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DETERMINATION OF KARYOTYPES AND NUCLEAR DNA CONTENT IN FROGS OF THE FAMILY LEPTODACTYLIDAE

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ABSTRACT

Nuclear DNA content and karyotypes were analyzed in 18 species of anurans from the family Leptodactylidae. The most frequent diploid number was 2n = 22 (14 species), although two species had 2n = 26 and two polyploid species had 4n = 44 and 8n = 104. The nuclear DNA content of these species was quite variable, ranging from 2.73 ± 0.03 pg/N to 26.92 ± 0.33 pg/N (picograms per nucleus), but the majority of the species (11) had values between 3 and 5 pg/N. Nuclear DNA content varied among species with the same chromosome number, and even between different populations of a given species. This indicates that the genetic content of anurans can change without altering the species' diploid number, and independently from alterations in chromosome morphology. These variations are associated with speciation, and could represent an intermediate stage of this process.

Key words : nuclear DNA, DNA content, cytogenetics, Leptodactylidae, Anura.

RESUMO

Foram estudados o conteúdo de DNA nuclear e a composição cariotípica de 18 espécies de anuros da família Leptodactylidae. O número diplóide mais freqüente foi 2n = 22 (14 espécies), embora duas espécies tivessem 2n = 26 e duas espécies poliplóides tivessem 4n = 44 e 8n = 104. O conteúdo de DNA nuclear destas espécies foi bastante variável, entre 2,73 ± 0,03 pg/N e 26,92 ± 0,33 pg/N (picogramas por núcleo), mas a maioria das espécies (11) tiveram valores entre 3 e 5 pg/N. O DNA nuclear variou entre as espécies com o mesmo número de cromossomos, e mesmo entre populações diferentes de mesma espécie. Isto indica que o conteúdo genético de anuros pode mudar sem alteração do número diplóide das espécies e independentemente de alterações morfológicas dos cromossomos. Estas variações estão associados à especiação, podendo representar um estágio intermediário deste processo.

Palavras–chave : DNA nuclear; conteúdo de DNA, citogenética, Leptodactylidae, Anura.

INTRODUCTION

The Leptodactylidae is one of the richest anuran families in terms of species number. However, few species have been studied with regard to karyotype or nuclear DNA content. Some species of this family, such as *Leptodactylus ocellatus*, are cultivated in frog farms, given the quality of their meat. A question of economic importance is whether there is a relationship between the body size of species and its nuclear DNA

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content. In leptodactylids, 2n = 22 is the most common diploid number, and 2n = 26 the second most common. Other diploid numbers are restricted to a few species or species groups (Beçak, 1968; Batistic, Beçak & Vizotto, 1969; Batistic *et al*., 1970; Batistic, 1970; Bogart, 1974).

Polyploid species also occur in this family. One is *Odontophynus americanus*, which has 4n = 44 (Beçak, Beçak & Rabello, 1966). Two species of *Pleurodema* present 4n = 44 (Barrio & de Chieri, 1970), *Ceratophrys dorsata* (today *C. aurita*) has 8n = 104 (Beçak, Beçak & Rabello, 1967) and *Ceratophrys ornata* (Bogart, 1967) is 8n = 104. Polyploidy is one of the most prominent examples of chromosomal rearrangements that can cause abrupt post-meiotic isolation in both animals and plants, which lead to the origin of new species (King 1993). Polyploidy is relatively common in anurans, having arisen independently in a number of different lineages. At present, at least 40 species of polyploid anurans have been identified. These species belong to seven families: Bufonidae, Hylidae, Leptodactylidae, Microhylidae, Myobatrichidae, Pipidae and Ranidae (King, 1990; Kuramoto, 1990).

Living organisms suffer constant environmental pressure, which molds the genetic alterations accumulated in the members of a given population. Mirsky & Ris (1949) and Boivin, Vendrely & Vendrely (1984) established that the quantity of DNA per nucleus is constant and characteristic, and can distinguish one species from another (Vendrely, 1955). Based on this premise, we analyzed the DNA content and karyotype of eighteen species of the family Leptodactylidae, belonging to distinct genera and subfamilies, and different populations of each species.

MATERIAL AND METHODS

The specimens were either collected or obtained from a number of localities in southern South America (see Table 1), from 1970 to 1980. Males predominate in our samples because collection was carried out at night, guided by the males' mating calls.

