Conservative genome size and rapid chromosomal evolution in the South American tuco-tucos (Rodentia: Ctenomyidae)

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Genome size (the amount of DNA per cell) was measured by flow-cytometric analysis in seven species of a chromosomally variable rodent genus: *Ctenomys boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. opimus*, and *C. steinbachi*. The mean genome size of these species was 7.19 pg DNA and little inter- and intra-specific variation was observed. Genome size was not correlated with diploid number, suggesting that chromosomal evolution at this level is independent of total DNA content. A hypothetical taxonomic unit optimization procedure was carried out using genome size change on a Wagner tree derived from allozyme data. Allozyme evolution and genome size change are linked by a weak, but significant, negative correlation suggestive of preferential genic evolution in the absence of genome size evolution.

Key words: genome size, Ctenomyidae, chromosomal evolution.

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La dimension des génomes (masse de l'ADN nucléaire) de sept espèces de rongeurs dans un genre chromosomiquement variable (*Ctenomys boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. opimus* et *C. steinbachi*) a été mesurée par cytométrie à flux. La masse moyenne de l'ADN pour les sept espèces est 7,19 pg, et très peu de variations, inter- comme intra-spécifiques, ont été observées. Aucune corrélation n'a été trouvée entre la masse d'ADN et le nombre de chromosomes, suggérant que l'évolution chromosomique à ce niveau est indépendante de celle affectant la teneur en ADN. Le procédé d'optimisation de l'unité taxonomique hypothétique a été appliqué aux teneurs en ADN sur un cladograme Wagner dérivé de données allozymiques. Il existe une faible, mais significative, corrélation négative entre le lot d'évolution des allozymes et les changements en teneur d'ADN. Cela suggère que l'évolution allozymique procède préférablement en l'absence de l'évolution en teneur d'ADN.

Mots clés : dimensions des génomes, Ctenomyidae, évolution chromosomique.

Considerable data are now accumulated on both genome size and chromosomal variation in higher organisms; however, few studies have focussed on the relationship between these two parameters. Flow-cytometric analysis has simplified comparative studies of the evolution of nuclear genome size (2C DNA content) in vertebrates, yet most of the genome size data are based on measurements of a single or small number of individuals representing a given species or family and the studies often are performed on specimens representing a very broad taxonomic spectrum (Bachmann et al. 1971; Sparrow et al. 1972; Shapiro 1975; Hinegardner 1976). The significance of these interspecific studies remains nebulous without an adequate assessment of intraspecific variation. In addition, few studies (e.g., Burton et al. 1989) have examined variation within a discrete, monophyletic lineage. Examples of vertebrate taxa for which intraspecific variation in genome size has been assessed include a few families of fish (Gold and Price 1985; Gold and Amemiya 1987; Johnson et al. 1987; Lockwood 1989; Ragland and Gold 1989; Gold et al. 1990; Lockwood and Bickham 1991; Lockwood et al. 1991), salamanders (Kelly et al. 1986; Sessions and Larson 1987), pocket gophers of the genus *Thomomys* (Sherwood and Patton 1982) and *Geomys* (Burton and Bickham 1989), and bats (Burton et al. 1989).

A suitable system to examine the putative relationships between genome size and chromosomal complement can be found in the mammalian order Rodentia. Subterranean rodents, because of their presumed demic population structure and notorious chromosomal variability, provide a frame-

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work for the testing of chromosomal evolutionary hypotheses. These rodents, in particular the geomyids and spalacids, have often been used as a paradigm for investigations of chromosomal evolution (Nevo 1979, 1982; Patton and Sherwood 1982, 1983; Sherwood and Patton 1982).

Tuco-tucos (genus *Ctenomys*) constitute another, phylogenetically independent, example of this pattern of extreme chromosomal variation in subterranean rodents. Tuco-tucos are relatively poorly known caviomorph rodents that are endemic to the southern cone of South America (Cabrera 1961; Anderson et al. 1987; Cook et al. 1990). The genus is speciose, with estimates of the number of species ranging to over 55 (Reig et al. 1990), and these species occur across a wide variety of habitats. In Bolivia, species of *Ctenomys* occur from 250 m (*C. conoveri*) in the Chaco to more than 4000 m (*C. opimus*) in the Puna of the altiplano (Anderson et al. 1987; Cook et al. 1990).

Tuco-tucos exhibit one of the greatest degrees of chromosomal variation known for any mammalian genus (Hsu and Bernischke 1977), with a diploid range of 10–70 (Anderson et al. 1987). Both inter- and intra-specific chromosomal variation have been described for this group (see citations in Cook et al. 1990). Within Bolivia alone, diploid numbers among seven species range from 10 to 56 (Cook et al. 1990). This explosive chromosomal variation, wide geographic distribution, and great taxonomic diversity suggest an explosive rate of speciation, as the oldest known fossils of *Ctenomys* are from the Early Pleistocene (Mones and Castiglioni 1979; Frailey et al. 1980; Reig 1989).

