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12. HETEROCHROMATIN VARIATION AMONG THE CHROMOSOMALLY DIVERSE TUCO-TUCOS (RODENTIA: CTENOMYIDAE) FROM BOLIVIA

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Abstract

Species of the genus *Ctenomys* (Rodentia: Ctenomyidae) exhibit one the greatest amounts of chromosomal variation known for a mammalian clade. We analyze the distribution of heterochromatin, as determined by C-banding, in 7 Bolivian species (*Ctenomys boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. opimus*, and *C. steinbachi*). Most chromosomes in all species have pericentric heterochromatin and some species also exhibit chromosomes with telomeric, interstitial, and heterochromatic whole arms. This variation was analyzed in light of phylogenetic hypotheses proposed for the genus, and recent analysis of the distribution of quantitative variation of satellite DNA. These combined analyses indicate that there is a weak phylogenetic component underlying the primary heterochromatic groups previously identified. Additionally, those clades that display high variable copy numbers of satellite DNA also have the most variable heterochromatic patterns. Although variation in heterochromatin is only one facet of chromosomal diversification, we suggest that it may be an important aspect to be further considered with more detailed molecular studies.

Keywords: Keywords: Bolivia, *Ctenomys*, chromosomal evolution, heterochromatin, RPCS, South America, satellite DNA.

Resumen

Las especies del género *Ctenomys* (Rodentia: Ctenomyidae) muestran una enorme variación de cromosomas conocido en los mamíferos. Nosotros analizamos la disitrbución de la heterocromoatina, determinado por el C-bandeo, en 7 especies bolivianas (*Ctenomys boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. opimus*, and *C. steinbachi*). La mayoría de los cromosomas en todas las especies tienen heterocromatina pericéntrica y algunas especies también mostraron cromosomas teoméricos, intersticiales y brazos heterocromáticos completos. Esta variación fue analizada bajo el esquema de hipótesis filogenéticos propuestas para el género y análisis recientes de la distribución de la variación cuantitativa del ADN satelital. Estos análisis combinados indican que existe un pobre componente filogenético en los grupos primarios de heterocromatina previamente identificados. Además, aquellos clados que muestran un número variable de copias de ADN satelital también tienen las tendencias más variables de heterocromatina. No obstante la variación en la heterocromatina es una faceta de la diversificación cromosómica, sugerimos que este aspecto es importante para considerarse en estudios moleculares más detallados.

Palabras clave: Bolivia, *Ctenomys*, evolución cromosómica, heterocromatina, RPCS, Sudamérica, ADN satelital.

Heterochromatic variation in mammals ranges from nonvariable and rare in some families (*e.g.*, felids, Pathak and Wurster-Hill 1977) to extremely variable and extensive in others (*e.g.*, geomyids, Patton and Sherwood 1982; Qumsiyeh *et al.* 1988). Even within a genus some species may exhibit extreme variation in the amount of heterochromatin among different populations, while other species exhibit little or no variation (*e.g.*, *Thomomys*, Patton and Sherwood 1982).

Constitutive heterochromatin (C-band positive regions) includes several classes of highly repetitive and satellite DNA (John and Miklos 1979, Schweizer 1983, Patton and Barros 1985, Schwarzacher-Robinson *et al.* 1988, King 1993). Variation in the distribution, quantity, and types of these repetitive sequences has not been adequately explained (Verma 1988). Hypotheses concerning the function of heterochromatin remain speculative at best (Brutlag 1980; Cavalier-Smith 1985; Sessions 1986; John 1988; Wallrath 1998). Gene loci are known to occur in some of these highly repetitive regions (Hilliker 1989), and structural properties of some heterochromatic blocks suggest that these may be involved in meiotic pairing (Irick 1994).

Regardless of function, variation in the amount or distribution of heterochromatin may be responsible for substantial amount of the chromosomal variation found within and among closely related species, including the addition and deletion of whole chromosome arms or even entire chromosomes (Duffey 1972; Pathak *et al.* 1973; Hatch *et al.* 1976; Patton and Sherwood 1982; Qumsiyeh *et al.* 1988; De Freitas 1994). Populations within a species that exhibit differences in the amount and organization of heterochromatin are often fully capable of interbreeding. Hybrid offspring from these matings generally show no perceptible abnormalities at meiosis and are fully fertile (Baverstock *et al.* 1982; Patton and Sherwood 1983) perhaps due to the mechanisms inferred by the meiotic modulation hypothesis (Bardhan and Sharma 2000). Assessments of chromosomal variation should be placed within a phylogenetic context and include details of the distribution and variation of constitutive heterochromatin across the genomes of interest.

One mammalian clade that has undergone an explosive amount of chromosomal evolution is the subterranean genus *Ctenomys* (tuco-tucos) with diploid chromosome numbers ranging from 10 to 70 chromosomes (Anderson *et al.* 1987). This degree of chromosome evolution has been unusually rapid for a mammal, as the earliest fossils of this South American genus are known from Pleistocene deposits (*e.g.*, Quintana 1994; Cook *et al.* 2000). Concomitant with the high level of chromosomal variation found in *Ctenomys* is a relatively high degree of cladogenesis. The number of species recognized fluctuates greatly from 38 (Woods 1993) to more than 56 (*e.g.*, Reig *et al.* 1990). Recently, new species have been described (De Freitas 2001; Kelt and Gallardo 1994; Pearson and Christie 1985). Many species of *Ctenomys* have not been karyotyped and differentially stained karyotypes, including C-bands, have been published for fewer than 50% of the species (Freitas and Lessa 1984; Vidal-Rioja 1985; Gallardo 1991). Table 1 includes information on the published chromosomes for species of *Ctenomys* known to date.

