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Occurrence of polyspermy in planarians

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

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Biologia. — *Occurrence of polyspermy in planarians.* Nota di GIUSEPPINA BENAZZI LENTATI (*) e PAOLO DERI (**), presentata (***) dal Socio M. BENAZZI.

ABSTRACT. — While carrying out cytological studies on two freshwater planarians, namely *Dugesia benazzii* (belonging to the «*D. gonocephala* group» and *D. polychroa* (belonging to the «*D. lugubris-polychroa* group»), the AA. observed that polyspermy may occasionally occur in them. Such an event appeared in natural polyploid populations showing peculiar genomic characteristics. It was never observed in natural diploid populations, whereas it occurred in diploid strains reared in laboratory as well as in strains obtained from interracial crosses. The AA. discuss the possible relationships between these different genotypic conditions and the appearance of polyspermy, on the basis of the concept that the eggs of planarians are, as a rule, monospermic, and that polyspermy is due to a block of the factors inhibiting supernumerary sperm penetration.

KEY WORDS: Planarians; Occasional polyspermy.

RIASSUNTO. — *Casi di polispermia osservati nelle planarie.* Gli AA., nel corso di ricerche citologiche su due planarie d'acqua dolce: *Dugesia benazzii* del «gruppo *D. gonocephala*» e *D. polychroa*, del «gruppo *D. lugubris-polychroa*», hanno riscontrato casi sporadici di polispermia. Tale evento si è manifestato in popolazioni naturali poliploidi con peculiari caratteristiche genomiche. Esso non è mai stato riscontrato in popolazioni naturali diploidi, mentre è stato osservato sia in stirpi allevate in laboratorio sia ottenute da incroci interrazziali. Gli AA. discutono le possibili relazioni tra tali differenti situazioni genotipiche e la comparsa della polispermia, in base al concetto che l'uovo delle planarie sia normalmente monospermico e che la polispermia sia attribuibile ad un blocco dei fattori che inibiscono la penetrazione di spermi supplementari.

INTRODUCTION.

Polyspermy is a very uncommon event in planarians, at least in the taxa studied by our team in Pisa for over forty years. Indeed, it was found in only two species, namely *Dugesia benazzii*, belonging to the «*Dugesia gonocephala* group» and *D. polychroa*, belonging to the «*D. lugubris-polychroa* group» [1]. In *D. benazzii* polyspermy occasionally occurred in certain natural polyploid populations, but it was also observed in diploid and polyploid hybrids reared in laboratory and in a diploid strain after a long period of culture. On the contrary, in *D. polychroa* it was only found in a single type of interracial hybrid obtained in laboratory conditions.

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[1] Before the revision of the group, this species was indicated as *D. lugubris* (Benazzi *et al.*, 1970).

In order to clarify these data, it must be pointed out that *D. benazzii* as well as *D. polychroa* present a diploid amphimictic biotype and two different polyploid pseudogamous biotypes, showing asynaptic and synatic oogenesis, respectively. Within each species, the diploid and polyploid biotypes are interfertile; sperm of the pseudogamous biotype evolves in pronucleous and, after its penetration into a diploid amphimictic egg, amphimixis takes place (2). We thus obtained interracial hybrids suitable for cytological and genetical investigations.

The aim of the present note is simply to report the occurrence of polyspermy in planarians and to attempt a comparative analysis of this event. It must be noted that our research was not specifically directed towards polyspermy, since this phenomenon was observed occasionally while studying other subjects.

Material and technique.

Eight strains of *D. benazzii*, collected in Sardinia and Corsica, were examined. The populations from Viglietu (Sardinia) and Paradojo (Corsica) are diploid, the others polyploid. Of the latter, two were collected in Sardinia (Giocca and Crisciuleddu) and four in Corsica (Prunelli, Corte, Loreto and Roccapina). The population from Giocca belongs to the biotype characterized by asynaptic oogenesis, whereas the strains from Crisciuleddu and from Corsica belong to the biotype with synaptic oogenesis. The specimens of *D. polychroa* were collected in various localities of the Italian peninsula: Lake Garda and Pisa as to the diploid biotype, Roma and Lake Iseo as to the polyploid biotypes, characterized by asynaptic and synaptic oogenesis, respectively.

