

Genome sizes and chromosomes in the basal metazoan *Hydra*

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Abstract

Hydras belong to one of the earliest eumetazoan animal groups, but to date very little is known about their genome sizes, gene numbers, and chromosomes. Here we provide genome size estimates and corresponding karyotypes for five *Hydra* species. Nuclear DNA contents were assessed by slide-based Feulgen microphotometry. *Hydra oligactis* possesses the largest genome of 1450 Mbp, followed by similar 1 C capacities in *H. carnea* (1350 Mbp), *H. vulgaris* (1250 Mbp) and *H. circumcincta* (1150 Mbp). The smallest genome of 380 Mbp was determined in *H. viridissima*. While the number of chromosomes is identical in all five *Hydra* species ($2n = 30$), the size of the chromosomes is strictly correlated to the size of the genome, with *H. viridissima* having conspicuously small chromosomes. The taxonomic and evolutionary significance of the C-value and chromosomal size variation in this ancient group of metazoans as well as its impact on genomic organization and forthcoming genome projects are discussed.

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Introduction

Animals with similar levels of cellular and developmental complexity can have very different genome sizes (Gregory, 2001, 2004). Differences in genome size can be due to variation in amplifications, deletions, and divergences of repetitive sequences, increase or reduction of heterochromatin, and the creation of accessory chromosomes. The significance and evolutionary implications of inter-species differences in genome size (also called the “C-value paradox”, Thomas, 1971) are unknown. Mere coincidence is commonly proposed to explain these differences (e.g. Pagel and Johnstone, 1992), but it may not be the only, or indeed the most likely, explanation. There is a general tendency for

genome sizes of multicellular species to exceed those of unicellular species. Therefore, some authors have proposed that expansions of genome size and complexity during eukaryotic evolution were essential for adaptive phenotypic diversification (Lynch and Conery, 2003). In any case, since the larger part of the DNA in all eukaryotes is nonencoding and this fraction can vary in size from species to species without affecting active genes, genome size and presumed gene number appear unrelated to each other (Mirsky and Ris, 1951). Genome size data (“C-values”, Swift, 1950) are currently available for nearly 4000 animal species (about 2500 vertebrates and 1300 invertebrates) at the Animal Genome Size Database (www.genomesize.com) (Gregory, 2001). Surprisingly, this database does not contain any data from one of the most ancient member of the animal kingdom, the phylum Cnidaria. Without detailed knowledge of these basal metazoans, however, it is

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impossible to provide an effective comparative framework for animal evolution.

Cnidaria are of monophyletic origin (Bridge et al., 1992, 1995) and exhibit considerable diversity in morphology. Symbiosis with photosynthetic algae contributes to the success of many cnidarians (Geller and Walton, 2001; Habetha et al., 2003). Within the cnidarians, the genus *Hydra* is a well established experimental model system in developmental biology and molecular research (Bosch and Fujisawa, 2001; Steele, 2002; Bosch and Khalturin, 2002; Bosch, 2003). The taxonomy of the genus is complicated and still not settled (Holstein et al., 1990; Stepanjants et al., 2000). Eight species of *Hydra* have been described and these have been placed in two subgenera (“groups”), the brown and green *Hydra*, respectively (Campbell, 1983; Holstein, 1995). The recent focus on genomics has intensified attempts towards elucidation of the transcriptomes of *Hydra* and other Cnidaria in the form of “expressed sequence tag” (EST) collections. First observations indicate that these basal metazoans are genetically much more complex than previously thought (Kortschak et al., 2003). Studies focused on cis-regulatory regions point in the same direction and indicate that the molecular mechanisms that direct spatially restricted gene expression in *Hydra* are surprisingly complex (Endl et al., 1999; Thomsen et al., 2004).

Although Cnidaria are crucial to comparative and evolutionary studies, little is known about genome sizes and chromosome structure in Cnidaria in general, and in *Hydra* in particular. In *Hydra vulgaris*, the diploid nuclear DNA content was determined previously to be 3.5 pg (David and Campbell, 1972). To date, there have been no such studies published for other *Hydra* species. For *H. vulgaris*, chromosome numbers ($2n$) of 32 (Niiyama, 1944; Datta, 1970, 1978) and 30 (Rahat et al., 1985; Ovanesyan and Kuznetsova, 1995) were reported. We previously described karyotypes for *H. oligactis* and *H. circumcincta* (Anokhin and Kuznetsova, 1999) as well as for *H. viridissima* (Kuznetsova et al., 2001) and an endemic *Hydra* species in Lake Baikal (Anokhin, 2002). Here we extend these studies and determine the genome sizes of five species of *Hydra* including brown and green ones. We show that the four brown species have very similar genome sizes while the genome of the symbiotic green *H. viridissima* is about 3 times smaller. Genome size differences correlate remarkably well with differences in size of the chromosomes whereas the number of chromosomes is identical in all species examined. These observations not only contribute to a better understanding of Cnidaria phylogenetics and genomics but may also enable further progress in understanding the organization and evolution of genomes.

