

Geographical distribution of sperm forms in the genus *Ctenomys* (Rodentia-Octodontidae)

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ABSTRACT

The argentophilic patterns of sperm in five species of the genus *Ctenomys*: *C. australis*, *C. sp.*, *C. minutus*, *C. flamarioni*, and *C. torquatus* are described. Sperm of *C. torquatus*, *C. sp.*, and *C. minutus* show symmetric, while these of *C. flamarioni* and *C. australis* exhibit simple-asymmetric forms. All the five species reveal different patterns of sperm under silver staining. This report discusses the geographic distribution of the sperm forms of species of the genus as well as evolutionary relationships.

INTRODUCTION

The polytypic genus *Ctenomys* is distributed between 15° and 55° South in the Patagonian sub-region at the southern portion of the Neotropical region (Cabrera, 1961; Pearson *et al.*, 1968). Three types of spermatozoa have been described in 25 species of this genus (Feito and Gallardo, 1976; Vitullo *et al.*, 1988 and Vittulo and Cook, 1991): symmetric, simple-asymmetric, and complex-asymmetric. The symmetric form presents normal head and tail, the simple-asymmetric a prolongation parallel to the tail but shorter than it, while the complex-asymmetric shows two prolongations located on each side of the tail. This structure was initially named postacrossomic process by Feito and Gallardo (1976). Later, using electron microscopy, Feito and Barros (1982) changed this name to nuclear caudal extension, since it is a prolongation of the nucleus. The geographic distribution of the species according to these three sperm forms was presented by Feito and

Gallardo (1982) and Vitullo *et al.* (1988): the asymmetric forms occur in the south and the symmetric in the Northern and Eastern regions of the Patagonian sub-region.

MATERIAL AND METHODS

In Brazil, *C. torquatus* Lichtenstein (1830) was collected from Laranjal, Pelotas (n=2); *C. minutus* Nehring, 1887 (n=3) from Torres; *C. flamarioni*, Travi, 1981 (n=3) from Cassino and Tramandaí, and *Ctenomys sp.* (n=2) from Lami, Porto Alegre. *C. australis* Rusconi, 1934 (n=2) was collected from Necochea, Argentina.

The spermatozoa were obtained from epididymides of *C. sp.*, *C. minutus*, and *C. flamarioni*, and also from testicles of *C. torquatus*, *C. flamarioni* and *C. australis*. The materials were fixed in methyl-alcohol and acetic acid (3:1). The suspensions were silver-stained according to Howell and Black's (1980) technique.

RESULTS

Two types of spermatozoa were observed in the five species studied (Figure 1). *C. minutus* (Figure 1a), *C. sp.* (Figure 1b), and *C. torquatus* (Figure 1c) all showed the same general symmetric form. Both *C. minutus* and *C. sp.* had a very dark band in the intermediate portion of the head. Moreover, *C. minutus* presented two distinguishable dots in the posterior region of the head.

C. flamarioni (Figure 1d) and *C. australis* (Figure 1e) present asymmetric forms. Silver staining did not mark the acrosomic region while the nucleus was sharply stained, as well as the nuclear caudal extension. In *C. flamarioni*, the midpiece was less marked than the tail, while the inverse pattern occurred in *C. australis*.

Observing the geographic distribution of 25 species of *Ctenomys* with sperm type data (Figure 2), two groups were identified: the first group has symmetric form sperm and ranges from 35° S to 15° S in a South-North direction; the second shows simple-asymmetric forms and is distributed from 55° to 25° S. The two groups share a zone between 35° S and 25° S at the eastern portion of the geographic distribution.

Table I shows a list of the 25 species in decreasing order of diploid number. Eleven species presented

