Z. Säugetierkunde 58 (1993) 244–251

ISSN 0044-3468

A major satellite DNA from the South American rodents of the genus *Ctenomys*

Quantitative and qualitative differences in species with different geographic distribution

By María Susana Rossi, O. A. Reig, and J. Zorzópulos

Grupo de Investigaciones en Biología Evolutiva, Departamento de Ciencias Biológicas and Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Unversidad de Buenos Aires, Buenos Aires, Argentina

Receipt of Ms. 31. 8. 1992
Acceptance of Ms. 7. 2. 1993

Abstract

The quantity and quality of the major satellite DNA present in South American rodents of the genus *Ctenomys* were analyzed in various species of the genus. The quantity was analyzed by genomic DNA hybridization with sequences of this major satellite DNA, in a dot-blot experiment. The quality was analyzed by hybridization of genomic DNA digested with restriction endonucleases, with sequences of this major satellite DNA, as well, in Southern-blot experimens. Quantitative and qualitative analyses were correlated with the geographic distribution of these species.

According to the dot-blot analysis performed under high-stringency conditions, *Ctenomys* species were classified in three groups containing none, low, and high amounts of this *Ctenomys* satelite DNA, respectively. The first group comprises only *C. opimus*. The second group comprises *C.* cf. *perrensi, C. tuconax,* and *C. occultus*. The last group includes *C. mendocinus, C. porteousi, C. azarae, C. australis,* and *C. talarum*. *C. latro* appears closely related to this last group but it shows some differences on its own. According to the quality of satellite *Ctenomys* DNA, *Ctenomys* species belonging to the last group are closely related, and all but one have a distinctive geographic distribution south of the 30°S latitude. In contrast, species of the second group seem to be more distantly related and all were found north of the 30°S latitude, as is also the case for *C. opimus*.

Introduction

A significant amount of the eucaryotic genome is composed of highly repetitive DNA sequences which are arranged tandemly over long stretches of DNA (Burtlag 1980). Common to most satellites are minor sequence variations between different repeat units, which in several cases have been shown to be clustered in segments of the satellite DNA (Hörz and Zachau 1977; Cooke and McKay 1978; Beauchamp et al. 1979). It is believed that the generation of these segments has involved independent amplification steps of single repeat units through a rolling-circle replication mechanism (Walsh 1987; Rossi et al. 1990). This particular replication mode may explain the significant variations observed in the satellite restriction patterns between animal species without extensive divergence in the overall repetitive sequence itself (Arnason 1982; Widegren et al. 1985; Bogenberger et al. 1987; Arnason et al. 1988; Rossi et al. 1990). Furthermore, random differences in the length of the segments generated during the satellite amplification may also result in large differences in the total amount of repetitive DNA of different species. Therefore, genomic DNA hybridization analysis before and after DNA digestion with restriction endonucleases, using as probe satellite DNA sequences (dot-blot and Southern-blot, respectively) to evaluate quantity and quality of satellite DNA, should result in valuable information on common ancestry of related groups.

Recently, a major satellite from the South American rodents of the genus *Ctenomys*, named RPCS (repetitive PvuII *Ctenomys* sequence), was described (Rossi et al. 1990). In the present study, the quantity and quality of this satellite DNA were investigated in several *Ctenomys* species by using both, dot-blot and Southern-blot analysis, respectively.

Material and methods

Specimens

We studied the DNA of an individual from each of 10 species of *Ctenomys*. All specimens were collected in the wild employing live traps. Skin and skull voucher specimens of all of them were deposited in the Collection of Mammals of the Municipal Museum of Natural History of Mar del Plata, Argentina. Geographic localities of the collected specimens, all from Argentina, are as follows (t.l. = type locality): *Ctenomys azarae* (Luan Toro, La Pampa), *Ctenomys australis* (Necochea, Buenos Aires, t.l.), *Ctenomys latro* (Tapia, Tucumán, t.l.), *Ctenomys talarum recessus* (Necochea, Buenos Aires), *Ctenomys tuconax* (El Infiernillo, Tucumán), *Ctenomys occultus* (Monteagudo, Tucumán, t.l.), *Ctenomys* cf. *perrensi* (2n = 54) (Saladas, Corrientes), *Ctenomys porteousi* (Bonifacio, Buenos Aires, t.l.), *Ctenomys mendocinus* (Tupungato, Mendoza), and *C. opimus* (Tres Cruces, Jujuy).

