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ON THE APPEARANCE OF FEMALE ASYNAPSIS AND POLYPLOIDY IN A POPULATION OF THE DIPLOID SYNAPTIC BIOTYPE OF *DUGESIA BENAZZII*: FIRST STEPS OF THE EVOLUTION TOWARDS THE POLYPLOID BIOTYPES (**)

Riassunto — *Sulla comparsa di asinapsi femminile e di poliploidia in una popolazione del biotipo diploide sinaptico di* Dugesia benazzii: *prime tappe della evoluzione verso i biotipi poliploidi. Dugesia benazzii* (planaria del gruppo *gonocephala*, trovata nelle Isole di Sardegna, Corsica e Capraia) presenta vari biotipi cariologici, dei quali sono stati studiati in modo particolare i seguenti: a) biotipo diploide anfimittico sinaptico nelle due linee germinali; b) biotipo tetraploide nelle linee somatica e germinale femminile, con ovogenesi asinaptica e sviluppo pseudogamico; c) biotipo triploide nella linea somatica, esaploide in quella femminile (per duplicazione del corredo cromosomico negli oogoni) con ovogenesi sinaptica e sviluppo pseudogamico.

I risultati di precedenti ricerche hanno suggerito l'ipotesi che le caratteristiche dei biotipi poliploidi (asinapsi o raddoppiamento del corredo cromosomco nella linea femminile) siano controllate da complessi multifattoriali e che nel biotipo diploide tali fattori possano essere presenti, ma in misura assai scarsa per cui ne è molto rara la espressione fenotipica.

Le attuali ricerche confermano, per il biotipo diploide, la presenza in condizione eterozigote di tali fattori, dimostrata dalla comparsa di ovociti asinaptici diploidi e tetraploidi e di ovociti sinaptici poliploidi in individui di una popolazione di detto biotipo, allevati per più di 30 anni in laboratorio.

L'A. ritiene che tali manifestazioni in esemplari del biotipo diploide rappresentino le prime tappe del processo che gradualmente ha portato alla evoluzione verso i biotipi poliploidi. I dati ricavati dalle presenti ricerche vengono discussi in base a reperti precedentemente ottenuti, sia dallo studio di ibridi fra il biotipo diploide (funzionante da femmina) ed i biotipi poliploidi, sia dall'esame di alcune popolazioni naturali.

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Abstract — The planarian *Dugesia benazzii* presents a diploid biotype which is synaptic and amphimictic and two polyploid biotypes which are characterized either i) by asynapsis in the female line or ii) by chromosome set doubling in the synaptic oocytes. Asynapsis and chromosome set doubling are characters controlled by multifactorial mechanisms. It has been suggested that these factors are also present in the diploid biotype, but in a very low number so that their phenotipic expression is extremely rare.

The present research confirms that the diploid biotype is heterozygous for these factors. This is evidenced by the appearance of asynaptic oocytes (which are either diploid or tetraploid) and of sinaptic polyploid oocytes. According to the A. these manifestations represent the first steps of the evolution towards polyploid biotypes.

Key words — Planarians - Evolution towards polyploid biotypes.

INTRODUCTION

Previous research has demonstrated peculiar characteristics of gametogenesis and of chromosome cycles in two planarians species, *Dugesia benazzii* and *D. polychroa* (of the *lugubris-polychroa* group). Both these species possess a diploid biotype with normal amphimictic development and two polyploid biotypes, in which either asynapsis with equational division or synapsis with chromosome set doubling occurs in the female line. Development is pseudogamic in these two biotypes. In *D. benazzii* an amphimictic biotype having triploid set in the somatic and female lines is also known. In all the polyploid biotypes the male line is generally diploid [1].

The characteristics of polyploid biotypes are independently inherited, as demonstrated by crossess between the diploid biotype (acting as female) and the two polyploid pseudogamic biotypes. Most of the F_1 is diploid in the somatic and germ lines, which are synaptic. In contrast, only a small percentage of the F_1 possess the characters of the paternal biotypes: either asynapsis with or without polyploidy, or synapsis with chromosome set doubling. In addition, these characters have, for the most part, partial expression (i.e. synaptic and asynaptic oocytes or diploid and tetraploid ones in the same individual) (BENAZZI LENTATI, 1970; BENAZZI LENTATI and MEZZANI, 1975).

