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Nuclear DNA and Remarks on Chromatin Diminution in Cyclopoid Copepods

Andrey Konstantinovich Grishanin[1,*], Hans-Uwe Dahms[2] and Alexey Pavlovitch Akifiev[1]

[1]*Vavilov Institute of General Genetics, Russian Academy of Sciences, ul. Gubkina 3, Moscow 117809, Russia*

[2]*AG Zoosystematik und Morphologie, University of Oldenburg, FB 7, D-26111 Oldenburg, Germany*

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Andrey Konstantinovich Grishanin, Hans-Uwe Dahms and Alexey Pavlovitch Akifiev (2004) Nuclear DNA and remarks on chromatin diminution in cyclopoid copepods. *Zoological Studies* **43**(2): 300-303. The sizes of chromosomes and nuclear DNA were determined for German populations of *Acanthocyclops viridis*, *Eucyclops macruroides*, *E. serrulatus*, *Cyclops insignis*, and *A. viridis* and Russian populations of *Macrocyclops albidus* and *Thermocyclops crassus*. In contrast to German populations, the Russian populations of *C. insignis* show no chromatin diminution (CD). The absence of CD in Russian populations of *C. insignis* elicited the supposition that these 2 populations are probably at the early stages of speciation on the path towards speciation events. In *P. affinis*, we noted a striking difference in the chromosomes and nuclear DNA contents of embryonic cells compared to somatic cells of adults. http://www.sinica.edu.tw/zool/zoolstud/43.2/300.pdf

Key words: Genome size, Chromatin diminution, Cryptic speciation.

Cytogenetic characters have become a valuable tool for phylogenetic reconstructions and for a general understanding of evolution processes (McLaren et al. 1988 1989, Einsle 1993, Grishanin and Akifiev 2000, Wyngaard 2000, Wyngaard and Rasch 2000, Akifiev et al. 2002). In addition to chromosome numbers, chromosomes, and nuclear DNA, the absence or presence of chromatin diminution (CD), its location, the amount of eliminated chromatin, and the timing of the eliminated nuclear DNA and other characteristics could serve as additional tools for understanding complex evolutionary questions.

Chromosomes and nuclear DNA of plants and animals can be important diagnostic or constitutive characters for taxonomy and phylogeny. However, there is increasing evidence that the chromosomes and nuclear DNA of some members of the Copepoda are not constant during ontogenesis due to CD. CD is found in some species of Copepoda (Beermann 1959 1966 1977, Einsle 1962 1964 1993, Robins and McLaren 1981, Standiford 1988 1989, Escribano et al. 1992, Grishanin et al. 1996, Leech and Wyngaard 1996, Dorward and Wyngaard 1997). Ulrich Einsle (1962) proposed to use the presence/absence of a CD picture for taxonomic purposes. The systematics of many freshwater copepods are difficult, as cryptic species are found in many groups. Interpopulation differences have also been recorded as far as the timing and amount of eliminated chromatin, the duration of CD, as well as the timing and location of the eliminated DNA. This also holds for Russian and German populations of *Cyclops kolensis* and *C. strenuus strenuus* (Einsle 1993, Grishanin and Akifiev 2000).

MATERIALS AND METHODS

We studied the chromosomes and nuclear DNA of 4 cyclopoid species (*Acanthocyclops viridis*, *Macrocyclops albidus*, *Eucyclops macruroides*, and *Paracyclops affinis*) from Germany (Oldenburg region) and 3 cyclopoids (*Cyclops insignis*, *Eucyclops serrulatus*, and

*To whom correspondence and reprint requests should be addressed. Tel: 7-096-212-8319. Fax: 7-095-132-8962.
E-mail:andreygrishanin@yahoo.com

Thermocyclops crassus) from Russia (Moscow and the Rybinsk reservoir region).

Acanthocyclops viridis Jurine and *Macrocyclops albidus* Jurine were collected from Dobbenteich, a pond within the town of Oldenburg (Germany) in June 1999 and *Eucyclops macruroides* Lill. (preliminary identification) and *Paracyclops affinis* Sars (preliminary identification) were collected from the same pond in September 1999. *Cyclops insignis* Claus was collected from a pond located in the Vorob'evy Mountains (Moscow) in Apr. 1999. *Eucyclops serrulatus* Fischer and *Thermocyclops crassus* Fischer were caught in ponds of the Borok Nature Reserve Vologodskaia obl. near the Rybinsk Reservoir (Russia) in June-July 1999. All cyclopoid species were identified according to Rylov (1948) and Monchenko (1974). The cleavage division was determined according to the number of cells within embryos.

