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Genome sizes of some Bivalvia species of the Peter the Great Bay of the Sea of Japan

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Abstract. By means of cytophotometry on squashed slides stained by Feulgen the 2C-value of DNA was determined in diploid nuclei of 12 bivalve species from 8 families of 2 subclasses (Pteriomorphia and Heterodonta) of the Peter the Great Bay of the Sea of Japan. It was found that 2C DNA mass of the species investigated varies from 1.82 ± 0.03 *pg* in *Crassostrea gigas* (Ostreidae) to 6.80 ± 0.06 *pg* in *Modiolus kurilensis* (Mytilidae). Average genome masses scarcely differ between two subclasses. However, within Pteriomorphia DNA content varies more significantly than within Heterodonta. In general, in subclass Pteriomorphia, a positive correlation between specialization of species and low genome size takes place supporting the hypothesis that evolution of Bivalvia occurred through a morphological specialization and was accompanied with decreasing of DNA amount.

Key words: Genome evolution, DNA content, DNA-fuchsine cytophotometry, computer image analysis, Bivalvia, Pteriomorphia, Heterodonta.

Introduction

Genome size is an important genetic characteristic of species and generally tends to increase through a progressive evolution. Although this tendency seems to be logical and expectable, the nuclear DNA content does not always correspond to gene number and morphological complexity of organisms (C-value paradox); furthermore there is a great variability of this parameter among representatives of related phylogenetic groups (Thomas, 1975; Cavalier-Smith, 1978; 1985; Vinogradov, 1999). Various concepts exist to explain the relationship between genome size and phenotypic features of organisms and in particular their adaptation abilities. It is supposed that genome size determines some cell parameters such as the cell and body size, the rate of cell metabolism, the length of cell cycle, the rate of development; all these parameters undoubtedly play a role in the processes of evolutionary adaptation of species (Hinegardner, 1974, 1976; John, Miklos, 1988; Xia, 1995; Vinogradov, 1999; Gregory, 2002). A negative correlation between DNA amount and degree of morphological specialization during radiation within some groups of animals lies at the base of one of the hypotheses (Hinegardner, 1974). According to this idea, more generalized species possess higher DNA content values in comparison with more specialized ones which have lost a part of their structures (together with a part of DNA) as the result of adaptation process. The Bivalvia is an extensive phylogenetic group characterized by long evolutionary history and extensive radiation (Rice et al., 1993; Morton, 1996), and the phenomenon of genome size variability was demonstrated widely for this class (Hinegardner, 1974; Cavalier-Smith, 1978; Ieyama et al., 1994; Rodriguez-Juiz et al., 1996; Gonzalez-Tizon et al., 2000). However, in spite of voluminous data concerning genome size in Bivalvia of the World, the

Far Eastern fauna including the one of the Sea of Japan still remains unexplored in this connection. Comparative analysis of DNA content of bivalves of this region and comparison with the species (or populations) investigated from other areas is the aim of the present study. It can be a contribution to study of the genome size variability among representatives of different taxonomic groups and of biological significance of C-value paradox. Up to now this problem has not been decided completely therefore such investigations are still of great importance.

Material and methods

12 species of bivalve mollusks from the Peter the Great Bay of the Sea of Japan belonging to 8 families from 2 subclasses (Pteriomorphia and Heterodonta) were analyzed for nuclear DNA content (2C-value). The species are listed in the Table.

