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GENOME SIZE AND AT-DNA CONTENT IN THIRTEEN SPECIES OF DECAPODA

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ABSTRACT

The genome size (GS) and AT base pair content of thirteen species of Decapoda belonging to different genera (*Nephrops, Homarus, Palinurus, Jasus, Scyllarides, Scyllarus*, and *Aristaeomorpha*) from four families (Nephropidae, Palinuridae, Scyllaridae, and Aristeidae) were determined by flow cytometry. A high variability of DNA content has been observed. The percentage content of AT in the studied species ranges from 34.92 in *Homarus americanus* to 47.46 in *Palinurus elephas* and most species have a value of about 40%. In the family Scyllaridae, the correlation between DNA content and chromosome number supports the hypothesis of polyploidization events during karyotype evolution.

INTRODUCTION

In Crustacea, the genome size (GS) varies from 0.37 to 22.6 pg (haploid value) with a mode of 2-3 pg. Also, the order Decapoda presents a wide range of GS values, from 1.3 to 22.6 pg (Lécher et al., 1995). Furthermore, Decapoda show some of the highest diploid chromosome numbers ever reported for organisms: their values vary from 2n = 54 in *Liocarcinus vernalis* (Risso, 1816) (cf. Trentini et al., 1989) to 254 in *Pagurus ochotensis* Brandt, 1851 (cf. Niiyama, 1959) [the previous number reported for *Pacifastacus trowbridgii* (Stimpson, 1857), i.e., 2n = 376 (cf. Niiyama, 1962), has been revised to 2n = 186 (Komagata & Komagata, 1992)].

In this study we present an evaluation, by flow cytometry, of the genome size and the adenine-thymine base pair DNA content of twelve species of Decapoda

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Pleocyemata, belonging to the families Nephropidae, Palinuridae, and Scyllaridae. The dendrobranchiate decapod *Aristaeomorpha foliacea* (Risso, 1827), from the family Aristeidae, was investigated as an outgroup species. Within the families, the correlation between the DNA values and karyotype features allowed some phylogenetic remarks.

MATERIALS AND METHODS

Samples of *Nephrops norvegicus* (Linnaeus, 1758), *Homarus gammarus* (Linnaeus, 1758), *Palinurus elephas* (Fabricius, 1787), *P. mauritanicus* Gruvel, 1911, *Scyllarides latus* (Latreille, 1802), *Scyllarus arctus* (Linnaeus, 1758), *S. pygmaeus* (Bate, 1888) and *Aristaeomorpha foliacea* were bought from commercial fisheries all around the island Sardinia (Mediterranean Sea). Live specimens of *Homarus americanus* H. Milne Edwards, 1837, *Jasus* (*Jasus*) *frontalis* (H. Milne Edwards, 1837), *J.* (*Jasus*) *edwardsii* (Hutton, 1975), *J.* (*Jasus*) *novaehollandiae* Holthuis, 1963, and *Scyllarides herklotsii* (Herklots, 1851) were obtained from available, imported, commercial stocks. Non-Mediterranean species were identified according to Holthuis (1991). The nuclear DNA content and AT base-pair contents were investigated by flow cytometric assays performed with a BRYTE HS flow cytometer (Bio-Rad Laboratories Inc.) equipped with a Xenon-Mercury lamp. As a source for cell suspension the antennal gland or, alternatively, the gills of at least two individuals from each species were employed. Samples were frozen and kept at −80°C until their use. The cell suspension was obtained by mincing the tissues with fine scissors in saline buffer; a proportioned amount of European eel *Anguilla anguilla* (Linnaeus, 1758) erythrocytes was added to the suspension as an internal reference of known diploid GS (3.20 pg) and AT-DNA amount (1.39 pg) (Ronchetti et al., 1995). The cell suspension was filtered through a 30 μm mesh.

Genome size evaluation was performed by suspending the cells in 1 ml of solution containing 0.12% sodium citrate, 0.005% propidium iodide, and 0.1% RNase, after centrifugation. RNA digestion was performed at 25°C for 30 min and the staining lasted overnight at 4°C. In order to determine the AT-DNA content, the cells were fixed overnight in 70% ethanol after centrifugation. After further centrifugation, the cells were washed in a saline buffer, centrifuged again, and suspended in a 7 μg/ml DAPI solution. The flow cytometer was set in order to give the reference fluorescence diploid peak around channel 50 on a 512 channel scale. For every sample of a species 2,500 cells were considered. DNA amounts of crustaceans were determined by comparison of the mean value of the diploid peak with that of the reference.

