Original article

Genome size in *Calomys laucha* and *Calomys musculinus* (Rodentia, Cricetidae)

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Summary – The DNA content of 2 related species, *Calomys laucha* Thomas (C l) ($2n = 64$, fundamental number = 74) and *Calomys musculinus* Fisher (C m) ($2n = 38$, fundamental number = 62) was studied using Feulgen microdensitometry using *Mus domesticus* as a control. Amounts of (haploid) DNA in the 2 species were significantly different (Cl: 6.940 pg; Cm: 6.202 pg; $P < 0.05$). The results were analyzed in relation to: the total diploid karyotype length measured from synaptonemal complexes with a light microscope (Cl: 735.55 μm; Cm: 446.30 μm; $P < 0.0001$) and from mitotic metaphase chromosomes (Cl: 222.074 μm; Cm: 102.651 μm; $P < 0.0001$), the metacentric-submetacentric autosome number (Cl: 8; Cm: 22) and the area of chromocenters showing positive staining with C-banding technique (heterochromatin) (Cl: 100%; Cm: 99.66%).

The DNA amount in pg per unit length of karyotype (measured from synaptonemal complexes) is higher in *Calomys musculinus* (0.028 pg/μm) than in *Calomys laucha* (0.019 pg/μm). This indicates that there is not a constant amount of DNA associated with a given length of karyotype, which suggests that the difference between the 2 species may involve differential packing of DNA. This could be due to: genic differences; differential interactions between genes and the cellular environment; and/or alteration of gene expression following the formation of new linkage groups due to chromosomal rearrangements.

Calomys musculinus / *Calomys laucha* / C-value / karyotype length / synaptonemal complex

Résumé – **Taille du génome chez *Calomys laucha* et *Calomys musculinus* (Rongeurs, Cricétidés).** *La teneur en ADN de 2 espèces apparentées Calomys laucha Thomas (Cl $2n = 64$, nombre fondamental = 74) et Calomys musculinus Fisher (Cm $2n = 38$, nombre fondamental = 62) a été étudiée par microdensitométrie avec Feulgen, en utilisant* Mus

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domesticus *comme témoin. Les teneurs en ADN (haploïde) des 2 espèces ont montré des différences significatives (Cl = 6,940 pg; Cm = 6,202 pg; P < 0,05). Les résultats ont été analysés en relation avec : la longueur totale (diploïde) du caryotype, mesurée en microscopie optique à partir des complexes synaptonémiques (Cl : 735,55 µm; Cm : 446,30 µm; P < 0,0001) et à partir des chromosomes en métaphase (Cl : 222,07 µm; Cm : 102,65 µm; P < 0,000 1), le nombre des autosomes métacentriques-submétacentriques (Cl : 8; Cm : 22) et la surface des chromocentres montrant une coloration positive avec la technique de bande C (hétérochromatine) (Cl : 100%; Cm : 99,66%).*

La quantité d'ADN exprimée en picogrammes par unité de longueur du caryotype (mesurée sur les complexes synaptonémiques) est plus élevée chez Calomys musculinus (0,028 pg/µm) que chez Calomys laucha (0,019 pg/µm). Cela indique et suggère que la différence entre ces 2 espèces pourrait impliquer un empaquetage différent de l'ADN. Cela pourrait être dû à des différences géniques, des interactions différentielles entre les gènes et le milieu cellulaire, et/ou des expressions de gènes modifiées suite à la formation de nouveaux groupes de liaison par suite de remaniements chromosomiques.

Calomys musculinus / Calomys laucha / valeur-C / longueur du caryotype / complexe synaptonémique

INTRODUCTION

The genus *Calomys* (Phyllotinae) has not been completely studied and the taxonomical and phylogenetical relationships between its species are still somewhat uncertain (Cabrera, 1961; Hershkovitz, 1962; Reig, 1984). The ancestral karyotype of the Phyllotinae is $2n = 70$, fundamental number (NF) = 68, most of the chromosomes being acrocentric (Pearson and Patton 1976). The present species of *Calomys* exhibit a range of chromosome numbers from $2n = 64$ (NF = 68) to $2n = 36$ (NF = 68) (Hurtado de Catalfo and Waimberg, 1974; Lisanti *et al*, 1976; Pearson and Patton, 1976; Gardenal *et al*, 1977; Forcone *et al*, 1980).

