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The role of sperm morphology in the evolution of Tuco-Tucos, *Ctenomys* (Rodentia, Ctenomyidae): confirmation of results from Bolivian species

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Abstract

Studied sperm morphology and dimensions in five species of caviomorph rodents of the genus *Ctenomys: C. boliviensis, C. frater, C. lewisi, C. opimus* and *C. steinbachi*, collected in Bolivia. All species showed symmetric spermatozoa with paddle-like heads. Dimensions of main sperm components did not vary greatly among species, with mean head and tail lengths of approximately 6.35 and 46 μm, respectively. General morphological characteristics, linear dimensions, and relative sizes of the spermatozoa of these five species were similar to those previously reported for other species of *Ctenomys* that also bear symmetric spermatozoa. The sperm type found in Bolivian *Ctenomys* is in concordance with previous observations which pointed out that symmetric sperm-bearing species inhabit the northern area of the geographic range of the genus, while southern species are characterized by asymmetric spermatozoa. The morphological characteristics of spermatozoa from the Bolivian *Ctenomys*, in conjunction with newly available information, support a previous hypothesis on the role of sperm variants in the reproductive isolation and speciation in this genus.

Introduction

Species of the genus *Ctenomys* Blainville, 1826 (Rodentia, Ctenomyidae) are some of the most specialized and morphologically diverse rodents in Souh America. These fossorial rodents occur in most major habitats south of 10° S latitude (Mares and Ojeda 1982), and since the beginning of the Pleistocene they have undergone an explosive radiation (Reig 1989). They exhibit one of the highest karyotypic diversifications known for mammals, with diploid numbers ranging from 10 to 70 chromosomes (Anderson et al. 1987; Ortells et al. 1990). More than 50 species have been described for the genus (Reig et al. 1990).

Ctenomyids also show many unusual morphological characters, among which the variation in their sperm morphology represents, as far as we know, a unique feature among mammals. Three morphological sperm types are currently recognized for the genus: symmetric, simple-asymmetric, and complex-asymmetric. The nuclear area of simple-asymmetric spermatozoa extends beyond the base of the head forming a "nuclear caudal extension" which runs parallel to the flagellum (see Figure). This peculiar sperm cell was first described for *C. maulinus* from southern Chile (Feito and Gallardo 1976), and later on found in many other species inhabiting the southern area of the geographic range of the genus (Feito and Gallardo 1982; Vitullo et al. 1988). The complex-asymmetric spermatozoon shows two nuclear caudal extensions, which has so far been described for a single species, *C. yolandae* (Vitullo and Roldán 1986a). Symmetric spermatozoa lack the nuclear caudal extension (see Figure) and characterize the species inhabiting the northern area of the geographic range of the genus. The association between sperm morphology and geographic distribution in *Ctenomys* was first noticed by Feito and

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Gallardo (1982) and confirmed in further studies (Altuna et al. 1985; Vitullo et al. 1988).

The clear-cut distribution of two groups of species bearing a given sperm type, and the low interspecific variability in morphological patterns and dimensions of sperm cells within each group have led to the supposition that sperm variants appeared at an early stage in evolution of *Ctenomys* and played a major role in the first steps of speciation by promoting the separation of two lineages, which underwent, independently, an explosive speciation related to high rates of chromosomal repatterning (Vitullo et al. 1988).

In this study, we report the analysis of spermatozoa from five species of *Ctenomys* collected in Bolivia, and discuss these results in the light of newly available information on other research fields, which support the hypothesis previously advanced on the role of sperm morphology in the evolution of *Ctenomys*.

Materials and methods

Morphological characteristics of spermatozoa were studied in five species of *Ctenomys* distributed in Bolivia. Species and trapping localities were as follows: *C. boliviensis* (N = 5), Department of Santa Cruz; *C. frater* (N = 2), Tarija, 8 km E Rancho Tambo; *C. lewisi* (N = 2), Tarija, 1 km E Iscayachi; *C. opimus*, Potosí, Laguna Colorado (N = 1), and Oruro, 7 km S, 3 km E Cruce Ventilla (N = 1), and Oruro, Huancaroma (N = 3); and *C. steinbachi* (N = 5), Department of Santa Cruz. Voucher specimens were preserved at the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico, and the American Museum of Natural History, New York, USA.

Spermatozoa were prepared as previously described (Vitullo et al. 1988). In brief, cauda epididimydes fixed in glutaraldehyde were minced with sharp scissors. Sperm suspensions were smeared onto cleaned slides, air-dried, rinsed in tap water, and stained with Giemsa according to Watson (1975).

