

Genome size in Polychaetes: relationship with body length and life habit

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ABSTRACT

Only a few species of polychaetes have previously been analyzed for nuclear DNA content (CONNER *et al.*, 1972), by means of microdensitometric analysis of the amount of Feulgen-stained DNA in interphase nuclei. We have correlated genome sizes of 47 species of Polychaeta with mean body length of adult worms. In the species examined genome sizes ranged from 0.07 to 1.2 pg of DNA per nucleus in interstitial species and from 0.4 to 7.2 pg in macrobenthic species. The lowest values are among the lowest genome sizes of invertebrates so far investigated. Genome size appeared to be significantly correlated with mean body length ($r = 0.30$; $df = 45$; $0.05 < P < 0.01$), which ranged from 0.9 mm to 250 mm in the species we examined.

RÉSUMÉ

Taille du génome chez les Polychètes : rapports avec la taille du corps et l'habitat

Le contenu nucléaire en ADN chez les Polychètes a été jusqu'à maintenant étudié chez très peu d'espèces (CONNER *et al.*, 1972). La taille du génome de 47 espèces de Polychètes a été mesurée à l'aide d'une analyse microdensitométrique de la quantité d'ADN des noyaux en repos colorés par la réaction de Feulgen. Cette taille a été corrélée avec la longueur moyenne du corps des annélides adultes de chaque espèce. La quantité d'ADN haploïde varie de 0,07 à 1,2 pg chez les espèces de la méiofaune et de 0,4 pg à 7,2 pg chez les espèces macrobenthiques. Les valeurs les plus petites sont parmi les plus faibles mesurées chez toutes les espèces d'invertébrés analysées jusqu'à maintenant. La longueur moyenne du corps des espèces examinées est comprise entre 0,9 mm et 250 mm, et est significativement corrélée avec la taille moyenne du génome ($r = 0,30$; $df = 45$; $0,05 < P < 0,01$), chez les espèces étudiées.

INTRODUCTION

Genome size (also known as the C-value) is defined as the mass of DNA in a haploid genome (HINEGARDNER,

1976). Genome size is known to be positively correlated with cell volume and size, length of cell cycle, duration of meiosis and developmental rates (CAVALIER-SMITH, 1985; MCKINNEY & McNAMARA, 1991). These correlations are believed not to be a direct function of genome size but to result from a balance between selective forces favoring larger cells, and hence larger genomes, and those favoring rapid cell multiplication (more easily achieved with small cells and small genome sizes).

Very small organisms, such as those in the interstitial marine fauna, have never been examined to test whether these relationships apply. In addition to their small size, interstitial organisms have so many biological and ecological adaptations to life in a harsh and variable environment, such as the sediment interstices, that they can be considered very specialized living forms (WESTHEIDE, 1984).

Among lower invertebrates, polychaetes show the highest variety in body sizes and morphology; this reflects their high diversity of niches and life histories. Their morphological variation is probably due to their plasticity and early evolutionary radiation (FAUCHALD, 1984). Among polychaetes, nine families are exclusively represented by interstitial taxa (WESTHEIDE, 1985, 1990) and many others have interstitial representatives (e.g. Hesionidae, Syllidae, Dorvilleidae). These organisms thus could represent good "biological tools" for studying relationships between genome size, body size and organization. Up to now data on genome size for the Polychaeta are scarce. Only CONNER *et al.* (1972) measured nuclear DNA amounts for several species of polychaetes by means of microdensitometric determinations of Feulgen dye content of somatic nuclei and fluorimetric quantitation of the DNA of sperm cells. However, among the species considered by CONNER *et al.* (1972), there were no interstitial taxa.

This report is a first attempt to test the hypothesis that, in polychaetes, a positive correlation between genome size and body size may be expected, especially when interstitial forms are included in the study.

MATERIALS AND METHODS

To the 27 species of polychaetes considered by CONNER *et al.* (1972) we added 20 species. They were chosen on the basis of their availability. Among the species considered, 13 belong to the meiofauna and 34 to the macrofauna. Among the interstitial species, three belong to exclusively interstitial families (*Saccocirrus papillocercus*, *Mesonerilla intermedia*, *Dinophilus gyrotilatus*).

