. For testing the chromosome variability between our results and published data of specimens from introduced populations, a karyological comparative analysis was performed. The difference obtained may be interpret as subspecific variations or as a chromosomal evolution process.

Key words: nutria, subspecific variation, chromosomal evolution

RESUMEN

Con el propósito de investigar la posición sistemática y evolución del roedor *Myocastor coypus*, se realizó un estudio citogenético de ejemplares uruguayos de fenotipo "silvestre" e "híbrido cognac". El cariotipo 2n=42 ; NF=80 estaba integrado por cromosomas bibraquiados. El X es metacéntrico, mientras que el Y es un acrocéntrico pequeño. Se aplicaron para el análisis de los cromosomas mitóticos, las técnicas de bandeo G, C y Alu I. Se localizaron las regiones NOR. Se analizaron también los cromosomas meióticos. Los resultados obtenidos en los individuos de fenotipo "silvestre" e " híbrido cognac" no mostraron diferencias notorias. Debido a que esta especie ha tenido una amplia introducción y con el fin de detectar la existencia de variabilidad cromosómica, se efectuó un estudio comparativo entre nuestros resultados y datos publicados. Las diferencias encontradas pueden interpretarse como variaciones subespecíficas o como consecuencia de un proceso de evolución cromosómica.

Palabras clave: nutria, variabilidad subspecífica, evolución cromosómica.

INTRODUCTION

Myocastor coypus is a hystricomorph rodent originally of exclusive South American distribution. It inhabits parts of Bolivia, Paraguay, southern Brazil, Argentina, Chile and Uruguay (Mares & Ojeda, 1982); it is usually called "nutria" or "coipo".

This species has been widely introduced in the United States since 1899, with the purpose of installing fur farms. Some authors support the idea that *Myocastor coypus bonariensis*, the subspecies that inhabits northeast Argentina and Uruguay, was the primary source of nutria introduced into North America (Atwood 1950, Evans 1970 cited by Willner 1982). Around the 1930's, it also began to be introduced in Europe and Asia with the same purposes.

Previous cytogenetic studies have been performed by Fredga (1966), Tsigalidou et al.(1966) and Kasumov et al. (1976). The specimens studied by the later author were from Azerdbaishan fur breeders, but the original source of the founders was not indicated.

The purpose of this study was to analyze the systematics and evolution of this species at the chromosome level. A cytogenetic study of mitotic chromosomes using G, C and Alu I endonuclease banding was carried out. Also were included NOR's

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localizations and examination of meiotic chromosomes. A comparative analysis of our results with available data published by other authors (Fredga, 1966) and Kasumov et al. (1976) was performed.

MATERIAL AND METHODS

Twenty two *Myocastor coypus* specimens were analyzed from three different Uruguayan populations; sixteen of "feral" phenotype (collected in Rocha and Maldonado Departments of Uruguay) and six of "cognac hybrid" phenotypes obtained in Facultad de Veterinaria from crosses between "feral" and "cognac" individuals (Table I).

Mitotic chromosome preparations were obtained following Moorhead et al. (1960) method, and bone marrow microculture as described by Fredga (1987). Meiotic preparations were obtained using the procedures of Evans (1964) and Meredith (1969).

To identify the chromosomes, we followed the nomenclature proposed by Levan et al. (1964) and Fredga (1966). We measured 12 metaphasic plates of "feral" and 11 of "cognac hybrid" phenotype (Table II). For performing the karyological comparative analysis two metaphase plates published by Fredga (1966) and the one published by Kasumov et al. (1976), were measured. The values were transformed into percentages of the total haploid plus X set and displayed in a karyoidiogram (Spotorno 1985). To establish statistical comparisons, Student's t test was applied.

To obtain G-banding, we followed Seabright (1971) and Chiarelli et al. (1972) methods. Silver staining (NORs) was induced, treating the slides with the procedure of Rufas & Gonsalves (1982). The constitutive heterochromatic chromosome regions were differentiated using Sumner's (1972) technique and endonuclease banding with Alu I, following Miller et al. (1983).

With the purpose of analyzing the recognition pattern of this enzyme in the genome, DNA was obtained from lymphocytes. The isolated DNA was digested with Alu I, according to Sambrook et al.(1989) method.

RESULTS

Karyotype

The *Myocastor coypus* karyotype had 2n = 42 and FN = 80 (Fig. 1a). Four groups of chromosomes were distinguished:
Group I, integrated by 6 pairs of large chromosomes (pairs 1-6), ranged from 8.81% to 5.85% of the female haploid set length.

Group II, consisting of 6 pairs of medium size chromosomes (pairs 7-12), ranged from 5.50 % to 3.82 % of the female haploid set.

Group III, had pairs of small chromosomes (pairs 13-18) representing 3.69% to 3.00% of the female haploid set.

Group IV included, according to Fredga (1966), 2 autosomic pairs and the sexual chromosomes. Chromosome pair 19, as a submetacentric element had 5.32 %. The chromosome 20 was a microchromosome of 1.39%. The X was a metacentric of 5.32%, and the Y chromosome, was an acrocentric chromosome of 1.80%.

Autosome pairs 5, 9, 10, 15 and 19 were submetacentric, being the remaining chromosome metacentrics. Pair 19 had a secondary constriction in the long arm (q), which showed a positive AgNOR reaction (Fig. 1c).

The comparisons performed among the measures obtained of the "feral" and "cognac hybrid" individuals through a Student t test, revealed significant differences ($p<0.05$) in the length of the chromosomes 5q, 9, 19q and 20. These results may be taken with caution because multiple comparisons were done. A karyoidiogram illustrated these data (Fig. 2).