In the laboratory, each specimen received an intraperitoneal injection of 1% colchicine, at 0.1 ml per 10 grams body weight (Beçak, Beçak & Rabello, 1966). After two hours, the animal was anesthetized with ether. Blood was then removed by heart puncture with heparin for the blood smear, which was used for the quantification of nuclear DNA. The animal was subsequently dissected, and the intestine, spleen, and gonads were removed for karyotyping. The specimens were preserved in 70% ethanol, and deposited in the collection of the Genetics Laboratory of the Butantan Institute.

Cytological preparations for chromosomal analysis (squashing technique)

Fragments of intestine, spleen, and male gonads were hypotonized in cool distilled water for 15 minutes, then fixed in 50% acetic acid for 15 minutes. Next, the fragments were spread onto a glass slide, covered with a coverslip, and placed between sheets of filter paper, onto which pressure was exerted. After immersion in dry ice and absolute ethanol, the coverslip was removed with the aid of a steel blade. Slides were dried at room temperature for at least 24 hours, then hydrolyzed for 10 minutes in HCl 1N, washed in tap water for 10 minutes, and finally stained with Giemsa 2% in phosphate buffer for 5 to 10 minutes.

Cytological preparations for cytophotometry

Eight slides were hydrolyzed for each specimen, and three control slides were prepared as follows: blood smear of *Bufo ictericus*, kidney of *Rattus norvegicus* and human lymphocytes. The *Rattus* kidney slides were made by cutting the kidney into fragments of 1 to 2 mm, which were fixed in 4:1 methanol:acetic acid solution for one hour. Gentle

squashing was then applied, in order to release the cells from the tissue. Only individual cells were considered for the cytophotometry measures. Human lymphocytes were obtained from pellet smears of leukocytes and fixed in 4:1 methanol:acetic acid solution. Anuran blood smears were prepared and air-dried, fixed with methanol for 3 minutes, and stored until use.

The eight slides taken from each specimen were hydrolyzed in HCl 1N at 60°C, in a water bath, for 2, 4, 6, 8, 10, 12, 14 and 16 minutes, respectively, dipped in freezing distilled water, then washed in tap water for 15 minutes (Soma, 1981), air dried for two hours and Feulgen stained in the dark, for one hour. Next, they were washed in 10% sulfurous water, with three 5-minutes baths, washed in tap water for 15 minutes, left to dry for 2 hours and mounted with Permount. The cytophotometric readings were carried out the following day in a Zeiss MPM scanning cytophotometer, at l = 540 nm with 0.5 mm steps, utilizing an immersion objective 100X/1,30 and optavar 1,6X.

Measurements were made in absorbance and the cytophotometer was connected to a Facit 4070 perforator. The punched tape was analyzed on an HP computer. The slide with the largest absorption value was chosen (most of the leptodactylid species had a hydrolysis peak in the 8 to 12 minutes range), and its hydrolysis time was used to repeat the process with another two or three slides, together with the control slides, varying hydrolysis time by about one minute. After analysis in the cytophotometer, the slide that presented the greatest absorption value in picogramas (pg) was chosen, given that known DNA values for the *Rattus* kidney and human lymphocytes are also in pg (Vendrely, 1955; McCarthy, 1969; Soma, 1981).

Thirty-six nuclei from each slide were analyzed, resulting in a spatial distribution of values (Figure 1) indicating the absorption values, that is, the distribution of DNA in the nucleus. Each value represents the mean of eight measurements of the field analyzed at 0.5 mm steps. A filter on the computer program eliminated the absorption values of the cytoplasm less than 0,009 units; a pilot experiment indicated those values to correspond to background levels.

RESULTS

Results are summarized in Table 1. Within each subfamily, there is a certain homogeneity with regard to the haploid number (n = 11 for Leptodactylinae, Eleutherodactylinae and Telmatobinae, n = 13 for Cycloramphinae and n = 11 or n = 13 for Ceratophryinae). Variation in chromosome number is due basically to the occurrence of polyploidy in two species, *Odontophrynus americanus*, with diploid (2n = 22) and tetraploid (4n = 44) populations and *Ceratophrys aurita*, which is octoploid (8n = 104).

