The Nuclear Content of DNA in Three Different Strains of *Artemia salina* Leach (Phyllopoda, Branchiopodidae)

In the brine shrimp, *Artemia salina*, diploid and polyploid strains have been described by many authors, which differ from each other morphologically as well as physiologically. Furthermore, several strains or species exist with differences in their cytology. The diploid strains develop by fertilization or parthenogenetically, whereas the polyploid strains develop only parthenogenetically. In the diploid biotype parthenogenesis is automictic, whereas tetraploid *Artemia* are autopolyploid and therefore an exact ploidy relationship has to be found in the DNA content of the diploid and tetraploid parthenogenetic race. Considerable differences exist among the diploid strains. Structural differences in the chromosomes of *A. salina parthenogenetica 2n* and *A. salina anfigonica*, make it very likely that there is a different DNA content in the nuclei of these strains[1]. Therefore we tested whether the differences are to be accounted due to modifications of physiological character or to diversities correlated to a different DNA content in the 2 forms.

Artemia salina is present in Cagliari (Sardinia) as 2 biotypes: the diploid anfigonic in the salt works of S. Bartolomeo, and the diploid parthenogenetic in the salt works of S. Gilla. *A. s. parthenogenetica 4n* which lives in the marshes of Comacchio. For our research studies we have collected *A. s. anfigonica* and *A. s. parthenogenetica 2n* directly from the salt marshes of S. Bartolomeo and S. Gilla. For the tetraploid parthenogenetic strain we have used a stock we bred in our laboratory. Preparations of nervous tissue were made by dissecting optical ganglia and, after fixation in Carnoy, they were squashed in acetic acid 45%. Before hydrolysis, each slide was frozen in liquid nitrogen and the cover-slide taken off. The time for hydrolysis was chosen, in some tests, as 6.8 and 10 min. After 8 min of hydrolysis, nuclei showed a maximum of absorption at the wave length of 546 nm. The method we used for Feulgen staining is that proposed for cytophotometric measurements[2]. To determine the DNA content in the interphase nuclei we used the 'Whole-nucleus'-method[3] and only for comparison did we take measurements through the 'Plug'-method[4]. Measurements were taken with the Zeiss Mikrospektralphotometer. In the diagrams, the extinction was plotted against the number of nuclei in percent.

The results of the measurements on interphase nuclei from diploid *A. s. anfigonica* and tetraploid *A. s. parthenogenetica* are given in the Figures 1 and 2. In both types various degrees of ploidy could be found. The diploid form shows 3 maxima at 2c, 4c and 8c and about 90% of the cells belong to the DNA classes 2c and 4c. Both of these DNA classes are to be expected in tissues of diploid cells at the stage of cell division, whereas the DNA value 8c is characteristic for endomitotic growth of the cells. The maxima for the tetraploid *Artemia* were found to be 4c and 8c in 95% and 16c in only 5% of the cells. Thus the ploidy relationship 1:2 between the diploid and the tetraploid form demonstrated by the cytological examination of the metaphase plates, was also confirmed by the DNA measurements.

Comparison of *A. s. parthenogenetica 2n* and *A. s. anfigonica* was made on cells of the optical ganglia of both forms in order to obtain exact ploidy classes. Figures 3 and 4 summarize the data in terms of the 2 forms. In *A. s. anfigonica* the mean maximum is 0.64 ± 0.07, in *A. s. parthenogenetica 2n* the mean maximum is 0.78 ± 0.12. Comparing the extinctions it may be deduced that there exist no different ploidy classes in the ratio 1:2, although it cannot be excluded that gene duplications are present as the result of evolutionary steps, leading to small differences in the DNA content. The total length of the chromosomes between both forms is 1.4:1 as shown by STEFANI[5], whereas our findings on the DNA measurements of both forms show about 1.2:1. Therefore it could be assumed that the differences found in the chromosomal measurements are not only due to differences in the DNA content but also due to RNA and nuclear proteins. Nevertheless, measurements by ultramicrospectrophotometry would be necessary.

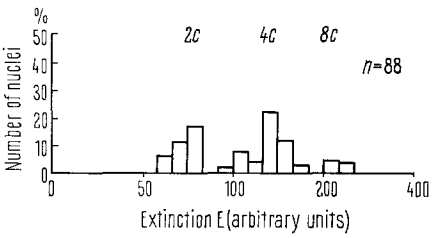

Fig. 1. Frequency distribution of DNA in interphase nuclei of *Artemia salina anfigonica*. The 2 maxima *2c* and *4c* are characteristic for diploid cells in different stages of DNA synthesis. The maximum *8c* is due to endomitotic cell growth.

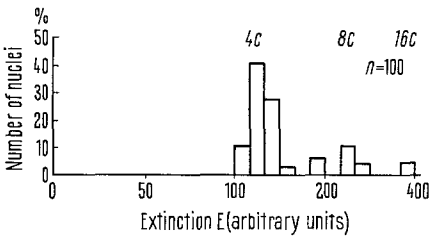

Fig. 2. Frequency distribution of DNA in interphase nuclei of *Artemia salina parthenogenetica 4n*. The maximum *2c* characteristic for diploid cells is absent. The *4c* and *8c* maxima are characteristic for tetraploid cells and the 16c maximum for cells with endomitotic cell growth.

