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G. SANTANGELO (*)

MACRONUCLEAR DNA CONTENT IN HETEROTRICHOUS CILIATES

Riassunto — *Il contenuto macronucleare di DNA nei ciliati eterotrichi.* Il contenuto macronucleare di DNA di due specie di ciliati etrotrichi è stato misurato con metodi microdensitometrici e calibrato rispetto a quello di cellule il cui contenuto di DNA è ben noto.

Il contenuto macronucleare di DNA, espresso in picogrammi, è risultato più elevato di quello di tutte le altre cellule.

Abstract — The total content of macronuclear DNA in two species of heterotrichous ciliates was measured by a microdensitometric evaluation on Feulgen-stained cells and calibrated against that of cells whose nuclear DNA amount is well known. The macronuclear content of the two heterotrich ciliates, expressed in picograms, resulted notable higher than that of all the other cells examined.

Zusammenfassung — Der makronukleare DNA - Gehalt zweier Ciliatenarten wurde mit microdensitometrischen Methoden gemessen und mit dem bekannten DNA - Gehalt anderer Zellen verglichen. Der DNA - Gehalt der beiden untersuchten Arter erwies sich als wesentlich hoher als der in anderen Zellen gefundene Wert.

Key words — DNA amount, ciliates, macronucleus, microspettrophotometry.

Genome size has been largely investigated in the main taxa of plants and animals in order to verify the existence of the so-called «nucleotypic effect», i.e. a relation between genome size and other parameters like nuclear and cell surface, volume, and cell growth rate (CAVALIER-SMITH 1978). In addition, genome size was considered to be a species-specific constant and a useful tool in the investigation of phylogenetic relationships among taxa and evolutionary processes based on karyotype relationships (CAVALIER-SMITH 1980, MORESCALCHI 1983). Eukariotic cell-organisms, like ciliated protozoa, were also investigated in this

(*) Dipartimento di Scienze dell'Ambiente e del Territorio, Via Volta 4, 56100 Pisa (Italy).

respect (TAYLOR and SHUTER 1981). Protozoa are provided with various nuclear systems; in particular, ciliates are characterized by nuclear dualism. The cell of ciliates containes two kinds of nuclei: a germinal one, termed micronucleus, and a large, vegetative one, called macronucleus, which presides over the majority of cell physiological activities. The macronucleus originates from the micronucleus after each sexual process (conjugation). It amplifies the information received from the micronucleus itself and is equipped with a large amount of DNA: indeed, its DNA content is hundreds or even a thousand time higher than that of the micronucleus. During cell fission, the macronucleus divides amitotically and is not organized into chromosomes (RAIKOV 1982).

On the basis of these characteristics, the macronucleus of ciliates has been widely investigated, but only a few measurements have been made in order to quantify its DNA content (RAIKOV 1982, KRAUT et Al. 1986).

However, this kind of measurement could be a remarkably useful tool in the evaluation of the amount of DNA at disposal of ciliate cells for their vegetative purposes, compared to nuclei of other eukaryotic cells.

The target of this research was to determine the total DNA content of the macronucleus of two heterotrichous ciliates of the genus *Blepharisma*. These two species are so closely related that of the two sexual pheromones produce by each, one is in common whith both (MIYAKE and BLEYMAN 1973). The macronuclei of ciliates of this genus are variously shaped: multinodal as in *B. americanum* or ribbon-like as in *B. japonicum* (GEISE 1973). In both species the macronucleus reaches 2/3 of the total cell length and its irregular shape does not facilitate morphometric measurement of nuclear area or volume. In order to measure macronuclear DNA content in *Blepharisma* the cytophotometric technique was chosen. Measurements of genome size have been obtained for many organisms by means of microdensitometric evaluation of the nuclear DNA content stained with specific DNA cytochemical reagents.

The macronuclear DNA content of *B. americanum* and *B. japonicum* was measured in arbitrary units and calibrated against that of chicken (*Gallus gallus*), newt (*Triturus cristatus*), frog (*Rana esculenta*) erythrocytes and planarian (*Dugesia benazzii*) regenerative blastemal cells, whose nuclear DNA contents are well known (OLMO 1983, PELLICCIARI et Al. 1986, respectively).

