

NUCLEAR DNA CONTENT AND KARYOSYSTEMATIC RELATIONSHIPS OF SPECIES GROUPED IN PRIMITIVE TRIBES OF LEPTODACTYLIDAE (AMPHIBIA-ANURA)

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ABSTRACT

The nuclear DNA content (DNA/N), of 12 anuran species belonging to three tribes of lower Leptodactylids is shown.

The amount of DNA/N determined by cytophotometric measurements in erythrocyte interphase nuclei ranged between 14.39 pg/N in *Caudiverbera caudiverbera* ($2n = 26$) to 5.81 pg/N in *Telmatobius pefauri* ($2n = 26$).

Intergeneric DNA/N variability is higher than intrageneric variability except in *Batrachyla*.

The DNA/N amount in these species is not clearly associated with diploid chromosome number variability except in the Telmatobiini, where high diploid number species ($2n = 28, 30$), have a larger genome size.

INTRODUCTION

Most anuran species distributed in Chile, belong to the Leptodactylidae and are grouped in the Telmatobiinae. They correspond to approximately 29 species distributed in 13 genera. These species represent an old diversified group in existence since the lower Tertiary of Patagonia (Lynch, 1978).

Karyosystematic studies in a number of these species demonstrated their intra and intergeneric karyotypic variability. This suggests the hypothesis of a tendency towards a reduction of chromosome number from karyotypes of high chromosome

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number and telocentric chromosomes (Bogart, 1970; Diaz and Veloso, 1979). Chromosome rearrangements such as centric fusions and translocations are responsible for this tendency. The distributional pattern of C bands including chromosomes with whole arms completely C+, suggests that heterochromatin addition also participates as a mechanism, modifying chromosome morphology (Díaz and Veloso, 1979).

Nuclear DNA (DNA/N) comparisons in different vertebrate groups except lungfishes and urodela, show that the more advanced species in a phylogenetic sense, have more DNA than primitive ones (Bachmann *et al.*, 1975). Anuran species studied are grouped around a mode value between 9 and 10 picograms (pg) (Olmo, 1973; Bachmann and Nishioka, 1978; Olmo and Morescalchi, 1978).

Karyosystematic studies of lower Telmatobiinae have been oriented to compare diploid number and chromosome morphology between species. Genome size measurements have not been incorporated as a cytogenetic parameter in these comparisons.

In this paper, the DNA/N content of 12 species of lower leptodactylids is showed. This parameter can be useful for the phylogenetic analysis of this species group, which according to Heyer (1975), Lynch (1971, 1978) and Díaz, (1986), shares a common genetic background.

MATERIALS AND METHODS

The twelve species studied were: *Telmatobius halli* (2 females, 1 male), *T. peruvianus* (1 female, 2 males), *T. marmoratus* (2 females, 1 male) *T. pefauri* (2 females, 1 male), *Eupsophus migueli* (1 female, 2 males), *E. roseus* (2 females, 1 male), *E. vertebralis* (1 female, 2 males), *Alsodes nodosus* (2 females, 1 male), *A. tumultuosus* (1 female, 2 males), *Batrachyla taeniata* (3 males), *B. leptopus* (1 female, 2 males) and *Caudiverbera caudiverbera* (1 female, 2 males). For comparative purposes, *Bufo chilensis* was included as a control species, establishing total DNA content in 10 specimens by the Diphenylamine method (Burton, 1956).

Blood smears of each species were obtained by cardiac puncture in anesthetized specimens. Smears were fixed with Alfac for one hour followed by a 10 min. in distilled water and stained by fluorescent Feulgen, using a modified Schiff reagent (Prenna *et al.*, 1974). In each case, blood smears were submitted to hydrolysis with 5 N HCl at 20°C (Decosse and Aiello, 1966; Kjellstrand, 1980). The maximum intensity of staining, was determined by subjecting blood smears of *B. chilensis* to different hydrolysis time between 5 to 240 min. Optimum hydrolysis time was reached at 30 min. and was maintained through 240 min. According to this, the selected hydrolysis time for the other species was 30, 60 and 120 min. including always a blood smear of *B. chilensis* as a control.

Cytophotometric measurements (arbitrary units) were taken in erythrocyte interphase nuclei by a Zeiss Cytophotometer with an excitation filter of 546 nm (BP 546), a barrier filter of 590 nm (LP 590) and a selector filter of 580 nm (FT 580).