C-Banded chromosomes

C-banding results obtained in all the analyzed sample were represented by an idiogram (Fig. 3). Small pericentromeric blocks appeared on the chromosomes of group I (1, 2, 4, 5, and 6). Pair 1 showed a

TABLE 1

Biological data of studied individuals: catalogue number (number), processed date (date), sex, origin, weight and total length (without tail) and methods for studying mitotic and meiotic processes.
Datos biológicos de los individuos estudiados: número de catálogo, fecha de procesamiento, sexo, origen, peso y largo total (sin cola) y métodos de estudio para los procesos mitóticos y meióticos.

Number Date	Sex	Orig.	Weight	Total Length	Micotic	Meiotic
801 Dec. 88	♂	Maldo-nado	5.105g Feral	56cm	lymphocyte culture C, G NOR	C NOR
802 Dec. 88	♂	Maldo-nado	6.000g Feral	57cm		C NOR
803 Dec. 88		Maldo-nado	4.925g Feral	51cm		C NOR
804 Nov. 89	♂	Maldo-nado	---- Feral	--	lymphocyte culture	
805 Nov. 89	♂	Vet. Breed.	---- Cognac	-- Hybrid	lymphocyte culture	C NOR
806 Giemsa Dec. 89	♂	Maldo-nado	4.650g Feral		--------	
807 Dec. 89	♂	Maldo-nado	4.000g Feral	49cm	--------	Giemsa
808 Dec. 89	♂	Maldo-nado	4.350g Feral		------	Giemsa
809 Dec. 89	♂	Maldo-nado	5.600g Feral		bone marrow culture C- GP	Giemsa C band
810 Aug. 89	♂	Rocha	3.770g Feral	44.5cm	------	Giemsa C NOR
811 Aug. 89	♂	Rocha	3.550g Feral	43.3cm	------	Giemsa C NOR
812 Aug. 89	♂	Rocha	3.100g Feral	44.2cm	------	Giemsa C NOR
813 Aug. 89	♂	Rocha	3.270g Fera	48.5cm	------	Giemsa C NOR
814 Nov. 89	♂	Vet. Breed.	Cognac	Hybrid	------	Giemsa C band
815 Nov. 89	♂	Vet. Breed.	Cognac	Hybrid	------	Giemsa C band
816 Nov. 89	♂	Vet. Breed.	Cognac	Hybrid	------	Giemsa C band
817 Nov. 89	♂	Vet. Breed.	Cognac	Hybrid	------	Giemsa C band
818 Apr. 90	♀	Vet. Breed.	3.505g Feral.		bone marrow	----- C G
819 Apr. 90		Vet. Breed.	4.310g Feral.		bone marrow C G NOR	Giemsa C NOR
821 Jun. 90	♂	Vet. Breed.	4.930g Feral.	53 cm Hybrid	bone marrow C G NOR Alu I banding	Giemsa C NOR
822 Jun. 90	♀	Vet. Breed.	3.900g Feral.	50 cm Hybrid	bone marrow C G NOR Alu I banding	------

TABLE 2

Chromosome lengths are expressed as percentage of the female haploid set. Asterisks indicate significant differences in the student test for $\alpha>0.05$ probability.

Las longitudes están expresadas como el porcentaje del set haploide femenino. Los asteriscos indican diferencias significativas en el test de t Student para una probabilidad $\alpha>0.05$.

	Feral				Cognac hybrid		
	$\bar{X}$	S	S^2		$\bar{X}$	S	S^2
1 P	4.04	0.253	0.0643	1 P	3.87	0.490	0.2469
q	4.77	0.317	0.1009	q	4.66	0.538	0.2902
2. p	3.44	0.343	0.1177	2. p	3.45	0.238	0.0565
q	4.04	0.310	0.0962	q	4.27	0.666	0.3201
3 p	2.99	0.339	0.1152	3 p	3.14	0.279	0.0779
q	3.88	0.289	0.0836	q	3.92	0.314	0.0988
4 p	2.78	0.304	0.0928	4 p	2.63	0.233	0.0541
q	3.56	0.521	0.2723	q	3.89	0.292	0.0854
5 p	2.32	0.483	0.2340	5 p	2.47	0.253	0.0639
q	3.86	0.620	0.3846	q	3.37	0.303	0.0920
6 p	2.50	0.366	0.1342	6 p	2.24	0.299	0.0899
q	3.35	0.311	0.0969	q	2.24	0.299	0.0899
7 p	2.35	0.202	0.0412	7 p	2.17	0.308	0.0946
q	3.15	0.305	0.0932	q	3.02	0.248	0.0613
8 p	2.19	0.261	0.0681	8 p	2.07	0.395	0.1560
q	3.23	0.288	0.0833	q	3.00	0.302	0.0910
9 p	2.08	0.320	0.1029	9 p	1.48	0.208	0.0435
q	3.11	0.261	0.0682	q	3.33	0.191	0.0363
10 p	1.68	0.375	0.1412	10 p	1.39	0.211	0.0444
q	2.85	0.311	0.0969	q	2.89	0.208	0.0432
11 p	1.66	0.298	0.0888	11 p	1.78	0.260	0.0677
q	2.22	0.282	0.0796	q	2.45	0.310	0.0958
12 p	1.69	0.267	0.0717	12 p	1.72	0.265	0.0705
q	2.13	0.242	0.0588	q	2.06	0.272	0.0740
13 p	1.71	0.187	0.0353	13 p	1.74	0.265	0.0505
q	1.98	0.208	0.0433	q	2.06	0.301	0.0904
14 p	1.69	0.188	0.0355	14 p	1.51	0.307	0.0941
q	1.84	0.258	0.0667	q	2.02	0.297	0.0883
15 p	1.28	0.125	0.0157	15 p	1.21	0.234	0.0546
q	1.97	0.268	0.0723	q	2.20	0.249	0.0620
16 p	1.49	0.143	0.0205	16 p	1.38	0.272	0.0741
q	1.78	0.202	0.0408	q	1.99	0.372	0.1385
17 p	1.37	0.213	0.0453	17 p	1.32	0.279	0.0780
q	1.70	0.203	0.0414	q	1.77	0.249	0.0621
18 p	1.31	0.159	0.0254	18 p	1.25	0.325	0.1058
q	1.69	0.188	0.0354	q	1.56	0.128	0.0164
19 p	1.38	0.267	0.0714	19 p	1.26	0.217	0.0472
q	2.26	0.275	0.0758	q	2.75	0.376	0.1416
20 p	0.69	0.217	0.0471	20 p	0.93	0.169	0.0284
q	0.70	0.225	0.0509	q	0.97	0.183	0.0334
X p	2.40	0.286	0.0822	X p	2.53	0.248	0.0613
q	2.92	0.243	0.0590	q	3.05	0.403	0.1624