This study is an examination of genome size variation in seven Bolivian species of tuco-tucos. Intra- and interspecific genome size variation is assessed and the relationship between genome size, diploid chromosomal variation, and allozyme evolution is examined. The evolutionary patterns of genome size are compared with those inferred for the same individuals from protein evolution by using the hypothetical taxonomic unit (HTU) optimization procedure of Farris (1970) on a Wagner tree. We consider whether a single process can explain the observed genome size variation across all taxa or whether different mechanisms are at work among different lineages. Finally, the implications of the observed genome size variation for testing the hypothesis that the genome size patterns and differences are due to changes in chromosome number or structure variation is tested using data from the genus *Ctenomys*.

Materials and methods

Seventy-six individuals of seven tuco-tuco species from Bolivia, *Ctenomys boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, *C. lewisi*, *C. opimus*, and *C. steinbachi*, were sampled for this work; 10 individuals from a University of New Mexico laboratory colony of *Octodon degus* (Rodentia: Octodontidae) were used as an outgroup. *Ctenomys leucodon*, *C. lewisi*, and *C. opimus* were all high elevation Puna species (Cook et al. 1990). *Ctenomys frater* is an intermediate elevation species; the remaining species examined here are restricted to elevations of 250–450 m (Anderson et al. 1987). Tissues were collected from freshly sacrificed specimens in the field, frozen in liquid nitrogen, then stored at −80°C until processed. Voucher specimens, frozen tissues, and cell suspensions were deposited in the American Museum of Natural History; Museum of Southwestern Biology at the University of New Mexico, Albuquerque; and Museo Nacional in La Paz, Bolivia.

Preparation of samples for flow cytometric analysis followed a modification of Burton et al. (1989). Small portions of kidney (ca. 2 mm^3) were allowed to thaw in fixative solution (1:1 absolute ethanol – Hanks balanced salt solution (BSS), pH 7.6). Once thawed, the samples were minced into fine pieces and ground in fixative for 6 s using a Tissue-tearror tissue homogenizer (Biospec Products; Bartlesville, Okla.) at the lowest setting. The resulting cell suspension was filtered through a 34-μm monofilament mesh (Tekto Inc., Elmsford, N.Y.) into a centrifuge tube, topped up to 6 mL with fresh Hanks' BSS, and centrifuged for 8 min at 2000 rpm. The resulting supernatant was discarded and the precipitate resuspended in 1.5 mL 4',6-diamidino-2-phenylindole stain (DAPI). The stain consisted of 15 mL DAPI stock solution (10 mg DAPI in 300 mL absolute ethanol) in 85 mL Tris–HCl buffer, pH 7.6 (method 1A; Otto et al. 1979). Cells were stained for 72 h at 4°C and run on a microscope-based Leitz MPV flow cytometer equipped with a 100 W mercury vapor bulb. Filter cube A was used, providing UV excitation in the 340–380 nm wavelength range.

At least 20 000 cells were counted for each *Ctenomys* G_1 peak (that portion of cells in the Gap 1 phase of the cell cycle). Relative fluorescence was measured against an internal standard consisting of erythrocytes from a highly inbred strain of chicken (*Gallus domesticus*) and a chicken of the Highline strain of White Leghorn. The use of chicken as a standard is well established (Bloch et al. 1978; Noguchi and Browne 1978; Tannenbaum et al. 1978), and its genome size, 2.54 pg, has been determined by independent chemical analysis (Rasch et al. 1971). The chicken G_1 peak was placed on channel 50 by adjusting the gain and current to the photometer (Fig. 1), and the relative DNA was calculated as a simple ratio based on the channel numbers of the chicken standard and the *Ctenomys* G_1 peaks.

Variation in relative fluorescence of tissues stained for varying periods of time is a common problem in flow cytometric studies (Lockwood and Bickham 1991). The protocol was strengthened by selection of a fluorochrome, DAPI, with stoichiometric binding properties (Manzini et al. 1983) and uniformly staining for 72 h to ensure complete saturation of the DNA with dye (Otto et al. 1981; Manzini et al. 1983; McBee and Bickham 1988; Lockwood and Bickham 1991). The *Ctenomys* tissues were stained on 2 days and run on 2 days, thus eliminating much of the day to day variation in machine performance. Instrumental variability was also controlled by standardizing the flow cytometer daily, and periodically between samples, with a double standard. Chicken red blood cells (RBCs) were run against tortoise RBCs (*Geochelone elephantopus*) and Kemp's Riddley sea turtle RBCs (*Lepidochelys kempi*), all stained at the same time as the *Ctenomys*; the ratio was always constant: chicken RBCs, channel 50; turtle RBCs, channel 199 (6.04 pg DNA); sea turtle, channel 85 (4.31 pg DNA). This is identical to the results (for tortoise) found by Lockwood and Bickham (1991). Coefficients of variation of the chicken RBCs run as internal standard varied between 1.6 and 2.3. Running two standards at constant channels thus controlled for nonlinearity or zero-shift error in the measurements (Vindeløv et al. 1983; Shapiro 1988). Because of the possibility of overlap of testudinate RBCs with either *Ctenomys* G_1 or G_2 peaks during the trial runs, chicken RBCs were preferred as a single standard, rather than running two concurrent internal biological standards; no improvements in genome size measurements were found using second standards in a number of DNA content studies (Johnson et al. 1987; Burton et al. 1989; Lockwood and Bickham 1991).