C laucha and *C musculinus* are 2 cricetid rodents, significant from a health point of view because they are vectors of the Junin virus which causes the Argentine haemorrhagic fever (Gardenal *et al*, 1977). These 2 species are synmorphic and sympatric species (Hershkovitz, 1962; Massoia *et al*, 1968; Gardenal *et al*, 1977; Reig, 1984) and it is very difficult to differentiate them in the field. However, they present very distinctive karyotypes since *C laucha* has $2n = 64$ (NF = 74) and *C musculinus* has $2n = 38$ (NF = 62) chromosomes (Pearson and Patton, 1976; Gardenal *et al*, 1977; Ciccioli, 1988, 1991).

The main mechanism involved in the chromosomal evolution of rodents is that of Robertsonian fusions (White, 1973; Capanna *et al*, 1976; Gropp and Winking, 1981). Although this mechanism would be the most parsimonious explanation in *Calomys* (Pearson and Patton 1976), other types of rearrangements are needed to explain the karyotype change between the 2 species, such as superimposed pericentric inversions (Forcone *et al*, 1980; Ciccioli, 1991). In *C musculinus* a double centromeric region was observed by electron microscopy (EM) on synaptonemal complexes (SC) (Ciccioli, 1991) in *ca* 12 of a total of 19 bivalents (Ciccioli and Rahn, 1984; Ciccioli, in preparation). Moens (1978) observed in *Neopodismopsis* that each of 2 submetacentric Robertsonian fusion have "a centric knob which is

double in size and structure". In house mice, Redi *et al* (1986) said that "it has been inferred (Gropp and Winking, 1981) that Rb translocation occurs with loss of the 2 shortest arms of the acrocentrics involved in translocation, probably followed by functional inactivation of a centromere (Hsu *et al*, 1975)". This mechanism does not necessarily imply a quantitative variation in total DNA content since in *Mus poschiavinus* there is apparently little or no quantitative change in genome size (C-value) (Manfredi-Romanini *et al*, 1971; Comings and Avelino, 1972; Redi *et al*, 1986).

The difference in genome size between *C laucha* and *C musculinus* are studied by Feulgen microdensitometry in the present paper. The aim of this work is focused on the relationships between DNA content and the total karyotype length (TKL) (measured on synaptonemal complexes (SC) and on metaphase mitotic chromosomes (MMC)), as well as other nucleotypical parameters (Bennett, 1987; Grant, 1987). Moreover, additional information on genome size will be discussed.

MATERIALS AND METHODS

Six male individuals from Laguna Larga (Province of Córdoba, Argentina) of each species (*C laucha* and *C musculinus*) and one individual of *Mus domesticus* (Province of Buenos Aires) were studied.

DNA content measurements (Feulgen microdensitometry)

Slides were prepared by dispersion and air-drying from specimens which had not been pretreated. In *C musculinus*, slides of meiosis (from testis) and mitosis (from bone marrow) were made. In *C laucha* and *M domesticus*, the same procedure was followed using only bone marrow.

Hydrolysis was carried out with 5 N HCl at 20°C. Different times of hydrolysis were tested (10, 20, 30, 35, 40, 50 and 60 min) and hydrolysis curves were determined. After hydrolysis, the slides were rinsed 3 times with distilled water for 10 min each.

Staining was carried out with Feulgen stain at pH 2.2 for 2 h. Slides were rinsed 3 times in SO_2–water for 10 min each time, and then in distilled water (10 min).

The slides were air-dried in the dark and mounted in Euparal.

In slides of testes, measurements were made on spermatids and sperm, and lymphocytes were measured in slides of bone marrow. The values obtained were expressed in arbitrary units (AU), or in absolute units (pg of DNA) using *Mus domesticus* as a control, the DNA content of which is known by chemical methods (C = 7 pg (Lewin, 1980)).

