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# Research on the hereditary mechanism of polyploidy in planarians

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**Biologia.** — *Research on the hereditary mechanism of polyploidy in planarians* (*). Nota (**) di GIUSEPPINA BENAZZI LENTATI e VERONETTA MEZZANI, presentata dal Socio M. BENAZZI.

RIASSUNTO. — Il raddoppiamento del corredo cromosomico nella linea femminile rappresenta la prima tappa nella successione degli eventi che determinano l'instaurarsi della poliploidia nelle planarie.

Con l'intento di indagare le basi genetiche di tale raddoppiamento sono stati compiuti in *Dugesia lugubris s.l.* incroci fra il biotipo diploide (funzionante da femmina) ed un biotipo poliploide. Tutti gli $F_1$ hanno corredo somatico diploide, ma gli ovociti possono essere diploidi o tetraploidi: precisamente, la maggioranza degli ibridi (67,9%) ha ovociti esclusivamente diploidi, un piccolo numero (1,1%) ovociti esclusivamente tetraploidi, i rimanenti (31%) presentano i due tipi di ovociti, con frequenze diverse nei vari individui. Tali differenze sono statisticamente significative.

Viene prospettata qualche ipotesi circa il meccanismo ereditario che porta al raddoppiamento del corredo cromosomico.

## INTRODUCTION

The research effected till now on two species of Triclads (Turbellaria Paludicola), which have diploid and polyploid biotypes, has permitted us to obtain a suggestion of the route that may lead to polyploidy (1). The first basic step is the capacity of chromosome set doubling, that is accomplished in the female line of the $F_1$ individuals born from the cross between diploid individuals (acting as female) and polyploid ones. This doubling is the 'character' which is transmitted by the sperm of polyploid biotype to a small percentage of the offspring. The neoblasts that transform themselves into oogonia undergo a nuclear division not followed by cytokinesis; the fusion of the two nuclei always occurs and, therefore, a peculiar 'restitution' of interphase or prophase nuclei takes place. It is to be pointed out that generally only some of the oogonia have a double set, so that, in the same individual there are both diploid and tetraploid oocytes.

The first observations on this fact were made many years ago, when Benazzi found, among the offspring of successive generations originating from a cross between individuals of the diploid biotype of *Dugesia lugubris s.l.* (2) (population of Pisa) and polyploid individuals (population of Pavia),

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(1) For further details on this question see Benazzi Lentati, 1970.

(2) In the previous papers of our team this planarian is named *D. lugubris*, because the taxonomic problem of the two allied species (*lugubris* and *polychroa*) described by O. Schmidt in 1860 was still unsolved. This question has now been definitely clarified by Benazzi

two specimens which had diploid and tetraploid oocytes (cfr. Benazzi and Benazzi Lentati, 1959). Similar results were obtained in hybrids of lst generation of *D. benazzii* born from a cross similar to the abovementioned; some specimens had oocytes with two ploidy levels (triploid and hexaploid), the former however do not generally leave the ovary, where they degenerate (Benazzi Lentati and Puccinelli, 1959).

Recently Benazzi and Giannini (1970) have found two populations of *D. benazzii* from Corsica with triploid and hexaploid oocytes; both types of oocytes are regularly laid. Statistical analysis has shown that the two populations present a significant difference in the frequencies relating to the two types of oocytes and that one population reveals marked individual differences in the relative frequencies of the two types of oocytes.

The study of the genetic determinism of the ' set doubling ' character was begun by one of us in 1962 on *D. lugubris s.l.*, by crossing, at first, diploid individuals of a Sardinian population with polyploid individuals from Pavia and Lake Iseo and then, diploid individuals from Lake Garda with polyploid individuals from Lake Iseo. From the cross between the Sardinian population and that from Pavia were obtained 15 specimens constantly diploid (50 oocytes were examined) and 3 whose oocytes (15 examined) were all tetraploid for two reproductive cycles, but then they also gave 6 diploid oocytes and 10 tetraploid ones. A similar result was obtained from the cross between the Sardinian population and that from Lake Iseo: 8 individuals with constant diploid oocytes (34 oocytes) and 6 with tetraploid oocytes (23 oocytes) for two reproductive cycles; in the 3rd cycle the latter individuals gave also 10 diploid oocytes besides 20 tetraploid ones. The animals died at the end of the 3rd reproductive cycle. Another specimen laid for eighth reproductive cycles 18 tetraploid oocytes. The cross of the populations from Lake Garda and Lake Iseo gave 15 offspring, 11 with diploid oocytes (45 oocytes) and 4 with diploid and tetraploid oocytes, probably with different frequencies between the two types of oocytes in the different animals, at least on the basis of the little evidence obtained.