To begin to understand the significance of heterochromatin in the diversification of tuco-tucos, we document these patterns of variation within a phylogenetic framework. Specifically, our objective is to describe the distribution of heterochromatin among several species of Bolivian *Ctenomys* that chromosome number ($2n$ = 10-56) and belong to different clades within the genus (*e.g.*, Lessa and Cook 1998; Mascheretti *et al.* 2000; Slamovits *et al.* 2001). Additionally, we place this heterochromatic variation in the context of the patterns of amplification/deletion of the Repetitive *Pvu* II *Ctenomys* Sequence (RPCS) satellite DNA (Rossi *et al.* 1990, Slamovits *et al.* 2001). Diploid variation among species of this genus is extensive and has been the subject of recent reports (Garcia *et al.* 2000b, 2000c; Gimenez *et al.* 1999; Gimenez *et al.* 1997; Justo and Contreras 1999). However, the last attempt to summarize information on C-banded chromosomes of this genus dates back to the early 1990s (Reig *et al.* 1992).

Materials and Methods

The general Bolivian distributions of the seven species of *Ctenomys* included in this study are detailed in Fig. 1. Animals were collected at various times between 1984 and 1990. Specimens examined are listed by *N*ew Mexico *K*aryotype number as follows (males/females): *Ctenomys conoveri* (1/1), 8 km E Carandayti, NK12573, 9 km E Carandayti NK12607; *C. frater* (1/1), Rancho Tambo NK14610,

Table 1. Diploid (DN) and fundamental (FN) numbers of extant species of *Ctenomys*. Woods (1993) is followed for the taxonomy of the genus with the addition of species described since 1992. (Continúa).

Species name	DN	FN	Ref	G-B	Ref	C-B	Ref
C. steinbachi	10	16	4	N		Y	3
C. occultus	22	38, 40	1	Y	14	Y	7
C. fulvus	26	48,52	10	N		Y	10
C. robustus	26	48	1	N		Y	10
C. maulinus brunneus	26	48	10	N		Y	10
C. maulinus maulinus	26	50	1	N		N	
C. o. luteolus	26	46	6, 7	N		Y	7
C. o. opimus	26	52	1, 4, 5,7	Y	14	Y	3
C. coyhaiquensis	28	42, 44	10	Y	9	Y	9,10
C. fodax	28	46	5	Y	14	N	
C. tucumanus	28	56	1	Y	14	Y	7
C. colburni	34	64	10	N		N	
C. magellanicus	34, 36	64	7, 10, 21	Y	14	Y	10
C. knightii	36	68	1	Y	14	Y	7
C. leucodon	36	68	4	N		Y	3
C. latro	40-42	46-47, 50	1, 7	Y	14	Y	7
C. minutus	42,45-50	74-78	17	Y	17	Y	17
C. boliviensis	42-46	64	4	N		Y	2
C. bol goodfellowi*	46	68	2, 4	N		N	
C. torquatus	44, 46	72	15	Y	15	Y	15
C. argentinus	44	50,52,54	1, 19	Y	14	Y	7
C. porteusi	46-48	71-73	5	Y	16	Y	16
C. azarae	47	71, 78	7	Y	16	Y	16
C. mendocinus	47, 48	80	1, 14	Y	14	Y	16
C. chasiquensis	47,48	?	8	N		N	
C. australis	48	76, 80	7, 16	Y	16	Y	16
C. conoveri	48	70	2,4,11	N		Y	3
C. flamarioni	48	50-78	8	Y	8	Y	8
C. roigi	48	76, 80	7	Y	14	Y	16
C. talarum	48, 50	65-86	4, 6, 7	Y	5,14	Y	13,16
C. rionegrensis	48,50,52,56	68-72,74	7, 13	Y	13	Y	7
C. pilarensis	48,50	50	20	Y	19	N	
C. haigi	50	66	1, 10	N		Y	10
C. pundti	50	66	7	N		Y	7
C. yolandae	50	74, 78	7, 12	N		N	
C. perrensis	50,54-56, 58	80, 84	7, 5, 12	Y	14	Y	7
C. olvaldoreigi	52	56	18	Y	18	Y	18
C. rosendopascuali	52	62, 64, 66	18	Y	18	Y	18
C. frater	52	76, 78	2, 7	N		Y	3
C. lewisi	56	74	2, 7	N		Y	3
C. sociabilis	56	72	5, 7, 10	N		Y	10
C. pearsoni	56, 64, 70	60, 84, 88	1,7, 12,13	Y	22	Y	22
C. tuconax	58-61	80	7	N		N	
C. d'orbignyi	70	80,84, 88	7, 12, 14	Y	14	Y	7
C. lami			20	Y	20	Y	20
C. dorsalis	?	?	11	N		N	
C. emilianus	?	?	7	N		N	
C. nattereri**	36	64	4	N		N	
C. peruanus	?	?	11	N		N	
C. pontifex	?	?	11	N		N	
C. saltarius	?	?	11	N		N	
C. sericeus	?	?	11	N		N	
C. validus	?	?	11	N		N	
C. bonettoi	?	?	11	N		N	
C. brasiliensis	?	?	11	N		N	
C. sp. (Monte)	24	40	23	N		N	
C. sp. (Lonquimay)	28	52	1, 5	N		N	
C. sp. (Pto. Madryn)	38	42	5	N		Y	7
C. sp. (Curuzu laurel)	42	80	12	Y	14	Y	7

Table 1. Diploid (DN) and fundamental (FN) numbers of extant species of *Ctenomys*. Woods (1993) is followed for the taxonomy of the genus with the addition of species described since 1992.