References: $\bar{X}$ = mean, S = Standard deviation and S^2 = variance. Feral: n = 12 metaphases. Cognac Hybrid: n = 11 metaphases.

heteromorphism, an element had a large heterochromatic block, while the other had a small heterochromatic block.

Other heteromorphism was observed in pair 9. One element had a large pericentromeric block in the short arm (p), while the other had an interstitial band on the long arm (q). The sexual XY chromosomes had small pericentromeric blocks.

The Alu I-banding showed positive reaction in pairs 1, 2, 4, 5, 9, 11 and the X chromosome. The comparison between Sumner's technique results and Alu I banding, revealed some differences. The C-like bands produced by Alu I were sometimes similar, in size and position, to those corresponding to C-band with saline solution, or smaller. This is the case of pairs 1, 2, 4, 5, 9 and X. However, in the other chromosomes the reaction was completely negative for Alu I reaction. The last technique revealed a heterochromatic block in one element of pair 5, meanwhile C-banding with Ba(OH)2 showed hetero-

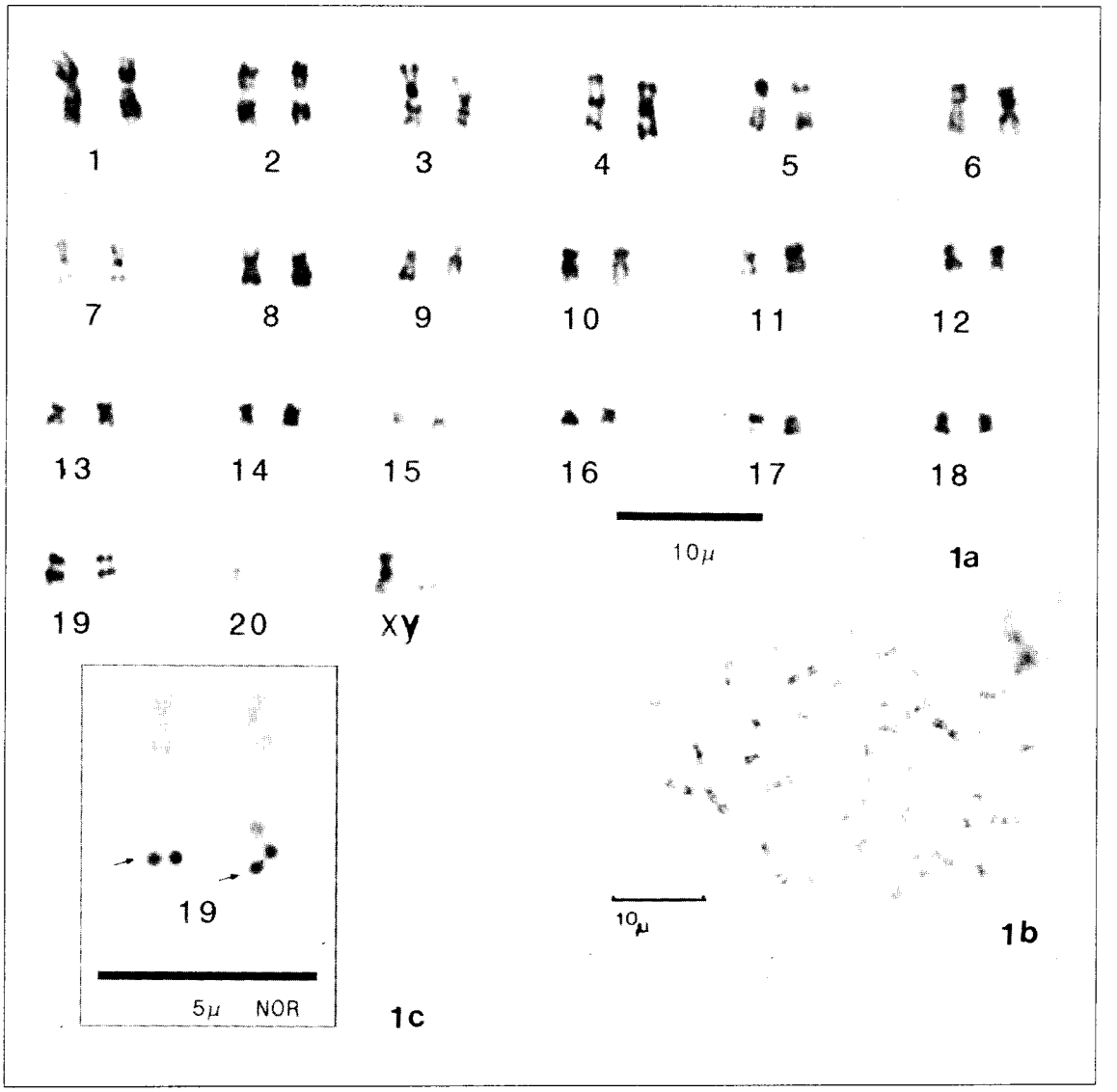

Fig. 1. a) G-banding karyotype of specimen N° 820. b) Metaphase plate. c) Pair N° 19 Giemsa stained. Arrows indicate secondary constrictions and nucleolar organizer regions (AGNOR).

a) Cariotipo con bandeo G del especimen N° 820. b) Placa metafásica. c) Par 19 teñido con Giemsa. Las flechas indican las constricciones secundarias y la localización de las regiones organizadoras nucleolares (AGNOR).

chromatic blocks in both chromosomes. Pair 6 showed heterochromatic regions when the chromosome was treated with saline method. Pair 11 only revealed a pericentromeric block with Alu I banding (Fig. 4).

The recognition pattern of Alu I in genomic DNA from a nutria specimen was also tested (Fig. 5b, lane 2), showing that it was highly repeated in the genome.

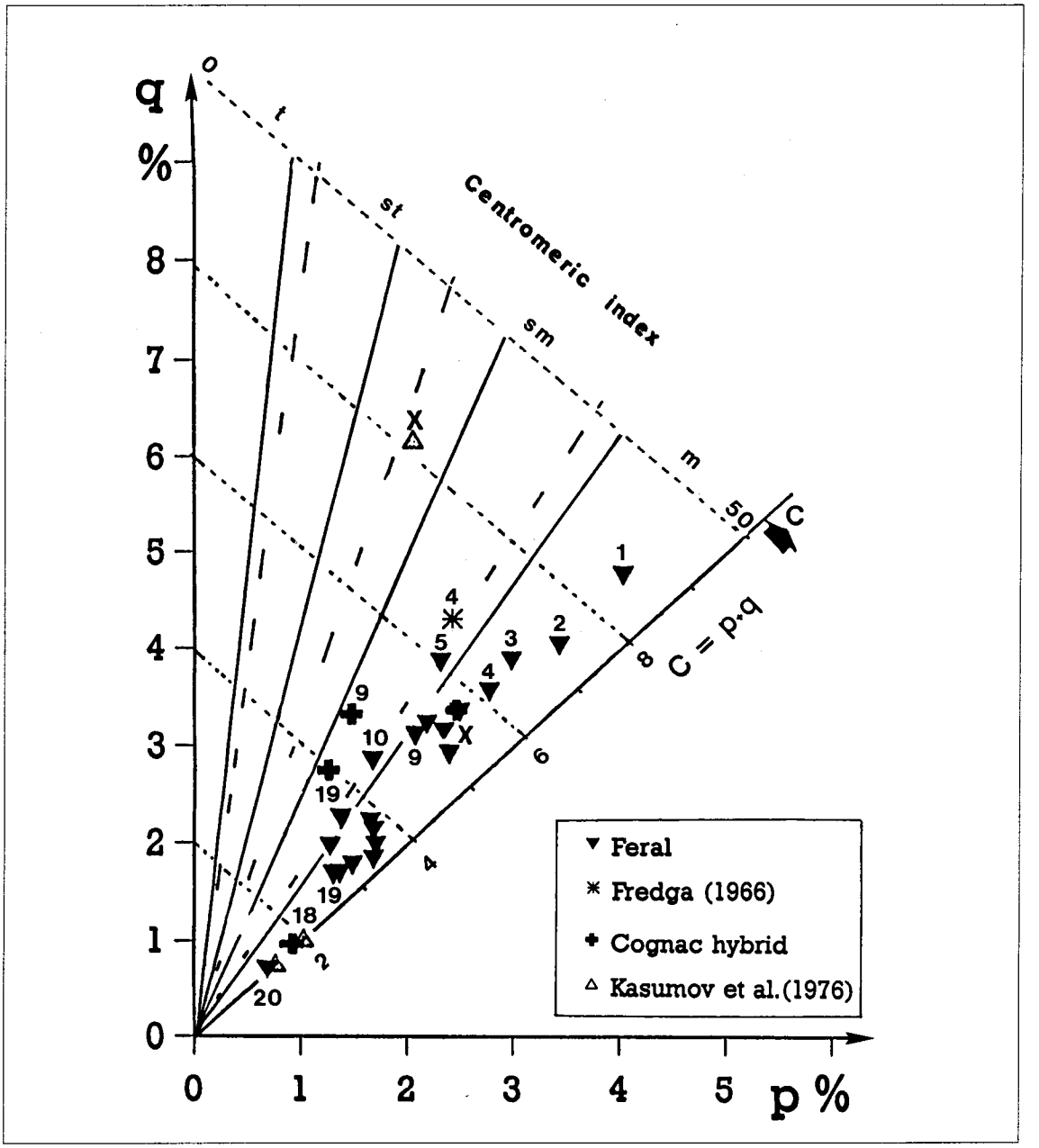

Fig. 2. Karyoidiogram.The nutria feral chromosome set (haploidfemale) is shown. Differences found among "Cognac hybrid" phenotype (5, 9, 19 and 20), element 4, (Fredga 1966) and X, 18 and 19 chromosomes (Kasumov et al. 1976) are indicated.