The data are not sufficient to make a deep analysis of the determinism of chromosome set doubling. It is only possible to deduce the following conclusions: *i*) the number of individuals which have oocytes with a double set is inferior to that with exclusively diploid oocytes; *ii*) the frequency of the tetraploid oocytes varies in relation to the diploid population used; *iii*) perhaps there is a certain difference in the ratio between diploid oocytes and tetra-

*et al.* (1970) and by Reynoldson and Bellamy (1970). We know that the diploid and polyploid biotypes referred to in this paper must be attributed to *D. polychroa.* However, Benazzi *et al.* (loc. cit.) have proposed to maintain the name *lugubris s.l.* (as superspecies) including all biotypes and we have thought convenient to follow this nomenclature.

We recall that there are three polyploid biotypes differing with regard to the ploidy level and the modalities of oogenesis. In the present research we have used biotype B, which is triploid in the somatic line and hexaploid in the female germ line.

ploid ones, among the various individuals coming from the same cross; *iv*) it appear clearly that the ' chromosome set doubling ', though appearing at $F_1$, is not a dominant character.

These data are similar to those obtained studying the hereditary behaviour of another character, also present in some polyploid biotypes, that is ' female asynapsis ' which as been widely studied and which is believed to be controlled by a multifactorial mechanism (Benazzi Lentati and Bertini, 1961).

With the aim of extending the research on the genetic bases of polyploidy, in these last years we have resumed the crosses between diploid individuals from Pisa and polyploid ones from Lake Iseo, obtaining a fairly good number of offspring which have been fertile, at least during the first reproductive cycles.

Some interesting data have also been collected in studying individuals of successive generations from a cross between two diploid populations, as we will see at the end of this paper.

## Material and Technique

Ten crosses were made between diploid individuals from Pisa and polyploid ones from Iseo; only four gave offpring. The 1st cross had 25 offspring, the 2nd cross 14, and the other two crosses had only 4.

The two diploid populations, whose cross also concerns the present study, are from Pisa and Lake Garda.

The bivalent count was effected, as usual, on the unfertilized oocytes coloured with aceto–carmine.

We point out that in the planarians of the ' *lugubris* group ' all, or nearly all, the oocytes at the end of prophase leave the ovary; therefore, we may be sure that the ratio between the diploid and tetraploid oocytes found in the cocoons is equal to that of the oocytes present in the ovaries.

In Table I the results of the 4 crosses have been kept separate so as to give as precise a picture as possible. The numbers with which the single individuals are labelled correspond to the order in which they began laying and, therefore, to the order in which we began to study them. However, we thought it useful, to give greater emphasis to the data collected, to divide the individuals into two groups: above ($a$, $a'$, $a''$) the individuals with only diploid oocytes and below ($b$, $b'$, $b''$) those with the two types of oocytes. Furthermore, in group $a$, $a'$, $a''$ the individuals have been arranged according to the number of oocytes laid, while in group $b$, $b'$, $b''$ they have been arranged according to the increasing percentage of tetraploid oocytes.

## Results

From Table I it can be seen that the number of oocytes laid by each individual is very different and in some cases it is very low. We point out that some animals died at the end of the second reproductive cycle and others

laid only a few cocoons, often without oocytes. Due to these breeding difficulties, we think it fit to publish the data collected, even though not very numerous, since they allow us to make some deductions regarding the problem we set ourselves and may be compared with those previously given.