Diploid DNA mass (2C-value) was estimated in CNS neurons nuclei that in bivalves do not undergo somatic polyploidization and so, remain diploid, as has been detected earlier (Tabakova et al., 2005). DNA-fuchsine cytophotometry was carried out at a wavelength of 550 nm by computer image analysis on the squashed slides of pedal ganglions, stained by Feulgen. Computer image analysis was applied successfully in analogous researches for C-value estimation in bivalve molluscs (Gonzalez-Tizon et al., 2000; Gallardo-Escarate et al., 2005). Image analysis was carried out using an Amplival light microscope (Carl Zeiss), a high-resolution video camera (MTC-5C23B, Hitron Systems Ink.) and personal computer. With the Adobe Photoshop CS2 the optical density of DNA-fuchsine per one pixel of image and the areas of nuclei estimated in pixels were determined. Then, these parameters were multiplied and this way the conditional DNA-fuchsine mass for each nucleus was obtained. 3-4 individuals of each species and 100 nuclei from each individual were taken for present study. For each individual the average values of conditional DNA-fuchsine mass with their standard errors were calculated. An analysis of variance of nuclei within individuals revealed low heterogeneity: standard errors of the average values of conditional DNA-fuchsine mass did not exceed 3%, and corresponding coefficients of variation were no more than 20%. Such variability can be accounted for method error as well as neurons DNA content alterations during cell cycle. Then, the values obtained were transformed to picograms according to the formula:

$$\text{abs.2C-value}_x = \text{abs.2C-value}_{\textit{Crassostrea gigas}} \cdot \text{cond.2C-value}_x / \text{cond.2C-value}_{\textit{Crassostrea gigas}}$$

Table. Diploid DNA mass (2C-values) in some Bivalvia species.

Taxa	DNA mass, *pg*
Subclass Pteriomorphia	
Family Arcidae Lamark, 1809	
Arca boucardi Jousseaume, 1894	3.31 ± 0.07
Anadara broughtoni (Schrenck, 1867)	2.89 ± 0.03
Family Mytilidae Rafinesque, 1815	
Mytilus trossulus Gould, 1850	2.57 ± 0.03
Crenomytilus grayanus (Dunker, 1853)	4.22 ± 0.06
Modiolus kurilensis Bernard, 1983	6.80 ± 0.06
Family Ostreidae Rafinesque, 1815	
Crassostrea gigas (Thunberg, 1793)	1.82 ± 0.03
Family Pectinidae Rafinesque, 1815	
Mizuhopecten yessoensis (Jay, 1857)	3.38 ± 0.03
Subclass Heterodonta	
Family Mactridae Lamark, 1809	
Spisula sachalinensis (Schrenck, 1862)	2.82 ± 0.03
Family Corbiculidae Gray, 1847	
Corbicula japonica Prime, 1864	3.86 ± 0.03
Family Veneridae Rafinesque, 1815	
Callithaca adamsi (Reeve, 1850)	3.94 ± 0.05
Dosinia japonica (Reeve, 1850)	4.03 ± 0.05
Family Myidae Lamark, 1809	
Mya arenaria Linnaeus, 1758	2.79 ± 0.02

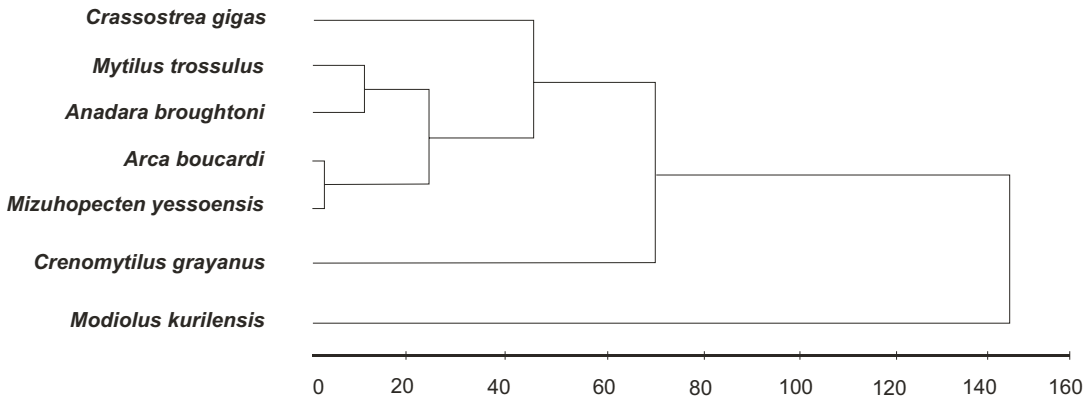

Fig. 1. Distribution of species by genome size in subclass Pteriomorphia based on DNA values of individuals. The scale under the tree reflects the Euclidean distance.