Species name	DN	FN	Ref	G-B	Ref	C-B	Ref
C. sp. (San Miguel)	42	80	1, 5	N		N	
C. sp. (Itahuaticua)	46	50	23	N		N	
C. sp. (Santa Rosa)	46	70	23	N		N	
C. sp. (E. Rios)	48-56	72-74	1, 5	N		N	
C. sp. (Parana)	52	74	12	Y	12	Y	12
C. sp. (Saladas)	54-56	84	12	Y	14	N	
C. sp. (Ubajay)	56	78	12	Y	12	Y	12
C. sp. (Mburucuya)	58	84	12	Y	12	Y	12
C. sp. (km 14 Saladas)	58	84	1, 5	Y	14	N	
C. sp. (MF Mantilla)	62	84	14	Y	14	N	
C. sp. (San Roque)	62	80, 84	1,5, 7, 12	Y	14	Y	12

**Ctenomys boliviensis goodfellowi*

**Anderson et al. (1984) considered specimens from Roboré (Bolivia) as *Ctenomys boliviensis nattereri*, but Mascheretti et al. (2000) suggested that these specimens should be recognized as *C. nattereri*

References: 1) Reig 1989; 2) Cook et al. 1990; 3) this paper; 4) Anderson et al. 1987; 5) Reig et al. 1990; 6) Reig and Kiblisky 1969; 7) Reig et al. 1992; 8) Freitas 1994; 9) Kelt and Gallardo 1994; 10) Gallardo 1991; 11) Woods 1993; 12) Ortells et al. 1990; 13) Garcia et al. 2000; 14) Ortells 1995; 15) Freitas and Lessa 1984; 16) Massarini et al. 1991; 17) Freitas 1997; 18) Gimenez et al. 1999; 19) Gimenez et al. 1997; 20) Freitas 2001; 21) Lizarralde et al. 2001; 22) Novello and Lessa 1986; 23) Cook, unpub.

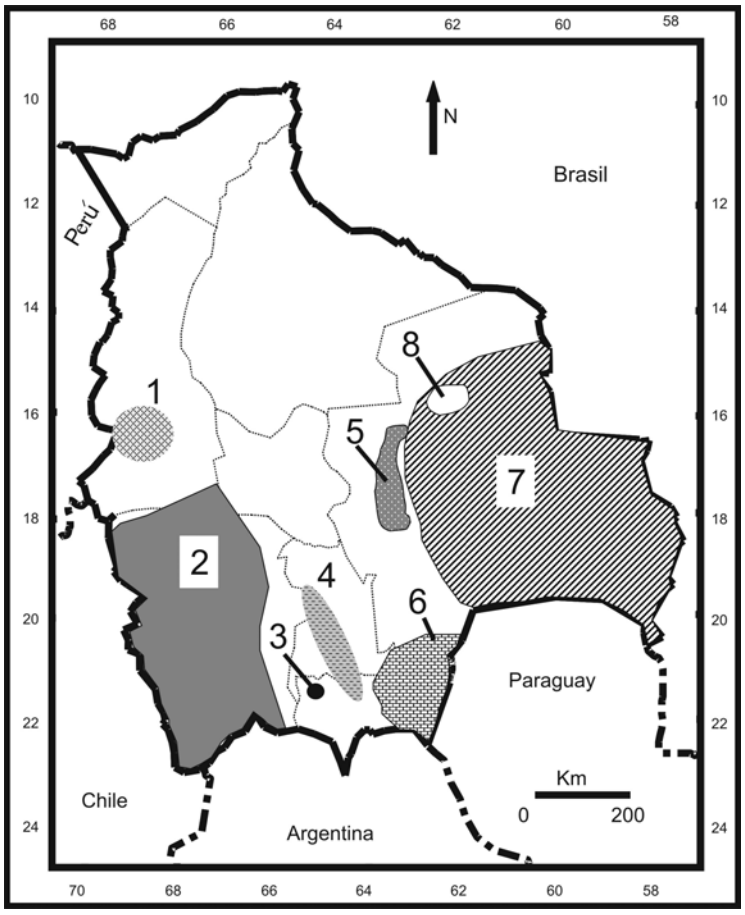

Fig. 1. Distributions of some of the species of *Ctenomys* known to occur in Bolivia and included or mentioned in this study: 1 = *Ctenomys leucodon*; 2 = *Ctenomys boliviensis*; 3 = *Ctenomys lewisi*; 4 = *Ctenomys frater*; 5 = *Ctenomys steinbachi*; 6 = *Ctenomys conoveri*; 7 = *Ctenomys boliviensis*, and 8 = *Ctenomys goodfellowi.*

8 km W Rancho Tambo, NK14622; *C. leucodon* (0/1), 16 km SW San Andres de Machaca, NK14793; *C. lewisi* (0/2), 1 km E Iscayachi, NK14650, NK14652; *C. opimus* (3/2), 3.5 km E Huancaroma, NK11513, NK11568, NK14557, NK14776, NK14782; *C. steinbachi* (1/1), 6 km N Buen Retiro, NK12136, NK12138; *C. boliviensis* (2/6), 12 km S Santa Cruz, NK11681; Estancia Cachuela Esperanza, NK11807, NK11808; 2 km SE Cotoca, NK15330, NK15347; 27 km SE Santa Cruz, NK15407; 3 km S Montero, NK15525; 8 km W Santa Cruz, NK15630.