Females of these cyclopoid species carrying egg sacs were fixed in ethanol/glacial acetic acid (3:1) for 1 h, with 2 changes of fluid. The female and embryos were separated on a subbed slide, softened in 45% glacial acetic acid, squashed gently with a coverslip, dipped into liquid nitrogen, and thawed in 2 rinses of 100% ethanol. Preparations were hydrolyzed in 5 N HCl at 25°C for 15 min, rinsed in 5 changes of water and placed into Feulgen stain for 2 h. Slides were then passed through a series of 3 rinses of 10% potassium bisulfite for 5 min, rinsed in 3 changes of water, dehydrated in 95% ethanol for 1 min, and subsequently dried. DNA content was measured using a scanning microdensitometer (Vickers M86) at a wavelength of 580 nm and an objective lens of 100x. We used standards prepared by Dr. E. M. Rasch and Dr. G. A. Wyngaard of chicken (*Gallus domesticus*) (2.5 pg DNA nucleus) and rainbow trout *Oncorhynchus mykiss* (*Salmo gairdneri*) (5.2 pg DNA nucleus). Only embryonic and adult nuclei which were distinctly separated were used for the measurement of genomic DNA. All embryonic measurements were made at the metaphase (4C), anaphase (4C), telophase (2C), or interphase stage (4C). The measurement for cells of adults were made using interphase cells (2C and 4C). The measurements for cells of the germ line were made at the prophase stage of the 1st meiotic division (4C).

Only embryonic and adult cells of the cyclopoid species that were separated were used for the measurement of DNA comprising their genomes. The quantity of DNA was only determined for germ line cells which were qualified for a cytophotometric investigation.

RESULTS

The genome sizes of *A. viridis* (Germany), *E. serrulatus* (Russia), *E. macruroides* (Germany), *C. insignis* (Russia), and *P. affinis* (Germany) were measured herein for the 1st time (Table 1). We also determined the genome sizes of German populations of *M. albidus* and Russian populations of

Table 1. Average DNA contents per cell (pg) in adult somatic, embryonic, and germ line cells of freshwater cyclopoid copepods. Cells of the germ line were measured at the prophase stage of the 1st meiotic division. Standard errors are given in parentheses, and *n* refers to the number of cells measured

Species	Adult 2C		Embryonic cleavage	2C		Germ line 4C	
	Somatic	*n*	Division		*n*		*n*
	DNA per cell (pg)			DNA per cell (pg)			
Eucyclops serrulatus	1.1 (0.02)	65	4th	1.1 (0.02)	95		
Cyclops insignis	4.2 (0.08)	154	2nd	4.3 (0.08)	35		
			3d	4.3 (0.06)	56		
			4th	4.3 (0.02)	182		
			10th	4.2 (0.06)	130		
Acanthocyclops viridis	4.3 (0.07)	121	6th	4.2 (0.02)	67		
Macrocyclops albidus	2.2 (0.03)	78	5th	2.1 (0.02)	72		
			10th	2.1 (0.03)	101		
Thermocyclops crassus	2.4 (0.04)	68				4.8 (0.06)	103
Paracyclops affinis	0.8 (0.02)	77	4th	1.4 (0.02)	58		

T. *crassus* (Table 1). The chromosomes and nuclear DNA of somatic cells (2C) in adult females and embryonic cells at the 6th cleavage division of *A*. *viridis* were equal (Table 1). The nuclear DNA quantity of somatic cells of adult females of *E*. *macruroides* and embryonic cells at the 5th and 6th cleavage divisions of this species did not differ either. Measurements of DNA content in cells of *M*. *albidus* showed almost equal quantities at the 5th and 10th cleavage divisions, and in cells of adult females (Table 1). Embryonic cells of *E*. *serrulatus*, and somatic cells of adult animals had almost identical nuclear DNA contents (Table 1). The nuclear DNA content in a haploid set of somatic cell chromosomes of adult *T*. *crassus* and the DNA content in a haploid set of chromosomes of germ line cells at the prophase stage of the 1st meiotic division were 1C = 1.2 pg. The nuclear DNA content of embryonic cells of *C*. *insignis* during the 2nd, 3rd, 4th, and 10th cleavage divisions and somatic cell lines of adult *C*. *insignis* were very similar (Table 1). The DNA content (2C) of embryonic nuclei of *P*. *affinis* was almost twice as high as that in somatic cells of adults (Table 1).