Despite the overall consistency in chromosome numbers, morphology is quite diverse, but is most similar between closely related species. By contrast, the nuclear DNA content varied among different populations of the same species, or even within a single population, as in the case of *Ceratophrys aurita* from Santo Amaro, a suburb of São Paulo. Intrageneric variation was also found. In *Eleutherodactylus*, for example, which was homogeneous in terms of chromosome number, there is a 350% difference in nuclear DNA content across species. The most common values in diploid leptodactylids are observed in the 3pg/N to 5pg/N range. The subfamilies Eleutherodactylinae and Telmatobinae presented the highest values.

Cellular cytoplasmatic and nuclear volumes were measured from erythrocytes of the polyploid species (*C. aurita* and *O. americanus*), diploid species *O. cultripes* and *O. americanus* (diploid populations). Taking the value of the diploid *O. americanus* from Serra do Cipó as the baseline, the relationship between these values was evaluated (Table 2). Cellular, nuclear

and cytoplasmic volumes all increase as nuclear DNA content increases, but in all cases, DNA content increases at a higher rate. The estimated value of DNA in picograms refers to the value known as 2C, because it was measured in somatic tissues (nucleated erythrocytes of circulating blood), therefore in diploid nuclei (or tetraploid or octoploid nuclei in the case of the polyploid species).

DISCUSSION

Genomic duplication is an important mechanism in evolution, which generates diversity and organism complexity (Keller & Gerhardt, 2001). The evidence suggests that two duplications occurred in the genome at the origin of the vertebrates, with a third duplication occurring in the Actinopterygean lineage, which gave rise to modern fish (Meyer & Schartl, 1999). However, as emphasized by Cavalier-Smith (1985), the forty-fold variation in the number of genes that code for proteins is insufficient to justify an increase in the size of the eukaryote genome of up to 80,000 times. The relationship between nuclear DNA content and the complexity of organisms is still under debate, as is its role in both macroevolutionary and microevolutionary mechanisms. DNA content varies considerably, even among closely related organisms. Up to a point, increased complexity of an organism is reflected in increased nuclear DNA. Vertebrates have at least one hundred times more DNA than bacteria, which in turn have at least one hundred times more than bacteriophages. The variation is also enormous within each group, however. In the amphibians, for example, *Amphiuma* presents 168 pc/N, in comparison with 8.9 pc/N in *Bufo regularis* (Rees & Jones, 1972).

In amphibians, there is considerable variation in DNA content, reaching high levels, mainly in Urodela, and slightly lower ones in the anurans. This variation may be linked to the fact that the amphibians were the first vertebrates to conquer the terrestrial environment, given that redundant DNA would be free to suffer mutations, some of which could favor adaptation to new environments. The long-term accumulation of mutations may have led to increased complexity of structures and metabolism, giving rise to new life forms. Species richness is greater in tropical regions, and Brazilian anurans are distributed in different environments with unique climatic characteristics and vegetation, which aid in the diversification of the species.

Carvalho *et al*. (2002) found up to 79% variation in the nuclear DNA content of fish of the genus *Astyanax* (Tetragonopterinae, Characidae). The species with the lowest content (*A. altiparanae*) presented a value of 2.09 ± 0.15 pg/N, whereas *A. scabripinnis* returned a value of 3.74 ± 0.13 pg/N, despite the fact that both species have the same chromosome number (2n = 50). In plants, Asif-Javed et al. (2001) recorded a nuclear DNA content of 1.41 ± 0.01pg/N in *Musa violascens* (2n = 20) and 1.13 ± 0.01 pg/N in *Musa balbisiana* (2n = 22), emphasizing the lack of a clear relationship between chromosome number and nuclear DNA.

We found a similar situation in the family Leptodactylidae. For example, *Physalaemus albonotatus* (2n = 22) and *Cycloramphus duseni* (2n=26) presented practically the same amount of nuclear DNA (3.96 $\pm$ 0.07 pg/N and 3.83 $\pm$ 0.06 pg/N, respectively), despite the difference in chromosome number. We also found no relationship between chromosome number, nuclear DNA content and body size. For example, diploid (2n = 22) and tetraploid (4n = 44) individuals of *Odontophrynus americanus* are indistinguishable in external morphology, even though the tetraploid individuals have double the amount of nuclear DNA.