Specimens were karyotyped as described by Anderson *et al.* (1987) and the frozen cell suspensions were returned to the laboratory for subsequent banding procedures. The use of C-banding techniques allows the identification of the amount and location of constitutive heterochromatin (C-band positive regions) in the genome. C-bands were obtained by a modification of the $Ba(OH)_2$ technique of Sumner (1972). Cell suspensions were thawed and dropped directly onto slides from a height of 2.2 m. Slides were warmed at 60 °C for 24 h. Some slides were C-banded at this time and some were stored for periods of up to 3 years and then C-banded without apparent loss of quality in the C-band preparations. Slides were placed in 0.2 N HCl for 30 min, rinsed with HOH, placed in $Ba(OH)_2$ for 3-6 minutes at 46 °C, rinsed with HOH, dried, treated with 1X SSC for 30 min at 60 °C, rinsed in HOH, dried, and stained for 10 min in 2 % Giemsa.

A minimum of ten metaphase spreads was scored in each specimen. Chromosomes were arranged by decreasing size and are numbered sequentially as previously published (Anderson *et al.* 1987; Cook *et al.* 1990).

All voucher specimens have been deposited in the Museum of Southwestern Biology, University of New Mexico; the American Museum of Natural History; the Colección Boliviana de Fauna in La Paz, Bolivia, or the Museo de Historia Natural "Noel Kempff Mercado" in Santa Cruz, Bolivia.

Results

A total of 21 individuals in 7 species of *Ctenomys* were examined and C-band variation is detailed for each. Quality of the C-band preparation and the number of scoreable spreads were lower for *C. conoveri, C. leucodon, C. lewisi,* and *C. conoveri* specimens, perhaps because frozen cell suspensions were inadvertently thawed twice prior to C-banding. Standard karyotypes have been presented in Anderson *et al.* (1987) or Cook *et al.* (1990). What follows is a description of the C-banded karyotypes.

Ctenomys steinbachi ($2n = 10$, FN = 16). The standard karyotype of this lowland species was reported by Anderson *et al.* (1987) and represents the lowest diploid number yet reported for the genus and for rodents in general (Matthey 1973, but see Silva and Yonenaga-Yassuda 1998). The completely biarmed autosome set (Fig. 2) has heavily stained blocks of heterochromatin restricted to the centromeric regions. The *X* and *Y* chromosomes are subtelocentric and have pericentromeric blocks of heterochromatin. Nearly the entire *Y* chromosome is heterochromatic.

Ctenomys opimus ($2n = 26$, FN = 48). The standard karyotype of this highland species has been reported elsewhere (*e.g.*, Reig and Kiblisky 1969; Gallardo 1979; Cook *et al.* 1990). It has a completely biarmed autosomal complement (Fig. 2) with heavily stained blocks of heterochromatin restricted to the centromere in pairs 4, 6, 7, and 8. Pairs 5, 10, and 11 have heterochromatic short arms and pair 12 appears to be predominantly heterochromatic. Pair 9 and the *X* chromosome lack C-bands and the *Y* chromosome was almost entirely C-band positive. In contrast to the results of Gallardo (1991), pairs 1, 2, and 3 exhibited very weak C-band positive centromeric regions.

Ctenomys leucodon ($2n = 36$, FN = 68). The standard karyotype of this high elevation form was reported by Cook *et al.* (1990). This species has a completely biarmed karyotype (Fig. 2) that exhibited C-band positive regions restricted to the centromeres in all pairs except 3, 5, 10, 15, and 18. The latter two are small metacentric chromosomes with heterochromatic whole arms and pair 3 has a heterochromatic short arm. An additional telomeric band was present in pair 5 and two large interstitial bands occur on the long arms of pair 10. A C-banded male karyotype was not available and the sex chromosomes have not been unequivocally identified in this species (Cook *et al.* 1990).

Ctenomys boliviensis ($2n = 42, 44, 45, 46$; FN = 64, 68). Standard karyotypes of this chromosomally polymorphic lowland species were reported by Anderson *et al.* (1987). This species has also been found to include a $2n = 43$ form and polymorphisms involving the 42-46 cytotypes are common in many