The research was carried out on fertilized oocytes. In order to obtain these oocytes, it was necessary to remove the cocoon from the genital antrum. The contents of the cocoon were then stained with acetic carmine and observed in hanging-drop or in normal slides. The most suitable oogenetic stages were metaphase I and anaphase I, because the supernumerary spermatic heads were expelled or degenerated precociously. However, since, as has already been pointed out, the main purpose of our study was not polyspermy, we took all the phases of maturation into account; for this reason, monospermic-looking oocytes during metaphase II and anaphase II, might have been polyspermic initially. Therefore it was not possible to ascertain the exact ratio between monospermic and polyspermic oocytes.

Observations.

The presentation of the results is divided into three sections. The first section exclusively concerns the observations on specimens belonging to populations collected in nature; the second section reports data on hybrids obtained in laboratory by crossing individuals of the diploid amphimictic biotype, acting as female, with samples of the polyploid biotypes; the third section, the most interesting in our opinion, concerns the recent appearance of polyspermic eggs in individuals of the diploid population from Paradojo, in which no cases of polyspermy had previously been observed in almost forty years of laboratory culture.

(2) For complete details of the characteristics of these planarians, see Benazzi Lentati (1970).

Section 1: natural populations

Dugesia benazzii. These observations refer to our early studies on planarians, when one of us (Benazzi Lentati, 1953) found specimens in a synaptic polyploid strain from Sardinia (Crisciuleddu stream) in which mostly monospermic eggs were present, but there were also a number of polyspermic eggs, with two or more (five in one case) spermatic heads. Other data were obtained from the Corsican populations, particularly from the one from Corte, which also belongs to the polyploid synaptic biotype. Only six specimens of this population were examined, but polyspermic eggs were found in all of them, sometimes with several spermatic nuclei. This population might have provided useful research material but unfortunately it died after a short period of laboratory culture. It was possible to ascertain that behaviour was not always the same in the polyspermic eggs of the various populations. Supernumerary spermatic heads might either degenerate or be expelled and, when several supernumerary nuclei are present, some of them might degenerate and others be expelled.

Observations by Giannini and Puccinelli (1970) on two other polyploid populations from Corsica (strains from Loreto and Roccapina) proved that the same cocoon can contain both monospermic and polyspermic eggs, the latter in very low number. Polyspermy was observed neither in the population from Viglietu, belonging to the diploid biotype nor in the polyploid population (asynaptic in the femal line) from Giocca, although both populations were bred for many years in laboratory.

Dugesia polychroa. Polyspermy was never observed either in the diploid or in the polyploid populations examined.

Section II: interracial hybrids

Dugesia benazzii. Polyspermy was occasionally observed in polyploid hybrids obtained from the cross between specimens of the diploid strain from Paradojo and specimens of the population from Giocca; the behaviour of the spermatic heads was similar to that described above in connection with the natural populations (Benazzi Lentati and Puccinelli, 1959). Very recent observations concerning diploid and polyploid hybrids, obtained from the cross between specimens of the population from Paradojo acting as female and specimens of the population from Roccapina (showing polyspermy also in nature, as stated above), have shown a low number of polyspermic eggs (Plate I: *a*).

Dugesia polychroa. Diploid hybrids obtained from the cross between specimens of the diploid populations from Pisa and Lake Garda and specimens of the polyploid populations from Rome and Lake Iseo (the former asynaptic and the latter synaptic) allowed one of us to obtain further details concerning the occurrence of polyspermy in this species (Benazzi Lentati, 1971). In the first generation of the cross between individuals of the diploid biotype and individuals of the polyploid synaptic biotype, no polyspermic oocyte was found out of 250; in the following generations 16 oocytes out of 350 were polyspermic. In the first generation of the cross between diploid and asynaptic polyploid biotypes, 6 polyspermic oocytes were found out of 720, whereas in the following generations the polyspermic oocytes turned out to be 11 out of 350. In all, in F_1 generation, 6 polyspermic oocytes (originating solely from the cross with the

asynaptic biotype) out of 970, and in the subsequent generations 27 polyspermic oocytes out of 700 were counted. In the backcross between the F_1 (obtained from the cross between the diploid biotype, acting as female, and the polyploid asynaptic biotype) and the paternal biotype, 2 oocytes out of 17 proved to be polyspermic. From inbreeding between individuals of this generation, 44 oocytes were produced, 9 of which were polyspermic (Plate I: *b*).