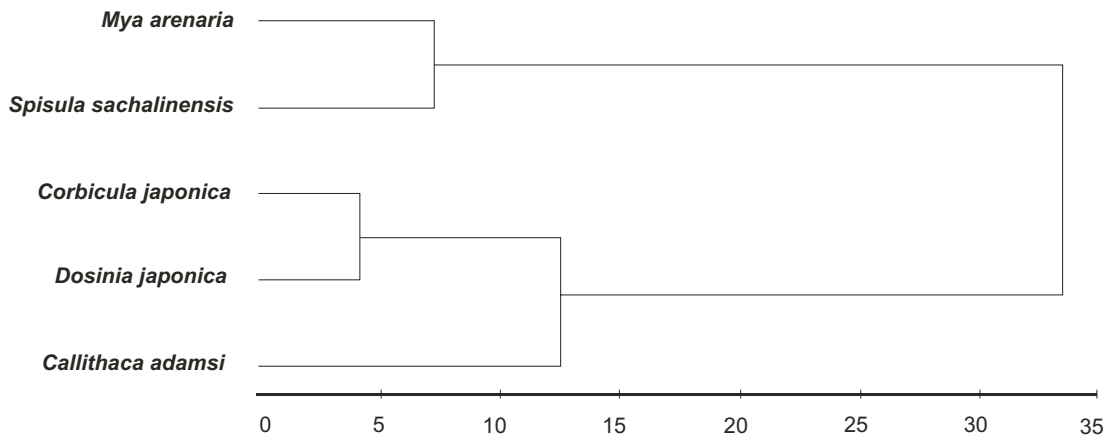

Fig. 2. Distribution of species by genome size in subclass Heterodonta based on DNA values of individuals. The scale under the tree reflects the Euclidean distance.

where x represents any of the species investigated. We used *Crassostrea gigas* (Thunberg, 1793) as a standard. This species has a diploid DNA content of 1.82 ± 0.04 *pg* (Gonzalez-Tizon et al., 2000). On the base of individual DNA values a calculation of average 2C-values for the species with their standard errors was made. In this case the standard errors reflect genome-size variation among individuals within species taking into account the variability among nuclei within each individual. It was calculated as a standard error of the average taken from several independent averages (Lakin, 1973). In order to visualize the DNA content distribution within subclasses and to demonstrate a degree of genome mass likeness between the species the cluster analysis was carried out with the program STATISTICA 6.0.

Results

The results of diploid DNA content measurement in nuclei of 12 bivalve species of Sea of Japan are shown in the table. Our data confirm that the values of DNA mass in class Bivalvia vary significantly even within families. For example, in

Mytilidae (Pteriomorphia) the 2C-values varied from 2.57 ± 0.03 *pg* for *Mytilus trossulus* to 6.80 ± 0.06 *pg* for *Modiolus kurilensis*. In general, within subclass Pteriomorphia the lowest genome size was found in the oyster *Crassostrea gigas* (Ostreidae) – 1.82 ± 0.03 *pg*, and the highest one – in the mussel *Modiolus kurilensis* (Mytilidae) – 6.80 ± 0.06 *pg*. Thus, there is a wide variation of DNA mass in subclass Pteriomorphia; the differences between the extreme points reach 4 times (Table).

In the species of subclass Heterodonta the values of DNA content varied in a less degree (Table). The lowest 2C-values in this subclass were found in *Mya arenaria* (Myidae) and *Spisula sachalinensis* (Mactridae) – they are 2.79 ± 0.02 *pg* and 2.82 ± 0.03 *pg*, correspondingly. The highest ones were obtained in *Callithaca adamsi* (3.94 ± 0.05 *pg*) and *Dosinia japonica* (4.03 ± 0.05 *pg*) from the family Veneridae. This way, the species in subclass Heterodonta were different between themselves no more than 1.5 times (Table).