TABLE I

| *a* (1° cross) | | | *a'* (2° cross) | | | *a''* (3°–4° cross) | | |
|---|---|---|---|---|---|---|---|---|
| ind. no. | ooc. 2 *n* | ooc. 4 *n* | ind. no. | ooc. 2 *n* | ooc. 4 *n* | ind. no. | ooc. 2 *n* | ooc. 4 *n* |
| 5 | 7 | — | 15 | 23 | — | 1 | 4 | — |
| 3 | 17 | — | 14 | 26 | — | 2 | 14 | — |
| 9 | 25 | — | 21 | 27 | — | — | — | — |
| 19 | 26 | — | 18 | 38 | — | — | — | — |
| 14 | 28 | — | 12 | 39 | — | — | — | — |
| 18 | 28 | — | 17 | 44 | — | — | — | — |
| 1 | 30 | — | 19 | 48 | — | — | — | — |
| 6 | 33 | — | 8 | 69 | — | — | — | — |
| 7 | 35 | — | 1 | 81 | — | — | — | — |
| 22 | 35 | — | — | — | — | — | — | — |
| 15 | 36 | — | — | — | — | — | — | — |
| 17 | 36 | — | — | — | — | — | — | — |
| 10 | 42 | — | — | — | — | — | — | — |
| 12 | 45 | — | — | — | — | — | — | — |
| 4 | 66 | — | — | — | — | — | — | — |
| 26 | 76 | — | — | — | — | — | — | — |
| *b* | | | *b'* | | | *b''* | | |
| 24 | 64 | 2 | 10 | 20 | 7 | 4 | 8 | 16 |
| 25 | 17 | 1 | 20 | 20 | 15 | 3 | 12 | 27 |
| 20 | 15 | 4 | 2 | 17 | 29 | — | — | — |
| 11 | 11 | 6 | 22 | 8 | 39 | — | — | — |
| 8 | 20 | 15 | 13 | 1 | 29 | — | — | — |
| 2 | 13 | 19 | — | — | — | — | — | — |
| 21 | 5 | 33 | — | — | — | — | — | — |

## TABLE II

a) *Percentage of hybrids with diploid and tetraploid oocytes*

| | | |
|---|---|---|
| Total no. of hybrids from all crosses: 41 | hybrids with 2 *n* oocytes . . . . | 27 (65,85%) |
| | hybrids with 2 *n* and 4*n* oocytes . | 14 (34,15%) |

b) *Percentage of diploid and tetraploid oocytes from all crosses*

| | | |
|---|---|---|
| Total no. of oocytes: 1451 | 2 *n* oocytes . . . . . | 1209 (83,32%) |
| | 4 *n* oocytes . . . . . | 242 (16,68%) |

c) *Percentage of diploid and tetraploid oocytes from the first and second cross*

| | | |
|---|---|---|
| Total no. of oocytes from 1° cross: 790 | 2 *n* oocytes . . . . . | 710 (89,87%) |
| | 4 *n* oocytes . . . . . | 80 (10,13%) |
| Total no. of oocytes from 2° cross: 580 | 2 *n* oocytes . . . . . | 461 (79,48 %) |
| | 4 *n* oocytes . . . . . | 119 (20,52%) |

Statistical check: $\chi^2$ 29,51 $P < 1\%$

d) *Percentage of diploid and tetraploid oocytes (only from specimens with both types of oocytes)*

| | | |
|---|---|---|
| Total no. of oocytes from 1° cross: 225 | 2 *n* oocytes . . . . . | 145 (64,45%) |
| | 4 *n* oocytes . . . . . | 80 (35,55%) |
| Total no. of oocytes from 2° cross: 185 | 2 *n* oocytes . . . . . | 66 (35,67%) |
| | 4 *n* . . . . . | 1119 (64,33%) |

Statistical check: $\chi^2$ 33,56 $P < 1\%$

## TABLE III

*Percentage of diploid and tetraploid oocytes laid by the single individuals.*

| 1° cross | | | 2° cross | | | 3°–4° cross | | |
|---|---|---|---|---|---|---|---|---|
| ind. no. | % ooc. 2 *n* | % occ. 4 *n* | ind. no. | % ooc. 2 *n* | % ooc. 4 *n* | ind. no. | % ooc. 2 *n* | % ooc. 2 *n* |
| 24 | 97% | 3% | — | — | — | — | — | — |
| 25 | 94,4% | 5,6% | — | — | — | — | — | — |
| 20 | 79% | 21% | 10 | 74% | 26% | — | — | — |
| 11 | 64,7% | 35,3% | — | — | — | — | — | — |
| 8 | 57,3% | 42,7% | 20 | 57,3% | 42,7% | — | — | — |
| 2 | 40,6% | 59,4% | 2 | 37% | 63% | 4 | 33,3% | 66,7% |
| — | — | — | — | — | — | 3 | 30,7% | 69,3% |
| 21 | 13,2% | 86,8% | 22 | 17% | 83% | — | — | — |
| — | — | — | 13 | 3,3% | 96,7% | — | — | — |

From Tables I and IIa it can be seen that 65,85 % of the offspring laid only diploid oocytes, while the remaining 34,15 % laid both diploid and tetraploid oocytes. Consequently the number of tetraploid oocytes is much lower than that of the diploid oocytes, representing only 16,68 % of the total (Table IIb). There is a significant difference between the first and second cross regarding the frequencies of the two types of oocytes, considering the oocytes laid by all individuals (Table IIc). The difference is significant, even limited to the individuals which laid the two types of oocytes (Table IId).