DISCUSSION

The nuclear DNA contents of somatic cells of the adults (2C) of 6 species corresponded to the embryonic genome size of these species and ranged between 1.1 and 4.3 pg. Our data on the chromosomes and nuclear DNA of German populations of *M*. *albidus* (2C = 2.1-2.2 pg) are close to those of *M*. *albidus* from a Louisiana population in the US (Wyngaard and Rasch 2000).

We did not study the CD for *M*. *albidus*, *E*. *serrulatus*, *E*. *macruroides*, *M*. *viridis*, or *P*. *affinis* because we had no germ cells or embryos of these species at the earliest cleavage division (CD occurs for Cyclopoida usually between the 4th and 7th cleavage divisions). The amount of DNA of embryonic nuclei exceeded that of somatic cell lines of adults by 43%, so we assumed that *P*. *affinis* underwent CD during ontogenesis. This result is very interesting because CD has been found in no other species of the Eucyclopinae. However, there is a need to measure the genome size of germ line cell nuclei and confirm the presence of granules of eliminated chromatin to verify the existence of CD.

Chromatin diminution was not found in *T*. *crassus* or *C*. *insignis*. We found no differences between the genome size of germ line cell nuclei and the genome size of somatic cell nuclei of adult specimens for *T*. *crassus* and there were no differences between the DNA contents of embryonic and adult cell nuclei of *C*. *insignis*. Einsle (1993) found granules of eliminated chromatin in the German population of *C*. *insignis* at the 5th cleavage division. We intensively explored preparations of *C*. *insignis*, but found no CD at the 5th cleavage division. The process of chromatin diminution is generally indicated by granules of eliminated chromatin and an appreciable difference between chromosomes and nuclear DNA of embryonic cells at early cleavage division and cells of the germ line and chromosomes and nuclear DNA of somatic cells of adult animals or embryonic somatic cells after chromatin diminution. We found no granules of eliminated chromatin during the 5th cleavage division or during other cleavage divisions. The difference between the chromosomes and nuclear DNA of somatic cells of adult animals and embryos at the 2nd to the 4th cleavage divisions was not significant. Consequently, we may conclude that Russian populations of *C*. *insignis* does not exhibit CD. The absence of CD in Russian populations of *C*. *insignis* elicited the supposition that these 2 populations of *C*. *insignis* are probably on the path towards cryptic speciation.

Another question is of interest. The genome sizes of the Russian populations of *M*. *viridis* and *C*. *insignis* are very close to that of *C*. *insignis* (2.3 pg) (Grishanin 1996). But embryonic somatic cells of *C*. *kolensis* lose 94% of their DNA during the 4th cleavage division, contrary to Russian populations of *C*. *insignis* and *A*. *viridis* which experience no CD. Hence, what is the role of the eliminated DNA? Perhaps the eliminated DNA is producing a barrier of reproductive isolation and therefore fostering speciation. The eliminated DNA is only functional during meiosis. Later during embryogenesis, the DNA is lost via CD. The same phenomenon may hold for *T*. *crassus*. *Thermocyclops crassus* (2C = 2.4 pg) shows a 3-fold genome size compared to a *T*. *crassus* population from Hanoi, Vietnam (2C = 0.84 pg) (Wyngaard and Rasch 2000). We know that the taxon *Thermocyclops* occurs more widely in the tropics of the eastern Eurasian region (Rylov 1948). We hypothesize that resettlement of *T*. *crassus* in the Palearctic was accompanied by an amplification of the chromosomes and nuclear DNA, and that could have changed some characteristics of the ecophysiological properties of this species (such as its development and ecology). It is possible that an increase in genome size of nuclei accompanies speciation.

The mechanism of this process may be comparable to quantum jumps in the genome sizes documented in *Pseudocalanus* and *Calanus* (McLaren et al. 1989).

Significant interpopulation differences in the DNA content of somatic nuclei of adult females of some marine copepods were revealed by Escribano et al. (1992). Interpopulation differences were also marked as to the timing and amount of eliminated chromatin, the duration of chromatin diminution, as well as the timing and location of the eliminated DNA between Russian and German populations of *Cyclops kolensis* and *Cyclops strenuus strenuus* (Grishanin and Akifiev 2000). In any case, further explorations of cyclopoid species by cytogenetics and cytophotometric methods will provide new additional information useful for the interpretation of evolutionary pathways within and phylogenetic relationships among the Copepoda.

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