The genome size data were organized into three groups and tested for normality using the g_1 and g_2 indices of skewness and kurtosis (Sokal and Rohlf 1981). The groupings were (*i*) all measurements of genome size within a species, (*ii*) all measure-

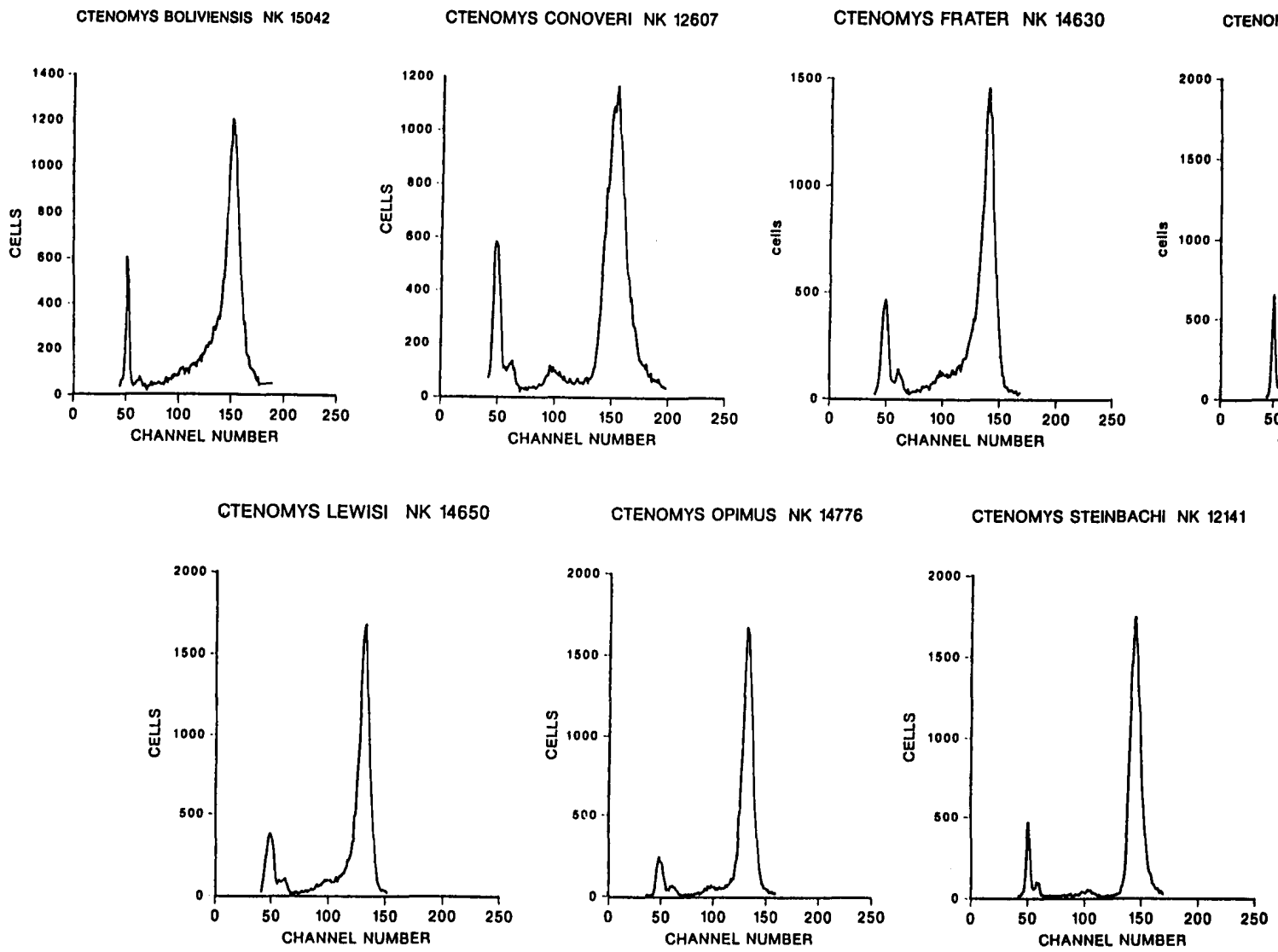

FIG. 1. Representative DNA frequency histograms for each of the *Ctenomys* species examined. The G_1 peak of the standard, *Gallus domesticus* erythrocytes, in each case is adjusted to channel 50, the genome size of the *Ctenomys* spleen G_1 peak being calculated as a simple proportion. For the specimens depicted above, the data are as follows: *C. boliviensis*, female, 7.52 pg, coefficient of variation (CV) = 3.764; *C. conoveri*, male, 8.15 pg, CV = 4.528; *C. frater*, male, 7.06 pg, CV = 3.673; *C. leucodon*, female, 7.52 pg, CV = 3.220; *C. lewisi*, female, 6.71 pg, CV = 3.238; *C. opimus*, female, 6.93 pg, CV = 3.316; *C. steinbachi*, male, 7.22 pg, CV = 2.973.

