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On the production of diploid offspring from specimens of the triplo-hexaploid biotype of the planarian Dugesia benazzii

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Biologia. — *On the production of diploid offspring from specimens of the triplo-hexaploid biotype of the planarian* Dugesia benazzii. Nota di GIUSEPPINA BENAZZI LENTATI (*) e PAOLO DERI (**), presentata (***) dal Socio M. BENAZZI.

RIASSUNTO. — Sono stati compiuti ulteriori studi su una popolazione del Triclade paludicolo *Dugesia benazzii* caratterizzata dalla contemporanea presenza, in percentuali circa eguali, di ovociti esaploidi e triploidi. Gli ovociti esaploidi producono per pseudogamia discendenti triploidi che a loro volta presentano di nuovo i due tipi di ovociti; gli ovociti triploidi invece danno uova aploidi dalle quali, dopo fecondazione, si sviluppano embrioni diploidi, che tuttavia sono colpiti da precoce letalità.

Le ricerche da noi recentemente compiute hanno messo in evidenza un totale cambiamento nel destino di tali embrioni diploidi, avendo ottenuto molti nati diploidi perfettamente vitali. Questo cambiamento è concomitante con variazioni insorte nella popolazione dopo un lungo periodo di allevamento in laboratorio, consistenti nella comparsa, in tutti gli esemplari, di B–cromosomi, nella insorgenza, in molti di essi, della riproduzione scissipara e nella forte riduzione del numero degli ovociti esaploidi negli individui rimasti sessuati.

INTRODUCTION

This paper makes a new contribution to the studies carried out on a planarian with peculiar characteristics belonging to the triplo–hexaploid biotype of *Dugesia benazzii*. In fact, the triplo-hexaploid biotype normally possesses triploid somatic cells ($3n = 24$), hexaploid female cells (owing to a chromosome set doubling) and diploid male cells (due to elimination of a haploid set) (1).

In the population from Roccapina (Corsica), however, only about 50 % of the oocytes are hexaploid, the others being triploid [3]. Previous research [4] has demonstrated that only the hexaploid oocytes are fertile; they develop pseudogamically and give rise to triploid pseudozygotes, as normally occurs in the triplo-hexaploid cycle. The triploid oocytes, on the contrary, are amphimictic (2), but do not generally produce offspring. Some of them present meiotic

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(1) For precise data regarding the biotypes of *D. benazzii*, the chromosome cycles and the characteristics of the hybrids derived from the crosses between these biotypes, see Benazzi Lentati, 1970 [1] and Benazzi and Benazzi Lentati, 1976 [2].

(2) This peculiar condition, for which the modalities of development seem to be related to the ploidy level, has been discussed in preceding papers [1], [5].

anomalies while others undergo a peculiar maturation process. The oocytes possess 8 bivalents and 8 univalents; the dyads and subsequently the univalents penetrate the first polocytes and therefore at metaphase II only 8 dyads are present in the oocytes and the ripe eggs will be haploid. The fertilization by haploid sperm gives rise to diploid zygotes. Their development, however, stops precociously, as proved by the presence in the cocoons of diploid embryos in degeneration. Diploid descendents are in fact extremely rare; among the great number of adult specimens examined in the first research [4], only one showed a diploid somatic set.

In two preceding papers [6], [7] some unexpected events, which occurred after a long period of laboratory culture, were reported, viz. the appearance in most specimens of the asexual propagation by fission and the presence of B-chromosomes in the complement of all individuals. Moreover, the few planarians still reproducing sexually present almost exclusively triploid oocytes (about 94 %) from which numerous offspring are derived. The latter fact, also completely unexpected on the basis of the first studies, is the object of the present paper.

MATERIAL AND METHODS

The specimens from Roccapina were bred together from 1970 until 1975. After the appearance of fissioning we immediately separated the cocoons (4) found in the culture vial and isolated the few individuals (13) still showing the morphological features of sexuality. From the 4 cocoons 10 offspring were obtained and the sexual individuals produced 8 offspring. In 1977 the 13 animals (previously isolated) were crossed with individuals of the diploid biotype of the same species. So far 27 descendents have been obtained.

