Genome and cell sizes in frogs: A comparison with salamanders[1]

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Summary. The nuclear volume (Nv), cell volume (Cv) and cell surface area (Cs) of erythrocytes from 26 anuran species show a linear and direct correlation with the nuclear DNA content; the Cs/Cv ratio tends to decrease as DNA increases. Analogous phenomena are found in urodeles possessing less than 70 pg/N of DNA, whereas in those with a larger genome there is a trend towards stabilization of the Cs/Cv ratio. The same mechanisms appear to control the ratios of genome sizes to cell sizes within the amphibians and possibly in other vertebrates as well. The former, however, seem to avail themselves to a greater extent of the adaptive properties inherent in their genome and cell size variations.

Among anuran amphibians, the nuclear DNA amount (which is constant in presynthetic nuclei in all species)[2] shows a high degree of interspecific variability, even between species belonging to the same genus. In these organisms, the range of such variability (from below 2 picograms for each nucleus (pg/N) up to above 20 pg/N) is wider than that displayed by other vertebrate orders or classes, except for the urodele amphibians (30 to above 160 pg/N), Dipnoi (160 to 284 pg/N) and possibly the Gymnophiona[3].

Since interspecific differences in the DNAs from anurans do not seem to be correlated with evolutionary factors, but rather with other factors of a physiological, developmental or ecological character, the hypothesis that the genome size of these forms may have per se an adaptive value has been repeatedly forwarded. The same might be true of the urodeles and many plants[3,4]. According to some investigators, natural selection would affect the nuclear and cellular sizes (upon which the genome size would depend) whereby their quantitative variability would influence the cell metabolism, and hence indirectly the general metabolism of the organism and its developmental rate[3,5].

In a previous note[6], the existence of a precise relationship between the genome size and that of some relevant cell parameters had been brought to light in several urodele species. In the present study, our observations were extended to a sample consisting of 26 anuran species belonging to the main families of the order. Among these, some species in which the nuclear DNA contents approach the minimum or maximum values found in these amphibians, were included. The purposes of the present research are: a) an analysis of the correlations between the genome size and several morphometric cell parameters in a group, like the anurans, possessing DNA amounts comparable to those of the majority of vertebrates (in fact many groups of fishes, the sauropsids and the mammals have interspecific variability ranges of DNA falling entirely within those characteristic of anurans); b) verification of whether in such a specialized order as the anurans the above correlations reach the same values already recorded for the urodeles.

Former techniques already reported were again used here[6]. DNA values were recorded histophotometrically in Feulgen-stained erythrocyte nuclei; the various cell parameters, e.g. the nuclear volume (Nv), cell volume (Cv), cell surface area (Cs) were calculated in the erythrocytes viewed in blood smears as cylinders with an elliptical base. The cylinder height (thickness) was estimated by means of a microinterpherometer. The species under study, listed according to their increasing nuclear DNA amounts, chromosome number (already known through former studies of ours) and various cell parameters, are shown in table 1.

Statistical analysis of the above data, graphically represented in figure 1, shows the existence of a direct, linear and significant correlation between Nv, Cv and Cs (up the ordinate) and DNA amounts (along the abscissa). Such a correlation is expressed by the regression lines *a* (relative to Nv), *b* (Cv) and *c* (Cs).