Materials and Methods

All the cells were simultaneously prepared; particular care was taken to standardize times and temperatures of the preparation phases. The nuclear DNA of fifty erythrocytes of the three vertebrate species and of fifty planarian regenerating blastema cells was Feulgen-stained and measured with a microspectrodensitometer (Barr and Stroud model GN5).

Planarians were cross-sectioned and, after four days their regenerative blastemas were removed, tranferred for ten minutes in distilled water, fixed in 3:1 metanol acetic acid, and disaggregated with a Pasteur pipette. The same number of clonal *B. japonicum* and *B. americanum* cells, fed on *Enterobacter aerogenes* in lettuce medium buffered at PH 6.8 (Miyake and Beyer 1973) were fixed and stained. The macronuclear DNA content was measured in cells of *Blepharisma* fixed two hours after their last division, a time in which they are in G1 phase (Santangelo and Barone 1987).

All the cells were processed with the same batch of reagents: hydrolized with 5N HCL for 60 min. and stained with Shiff's reagent for about 45 min. washed, dehydrated and mounted with Eukitt according to the methods used by Pellicciari *et Al. (1987) and* Santangelo and Barone (1987). All the experimental cells were treated as described above.

Finally, a convertion factor from arbitrary units into picograms was calculated as the average of the ratios between experimental values of nuclear DNA and those reported in literature for the same cells. The values concerning the erythrocytes of *Gallus gallus, Triturus cristatus, Rana esculenta* were taken from Olmo (1983) and those concerning *Dugesia benazzi* from Pellicciari et Al. (1986).

Results

The convertion factor (average of the ratios between measured and reported DNA values) was found to be 7.19. This value was multiplied by the number of arbitrary units measured for the macronuclei of the two species of ciliates. The data on nuclear Feulgen-DNA content of the examined species and their distribution histograms are reported in Tab. 1 and Fig. 1, both in arbitrary units and in picograms.

The macronuclear DNA amount of *B. americanum* and *B. japoni-*

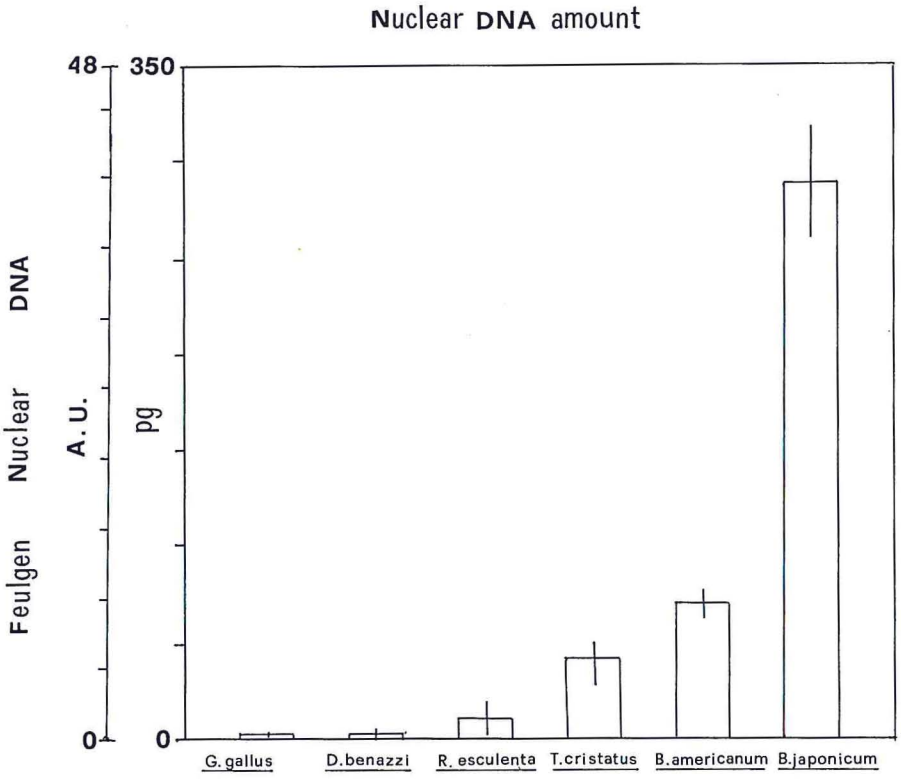

Fig. 1 - Distribution histograms of the Feulgen-DNA content of the nuclei of all the examined species.