A karyological examination of the asexual descendents was performed on the regenerative blastemata, while in the animals which became sexual we examined the unfertilized oocytes removed from the genital antrum.

The cytological methods were the same as those reported in our previous papers.

RESULTS

From a cocoon found in culture we obtained 2 triploid and 1 diploid offspring; from another cocoon 1 triploid and 2 diploid (Fig. 1, *a* and *b*) and from the other two cocoons 4 diploid offspring. The 13 individuals bred together produced 8 descendents, 4 triploid and 4 diploid. Summing up, therefore, we obtained 11 diploid (61 %) vs. 7 triploid offspring. The number of diploids is surprising, bearing in mind that in the first investigation [4] only one diploid individual was found in the very great number of planarians studied.

Even more numerous (89 % ca.) are the diploid offspring born from the crosses between the 13 Roccapina individuals (acting as females) and individuals of the diploid biotype: in fact, till now we have obtained 24 diploid and 3 triploid offspring.

The diploid offspring prove that the ripe eggs of Roccapina individuals were haploid and after fertilization by haploid sperm gave rise to diploid zygotes. The triploid offspring, on the contrary, were produced by the few hexaploid oocytes still present in these specimens. In fact, after maturation, the hexaploid oocytes, as already stated, give triploid eggs which develop pseudogamically.

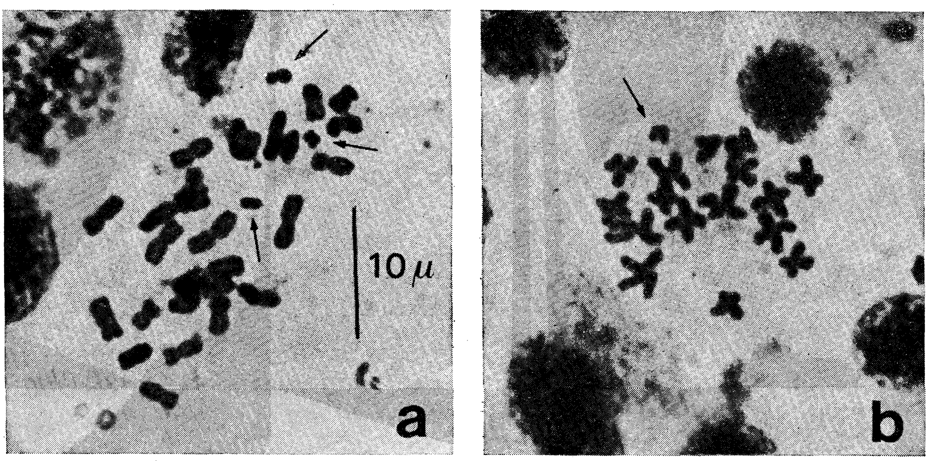

Fig. 1. – *Dugesia benazzii*, population from Roccapina. Somatic mitoses with: *a*) triploid complement with 3 B-chromosomes (arrows); *b*) diploid complement with 1 B-chromosome (arrow).

The meiotic mechanism of the triploid oocytes giving rise to haploid eggs was not thoroughly examined, since we were interested in establishing the fertility of these individuals which present such a high number of triploid oocytes. However, the few oocytes found at anaphase II showed a haploid set. We think, therefore, that the maturation mechanism of the triploid oocytes may correspond to the one illustrated in the first study [4].

It may be assumed that the birth of diploid offspring, which were not present in the population from Roccapina before the variations in the culture, is due to the disappearance or strong reduction of the diploid embryo lethality. On the other hand, the high frequency of these diploid offspring compared with that of the triploids may be due to the reduction of the number of hexaploid oocytes. This reduction appears to have gradually increased, because in the offspring coming from inbreeding of the Roccapina individuals diploids accounted for about 61 %, while in the offspring born from the outcrosses with specimens of the diploid biotype about 89 % is reached. This high value could also be attributed to the presence of the paternal haploid set, in which lethality is lacking. However, we think it more likely that the viability of the diploid embryos rose in correlation with the new genetic background. As a matter of fact, even the percentage (61 %) of the diploid offspring born from inbreeding of the Roccapina individuals is rather high.