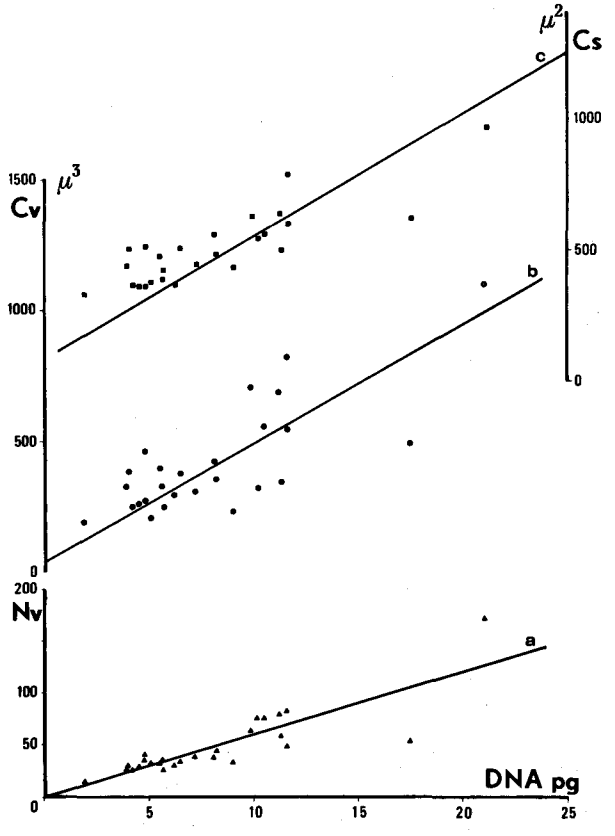

Fig. 1. Nuclear volume (Nv: triangles), cell volume (Cv: circles) and Cell surface area (Cs: squares) up the ordinate, plotted against the nuclear DNA content, along the abscissa, in 26 anuran species (table 1). Regression lines (*a* relative to Nv; *b* to Cv; and *c* to Cs) are highly significant.

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Table 1

Species	Family	2n	DNA (pg/N)	Nv (μ^3)	Cv (μ^3)	Cs (μ^2)	Cs/Cv
Limnodynastes ornatus	Leptodactylidae	22	1.9	14	192	330	1.72
Limnodynastes peronii	Leptodactylidae	24	2.6	29	263	359	1.37
Limnodynastes terraereginae	Leptodactylidae	22	3.9	27	329	441	1.34
Uperodon systoma	Microhylidae	26	4.0	30	385	505	1.31
Adelotus brevis	Leptodactylidae	24	4.2	26	253	366	1.44
Hyla arborea	Hylidae	24	4.8	35	275	362	1.32
Pipa pipa	Pipidae	22	4.8	41	461	514	1.11
Pleurodema bibronii	Leptodactylidae	22	5.1	32	205	378	1.84
Ceratophrys calcarata	Leptodactylidae	26	5.5	32	395	477	1.21
Megophrys nasuta	Pelobatidae	26	5.6	35	330	389	1.18
Cyclorana alboguttatus	Leptodactylidae	26	5.7	25	250	425	1.70
Xenopus laevis	Pipidae	36	6.2	29	295	367	1.25
Limnodynastes dumerilii	Leptodactylidae	22	6.5	33	376	506	1.35
Bufo koyanoiensis	Bufonidae	22	7.2	39	308	444	1.44
Acris crepitans	Hylidae	22	8.1	38	420	559	1.33
Pelobates fuscus	Pelobatidae	26	8.2	44	357	480	1.34
Pelobates syriacus	Pelobatidae	26	9.0	33	231	431	1.86
Bufo viridis	Bufonidae	22	9.9	64	709	627	0.88
Hyperolius concolor	Rhacophoridae	24	10.2	75	324	540	1.67
Discoglossus pictus	Discoglossidae	28	10.5	75	557	562	1.01
Rana esculenta	Ranidae	26	11.2	79	689	639	0.93
Rana graeca	Ranidae	26	11.3	58	343	501	1.46
Rana pipiens	Ranidae	26	11.6	48	544	597	1.10
Bufo bufo	Bufonidae	22	11.6	82	819	792	0.97
Pseudophryne bibronii	Leptodactylidae	24	17.5	53	488	622	1.28
Bobina variegata	Discoglossidae	24	21.1	171	1102	972	0.88

Table 2. Mean increase in the cell size parameters vs the unit (1 pg/N) DNA increase, in the 3 amphibian groups under study

	DNA (pg/N)	Nv (μ^3)	Cv (μ^3)	Cs (μ^2)	Cs/Cv
Anurans	1	8	47	33	0.050
Urodeles (less than 70 pg/N of DNA)	1	11	58	40	0.040
'Paedogenetic' urodeles	1	9	28	19	0.006

