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Nuclear DNA content in Gastrotricha

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Abstract. A cytofluorometric evaluation of nuclear DNA content was carried out on fifteen species of Gastrotricha. Genome size, ranging from 0.05 to 0.63 pg, appears uniform in this group and rather low compared to values found in other lower Metazoa. Differences in DNA content between the two orders of the phylum, Macrodasyida and Chaetonotida, which differ greatly in morphology and reproductive biology, are not evident. From these data, a polyploid condition of obligatory parthenogenetic Chaetonotida seems unlikely.
Key words. Gastrotricha; DNA content; genome size.

Almost no karyological data on the phylum Gastrotricha are available, mainly due to its euthelic cellular condition which makes the study of chromosomes very difficult. The opportunity to prepare metaphasic plates is limited to the brief phase of cellular proliferation during the first hours of embryonic development. Mitotic chromosomes have only been observed in two species of the Chaetonotida order[1] (Balsamo, pers. observ.). However, the chromosomes are so small, numerous and dispersed in such a large amount of yolk that they are hardly visible. Consequently, this characteristic cannot be used for cytotaxonomic purposes in this phylum, and taxonomy is based on morphological criteria alone. Meiotic chromosomes have been observed in oocytes of a few hermaphroditic species of the Macrodasyida order ($n = 8$ in *Diplodasys ankeli* and *Thaumastoderma heideri*, $n = 7$ in *Platydasys* cf. *ocellatus*)[2], whereas no data are available on hermaphroditic and parthenogenetic species of Chaetonotida. The cytological mechanism of parthogenesis in gastrotrichs is thus still unknown, even if there is some indirect evidence of apomictic thelytoky[3], a type of parthenogenesis widespread among freshwater animals[4].

Since the study of chromosomes of Gastrotricha is so difficult, we began our analysis of the chromatin by measuring the nuclear DNA content to establish genome size in a number of species. Indeed, genome size has proved to be of cytotaxonomic value and has been used repeatedly in the study of the phylogenetic relationships within a phylum[5–7]. Moreover, these data allow genome size to be compared with those of Gnathostomulida, Rotifera and Nematoda, three taxa which are phylogenetically close to gastrotrichs, according with different presumed sinapomorphies[9–11]. We considered 15 species of Gastrotricha belonging to both orders of the phylum, Macrodasyida and Chaetonotida, which exhibit major differences in morphology and reproductive biology[8].

Materials and methods

Sexually mature specimens of marine Macrodasyida and Chaetonotida were extracted by narcotization with $MgCl_2$ 7% aqueous solution[12] from sandy sediments collected in several Italian and foreign localities. Specimens of *Heterolepidoderma ocellatum*, the only freshwater species examined, were taken from laboratory cultures kept in our department since 1992. Spermatozoa and somatic nuclei were isolated from 4–6 animals at a time either by squashing under a coverslip or by dissecting with tungsten microneedles in a drop of physiological saline solution. Most slides had been previously treated with polysine aqueous solution to improve cell adhesion. Slides were air dried at room temperature, frozen at −80 °C and kept at this temperature until fixation (methanol-acetic acid 3:1 for 5 min) and staining.

To evaluate DNA content, slides were stained with ethidium bromide (EB), 4′-6 diamidino-2-phenylindole (DAPI) or acriflavine feulgen (AF). In the first case samples were treated with a 0.04% solution of EB in phosphate buffer (PBS, pH 7.4) for 30 min, rinsed in distilled water, air dried and mounted in PBS. DAPI staining was carried out by treating each slide with 100 µl of DAPI solution (1 µg/ml in McIlvaine buffer, pH 7.0) for 20 min, rinsing in PBS and mounting in buffered glycerol. The acriflavine feulgen reaction was performed according to Crissmann et al.[13].

DNA content was estimated in arbitrary units with a Zeiss Photomicroskop III, equipped with a Photometer 03 microfluorometer. To convert arbitrary units to picograms of DNA, erythrocytes of *Gallus domesticus* were used, taking 2.5 pg as the value for the diploid chicken genome[14]. The combination of exciter dichroic: barrier filters used were BP 546/12: FT 580: LP 590 for EB; BP 365/11: FT 395: LP 397 for DAPI, and BP 485: FT 510: LP 520 for AF.