There was little variation in chromosome number in the diploid species, although nuclear DNA content increased from 2.73 pg/N in *Leptodactylus ocellatus* to 26.92 pg/N in *Eleutherodactylus binotatus*. The latter species presents correspondingly

larger chromosomes, given that both species are 2n = 22. There is little variation, overall, in the diploid number of anurans, although morphological rearrangements and changes in the genomic constitution may be commom. Karyotypic analysis shows morphological variation, which indicates the loss or gain of fragments (deletions, duplication of segments, with or without translocations) as well as centric fusion and fission.

Of the leptodactylid subfamilies, Telmatobinae is the most stable, karyotypically, and Eleutherodactylinae the most variable. The diploid number in the Eleutherodactylinae varies progressively from 18 to 36 (Bogart, 1970, 1973, 1981; Beçak & Beçak, 1974). Three species of *Eleutherodactylus* studied here, all 2n = 22, presented considerable variation in nuclear DNA content. *Eleutherodactylus cubanus* had the lowest value of 7.63±0.27 pg/N, *E. guentheri* a slightly higher value of 8.21±0.12 pg/N, whereas *E. binotatus* presented a relatively high value, of 26.92±0.33 pg/N. The presence of a multivalent chromosome in meiosis of specimens from the continent, together with the high amount of nuclear DNA, led Beçak & Beçak (1974) to consider this a polyploid species that had lost most of the characteristics of the polyploidization process. However, other processes of DNA duplication could have occurred. Segments of DNA could have suffered intrachromosome duplications, altering their morphology, but it is also possible that some segments were duplicated in more than one chromosome and multivalent chromosomes formed by pairing.

Several studies suggest that 2n = 26 is the ancestral diploid number in Leptodactylidae (Morescalchi, 1973, Bogart, 1974), and all other modern anuran families. Even if the hypothesis of an ancestral number of 2n = 26 for modern Anura is accepted, the possibility that Leptodactylidae had an ancestral number of 2n = 22 cannot be discarded as a karyotype of 2n = 26 could have been recomposed with posterior modifications. The almost total absence of 2n = 24 in the family is intriguing, although an early dichotomy is unlikely because there are 2n = 22 and 2n = 26 species in practically all leptodactylid subfamilies. This indicates that these dichotomies occurred later, repeatedly, in each subfamily.

The Eleutherodactylinae are especially interesting, because the group has undergone an extensive adaptive radiation, and is currently distributed throughout the New World. It is difficult not to relate this dispersion to the exceptional karyotypic plasticity of the subfamily – shown in the present study and other data – in terms of chromosome morphology or DNA content.

According to Roth, Dicke & Wishikawa (1992), alterations in the size of the amphibian genome mainly involve satellite DNA, factors of metabolic and ontogenetic importance in their adaptive reproductive strategies. Amphibians that live in warmer regions have smaller amounts of DNA to allow for quicker development. For example, *L. ocellatus* from Recife, PE has 2.73 pg, whereas the individual from Praia Grande (SP) has 3.21 pg (Table 1). Goin, Goin & Bachmann (1968) and Oeldorf, Nishioka & Backmann (1978) observed a reasonably constant relationship between the amount of nuclear DNA and the duration of a species's tadpole stages. Species with reduced DNA content usually live in environments with little water and develop faster than those that inhabit more humid regions. Van't Hof & Sparrow (1963) proposed that, with increased DNA, the duration of the mitotic cycle also increases, so that there is an inverse relationship between DNA content and the rate of oxidative metabolism. Batistic, Beçak & Beçak (1973) also found an increase in the duration of replication in the polyploid anuran *O. americanus* compared to the diploid species.

Other explanations – such as telomeric repetitions (Shippen & McKnight, 1998) – have been presented for the variation in DNA content, the so-called C content paradox. Grime & Mowforth (1982) suggested a relationship between genomic DNA

content and ecological variation in British flora. They suggested that the selective force determining the relationship between genome size and growth season results in a differential cellular division and expansion at low temperatures. Species that grow in low temperatures have large cells and nuclear volumes and high DNA content, although the mechanism that determines the relationship between cell size and nuclear volume remains unknown (Asif-Javed, Mark & Othman, 2001). In the present study, nuclear volumes were larger in *O. americanus* 4n from Uruguay and *O. americanus* 2n from Serra do Cipó than in the same species (2n and 4n) from Poços de Caldas, which has a much warmer climate. There was little variation among sites in cytoplasmatic and cell volumes, however.