TABLE 1. Descriptive statistics for each species of *Ctenomys* and *Octodon degus*

Species	*n*	Mean ± SD	Species group	Range	Normality	Skewness (g_1)	Kurtosis (g_2)
C. boliviensis	18	7.53±0.62	*ab*	6.85–8.03	—	−0.450	−0.113
C. conoveri	4	7.80±0.33	*b*	7.41–8.15	—	−0.291	−2.057
C. frater	6	7.53±0.48	*ab*	6.85–8.07	—	−0.503	−1.541
C. leucodon	4	7.75±0.42	*b*	7.52–8.38	—	1.981	3.933
C. lewisi	9	7.12±0.42	*a*	6.35–7.62	—	−0.594	−0.306
C. opimus	18	6.32±0.39	*c*	5.23–6.99	—	−0.607	1.866
C. steinbachi	17	7.46±0.20	*ab*	7.21–7.78	*	0.185	−1.127
O. degus	10	8.64±0.11	*d*	8.53–8.89	—	1.015	1.461
Species groups							
Group *a*	49	7.39±0.46		7.12–8.07	—	−0.685	0.807
Group *b*	48	7.50±0.46		7.40–8.38	—	0.141	0.295
Group *c* (see *C. opimus* above)							
Group *d* (see *O. degus* above)							
All *Ctenomys*	76	7.19±0.62		5.23–8.38	**	−0.797 **	0.210

NOTE: "Species group" refers to results of the Student–Newman–Keuls multiple range test, performed at $\alpha = 0.05$ (*O. degus* not included in this analysis). Letters under the group heading designate assemblages of species not differing significantly. Tests of normality on multispecies assemblages were carried out on the rankit values of the individuals (Gold and Amemiya 1987) to minimize the scaling effects owing to individuals being drawn from different populations (species). Normality indicates the results of the SAS procedure PROC UNIVARIATE NORMAL, significance indicates departure from normality. Negative g_1 values indicate skewness to the left, positive g_1 values indicate skewness to the right; a negative g_2 indicates platykurtosis, a positive g_2 leptokurtosis (Sokal and Rohlf 1981); —, not significant.

*$P < 0.05$.

**$P < 0.01$.

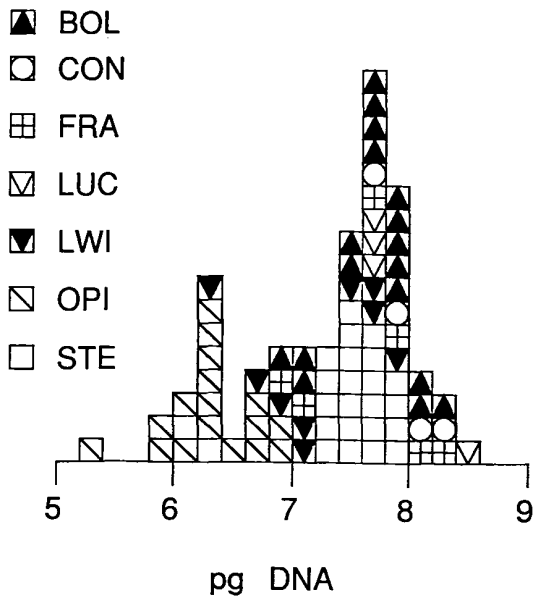

FIG. 2. Distribution of genome size values for all specimens of *Ctenomys* examined in the study. Abbreviations are listed in order as in Fig. 1. The distribution of values shown indicates the contribution of *C. opimus* to the abnormal, skewed distribution of genome size values of all *Ctenomys* species in this study.

ments within nonsignificantly differing species groups, and (*iii*) all measurements across all *Ctenomys* species. The last test was carried out on rankit scores of individuals generated according to equation 1 of Gold and Amemiya (1987). Transformation to rankit values removes scaling effects owing to individuals being drawn from different populations and thus increases sample size in testing for normality of genome size values of individuals within species across all species (Gold and Amemiya 1987). The biological significance of skewness is not clear; however, platykurtosis may indicate that individuals sampled from the species showing this pattern of distribution are partial sibs, or inbred to a certain degree, but sampled from a variety of parental stocks (i.e., the distribution approaches bimodality). Likewise, significant leptokurtosis may indicate that all the individuals sampled are from a single, closely related lineage, and thus that insufficient sampling has been carried out for the species exhibiting this distributional pattern.

Statistical analyses were performed using SuperCalc4, the Biostat 1.0 computer package (Sigmasoft; Placentia, Calif.), mainframe SAS (SAS Institute 1985), and PC-SAS, version 6 (SAS Institute 1986). The level of significance for all analyses was set at $\alpha = 0.05$. A model I double classification analysis of variance followed by a Student–Newman–Keuls multiple range test (Biostat procedure AOV2-3) assessed heterogeneity owing to species, sex, or species by sex interaction. Pearson's product moment correlation coefficient was obtained using the COR procedure of Biostat to obtain an assessment of the amount of association between diploid number and genome size. The significance level of the correlation between branch length in the electrophoretic distance derived Wagner tree and genome size change along each branch was obtained using pc-SAS procedure REG.

Estimates of within- and among-species genome size distances (GSD_k and GSD_{kl}) were calculated using the genome size distance equations of Gold and Amemiya (1987), which represent the mean of all pairwise distances among individuals within species (GSD_k) and between species (GSD_{kl}).

