DNA Characteristics in Species of Calomys (Rodentia, Cricetidae)

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The vole mice Calomys (Cricetidae) has been suspected to be the natural reservoir of some Arenavirus such as the Junin virus ethyological agent of the endemic disease Argentine Hae-
morragic Fever (Parodi et al. 1959, Sabattini et al. 1967). Besides their sanitary importance species of the genus Calomys constitute an appropriate group for evolutionary studies on ac-
count of the systematic position they occupy.

The South American theriological fauna comprise a high percentage of rodents from which the cricetids represent about 40% of the species in the Order. Recently, the Family Cricetidae has been divided in two Subfamilies: Neotominae and Sigmodontinae, the former with pre-
ponderant North American and the latter with preponderant South American distribution. The Family Sigmodontinae is composed by seven tribes. Oryzomyni, Akodontini and Phyl-
lotini are the tribes comprising the largest number of species. Moreover, Phylotini is the most
specialized of the seven tribes and Calomys is the most generalized genus within the Phylotini
tribe (Reig 1981). Therefore, the evolutionary interest of this genus seems evident since it
constitutes the most generalized form of the most specialized tribe.

Initially, Calomys laucha was considered to have two subspecies: C. l. laucha and C. l.
musculinus. However, in the middle of the sixties Massoia et al. (1968) established the full
species status for the two forms C. musculinus and C. laucha on the basis of morphometrical
and cytotaxonomic studies. Karyotypes of the two species are clearly different. C. musculinus
has a diploid number (2n) of 38 chromosomes and large C-bands in the centromeric region of
autosomes and X chromosomes (Forcone et al. 1980). On the other hand, C. laucha lacks
C-bands and has a 2n=64 (Gardenal et al. 1977b). Moreover, allozymic pattern differences
between C. musculinus, C. laucha and C. callosus have been identified by Gardenal et al. (1977a).

The aim of this work was to characterize the DNA of C. musculinus and C. laucha. Analy-
chetical ultracentrifugation in neutral CsCl gradients allowed to determine the percentage of
GC content, no satellite DNA could be identified by this method. Thermal denaturation
studies showed that C. musculinus has a thermostable component formed by moderately to
highly repetitive DNA sequences. Additional interspecific differences came to light by the
analysis of reassociation kinetics and by DNA cleavage with restriction endonucleases.

Materials and methods

The C. musculinus and Mus musculus specimens employed were obtained from the colonies
maintained in the IMBICE. The specimens of C. laucha were captured in the field in the loca-
tiy of Pila (Buenos Aires Province, Argentina).

DNA from liver, kidney, heart and spleen tissues was prepared as described elsewhere
(Vidal-Rioja et al. 1982). DNA purity criteria were based on the spectrophotometrical ratios
A$_{260}$/A$_{280}$ 1.85 and A$_{260}$/A$_{230}$ 2.3.

Analytical ultracentrifugations were performed in a Beckman Model E centrifuge. Each
gradient containing about 4 μg DNA was adjusted to the initial density 1.700 g/ml with solid

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CsCl. Centrifugation was performed at 42040 rpm at 22°C for 24 h. Reference DNA from bacteriophage SPO-1 (1.742 g/ml buoyant density) was included in each gradient. Densitometric tracings of the ultraviolet photographs were obtained in a Beckman Analytrol. Buoyant densities were calculated as described by Mandel et al. (1968) and the GC content by the Schildkraut equation.

Thermal denaturation assays were performed in a Gilford 250 spectrophotometer supplied with thermoprogrammer. The temperature increment applied to the samples was 0.5°C/min. DNA samples were dissolved in either 1 x or 0.1 x SSC (SSC=0.15 M ClNa, 0.015 M sodium citrate). M. musculus DNA and the synthetic polynucleotide Poly dAT (Sigma) were employed as standards. Melting temperature (Tm) and GC content were calculated from the integral (experimental) curve according to Mandel and Marmur (1968). Thermal subtransitions were visualized by the numerical derivative curve from the experimental data. The numerical derivation is based on the fitting of five successive experimental points to a quadratic polynomial \( A = a + bT + cT^2 \), where A is absorbance, T temperature and a, b, c the polynomial parameters. The determination of the parameters a, b and c was achieved by the method of the least squares and finally the derivative with respect to b was obtained (Spiridonov and Lopatkin 1973).