Materials and methods

Animals and culture conditions

Experiments were carried out with *H. vulgaris* (strain AEP), *H. carnea*, *H. circumcincta*, *H. oligactis*, and *H. viridissima*. *H. vulgaris* strain AEP was obtained from Dr. Ulrich Technau, Darmstadt, and maintained as stock culture as described previously (Fröblius et al., 2003).

AEP males exhibit constitutive sexual activity (Kuznetsov et al., 2002) and therefore were used to study nuclear DNA contents in spermatogenesis. All *Hydra* species were cultured using standard conditions in a constant temperature room maintained at $18 \pm 0.5^\circ\text{C}$. The animals were fed regularly with freshly hatched *Artemia* nauplii. A cockerel of the *Bankiva* chicken (*Gallus gallus*) provided 2 ml venal blood that was diluted 1:10 with 4% sodium citrate solution. Interphase nuclei of the red blood cells contain 2.53 pg DNA each (Mulligan and Rasch, 1985) and served as an adequate standard to calibrate the DNA capacity of nuclei in the *Hydra* specimens (Fig. 1).

Chromosome preparation

Hydra polyps were subjected to a hypoosmotic shock with 0.4% trisodium citrate for 30 min, followed by fixation in ethanol and acetic acid (3:1) for 15 min. Specimens were transferred in a drop of 70% ethanol to glass slides and dissected with needles. The cell suspension was spread by drying with a hot fan and stained in

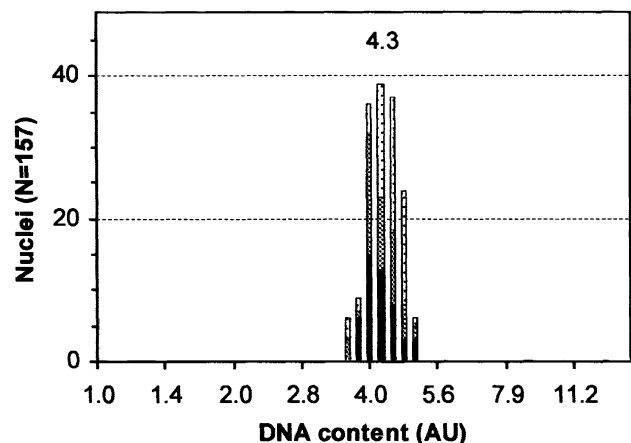


Fig. 1. DNA standard from erythrocyte 2C nuclei of male domestic fowl, *Gallus gallus*. Three slides from different Feulgen batches were analyzed. The overall mean 4.34 ± 0.03 AU (area absorption units) from 157 microphotometric records is equivalent to 2.53 pg DNA (Mulligan and Rasch, 1985).

6% Giemsa in Sørensen's phosphate buffer at pH 6.8 as described previously (Anokhin and Kuznetsova, 1999).

Feulgen procedure

Hydra cell suspensions obtained by maceration were fixed with 4% formaldehyde and spread on gelatin-coated glass slides as described (David, 1973). Blood smears from the cockerel were included in each staining run. For Feulgen staining, the crucial step of hydrolysis was done simultaneously with the *Hydra* specimens and the chicken DNA standard in 5 M HCl at 23 °C for 50 min. After a short rinse in water, staining was carried out with colorless Schiff's reagent (Merck 109033.0500) at room temperature for 50 min. The specimens were bleached in 2 mM sulfurous acid (3×5 min), dehydrated, and mounted in Euparal (Chroma 3C239, Köngen, Germany).