Sperm cells were examined under bright field microscopy and measured with an ocular micrometer at a magnification of 630×. The following sperm components were measured and expressed as mean values: head length, head width, midpiece length, tail length and total length (head plus flagellum).

Results and discussion

The spermatozoa of *C. boliviensis, C. frater, C. lewisi, C. opimus* and *C. steinbachi* were found to belong to the symmetric type, lacking the nuclear caudal extension. The cells showed paddle-like, caudally truncated heads with tails inserting centrally into the base of the head (Fig. 1a). Alterations to this pattern were not found among individuals or between species. The sperm type found in Bolivian species agrees with previous observations that the species bearing symmetric spermatozoa are distributed in the northern geographic range of the genus (Feito and Gallardo 1982; Vitullo et al. 1988).

Sperm dimensions (Tab. 1) did not vary among the five species studied, with mean head and tail lengths of approximately 6.35 and 46.0 μm, respectively. Linear dimensions of main sperm components were found to be very close to those previously reported for other species of *Ctenomys* with the same sperm type (Vitullo et al. 1988). Of the five species studied, previous information on sperm morphology has only been reported for *C. opimus* from Chile (Feito and Gallardo 1982). Differences were not found in sperm dimensions between Chilean and Bolivian *C. opimus*.

Although sperm morphotypes are strikingly different (see Figure), and symmetric spermatozoa are shorter than the asymmetric forms, a very low interspecific morphometric variability has been shown to exist within each group, and relative sizes of head, midpiece, and tail were found to be invariable in the three sperm types (Vitullo et al. 1988). Results on Bolivian *Ctenomys* confirm these observations. Sperm dimensions do not show interspecific variability (Tab. 1), and relative sizes of the main sperm components are within the range of other species bearing the symmetric sperm type (Tab. 2).

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(a) Symmetric spermatozoa from *C. boliviensis*. The same sperm type was found in *C. frater, C. lewisi, C. opimus* and *C. steinbachi*. Currently recognized sperm types in *Ctenomys:* (b) symmetric, (c) simple-asymmetric, (d) complex-asymmetric. Arrowheads show the nuclear caudal extension(s) characterizing asymmetric spermatozoa. (Bar = 10 μm)

No association between a given sperm type and low or high chromosomal numbers has been previously found (Vitullo et al. 1988). Diploid numbers in Bolivian *Ctenomys* range from 2n = 10 in *C. steinbachi* to 2n = 56 in *C. lewisii* (Anderson et al. 1987; Cook et al. 1990, confirming the lack of association between chromosomal variation and sperm types. In addition, alterations either in morphology or linear dimensions of symmetric spermatozoa among individuals of *C. boliviensis* (a chromosomally polymorphic species, 2n = 36–46, cf. Anderson et al. 1987) were not found.

Table 1. **Sperm dimensions (μm) in Bolivian *Ctenomys***

Species	Head length	Head width	Midpiece length	Tail length	Total length
C. boliviensis	6.35 ± 0.51	3.21 ± 0.47	6.18 ± 0.53	46.04 ± 1.04	52.39 ± 1.18
C. frater	6.35 ± 0.57	3.12 ± 0.49	6.12 ± 0.53	45.91 ± 1.26	52.24 ± 1.44
C. lewisi	6.34 ± 0.56	3.16 ± 0.51	6.26 ± 0.56	46.17 ± 1.27	52.46 ± 1.34
C. opimus	6.34 ± 0.51	3.20 ± 0.48	6.10 ± 0.54	46.30 ± 1.02	52.65 ± 1.24
C. steinbachi	6.33 ± 0.52	2.85 ± 0.65	5.97 ± 0.56	46.03 ± 1.26	52.32 ± 1.24

Due to the clear-cut distribution of species of *Ctenomys* bearing symmetric and asymmetric spermatozoa, the interspecific constancy of both morphology and dimensions of the sperm types, and the lack of association of sperm types with chromosomal characteristics, Vitullo et al. (1988) proposed the following scenario for the evolution of

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Table 2. **Mean relative sizes of sperm components in Bolivian *Ctenomys***

Species	Percentage of mean total length		
	Head	Midpiece	Tail
C. boliviensis	12.12	11.80	87.88
C. frater	12.15	11.71	87.88
C. lewisi	12.08	11.94	88.02
C. opimus	12.04	11.58	87.94
C. steinbachi	12.10	11.41	87.97
Symmetric Sperm[1]	12.31 (12.02–12.81)	11.50 (11.41–11.76)	87.47 (86.89–87.98)

[1] Mean and range of relative size for symmetric sperm type taken from Vitullo et al. (1988).