Individuals of *O. labronica* and *O. puerilis* were collected in 1991 in the harbour of Genoa (Italy) and individuals of *D. gyrotilatus* were collected in 1992 in the aquarium of Leghorn (Italy). Specimens of *O. robusta* and *O. macrovifera* came from strains set up in 1988 with animals collected in the harbour of Genoa. Specimens of *O. gracilis* came from a strain set up with animals collected at the isle of Sylt (Germany) in 1987, specimens of *O. diadema* came from a collection made in 1989 at Long Beach (U.S.A.), specimens of *O. hartmanni* came from individuals collected in 1990 in Algeciras (Spain). Individuals of *O. l. pacifica* were collected from Woods Hole aquariums in 1984 and individuals of *O. costlowi* from aquariums of Tampa (Florida) in 1986. *O. notoglandulata* individuals came from a 1979 collection in the Sagami Bay (Japan). All these strains of *Ophryotrocha* were kindly provided to us by Prof. B. Åkesson. Individuals of the other species were collected in autumn 1990 and spring 1991 in two coastal biotopes of the island of Ischia (Gulf of Naples, Italy).

The interstitial taxa as well as *P. kefersteini* and *O. flexuosus* (see Table 1) were collected in very coarse sands located at around 8-10 m depth and characterized by a typical "*Amphioxus*-sand" community (PICARD, 1965). The other macrofaunal species (*S. prolifera*, *P. dumerilii*, *A. mediterranea*, *S. spallanzani* and *B. luctuosum*) were collected in very shallow (1-3 m depth) hard bottoms covered by photophylic algae, mainly the brown macroalgae *Cystoseira crinita* and *Halopteris scoparia*. To measure genome sizes, cellular suspensions of whole animals were splashed on cool slides, subsequently air-dried and stained with the Feulgen reaction according to the procedure of ITIKAWA & OGURA (1954). Evaluation of the Schiff-positive material was carried out with a Vickers M86 microdensitometer at a wavelength of 545 ± 5 nm. For absolute DNA calibration mouse sperm and lymphocyte preparations were stained together with polychaete preparations. For each species several slide preparations were obtained with 2 to 20 individuals, according to the availability of live material. At least 50 nuclei per species were measured from different slides (including both spermatozoa and spermatids, when present) except for *O. flexuosus* and *M. intermedia* where only 20 nuclei could be measured. The absorption values obtained as arbitrary units were plotted as percent frequency distribution histograms in order to identify the 1C, 2C, 4C classes of DNA. When the DNA values of sperm were not available, the 1C class value was inferred by halving the 2C class. For the evaluation of absolute DNA amounts, values obtained as arbitrary units were

TABLE 1. — Range and mean of body length (mm) and haploid nuclear DNA content (in pg) by microdensitometric and fluorimetric determination in 47 species of polychaetes. Fluorimetric data are indicated without standard error. * = meiofaunal species; a = present paper; b = CONNER *et al.* (1972).