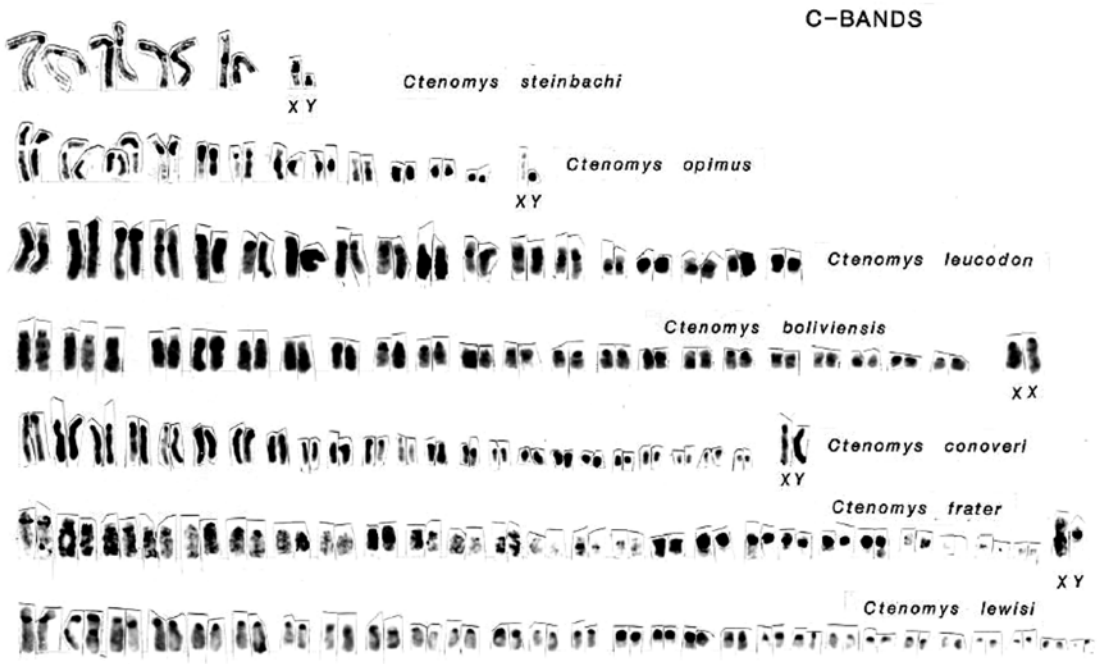

Fig. 2. The C-banded karyotypes of the 7 species of Bolivian tuco-tucos presented in this paper.

of the populations near Santa Cruz, Bolivia (Cook *et al.*, unpublished data). Mascheretti *et al.* (2000) suggested that specimens from Robore ($2n = 36$) assigned to *C. bolivienis* by Anderson *et al.* (1987) should better assigned to *C. nattereri*, although it is unclear to us whether or not they studied the type material of the later. Regardless, we have not analyzed C-banded chromosomes of the $2n = 36$ form. The $2n = 45$ cytotype (Fig. 2) and the 44 and 46 form individuals examined have a similar banding pattern. This lowland species or species complex has little heterochromatin, which is found in lightly staining centromeric blocks in all chromosome pairs. Pair 5 had an additional positive interstitial band.

Ctenomys conoveri ($2n = 48$, FN = 70). The standard karyotype of this species has been reported by Anderson *et al.* (1987). This karyotype (Fig. 2) shows C-band positive blocks occurring in centromeric regions, except in pair 12 which did not show C-band positive heterochromatin. Heterochromatin also appears to be dispersed throughout pairs 2, 3, and 6. Although no males were collected, the structure of the Y chromosome was inferred based on the banding patterns of the paired chromosomes and, thus it appears that an entire arm of the metacentric Y chromosome is heterochromatic.

Ctenomys frater ($2n = 52$, FN = 78). This species apparently inhabits a wide elevational range throughout the southern Andes of Bolivia and probably Argentina (Cook *et al.* 1990). Centromeric blocks of heterochromatin were present in most chromosome pairs (Fig. 2), although pairs 8, 13, and 23 showed no C-band positive material. Pair 12 had two additional interstitial bands. Large heavily stained blocks that appear to be heterochromatic short arms were present in pairs 16-21. The *X* and *Y* chromosomes have heterochromatin restricted to the centromere.

Ctenomys lewisi ($2n = 56$, FN = 74). This high elevation species had the standard karyotype reported by Cook *et al.* (1990). All chromosomes have C-band positive centromeres (Fig. 2). In addition, pairs 15, 16, and 17 have heavily stained heterochromatic short arms and an additional telomeric band is prominent in pair 2. A male *C. lewisi* was not available for analysis, so the sex chromosomes were not identified.

Discussion

An early attempt by George and Weir (1974) to outline major chromosomal trends within hystricomorph rodents based solely on standard karyotypes is overly simplified and emphasizes the inherent problems of inferring phylogenetic relationships from non-differentially stained chromosomes (Baker *et al.*, 1987). George and Weir (1974) conclude, for example, that "cytotaxonomy confirms .... the homogeneous nature of the Octodontoidea". We now know that this group spans nearly the entire range of mammalian chromosome variation ($2n$ = 10-102) and a number of differentially stained karyotypes further confirm that extensive structural and numerical rearrangements occurred during the evolution of this group.

Data from C-banded karyotypes may have limited value for determining evolutionary relationships among taxa due to potentially high levels of homoplasy (Qumsiyeh 1988). Nevertheless a first approach to comprehend the pattern of chromosomal evolution in this family should be documenting the distribution of heterochromatin across taxa because variation in the amount and location of heterochromatin is the basis for, at least some karyotypic variation. C-band analyses clearly indicate that substantial structural rearrangements in tuco-tucos are also common.

Heterochromatin variation and chromosomal polymorphisms

Heterochromatin has been suspected to play a role in facilitating chromosome rearrangements (*e.g.*, Hatch *et al.* 1976; Pathak and Wurster-Hill 1977; Holmquist 1992). Redi *et al.* (1990) suggested a direct relationship between the amount of heterochromatin variation and frequency of chromosome rearrangements in *Mus musculus*. Others (*e.g.*, Garagna *et al.* 2001) have demonstrated that the ultrastructure of pericentric satellite DNA in *M. musculus* (a species with a high frequency of Robertsonian translocations) is highly organized in orientation and polarity with respect to the centromere, an area characteristically heterochromatic. In contrast, Miklos *et al.* (1980) and John (1988) summarizing the existing evidence for this suspected relationship concluded that the amount of heterochromatin and the degree of chromosomal variation was not tied closely together. Fundia *et al.* (2000) found a low coincidence between the location of fragile sites and heterochromatic blocks on individual chromosomes for two genera of ceboid monkeys. Patton and Sherwood (1982) have also suggested that large amounts of heterochromatin did not necessarily promote chromosomal rearrangements in species of *Thomomys*.