Ctenomys: 1. sperm variants appeared at an early stage in evolution, 2. the early appearance of sperm variants played a major role in reproductive isolation during early speciation prior to geographical dispersal, and 3. chromosomal events leading to high speciation in the genus occurred after the original separation of two groups of species as a consequence of the reproductive isolation promoted by the early appearance of sperm variants. Symmetric spermatozoa were considered to represent the ancestral or plesiomorphic condition for *Ctenomys* because they are very close to those of other Caviomorpha, and to the eutherian ancestral type (Vitullo and Roldán 1986b; Vitullo et al. 1988; Roldán et al. 1991). On the other hand, the longer asymmetric sperm was considered to be the apomorphic condition. The major predictions of our previous hypothesis are supported by the present results as discussed above, and by newly available information form other research fields.

Recently, Rossi et al. (1990) have isolated a highly repetitive DNA sequence of about 370 kbp from *C. porteousi*, a species bearing simple asymmetric spermatozoa. Heterologous hybridization of this sequence with DNA from many other species of *Ctenomys* has shown that the sequence is widespread in the genus. Nevertheless, hybridization patterns are species-specific and, significantly, *C. opimus* displays a weak hybridization signal only under permissive conditions (Rossi et al. 1990). The low intensity of the signal found in *C. opimus* is quite similar to that of other Octodontidae, suggesting that the expansion of the sequence occurred in species with asymmetric spermatozoa after the divergence of this phylogenetic lineage from species with symmetric sperm cells. These results support our hypothesis that a symmetric spermatozoon represents the ancestral condition for the genus. In addition, a detailed analysis of the trends in evolution of sperm shape and size in rodents pointed out that simple symmetric spermatozoa represent the ancestral type not only for Caviomorpha but for Muridae and Cricetidae, and eutherian mammals in general (Roldán et al. 1991).

Rossi et al. (1990) suggested that at least two genomic lines may have evolved in *Ctenomys* with regard to the patterns and the degree of amplification of the highly repetitive DNA sequence. While not mentioned by the authors these lines are characterized by a different sperm type, further supporting the idea of an early appearance of sperm morphotypes in the evolution of *Ctenomys* (Vitullo et al. 1988).

Based on chromosome affinities, it was recently shown that the different species of *Ctenomys* may be grouped in five well-defined clusters which probably represent evolutionary units (Ortells 1990). Every cluster is characterized by a sperm morphotype, with minor exceptions (Ortells 1990; Reig pers. comm.), reinforcing the idea that explosive chromosomal evolution took place after the original separation of two groups of species as a consequence of reproductive isolation promoted by the early appearance of sperm variants (Vitullo et al. 1988).

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Finally, it is important to note that the hypothesis involving sperm morphotypes in the evolution of *Ctenomys* represents a novelty for speciation in rodents. The proposal that sperm variants triggered a mechanism of strong reproductive isolation which determined the separation of two lineages, implies as a corollary the necessity of some kind of gametic incompatibility. Significantly, it was argued that a main function of the nuclear caudal extension is to increase the contact area between sperm and egg at fertilization (Feito and Barros 1982). Studies on in vitro and in vivo cross fertilizations may shed light on the cellular and molecular basis of such a mechanism of gametic incompatibility.

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Zusammenfassung

Die Rolle von Spermienmorphologie in der Evolution der Kammratten, Ctenomys (Rodentia, Ctenomyidae)

Untersucht wurde die Spermienmorphologie von 5 caviomorphen Rodentia-Arten der Gattung *Ctenomys* aus Bolivien: *C. boliviensis, C. frater, C. lewisi, C. opimus* und *C. steinbachi*. Alle Arten verfügen über relativ einheitlich gebaute symmetrische Spermien mit paddelförmigen Köpfen. Für morphometrische Daten ergaben sich bei durchschnittlicher Länge der Kopf- und Schwanzregion von 6,35 bzw. 46 µm nur geringe Unterschiede im zwischenartlichen Vergleich. In morphologischen Kennzeichen und Maßen ähneln die Spermien dieser fünf Arten ferner anderen *Ctenomys*-Vertretern mit symmetrischen Spermien. Damit wird erneut bestätigt, daß Kammrattenarten aus dem nördlichen Teil der Gesamtverbreitung symmetrisch gebaute Spermien haben. Für Arten aus dem südlichen Teil sind hingegen asymmetrische Spermien kennzeichnend. In Übereinstimmung mit anderen Befunden werden diese Unterschiede aus evolutionsbiologischer Sicht diskutiert.

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