Taxa	body length range (mm)	mean body length (mm)	haploid DNA content	SE (%)	reference
Order Phyllodocida					
Fam. Phyllodocidae					
<i>Nereiphylla paretti</i> (Blainville, 1828)	15-30	22.50	2.70	1.60	b
Fam. Glyceridae					
<i>Glycera americana</i> Leidy, 1855	150-350	250	3.50	1.90	b
Fam. Hesionidae					
° <i>Ophiodromus obscurus</i> (Verrill, 1873)	13-20	16.50	1.60	9.90	b
<i>Ophiodromus flexuosus</i> (Delle Chiaje, 1825)	35-70	58.0	0.35	0.40	a
Fam. Syllidae					
<i>Syllis prolifera</i> Krohn, 1852	10-25	15	0.45	2.30	a
Fam. Nereididae					
<i>Platynereis dumerilii</i> (Aud. & M. Edws, 1833)	20-100	50	1.00	3.00	a
<i>Laeonereis culveri</i> (Webster, 1979)	55-75	65	0.80	10.10	b
<i>Nereis succinea</i> Frey & Leuckart, 1847	80-190	130	2.20	4.00	b
Fam. Nephtyidae					
<i>Nephtys incisa</i> Malmgren, 1865	25-150	80	7.20		b
Fam. Polynoidae					
<i>Lepidonotus sublaevi</i> s Verrill, 1873	13-34	23.50	2.20		b
<i>Lepidonotus squamatus</i> (Linnaeus, 1767)	15-50	36	1.50		b
Order Amphinomida					
Fam. Amphinomidae					
° <i>Linopherus ambigua</i> (Monrò, 1933)	47	47	2.40	5.50	b
Order Eunicida					
Fam. Onuphidae					
<i>Onuphis eremita oculata</i> Hartman, 1951	30-120	76	1.70	6.70	b
<i>Diopatra cuprea cuprea</i> (Bosc, 1802)	50-300	170	2.00	3.00	b
° <i>Americonuphis magna</i> (Andrews, 1891)	150-350	250	1.20		b
Fam. Lumbrineridae					
<i>Lumbrineris tenuis</i> (Verrill, 1873)	70-150	110	2.40	6.60	b
<i>Ninoe nigripes</i> Verrill, 1873	100	100	5.30		b
Fam. Dorvilleidae					
* <i>Ophryotrocha hartmanni</i> Huth, 1934	1.8-4.0	2.50	1.20	5.05	a
* <i>Ophryotrocha notoglandulata</i> Pfannenstiel, 1972	2.2-8.0	2.50	0.36	2.90	a
* <i>Ophryotrocha costlowi</i> Åkesson, 1978	2.2-5.0	3	0.45	2.20	a
* <i>Ophryotrocha</i> sp. (<i>labronica pacifica</i>)*	2.2-5.0	3	0.40	2.50	a
* <i>Ophryotrocha diadema</i> Åkesson, 1976	1.5-4.6	3	0.44	1.40	a
* <i>Ophryotrocha labronica</i> Bacci & La Greca, 1962	2.2-4.9	3	0.44	3.00	a
* <i>Ophryotrocha</i> sp. (<i>robusta</i>)**	2.6-5.2	3.50	0.40	5.60	a
* <i>Ophryotrocha</i> sp. (<i>macrovifera</i>)**	2.4-5.0	3.50	0.80	2.30	a
* <i>Ophryotrocha gracilis</i> Huth, 1934	2.5-4.0	3.50	0.40	3.70	a
* <i>Ophryotrocha puerilis</i> Clap. & Meczn., 1869	3.0-7.0	5	0.45	2.80	a
<i>Protodorrvillea kefersteini</i> (McIntosh, 1869)	10-15	12.50	0.36	3.13	a
Order Orbiniida					
Fam. Orbiniidae					
<i>Scoloplos rubra</i> (Webster, 1879)	70	70	3.10	3.40	b
° <i>Leioscoloplos fragilis</i> (Verrill, 1873)	40-150	90	2.30	4.70	b

TABLE 1 (continuation). — Range and mean of body length (mm) and haploid nuclear DNA content (in pg) by microdensitometric and fluorimetric determination in 47 species of polychaetes. Fluorimetric data are indicated without standard error. * = meiofaunal species; a = present paper; b = CONNER *et al.* (1972).