Table 1. Mean values (±SD) of the haploid and somatic DNA content (in arbitrary units [AU] and in pg) in fifteen species of Gastrotricha after staining with ethidium bromide (EB), DAPI or acriflavine feulgen (AF). Higher somatic DNA content values observed in six species are reported.

Species	Fluorochrome	Haploid DNA content			Somatic DNA content		
		AU ± SD	n	pg	AU ± SD	n	pg
Macrodasyida							
Turbanella cornuta	AF	15.16 ± 1.70	45	0.19	33.21 ± 2.20	25	0.41
Turbanella sp. 1	EB	36.30 ± 4.87	13	0.13			
	DAPI	101.05 ± 16.15	39	0.13			
Turbanella sp. 2	EB	9.86 ± 2.58	20	0.14	22.10 ± 0.78	27	0.27
					53.33 ± 2.30	15	0.66
	DAPI	5.96 ± 4.01	29	0.14	10.66 ± 3.50	16	0.22
Paraturbanella dohrni	EB	31.20 ± 1.80	20	0.20	62.70 ± 3.70	20	0.40
	AF	16.70 ± 1.30	22	0.21	34.40 ± 2.20	25	0.43
Paraturbanella teissieri	EB	11.11 ± 1.99	17	0.05			
Mesodasys adenotubulatus	EB	6.21 ± 1.80	20	0.28			
Mesodasys laticaudatus	EB	29.80 ± 2.40	21	0.29	68.50 ± 6.10	22	0.66
	AF	21.04 ± 0.80	32	0.26	43.31 ± 1.90	23	0.53
					124.75 ± 2.36	8	1.54
Acanthodasys aculeatus	EB	10.25 ± 1.25	20	0.26			
	DAPI	190.90 ± 5.30	18	0.25			
Diplodasys ankeli	DAPI	5.40 ± 1.00	27	0.20			
Diplodasys meloriae	DAPI	4.95 ± 0.97	34	0.18	11.02 ± 1.10	18	0.44
					41.66 ± 2.88	12	1.52
Ptychostomella mediterranea	EB	9.86 ± 2.58	22	0.14			
	DAPI	6.10 ± 1.41	26	0.16	20.01 ± 2.82	17	0.29
					31.50 ± 3.42	15	0.46
					65.01 ± 1.10	8	0.96
Tetranchyroderma polypodium	EB	24.88 ± 3.40	22	0.63			
	DAPI				49.80 ± 2.74	18	1.28
					101.66 ± 1.52	6	2.61
Chaetonotida							
Xenotrichula intermedia	DAPI	11.53 ± 1.61	33	0.31	19.50 ± 2.12	26	0.52
					39.02 ± 1.41	9	1.05
Xenotrichula punctata	DAPI	8.38 ± 2.82	28	0.30	15.01 ± 0.81	20	0.54
Heterolepidoderma ocellatum	AF				12.10 ± 0.90	26	0.15

Results

The DNA content (in arbitrary units [AU] and pg) measured in 15 species is reported in table 1. The C value measured in spermatozoa ranged between 0.05 and 0.63 pg in twelve species of Macrodasyida and was 0.31 in two Chaetonotida. In somatic nuclei the lowest class of DNA content, probably 2C, ranged from 0.22 to 1.28 pg in seven species of Macrodasyida and from 0.15 to 0.52 in three Chaetonotida. In six species of both orders higher values of somatic DNA content (up to 2.61 pg), not always corresponding to doubling classes, were observed.

Discussion

The haploid DNA content appears relatively uniform within the phylum (table 1). It is noteworthy that when spermatozoa of the same species are stained with fluorochromes which are differently influenced by chromatin condensation, C values are remarkably similar (table 2). The correspondence of C values in gastrotrich spermatozoa following staining with EB, DAPI or AF confirms the reliability of the data. At the same time, these findings suggest that chromatin organization differs from that found in mammal sperm, as the DNA content of mature spermatozoa of mammals is known to be underestimated by staining with intercalating agents such as ethidium bromide[15,16].

Interestingly, C values are similar in macrodasyids and chetonotids, although the morphological and biological differences between the two orders are so great that even the unity of the phylum has recently been doubted[9].