Carioidiograma. El set cromosómico haploide femenino de la nutria silvestre está representado. Se indican las diferencias encontradas con el fenotipo "hibrido cognac" (5, 9, 19 y 20), el elemento 4 (Fredga 1966) y los cromosomas X, 18 y 19 (Kasumov et al. 1976).

Meiosis

Meiotic process was studied considering Oud et al.'s (1979) classification of stages and heterochromatin behavior.

In early prophase, several C-positive blocks were observed with Ba (OH)2-treatment (Fig. 6a). Seven heterocromatic blocks were scored, in agreement with the positive blocks found in mitotic metaphases (Fig. 6b). In middle pachytene, the bivalents and the sex vesicle appeared stained (Fig 6c). In late pachytene, two nucleolar masses were detected (Fig 6d).

At the diffuse stage, the autosomes had been separated and chromatin despiralization occurred (Fig 6e). The nucleolus was then filled up with a network of fine threads.

At diplotene stage (Fig. 6f) the X and Y sex chromosomes were associated end to end. In this stage, some bivalents appeared with two or three chiasmata.

DISCUSSION

Karyological comparative analysis

A comparative study between Fredga's (1966) and our results through a karyoidiogram showed difference in the chromosome pair 4. It also reveals differences between ours and Kasumov's et al. (1976) data, in the morphology and size of chromosome 18, 19 and X. Specifically, the X chromosome has a notorious difference, since in all the sample analyzed by us, this element appeared as metacentric and medium size (5.37%), while in the material studied by these authors, is a large submetacentric chromosome (8.37%). These morphological differences were confirmed in the meiotic chromosomes, analyzing the diplotene stage from both materials.

Banding pattern

C and Alu I banding patterns showed different amount of heterochromatic blocks. Similar results have been found in human and other mammalian species, suggesting that Alu I reveals different heterochromatin regions (Miller et al. 1983). Alu I enzyme recognizes 5'...AG CT...3' DNA sequences which are highly repeated in human and other rodent DNA (Holmquist 1989). In nutria DNA, the presence of a smear in DNA digested with Alu I, confirmed that it contains the Alu I repeated sequences, as was previously reported in other rodent species.

Comparative analysis between our C-banding pattern and the one reported by Kasumov et al. (1976), showed differences in the amount and location of constitutive heterochromatin. They found large

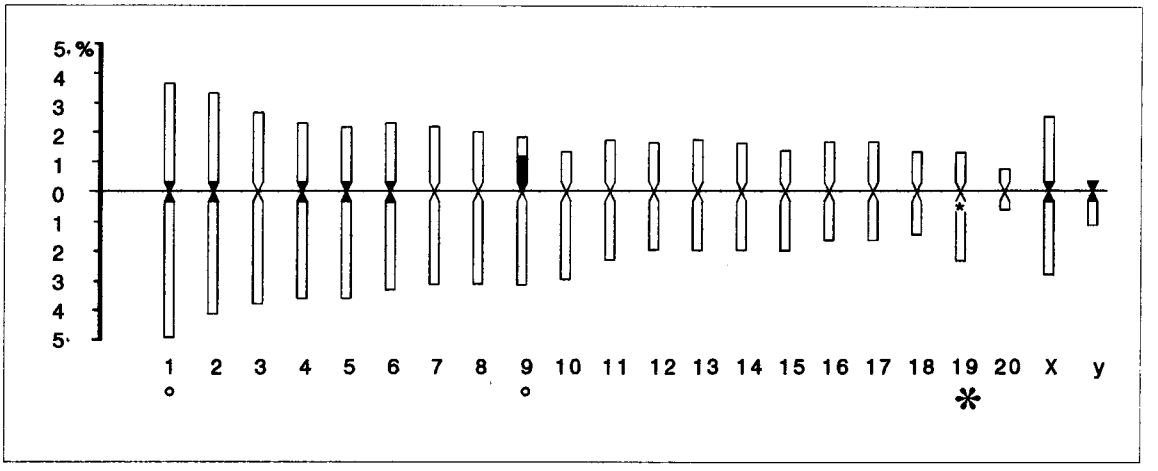

Fig. 3. C- banding idiogram. In the autosome pairs n° 1 and 9 the points (O) indicate heteromorphism. Asterisk (*) shows NOR's location.

Idiograma con bandeo C. En los autosomas n° 1 y 9 los puntos (O) indican heteromorfismo. Asterisco (*) muestra la localización del NOR.

pericentromeric blocks in pairs, 1 to 9 and X, appearing also telomeric and pericentromeric bands in other chromosomes. In the Uruguayan sample, we only found small pericentromeric blocks in six autosomic pairs and in the X chromosome. One fourth of this element was represented by the heterochromatin region.

G-banding pattern comparison between our results and Kasumov's et al. (1976) showed some differences in pairs 5, 7, 9, 11, 18 and 19. A notorious variation was detected in the G-banding pattern of X-chromosome (Fig. 7).

In conclusion significant differences were found in the comparison of the X-chromosome in morphology, size, G and C banding patterns.

Systematic and evolution

In relation to *Myocastor coypus* chromosome evolution, George & Weir (1974) have postulated two possible theories, "Robertsonian" and "Stebbin's", for establishing karyotypical relationships within hystricomorph rodents. The ancestral hystricomorph karyotype considered by George & Weir (1974), would be 2n=98 and FN=136, composed by 30 pairs of acrocentrics and 18 pairs of small metacentrics. Thus species with a high diploid number of acrocentrics would be more primitive than those, having a low diploid number with biarmed chromosomes. The latter is the case of *Myocastor coypus* karyotype that has all biarm chromosomes.