Evolutionary patterns of genome size change along phylogenetic lineages were inferred using the HTU optimizing procedure of Farris (1970) as applied by Sessions and Larson (1987). The procedure is used "to assign an optimal set of HTUs to the dendrogram generated by any sort of ... clustering procedure" (Farris 1970, p. 92); in this case we used a Wagner tree generated from allozyme variation data (Cook 1990) because a Wagner tree also estimates evolutionary rates. Thus, it is possible to assess the evolutionary rate based on allozyme data and superimpose the genome size data on the phylogeny to infer

TABLE 2. Matrix of genome size distances calculated according to the genome size equations of Gold and Amemiya (1987)

	C. boliviensis	*C. conoveri*	*C. frater*	*C. leucodon*	*C. lewisi*	*C. opimus*	*C. steinbachi*	*Octodon*
C. boliviensis	0.36364							
C. conoveri	0.40192	0.42104						
C. frater	0.43956	0.47498	0.58510					
C. leucodon	0.03735	0.39224	0.49993	0.44027				
C. lewisi	0.51712	0.70644	0.59159	0.64814	0.50248			
C. opimus	1.21356	1.48548	1.21576	1.43125	0.84098	0.44009		
C. steinbachi	0.29951	0.39995	0.41920	0.36026	0.43942	1.14785	0.22997	
Octodon	1.10793	0.83477	1.10801	0.88900	1.51739	2.32025	1.17163	0.13099

The values along the diagonal (underlined) represent the mean genome size distance, in picograms of DNA, between individuals within the species (GSD_k, the mean of all within species pairwise comparisons), while values below the diagonal represent the mean between species genome size distance (GSD_{min}, the mean of all pairwise between species comparisons). The GSD_{min} values can be visualized as a phenogram, as depicted in Fig. 4, where it can be appreciated that only one species, *Ctenomys leucodon*, is misplaced from its position as inferred by a Wagner tree generated from protein distance (Cook 1990) shown in Fig. 5.

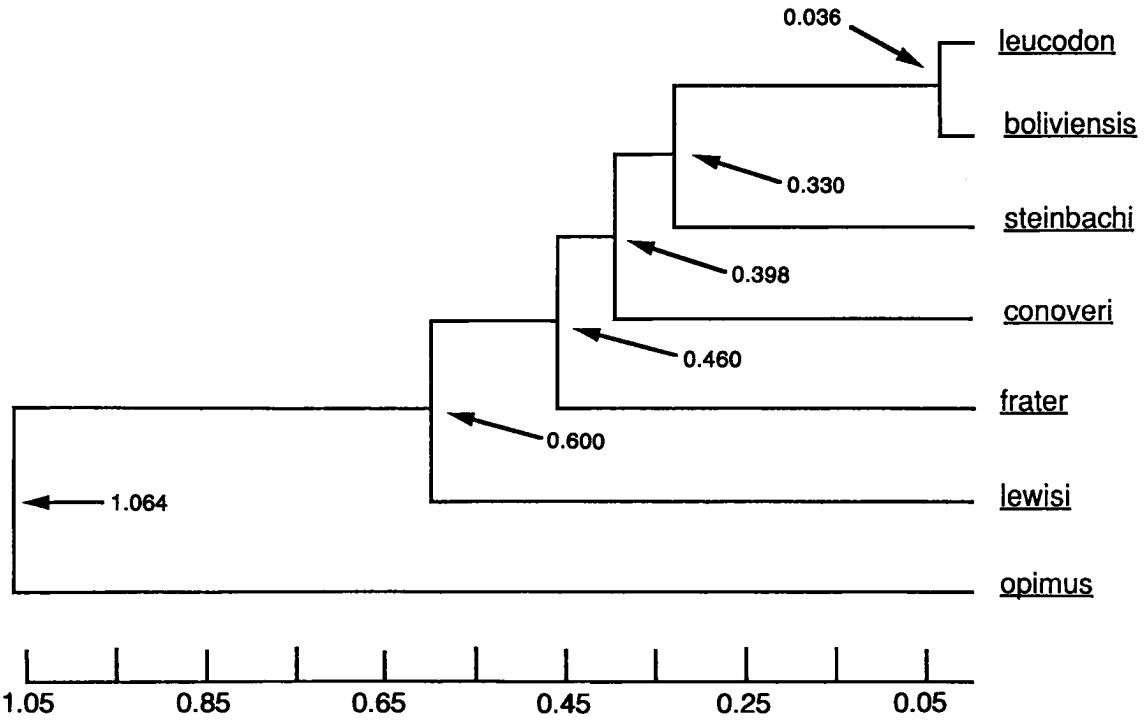

FIG. 3. Phenogram derived from the GSD_{kl} values (Table 2) by the unweighted pair group method of averages (UPGMA) technique. The phenogram visualizes the mean genome size distance values (in pg DNA) among species and shows a certain heuristic taxonomic value, as can be appreciated by comparison with the Wagner tree derived from allozyme data (Cook 1990) shown in Fig. 4.

genome size changes along phylogenetic lineages, thus arriving at an independent estimate of genome size evolution in the same lineage.