Microphotometry

Measurements were carried out using a scanning densitometer MPM 03 with APAMOS T3 software (Zeiss, Oberkochen). All nuclei were examined at $\times 100$ magnification. The diameter of the measuring diaphragm was set to $1.0 \mu\text{m}$ and the scanning steps were chosen to be $1.0 \mu\text{m}$ in *X*-axis as well as in *Y*-axis. Monochromatic light was adjusted to 560 nm using a spectral interference filter. The nuclear contents of Feulgen-DNA resulted in area absorption units (AU), which represented the sum of absorbance in pixel records of individual scanning procedures. Control measurements within the (colorless) cytoplasm of the specimens averaged to negligible absorbance. Since measuring fields were adjusted most closely to each nuclear projection area, the conditions for APAMOS data output were used within the default thresholds $0.022 < A < 1.301$, that is $95\% > T > 5\%$ for transmittance. Data scatter was expressed as standard error of means throughout this paper. Interpretation of DNA records from somatic cells depends on the assessment of standard nuclei with certain DNA contents. Since spermatogenesis does provide such nuclei at 4 C, 2 C and 1 C (Swift, 1950; Zacharias et al., 1988), *H. vulgaris* strain AEP which continuously produces male gametes was used to analyze spermatocytes I, spermatocytes II and spermatids, respectively (Figs. 2a–c). Measurements included 178 meiocytes in total. They formed, as expected, three consecutive classes of DNA contents reflecting their status and progress in meiosis from 4 C via 2 C to the final 1 C value (Fig. 2). The major peak at 7.33 ± 0.08 AU from $N = 70$ nuclei in prophase I, most probably in zygotene and pachytene, indicated 4 C DNA. Interkinetic 2 C nuclei of spermatocytes II averaged at 4.27 ± 0.06 AU ($N = 50$). Early spermatids

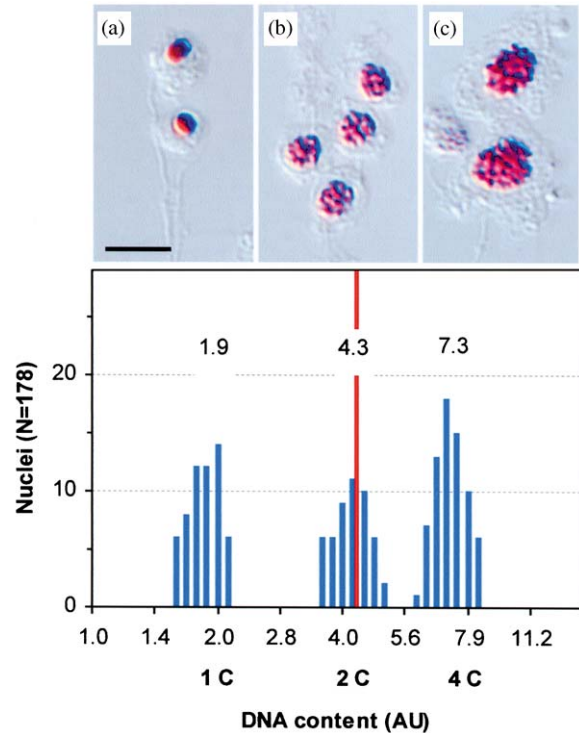


Fig. 2. Nuclei from spermatogenesis in AEP strain of *H. vulgaris*: (a) Early spermatids. (b) Interkinetic spermatocytes II. (c) Spermatocytes I in zygotene or pachytene. Feulgen. Scale bar: $5 \mu\text{m}$. Plot: Microphotometric records from spermatogenesis (from right): 70 spermatocytes I: 7.33 ± 0.08 AU with 4 C DNA; 50 spermatocytes II: 4.27 ± 0.06 AU at 2 C; 58 spermatids: 1.86 ± 0.02 AU possessing the DNA amount of the genome (1 C). Vertical line (red in web version): DNA standard 4.34 AU from Bankiva cockerel (Fig. 1).

showing rounded nuclei were chosen to determine the 1 C level at 1.86 ± 0.02 AU ($N = 58$). Elongated spermatids were not taken into consideration because control measurements revealed their nuclei to be much more negatively biased by enhanced chromatin condensation and surface reduction. Conversion of picograms into mega-base pairs was based on the calculation by Doležel et al. (2003) according to which 1 pg DNA is equivalent to 978 Mbp.

