### ATTI ACCADEMIA NAZIONALE DEI LINCEI

CLASSE SCIENZE FISICHE MATEMATICHE NATURALI

## Rendiconti

Claudio Barigozzi, Laura Baratelli Zambruni

# Presence and absence of chromocenters in populations of Artemia

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **71** (1981), n.5, p. 122–125. Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA\_1981\_8\_71\_5\_122\_0>

L'utilizzo e la stampa di questo documento digitale è consentito liberamente per motivi di ricerca e studio. Non è consentito l'utilizzo dello stesso per motivi commerciali. Tutte le copie di questo documento devono riportare questo avvertimento.

Articolo digitalizzato nel quadro del programma bdim (Biblioteca Digitale Italiana di Matematica) SIMAI & UMI http://www.bdim.eu/ Genetica. — Presence and absence of chromocenters in populations of Artemia. Nota di Claudio Barigozzi e Laura Baratelli Zambruni, presentata (\*) dal Socio C. Barigozzi.

RIASSUNTO. — Gli autori descrivono la presenza di cromocentri in nuclei somatici in riposo di naupli di Artemia.

Sembra che queste formazioni siano limitate alla specie A. franciscana. I risultati vengono discussi in relazione alla evoluzione del genere e alla possibilità di valutare la presenza di specie diversa nello stesso biotopo.

The microscopical structure of the resting nucleus of Artemia exhibited by the Nauplius cells consists of thin filaments of chromatin (Fig. 1) and, only in exceptional cases, of one or two chromocenters attached to the nucleolus. This corresponds to the description given by Barigozzi (1941) utilizing diploid material from Cagliari (Sardinia) and tetraploid material from the Istrian coast.

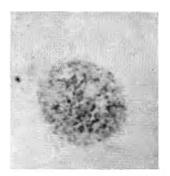


Fig. 1. – Resting nucleus without chromocenters.  $\times 2600$ .

Recent opportunities made available samples from a number of Artemia populations, located in all continents, and, therefore, new possibilities were opened by comparing the microscopical structure of the resting nucleus of individuals living in different biotopes.

The present paper aims to summarize the results obtained from Nauplii hatched in the laboratory from cysts collected in several localities, and not yet studied.

#### MATERIAL AND METHODS

It is well known that Artemia can be diploid (42 chrom.), tetraploid (84 chrom.), triploid (63 chrom.) and heteroploid (44, 46, 48, 50 chromosomes). Reproduction can be bisexual or parthenogenetic. The Nauplii investigated

(\*) Nella seduta del 21 novembre 1981.

belong to the following populations: Poetto, Sardinia Italy (diploid bisexual) – Tarquinia, Latium Italy (diploid bisexual) – Margherita di Savoia, Apulia Italy (diploid and tetraploid parthenogenetic) – Tien Tsin, China (predominantly diploid, exceptionally tetraploid) – Shark Bay, Australia (diploid bisexual) – Tuticorin, Kerala India (predominantly triploid, rare individuals with less than 60 chromosomes parthenogenetic) – Calpe, South of Spain (heteroploid and diploid, parthenogenetic) – Barbanera, South of Spain (predominantly diploid, rare heteroploid, bisexual) – Saelices, Central Spain (tetraploid parthenogenetic).

All these samples belong to *Artemia* formerly called *salina* (Leach) or to parthenogenetic populations traditionally attributed to *A. salina* (Barigozzi 1981).

Other populations were investigated: San Francisco Bay California, U.S.A. from two undetermined sources (diploid bisexual) – Great Salt Lake, Utah U.S.A. (diploid partenogenetic) – Macau, Brazil (diploid bisexual).

The latter four populations belong to the species Artemia franciscana (Barigozzi 1974). The population of Macau was introduced from the United States.

The freshly hatched Nauplii were treated as follows:

1) put in hypotonic solution (tap water or 0.5 % sodium citrate) for 30–40 min.