In the present study, polyploidy was recorded only in species of the subfamily Ceratophryinae, although as these species have different haploid numbers, it seems likely that at least two events of polyploidy occurred independently. The two individuals of *C. aurita* from Santo Amaro – presumably from the same population – are surprisingly different from each other, with a 39% difference in the DNA per nucleus. As the number of chromosomes is the same, and no differentiated sex chromosomes are present in the species, DNA fragments must have been either lost or gained through rearrangements. As no values of nuclear DNA content are available for the *Ceratophrys* species with 2n = 26, it is not possible to identify the original DNA content duplicated by polyploidy. This discrepancy may be related to the excess of DNA in this species, which implies in greater potential for the loss of segments. The loss of a given segment would be less deleterious because other homologous segments would be present, guaranteeing the metabolic functions of the individual.

This interpretation has important evolutionary implications. Once polyploidy is established, and individuals can breed, the availability of excess DNA may be adaptively advantageous, especially in the context of the colonization of new habitats. This conclusion is supported by *O. americanus*, given that the tetraploid species has a wide geographical distribution in comparison with the restricted range of the diploid species (Ruiz, 1980; Ruiz, Soma & Beçak, 1981). None of the other classes of vertebrates have species that can be identified reliably as polyploid, although there is evidence of remnants in fish. While polyploidy has likely occurred in other vertebrates, more rigid regulatory mechanisms may have limited its establishment. Polyploid fetuses are known to be unviable in humans, for example.

Despite the small number of leptodactylid species analyzed here, considerable variation in DNA content was found, even in individuals of the same species. In *L. ocellatus*, the specimen from Praia Grande had 17.5% more DNA than the one from Recife. The individuals of *O. americanus* 2n from Guapiara and Paranapanema had about 26.5% more DNA than those from Córdoba in Argentina. Variation was even more accentuated in *O. americanus* 4n. Uruguayan specimens from Santa Lúcia and Montevidéo had 39% and 25% more DNA, respectively, than those from Cotia and Poços de Caldas. The greatest difference of all – 253% – was recorded in the genus *Eleutherodactylus*, between *E. cubanus* and *E. binotatus* (both 2n = 22) from Boraceia. Surprisingly, the amount of DNA in *E. binotatus*, a diploid species, is larger than that in the octoploid species, which suggests that mechanisms other than polyploidy may increase DNA content in anurans, without altering chromosome numbers. The C banding, applied to *E. guentheri* and *E. binotatus*, revealed large heterochromatic blocks in all the centromeres and pericentromeric regions of the former, and relatively little heterochromatine in the latter, restricted to the centromeres and some of the interstitial regions (R.F. Batistic, unpublished data). Cytologically, then, this excess DNA is not inactivated. The diploid chromosome number of the most widely

distributed species also varied in association with this variation in DNA content.

Heteromorphic sexual chromosomes are rare in anurans, and were not found in any of the species analyzed here. It is likely that an absence of differentiation in the sexual chromosomes is an important mechanism for the establishment of polyploidy, by avoiding sexual anomalies in the offspring.

We propose that the mechanisms determining the considerable variation in DNA content in anurans are adaptive because of the evolutionary plasticity it offers, hindering the barriers imposed by the chromosome number stability. While there is not as yet an explanation for this behavior (the chromosome number stability), once this restriction is slackened, a great number of variants of the modal number of the genus or family appear. The best example here is the genus *Eleutherodactylus*, but there are other examples in the Leptodactylidae, such as *Pseudopaludicola* (Batistic, Beçak & Vizotto, 1969, Batistic *et al.*, 1970; Batistic, 1970), in which the diploid number varies progressively from 2n = 16 to 2n = 22. Alterations in chromosome numbers may result in a range of evolutionary possibilities for a species. This is based on the increase in recombination events propitiated by both the numeric variation of chromosomes and the rearrangement of genes. Recent advances in our knowledge of gene function, organization and regulation support this interpretation.

Further understanding of genetic mechanisms will permit more detailed analysis of the evolutionary implications of alterations in DNA content. What does seem clear at this point is that the mechanisms are different in different types of organisms, perhaps even at the intra-generic level.