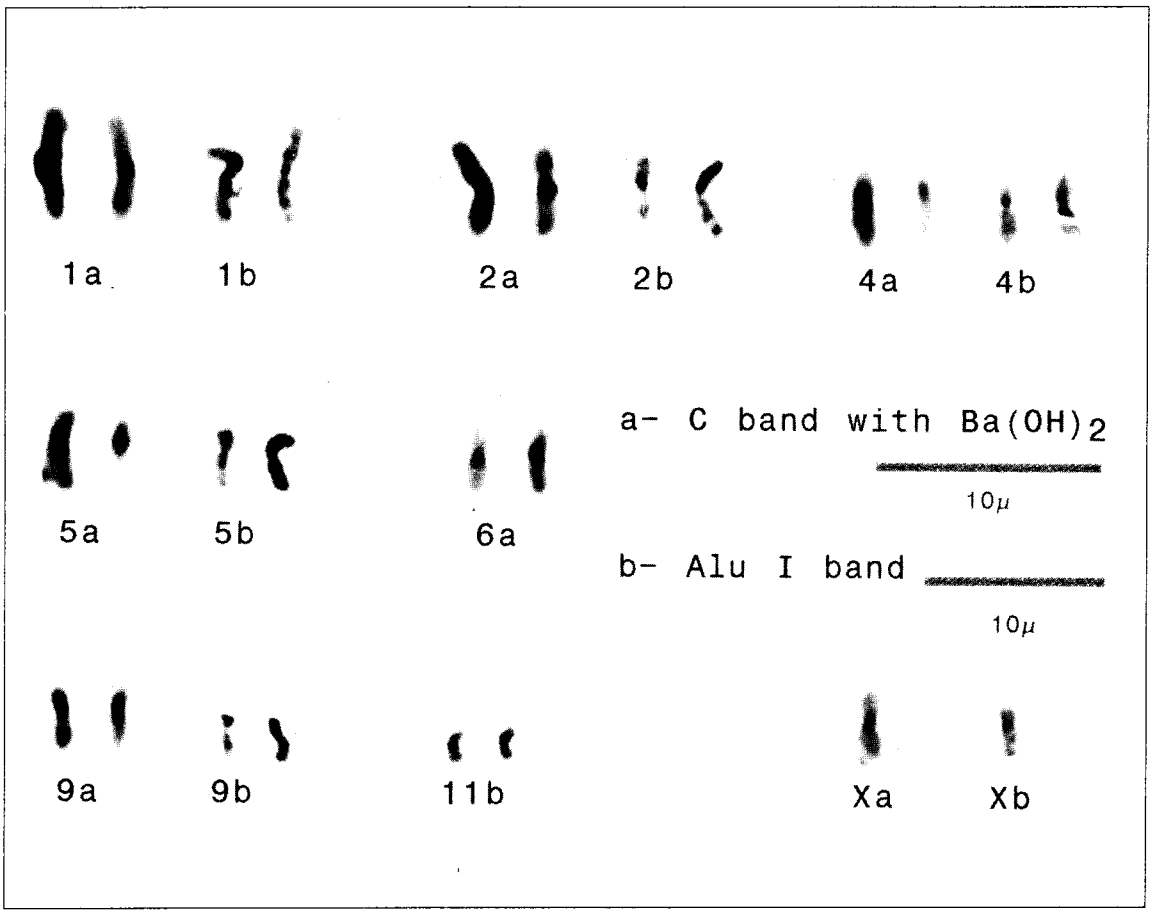

Fig. 4. Comparison of heterochromatic regions of a partial karyotype (specimen N° 822) among: a) C-banding with Ba(OH)2 and b) Alu I banding.

Comparación de las regiones heterocromáticas de un cariotipo parcial (especimen n° 822) entre: a) bandeo C con Ba(OH)2 y b) bandeo con Alu I.

Hystricomorphs have characteristic chromosomes with secondary constrictions, which are considered "markers" of the group. Two types of these chromosomes were found: "chinchillid" and "octodontid" (George & Weir 1974). *M. coypus* has a chromosome pair (n°19) carrying secondary constrictions similar to the octodontid type.

Many authors, considering other taxonomic features such as anatomy, paleontology and ecology, have reached different conclusions in establishing hystricomorph relationships. For example, *Myocastor coypus* was included in the family Capromyidae by Simpson (1945). Patterson & Pascual (1968) suggested that *Myocastor* is more closely related to the Echimyidae than to the Capromyidae. Woods (1982) performed a review of this complex group and by considering serologic data, suggested that this genus must be placed into a separated family, Myocastoridae.

Considering that nutria has a specialized karyotype, certain anatomical-physiological adaptations and serologic data like those provided by Woods (1982), we agree that this species should be in an isolated family, Myocastoridae as Miller & Gidley have proposed in 1918.