Results

Genome sizes in different *Hydra* species

Genome sizes were recorded for five *Hydra* species. Chicken erythrocytes were used as a reference (Fig. 1). Spermatocytes and spermatids of *H. vulgaris* strain AEP provided standard nuclei at 4 C, 2 C and 1 C (Fig. 2). 2 C nuclei of spermatocytes II averaged at 4.27 ± 0.06 AU

($N = 50$). Early spermatids showing rounded nuclei (Fig. 2c) were chosen to determine the 1 C level at 1.86 ± 0.02 AU ($N = 58$). The nuclear DNA content of *H. vulgaris* was determined using interphase nuclei from both interstitial cells and epithelial cells (Figs. 3a, c, e). About half of the large interstitial cell nuclei (34 out of 57) yielded 4.15 ± 0.04 AU, indicating 2 C DNA content (Fig. 4a). The majority of *H. vulgaris* epithelial cell nuclei (62/72) showed the 4 C level at 9.34 ± 0.09 AU (Fig. 4b). This is in agreement with previous observations and indicates that in contrast to interstitial cells, the majority of epithelial cells in *Hydra* are in the G2 period of the cell cycle (David and Campbell, 1972). Interestingly, measurements of two epithelial cell nuclei resulted in 14.6 and 17.4 AU indicating excessive DNA endoreplication in the epithelium (data not shown). For determination of the nuclear DNA content of *H. oligactis*, a total of 67 interstitial cells were investigated. While the majority of these nuclei (51/67) yielded 4.63 ± 0.05 AU (Fig. 5a), most *H. oligactis* epithelial cell nuclei (32/38) yielded 11.04 ± 0.17 AU. Thus, the observed values of 2 C and 4 C nuclear DNA were 12% and 18% above the respective values in *H. vulgaris* (Table 1). Measurements of 52 interphase nuclei from *H. carnea* interstitial cells (Fig. 5b) yielded a

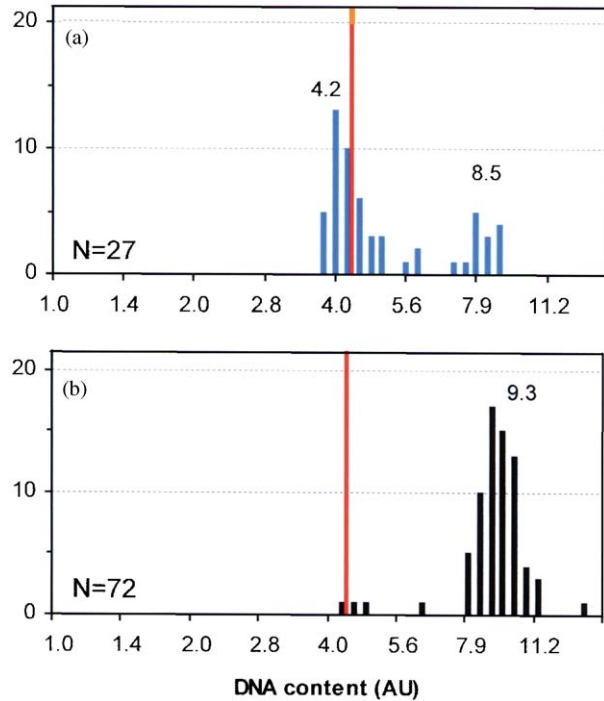


Fig. 4. Nuclear DNA contents in *H. vulgaris* AEP: (a) Interstitial cells show 2 C DNA content at 4.15 ± 0.04 AU. (b) Epithelial cells with 4 C DNA at 9.33 ± 0.09 AU. Apart from the extreme nucleus with 14.6 AU, there was another one with 17.4 AU (not shown). Red: DNA standard 4.34 AU from *Bankiva cockerel* (Fig. 1).

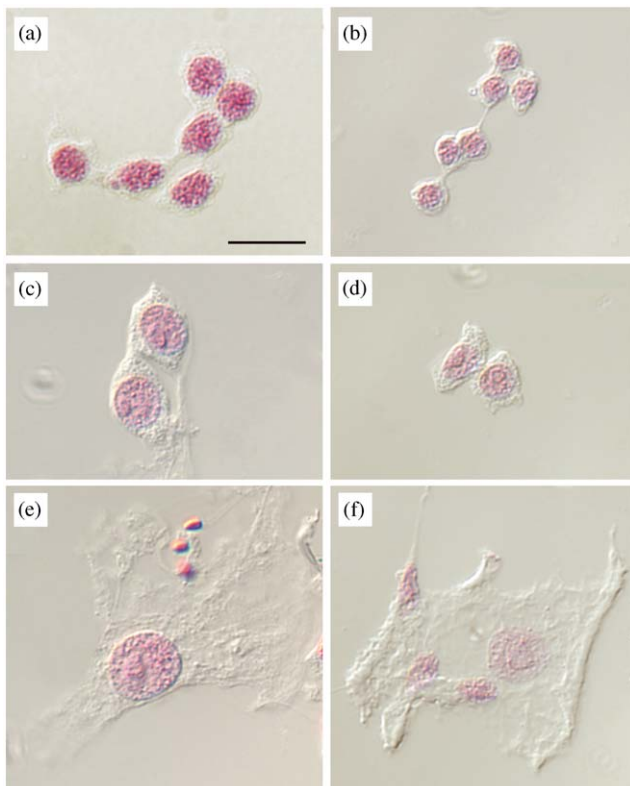


Fig. 3. Cytology of somatic nuclei from *H. vulgaris* AEP (a, c, e) and *H. viridissima* (b, d, f). (a–d): interstitial cells; (e–f): epithelium. Feulgen. Scale bar: 10 μ m.