TABLE 1 - *Feulgen-DNA amount measured both in arbitrary units and in picograms in the cell nuclei of four metazoan and in two heterotrich ciliate species.*

Species:					
G. gallus	*D. benazzii*	*R.esculenta*	*T. cristatus*	*B. americanum*	*B. japonicum*
Experimental values:					
A.U. X DS					
0.3 ± 0.1	0.4 ± 0.1	1.50.1	5.80.5	9.6 ± 1	40 ± 4
Values found in literature:					
pgs.					
2.4	2.36	11.2	43.6	—	—
Experimental values converted in pgs.:					
X DS					
2.2 ± 0.7	2.9 ± 0.7	11 ± 7.2	42 ± 7.2	69 ± 7.2	288 ± 29

The average conversion factor from A.U. to pgs. was found to be 7.19.

cum and that of the nuclei of the other examined species are extremely different from each other (Fig. 1); the two ciliated congeneric species showed a MaDNA content higher than that of vertebrate erythrocyte cells, of planarian regenerative blastemal cells and, according to reports in literature (Olmo 1983), of the majority of vertebrate erythrocyte nuclei. The two *Blepharisma* species show different macronuclear DNA amounts, reaching in the smaller *B. americanum* a mean value of 69.02 pgs and in the larger *B. japonicum* a mean value of 287.84 pgs. These results agree with the rule that larger ciliates have higher macronuclear DNA content (Taylor and Shuter 1981, Santangelo and Barone 1987). The standard deviation indicates intraclonal heterogeneity in macronuclear DNA amount, which, all the examined cells being in G1, cannot be ascribed to different phases of DNA synthesis; this heterogeneity could be presumably due to the irregular distribution of DNA content during the amitotic macronuclear division (Bergher 1979, Takagy and Kanazawa 1982).

These findings align to the few quantitative data available for the genome size of ciliates and are comparable with those reported by Kraut et al. (Kraut et Al. 1982) for three species of hypotrichs, which evidentiate the macronuclear DNA richness of ciliates.

Discussion

It is well known that in the macronucleus of ciliates, which presides over nearly all phenotypic expression, many of the genes, like that codifyng for rDNA, are highly amplified and that some others may be underrepresented (Raikov 1982). On this nuclear structure, generally coinsidered as «rich in DNA», few measurements have been made. For these reasons it is interesting to quantify the total DNA content of these nuclei and compare it with that of cells of other organisms.

The macronuclei of ciliates are very large and they usually have a complex shape; it is difficult to measure and compare their volume with that of other nuclei, but the Feulgen reaction stains them heavily and so it is possible to make measurements with microdensitometric tools and compare them with whose of other nuclei. The measured macronuclear DNA amount resulted notably high in both the examined ciliated species, higher than that found in the examined metazoan cells and higher than the majority of the measurements

reported in literature for Feulgen-stained erytrhocite nuclei of vertebrates. This result agrees with the few data available on macronuclear DNA content of hypotrichous ciliates (KRAUT et Al. 1982).

The macronuclear DNA content of ciliates can change, within a certain interval, in response to food changes that induce cell volume changes (SANTANGELO 1986, SANTANGELO and BARONE 1987). The cells of *B. japonicum* examined are normal sized cells (fed on bacteria) but, in this species, giant cells have the highest macronuclear DNA amount; these cells, that feed on conspecifics, show a macronuclear DNA content about six-fold that of normal cells, as reported in previous papers by SANTANGELO (1986) and SANTANGELO and BARONE (1987).

All these findings are evidence to the large macronuclear DNA content of heterotrichous ciliates as well as *B. americanum* and *B. japonicum*; the large DNA quantities found could be connected with the polyploid, or rather, highly repetitive structure of the macronucleus of ciliates in which many isolated gene copies are selectively amplified with a process that is similar to gene amplification.

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