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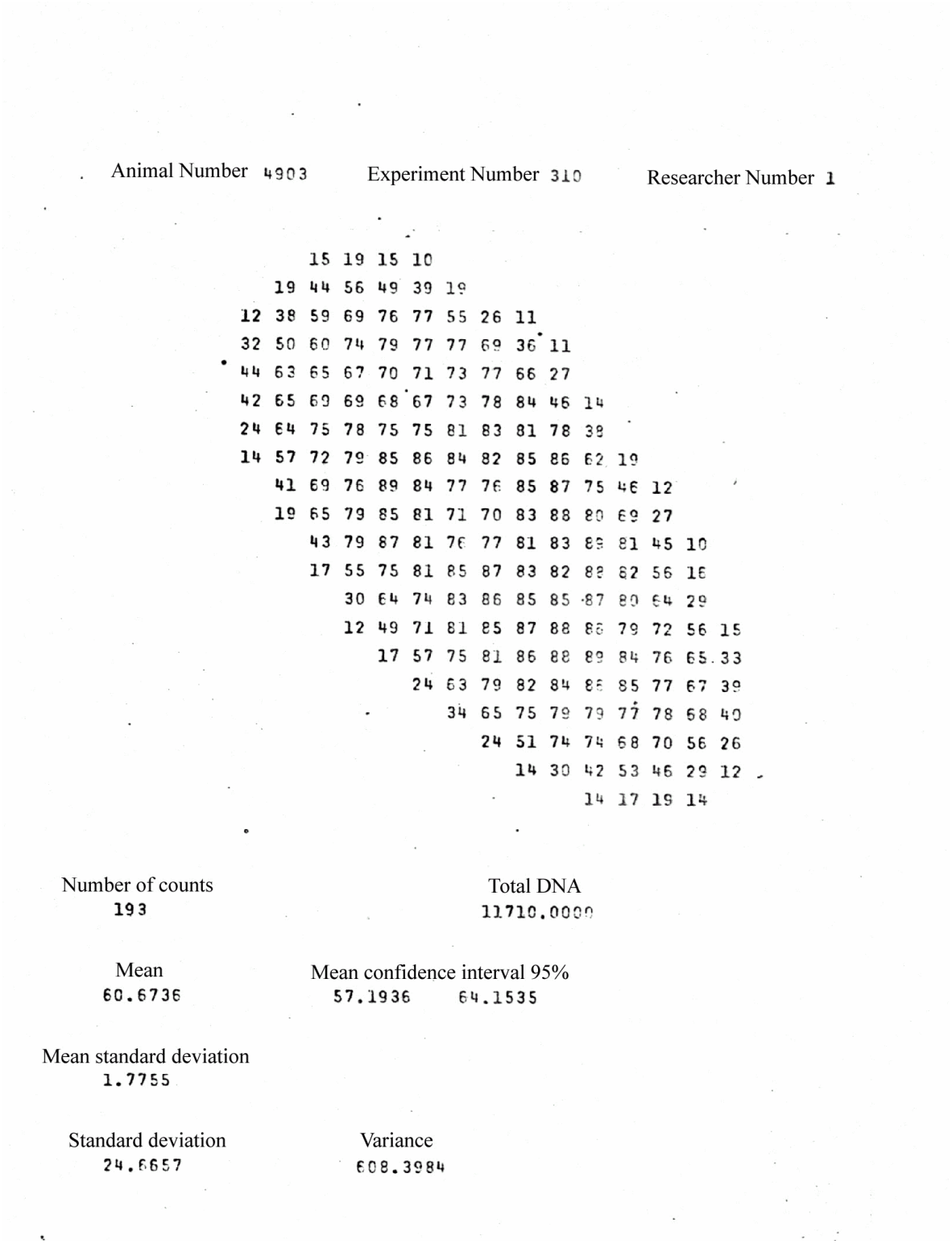

Figure 1. Numerical representation of the nuclear DNA distribution inside a nucleus, after a punch sheet decodification by HP or IBM –1130 Computer.

Table 1. Nuclear DNA content in the eighteen species of the family Leptodactylidae (Amphibia Anura).