Since the values of DNA content of species from both subclasses overlap, cluster analysis was carried out for each subclass separately. Grouping of species within Pteriomorphia and Heterodonta based on 2C-values of individuals is shown in Figs 1 and 2. Character of the distribution demonstrated again that there was more significant heterogeneity of genome size within subclass Pteriomorphia in comparison with subclass Heterodonta. It can be seen from Euclidean distance values on the diagrams that it reached about 145 between the most distant species for Pteriomorphia while for Heterodonta it was only 33. In Pteriomorphia *Mytilus trossulus* (Mytilidae) was grouped together with *Anadara broughtoni* (Arcidae), and *Arca boucardi* (Arcidae) was grouped with *Mizuhopecten yessoensis* (Pectinidae). *Crassostrea gigas* had the lowest value of DNA in this subclass and formed a separated stem. Also, *Crenomytilus grayanus* and *Modiolus kurilensis* (Mytilidae) formed separated stems, *Modiolus kurilensis* being far distant from other representatives of the subclass (Fig. 1). In Heterodonta two clusters stood clearly apart, one of them including *Mya arenaria* (Myidae) and *Spisula sachalinensis* (Mactridae), another including *Corbicula japonica* (Corbiculidae), *Dosinia japonica*, and *Callithaca adamsi* (Veneridae). It should be noted that species belonging to the same family Veneridae – *Dosinia japonica* and *Callithaca adamsi* – were not clustered together, but found themselves in different stems (Fig. 2). Thus, in both subclasses species from the same family can be found in different clusters while species belonging to the different families are grouped together. Moreover, as stated above, the whole subclasses present the clusters overlapping one with another (see Table).

Discussion

Comparative analysis of genome size in bivalves started as long ago as in the 1970s. As the result of these studies it was established that in bivalve molluscs C-values range from 0.43 *pg* to 5.40 *pg*, demonstrating more than 12-fold difference in genome size (Hinegardner, 1974; Cavalier-Smith, 1978). Our data the same as data of other recent researchers are in line with these conclusions (Ieyama et al., 1994; Rodriguez-Juiz et al., 1996; Gonzalez-Tizon et al., 2000).

Genome size values determined for the same species by different authors sometimes differ. Some species investigated in the present work (*Crassostrea gigas*, *Mytilus trossulus*, and *Mya arenaria*) were studied earlier. *C. gigas* was used by us as a standard for absolute DNA mass estimation; its 2C-value was expected to be 1.82 *pg* (Gonzalez-Tizon et al., 2000). The 2C-value obtained according to this proportion for *M. arenaria* is 2.79 *pg* and coincides with the value 2.8 *pg* detected by Hinegardner (1974). However, in another work devoted to learning of cytogenetics characteristics of this species in norm and with disseminated neoplasia the normal 2C-value of

DNA was fixed as 3.2 *pg* (Reno et al., 1994). Such discrepancies are usual for the genus *Mytilus*. For example, Hinegardner (1974) determined 2C-value for *M. edulis* as 3.2 *pg* while Rodrigues-Juiz with co-authors (1996) as 3.4 *pg*. Even more significant disparity was derived for *M. californianus* – 2C-values were measured in one case as 3.8 *pg* (Hinegardner, 1974), in another as 3.2 *pg* (Gonzalez-Tizon et al., 2000). 2C-value obtained in the present work for *M. trossulus* was 2.57 *pg*; in analogous study (Gonzalez-Tizon et al., 2000) the same parameter for the same species but from American Pacific coast was 3.02 *pg*. As already stated by previous researches such contradictions can be caused, on the one hand, by method error, and on the other hand, by real intraspecific distinctions of DNA mass between different populations (Vinogradov, 1998; Vieira et al., 2002; Gallardo-Escarate et al., 2005). As to *Mytilus trossulus*, the remarkable differences were found in karyotype of the populations from Pacific and Atlantic oceans (Martinez-Lage et al., 1997).