Chromosomal variations is frequently observed in the kariological analysis of rodents. In general, this fact has been interpreted as subspecific variations or as chromosomal evolution processes. If we consider that there is not available information about which was the original

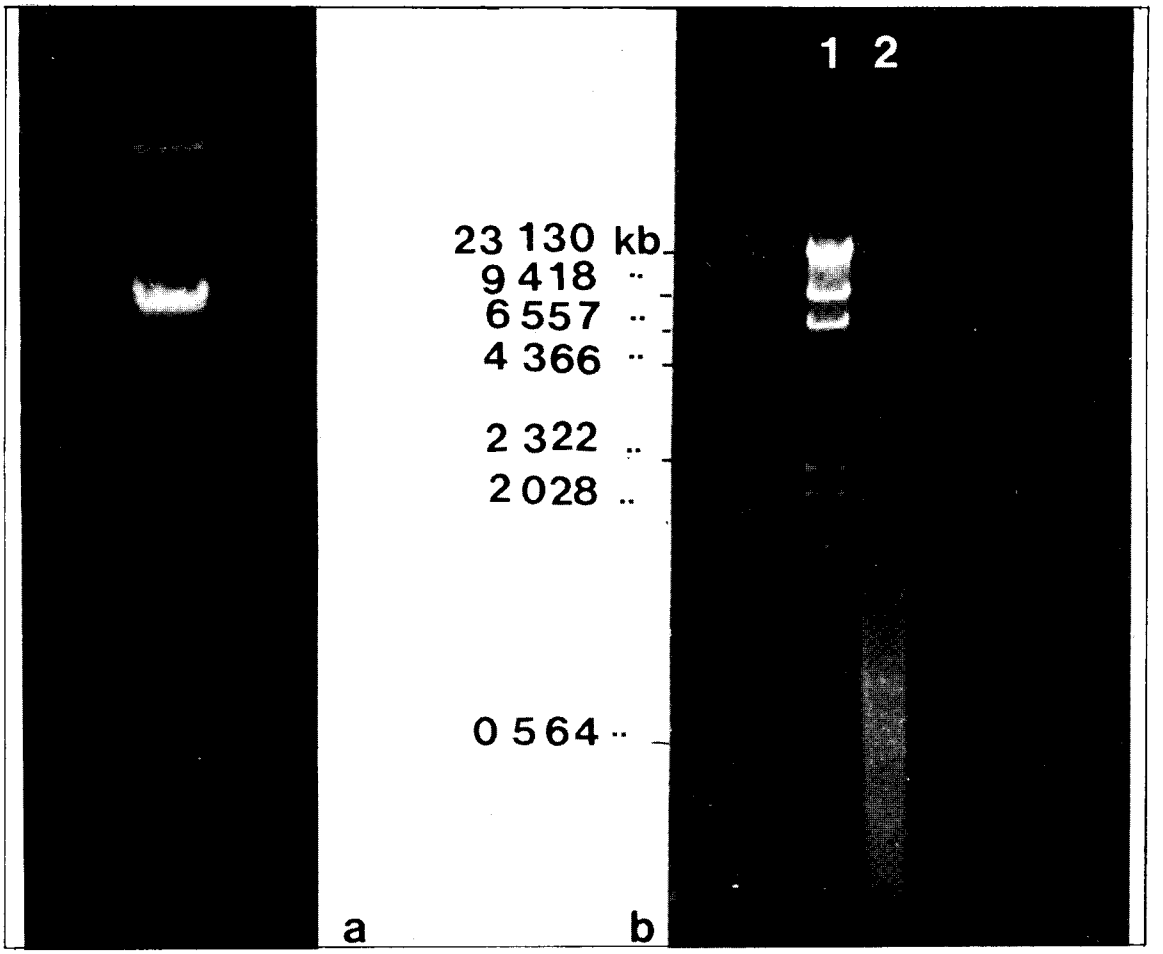

Fig. 5. a) Genomic *Myocastor coypus* DNA isolated from specimen N° 820. b) Lane 1 Lambda DNA marker digested with Hind I. Lane 2 *Myocastor coypus* DNA (820) digested with Alu I.

a) ADN genómico de *Myocastor coypus* extraido del especimen N° 820. b) Carril 1 Marcador de tamaño, ADN de lambda digerido con Hind I Carril 2 *Myocastor coypus* ADN (N° 820) digerido con Alu I.

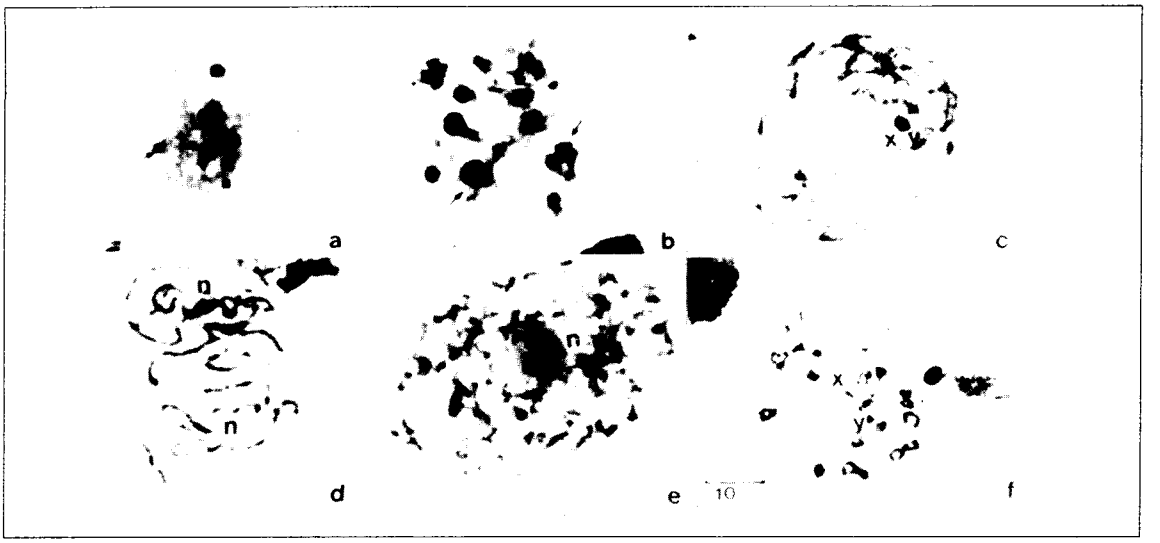

Fig. 6. Meiosis stages: a) early prophase. The arrow indicates heterochromatic block. b) pachytene, arrows show heterochromatic blocks. c) middle pachytene. d) final pachytene. e) diffuse stage. f) diplotene. XY = sexual bivalent. n= nucleolus.