Subfamíly	Species	Nr.	ploidy / Chrs Nr	DNA/N	Collecting locality
Ceratophryinae	*Ceratophrys aurita*	1M	8n=104	16,28 ± 0,09	Paralheiros, Santo Amaro, SP
	Ceratophrys aurita	1F	8n=104	22,71± 0,06	Jd. Das Palmas, Santo Amaro, SP
	Macrogenioglotus alipioi	1M	2n=22	3,38 ± 0,04	Juquiá, SP
	Odontophrynus americanus	2M	2n = 22	3,52 ± 0,03	Serra do Cipó, MG
	O. americanus	4M	2n = 22	4,02 ± 0,03	Guapiara, SP
	O. americanus	5M	2n = 22	4,02 ± 0,06	Holambra, Paranapanema, SP
	O. americanus	3M	2n = 22	3,18 ± 0,07	Córdoba, Argentina
	O. americanus	3M	4n = 44	7,10 ± 0,07	Poços de Caldas, MG
	O. americanus	2M	4n = 44	7,08 ± 0,10	Cotia, SP
	O. americanus	3M	4n = 44	8,84 ± 0,31	Montevideo, Uruguay
	O. americanus	3F	4n = 44	9,85 ± 0,25	Santa Lucia, Uruguay
	O. carvalhoi	2M	2n = 22	4,46 ± 0,05	Maracás, BA
	O. cultripes	4M	2n = 22	3,65 ± 0,11	Belo Horizonte MG
	O. moratoi	2M	2n = 22	5,45 ± 0,06	Rubião Jr., Botucatu, SP
	O. occidentalis	3F	2n = 22	3,02 ± 0,03	La Rioja, Argentina
	O. occidentalis	3M	2n = 22	3,76 ± 0,04	Mendoza, Argentina
Cycloramphinae	*Cycloramphus duseni*	1F	2n = 26	3,83 ± 0,06	Gruta Gurutuva, Iporanga, SP
	Zachaenus parvulus	1M	2n = 26	3,41 ± 0,03	Nova Iguaçu, RJ
Eleutherodactylinae	*Eleutherodactylus binotatus*	1F	2n = 22	26,92 ± 0,33	Boracéia, SP
	E. güentheri	1	2n = 22	8,21 ± 0,12	Boracéia, SP
	E. cubanus	1	2n = 22	7,63 ± 0,27	Boracéia, SP
Leptodactylinae	*Leptodactylus mystaceus*	3M	2n = 22	4,02 ± 0,08	Botucatu, SP
	L. ocellatus	3M	2n = 22	2,84 ± 0,12	Lajeado, Botucatu, SP
	L. ocellatus	2M	2n = 22	2,73 ± 0,03	Cid. Universitária, Recife, PE
	L. ocellatus	1F	2n = 22	3,21 ± 0,02	Praia Grande, SP
	L. podicipinus	3F	2n = 22	3,30 ± 0,03	Avaré, SP
	Physalaemus albonotatus	3M	2n = 22	3,96 ± 0,07	Imbituva, PR
Telmatobiinae	*Alsodes montanus*	1M	2n = 22	20,75 ± 0,20	La Paira Favellones, Chile

Nr = number and sex (M= male, F= female) of the animals analysed; ploid y/Chrs Nr = ploidy and chromosome number; DNA/N = nuclear DNA content in picograms per nuclei.

Table 2. Nuclear, cellular and cytoplasmatic volume ratios among polyploid and diploid anuran species.

Species	Sex	Ploidy	VN / VN	Vce / Vce	Vci / Vci	DNA / DNA	Locality
Ceratophrys dorsata	M	8n = 104	3,9	3,3	3,3	4,6	Parelheiros
Odontophrynus americanus	M	2n = 22	~4,0	~3,5	~3,5	~4,5	Serra do Cipó
O. americanus	M	4n = 44	2,1				Montevideo
O. americanus	M	2n = 22	~2,0	1,5	1,5	2,5	Serra do Cipó
O. americanus	M	4n = 44	1,6				Poços de Caldas
O. americanus	M	2n = 22	~1,5	1,5	1,5	2,0	Serra do Cipó
O. cultripes	M	2n = 22	1,1	1,2	1,2		Belo Horizonte
O. americanus	M	2n = 22	~1,0	~1,0	~1,0	1,0	Serra do Cipó

M= male, VN= nuclear volume, Vce= cell volume, Vci= cytoplasmic volume, DNA= nuclear DNA content in