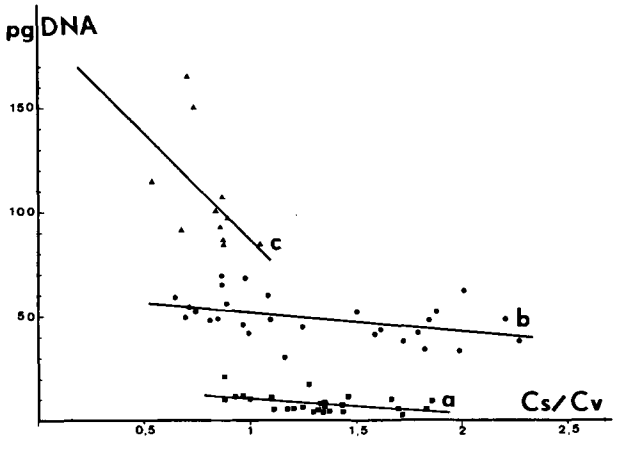

Fig. 2. Relative cell surface area (Cs/Cv), along the abscissa, plotted against the nuclear DNA content, up the ordinate, in the anurans (squares) in the urodeles with less than 70 pg/N of DNA (circles) and in 'paedogenetic' urodeles (triangles). Regression lines (*a* relative, to the anurans; *b* and *c*, respectively, to the 2 groups of urodeles) are highly significant. Statistical analysis of their parallelism demonstrates that *a* and *b* do not diverge from each other, whereas the *c* line exhibits a highly significant difference in parallelism with respect to both of them ('t' test significant at a level of $p = 0.01$).

In our sample, a mean Cs increase, lower than the Cv mean increase, is seen to correspond to each mean pg increase in nuclear DNA (table 2). Hence, in the anurans the ratio Cs/Cv, (namely, the relative cell surface area) would tend to decrease as nuclear DNA increases.

Analogous correlations have already been described by us in the urodeles. The similarities in the pattern of these phenomena, between anurans and urodeles, are enhanced if within the urodeles the species with less than 70 pg/N of DNA are set apart from those possessing larger amounts of genetic material (in our urodele sample, all the species with more than 70 pg/N, being represented by paedogenetic forms, will be labelled 'paedogenetic urodeles', even though we believe that within this group displaying a hypertrophic genome some not permanently larval species may perhaps be included).

Actually, as shown by table 2, the mean Nv increase with respect to a unit DNA increase, is the same in the 3 amphibian groups under inspection (i.e. anurans, urodeles with less than 70 pg/N of DNA and paedogenetic urodeles), while the Cv and Cs increases are more or less alike in the first 2 groups, with much lower values in the paedogenetic urodeles.

The study of the Cs/Cv ratios within the 3 amphibian groups (again with respect to DNA amounts) is of particular interest, in view of the fact that the relative cell surface area is known to be a critical factor governing the cell oxidative metabolism[7]. The pattern of the Cs/Cv ratio is represented in figure 2, as a Cartesian system in which the DNA amounts are reported up the ordinate and the Cs/Cv values along the abscissa. Notwithstanding the marked differences in DNA amounts between the 2 groups, the regression lines of the values concerning the anurans *a* and the urodeles with less than 70 pg/N *b* ex-

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hibit a significantly parallel course. However, in these urodeles a greater extension in the range of Cs/Cv values is found. On the other hand, paedogenetic urodeles display broad nuclear DNA variations associated with changes, however small, in the relative cell surface area (whose values lie around the minimal Cs/Cv values attained by the other urodeles). Hence, the slope of the regression line of these values *c* is much steeper than in the other 2 amphibian groups (figure 2).

Other investigators[8], though confirming the general hypothesis of a correlation between cell size and the rate of respiratory metabolism, have seen that in some amphibian species the erythrocytes (studied in vivo) tend to retain the same relative surface area despite their different sizes: according to these workers, this fact would depend upon the decrease in erythrocyte thickness as the cell volume increases. In the blood smears under study here, erythrocyte thickness increases according to the cell volume, whereby, as stated earlier, larger erythrocytes tend to decrease in their relative surface area in both the anurans and urodeles. In these cells, too, as in those of some tissues from various amphibian species, a reverse correlation between cell size and metabolic rate, as reported by several workers[9], seems to be valid.