In *Ctenomys,* however, there appears to be some relationship between chromosomal polymorphism and the distribution of heterochromatin. For example, Vidal Rioja (1985) confirmed the heterochromatic nature of the chromosomal regions involved in polymorphisms reported for *C. talarum*. In *C. minutus* of Brasil, De Freitas (1997) found that constitutive heterochromatin varied in relation to the distribution of karyotypes from north to south, with the highest chromosomal numbers ($2n$ = 50) in the north, having greater amounts of constitutive heterochromatin than southern populations (*e.g.*, $2n$ = 42 morph).

However, Massarini *et al.* (1995) showed that chromosomal polymorphims in *C. talarum* from Argentina were accompanied by the presence of three C-banding patterns not coincident with geographic location. Furthermore, De Freitas (1994) indicated the number of autosomal arms as well as the distribution of constitutive heterochromatin varied across the geographic distribution of *C. flamarioni*. These examples indicate that some species of *Ctenomys* may not only exhibit a high degree of chromosomal polymorphisms, but also a high degree of polytipism (*i.e.*, different types of C-bandings), and in many cases these appear to be associated events**.** That is to say, species with higher degrees of chromosomal polymorphism are also those with higher diversity of C-banding types.

Heterochromatin variation and average DNA-contents

Ruedas *et al.* (1993) used flow cytometry to measure the total amount of genomic DNA in 7 species of Bolivian tuco-tucos to test whether the chromosomal variation found among these species is due to differences in the total amount of DNA per cell (2C value). These authors identified three clusters of species based on total DNA content. *Ctenomys opimus* had significantly less DNA than the other species, while the remaining 6 species were placed into

two broadly overlapping groups. They did not find a relationship between diploid number and total amount of DNA. Relatively small differences among these species in total DNA content corroborates the conclusion that chromosomal variation found among Bolivian tuco-tucos is not due primarily to variation in the amount of heterochromatin. These species therefore contrast strongly with patterns of high variation in total DNA content and heterochromatin found in some other chromosomally variable mammals such as *Thomomys bottae* (Patton and Sherwood 1982; Sherwood and Patton 1982) and among some species of *Peromyscus* (Deaven *et al.* 1977). Whatever the role of heterochromatin is in chromosome evolution, it is clear that heterochromatic additions and deletions are not responsible for many of the chromosomal rearrangements present among species of the genus *Ctenomys*. Althouhg a high degree of intra-population and intra-specific variation is due to heterochromatin polymorphisms, most chromosomal variation appears to be due to structural re-arrangements (inversions and translocations).

Patterns of heterochromatin distribution

Karyotypic evolution within the genus has been extensive and apparently involves several mechanisms. Variation in fundamental number among karyotypes of Bolivian tucos ranges from 16 in *C. steinbachi* to 78 in *C. frater* indicating that non-Robertsonian events were responsible for many chromosomal rearrangements in this group. A Robertsonian fission or fusion is, however, responsible for the 44-46 polymorphism found within populations of *Ctenomys boliviensis* (Cook *et al.*, in prep.).

Variation in the amount and distribution of heterochromatin was evident among all the tuco-tuco species that have been C-banded (*e.g.*, Reig *et al.* 1992). Variation was also present among the Bolivian species, although all had C-band positive regions associated with the centromeres of most chromosome pairs. This is a pattern similar to that found by Reig *et al.* (1992) and Gallardo (1991). The amount and staining intensity of these heterochromatic regions varied considerably among species. In *C. steinbachi* ($2n = 10$), heterochromatin was restricted to large pericentromeric blocks, perhaps indicative of a succession of past fusion events. Heterochromatic short arms occurred in *C. opimus, C. leucodon, C. frater,* and *C. lewisi,* while *C. leucodon, C. boliviensis,* and *C. frater* exhibited interstitial heterochromatic blocks. Telomeric bands were found in pair 2 of *C. lewisi* and pair 5 of *C. leucodon.* Chromosome pair 12 in *C. opimus* appears to be fully heterochromatic. The majority of the chromosomal variation found among the species, however, appeared to be due primarily to substantial structural rearrangements (*i.e.*, Robertsonian events, inversions, and translocations).

Intraspecific variation in the distribution of heterochromatin was not noted among the Bolivian species. However, increased sampling of individuals may reveal intrapopulation polymorphism as found in other species of the genus (Braggio *et al.* 1999; De Freitas 1997, 1994; Garcia *et al.* 1996; Massarini *et al.* 1995; Massarini *et al.* 1991; Massarini *et al.* 1998)

Reig *et al.* (1992) reviewed the then existing information on C-bands for *Ctenomys* including unpublished data for 5 species. Those authors updated an earlier classification (Reig *et al.* 1990) of C-banded chromosomal complements and identified 4 primary patterns of heterochromatin distribution in the genus. In Pattern I, C-banding is fully negative in most chromosomes, or scarcely positive at the centromeric region of biarmed chromosomes. Other C-band types may also be present, but involve less than 20% of the chromosomes. In Pattern II, C-banding is normally positive in conspicuous pericentromeric blocks, rarely in a full short arm. In Pattern III, C-banding is strongly positive in conspicuous blocks in most short arms and also in a few full chromosomes, also present in pericentromeric blocks. In Pattern IV, C+ bands occur in telomeric or interstitial bands, and/or combinations with the previous 3 patterns. The Bolivian species would be classified into two of these heterochromatin patterns. *Ctenomys lewisi* and *C. boliviensis* represent Pattern I, whereas the remaining species of Bolivian *Ctenomys* show Pattern II.