DNA extraction, enzyme digestion, and gels

DNA was extracted as described elsewhere (Rossi et al. 1990). The DNA was digested with restriction endonucleases (Bethesda Research Laboratories) according to the instructions of the manufacturer. The DNA fragments were separated in 1.2 % agarose gels (Maniatis et al. 1982).

Blotting and hybridization

For Southern-blot analysis, agarose gels were denatured, neutralized, and transferred nylon membranes (Pall Byodine), according to the method described by Southern (1975). For dot-blot analysis, DNA was denatured in 0.2 N NaOH, 10× standard saline citrate (SSC) (1× SSC = 0.15 M NaCl, 0.015 M trisodium citrate, pH 7) during 30 minutes at room temperature, and spotted onto the nylon membrane.

In both cases, prehybridization and hybridization were performed in a solution containing 6× SSC, 100 µg of salmon DNA (Sigma, USA)/ml, 0.5 % SDS and 0.3 % nonfat dry milk, and labeled DNA probe was hybridized with the DNA immobilized on the membranes for 18 h at 60 °C. Then the membranes were washed five times at 65 °C in 0.1× SSC (high stringency) and 1 % SDS. Hybrid DNA was detected by autoradiography, using 3M X-ray films and DuPont Lightening Plus screens.

Labeling of DNA

The RPCS probe was labeled radioactively to a specific activity of 10^8 cpm/µg by using the nick translation procedure (Rigby et al. 1977).

Isolation and cloning of the satellite DNA

The isolation, cloning, and characterization of the monomer of the major satellite DNA of *Ctenomys* (RPCS), as well as its nucleotide sequence, were described elsewhere (Rossi et al. 1990).

Results

Relative amounts of the major satellite DNA in several *Ctenomys* species

The monomer of the major satellite DNA of *Ctenomys*, RPCS, is 337 base pairs long, and ~ 42 % C+G (Rossi et al. 1990). This monomer was originally cloned from *Ctenomys porteousi*, and we estimate that this species has ~ 3×10^6 copies of the monomer per haploid genome.

Genomic DNA was isolated from liver of several species of *Ctenomys*. Equal amounts

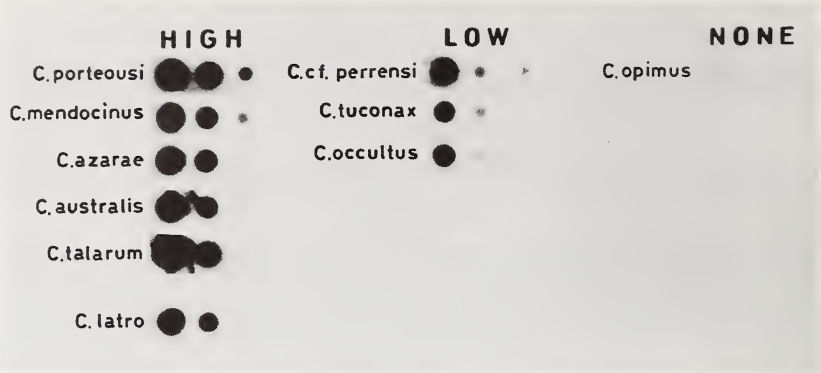

Fig. 1. Quantitative analysis of RPCS in different *Ctenomys* species by dot-blot experiment. Hybridization to labeled RPCS was performed under high-stringency conditions. In all cases the amounts of DNA were 1000, 100, and 10 ng

of these DNAs were spotted on nylon membranes and hybridizated to labeled RPCS (dot-blot technique). The hybridization was carried out under conditions where 95 % identity would be expected to generate a signal. Hybridization was positive for every spot containing *Ctenomys* DNA, except in the one containing *Ctenomys opimus* DNA (Fig. 1). According to these results, the species of *Ctenomys* were classified into three major groups (Table).