To ensure the accuracy of the measurements in the case of *C musculinus*, the relationships between DNA content measurements at prophase (4C) and telophase (2C) was checked to correspond to the ratio 2:1 in mitotic lymphocytes (AU 35.23 and AU 16.85 respectively) and 4:1 in prophase I (4C) and telophase II (1C) of meiotic cells (AU 36.24; AU 9.63).

The amount of Feulgen staining per nucleus was measured at a wavelength of 570 nm using the scanning method in a Zeiss Cytoscan. In both species, the readings were made in the same individuals in which synaptonemal complexes were

measured. The differences in DNA content between species were tested with a Student t-test.

Synaptonemal complexes using light microscopy (LM)

Synaptonemal complexes (SC) were studied using the method described by Solari (1983) for electron microscopy, adapted by modifying the stain to 50% (W/V) of $AgNO_3$ in distilled water. Two or 3 drops of silver nitrate solution were placed on previously air-dried slides. Floating coverslips were put on the slides, which were incubated in a moist chamber at 60°C for 3–5h. Staining was monitored under a phase contrast objective until yellowish pachytene nuclei were seen with dark brown SCs. The process was stopped by washing with distilled water. Slides were air-dried and mounted in DEPEX.

The total karyotype length (TKL) was measured from optical micro-photographs. Five mid-pachytene nuclei were measured for each species. The length of the SC was measured 3 times and an average value was determined for each autosomic bivalent. The same procedure was followed for the lateral elements of the X and Y chromosomes. The TKL (SC) was calculated by doubling the average value for each autosome and adding that of the lateral elements of the sexual pair. All measurements were carried out using a Mini-Mop (Kontron) Image Analyzer. The differences in TKL length measured on SC (LM) between species were tested with a Student t-test.

Conventional karyotypes

The animals were injected with a yeast solution on 2 successive days to increase the mitotic index (Lee and Elder, 1980). On the third day they were injected with a colchicine solution (0.0025%). Two hours later, they were etherized and the bone marrow extracted according to routine techniques (Evans *et al*, 1964). The preparations were made by dispersion and air-drying.

The karyotypes were described according to the nomenclature proposed by Levan *et al* (1964) (m, sm and st: chromosomes with centromeres in the median, submedian and subterminal region, respectively). The average centromeric indexes, short arms, long arms, total chromosome length and chromatid width were measured and calculated in 3 cells. The total chromosome volume (TCV) was obtained by considering each chromosome as 2 cylinders. The formula used was $(\widetilde{\Pi} \times r^2 \times h) \times 2$ (r = half the chromatid width; h = chromosome length). Measurements were carried out using a Mini-Mop (Kontron) Image Analyzer. The differences in TKL length on mitotic metaphase chromosomes between species were tested with an approximate Student t-test (Games and Howell, 1976), on the assumption of heterogeneity of variances (Sokal and Rohlf, 1981).

C-banding

The C-banding technique was performed on conventionally prepared slides as follows: a) 60% acetic acid for 30 min; b) 0.2 N HCl for 1 h; c) solution $(OH)_2$ Ba sat in distilled water, 12-15 min at 20°C; d) 2 × SSC for 45–60 min at 60°C;

e) 2% Giemsa in buffer phosphate ph 6.8 for 10–12 min. The heterochromatin area per interphase nucleus was obtained by measuring the area of each C positive chromocenter within each nucleus. The total area of chromocenters from 10 nuclei was averaged in each species. The values obtained are expressed in table I where *C laucha* is given the 100% value. The measurements were carried out using a Mini-Mop (Kontron) Image Analyzer, working with photomicrographs with similar exposure time, development procedure and enlargement in both species.

Table I.