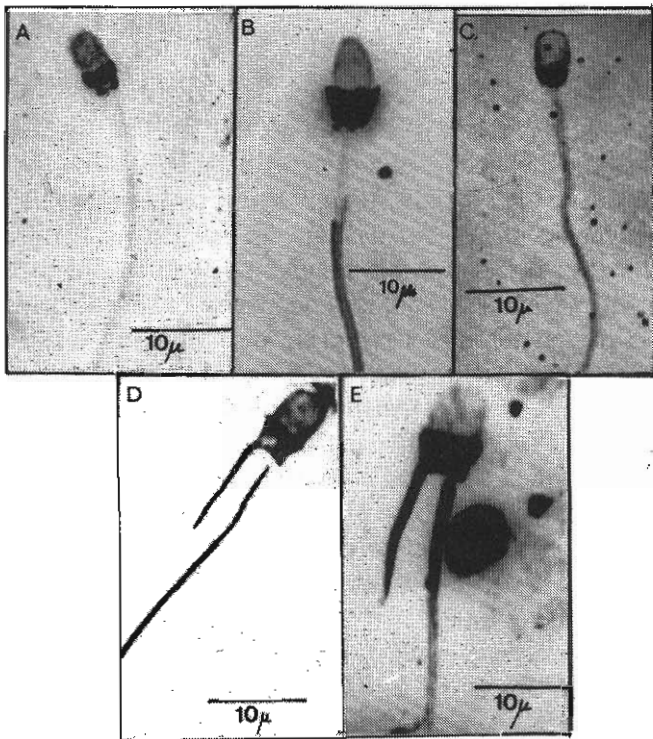


Figure 1 - Symmetric form of sperm in *Ctenomys minutus* (a), *C. sp.* (b), and *C. torquatus* (c). Simple-asymmetric forms of *C. flamarioni* (d) and *C. australis* (e). The bar indicates 10 microns.

simple-asymmetric sperms, 13 had symmetric forms and only one had a complex-asymmetric type. The symmetric form is distributed among $2n=10$, 26, and 36 to 46. Of the six species with diploid number higher than 48 five presented the symmetric form, while one showed a complex-asymmetric sperm. The modal diploid number in this genus is $2n=48$, found in eight species. In this group, six species had simple-asymmetric sperms and only two (*C. talarun* and *C. conoveri*) presented a symmetric form.

DISCUSSION

The spermiatic forms of 22 species of *Ctenomys* have been studied using stained basic fuchsin only (Feito and Gallardo, 1976; Gallardo, 1979; Feito and Gallardo, 1982; Altuna *et al.*, 1986; Vitullo *et al.*, 1988;

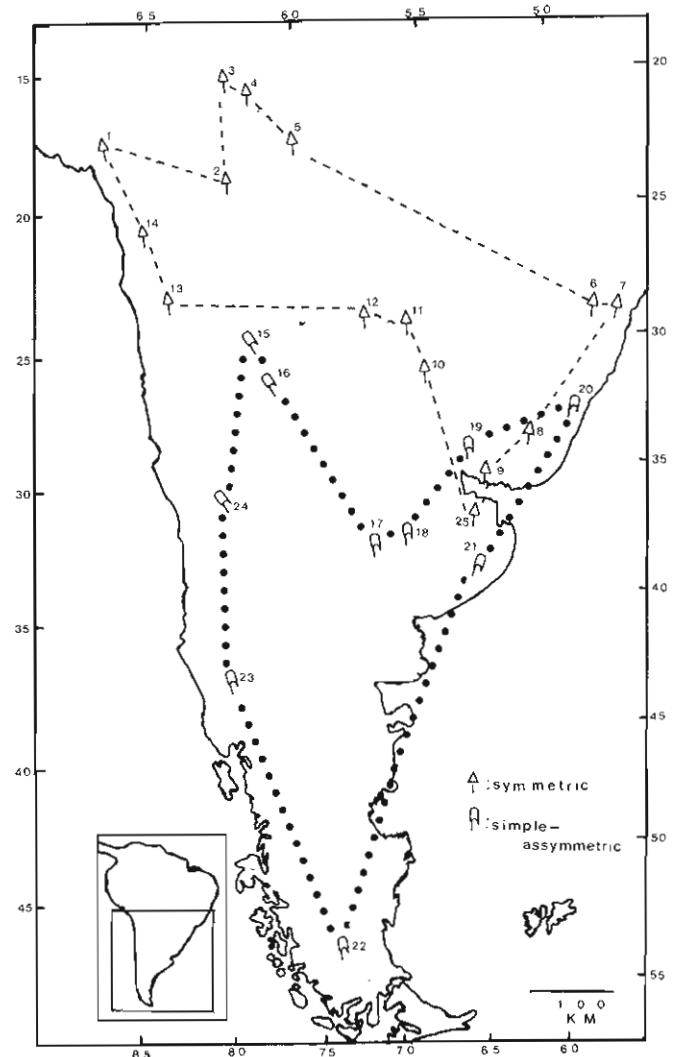


Figure 2 - Geographic distribution in South America of *Ctenomys* species with symmetric and asymmetric forms.

Table I - Diploid number and sperm form of 25 *Ctenomys* species.