peak (43/52 nuclei) at 4.43 ± 0.04 AU. In the sample of 47 *H. carnea* epithelial cells, 38 showed a mean of 9.77 ± 0.12 AU nuclear DNA. These values exceeded the 2 C and 4 C levels in *H. vulgaris* by 7% and 5%, respectively. The sample of 70 *H. circumcincta* interstitial cells (Fig. 5c) revealed a majority of 48 nuclei at 4.03 ± 0.04 AU. Of the 69 *H. circumcincta* epithelial cells measured, the mean of 60 nuclei was at 8.70 ± 0.08 AU. Thus, these 2 C and 4 C results were very similar to the respective data in *H. vulgaris* (Table 1). In addition to the somatic cells, in *H. circumcincta* we also measured a telophase II nucleus in the vicinity of spermatids. The double reading on both halves was 4.43 ± 0.08 AU confirming its 2 C DNA content. Moreover, microphotometric analysis of 20 condensed nuclei from *H. circumcincta* spermatids averaged at 1.73 ± 0.03 AU, corresponding to the 1 C level determined for *H. vulgaris* AEP spermatids.

In order to include a green *Hydra* species living in symbiosis with *Chlorella* algae in our analysis, we also investigated *H. viridissima*. Microphotometry on a total of 102 *H. viridissima* interstitial cells (Fig. 5d) showed the presence of two classes of nuclei. The majority of 72 interstitial cell nuclei had 1.30 ± 0.01 AU indicating 2 C, while 19 larger nuclei were observed