Species	C laucha	C musculinus
2*n*	64	38
NF	74	62
m − sm autosome number	8	22
DNA content C(pg); $\overline{X} \pm SE$	6.940 ± 0.21	6.202 ± 0.20
Sample size	18	30
t-test value, significance	$t = 2.331, P < 0.05$	
TKL (μm) (SC) $\overline{X} \pm SE$	735.55 ± 32.50	446.30 ± 17.02
Sample size	5	5
t-test value, significance	$t = 7.88, P < 0.0001$	
TKL (μm) (MMC) $\overline{X} \pm SE$	222.074 ± 0.923	102.651 ± 3.068
Sample size	5	5
t-test value, significance	$t = 37.27, P < 0.0001$	
DNA (2C;pg)/TKL SC (μm)	0.019	0.028
NA (%)	100	54.43
HA (%)	100	99.66

2*n*: diploid number. NF: fundamental number. TKL: total karyotype length. SC: synaptonemal complex. MMC: mitotic metaphase chromosome. TCV: total chromosomal volume. NA: nuclear area. HA: heterochromatin C(+) area. m: chromosomes with centromere in the median region. sm: chromosomes with centromeres in the submedian region. SE: standard error.

RESULTS AND DISCUSSION

The karyotype of *C laucha* (2*n* = 64; NF = 74) comprised 8 m–sm (pairs 1–4) + 54 st–t + X (m) Y (m) (fig 1A). *C musculinus* (2*n* = 38; NF = 62) had a karyotype with 22 m–sm (pairs 1 to 11) + 14 st + X(m) Y (m) (fig 1B).

The species were measured at their optimum hydrolysis time, *ie*: *C laucha*: lymphocytes 30 min; *C musculinus*: spermatids 35 min, sperm 40 min and lymphocytes 35 min (fig 2). The differences related to hydrolysis time and DNA content observed
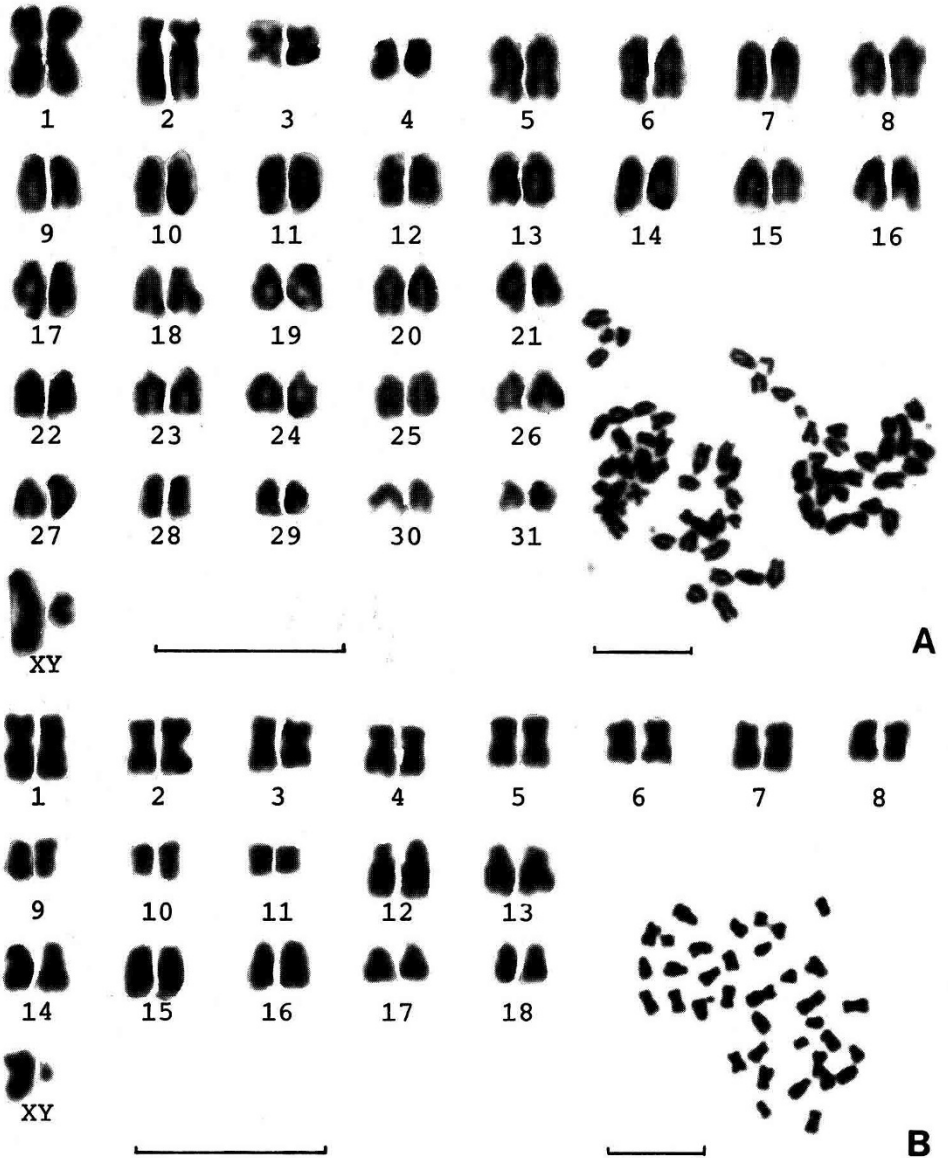
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Fig 1. Mitotic metaphase karyotypes. (A) *C laucha*; (B) *C musculinus*. The scale represents 10 μm.