Analysis of genome size variability carried out by us revealed that in subclass Pteriomorphia the lowest 2C-values of DNA are found in oyster *Crassostrea gigas* and mussel *Mytilus trossulus*, the highest ones – in mussels *Modiolus kurilensis* and *Crenomytilus grayanus*. Intervening DNA values are known in species of the families Arcidae (*Anadara broughtoni* and *Arca boucardi*), and Pectinidae – *Mizuhopecten yessoensis*. As it was demonstrated in previous works (Hinegardner, 1974; Rodriguez-Juiz et al., 1996; Gonzalez-Tizon et al., 2000) the species of Ostreidae and Pectinidae in general have a lower DNA mass than the mollusks from other Pteriomorphia families, such as Mytilidae. These observations are in line with Hinegardner's (1974) hypothesis that species evolutionary specialization in Bivalvia was accompanied with a lost of structures and some part of genetic material, because families Ostreidae and Pectinidae are considered to be more specialized in comparison with Mytilidae (Waller, 1978; Rice et al., 1993; Morton, 1996). However, some data do not conform to this hypothesis: Gonzalez-Tizon and co-authors (2000) estimated 2C-value in scallop *Chlamys hastata* to be as high as 3.28 *pg*, while we obtained similarly high value for *M. yessoensis* (3.38 *pg*).

It is well known that as well as a decrease in DNA amount during evolution, there is sometimes also an increase as a result of reduplication of genes, chromosomes and whole genomes. For example, an opinion was expressed that in the family Pectinidae the modal karyotype, n = 19, is tetraploid; karyotypes with chromosome number less than the modal one came later and were the result of chromosome rearrangements (Wang, Guo, 2004). When holding this conception it may be supposed that *M. kurilensis,* which had the maximal value of DNA content, might be such a polyploid predecessor in the family Mytilidae. In this context it seems interesting that 2C-value of *Modiolus* sp. was found to be 3.8 *pg* (Hinegardner, 1974), i.e. about 2 time less than the one of *M. kurilensis* examined by us (6.8 pg). It also gives a reason to suppose the *M. kurilensis* genome to be tetraploid. However, to draw such conclusions a chromosome analysis is required.

In the subclass Heterodonta the lowest 2C-values were found for *Mya arenaria* (Myidae) and *Spisula sachalinensis* (Mactridae), the highest ones for *Dosinia japonica* (Veneridae) and *Callithaca adamsi* (Veneridae). In a number of studies it was recorded that the representatives of Veneridae as a rule demonstrate a higher DNA content than, for example, the species belonging to Mactridae (Hinegardner, 1974; Rodriguez-Juiz et al., 1996; Gonzalez-Tizon et al., 2000). This could be explained once again by the Hinegardner's (1974) hypothesis because family Veneridae is known as evolutionary conservative taxon despite its great radiation (Rice et al., 1993; Morton, 1996).

The last result to be discussed is the higher

homogeneity in DNA content within Heterodonta in comparison with Pteriomorphia. This phenomenon, again, was already recorded by researches on bivalves from other regions (Rodriguez-Juiz et al., 1996; Gonzalez-Tizon et al., 2000). Within the hypotheses listed above we can surmise that the presence of highly specialized species from one side and ability for duplication of (parts of) genome from another could be the cause of high interspecific genome size variability within Pteriomorphia.

So, like another authors we did not reveal any precise relationship between DNA mass and phylogenetic status of species in class Bivalvia: the genome size varies significantly even within individual families and species with the same genome size often belong to the families far distant from each other. Furthermore, our data demonstrate that the mean value of genome size does not differ practically for Pteriomorphia and Heterodonta, nevertheless different level of heterogeneity within subclasses. In general, more specialized species of both subclasses demonstrated the DNA values to be less than the ones of more generalized species. Although the relationship between species specialization and low DNA content is not universal, most of data (including ours) confirm the existence of such tendency. In spite of increasing of data concerning the genome size evolution in mollusks the problem didn't exceed the bounds of assumptions and hypotheses yet. A correct interpretation of these data requires further research of mechanisms of genome changes and expansion of species amount.

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