(1) For more detailed particulars on the gametogenesis and chromosome cycles, see BENAZZI and BENAZZI LENTATI (1976).

The study of hybrids has given an insight into the successive steps that may lead to the appearance of asynaptic or synaptic polyploid cycles. In *D. benazzii* a normal polyploid synaptic cycle occurs in few individuals F_1, whereas in *D. polychroa* it only occurs in successive generations. In a part of hybrids of both species pseudogamic development takes place and this event is essential in establishing the rise of polyploid cycles similar to the ones present in nature (BENAZZI LENTATI, 1966).

The appearance of asynapsis and chromosome set doubling in a small portion of the F_1 has suggested the hypothesis that the factors controlling these characters are also present in the synaptic diploid biotype. It is, moreover, worth remembering that these two characters appear in the offspring whatever was the polyploid biotype acting as male in the crosses. All the biotypes are, therefore, heterozygous for these characters (BENAZZI LENTATI, 1966 l.c., 1968). Factors for pseudogamy are perhaps also present in the diploid amphimictic biotype; at present I have only demonstrated that the polyploid biotypes possess factors for amphimixis (BENAZZI LENTATI, 1979). However, a further discussion of this interesting question is beyond the scope of this paper.

Asynapsis and chromosome set doubling are controlled by a multifactorial mechanisms; in previous works it has been suggested that in the diploid biotype the number of the factors is low. Therefore, they are never expressed no matter how they are combined in the mating among individuals of this biotype. However, in new genic combinations resulting from crossing, these factors seem to lead to the production of novel phenotipic expression. Successively, a fortunate chance has permitted an experimental verification of the heterozygosity of the diploid biotype and the influence of repeated incrosses within two different populations of the diploid biotype. In fact, descendents from consecutive incrosses of two diploid populations (from Lake Garda and Pisa) of *D. polychroa* revealed the occasional production of tetraploid oocytes (BENAZZI and BENAZZI LENTATI, 1976). Another set of data has been collected in *Planaria torva*, which normally presents only synaptic diploid oocytes: in a single specimen of a natural population either totally synaptic or totally asynaptic oocytes have been found (PUCCINELLI, 1968).

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In this paper I present some data collected on *D. benazzii,* which decisively proves the heterozigosity of the diploid biotype, and, more important, allows one to outline the successive steps that may lead to the establishment of polyploid biotypes, starting with a single population of the diploid biotype [2].

Material and methods

The individuals of the population of diploid biotype, the object of this study, were collected in Corsica (Paradojo stream near Bastia) in 1949. Unfortunately all individuals but one died in a few days. The only surviving specimen has laid some cocoons with fertilized oocytes, from which it has been possible to obtain a riche culture which has provided all animals used for the various crosses with individuals of polyploid biotypes, as already pointed out in the Introduction. For this research works it was necessary to check the chromosome complement of the oocytes. This examination must be made in unfertilized oocytes, stopped in a stage which may correspond to the prometaphase: in fact, the nuclear envelope is disrupted, but the bivalents (or univalents) appear scattered along the spindle. In this species the normal orientation and the metaphase congression occur only after sperm penetration.

In order to obtain the oocytes stopped at the prometaphase one must remove the cocoons from the genital antrum.

The oocytes have been treated following the usual technique adopted in our previous research.

Results

The examination of unfertilized oocytes of the individuals of the Paradojo population, conducted since 1950, was repeated time to time on many specimens, which have always shown synaptic oocytes with diploid complement (8 bivalents) (Fig. 1) and with an average chiasma number of 24. According to the more or less

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(2) A summary of these data has been presented in the relation read by BENAZZI at the International Meeting on « Mechanisms of Speciation » organized by the Accademia Nazionale dei Lincei (Rome, may 1981).