Unlike the uncoiled and generally small nucleus of chetonotid sperm, spermatozoa of macrodasyids generally show a thin, very long nucleus coiled around a mitochondrial axis. Thus, in accordance with ultrastructural data indicating a more compact chromatin structure in macrodasyid than in chetonotid spermatozoa (Balsamo and Ferraguti, in preparation), comparable genome size could be related to a different organization of the sperm chromatin in each order.

Both in macrodasyids and chetonotids, the lowest class of DNA content in somatic nuclei fits a diploid cellular condition. The significance of DNA content above 2C in five macrodasyids and *Xenotrichula intermedia* remains unclear, in view of the euthely of the phylum and

Table 2. Comparison of the haploid and somatic DNA content values (in pg) in fifteen species of Gastrotricha after staining with ethidium bromide (EB), DAPI or acriflavine feulgen (AF).

Species	Haploid DNA content (pg)			Somatic DNA content (pg)		
	EB	DAPI	AF	EB	DAPI	AF
Macrodasyida						
Turbanella cornuta			0.19			0.41
Turbanella sp. 1	0.13	0.13				
Turbanella sp. 2	0.14	0.14		0.27	0.22	
				0.66		
Paraturbanella dohrni	0.20		0.21	0.40		0.43
Paraturbanella teissieri	0.05					
Mesodasys adenotubulatus	0.28					
Mesodasys laticaudatus	0.29		0.26	0.66		0.53
						1.54
Acanthodasys aculeatus	0.26	0.25				
Diplodasys ankeli		0.20				
Diplodasys meloriae		0.25			0.44	
					1.52	
Ptychostomella mediterranea	0.14	0.16			0.29	
					0.46	
					0.96	
Tetranchyroderma polypodium	0.64			1.28		
				2.61		
Chaetonotida						
Xenotrichula intermedia		0.31			0.52	
					1.05	
Xenotrichula punctata		0.31			0.54	
Heterolepidoderma ocellatum						0.15

the lack of data on polyploidy. However, different classes of ploidy have been found in single tissues and organs of other euthelic taxa, mainly in the nuclei of secretory cells[17,18]. The freshwater parthenogenetic chetonotid *Heterolepidoderma ocellatum* appears to be a special case: its somatic DNA value content is very low, below even the genome size of the marine hermaphroditic chetonotids. Thus a possible somatic polyploidy of chetonotids, related, by analogy to many plant and animal taxa[19], to their obligatory parthenogenesis, seems unlikely, at least in this species. Measurement of C value in the few, morphologically aberrant spermatozoa with uncertain functional capacity[20] which appear in many chetonotid species after the parthenogenetic phase, will be useful for a comparison with hermaphroditic species.

Gastrotricha C value is very low, one of the lowest found in invertebrates to date[7,21,22]. Similar values have been reported in sponges, cnidaria, tardigrades, some insects and tunicates[22–24,28]. As far as taxa which are phylogenetically close to Gastrotricha are concerned, genome size has not yet been determined in Gnathostomulida, whereas in a parthenogenetic bdelloid Rotifer it is 0.75 pg, a value not far from those obtained in the present study[25]. Other data on C values of Rotifera, expressed only in arbitrary units, do not allow comparison with gastrotrichs[26]. Somatic DNA content in Rotifera can vary in relation to the polyploidy of some morphotypes as well as of different cellular types[17,27]. Nematoda, the phylum closest to Gastrotricha, show a comparable minimum genome size, 0.08 pg, but range up to 2.5 pg[28].

The uniformity of the genome size in Gastrotricha, evidenced by its narrow range around a low value, favours the basic unity of the phylum in spite of the differences between the two orders. A relation between the low genome size of Gastrotricha and their stem position in the phylogenesis of lower Metazoa would seem reasonable, though the complexity of an organism is often not strictly related to its genome size[21,22,28]. Many taxa of Platyhelminthes, the stem group from which Gastrotricha probably arose, show higher and more variable genome sizes[29,30], thus the low C value in gastrotrichs may not be a primitive feature but rather the result of selection of a small cell size and body size, as well as short life cycles and rapid reproductive rates, all features fitting the r-strategy of this phylum[22,31].

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