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The Possible Role of Genome Activity Changes in the Sex Determination of *Daphnia pulex*

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Summary. Sex-dependent differences in the state of the nuclear chromatin of somatic cells were found in *Daphnia pulex*. It is suggested that the genome of *Daphnia pulex* has two developmental programmes based on identical chromosome sets. The female programme consistently functions under a wide range of ecological conditions, whereas the male programme is turned on by specific ecological stimuli. The genes controlling the activation and function of the male programme may be phenotypically latent for many parthenogenetic generations.

Key words: Daphnia Pulex – Development – Sex Determination – Ecological factors – Identical Chromosome Sets

Introduction

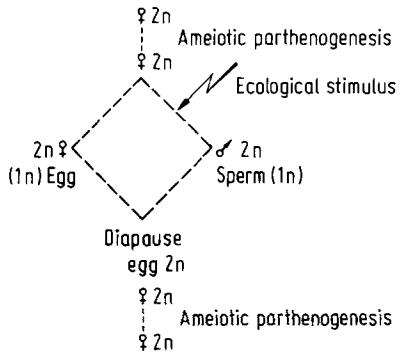
It is well known that many *Cladocera* species (*Crustacea*) reproduce parthenogenetically and that successive generations may be presented by females only. However, under the effect of stressing ecological factors (increased population density, changes in thermal and photoperiodic conditions, CO_2 concentrations) some parthenogenetic eggs may give rise to males (Banta 1939; Stross 1966). The cytogenetic mechanism controlling the appearance of males in populations of *Cladocera* is unknown. Herein is presented an attempt to obtain data clarifying this mechanism.

Materials and Methods

Experiments were carried out with *Daphnia pulex*. Males were produced by crowding parthenogenetic females (approximately 100 females per 50 ml of water) for 36-40 h. The preparations of somatic cells were obtained according to the standard method. Fluorescence cytophotometry of nuclear Feulgen-DNA (Böhm et Sprenger 1968) was done after auramine-00-Schiff staining (Ruch et Bosshard 1963). To identify heterochromatin in interphase nuclei, Sumner's technique (1972), as well the fluorescent stain propyl-quinacrine mustard (Kulikov and Bondarev 1976), were used. The preparations were examined with the light microscope ('Ergaval' Carl Zeiss Jena DDR) and with the fluorescent microscope ('ML-2B' to which a fluorescent device was added, FMEL LOMO USSR).

Results and Discussion

In females the diploid chromosome number is 2n = 24 (Zaffagnini and Sabelli 1972). In principle, the germ cells of male *Daphnia pulex* should also have at least 24 chromosomes in order to maintain constancy of chromosome number within the species:



The chromosome counts of male proliferating intestinal epithelial cells confirmed this expectation. Fluorescence measurements show that the content of DNA-auramine in cells of both sexes is the same (Fig. 1). Thus, females and males develop from eggs with identical chromosome sets.

Banta and Brown (1929) have demonstrated that the critical period for sex determination in a similar species lasts 3-4 h and terminates shortly before the beginning of cleavage. Sex determination occurred during this period

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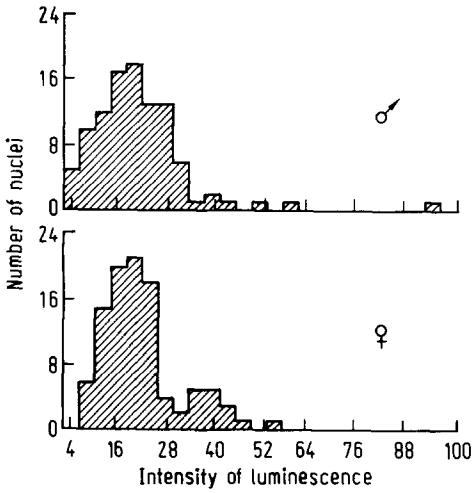

Fig. 1. The content of DNA-auramine in the nuclei of the intestinal epithelial cells of male and female *Daphnia pulex* measured by cytofluorometry

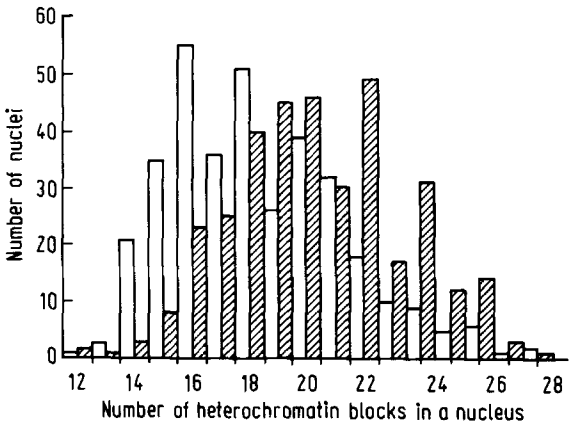

Fig. 2. The distribution of heterochromatin blocks in the nuclei of the intestinal epithelial cells in male (white bars) and female (black bars) *Daphnia pulex*

under the effects of ecological stimuli. One could suppose that the crucial event in determination is an alteration of gene activity which is irreversible during subsequent development.