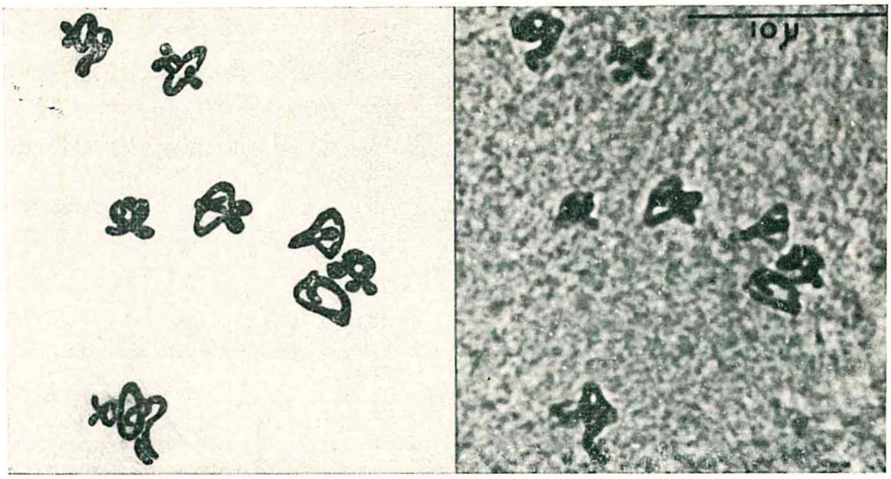

Fig. 1 - Diploid oocyte with 8 bivalents (in all the Figs. corresponding outline drawings have been added to represent the exact morphology of the bivalents and univalents).

precocious stage in which the cocoons were removed from the genital antrum, the number of chiasmata decreases from 28-30 to 20-22 and many of them appear terminalized, particularly in the little chromosomes. Bivalents with a single chiasma are extremely rare or absent.

In 1977, while carring out some crosses for a new research, I had, as usual, examined unfertilized oocytes of 6 isolated individuals. To my surprise, I found, in two of them, some oocytes with bivalents and univalents, i.e. possessing the characteristics of the oocytes laid by the hybrids between different biotypes, as mentioned in the Introduction.

I then separated all the sexually ripe individuals (first group of 45 specimens) present in the same culture, which, however, laid only fertilized oocytes. It has, therefore, been necessary to wait the successive reproductive period for the laying of unfertilized oocytes. Although only a part of these individuals laid cocoons, the data collected from them may provide some useful indications.

At the same time I have collected and bred in isolation each planarian hatched from the cocoons laid by the fertilized individuals of the first group. Thus it was possible to obtain unfertilized planarians, and their oocytes were also examined (second group of 52 individuals).

Individuals of the first group:

Total number of individuals		45	
Number of individuals studied		26	
Ind. with	only synaptic oocytes (minimum two chiasmas per bivalent)	14	(No. oocyt. 110 - Aver. No. chias. 24) (*)
	only synaptic oocytes (but some bivalents with a single chiasma)	6	(No. oocyt. 20 - Aver. No. chias. 20) (*)
	bivalents and univalents in the same oocyte or totally synaptic and totally asynaptic oocytes in the same individual	4	(No. oocyt. 13)
	tetraploid synaptic oocytes	2	(No. oocyt. 3 - Aver. No. chias. 52) (*)

Individuals of the second group:

Total number of individuals		52	
Number of individuals studied		40	
Ind. with	only synaptic oocytes (minimum two chiasmas per bivalent)	24	(No. oocyt. 130 - Aver. No. chias. 24) (*)
	only synaptic oocytes (but some bivalents with a single chiasma	5	(No. oocyt. 11 - Aver. No. chias. 18) (*)
	bivalents and univalents in the same oocyte or totally synaptic and totally asynaptic oocytes in the same individual	9	(No. oocyt. 19)
	tetraploid synaptic oocytes	0	
	tetraploid asynaptic oocytes	1	(No. oocyt. 2)
	triploid oocytes with 8 bivalents and 8 univalents	1	(No. oocyt. 6)

(*) Total number of oocytes - Average chiasma number.