Estadios meióticos: a) profase temprana. La flecha indica bloque heterocromático. b) paquiteno, las flechas muestran los bloques heterocromáticos. c) paquiteno medio. d) paquiteno final. e) estadio difuso. f) diploteno. XY =bivalente sexual. n = nucléolo

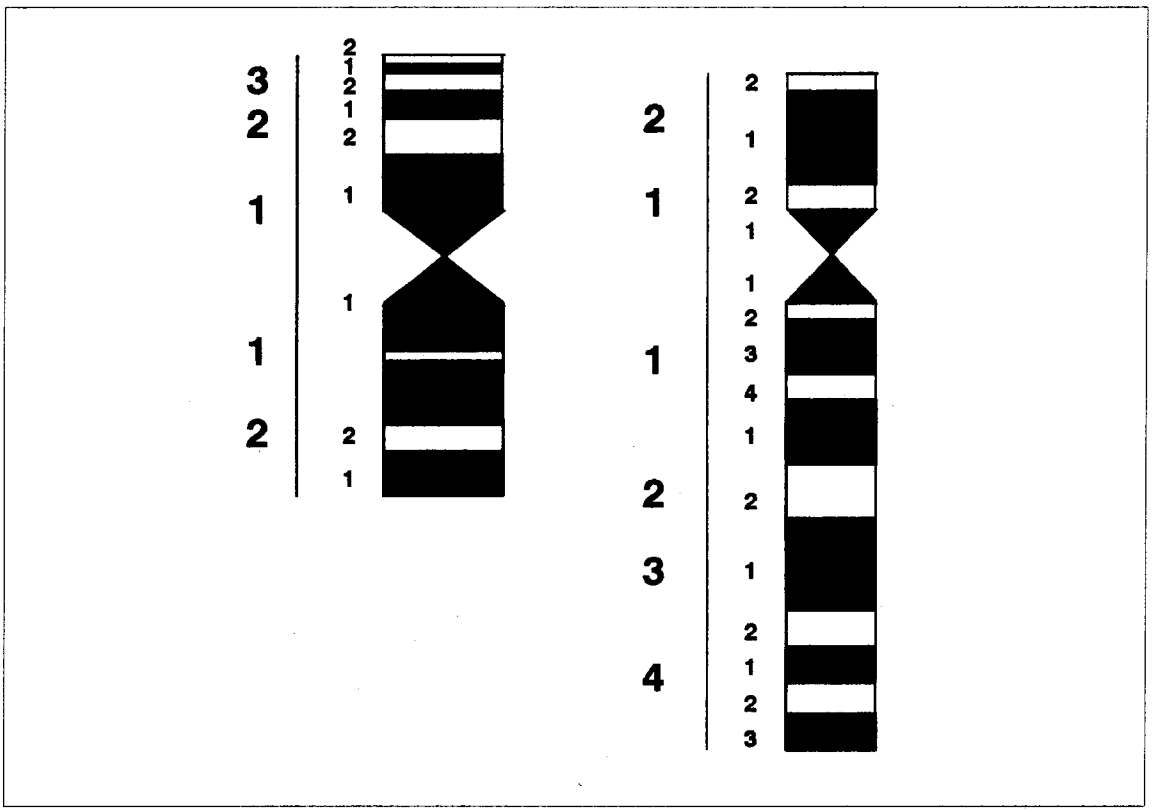

Fig. 7. Idiogram showing G-banding pattern of X chromosome. X-chromosome of Uruguayan feral nutria (left) and (right), X chromosome of Azerdbaishan nutria.

Idiograma que muestra el patrón de bandeo G del cromosoma X. Cromosoma X de nutrias silvestres uruguayas (izquierda) y a la (derecha) cromosoma X de nutrias de Azerdbaishan.

subspecies introduced in Abzerdbaishan, nutria subspecific or intraspecific heterochromatin variation may explain the differences reported here.

Intraspecific polymorphisms in the size of sex chromosomes due addition of heterochromatin has been observed in other rodent species (Baverstock et al. 1977, Brum-Zorrilla et al. 1988). If we interpret these variations as a restricted chromosomal evolution phenomenon, we must take into account that this not necessarily should be related with morphological changes, such as the quality and coloration of skin; speciation processes may not yet be involved (Aguilera 1980, Reig 1989). In the "cognac hybrid" individuals, where chromosome and morphological changes had appeared, reproductive isolation seems to have not occurred.

If we suppose that the nutrias studied by Kasumov et al. (1976) have been carried out from Uruguay to Azerdbaishan for fur farming, we can postulate that in nearly fifty years of captive breeding, the artificial selection favoring quality and skin coloration could have lead to secondary chromosome rearrangements. It is most probable that the initial population exported had small size, and that high levels of inbreeding must have occurred, eventually producing fixation of random chromosome rearrangements. This process is known as "founder effect" (Templeton 1980, Reig 1989). To test whether such chromosome evolution has played an important role in divergence and perhaps to speciation, it would be necessary to carry out crossings between these two allopatric populations and to analyze the meiotic processes in the eventual hybrid.

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LITERATURE CITED

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