Classes of *Ctenomys* species according to its content of RPCS

The amount of RPCS was estimated by dot-blot hybridization performed under high-stringency conditions (see Fig. 1)

High	Low	None
C. porteousi	*C.* cf. *perrensi*	*C. opimus*
C. mendocinus	*C. tuconax*	
C. azarae	*C. occultus*	
C. australis		
C. talarum		
C. latro		

Qualitative analysis of the RPCS satellite in several *Ctenomys* species

Figure 2 shows several restriction endonucleases hybridization patterns of RPCS satellite sequences present in several *Ctenomys* species. The restriction endonucleases employed were EcoR I, Pst I, Hinf I, and Ava II.

As previously reported (Rossi et al. 1990), these patterns demonstrate the existence of interspecies differences in the proportion of monomers of RPCS with restriction sites for different restriction endonucleases. However, the patterns corresponding to RPCS sequences present in *C. porteousi, C. australis, C. azarae, C. mendocinus*, and *C. talarum* were identical, while *C. latro* presented some differences, mainly in the EcoR I and Pst I patterns. This group of species is the same which results from the quantitative analysis of figure 1, as the one with high content of RPCS.

In contrast, the species of the group containing low amounts of RPCS possess different restriction patterns. Furthermore, these patterns are clearly different from the restriction patterns of the species belonging to the group with high content of RPCS.