These data show that the individuals with deviant oocytes were already present in the original culture and that a reduction of chiasma frequency occurs in the synaptic oocytes, due to the presence of bivalents with a single chiasma. In the cases with partial asynapsis it is possible to find a graduation from 7 bivalents and 2 univalents to 1 bivalent and 14 univalents in the oocytes (Fig. 2a). Likewise one can find oocytes with either 8 bivalents or 16 univalents in the same individual (Fig. 2b). All these facts prove that these individuals have an unbalanced genotype similar to that of the majority of hybrids derived from crosses between different biotypes. The tetraploid oocytes (Fig. 3a, b) arose from a doubling of the chromosome set in the diploid neoblasts. The single indivi-

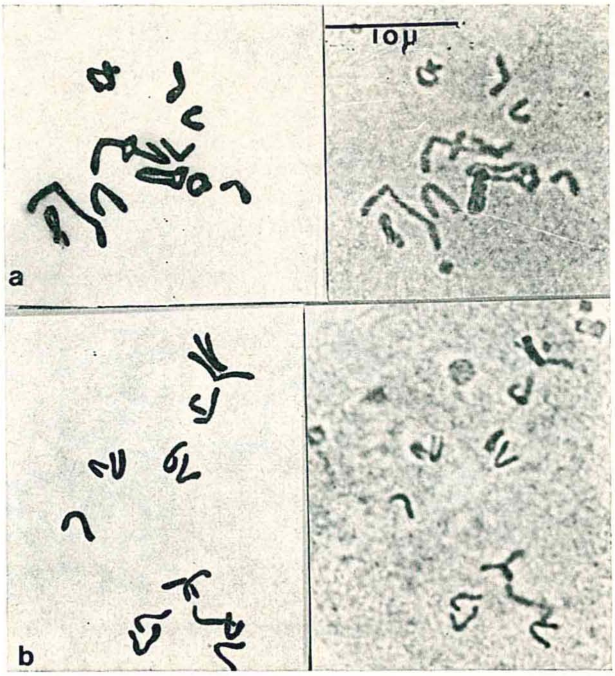

Fig. 2 - a) diploid oocyte with partial asynapsis: 5 bivalents and 6 univalents, b) totally asynaptic oocyte with 16 univalents.

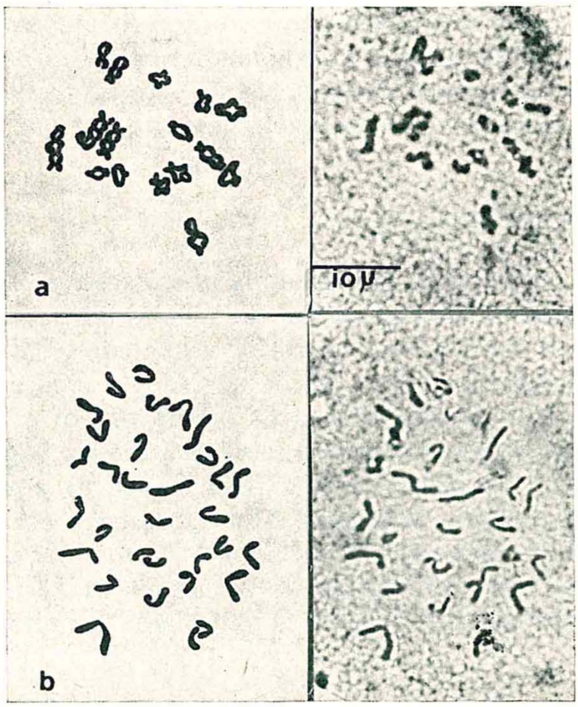

Fig. 3 - a) tetraploid synaptic oocyte with 16 bivalents, b) tetraploid asynaptic oocyte with 32 univalents.

dual with triploid oocytes (Fig. 4) derived from a tetraploid synaptic oocyte which yielded a triploid zygote after meiosis and amphimixis.