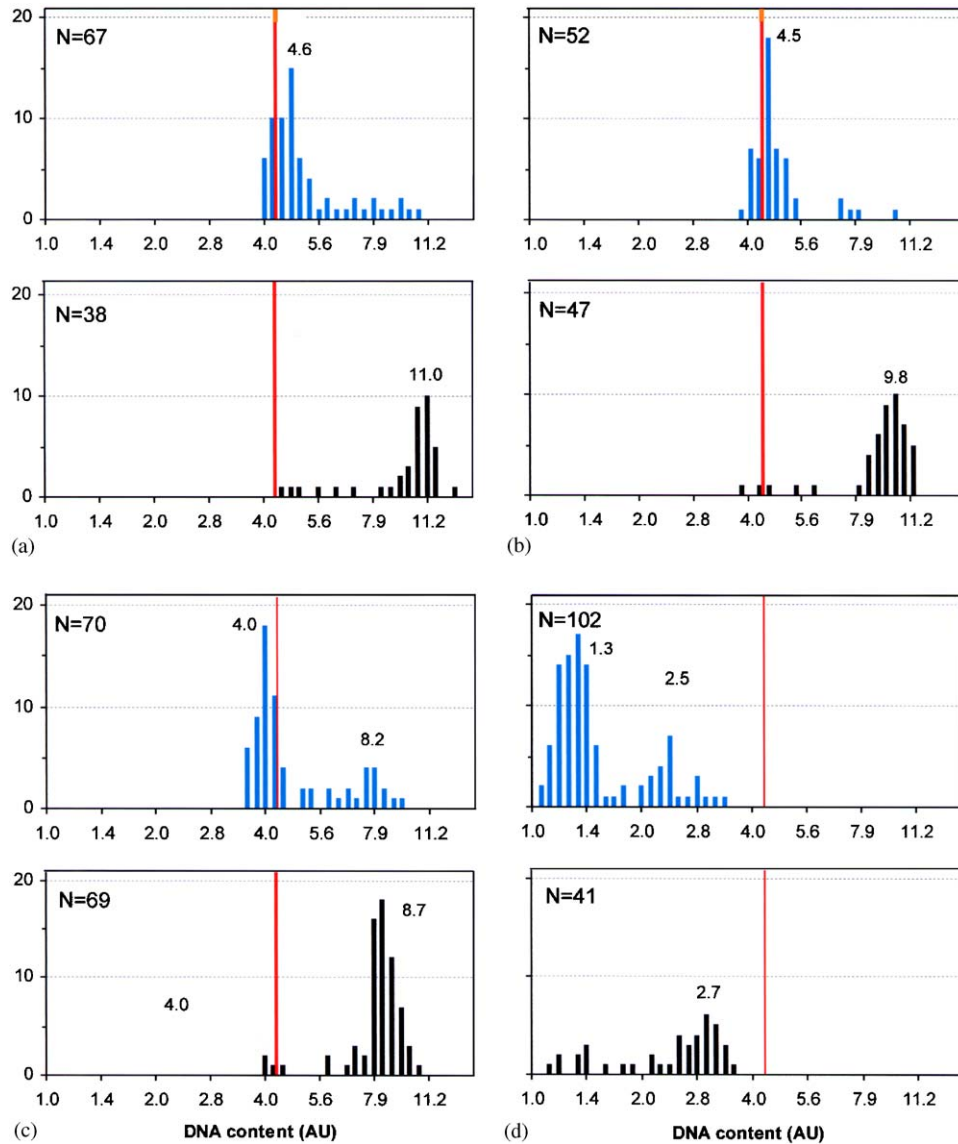


Fig. 5. Frequency distribution of nuclear Feulgen DNA contents in somatic nuclei from interstitial cells (i-cells, grey, blue in web version) and epithelial cells (epi-cells, black): (a) *H. oligactis*: i-cells peak at 4.63 ± 0.05 AU and epi-cells at 11.04 ± 0.17 AU. (b) *H. carnea*: i-cells, 4.56 ± 0.04 AU; epi-cells, 9.77 ± 0.17 AU. (c) *H. circumcincta*: i-cells, 4.03 ± 0.04 AU; epi-cells, 8.70 ± 0.08 AU. (d) *H. viridissima*: i-cells, 1.3 ± 0.05 AU; epi-cells 2.72 ± 0.06 AU.

at 2.53 ± 0.07 AU and most likely contained 4 C DNA. Measurements of epithelial cell nuclei indicated that half of them (21/41) had DNA contents of 2.72 ± 0.06 AU. These values were 31% and 33% below the respective values in *H. vulgaris*. Thus, the DNA content in *H. viridissima* is considerably smaller than in any of the other *Hydra* species. Table 1 shows the DNA contents of the five *Hydra* species in picograms and mega base-pairs. The data indicate genome sizes of about 380 Mbp for *H. viridissima*, 1150 Mbp for *H. circumcincta*, 1250 Mbp for *H. vulgaris*, and 1350 Mbp for *H. carnea*. *H. oligactis* possesses the largest genome of 1450 Mbp.

Chromosomes of *Hydra* species

The drastic differences in genome sizes between green and brown *Hydra* species led us to examine the chromosomes in these species. Karyotypes were recorded for the two brown species *H. vulgaris* strain AEP and *H. oligactis* as well as for the green *H. viridissima*. The metaphase chromosome spreads shown in Fig. 6 show that all three species have chromosome numbers of $2n = 30$. This is in agreement with the previous observation of $2n = 30$ in *H. circumcincta* (Anokhin and Kuznetsova, 1999) and in an endemic *Hydra* species in Lake Baikal (Anokhin, 2002). Thus, the genus

Table 1. Genome sizes in *Hydra* species, calculated after slide-based microphotometry of Feulgen-stained nuclei

Specimen	Microphotometric data					Genome (1 C)	
	Cells	C	AU ± SEM	N	pg	pg	Mbp
<i>H. oligactis</i>	i-cell	2	4.63 ± 0.05	51	2.70	1.35	1320
	epi	4	11.04 ± 0.17	32	6.44	1.61	1574
<i>H. carnea</i>	i-cell	2	4.56 ± 0.04	43	2.66	1.33	1300
	epi	4	9.77 ± 0.12	38	5.70	1.42	1393
<i>Hydra AEP</i>	sptid	1	1.86 ± 0.02	58	1.08	1.08	1060
	spcyt 2	2	4.27 ± 0.06	50	2.49	1.24	1217
	spcyt 1	4	7.33 ± 0.08	70	4.27	1.07	1045
<i>H. vulgaris</i>	i-cell	2	4.15 ± 0.04	34	2.42	1.21	1183
	epi	4	9.33 ± 0.09	56	5.44	1.36	1330
<i>H. circumcincta</i>	sptid	1	1.73 ± 0.03	20	1.01	1.01	986
	i-cell	2	4.03 ± 0.04	48	2.35	1.17	1149
	epi	4	8.70 ± 0.08	60	5.07	1.27	1240
<i>H. viridissima</i>	i-cell	2	1.30 ± 0.01	72	0.76	0.38	371
	epi	4	2.72 ± 0.06	21	1.59	0.40	388