Every cytophotometric determination represents the average of measurements of at least 300 nuclei of each specimen. Absolute quantity of DNA/N in picograms per nuclei (pg/N), was estimated transforming cytophotometric arbitrary units (AU) according to: $\text{pg "unknown" species} = \text{pg/AU "reference" species} \times \text{AU "unknown" species}$ (Hatch *et al.*, 1976).

Statistical procedures were used to test normality of cytophotometric measurements. The *a posteriori* test of Student, Newman, Keuls (Sokal and Rohlf, 1969) was performed to estimate mean DNA/n species differences.

All studied specimens are located in the Colección Herpetológica (DBGUCH), Unidad de Biología de Vertebrados, Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile.

RESULTS

The DNA/N content of *B. chilensis* measured by cytophotometry was 62.48 ± 1.039 AU and its absolute quantity of DNA determined by the Diphenylamine method 11.32 pg/N. This last value is in the range of previous DNA determinations for the genus (Bachmann, 1970).

The amounts of DNA/N by cytophotometric measurements (AU) are showed in Table I, where the $2n$ number of each species is also presented. DNA/N determinations ranges between 32.09 ± 2.08 in *Telmatobius pefauri* and 79.43 ± 1.08 in *Caudiverbera caudiverbera* and correspond to 5.81 pg/N and 14.39 pg/N, respectively. Intrageneric differences of DNA/N contents are: *Batrachyla* 27%, *Telmatobius* 20%, *Alsodes* 10% and *Eupsophus* 9%.

The Student, Newman, Keuls test grouped DNA/N measurements of the 12 species in 4 subgroups corresponding to: a) *Telmatobius pefauri*, *T. marmoratus*, *T. peruvianus* and *Batrachyla taeniata*, with a DNA/N range between 5.81 pg/N (32.09 AU) and 6.61 pg/N (36.52 AU), b) *Alsodes nodosus*, *B. leptopus* and *A. tumultuosus* with DNA/N range between 7.87 pg/N (43.44 AU) and 8.67 pg/N (47.87 AU), c) *Eupsophus* species with DNA/N ranging from 11.65 pg/N (64.35 AU) to 12.86 pg/N (70.98 AU) and d) fourth subgroup corresponding to *Caudiverbera caudiverbera* with 14.39 pg/N (79.43 AU). (Table II).

DISCUSSION

Observed DNA variations of the 12 studied species are in the range found in diploid Leptodactylidae (Beçak *et al.*, 1970; Olmo, 1973; Olmo and Morescalchi, 1978).

Table I - Chromosome number (2n), DNA/N cytophotometric measurements (AU) and estimated DNA (pg) in 12 leptodactylid species.

Species	2n*	DNA (AU) ± SD	DNA (pg/N)
<i>Telmatobius halli</i>	26	39.97 ± 2.39	7.24
<i>Telmatobius peruvianus</i>	26	36.52 ± 3.41	6.61
<i>Telmatobius marmoratus</i>	26	32.30 ± 3.07	5.85
<i>Telmatobius pefauri</i>	26	32.09 ± 2.08	5.81
<i>Eupsophus migueli</i> **	30	70.98 ± 3.26	12.86
<i>Eupsophus roseus</i>	30	64.35 ± 2.39	11.65
<i>Eupsophus vertebralis</i>	28	69.17 ± 4.00	12.53
<i>Alsodes nodosus</i>	22	43.44 ± 1.80	7.87
<i>Alsodes tumultuosus</i>	26	47.87 ± 1.97	8.67
<i>Batrachyla taeniata</i>	26	34.69 ± 2.11	6.29
<i>Batrachyla leptopus</i>	26	44.20 ± 1.02	8.00
<i>Caudiverbera caudiverbera</i>	26	79.43 ± 1.08	14.39

*Díaz y Veloso, 1979.

**Iturra y Veloso, 1981.

Table II - Cluster of 12 leptodactylid species according to DNA/N (UA) measurements (Student-Newman-Heuls test).

DNA (UA)	Species
32-36	<i>Telmatobius pefauri</i> <i>Telmatobius marmoratus</i> <i>Batrachyla taeniata</i> <i>Telmatobius peruvians</i>
34-39	
39-44	<i>Telmatobius halli</i> <i>Alsodes nodosus</i> <i>Batrachyla leptopus</i> <i>Alsodes tumultuosus</i>
43-47	
64-70	<i>Eupsophus roseus</i> <i>Eupsophus vertebralis</i> <i>Eupsophus migueli</i>
79	<i>Caudiverbera caudiverbera</i>

Comparisons of DNA/N are relevant only when they are performed in species groups closely related in their phylogeny.