Phylogenetic perspective on patterns of heterochromatin distribution

South American octodontids are closely allied with ctenomyids (*e.g.*, Ellerman 1940; Reig *et al.* 1990). However, recent work on the suprageneric relationships of the Octodontids has not unequivocally resolved the putative sister-taxa relationship bet-

ween these two groups (Gallardo and Kirsch 2001). In fact, in a recent analysis (Kohler *et al.* 2000) a closer relationship between octodonts and abrocomids has been suggested, although methodological (assumption of a molecular clock) or technical (reduced sample size of abrocomids) may have hampered the resolution of their analyses.

Early chromosome investigations of the octodontids (Fernandez 1966, 1968; George and Weir 1972; Venegas 1974; 1975) indicated that they are chromosomally diverse, and the level of chromosomal diversity for the subterranean forms appears to be higher. This led Reig (1989) to propose a causal relationship between the subterranean lifestyle and speciation. He hypothesized that subterranean forms exhibited reduced dispersal and gene flow among populations, which would result in more rapid fixation of mutations. The report of the second highest known mammalian diploid number ($2n = 102$) in the octodontid *Tympanoctomys barrerae* (Contreras *et al.* 1990; Dunnum *et al.* 2001), and its postulated tetraploidy (Gallardo *et al.* 1999) a species that lives above ground, does not substantiate that idea.

If the sister taxa relationship between ctenomyids and octodontids is accepted, it could be inferred that the primitive karyotypic condition for *Ctenomys* would exhibit small amounts of heterochromatin (Gallardo 1991). This due to the reported small amounts of weakly stained, mostly centromeric heterochromatin in the octodonts (Gallardo 1992; Contreras *et al.* 1994). Gallardo (1992) and Gallardo and Kirsch (2001), point out that the directionality of chromosomal evolution in the octodonts will depend on the unambiguous specification of their sister taxon. Until then, the primitive condition for the distribution of heterochromatin within this group remains unknown.

We attempted to map the distribution of C-banded karyotypes on the phylogenetic hypotheses proposed by Lessa and Cook (1998) and Mascheretti *et al.* (2000) with minor degree of success due to the unresolved nature of the basal polytomy found in this genus based on the sequence of the cytochrome b. Both phylogenies identified a basal group of species composed by *C. frater, C. lewisi* and *C. conoveri*, forming what Mascheretti *et al.* (2000) identified as the Boliviano-Paraguayo group of species. These species are chromosomally variable with regard to heterochromatic distribution. On the one hand *C. conoveri* has the most heavily heterochromatic complement of the Bolivian species, but on the other, *C. lewisi* and *C. frater* are not significantly different from the remaining species analyzed in their distribution of heterochromatin. Therefore, it appears that some basal members of the *Ctenomys* radiation have chromosomal complements that are heavily heterochromatic while others do not.

Patterns of heterochromatin distribution and satellite DNA

Recently Slamovits et al (2001) expanded upon previous phylogenies derived for the group (*e.g.*, Lessa and Cook 1998; Mascheretti *et al.* 2000) and then examined the evolutionary history of the RPCS (Rossi *et al.*, 1995) satellite DNA. By mapping quantitative estimates of the RPCS along a phylogenetic tree they noted that amplifications and deletions of this satellite DNA accompanied bursts of chromosomal diversification in *Ctenomys*.

Using the classification proposed by Reig *et al.* (1992), we mapped the distribution of heterochromatin patterns on the phylogeny proposed by Slamovits *et al.* (2001) to examine the association of the primary heterochromatic patterns with variation in the quantity of the RPCS satellite DNA. We found a high correspondence between sister taxa and their primary heterochromatic patterns (Fig. 3). For example, the fulvus-opimus clade showing virtually no copy numbers of RPCS, and all the members of this clade had heterochromatin pattern II. Interestingly, other species purportedly related to this clade (*C. frater* and *C. conoveri*) also show pattern II of heterochromatin. The only exception is *C. lewisi* with Pattern I.

Similarly, the clade formed by *C. porteousi*, *C. australis*, *C. mendocinus* and *C. rionegrensis*, is characterized by a high and relatively constant number of RPCS copies, with most species characterized as Pattern III. An exception is *C. rionegrensis*, characterized as Pattern II.

Finally, the only clade with a species that has Pattern IV includes the Patagonian and Argentinean species: *C. haigi, C. coyhaiquensis, C. magellanicus, C. argentinus, C. latro, C. tucumanus*. This clade, characterized by extreme variability in RPCS copy numbers (both deletions and amplifications) includes species with either Pattern I (*C. magellanicus, C. argentinus,* and *C. tucumanus*) or Pattern IV (*C. hagi, C. coyhaiquensis,* and *C. latro*).