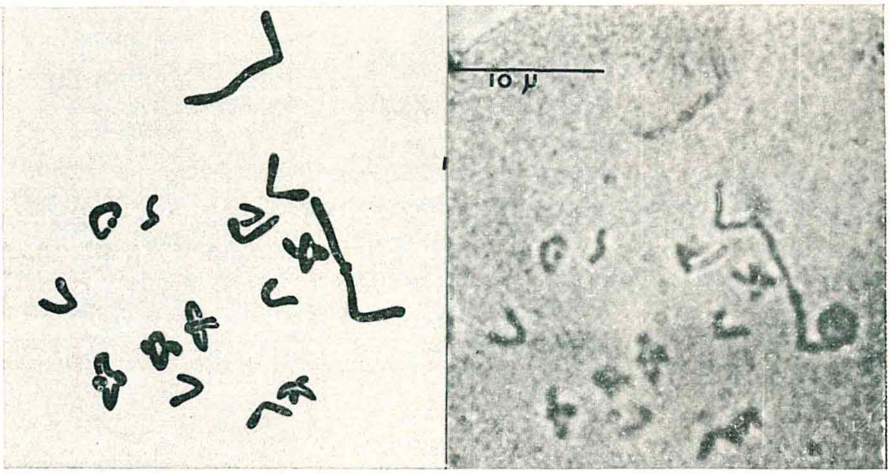

Fig. 4 - Triploid oocyte with 8 bivalents and 8 univalents.

These results are only indicative of the phenomena, which require more exhaustive studies. At present, owing to the scarcity of observations, it is not appropriate to compare the two groups of planarians studied, but it may be interesting to point out the evolutionary meaning of these events.

CONCLUSIONS

The data collected on the Paradojo population undoubtedly confirm the presence in the diploid biotype of factors for asynapsis and for doubling of chromosome complement, which may lead to polyploid cycles. The occurrence in the genotype of either a sufficient number of factors or the rise of a particular genic combination may determine (also in a unique population) the expression of i) asynapsis with diploid set or ii) polyploidy with asynaptic or sinaptic oogenesis. The appearance of asynaptic oocytes with chromosome set doubling may be the first step towards an asynaptic tetraploid strain. As a matter of fact, the natural asynaptic biotype of *D. benazzii* is tetraploid. The tetraploid synaptic oocytes, on the other hand, may represent the first step toward a polyploid synaptic strain; these oocytes are amphimictic, as may be deduced from the only descendent so far obtained, which possess triploid set in somatic and female line, the latter with 8 bivalents and 8 univalents (Fig. 4). It is to be noted that a biotype with similar chromosome complement has also been found in nature (BENAZZI, 1968).

Research on the modalities of oogenesis and on the type of development of the various deviant oocytes of the Paradojo population has begun, but the results are, so far, inadequate. On the other hand, I think it is more important at present to ascertain the extension of the phenomenon in each deviant individual. This require the examination of a great number of unfertilized oocytes during many reproductive periods, since the deposition are scarce and, in some individuals, the deviant oocytes are rather rare.

I shall, for now, limit myself to emphasize the data which suggest the probable route followed by this species in its evolution towards polyploidy with asynapsis or with chromosome set doubling in the female line.

These observations deserve to be taken into consideration also in order to interpret two data presented in previous research. First one, must remember that the manifestations of asynapsis and doubling of chromosome set take place in offspring born from initial crosses between the diploid biotype and the two pseudogamic polyploid biotypes (acting as males) whatever the polyploid biotype was used. This implies that each polyploid biotype must possess factors for both the characters. In fact, offspring from crossing diploid synaptic individuals with asynaptic polyploid ones may have synaptic oogenesis, with chromosome set doubling. Conversely, offspring from crossing diploid synaptic individuals with synaptic polyploid ones may be asynaptic in the female line. Hence the expression of these two characters in a single hybrid strain could result from a different balance between the respective factors, which are present in both the natural polyploid biotypes. This last peculiar fact can be easily explained by the existence, also in the diploid biotype from which the polyploid ones derive, of genes responsible for the manifestation of the two characters. Second one, BENAZZI (1968 l.c.) and BENAZZI and GIANNINI (1970) found that two populations of *D. benazzii* from Corsica are characterized by the fact that each individual produces two types of oocytes:

triploid and hexaploid, the relative frequencies of which are different in the two populations. Previous study by BENAZZI LENTATI and PUCCINELLI (1959) in hybrids between diploid amphimictic and polyploid asynaptic pseudogamic biotypes demonstrated the presence, in the same individual, of triploid and hexaploid oocytes. This fact allowed BENAZZI and GIANNINI to suggest a hybrid origin of the two population from Corsica. The different ratio of the two types of oocytes likely depend on differences in the frequencies of the genes responsible for chromosome set doubling. However, the observations illustrated in the present paper may suggest another interpretation, according to which the two population from Corsica would be derived directly from the diploid biotype during its gradual evolution toward polyploidy.

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