One approach to verify this suggestion was to compare the structure of the interphase nuclei of somatic cells, since it is well known that gene activity often correlates with chromatin structure. These comparisons were made on preparations of isolated epithelial cells of the intestine of male and female *Daphnia pulex*. Summer's technique based on Giemsa staining was used for demonstrating heterochromatin. The number of heterochromatin blocks was counted in 50 randomly chosen nuclei from each preparation. As Fig. 2 shows, males differed significantly from females in the distribution of heterochromatin blocks per nucleus ($\chi^2 = 81$; $P > 0{,}999$; $\nu = 16$). As a rule, the nuclei of the intestinal epithelial cells of males contained fewer heterochromatin blocks located at the periphery of the nucleus.

Differences in heterochromatin structure between males and females were made more pronounced by the fluorescent stain propyl-quinacrine mustard, the binding of which seems to depend on the conformational specificities of chromatin. The nuclei of the intestinal epithelial cells of males had a clear-cut granular appearance, while those females fluoresced homogeneously and their heterochromatin blocks contrasted against the background of some nuclei (Fig. 3a). The same differences in the structure of nuclear heterochromatin were observed in haemolymph cells (Fig. 3b).

Differences in chromatin structure were also established quantitatively between males and females with propyl – quinacrine mustard. Cytofluorometry of the stained preparations demonstrated that most cell nuclei of females fluoresced much stronger than the brightest ones of males (Fig. 4). In females, the unusual distribution of fluorescence intensity may be due to the intense proliferation of the intestinal epithelial cells, and the left-hand portion of the histogram reflects the presence of dying cells. In males, these processes do not affect the distribution pattern because the modal class lies near the origin of the coordinates.

The experimental data suggest that males and females develop from eggs with identical chromosome sets which

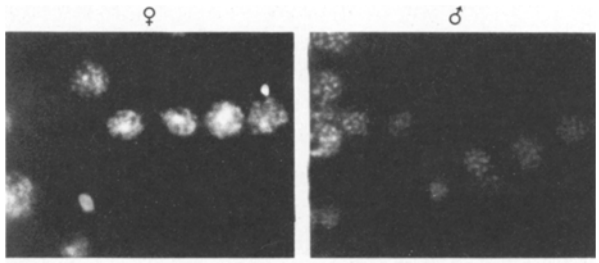

Fig. 3a.

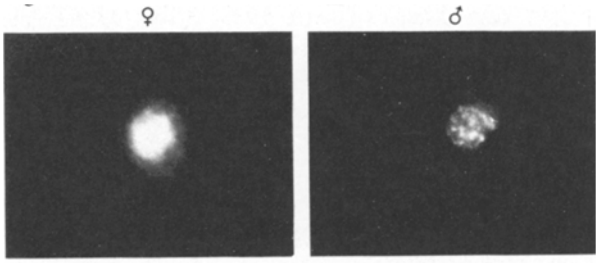

Fig. 3b.

Fig. 3. The nuclei of the intestinal epithelial cells (a) and the nuclei of the haemolymph cells (b) in male and female *Daphnia pulex* stained with propyl-quinacrine mustard (× 1000)

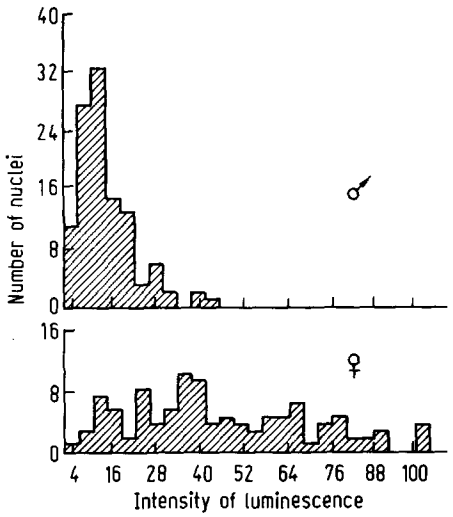

Fig. 4. The distribution of fluorescence intensity in the nuclei of the intestinal epithelial cells in male and female *Daphnia pulex* (Stained with propyl-quinacrine mustard)

function differently in the opposite sexes. The judgements made about the differences in genome function between males and females were based on the structural differences between the heterochromatin regions of the interphase nuclei. The structural differences between these nuclei may be regarded as the presumptive result of sex determination initiated by a maternal stimulus provided before the beginning of cleavage. The issue of the determination process is egg competence to conform to either the male or female developmental pathway. As confirmed by the structural similarities between the cell nuclei of various tissues of individuals of the same sex, the morphological and functional features of the interphase nuclei are conceivably patterned at an early stage of differentiation.

Potential bisexuality of living organisms has been well demonstrated (Hartman 1925; Myasoedov 1935). Its existence implies two genetic developmental programmes in the same organism. The accomplishment of a definite programme in most animal species is determined by various chromosome mechanisms resulting in qualitative and quantitative differences of chromosome sets in individuals of the opposite sex.

The genome of *Daphnia pulex*, and possibly that of other *Cladocera*, possess two genetic developmental programmes. However, their sex determination is not based on chromosomal differences, but on the changes in chromatin structure which are stably transmitted to the subsequent generations of somatic cells. It should be noted that the two developmental programmes in *Daphnia pulex* play different roles. Being functionally more ubiquitous, the female programme operates under a wide range of ecological conditions, whereas the male programme is switched on by a signal under specific conditions, when passage to sexual reproduction is needed for adaptation to extreme conditions.

Males fail to appear and hence, the male programme and consequently respective genes, are switched off for many hundreds, even thousands, of parthenogenetic generations (Banta 1939). Under the effect of an ecological stimulus, presumably mediated through the maternal organism, the male programme may be activated. This entails the activation of respective genes phenotypically latent for many parthenogenetic generations.

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