In this approach genome size is averaged within a species (Sessions and Larson 1987) and the change in values partitioned along branches of a phylogenetic tree and the HTU optimization procedure (Farris 1970, pp. 90–92) performed on the data. In the HTU approach used in this study, a median state optimization was carried out, that is, inferred nodal values were only used if they were present in the terminal taxa. The terminal genome size values, although varying continuously, were treated as discrete character states. If the genome sizes were equivalent in two sister taxa, then the value at the node uniting the clade was deemed to be equivalent to the terminal values (rule R-1 of Farris 1970, use of the intersection of the character states). In most cases, however, since genome size values either in terminal taxa or in hypothetical taxonomic units did not intersect, the nodal value was inferred from outgroup analysis to be that which minimized the amount of change along each lineage from the outgroup to each terminal clade of the ingroup (maximum parsimony criterion; rule R-2 of Farris 1970). In each case, two passes were done on the tree, one from the terminal taxa to the hypothesized ancestor and one in the opposite direction. The analysis was based on all the species examined in this study and used *Octodon degus* as the outgroup; *C. steinbachi* was split into two populations, as two allopatric populations were distinct at the allozyme level (but not significantly at the genome size level); the mean genome size of each of the allopatric populations was used as the terminal value for HTU analysis, as in all the other *Ctenomys* species.

The use of a mean value of genome size for a species in any given analysis entails some philosophical difficulties because of the typological nature of assigning a particular genome size to a given species; this sort of analysis is compounded further by the fact that there are no significant differences in genome size among a number of these species. However, the analysis is

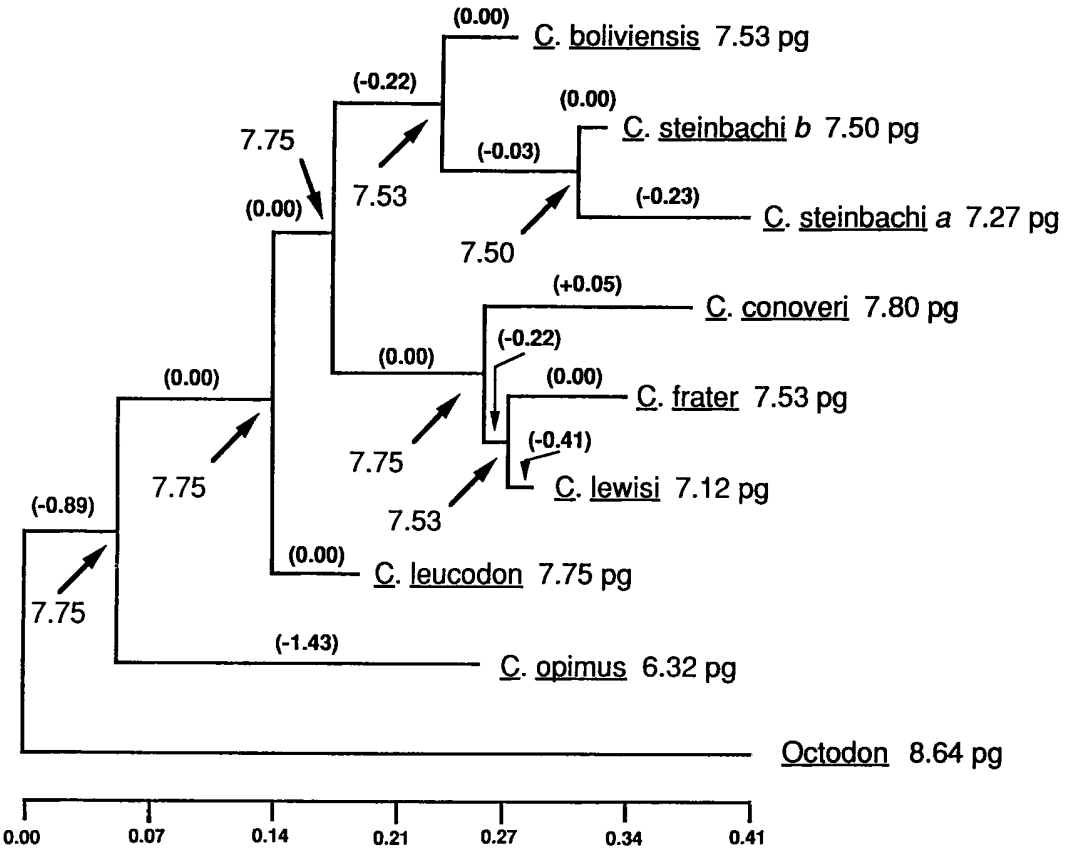

FIG. 4. Genome size change in each phylogenetic lineage of *Ctenomys* (lineages inferred from allozyme variation) as revealed by the HTU optimization procedure of Farris (1970; applied *sensu* Sessions and Larson 1987). Terminal genome size values are averaged within a species (except in *C. steinbachi*, see text). The nodal values are inferred by comparison with the closest outgroup, thereby yielding an estimate of genome size change along each lineage (values in parentheses), demonstrating that the history of genome size change in the *Ctenomys* species examined is one of loss of DNA.

valuable because it allows direct comparison of data sets from the same individuals that have been analyzed in the same manner.