between spermatids and sperm in *C musculinus* (arbitrary units) are remarkable and may be explained by the higher degree of chromatin condensation in sperm. This could be due to the chromatin condensation gradient which could have reduced the possibility of eliminating the depurinated DNA fragments during acid
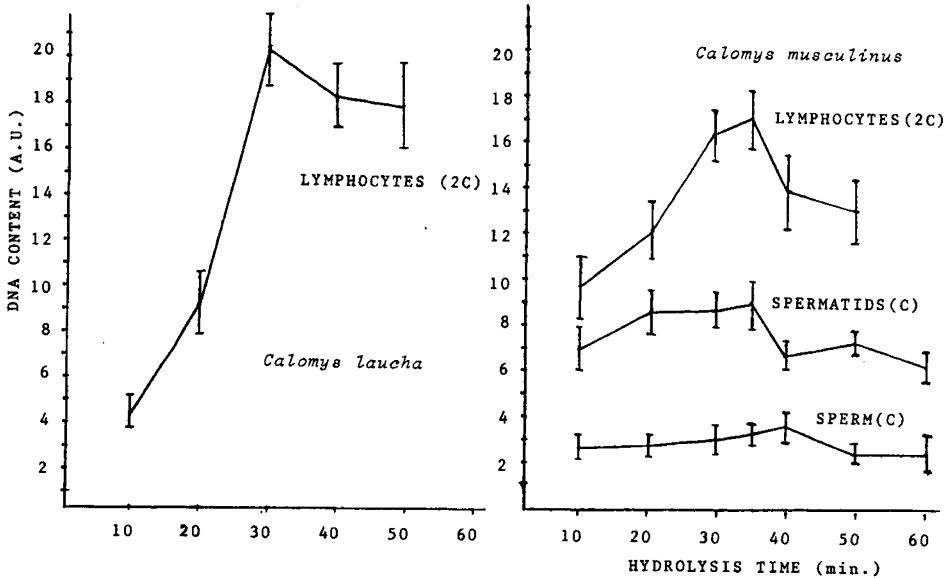
Fig 2. Hydrolysis curves in *Calomys laucha* (lymphocytes 2C) and *Calomys musculinus* (lymphocytes 2C, spermatids C, sperm C).

hydrolysis (Holmquist, 1979). This could also explain the small differences found in the optimum hydrolysis time between both species.

Table I shows the DNA content expressed in absolute values (pg) in both species. The differences in C-values between *C musculinus* and *C laucha* were significant: t $(46) = 2.331$, $P = 0.0226$) (Bartlett test for homogeneity of variances, $\chi^2 = 1.8877$, DF = 1, $P = 0.1656$). The DNA content and TKL presented a positive relationship with chromosome number (table I).

Synaptonemal complexes in mid-pachytene nuclei of *C laucha* and *C musculinus* prepared for the light microscope (LM) are shown in figure 3. The difference in the total karyotype length (TKL) between *C laucha* and *C musculinus*, as measured from SCs, was highly significant: $t(8) = 7.88$, $P = 0.000076$ (Bartlett test for homogeneity of variances $\chi^2 = 1.3288$, DF = 1, $P = 0.2475$) (table I).