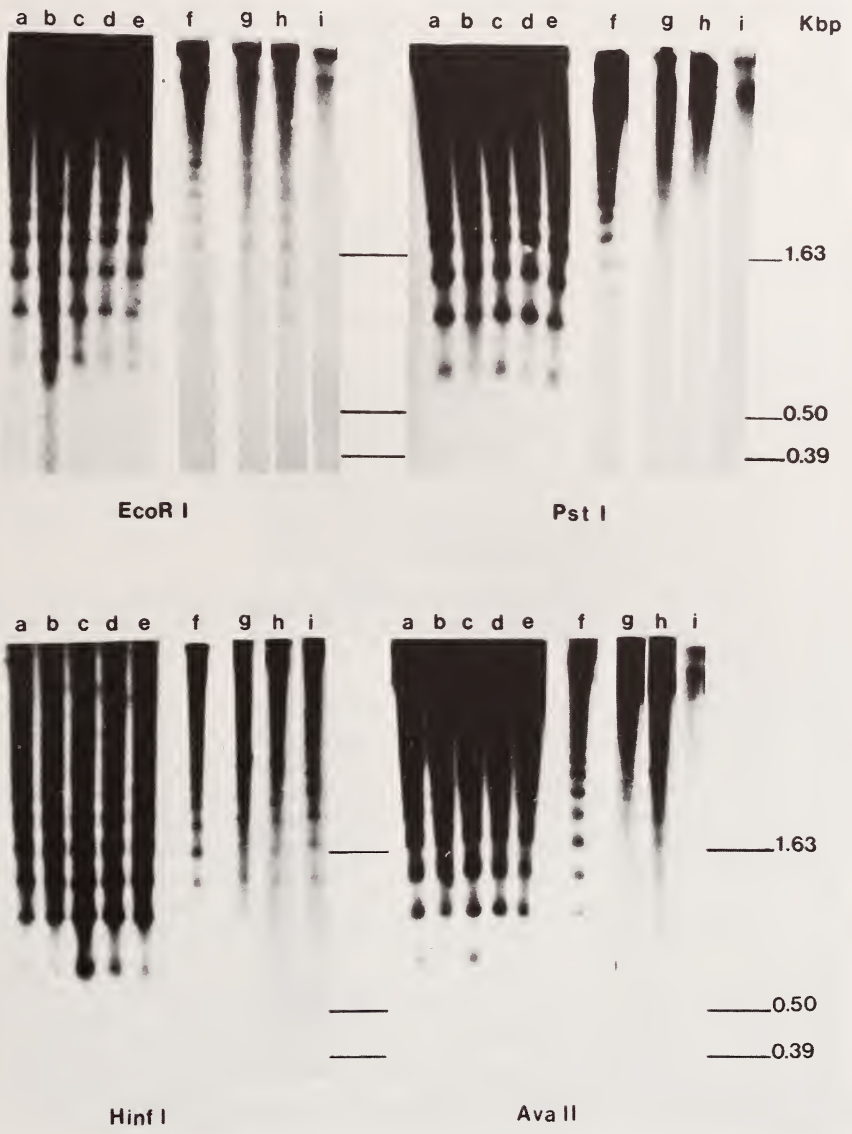

Fig. 2. Qualitative analysis of RPCS in different species of *Ctenomys* by Southern-blot experiments. The genomic DNAs were cleaved with EcoR I, Pst I, Hinf I, and Ava II. Hybridization to labeled RPCS was performed under high-stringency conditions. a: *C. mendocinus*, b: *C. porteousi*, c: *C. australis*, d: *C. talarum*, e: *C. azarae*, f: *C. latro*, g: *C.* cf. *perrensi* (2n = 54), h: *C. tuconax*, and i: *C. occultus*

Correlation between geographic distribution and the quantity and quality of RPCS in the studied species of *Ctenomys*

Figure 3 shows the geographic distribution of the *Ctenomys* species here studied. It can be observed that species containing high amounts of RPCS and a typical satellite restriction pattern described above also have a distinctive geographic distribution. All of them, with the exception of *C. latro*, are distributed south of the 30°S latitude. North of this latitude all the members of the group are distributed containing low amounts of RPCS and also *C. opimus*, the only member of the group whose DNA does not cross-hybridize with the RPCS under high-stringency conditions.

Discussion

Rodents of the genus *Ctenomys* are members of the family Octodontidae, which occur in the southern cone of south America. *Ctenomys* is included in the subfamily Ctenomyinae (Reig 1986; Reig et al. 1990), characterized by advanced adaptations to a herbivorous subterranean life. A major satellite DNA present in members of the genus *Ctenomys*, whose monomer was named RPCS, was recently described (Rossi et al. 1990).

In the present work members of the genus *Ctenomys* were classified in three major groups according to their contents of RPCS measured by quantitative analysis performed under high-stringency conditions. In this conditions, *C. opimus* was the only species of the genus analyzed that gave a negative result. RPCS-related sequences were only detected in this species under relaxed hybridization conditions, and the Pvu II pattern for this *Ctenomys* species was also very different from the Pvu II pattern observed in other species of *Ctenomys* (see also Rossi et al. 1990).

A second group includes the species *C.* cf. *perrensi*, *C. tuconax*, and *C. occultus*. This group is characterized by its relative low content in RPCS. However, the species of this group are heterogeneous according to qualitative analysis of RPCS satellite DNA (Fig. 2) and show different karyotypes (Reig and Kiblisky 1969; Ortells et al. 1991).

The third group contains the species *C. porteousi*, *C. australis*, *C. azarae*, *C. mendocinus*, and *C. talarum*. These species are very homogeneous according to qualitative analysis (Fig. 2). They also share the same 2n = 48 diploid number. However, *C. talarum* differs from the remaining in G- and C-banding patterns (Massarini et al. 1991). *C. porteousi*, *C. australis*, *C. azarae*, and *C. mendocinus* have been clumped together in the *mendocinus* group because of their sharing very similar banding patterns and the presence of heterochromatin in full arm blocks (Massarini et al. 1991). We also include in this third group *C. latro*, which shows some minor differences in the qualitative analysis as compared to the former species. It also has a different 2n = 42 karyotype and a symmetrical sperm type.

All the species included in the third group with the exception of *C. latro* are distributed south of the 30°S latitude. North of this latitude are distributed all the members of the group containing low amounts of RPCS and also *C. latro* and *C. opimus*. This suggests that *C. latro* may be in some way related to a stock which colonized the southern part of Argentina and that most (if not all) of the living species located in this area are derived from this stock.