The interphase nuclei of cockerel red blood cells averaged at 4.34 ± 0.03 AU ($N = 157$) and served as calibration standard $2C = 2.53$ pg DNA (Mulligan and Rasch 1985). Thus, 1 AU (area absorption unit) is equivalent to 0.5829 pg DNA or 978 Mbp (Doležel et al., 2003). Only rounded nuclei of early spermatids (sptid) prior to elongation were measured. N, nuclei in DNA class C; epi, epithelial cells; i-cell, interstitial cells; spcyt, spermatocytes.

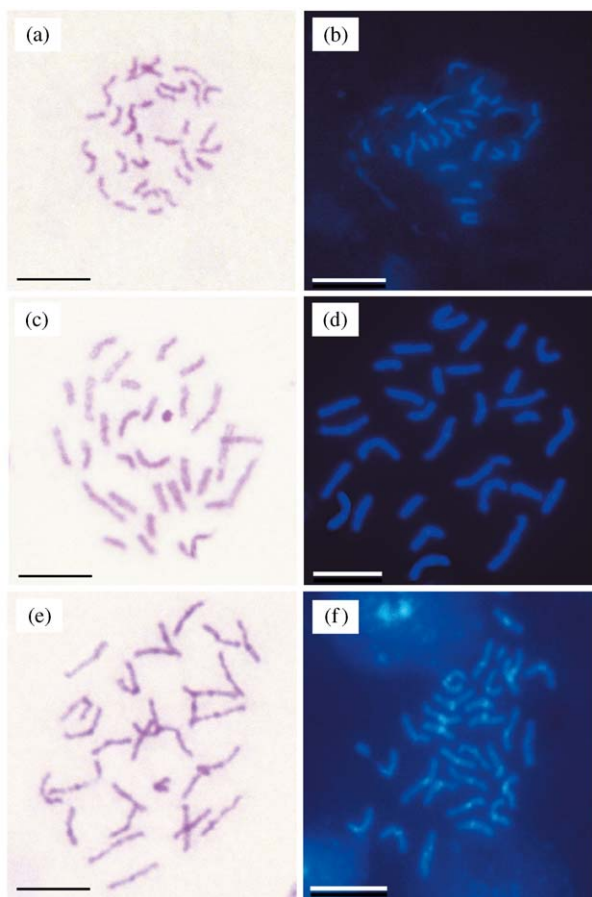


Fig. 6. Karyotype display in the *Hydra* group: metaphase chromosome spreads of somatic mitotic cells from three species of *Hydra*. $2n = 30$ in each case: (a, b): *H. viridissima*; (c, d): *H. vulgaris* AEP; (e, f): *H. oligactis*. Giemsa, DAPI. Scale bar: 10 μ m.

appears to have 15 pairs of chromosomes with no variation detectable. In contrast to the similarity in the number of chromosomes, Fig. 6 also shows that the sizes of the individual chromosomes of *H. viridissima* are drastically different when compared to the two brown *Hydra* species. The observed variations in genome size and chromosomal length may be of taxonomic significance, providing a clearer picture of the processes that account for hydroid phylogeny. A phylogenetic tree of the genus *Hydra* (Fig. 7) based on nuclear ribosomal sequences (provided by D. Martinez, Claremont) suggests that *H. oligactis* and *H. vulgaris* are closely related, whereas *H. viridissima* appears as the ancestral form; *H. magnipapillata* is considered as the Japanese *H. vulgaris*. The finding that *H. viridissima* has a conspicuously small genome in contrast to the other *Hydra* species supports a positioning of *H. viridissima* well apart from the brown *Hydra* species.

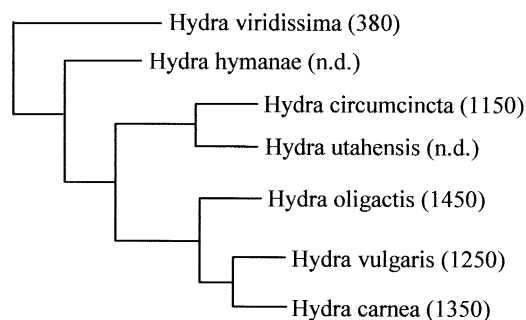


Fig. 7. Phylogenetic tree (based on ribosomal sequences and provided by D. Martinez, Claremont) and corresponding genome sizes in Mbp.