Reassociation kinetic studies were performed on DNA samples reduced to 400-500 base pairs (bp) in length by ultrasound. The fragment size was determined by agarose gel electrophoresis. Samples were denatured at 100°C for 7 min. Incubations were performed at 60°C in the case of samples dissolved in 0.12 M phosphate buffer (PB), and at 68°C for samples in 0.48 M PB. The range of Cot (Co concentration of nucleotides moles per liter and t time in seconds (Britten and Kohne 1968)) analysed was from \( 10^{-2} \) to \( 10^4 \). Single and double stranded DNA fragments were separated by hydroxylapatite (HTP DNA-grade, Bio-Rad) chromatography at 60°C. The DNA concentration in the eluted fractions was determined by readings at \( A_{260} \). In order to detect the number and characteristics of the kinetics components the experimental data were fitted to an ideal multicomponent curve according to the procedure of Ordahl (1977).

In order to obtain fractions with different degree of thermostability sonicated C. musculinus DNA (2 mg) dissolved in 0.12 M PB was poured onto a HTP column equilibrated at 50°C and then chromatographed at increasing temperatures. In the range 50-89°C the column temperature was incremented by 5°C steps and in the range 89-98°C the increments were reduced to 1°C steps.

Results

The buoyant density of the DNAs from six C. musculinus and five C. laucha was determined. The DNA of both species exhibited a unique and homogeneous banding pattern; neither shoulders nor discrete satellite DNA peaks were found (Fig. 1). In both species buoyant density values were 1.701 ±0.001 g/ml, corresponding to a GC content of 41.8 ±0.2%. M. musculus DNA, used as standard showed banding profiles, buoyant density and GC content identical to those reported previously (Bond et al. 1967).

Tm values and GC content of DNA from both Calomys species and also from standards are shown in Table 1. Values are similar for the two Calomys and for the M. musculus DNA used as standard. Nevertheless, the observation of the curves either experimental or derivative, reveals an interspecies difference (Fig. 2). Using 1 x SSC as solvent the C. musculinus DNA shows a thermal subtransition with an individual melting temperature (Tm,i) of 94°C, in the experimental curve, and a discrete peak, at the same temperature in the derivative curve. The area of the thermostable peak under the derivative curve amounts to about 9% of the total
melting derivative area. To corroborate this observation and to rule out the possible artifactual effect of evaporation of the solvent thermal denaturations were carried out in 0.1×SSC. Such as expected, Tm values decrease 15.4°C under this conditions (Mandel and Marmur 1968). Therefore, the presence of the thermostable component of C. musculinus DNA is confirmed. The same analysis performed with DNA isolated from purified nuclei shows the same results.

Figs. 1-2. 1, CsCl ultracentrifugation profiles and buoyant densities of total DNA of Calomys musculinus (a) and Calomys laucha (b). In both tracings the reference DNA is SPO-I bacteriophage (1.742 g/ml). 2, integral (●) and derivative (○) thermal denaturation curves of total DNA. Solvent 0.1×SSC.

Figs. 3-5. 3-4, reassociation kinetics of the DNA of Calomys musculinus and Calomys laucha. The solid line represents the hypothetical multicomponent second order kinetics which best fits the experimental points. 5, reassociation kinetics, spectrophotometrically monitored, of DNA fractions eluted from the genome of Calomys musculinus at 60°C, 85°C and 94°C.

discarding the possible mitochondrial nature of the thermostable component. No comparable feature is detected in C. laucha DNA.