From Tables I *b*, *b'* *b''* and III it can also be seen that there are differences in the frequencies of the two types of oocytes according to the individuals. Due to the low number of data, a deeply analysis is not profitable; we observe only that the values of the frequencies of the two types of oocytes seem to form a gradual series, hence a possible arrangement of these individuals in separate groups is questionable. It is to point out nevertheless that the percentages of diploid and tetraploid oocytes of some individuals are markedly different and that these differences appear to be significant; we propose some data which we believe to be among the most evident:

1° cross:

| Inds. no. | 24–20 | 24–11 | 24–8 | 24–2 | 24–21 | 20–2 | 11–21 | 8–21 | 2–21 |
|---|---|---|---|---|---|---|---|---|---|
| $\chi^2$ . . . . . | 4,431 | 13,14 | 22,98 | 37,7 | 71,9 | 5,71 | 12,9 | 13,69 | 5,50 |
| P . . . . . | 5%–2% | < 1% | < 1% | < 1% | < 1% | 2%–1% | < 1% | < 1% | < 1% |

2° cross:

| Inds. no. | 10–2 | 10–22 | 10–13 | 2–22 | 2–13 |
|---|---|---|---|---|---|
| $\chi^2$ . . . . . | 7,80 | 21,2 | 27,7 | 3,73 | 9,4 |
| P . . . . . | < 1% | < 1% | < 1% | 5 % | < 1% |

On the whole, considering also the presence of the individuals with only diploid oocytes, it is possible to assert that the $F_1$ from the cross between diploid and polyploid biotypes is represented by a large group of diploid individuals genetically rather uniform and by others that are strong differentiated. These data confirm what has already been deduced from the previous studies, according to which, moreover, there may be individuals with exclusively tetraploid oocytes.

Therefore, keeping in mind all data collected, we should have two opposite groups, the first being formed by approximatively 67,9 % of individuals with diploid oocytes, the second by 1,1 % of individuals with tetraploid oocytes, and one intermediate group represented by 31 % of individuals, with both types of oocytes; some of the latter individuals present significant differences in the percentage of the two types of oocytes.

The hereditary behaviour of the character in question, that is the doubling of the chromosome set, is similar to that of ' female asynapsis ' confirming

what was first postulated. Both the characters would be controlled by a multifactorial mechanism.

The appearence of set doubling at $F_1$ may be a result of heterozygosis of the diploid parent, similar to what postulated for ' female asynapsis '. In the diploid biotype asynapsis is never manifest, no matter how these factors are combined among the individuals of this biotype, either because the number of genes does not exceed a determined ' threshold ' or for other conditions, for example the absence of co-operators.

With regard to the ' set doubling ', a fortunate chance as permitted us to verify experimentally the validity of the hypothesis of the presence of factors for this character in the diploid biotype. In three individuals out of 30 examined, coming from generations following a cross between the two diploid populations from Pisa and Lake Garda, tetraploid oocytes have been found sporadically. This chromosome complement is never manifest in either of the two diploid populations: therefore, we must believe that it may appear in consequence of the new genic combination. This fact may therefore indicate the heterozygosity of the diploid biotype.

The multifactorial hypotesis presents, however, some gaps which for the moment we are not able to fill. In fact, with regard to ' asynapsis ' we recall that the same $F_1$ individual may laid asynaptic and synaptic oocytes, and likeweise with regard to ' set doubling ' the same individual presents two types of oocytes; moreover, some individuals with exclusively tetraploid oocytes for two reproductive cycles, began to produce diploid oocytes at the end of the third reproductive cycle.

Probably all these facts may be an indication of unbalanced genotypes; the manifestation of the characters is dependent upon different numbers of specific genes in each individual and perhaps also on different combination with other factors. It is, thus, evident that cells with the same genotype are able to behave in an independent way.

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