Discussion

Comparative observations of genome sequences, EST collections and gene functions across the metazoa are providing us with information on the quality and degree of evolutionary change that occurred during phylogeny. Cnidaria branched off the metazoan tree before Urbilateria came into existence. Surprisingly, they appear to share a large part of their gene repertoire with vertebrates (Kortschak et al., 2003). For that reason, Cnidaria are a good choice for comparative evolutionary research. Since little has so far been known about genome sizes and chromosomes in Cnidaria, and since the advent of genomics requires such information, we determined the genome sizes (C-values) of a variety of *Hydra* species. The results of this study demonstrate large and to some extent variable genome sizes within this ancient animal genus. While there are substantial similarities in genome size in the four brown species of *Hydra*, the only symbiotic species within this genus, *H. viridissima*, has a threefold smaller genome. Interestingly, a direct correlation emerged between genome size and size of chromosomes. The reasons for the small genome of *H. viridissima* are not known. It may be due to quantitative differences in single copy DNA or in repetitive fractions. In mammals, the modulation of DNA content has been proposed to be a means by which they fine-tune metabolic rates via the intermediate of cell size (Vinogradov, 1995; Gregory and Hebert, 1999; Gregory, 2001). Small cell sizes appear to require small genomes in both birds and mammals (Gregory, 2002). Interestingly, in *H. viridissima* the size of interstitial cells is conspicuously smaller than in brown polyps (see Fig. 3). Since symbiotic *Chlorella* algae contribute considerably to the metabolism of *H. viridissima* polyps (Habetha et al., 2003), it is tempting to speculate that the small genome size in *H. viridissima* is a consequence of the symbiosis. Furthermore, it has been proposed (Johnston et al., 1996) that genome size variation may be an adaptation to a changing environment. In particular, temperature has been reported as a factor to directly or indirectly influence genome size in reptilians (Olmo, 2003; Bernardi et al., 1985; Vinogradov, 1997). Reptiles with larger genome sizes have greater tolerance to critical maximum and lethal temperatures (Olmo, 2003). Although the ecological and environmental preferences of the five *Hydra* species used in this study are not known in detail, it has been reported that in contrast to *H. viridissima*, *H. oligactis* is strictly stenotherm and that “it is unlikely that *H. oligactis* is living in the same habitat as *H. viridissima*” (cited from p. 77, Holstein, 1995). Does the microenvironment of *H. viridissima* entail high metabolic costs and thus resulted in adaptations such as small genome and cell size? Whatever the processes by which its small genome

arose, *H. viridissima* appears to be the *Hydra* species of choice for genome sequencing projects.

What can *Hydra* chromosomes tell us?

A comprehensive survey of genome sizes and karyotypes may help to understand processes of evolution and can sometimes be taxonomically useful to clarify the phylogenetic relations between species. As discussed above, all five *Hydra* species analyzed show a partition of their genomes into $n = 15$ chromosomes. This fact supports the view (Blackman et al., 2003) that chromosome numbers within genera usually vary little, indicating that normally there is a strong stabilizing natural selection. While most of the genome sizes were found to be in the range of 1000–1400 Mpb, the haploid set of *H. viridissima* was observed to be just below 400 Mpb (Table 1). Thus, the DNA capacity of an average chromosome may amount to 25 Mbp in *H. viridissima*, but to 100 Mbp in *H. oligactis*. Two, partly complementary, causes have been proposed to explain chromosome sizes of significant difference: (i) Species with larger chromosomes may have more active genes than species with smaller chromosomes. (ii) Species with large chromosomes may contain additional amounts of noncoding DNA and repetitive elements resulting in domains of heterochromatin (Redi et al., 2001). Support for this view comes from both C-banding (B.A., pers. observation) as well as from DAPI-staining (Anokhin and Nokkala, 2004) of *Hydra* chromosomes which reveals AT-rich regions of heterochromatin in centromeric regions in chromosomes of all *Hydra* species except *H. viridissima*.

In sum, the data presented here add to the considerable body of other evidence that organismal complexity is not related to genome size. The results have identified *H. viridissima* as the species with the smallest genome in the genus *Hydra*. The comparative survey has also shown that, while the number of chromosomes is identical in all species, genome sizes are correlated with the sizes of chromosomes. Thus, the comparative study of genome sizes in the basal metazoan genus *Hydra*, especially in relation to their association with symbionts, has offered and will continue to offer important insights into the evolutionary forces shaping genomes and chromosomes.

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