By reassociation kinetic studies it is possible to identify four components in the DNA of both Calomys species as well as in the M. musculus DNA used as standard. Figures 3 and 4 show the DNA reassociation curves of each Calomys DNA; Table 2 summarizes the percentage
of each component, the Cot 1/2 value and the degree of reiteration of the sequences. *C. musculinus* and *C. laucha* present a rapid reassociation component amounting 9 and 6% of the haploid genome, respectively. This component reassociates at Cot 10^{-2} and probably includes palindromic and highly repetitive sequences. Sequences reassociating at 10^{-2} to 10 Cot values were identified as the Intermediate I component. Intermediate I comprises 12% of the *C. musculinus* and 14% of the *C. laucha* haploid genome. Moreover, copy number of this component was twice as large in *C. musculinus* than in *C. laucha*. The third component, Intermediate II reassociates between 10^0-10^1 Cot values. It represents 10% and 17% of *C. musculinus* and *C. laucha* haploid genome, respectively. Moreover, Intermediate II has less than 100 copies in both species. The Slow component has Cot values above 10^9. Cot 1/2 and percentage of the haploid genome included in the Slow component are 2200 and 69% for *C. musculinus* and 1500 and 63% for *C. laucha*. Our results on the reassociation kinetics of *M. musculus* DNA are, in general, in good agreement with previously published data. The main difference resides in the fact that we identified four components while other authors reported five. This difference may result from the fact that foldback and satellite DNA sequences of *M. musculus* reported in the literature as separate components are probably included in our Rapid component (Cot 10^{-2}) (Cech and Hearst 1976).

In order to detect the eventual presence of repeated DNA sequences in the thermostable *C. musculinus* DNA component, three fractions with differential thermostability were isolated (60, 85 and 94°C). After denaturation at 98°C in 0.12 M PB the reassociation kinetic of each fraction was spectrophotometrically followed. The figure 5 shows the reassociation curves of these fractions. For the same Cot value the 94°C fraction reanneals more precisely than the others. Hyperchromicity values obtained are 4.9%, 7.7% and 18.1% for the fractions eluted at 60, 85 and 94°C, respectively. Tm values for the same fractions are 75°C, 74°C and 78.1°C. Since the Tm value of the native *C. musculinus* DNA is 86°C in 0.12 M PB the Tm for the 60, 85 and 94°C fractions are 10.8°C, 12°C and 7.7°C, respectively. Tms are directly related with the extension of mismatching in reassociated DNA.

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**Table 1. Melting analysis of rodent DNAs**

<table>
<thead>
<tr>
<th>DNA</th>
<th>SSC 1× Tm °C</th>
<th>GC%</th>
<th>SSC 0.1× Tm °C</th>
<th>GC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poli dAT</td>
<td>64.6±0.5</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>M. musculus</em></td>
<td>86.2±0.7</td>
<td>41.2</td>
<td>—</td>
<td>41.7</td>
</tr>
<tr>
<td><em>C. laucha</em></td>
<td>86.2±0.3</td>
<td>41.2</td>
<td>71.0±1.2</td>
<td>41.7</td>
</tr>
<tr>
<td><em>C. musculinus</em></td>
<td>86.2±0.8</td>
<td>41.2</td>
<td>71.1±1.2</td>
<td>41.9</td>
</tr>
</tbody>
</table>

**Table 2. Kinetics components in the DNA of *C. musculinus, C. laucha* and *M. musculus***

<table>
<thead>
<tr>
<th>DNA</th>
<th>Rapid (Cot&lt; 10^{-2})*</th>
<th>%</th>
<th>Intermediate I (Cot 10^{-2}–10^0)</th>
<th>%</th>
<th>Number of copies</th>
<th>Intermediate II (Cot 10^0–10^3)</th>
<th>%</th>
<th>Number of copies</th>
<th>Slow (Cot&gt;10^3)</th>
<th>%</th>
<th>Cot 1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. musculinus</em></td>
<td>9</td>
<td>12</td>
<td>0.093</td>
<td>23</td>
<td>892</td>
<td>10</td>
<td>33</td>
<td>67</td>
<td>69</td>
<td>222</td>
<td>222</td>
</tr>
<tr>
<td><em>C. laucha</em></td>
<td>6</td>
<td>14</td>
<td>0.125</td>
<td>12</td>
<td>000</td>
<td>17</td>
<td>20</td>
<td>75</td>
<td>63</td>
<td>1500</td>
<td>500</td>
</tr>
<tr>
<td><em>M. musculus</em></td>
<td>25</td>
<td>6.5</td>
<td>0.142</td>
<td>11</td>
<td>732</td>
<td>13</td>
<td>6.25</td>
<td>267</td>
<td>55</td>
<td>1666</td>
<td>666</td>
</tr>
</tbody>
</table>

* (M. seg).