In the anurans and nonpaedogenetic urodeles, therefore, the correlation between the genome size and cell size seems to be controled by rather similar mechanisms, suggesting that such mechanisms may also be operative in the majority of vertebrates, possessing DNA amounts comparable to those typical of the anurans. Despite their hypertrophic genomes, paedogenetic urodeles (and possibly the dipnoans) succeed in retaining Cs/Cv ratios which do not diverge from the minimal ones in other amphibians by virtue of a lowered increase in both Cs and Cv versus DNA and Nv (table 2). The possible metabolic significance of these phenomena has already been discussed[4,6].

The conclusion may be drawn that the amphibians seem to avail themselves of the adaptive opportunities offered by genome and cell size variations to a greater extent than other vertebrates. This is perhaps related to the poor efficiency, in the amphibia, of the systems of internal homeostasis, which conversely make other vertebrates (especially terrestrial ones) more independent of changes in various environmental conditions[10]. Qualitatively, the interspecific differences in cellular and nuclear sizes in the amphibians seem to concern chiefly the more repetitive (nongenic) DNA fractions[4,11]. Besides affecting the cell metabolic rate, these differences appear to be correlated with the length of the S phase (DNA autosynthesis)[12], the duration of embryonic and larval developments[3] and perhaps with other physiological characters of high adaptive significance for the amphibians[10].

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Chemical mediators in the oviposition behaviour of the house longhorn beetle, *Hylotrupes bajulus*

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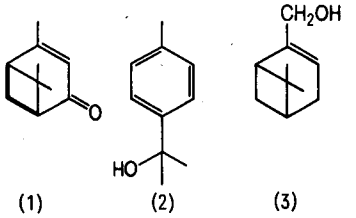
Summary. Oviposition behaviour in the timber pest *Hylotrupes bajulus* is mediated by pheromones ((–)-verbenone and p-cymen-8-ol) produced in the frass of the wood-boring larvae of the species.

In our studies of the role of chemosensory substances in the behaviour of the house longhorn beetle *Hylotrupes bajulus* (Coleoptera, Cerambycidae)[2], a serious pest of coniferous softwoods, we observed that mated females showed a preference for oviposition on actively-infested wood rather than on uninfested material. In this context, *H. bajulus* cultures were established artifically on blocks of untreated *Pinus sylvestis* pine wood obtained from a single source. Newly-hatched larvae were introduced into blocks of wood impregnated with yeast extract[3], and after 6 months development were transferred to untreated blocks for at least 3 months. Cold-treatment of these blocks for approximately 4 weeks at 5 °C results after pupation in the emergence of adult beetles some 5–7 weeks later. Within several minutes of emergence, the male becomes attracted to the female and after location and copulation, the mated female immediately seeks suitable sites for egg-laying. At close range, tactile stimuli predominate and the female probes the wood surface until a suitable fissure is located. However, the possibility of the involvement of chemical stimuli in oviposition was suggested by the observation that egg-laying on infested culture blocks was preferred to sound pine wood. In order to investigate the precise chemical effects in operation, we extracted the frass (predominantly fecal pellets) produced by the tunnelling of the larvae and fractionated the extract in conjunction with an electrophysiological bioassay using electroantennography (EAG)[4]. Microscale analysis of the fraction which produced the major EAG response allowed identification of several monooxygenated monoterpenes, the most abundant of which were (–)-verbenone (**1**), p-cymen-8-ol (**2**) and myrtenol)**3**)[2]. We present here the results of testing the importance in oviposition behaviour of **1** and **2**, both of which were individually EAG-active, and also report preliminary data for the testing of **3**[5].



(1) (–)-verbenone (2) p-cymen-8-ol (3) Myrtenol