Results

Tests of normality carried out within species among non-significantly different groups of species indicated that the distribution of genome size values throughout were generally normally distributed. A two-way analysis of variance indicated there were significant differences among the means of the species examined ($P < 0.0001$). Three different species groups were subsequently determined to exist by a Student–Newman–Keuls multiple range test (Table 1). Tests of normality, g_1, and g_2 indices in all multispecies assemblages were carried out on the rankit values of the individuals. The first group included only *C. opimus*. The second group was composed of *C. boliviensis*, *C. frater*, *C. lewisi*, and *C. steinbachi*. Distribution of genome size values within this group was normal ($P > 0.05$), and g_1 and g_2 indices were not significant. There was considerable overlap between components of the second and third group, which included *C. boliviensis*, *C. conoveri*, *C. frater*, *C. leucodon*, and *C. steinbachi*. Not surprisingly, distribution of genome size values in this latter group were also found to be normal ($P > 0.05$), as were g_1 and g_2 indices. The last test of normality was carried out on the rankit values across all ctenomyid species. The distribution of rankit values was abnormal ($P < 0.01$) and skewed right ($0.01 > P > 0.001$), probably owing to the contribution of *C. opimus* (Fig. 2). The mean genome size across all ctenomyids examined, summarized in Table 1, was 7.19 pg DNA (SD = 0.62, range 5.23–8.38 pg). Because the *O. degus* sampled originated from a laboratory colony, the low coefficient of variation among individuals of this species might be the result of inbreeding, reflecting the heritability of genome size (Price et al. 1983).

Diploid number ($2n$) was available for 47 of the *Ctenomys* examined in this paper (9 *C. boliviensis*, $2n$ = 45–46; 3 *C. conoveri*, $2n$ = 48; 5 *C. frater*, $2n$ = 52; 3 *C. leucodon*, $2n$ = 35–36; 4 *C. lewisi*, $2n$ = 56; 12 *C. opimus*, $2n$ = 26; 11 *C. steinbachi*, $2n$ = 10). A correlation between genome size and diploid number was not significant.

The GSD_k and GSD_{kl} values (Table 2) are graphically represented using a phenogram generated using the unweighted pair group method of averages (UPGMA; Fig. 3). It can be appreciated that the within-species variation in groups "a" and "b" are as great as or greater than between species variation, thus confirming the results of the multiple range test; only *C. opimus* greatly differs from remaining *Ctenomys*. It can also be appreciated from Fig. 3 that genome size has a certain heuristic taxonomic value: only one species, *C. leucodon*, is misplaced relative to its position as determined by allozyme variation (Cook 1990).

The HTU optimization (Fig. 4) shows that the history of genome size in Ctenomyidae has been one of loss of DNA from a presumed ancestral condition of 7.75 pg DNA, inferred from the 8.64 pg of *O. degus*, a member of the closely related Octodontidae (Cook 1990). A weak negative correlation (r = -0.28; P = 0.05) exists between branch length inferred from allozyme variation and genome size change.

Discussion

Within a single phylogenetic lineage, the genus *Ctenomys*, we have examined the relationship between genome size and extreme chromosomal evolution. The demonstration of a lack of correlation between these parameters within a single monophyletic group eliminates the confounding effect of different phyletic histories on the evolution of these characters and provides convincing evidence that genome size and chromosomal evolution are not related.

The intriguing correlation between elevated levels of chromosomal variability and the subterranean lifestyle as summarized by Nevo (1979) has been further examined in this study of genome size and chromosomal evolution in tuco-tucos. Our results differ dramatically from those of Sherwood and Patton (1982), who found two distinctly evolving lineages within the geomyid rodent *Thomomys*. The *Thomomys talpoides* species group was characterized by low genome sizes and largely Robertsonian rearrangements among species. The *T. bottae* species group was characterized by large genome sizes and primarily non-Robertsonian rearrangements. *Thomomys bottae* group species showed large amounts of inter- and intra-specific genome size variation, while *T. talpoides* group species hardly differed. In contrast, *Ctenomys* appear to show large amounts of both Robertsonian and non-Robertsonian rearrangements (Cook 1990), intermediate genome sizes, and little inter- and intra-specific genome size variation (with the exception of *C. opimus*). Upon closer scrutiny of various genetic parameters of the subterranean taxa, investigators have discovered considerable latitude in the actual mechanisms that produce these superficially convergent systems (Sage et al. 1986; Nevo and Reig 1990).

Genome size has been found to be correlated with a number of traits, including the volume and mass of metaphase chromosomes, nucleoli, nuclei, and cells; content of nuclear RNA and protein; length and duration of the cell cycle, meiosis, and DNA synthesis (S-phase); development rate and regeneration rate in amphibians; and seed weight, pollen maturation time, and minimum generation time in plants (Bachmann et al. 1979; Cavalier-Smith 1982, 1985*a*; Olmo 1983; Sessions and Larson 1987). Hypotheses attempting to explain the variation in, and the evolution of, genome size fall into adaptive and nonadaptive categories (Cavalier-Smith 1985*a*; Sessions and Larson 1987).