Taxa	body length range (mm)	mean body length (mm)	haploid DNA content	SE (%)	reference
Order Chaetoptera					
Fam. Chaetopteridae					
<i>Chaetopterus variopedatus</i> (Renier, 1804)	100-250	170	1.00		b
Order Cirratulida					
Fam. Cirratulidae					
<i>Cirriformia luxuriosa</i> (Moore, 1904)	110	110	3.40		b
<i>°Cirriformia grandis</i> (Verrill, 1874)	150	150	0.70		b
<i>Cirriformia filigera</i> (Delle Chiaje, 1828)	200-250	225	1.00	2.20	b
Fam. Paraonidae					
<i>Aricidea fragilis</i> Webster, 1879	70-100	85	4.60	2.30	b
Order Ophelida					
Fam. Scalibregmidae					
<i>Scalibregma inflatum</i> Rathke, 1843	60-100	80	4.00		b
Order Capitellida					
Fam. Maldanidae					
<i>°Axiothella mucosa</i> (Andrews, 1891)	70	70	2.70	2.20	b
Fam. Arenicolidae					
<i>Arenicola cristata</i> Stimpson, 1856	120-250	136	0.90	3.30	b
Order Terebellida					
Fam. Pectinariidae					
<i>Pectinaria gouldii</i> (Verrill, 1873)	20-45	32	1.30	7.20	b
Order Sabellida					
Fam. Sabellidae					
<i>Amphiglena mediterranea</i> (Leydig, 1851)	5-15	11	0.39	1.93	a
<i>Branchiommia luctuosum</i> (Grube, 1869)	20-45	34	1.20	4.34	a
<i>°Branchiommia crispum</i> (Kroyer, 1856)	30-70	45	1.30	1.20	b
<i>Myxicola infundibulum</i> (Renier, 1804)	150-200	175	3.10		b
<i>Sabella spallanzanii</i> (Gmelin, 1791)	200-300	250	0.65	3.30	a
Order Dinophilida					
Fam. Dinophilidae					
<i>*Dinophilus gyrotilatus</i> O.Schmidt, 1857	0.7-1.30	0.96	0.07	0.62	a
Order Nerillida					
Fam. Nerillidae					
<i>*Mesonerilla intermedia</i> Wilke, 1953	1-2	1.40	0.37	2.70	a
Order Protodrilida					
Fam. Saccocirridae					
<i>*Saccocirrus papillocercus</i> Bobretzky, 1871	25-30	27.5	0.54	1.50	a

° O. obscurus, reported as *Podarke obscura* in Conner *et al.* (1972)

° L. ambigua, reported as *Pseudeurythoe ambigua* in Conner *et al.* (1972)

° A. magna, reported as *Onuphis magna* in Conner *et al.* (1972)

** O. labronica pacifica*, not yet formally described, Åkesson (1984)

*** O. robusta* and *macrofifera*, not yet formally described, Åkesson (1975)

° L. fragilis, reported as *Scoloplos fragilis* in Conner *et al.* (1972)

° C. grandis, reported as *Cirratulus grandis* in Conner *et al.* (1972)

° A. mucosa, reported as *Clymenella mucosa* in Conner *et al.* (1972)

° B. crispum, reported as *Branchiommia nigromaculata* in Conner *et al.* (1972)

converted into picograms, taking the mouse genome size as 3.9 picograms (SPARROW *et al.*, 1972). To calculate the correlation between genome size and organism size, we considered body length as an estimate of body size. We are aware that in polychaetes body length is a simplistic measure of body size, and that not always is this parameter positively correlated with the actual size of the worm (DUCHÊNE, 1982). Besides, body length can greatly change in different populations of the same species according to geographic location (climatic), local environment and trophic conditions, and laboratory conditions in the case of laboratory reared populations. High body length variability in polychaetes notwithstanding, in order to compare our data with those of CONNER *et al.* (1972), we used the body length variable because it was the only size-parameter available from the literature. An indicative mean body length for each of the species considered was estimated by comparing and averaging body length data given by different authors. In Table 1 we reported the range of adult body length values, as well as a mean value obtained by averaging values obtained from literature. For the species in our study, we integrated the literature data with the values obtained by measuring at least 10 adult individuals from sampled populations. For the gonochoric species of the genus *Ophryotrocha* 10 sexually mature males and mature females were considered. In *D. gyrociliatus* only females were measured, in simultaneously hermaphroditic species only sexually mature individuals and in protandric sequential hermaphroditic species, worms were measured when they reached the female phase.