It is interesting to note that 1 triploid and 2 diploid descendents from inbred individuals became suddenly sexual and among the hybrids, till now, 4 triploid and 17 diploid individuals laid numerous cocoons. Two diploid individuals born from inbreeding and two diploids born from outcross immediately manifested fissioning.

DISCUSSION

The research carried out on the planarians from Roccapina has given some results which are worthy of note:

1) Appearance, after a long period of laboratory culture, of fissioning and of B-chromosomes.

2) Strong numerical increase (in the individuals remaining sexual or returning sexual) of the triploid oocytes in comparison with the hexaploid ones; in fact, triploid and hexaploid oocytes, both present in the same individual, were at first in the same ratio, while at present the triploids reach 94 %.

3) Capacity of these triploids to produce viable diploid offspring.

The last point appears to be particularly interesting because it strongly contrasts with the almost total lethality which was peculiar to the diploid embryos before the transformations in the laboratory culture. The activation of the fission-controlling genes and the appearance of B-chromosomes are certainly the expression of a new genetic background, which is concomitant with the numerical increase of the triploid oocytes and the disappearance of the diploid embryo lethality.

Therefore, a total change in the fate of the diploid embryos has taken place and it is worth mentioning its unique behaviour (an almost total degeneration in the first period of laboratory culture versus a normal development in these last years). Such behaviour is not easy to explain because the diploid embryos are almost certainly not genetically identical. In fact, the haploid eggs derive from triploid oocytes which have undergone the elimination of one haploid set and at the same time a normal segregation. Moreover, they are fertilized by haploid sperm which also derive from a normal meiosis after elimination of one haploid set. With regard to the hexaploid oocytes, which derive from triploid cells after a chromosome set doubling, it is to be noted that they produce viable offspring in all cases.

Owing to the viability of the diploid embryos born from inbreeding a diploid strain with B-chromosomes has been produced. Individuals with this chromosomal characteristic, as far as is known, have not been found in nature, either in *D. benazzii* or in other forms of the "*D. gonocephala* group". However, bearing in mind that one diploid specimen was present among the triploids studied in the previous research [4] (in a period in which B-chromosomes were not yet present), it seems justified to assume that the genic conditions which determine the viability of the diploid embryos may take place even in nature. Therefore, the finding in nature of diploid individuals sympatric with individuals of the triplo-hexaploid biotype would suggest their origin from the latter.

The present research finally confirms previous data according to which the appearance of fissioning is independent of the ploidy level. In fact, triploids and diploids may be either sexual or fissiparous and it is to be remembered that fission may also appear in the diploid hybrids which possess one haploid set of the diploid biotype, which never presents asexual reproduction.

Bromley ([8] and personal communication to Prof. Benazzi) found in two subspecies of *Dugesia biblica* some facts which show peculiar analogies with those observed in the Roccapina population; *D. biblica biblica* is triploid ($3n = 27$) with B-chromosomes and generally reproduces by fission, but a few individuals may become sexual and their offspring may present either triploid or diploid sets. *D. b. montana* is generally diploid ($2n = 18$) but in some individuals both triploid and diploid cells may be found. This fact has been observed also in Roccapina specimens [6].

In our opinion such chromosome complements suggest the existence of a particular chromosome cycle. Bromley did not investigate this aspect of the problem, probably because of the rarity of the sexual specimens. The chromosome complement of the female line is also unknown. The grounds for establishing a relationship with the data collected in the Roccapina population are, therefore, inadequate; however, it is perhaps not unlikely that in *D. biblica* diploid and triplo-hexaploid biotypes coexist (although with a different basic chromosome number with respect to *D. benazzii*), showing sexual and fissiparous modalities of development, with triploid individuals able to produce two types of offspring characterized by diploid or triploid sets. It would be therefore extremely interesting to carry out a comparative analysis of the two species mentioned above.

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