Comparisons of TKL based on SC measurement can be inaccurate, because of at least 2 possible sources of error (Anderson *et al*, 1985). One is the biological variability among different substages of pachytene. This variation must be discarded in the present study because only nuclei in mid-pachytene were chosen. The other is the physical stretching of SCs during dispersion in the hypotonic hypophase. In the present work, those nuclei which showed evidence of stretching were discarded. Still another source of error, when different species are compared, involves the quantity of heterochromatin. Compared to euchromatin, heterochromatin is, on average, 2 to 5 times under-represented in the length of pachytene chromosomes, due to its different condensation state in pachytene and metaphase (Stack 1984).

In both species of *Calomys* C-banding revealed that the amount of heterochromatin measured in interphasic C+ chromocenters is similar (*Calomys laucha* =
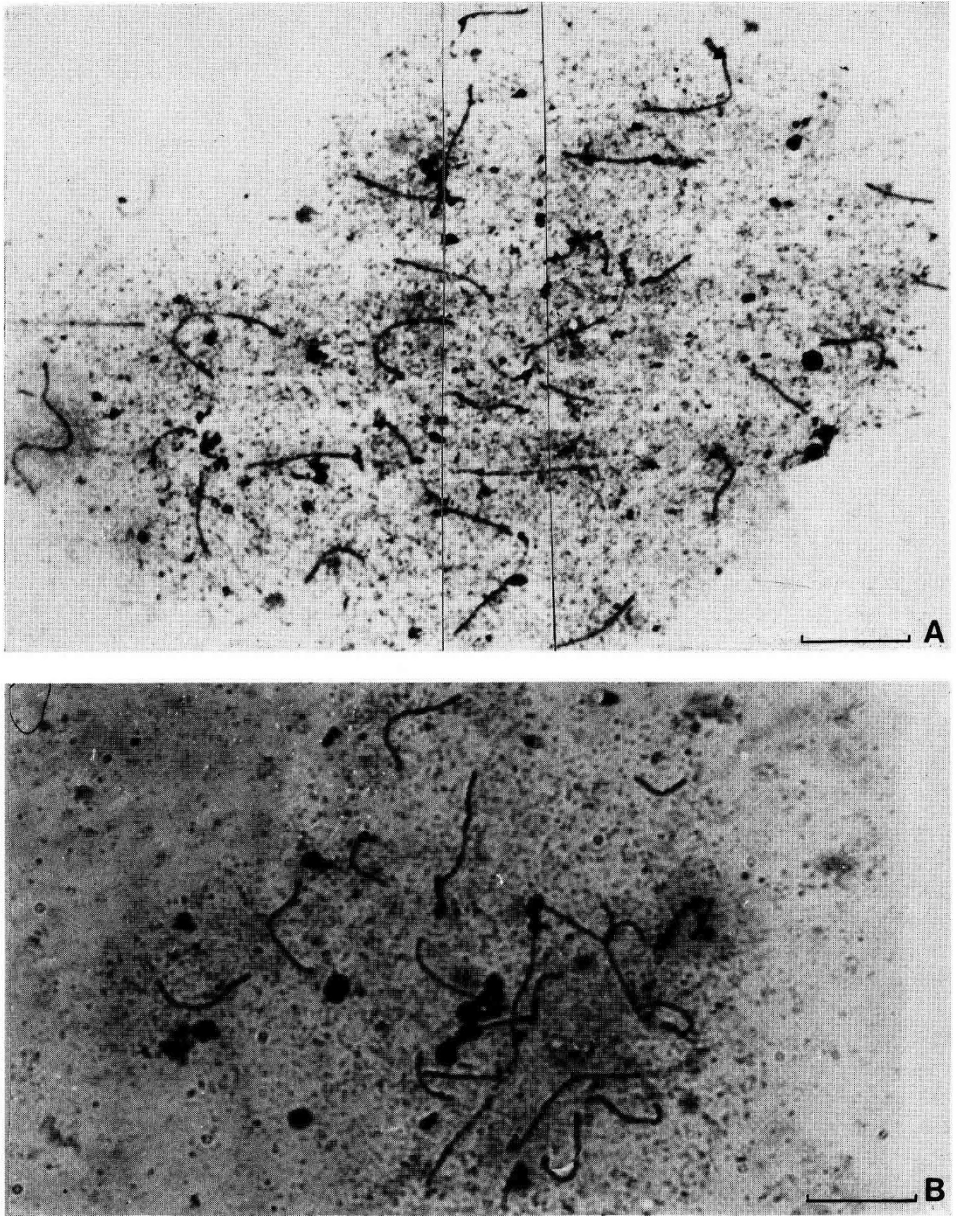
Fig 3. Synaptonemal complexes (SC) under LM. (A) *C laucha* ($2n = 64$). (B) *C musculinus* ($2n = 38$). The scale represents 10 μm.