This approach allowed us to assess at least the actual "order of magnitude" of body length reached within each single species.

RESULTS AND DISCUSSION

Genome sizes expressed as pg of DNA per haploid nucleus and mean body lengths of the species examined by us and by CONNER *et al.* (1972) are listed in Table 1, ranking them by orders and families according to PETTIBONE classification (1982). A total of 47 genome sizes are listed, 10 of which were measured by CONNER *et al.* (1972) with the fluorimetric method. The species examined belong to 23 families and 12 orders and are therefore representative of a broad evolutionary range within the class and of a wide spectrum of life habits, mainly represented by shallow-water species.

Genome sizes varied among species from 0.07 pg to 7.2 pg DNA per nucleus. Such a 100-fold range in genome size is comparable only to the ranges found in insects and teleosts. All other animal classes are much more conservative in their genome sizes (JOHN & MIKLOS, 1987). Some of the genome sizes observed in polychaetes are among the lowest found in invertebrates so far investigated. Only Eutardigrada (REDI & GARAGNA, 1987) and some nematodes (JOHN & MIKLOS, 1987) have the same or smaller amounts of DNA.

Genome size appeared to be significantly positively correlated with body length ($r = 0.30$; $df = 45$; $0.05 < P < 0.01$). However, only 9 % (i.e. r^2) of the variation in genome size is explained by its relationship to body length. In the 13 interstitial species included in the sample (*Ophryotrocha* spp., *S. papillocercus*, *D. gyrociliatus*, *M. intermedia*) no significant correlation was observed between genome size and body length, but by their small body size and small genome size, they greatly contributed to the significance of the overall r . If they were excluded, the correlation was no longer significant.

Different and contrasting patterns arise in comparing from an evolutionary view point the DNA content in polychaetes. At the generic level, the 10 species of *Ophryotrocha* showed quite homogeneous values, except *O. hartmanni*, as discussed by SELLA *et al.* (1993). Furthermore, *P. kefersteini*, that, like *Ophryotrocha*, belongs to the Dorvilleidae family, showed a comparable value. On the other hand, the three species of the genus *Cirriformia* showed quite different DNA amounts (Table 1). At the family level, the Sabellidae (5 species) demonstrated a high variability in genome size, while the Onuphidae (3 species) and the Polynoidae (2 species) showed more homogeneous values. The species belonging to exclusively interstitial families (*D. gyrociliatus*, *M. intermedia* and *S. papillocercus*) have among the smallest DNA amounts. At the order level, one may note that within the Eunicida, the macrofaunal taxa (Onuphidae, Lumbrineridae) have higher DNA amount than the interstitial ones (Dorvilleidae) (Table 1).

Although the sample of interstitial polychaetes is still quite small, these first results suggest that meiofaunal taxa have on the whole smaller genome sizes than macrofaunal ones.

We speculate that the small genome sizes characteristic of meiofaunal species may be partially correlated, not only with body size (not all the interstitial species have necessarily very small dimensions, e.g. *S. papillocercus*) but also with those biological features deriving from an interstitial life habit, such as progenesis (i.e. retention of

juvenile characters produced by a genetically fixed precocious sexual maturation (GOULD, 1977), short life-span, rapid sexual maturation, rapid cycling of generations, few eggs of large size, and brooding.

These features, which are well documented in *Ophryotrocha* spp., *Dinophilus* spp. and in many other meiofaunal polychaetes (SWEDMARK, 1964; WESTHEIDE, 1984), make up life histories with peculiar mosaics of "r" and "K" selected parameters. One may also speculate that small genome sizes can be a prerequisite to interstitial life.

The above considerations are still quite speculative due to the small amount of data to date available on this subject; further analyses on genome sizes in other species of macrofaunal and meiofaunal polychaetes are necessary to corroborate the hypothesis that interstitial polychaetes have smaller genomes than macrofaunal polychaetes. On the whole, the DNA content in polychaetes showed a wide range of values and a large variability. Such features are common to many other biological properties of this highly diversified group of marine organisms.

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