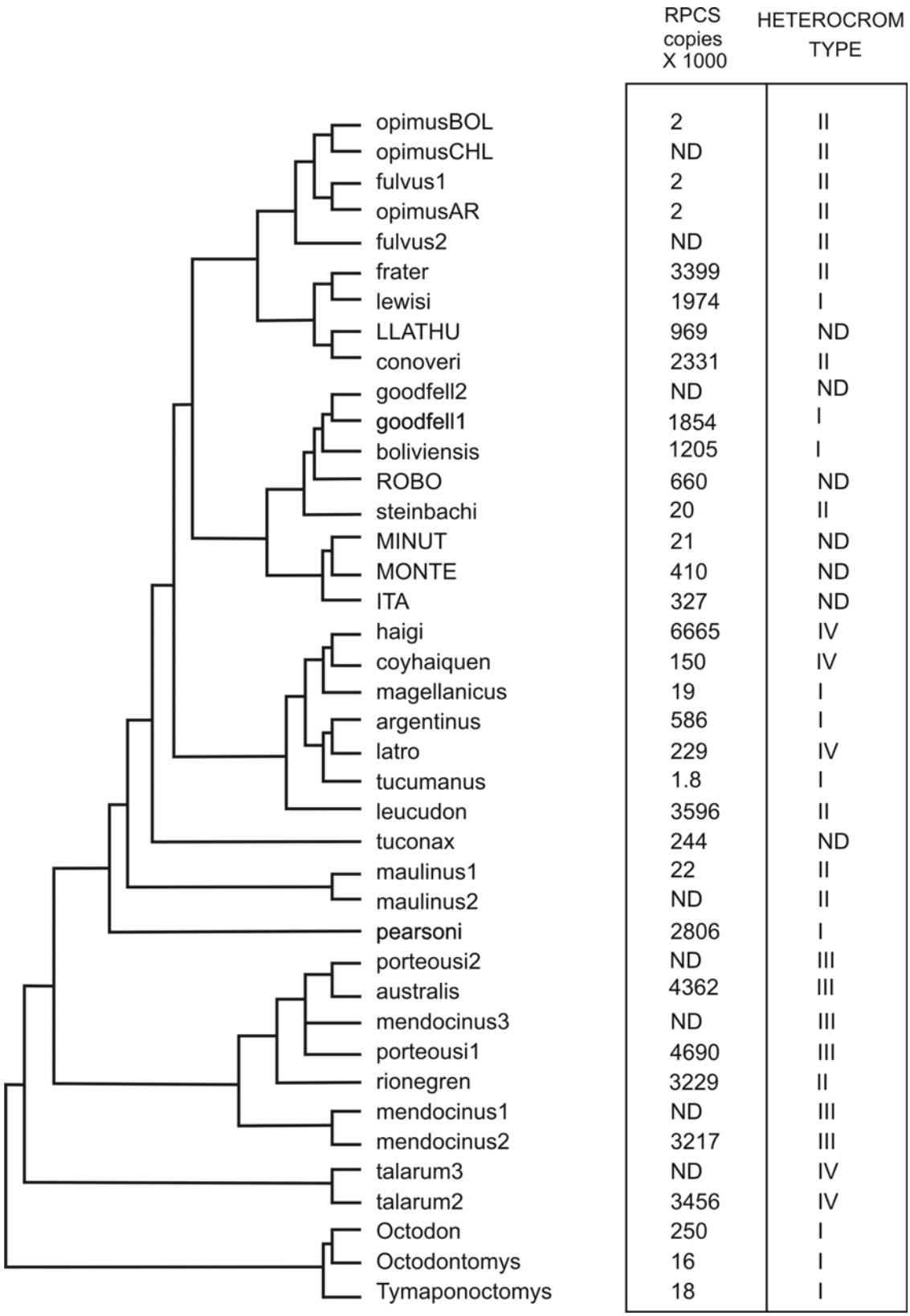

Fig. 3. A ML likelihood phylogeny for the genus *Ctenomys* based on Slamovits *et al.* (2001). The last column to the right includes the heterochromatin distribution type as discussed in text.

Although the correspondence is not complete (*e.g.*, *C. talarum* has Pattern IV, while most of the "mendocinus" group has Pattern III), the analysis shows that there is a correspondence between the pattern of heterocromatiic variation and phylogeny.

Like Slamovits *et al.* (2001), we also found 2 distinct patterns when analyzing heterochromatin type and RPCS copy number. First, high variability in RPCS copy number between closely related groups of species and variability of heterochromatin types characterized one pattern. Second, stable copy numbers of RPCS are found within clades with mostly uniform patterns of heterochromatin variation. The close correspondence between pattern of heterochromatic variation and the distribution of RPCS copy numbers is not surprising because RPCS are associated with heterochromatic areas (Rossi *et al.* 1995).

This analysis shows that interpretation on the polarity of chromosomal evolution needs to be analyzed in relation to independent dataset and that earlier claims (*e.g.*, Garcia *et al.* 2000a) must be viewed with caution. Parenthetically, the phylogeny recovered by Slamovits *et al.* (2001) does not support Justo and Contreras' (1999) suggestion that the genus *Ctenomys* had its origin in the Bolivian plateau.

The rapid and extreme cladogenesis of species of *Ctenomys* has been accompanied by one of the most significant examples of chromosomal evolution known in mammals. The direct role of chromosome change in speciation in tuco-tucos is unclear and although a number of chromosome speciation models have been proposed (Sites and Moritz 1987), none of these has been tested with *Ctenomys*. Population level studies, especially of chromosomally polymorphic species, that include meiotic analyses, as well as ecologic and genetic assessment of population structure should provide the necessary genetic correlates to test the predictions of several of these models (Sites and Moritz 1987).

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