RESULTS

The antennal gland and the gill tissues proved to be an effective source of cell suspension for flow cytometry: the cellular debris was few, crustacean and reference peaks were clearly distinguishable, and coefficients of variation were very low, ranging from 1.5 to 5%. No appreciable differences in DNA amounts were found when both antennal gland and gill tissue of *Scyllarus arctus* and *S. pygmaeus* were used.

Evaluated genome size, AT base-pair content, and AT-DNA/GS percentage ratio (% AT) in the thirteen species studied are listed in table I.

DISCUSSION

The results show a high variability of DNA content (GS and AT-DNA) among the species. Diploid GS ranges from 3.88 pg in *Scyllarus pygmaeus* to 13.98 pg in *Scyllarides latus*, and AT-DNA from 1.64 to 5.96 pg in the same species (table I). These GS values are consistent with those previously obtained by flow or static cytometry and by the reassociation kinetics method (see reference in Lécher et al., 1995). The % AT of the species studied ranges from 34.9% in *Homarus americanus* to 47.5% in *Palinurus elephas*, and most species have a value of about 40% (table I). No other data on AT-DNA in shrimps and lobsters are available from the literature.

TABLE I

Diploid genome size and AT-DNA content (in pg and +/− the standard error) and AT-DNA percentage of thirteen species of Decapoda

Species	Genome size	AT-DNA	% AT
Scyllaridae			
Scyllarus pygmaeus	3.88 ± 0.06	1.64 ± 0.02	42.27
Scyllarus arctus	4.03 ± 0.04	1.69 ± 0.06	41.90
Scyllarides herklotsii	13.63 ± 0.25	– –	–
Scyllarides latus	13.98 ± 0.41	5.96 ± 0.33	42.64
Palinuridae			
Palinurus mauritanicus	6.29 ± 0.13	2.79 ± 0.02	44.32
Palinurus elephas	8.53 ± 0.15	4.05 ± 0.05	47.46
Jasus (Jasus) frontalis	9.31 ± 0.18	3.56 ± 0.11	38.28
Jasus (Jasus) edwardsii	10.02 ± 0.13	3.84 –	38.30
Jasus (Jasus) novaehollandiae	10.65 ± 0.22	3.99 ± 0.11	37.49
Nephropidae			
Homarus gammarus	8.50 ± 0.12	3.31 ± 0.01	39.00
Homarus americanus	9.49 ± 0.20	3.31 ± 0.15	34.92
Nephrops norvegicus	9.80 ± 0.05	4.51 ± 0.05	46.00
Aristaeidae			
Aristaeomorpha foliacea	10.46 ± 0.15	4.37 ± 0.07	41.77

It is noteworthy that the highest and lowest values of GS and AT-DNA were both found in the family Scyllaridae. The genome sizes of *Scyllarides* species were more than three times those of *Scyllarus*. From a karyological point of view, the comparison among the three species studied shows that the chromosome number of *Scyllarides latus* (2n = 126-140, Deiana et al., 1997) is about twice that of *Scyllarus arctus* (2n ≅ 70, Deiana et al., 1992), and *S. pygmaeus* (2n ≅ 76, unpubl. data). The occurrence of polyploidization events may be hypothesized in this family. Furthermore, the AT-DNA percentage content is similar in all Scyllaridae studied (close to 42%), therefore genome differentiation between the two genera apparently occurred without changing the AT/GC ratio.

A different situation was found among the Palinuridae; the species of the genus *Palinurus* have lower GSs and higher AT percentages than *Jasus*, therefore a preferential increase of the AT-DNA fraction (or decrease of the GC-DNA) could be involved in the GS diversification between the two genera. Furthermore, the three *Jasus* species show very similar GS and AT percentages, whereas the values of the Mediterranean *Palinurus* species are quite different from each other. The only other GS datum reported for Palinuridae, 8.82 pg in *Panulirus interruptus* (Randall, 1840) (cf. Vaughn, 1976) is intermediate between *Jasus* and *Palinurus*.

In the family Nephropidae, despite of a relatively low variation of GS, the % AT largely differs among species and *Nephrops norvegicus* has one of the highest % AT values of the decapods analyzed. Whithin the genus *Homarus*, the two species studied have the same AT-DNA content but the GS is higher in *H. americanus* than in *H. gammarus*, therefore preferential changes of the GC fraction must have determined the DNA content diversification between these species.

The outgroup species, *Aristaeomorpha foliacea* (Decapoda, Dendrobranchiata) is characterized by intermediate values in GS and % AT in comparison with the Pleocyemata studied here. Among the Penaeidea, *A. foliacea* has a GS value (10.46) twice that encountered in all the other species analyzed so far (range 4.8-5.3 pg, Chow et al., 1990).

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