** Cot (M. seg) required for half-reassociation.
Accordingly, the lower Tm shown by the 94°C fraction indicates a more efficient reassociation probably resulting from an enrichment of repeated sequences in this fraction.

DNA digestions with Eco RI, Bam HI, Hind III, Taq I and Hae III restriction enzymes produced similar electrophoretic patterns in both Calomys species. On the other hand, Alu I produces a species specific pattern. This endonuclease generates two conspicuous bands at about 828 and 435 bp in C. musculinus and four bands of 504, 266, 209 and 153 bp in C. laucha (Fig. 6).

**Discussion**

The isopycnic centrifugation of C. musculinus and C. laucha DNAs showed similar density values and banding profiles and the lack of satellite peaks or shoulders. Thermal denaturation assays with DNAs from both species also showed equivalent Tm values yet, a thermostable component was detected in the C. musculinus DNA. Preliminary results with Southern blot assays showed hybridization of this component, used as probe, with C. musculinus but not with C. laucha DNA, this confirms the species specificity of the thermostable fraction.
The thermostable component of *C. musculinus* was found to be rich in highly repeated DNA sequences. Yet, with the methodology employed it is not possible to decide whether the foregoing component corresponds to a satellite fraction (not showing in neutral CsCl analytical ultracentrifugation) or to interspersed repeated sequences. Since *C. musculinus* has large masses of C-banding material in all autosomes and X chromosomes (Fig. 7) it is tempting to assume that the thermostable component is a satellite fraction responsible for the appearance of C-bands. This assumption, however, needs to be confirmed.

Additional differences in the *Calomys* DNAs were detected by the reassociation kinetics of whole DNA from both species. Slow component (single copy) Cot 1/2 amounted 2200 M. sec. in *C. musculinus* and 1500 M. sec. in *C. laucha* DNA. Such a result suggests a higher degree of complexity in the single copy component of the former species. Another kinetic difference resided in the repetition frequency of the Intermediate I component which has twice the copy number in *C. musculinus* than in *C. laucha* DNA.

*C. musculinus* and *C. laucha* are morphologically very similar; in fact, the state of full species of these two forms was mainly based on karyological differences (Massoia *et al.* 1968). Pearson and Patton (1976) proposed that chromosome numbers in cricetid rodents show a tendency to decrease coincidentally with the increase of species specialization. Moreover, these authors considered *C. sorellus* (2n=64) as the most generalized and primitive species of the genus. Accordingly, *C. laucha* with a diploid number of 64 seems to have conserved a primitive karyotype while *C. musculinus* chromosomes would have evolved to give a chromosome number 38. Thus, it is probable that the thermostable DNA component of *C. musculinus* (and perhaps also the other DNA differences detected) is a evolutionary novelty arising coincidentally with the changes which led to the karyotype specialization of this species.

**Summary**

The main DNA characteristics of the South American cricetid rodents *Calomys musculinus* and *C. laucha* were investigated and compared. The two DNAs were found similar in CsCl buoyant density and Tm values. Reassociation kinetics analysis showed three repetitive and one single copy DNA components in both species. Interspecific differences in the repetition frequency of Intermediate I component and in the complexity of the Slow component were revealed by the later approach. Relevant to the species difference was the finding of a high melting fraction in the DNA of *C. musculinus*. This fraction isolable by chromatographic thermal elution procedures was found to be enriched in highly repetitive DNA sequences. For each DNA specific electrophoretic patterns were obtained after Alu I digestions.

**Acknowledgements**

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**References**