The hypotheses of genome size as selectively neutral "junk DNA" (Ohno 1972) hold that DNA increases by the spread of noncoding DNA sequences in the genome through duplication, unequal crossing-over, or other mechanisms of intragenomic selection (Cavalier-Smith 1980; Doolittle and Sapienza 1980; Orgel and Crick 1980; Doolittle 1982, 1985). In contrast, the adaptive hypotheses state that genome size is an adaptive trait adjusted to an optimum by natural selection (Bennett 1972, 1973, 1976, 1977, 1982; Stebbins 1976; Morescalchi and Olmo 1982; Cavalier-Smith 1985*b*, 1985*c*). Tests of these hypotheses in vertebrate (Sessions and Larson 1987) and invertebrate (Ferrari and Rai 1989) animals have been inconclusive.

The tests of normality within and among the seven species of *Ctenomys* examined in this study can be interpreted as indicating that two phenomena are at work affecting genome size. If genome size distribution in a species or group of species varies in accordance with the precepts of the normal probability density function, it follows that the factors affecting genome size do so as well (Gold and Amemiya 1987). This means that genome size is affected by many small changes (gains or losses), that these changes are independent in occurrence, and that their effects are additive (Sokal and Rohlf 1981). However, two patterns were found in the Ctenomyidae studied. *Ctenomys steinbachi* showed a nonnormal distribution of genome size values; the remaining species examined all had normally distributed genome size values. While considering that g_1 and g_2 indices are extremely sensitive to sample size, this finding suggests that most *Ctenomys* species studied show a normal distribution of genome size values.

Within nonsignificantly differing species groups, genome size distribution appeared continuous and overlapping, again suggesting that differences among these species accrue by small, cumulative change (Gold and Amemiya 1987). This observation is hard to reconcile with the parallel observation of phenomenal diploid number variation in the species studied herein ($2n$ = 10–56). This variation involves both numerical and structural chromosomal changes (Cook et al. 1990).

The single exception to continuous variation in genome size within ctenomyids is *C. opimus*, which differs significantly ($P < 0.05$) from remaining species. With the inclusion of *C. opimus*, the distribution of genome-size values appears bimodal (Fig. 2). Indeed, the test of normality carried out on rankit values across all species of *Ctenomys* in this study indicates that the distribution of values is abnormal ($P < 0.01$) and significantly skewed toward higher values ($P < 0.01$), no doubt because of *C. opimus*. Although extinct or extant species of intermediate or unknown genome size cannot be ruled out, the presence of a quantum break between *C. opimus* and the remaining *Ctenomys* species is difficult to explain by the precepts of the normal probability density function, suggesting the causes of this difference are other than small, cumulative change.

Ctenomys opimus shows marked differences to other ctenomyids examined in additional aspects of its genome organization. As resolved by allozyme electrophoresis, *C. opimus* represents the most divergent ctenomyid species (Cook 1990). *Ctenomys opimus* has also retained the large submetacentric X and small Y chromosomes characteristic of octodontids (Gallardo 1991). Finally, repetitive DNA sequences in ctenomyids were studied by Rossi et al. (1990). A 337 bp long major satellite, named RPCS (repetitive *Pvu*II *Ctenomys* sequence), was found in 12 of 13 *Ctenomys* examined, the exception being *C. opimus* (Rossi et al. 1990). The absence of RPCS points not only to a different genome organization in *C. opimus* in contrast with remaining ctenomyids, but is also immediately suggestive of an explanation for the reduced genome size of *C. opimus* relative to remaining *Ctenomys* species, although the other Bolivian species were not included in Rossi et al. (1990). All the data therefore point to a large degree of difference between *C. opimus* and remaining ctenomyids.

An additional consideration of this study regards the presence of a large amount of intraspecific variation. The original precept that there is a taxonomic level constancy in genome size, that is, a "mammal DNA content" (Ohno et al. 1967; Ohno 1970; Bachmann et al. 1971; Bachmann 1972), although clearly refutable, has recently been typologically extended to a broad spectrum of species (Cavalier-Smith 1985*a*). As is evidenced by any of the *Ctenomys* species or *Thomomys bottae* (Sherwood and Patton 1982), one must be wary of stating unconditionally that the genome size of a given species is absolute.

Although caution must be exercised in the interpretation of correlation analyses (Hale 1989), the results of the analysis between branch length, as inferred from allozyme variation and genome size change, are rather provocative. The fact that there exists a weak, but significant, negative correlation between the two suggests that allozyme evolution proceeds preferentially in the absence of genome size change, implying that there may be a link between the two phenomena.

Within species of the genus *Ctenomys*, genome size varies in accordance with the postulates of the normal probability density function. This suggests that changes in genome size are affected by several factors, that the factors effecting changes in genome size are independent, and that the effects of the various factors are additive (Sokal and Rohlf 1981). This is not surprising in view of previous studies of genome size data (Gold and Amemiya 1987). There are, however, discrete differences separating some taxa (i.e., *C. opimus* from the remaining species of *Ctenomys*), as well as taxa that show an abnormal distribution of intraspecific values (i.e., *C. steinbachi*). This study shows that variation in genome size is a ubiquitous phenomenon. Whether or not changes in the genome size of an organism are of an adaptive nature remains an open question; a comprehensive model of genome evolution, therefore, remains elusive.

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