2) fixed in 1:1 methanol and acetic acid for 3 minutes.

3) after a 30 second exposure to 60 % acetic acid at ca. 40° temperature, stained in 2 % acetic orcein for 20 min. at room temperature;

4) rinsed twice in 95 % alcohol, transferred to absolute alcohol for 5 minutes and mounted in Euparal. Some samples were stained for 7 minutes with 0.5 % quinacrine.

#### RESULTS

The observations were made on 6 Nauplii for each population, considering a minimum of 10 nuclei per Nauplius.

All populations of Artemia from Europe, Asia and Australia (see the list under Material and Methods) confirmed the previous observations (Barigozzi 1941), while the four American populations belonging to *Artemia franciscana* exhibited a new type of structure: in every nucleus there is a number of chromocenters of different size, not very easy to count unambiguously (Fig. 2).

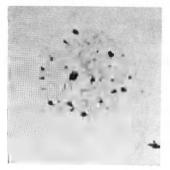


Fig. 2. – Resting nucleus with chromocenters (orcein).  $\times 2600$ .

Owing to difficulties in counting the chromocenters, which lie at different levels of the nucleus and sometimes are very close to each other, the observations were subjected to statistical evaluation.

	San Francisco Bay A	San Francisco Bay B	Great Salt Lake	Macau
Mean of chro- mocenters	14.8±1.8	14.4±1.3	14.4±1.9	15.1±1.7
Gen	eral Mean: 14.	$7 \pm 1.7$ F =	2.005 $P = 0$ .	11

Τ	ABLE	Ι

The chromocenters were counted in 10 nuclei, belonging to 6 Nauplii. These were selected on three different slides (hence 2 per slide), in order to assess whether the chromocenter number could be influenced by the technique. In all cases there was a sufficient homogeneity between slides of each population, therefore the numbers found can be considered as inherent to the genotype. The data are shown in Table I. The general mean (14.7  $\pm$  1.7) is very close to the mean of the single populations, and the analysis of variance indicates a strong homogeneity between them (F = 2,005 for 3 and 236 degrees of freedom, corresponding to a probability of 0.111).

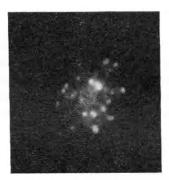


Fig. 3. – Resting nucleus with chromocenters (Quinacrine).  $\times$  260.

All counts, collected and classified in a histogram, point to a normal distribution with no evidence of kurtosis.

The nature of the chromocenters was investigated further by means of fluorescence. Stained with quinacrine the chromocenters were bright (Fig. 3). The structure of their chromatin is, thus, highly repetitive, being rich in AT.

#### DISCUSSION

The conclusions can be discussed as follows:

1) the chromocenters could correspond to entire heterochromatic chromosomes or to heterochromatic sections of some chromosomes. Up to the present stage of the investigation, there is no indication of the distribution of the heterochromatin in the mitotic chromosomes, thus no conclusion can be drawn. The deviation from the modal frequency can be explained either as a result of inaccuracy in counting due to overlapping or as a different degree of stretching of different chromocenters. In principle the chromocenter number should not fluctuate, unless supernumerary chromosomes are present, the existence of which is not yet known.

2) The presence of repetitive DNA in *A. franciscana* can be relevant for discussing speciation in Artemia.

3) The presence of chromocenters (if it is demonstrated, with further observations, that they are exclusive of A. franciscana) may provide a means for checking in the easiest way the situation in populations mixed with A. franciscana.

#### References

BARIGOZZI C. (1941) – I fenomeni cromosomici nelle cellule somatiche di Artemia salina Leach. « Chromosoma » (Berl.), 2, 251–307.

BARIGOZZI C. (1980) - Genus Artemia: problems of systematics. « The Brine Shrimp Artemia », Vol. 1, 146-153. Univ. Press., Wetteren Belgium.