The correlation between geographic distribution and quantity and quality of the RPCS – with the exception of *C. latro* above discussed – is in agreement with the previous described correlation between sperm morphology and geographic location (Feito and Gallardo 1982; Vitullo et al. 1988; Vitullo and Cook 1991). For that reason there is the hope that the RPCS periodicities may afford valuable clues to understand the evolution of *Ctenomys*.

Fig. 3. Geographic distribution of the *Ctenomys* species studied in this work. The shady area denotes geographic distribution of the family Ctenomyidae according to VITULLO et al. (1988). ●, ◒, and ○ denote high, low and none content of RPCS, respectively, according to the quantitative analysis performed under high-stringency conditions. In parenthesis are included the type of sperm and the diploid number. S = symmetrical sperm, A = asymmetrical sperm

Acknowledgements

This paper is dedicated to the memory of Professor Osvaldo A. Reig, who died in Buenos Aires in March 1992.

Zusammenfassung

Eine bedeutende Satelliten-DNA von südamerikanischen Nagetieren der Gattung Ctenomys. Quantitative und qualitative Unterschiede von Arten mit verschiedener geographischer Verbreitung

Quantität und Qualität einer bedeutenden Satelliten-DNA wurden bei mehreren Arten südamerikanischer Nagetiere der Gattung *Ctenomys* untersucht. Die Quantität wurde mittels genomischer DNA-Hybridisations-Analyse mit Sequenzen dieser bedeutenden Satelliten-DNA in einem dot-blot Experiment, die Qualität mittels genomischer DNA-Hybridisations-Analyse nach Verdauung mit Restriktions-Endonukleasen, mit Sequenzen dieser bedeutenden Satelliten-DNA in einem Southern-blot Experiment, untersucht. Die quantitative und qualitative Analyse wurde mit der geographischen Verbreitung dieser Arten in Zusammenhang gebracht.

Nach der unter hohen Stranghybridisations-Bedingungen vorgenommenen dot-blot Analyse, werden die *Ctenomys*-Arten in 3 Gruppen gestellt, je nachdem, ob sie gar kein, wenig oder viel dieser *Ctenomys*-Satelliten-DNA enthalten. Die erste Gruppe enthält nur *C. opimus*. Die zweite Gruppe enthält *C.* cf. *perrensi*, *C. tuconax* und *C. occultus*. Die dritte Gruppe enthält *C. mendocinus*, *C. porteousi*, *C. azarae*, *C. australis* und *C. talarum*. *C. latro* scheint nah verwandt mit dieser letzten Gruppe zu sein, zeigt aber eigene Unterschiede. Je nach der Qualität dieser *Ctenomys*-Satelliten-DNA, sind die *Ctenomys*-Arten der dritten Gruppe nah miteinander verwandt und alle, außer einer, haben eine bestimmte geographische Verbreitung südlich des 30. Breitengrades. Im Gegensatz dazu scheinen die Arten der zweiten Gruppe entfernter verwandt zu sein und befinden sich alle nördlich des 30. Breitengrades. Das gleiche gilt für *C. opimus*.

References

Arnason, U. (1982): Southern blot hybridizations in cetaceans, using killer whale restriction fragment as a probe. Hereditas **97**, 47–49.

Arnason, U.; Allderdice, P. W.; Lien, J.; Widegren, B. (1988): Highly repetitive DNA in the baleen whale genera *Balaenoptera* and *Megapera*. J. Mol. Evol. **27**, 217–221.

Beauchamp, R. S.; Michell, A. R.; Buckland, R. A.; Bostock, C. J. (1979): Specific arrangements of human satellite III DNA sequences in human chromosomes. Chromosoma **71**, 153–166.

Bogenberger, J. M.; Neitzel, N.; Fitter, F. (1987): A highly repetitive DNA component common to all Cervidae: its organization and chromosomal distribution during evolution. Chromosoma **95**, 154–161.