In this work, at least three monophyletic groups of an early radiation of Leptodactylidae are represented. They correspond to species of the genera *Eupsophus*, *Telmatobius* and *Alsodes*, grouped in the tribe Telmatobiini Fitzinger, *Caudiverbera caudiverbera* from the tribe Calyptocephalellini Reig and 2 species of *Batrachyla* from the tribe Batrachylini Gallardo (Lynch, 1978).

Fossil evidence corresponding to *Caudiverbera* is known from the early Eocene and late Miocene of Patagonia, while species of *Eupsophus* were found at the lower Oligocene, indicating their primitiveness among Leptodactylidae (Schaeffer, 1949). There is also molecular evidences reinforcing this primitiveness, Ureta *et al.* (1978), found that the four hexokynases present in vertebrates are A, B, C and D. The primitive pattern CBD is founded in *Eupsophus* and *Caudiverbera* while in *Alsodes* and *Telmatobius* only the derived pattern ABD is represented (Díaz, 1986).

The DNA/N from *C. caudiverbera*, *E. roseus*, *E. migueli* and *E. vertebralis* is larger than in the other species.

The DNA/N quantity of the 12 species is not clearly associated with diploid chromosome number variability (Table I). However, if Telmatobiini species are taken separately, a relationship between diploid number and genome size seems to emerge. *Eupsophus* species with high diploid number and telocentric chromosomes (Veloso, 1979), also have a larger genome size than *Alsodes* and *Telmatobius*. In this tribe, chromosome rearrangements and DNA deletions determine a reduction of diploid chromosome number and genome size respectively.

There is a strong evidence for a correspondence between repetitive DNA and constitutive heterochromatin (Mizuno and MacGregor, 1974; Hatch *et al.*, 1976). Differences in the amount of DNA/N between related species can be caused in part by different amounts of constitutive heterochromatin. However, single DNA duplications in certain chromosome regions can also be important (Ohno, 1970; Beçak *et al.*, 1970).

The constitutive heterochromatin pattern in Telmatobiini chromosomes is similar, but differences in quantity seem to be present (Díaz and Veloso, 1979). Although we lack at present information concerning the molecular composition of this constitutive heterochromatin, we cannot reject the hypothesis that changes of the genome size, can be mediated by changes in the amount of constitutive heterochromatin.

According to our results, there are no relationships between chromosome number and genome size at the genera level. However, there is some correspondence between DNA/N amounts and species grouped in different genera. Our results showed that the intergeneric DNA/N variability is higher than the intrageneric variability except in *Batrachyla*.

In other words, evolutionary factors that determine changes in chromosome numbers of related species are probably different than those determining intrageneric genome size variability. The variability in genome size due to HC variation seems to be related with changes in chromosome size and/or chromosome morphology (Velo and Iturra, 1979), instead changes in chromosome number.

To explain genome size variability, several authors (Goin *et al.*, 1968; Szarski, 1970; Bachmann and Nishioka, 1978; Cavalier-Smith, 1978, 1982), pointed out that natural selection acts over cell and nuclear sizes and this affects genome size. In the group of species we examined there are differences in blood values including cell size and volume of erythrocytes (Ruiz *et al.*, 1987; Northland, unpublished data). Genome size in *Telmatobius* and *Alsodes*, can be partially explained as adaptive responses to different ecological constraints, for which cells of small sizes represent a comparative advantage from the physiological point of view (Ruiz *et al.*, 1983).

It is possible that genome size variability in lower leptodactylids, can be related to different and possibly antagonistic evolutionary mechanisms. These may include chromosome deletions determining changes in chromosome number and increase in DNA (possibly non genic fractions), of adaptive significance.

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RESUMO

O conteúdo de DNA nuclear (DNA/N) de 12 espécies de anura, que fazem parte de três tribos dos Leptodactylids primitivos, é demonstrado.

A quantidade de DNA/N determinada por medidas citofotométricas em núcleos interfásicos de eritrócitos variou de 14,39 pg/N em *Caudiverbera caudiverbera* ($2n = 26$) a 5,81 pg/N em *Telmatobius pefauri* ($2n = 26$).

A variabilidade intergenética de DNA/N é maior do que a intragenética, exceto em *Barachyla*.

A quantidade de DNA/N nestas espécies não é claramente associada com a variabilidade no número diplóide de cromossomos, exceto em *Telmatobiini*, onde espécies com um alto número diplóide ($2n = 28,30$) tem um tamanho de genoma maior.

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