Section III: appearance of polyspermy in oocytes of specimens belonging to the synaptic and meiotic diploid biotype of *D. benazzii*.

The first specimens were collected in 1949 in the Paradojo stream. Unfortunately, all but one died within a few days. The only surviving specimen laid some cocoons with fertilized oocytes, from which it was possible to obtain a rich culture which provided all the animals for various crosses over forty years. However, during this long period, polyspermy was never observed. In 1977, while carrying out crosses for new research, one of us examined unfertilized oocytes of 6 isolated individuals. She was surprised to find oocytes with bivalents and univalents in two of them, *i.e.* oocytes with the characteristics of the oocytes laid by hybrids between different biotypes. She studied samples of this culture and their offspring, 66 in all. Of the oocytes laid by these specimens, 49 proved to be diploid, with bivalents usually with two or more chiasmata and occasionally with a single chiasma; 13 were diploid, partially or totally asynaptic and 3 polyploid, one of which was synaptic and two asynaptic (Benazzi Lentati, 1982). In order to obtain further information about these deviant oocytes, we reared the individuals that had laid them. It was thus possible to ascertain that polyspermy had recently appeared in these specimens: namely 6 polyspermic (Plate I: *c*) and 20 monospermic oocytes were found in three individuals.

Discussion.

The data reported above provide evidence that polyspermy never appeared in natural strains belonging to the diploid biotype of the two species examined (*D. benazzii* and *D. polychroa*), as is also the case with many populations belonging to the polyploid biotypes, with either synaptic or asynaptic oogenesis. On the contrary, polyspermy was observed in the following cases: i) in other polyploid populations of *D. benazzii* characterized by synaptic oogenesis; ii) in interracial hybrids of both species; iii) in specimens of the diploid population of *D. benazzii* from Paradojo, after a long period of laboratory culture.

We think it worthwhile to draw attention to the appearance of polyspermy in these groups and to point out that they have certain characteristics in common. The polyploid strains of *D. benazzii* possess oocytes whose chromosome set and pattern of development are similar to those of the hybrids obtained in laboratory with a chromosome cycle which is deviant in comparison with that of the other natural populations. The hypothesis has been advanced that these specimens originated through a hybridization process (which took place in nature) between diploid and polyploid biotypes (Benazzi and Giannini, 1968). The deviant specimens from Paradojo also acquired the same characteristics as the interracial hybrids, with diploid

asynaptic, polyploid asynaptic and polyploid synaptic oocytes (for the genetical mechanisms controlling the appearance of these phenotypes, see Benazzi Lentati, 1970).

Therefore, polyspermy appears in specimens characterized by the above mentioned genotypes, and it occurs similarly as other characters, namely polyploidy and synapsis or asynapsis. Indeed, individuals showing these deviant patterns in all the eggs of a single cocoon and even more so in all their eggs are very scant. Moreover, it must be pointed out that, in any case, if polyspermic eggs are present in the same cocoon, they are much less numerous than the monospermic eggs.

Eggs of planarians must be considered, as a rule, to be monospermic, *i.e.* provided with factors inhibiting supernumerary sperm penetration. At present, it is not possible to establish whether the lack of this block is induced directly by peculiar genotypic variations or it only represents a secondary event related to these variations.

The observations performed on Paradojo population seem to exclude a cytoplasmic effect.

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Explanation of Plate I

a) *Dugesia benazzii*: F_1 (Paradojo ♀ × Roccapina). Diploid oocyte in anaphase I with dyads at the opposite poles of the spindle (arrow). (mp): male pronucleus; (sph) spermatic head in advanced stage of degeneration.

b) *Dugesia polychroa*: individual belonging to subsequent generations from the cross between diploid (acting as female) and polyploid specimens. Diploid oocyte in anaphase I, showing four dyads (arrow) at the poles of the spindle. (mp): male pronucleous; (sph) degenerating spermatic head.

c) *Dugesia benazzii*: diploid specimen of the population from Paradojo. Oocyte in anaphase I, showing the dyads (arrow). (mp): male pronucleous in advanced evolution; (sph): spermatic head in degeneration.

PLATE I