100%; *Calomys musculinus* = 99.66%) (table I). Thus, the effect of differences in the quantity of heterochromatin on SC length between the species is negligble. The heterochromatin was expressed in absolute value and not in relation to the nuclear area of lymphocytes since both species differ significantly in this parameter (Cl = 100%, Cm = 54.43%). This difference follows the same pattern of variation as the TKL. Such differences in TKL measured on mitotic metaphase chromosomes are larger than those found on SCs (table I). Differences in the TKL between *C laucha* and *C musculinus* measured on mitotic metaphase chromosomes was highly significant: $t'(5) = 37.27$, DF $= 4.72 \approx 5$, $P = 0.00002$ (Bartlett test for heterogeneity of variances $\chi^2 = 4.2227$, DF $= 1$, $P = 0.0375$). The use of mitotic arresting agents such as colchicine may also lead to errors because they produce dose-related variation in chromatin contraction. In the present work, however, this should not be an important source of error since the same dose concentration and exposure were used during the experiments.

Variations in TKL for both sets of data (SC, MMC) show the same pattern of variation which suggests that the highly significant differences between the species are not an artefact but have a high genetic component.

Anderson *et al* (1985) showed that there is a strong correlation between TKL (SC length) and genome size in higher plants, indicating that a constant amount of DNA is associated with a given length of SC, at least when averaged over the whole genome. Whereas this statement is convincing when species within the same genus are compared (*eg Allium*) indicating that they have a similar chromosomal organization, the range in variation in DNA content per unit of SC length is much larger when other genera are included (*eg Solanum sparsipilum*). *C laucha* and *C musculinus* also present a positive relationships between DNA content and SC length. However, in spite of being closely related species, the TKL of *C musculinus* is 39.3% (SC) and 54.3% (MMC) lower than that of *C laucha*, while the difference in DNA content is only 10.6%. It may be worth mentioning that the difference of chromosome volume in mitotic chromosomes – though not accurately measured because chromosome width was near the resolution limit of our Mini-Mop Image Analyzer – is of the same order (6.5%, Cl $= 318 \ \mu m^3$; Cm $= 297 \ \mu m^3$) as the difference in DNA content.

Consequently, the amount of DNA per unit length of karyotype is much higher in *C musculinus* than in *C laucha* (Table I), and in these species a given length of karyotype does not contain a constant amount of DNA. This may be explained by the existence of different interactions between the nucleotypical parameters such as SC length and DNA amount. This could indicate that differential packing of DNA is an important difference between the species, due to, among other reasons: a) genetic differences, b) differential interaction between genes and the cellular environment, and/or c) alteration of gene expression due to the formation of new linkage groups following chromosome rearrangements. The TKL variation pattern is similar to that of the nuclear area (table I). Cavalier-Smith (1983) states that "the nuclear volume is jointly determined by: 1) nuclear DNA content; 2) the degree of folding of the DNA and its pattern of attachment to the nuclear envelope". In the present work, the main factor responsible for the significant differences in nuclear area and TKL could be the degree of folding as suggested by the differences found in hydrolysis times between the two species, and the pattern of attachment to the

nuclear envelope, probably due to the decrease in telomere number in *Calomys musculinus*. These interactions could be considered part of the genome ecology of the genus (Bennett 1987).

ACKNOWLEDGMENTS

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