Species	Diploid number	Spermatozoa form	No. in Figure 1
<i>C. steinbacki</i>	10	Symmetric ⁵	4
<i>C. opimus</i>	26	Symmetric ²	14
<i>C. fulvus</i>	26	Symmetric ²	1
<i>C. maulinus</i>	26	Simple-asymmetric ²	23
<i>C. tucumanus</i>	28	Simple-asymmetric ²	16
<i>C. magelanicus</i>	34	Simple-asymmetric ²	22
<i>C. boliviensis</i>	36-46	Symmetric ⁵	3
<i>C. latro</i>	42	Simple-asymmetric ²	15
<i>C. minutus</i>	42-50	Symmetric ⁶	7
<i>C. torquatus</i>	44-46	Symmetric ⁶	8
<i>C. argentinus</i>	44	Symmetric ⁴	12
<i>C. flamarioni</i>	48	Simple-asymmetric ⁶	20
<i>C. azarae</i>	48	Simple-asymmetric ²	17
<i>C. australis</i>	48	Simple-asymmetric ^{4,6}	21
<i>C. porteousi</i>	48	Simple-asymmetric ⁴	18
<i>C. rionegrensis</i>	48	Simple-asymmetric ³	19
<i>C. mendocinus</i>	48	Simple-asymmetric ⁴	24
<i>C. talarum</i>	48	Symmetric ¹	9
<i>C. conoveri</i>	48	Symmetric ⁴	5
<i>C. perrensis</i>	50	Symmetric ⁴	11
<i>C. yolandae</i>	50	Complex-asymmetric ⁴	-
<i>C. sp.</i>	54-58	Symmetric ⁶	6
<i>C. pearsoni</i>	56	Symmetric ³	25
<i>C. lewisi</i>	56	Symmetric ⁵	13
<i>C. dorbignyi</i>	70	Symmetric ⁴	10

The exponents indicate the references: 1, Jones (1975); 2, Feito and Gallardo (1982); 3, Altuna *et al.* (1986); 4, Vitullo *et al.* (1988); 5, Vitullo and Cook (1991) and 6, this study.

Vitullo and Cook, 1991). *C. maulinus*, which presents a simple- asymmetric form, was also studied by using the electron microscope (Feito and Barros, 1982).

The species with asymmetric forms, *C. flamarioni* and *C. australis*, occur along the sandy coastal region in Brazil and Argentina, respectively, while those with symmetric configuration (*C. minutus*, *C. sp.* and *C. torquatus*) inhabit the interior (Figure 2). The present results extend the distribution of the species with asymmetric forms further north than the Patagonian region (see *C. flamarioni*, Figure 2, Table I) and that of the species with symmetric forms up to the eastern Atlantic coast (see *C. sp.*, *C. minutus* and *C. torquatus*, Figure 2, Table I). The extension of the distribution of the asymmetric forms, due to *C. flamarioni*, emphasizes the area shared with the symmetric forms. *C. flamarioni* differentiated from the Argentinian species through migration and subsequent isolation (Freitas, 1994). This

is strengthened by the fact that this species inhabits the most recent marine and eolic deposits of Rio Grande do Sul (Villwock *et al.*, 1986; Freitas, 1994). The north-eastern migration of *C. flamarioni* could have occurred when the Coastal Plain was 100 km wider than at present and the Prata river was not such an important geographical barrier (Correa *et al.*, 1992).

Many factors are likely responsible for the speciation process of *Ctenomys* in South America: the environmental changes in the past (Webb, 1977), several different karyotype numbers (about 20), and the three different types of sperm, which are a reproduction barrier (Vitullo *et al.*, 1988). An association between karyotypic characteristics and sperm morphology was observed in a group of species named the *mendocinus*-group. This group was initially formed by *C. azarae*, *C. australis*, *C. porteousi*, and *C. mendocinus* (Massarini *et al.*, 1991). The species of the *mendocinus*-group are morphologically similar (Massarini *et al.*, 1986), present simple- asymmetric spermatozoa (Vitullo *et al.*, 1988), the same karyotype, with $2n=48$, and a large amount of heterochromatin (Massarini *et al.*, 1991). Recently Rossi *et al.* (1993) found a large amount of major satellite DNA named Repetitive Pvu II *Ctenomys* Sequences (RPCS) in Argentinian species of this group. Recent data included two more species in the *mendocinus*-group: *C. flamarioni*, found in Brazil, and *C. rionegrensis*, from Uruguay (Freitas, 1994). These findings suggest that this genus has several species lineages, with different ancestors; in the *mendocinus*-group, for example, *C. mendocinus* is the ancestor, as suggested by Massarini *et al.* (1991).

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RESUMO

Foram estudados os padrões de coloração com prata em espermatozóides de cinco espécies do gênero *Ctenomys*: *C. australis*, *C. sp.*, *C. minutus*, *C. flamarioni* e *C. torquatus*. *C. torquatus*, *C. sp.* e *C. minutus* possuem espermatozóide simétrico,

enquanto *C. flamarioni* e *C. australis* apresentam a forma simples-assimétrica. Todas as cinco espécies podem ser distingüidas pelo padrão de coloração de seus espermatozoides. O trabalho discute também a distribuição geográfica das formas de espermatozoides das outras espécies do gênero e suas relações evolutivas.