Burtlag, D. L. (1980): Molecular arrangement and evolution of heterochromatic DNA. Ann. Rev. Genet. **14**, 121–144.

Cooke, H. J.; McKay, R. D. G. (1978): Evolution of a human Y-chromosome specific repeated sequences. Cell **13**, 453–460.

Dennis, E. S.; Dunsmuir, P.; Peacock, W. J. (1980): Segmental amplification in a satellite DNA: Restriction enzyme analysis of the major satellite of *Macropus rufogriseus*. Chromosoma **79**, 179–198.

Feito, R.; Gallardo, M. (1982): Sperm morphology of the Chilean species of *Ctenomys* (Octodontidae). J. Mammalogy **63**, 658–661.

Hörz, W.; Zachau, H. G. (1977): Characterization of distinct segments in mouse satellite DNA by restriction nucleases. Eur. J. Biochem. **73**, 383–392.

Maniatis, R.; Fritsch, E. F.; Stambrook, J. (1982): Molecular cloning: a laboratory manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.

Massarini, A. I.; Barros, M. A.; Ortells, M. O.; Reig, O. A. (1991): Chromosomal polymorphism and small karyotypic differentiation in a group of *Ctenomys* from central Argentina (Rodentia, Octodontidae). Genetica **83**, 131–144.

Ortells, M. O.; Contreras, J. R.; Reig, O. A. (1990): New *Ctenomys* karyotypes (Rodentia: Octodontidae) from north-eastern Argentina and from Paraguay confirm the extreme chromosomal multiformity of the genus. Genetica **82**, 189–201.

Reig, O. A. (1986): Diversity patterns and differentiation of high Andean rodents. In: High Altitude Tropical Biogeography. Ed. by F. Vuilleumier and M. Monasterio. New York, Oxford: Oxford University Press, Pp. 404–439.

Reig, O. A.; Busch, C.; Ortells, M. O.; Contreras, J. R. (1990): An overview of evolution systematics, population biology and speciation in *Ctenomys*. In: Evolutionary Biology of Subterranean Mammals. Ed. by E. Nevo and O. A. Reig. New York: Alan R. Liss. Pp. 71–96.

Reig, O. A.; Kiblisky, P. (1969): Chromosome multiformity in the genus *Ctenomys* (Rodentia: Octodontidae). A progress report. Chromosoma **28**, 211–244.

Rigby, P. W.; Dieckmann, M.; Rhodes, C.; Berg, P. (1977): Labeling desoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**, 236–251.

Rossi, M. S.; Reig, O. A.; Zorzópulos, J. (1990): Evidence for rolling-circle replication in a major satellite DNA from the South American rodents of the genus *Ctenomys*. Mol. Biol. Evol. **7**, 340–350.

Southern, E. M. (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**, 503–517.

Vitullo, A. D.; Roldán, E. R. S.; Merani, M. S. (1988): On the morphology of spermatozoa of tuco-tucos, *Ctenomys* (Rodentia: Ctenomyidae): New data and its implications for the evolution of the genus. J. Zool. London **215**, 675–683.

Vitullo, A. D.; Cook, J. A. (1991): The role of sperm morphology in the evolution of tuco-tucos, *Ctenomys* (Rodentia, Ctenomyidae): Confirmation of results from Bolivian species. Z. Säugetierkunde **56**, 359–364.

Walsh, J. B. (1987): Persistence of tandem arrays: implications for satellite and simple-sequence DNA. Genetics **115**, 553–567.

Widegren, B.; Arnason, U.; Aküsjarvi, G. (1985): Characteristics of a conserved 1579-bp highly repetitive component in the killer whale *Orcinus orca*. Mol. Biol. Evol. **2**, 411–419.

Authors' addresses: María Susana Rossi, present address: Instituto de Investigaciones en Biología Molecular y Biotecnología (INGEBI), Vuelta de Obligado 2490-2do. Piso, RA-1428 Buenos Aires, Argentina; Jorge Zorzópulos, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2–4to. Piso, Ciudad Universitaria, RA-1428 Buenos Aires, Argentina