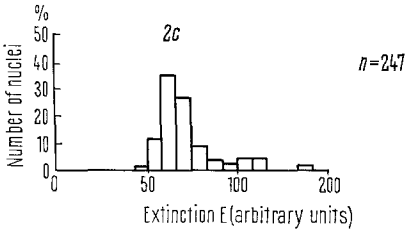

Fig. 3. Frequency distribution of DNA in the cells of the optical ganglia of *Artemia salina anfigonica*. 90% of the cells belong to the maximum *2c* with the DNA extinction mean 0.64 ± 0.07 (arbitrary units).

[1] R. STEFANI, Caryologia *16*, 625 (1963).
[2] T. BARKA and P. J. ANDERSON, *Histochemistry* (Harper and Row Publishers Inc., New York 1965).
[3] R. W. MERRIAM and H. RIS, Chromosoma *6*, 522 (1954).
[4] H. H. SWIFT, Physiol. Zool. *23*, 169 (1950).
[5] R. STEFANI, Caryologia *16*, 625 (1963).

As is shown by the cytological examination of the parthenogenesis of the diploid *Artemia salina*, the development starts with irregular meiosis, in that initially 2 meiotic divisions take place and then follows the fusion of the pronucleus and the polocyte I[6]. Thereby a diploid nucleus is produced which starts the cleavage divisions as usual and the development leads to a pure diploidy. These results could be verified through the DNA measurements, because most of the nuclei possess the DNA content of 2c while DNA classes of c and 3c are absent. Because in the tetraploid form the maxima of extinction are shifted towards 2c, and the composition of the cell material is similar to that of the diploid type, it can be concluded that in the obligatory parthenogenesis of diploid and tetraploid *Artemia* only one type of fusion of the descendants of the meiosis is present and a mosaic of haploid, diploid and polyploid cells is not established. In addition, the transition parthenogenetic *2n* to parthenogenetic *4n* is in agreement with the relation 1:2 in the DNA content.

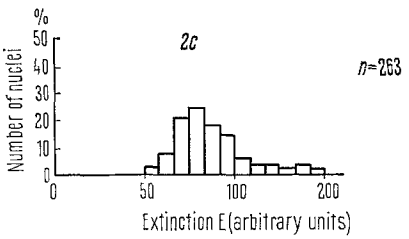

Fig. 4. Frequency distribution of DNA in the cells of the optical ganglia of *Artemia salina parthenogenetica 2n*. The DNA extinction mean is 0.78 ± 0.12 (arbitrary units).

Zusammenfassung. DNS-Messungen an Interphasekernen von *Artemia salina parthenogenetica 2n* und *Artemia salina anfigonica* zeigen ein Mengenverhältnis von 1.2:1, während zwischen *Artemia salina parthenogenetica 4n* und *Artemia salina anfigonica* DNS Werte im Verhältnis 2:1 gefunden wurden.

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26 January 1970.

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Thalidomide as a Mutagenic Agent in the Mosquito (*Culex pipiens molestus*)

During the past decade several attempts have been made to detect radiation-induced, as well as spontaneously occurring, mutations in mosquitoes: the pioneer experiments of LAVEN[1] and KITZMILLER[2], with X-ray induced mutations in *Culex*, have played a useful part in the recognition of phenotypic expression of certain mutants in mosquitoes. Nevertheless, the application of inbreeding techniques of CRAIG[3] and VANDEHEY[4,5], by single-pair matings for mutant isolation and other types of genetic research, imply that there are, perhaps, no proved cases of radiation-induced mutations, at least in *Aedes aegypti*, although a few such strains have been produced by irradiation techniques.

In view of previous findings related to the chromosome injuries produced by thalidomide (α-phthalimidoglutarimide)[6,7], it became of interest to test whether it could induce germinal mutations in *Culex*. The present communication presents part of the results of mutagenetic studies with *Culex*, where thalidomide has been used in the production of dominant lethal mutations, semilethals as well as induction of some phenotypic anomalies, the occurrence of which in repeated experiments has led to the presumption that the chemical might be useful in future mutagenesis studies and a possible means of inducing lethal genes in the control of mosquitoes.

Materials and methods. Egg-rafts of *Culex pipiens molestus* from our autogenous laboratory stock, were collected randomly. The larvae from each egg-raft were reared in separate enamel wash-basins. Half of the larvae from each basin were chosen for tests and the other half reared as controls. The newly emerged adult males, which were fed on the drug at prepupal stage, were starved in a less humid atmosphere for at least 24 h and further fed on the drug mixed with 10% sugar for 72 h. They were then crossed with normal virgin females from the same egg-raft, which were segregated according to size (female pupae larger than male pupae). Each egg-raft obtained from a parental pair (P) was separated and the F_1 adults which emerged were mated among themselves. The F_2 rafts were isolated and adults examined for any phenotypic deviations from the normal. For each experiment 5 lines were maintained, which were reared up to F_3 generation. At F_3 only 5 rafts from each line were tested for possible phenotypic changes. The results are demonstrated in Table I. The variant phenotypes in the F_1, F_2 and F_3 generations were scored as genetic mutants if they were similar in appearance to the previously established mutants. To be certain that they were not phenocopies, whenever possible they were tested for heritibility by sib-matings for subsequent generations.

In the F_3 generation of the treated group, phenotypically female mosquitoes with partially transformed male appendages, together with gynandromorphs (sex mosaics), also mosaics with morphological traits not related to sex such as (Kuf/+) were obtained in high frequencies, which is in agreement with the results obtained with chemical mutagens as demonstrated by AUERBACH[8].

The occurrence of fused (fu) with knobbed protrusions on antennal segments and (SpW) in high frequencies of

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