REFERENCES

- Altuna, C.A., Novello, A.F. and Lessa, E.P.** (1986). Nota sobre la morfología espermatocítica de *Ctenomys rionegrensis* (Rodentia-Octodontidae) del Uruguay. *Brenesia* 24: 397-401.
- Cabrera, A.** (1961). Catalogo de los mamíferos de America del Sur. *Rev. Mus. Ar. Cs. Nat. Zool.* 4: 309-732.
- Correa, I.C.S., Baitelli, R., Ketzer, J.M. and Martins, R.** (1992). Translação horizontal e vertical do nível do mar sobre a plataforma continental do Rio Grande do Sul nos últimos 17.500 anos bp. *Anais III Congresso ABEQUA*. Belo Horizonte, 1992, pp. 225-240.
- Feito, R. and Barros, C.** (1982). Ultrastructure of the head of *Ctenomys maulinus* spermatozoon with special reference to the nucleus. *Gamete Res.* 5: 317-321.
- Feito, R. and Gallardo, M.** (1976). Notes on the sperm morphology of *Ctenomys maulinus* (Rodentia-Octodontidae). *Experientia* 32: 743-735.
- Feito, R. and Gallardo, M.** (1982). Sperm morphology of Chilean species of *Ctenomys* (Octodontidae). *J. Mamm.* 63: 658-661.
- Freitas, T.R.O.** (1994). Geographical variation of heterochromatin in *Ctenomys flamarioni* (Rodentia-Octodontidae) and its cytogenetic relationships with other species of the genus. *Cytogenet. Cell Genetics* 67: 193-198.
- Gallardo, M.** (1979). Las especies chilenas de *Ctenomys* (Rodentia-Octodontidae). I. Estabilidad cariotípica. *Arch. Biol. Med. Exper.* 12: 71-82.
- Howell, W.M. and Black, D.A.** (1980). Controlled silver-staining of nucleolus organizer region with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.
- Jones, R.C.** (1975). The ultrastructure of spermatozoa from hystriocomorph rodents. In: *The Functional anatomy of the Spermatozoon* (Afzelius, B., ed.). Pergamon Press, pp. 251-258.
- Massarini, A., Coccia, M., Barros, M.A., Torno, A., Roig, V. and Reig, O.A.** (1986). Relaciones cromosómicas y morfométricas en *Ctenomys* de Argentina (Rodentia-Octodontidae). Programa y Resúmenes, Segundas Jornadas Argentinas de Mastozoología. Buenos Aires, 10-12 de Noviembre: 33.
- Massarini, A., Barros, M.A., Ortells, M.O. and Reig, O.A.** (1991). Evolutionary biology of fossorial Ctenomyine rodents (Caviomorph: Octodontidae). I. Chromosomal polymorphism and small karyotypic differentiation in Central Argentinian populations of tuco-tucos. *Genetica* 83: 131-144.
- Pearson, O., Binsztein, N., Boiry, L., Busch, C., Dipace, M., Gallopin, G., Penchszadeh, P. and Piantanida, M.** (1968). Estructura, social distribución espacial y comparación por edades de una población de tuco-tucos (*Ctenomys talarum*). *Investines. Zool. Chil.* 13: 47-80.
- Rossi, M.S., Reig, O.A. and Zorzópulos, J.** (1993). A major satellite DNA from South American rodents of the genus *Ctenomys*. *Z. Säugetierkund* 58: 244-251.
- Villwock, J.A., Tomazelli, L.J., Loss, E.L., Dehnhardt, E.A., Horn Filho, N.O., Bachi, F.A. and Dehnhardt, B.A.** (1986). Geology of the Rio Grande do Sul coastal province. In: *Quaternary of South America and Antarctic Peninsula* (Rabassa, J.A.A., ed.). Balkema, Rotterdam.
- Vitullo, A.D. and Cook, J.A.** (1991). The role of sperm morphology in the evolution of tuco-tuco, *Ctenomys* (Rodentia-Ctenomyidae): confirmation of results from Bolivian species. *Z. Säugetierkund* 56: 359-364.
- Vitullo, A.D., Roldan, E.R.S. and Merani, M.S.** (1988). On the morphology of spermatozoa of tuco-tucos, *Ctenomys* (Rodentia): New data and its implications for the evolution of the genus. *J. Zool. Land.* 215: 675-683.
- Webb, S.B.** (1977). A history of savanna vertebrates in the New World. Parts I. *Ann. Rev. Ecol. Syst.* 8: 355-380.

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