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On the cytological mechanism of pseudogamy in synaptic diploid hybrids of the planarian Dugesia lugubris

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Zoologia. — *On the cytological mechanism of pseudogamy in synaptic diploid hybrids of the planarian* Dugesia lugubris (*). Nota di
GIUSEPPINA BENAZZI LENTATI, presentata (**) dal Corrisp. M. BENAZZI.

RIASSUNTO. — Nella planaria *Dugesia lugubris* gli ibridi diploidi provenienti da incrocio fra il biotipo diploide sinaptico anfimittico, usato come femmina, ed i biotipi poliploidi (ad ovociti sinaptici oppure asinaptici) pseudogamici, possono ereditare la pseudogamia: lo spermio non si rigonfia nell'ovocita ma degenera alla fine della maturazione. Tale modalità era finora risultata peculiare dei biotipi poliploidi asinaplici naturali e degli ibridi pure poliploidi asinaptici, poichè in quelli poliploidi sinaptici lo spermio viene espulso. La pseudogamia è quindi un carattere trasmissibile dallo spermio ma la sua manifestazione è correlata al tipo di maturazione (ameiotico o meiotico) dell'uovo ed al grado di ploidia, indipendentemente dal biotipo pseudogamico che ha funzionato da padre.

Previous investigations have demonstrated that pseudogamy, which is characteristic of natural polyploid biotypes of some planarian species, is achieved by means of different cytological mechanisms. (For general references see Benazzi Lentati, 1970 [1]). In *Dugesia lugubris* and *D. benazzii*, in which polyploid biotypes with meiotic and ameiotic oogenesis occur, a correlation between the type of oocyte maturation and the pseudogamy pattern was found; similar correlation also manifests itself in the polyploid hybrids obtained, in both species, by crosses between specimens of the amphimictic diploid biotype (acting as female) and individuals of the pseudogamic polyploid biotypes.

The present study, carried out on *D. lugubris* alone, concerns the synaptic diploid hybrids. In this species the following biotypes are recognized: 1) diploid biotype with amphimictic synaptic oocytes; 2, 3) triploid and tetraploid biotypes both with pseudogamic ameiotic asynaptic oocytes; 4) biotype with a triploid somatic set but with pseudogamic meiotic synaptic hexaploid oocytes. Pseudogamy manifests itself by different ways according to whether the oocytes are synaptic or asynaptic, hence meiotic or ameiotic. In synaptic oocytes the sperm head undergoes swelling as soon as it penetrates the oocyte, but is expelled with a polocyte (generally the first one); on the contrary, in asynaptic oocytes the sperm head does not evolve and degenerates either within the zygote or in the early blastomeres. Crosses between the diploid biotype (acting as female) and the asynaptic triploid one have given origin to diploid and polyploid hybrids which may have both synaptic and asynaptic oocytes. In most cases the diploid hybrids have shown an amphimictic development while the polyploid ones may display both an

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amphimictic and a pseudogamic development. When the oocytes of the polyploid hybrids are asynaptic, pseudogamy is realized as in the paternal biotype, namely, with sperm degeneration; on the contrary, when the oocytes are synaptic the sperm is expelled, as in the synaptic polyploid biotype, despite the fact that the father is asynaptic. Therefore the hybrid inherits the character *pseudogamy* from the father, but the mode by which this manifests itself is correlated with the type of oocyte maturation (Benazzi Lentati, 1965, 1966) [2, 3].

Only very scanty information I was able to collect, up to a year ago at least, on the diploid hybrids with pseudogamic synaptic oocytes (1). As just stated, these hybrids are almost all amphimictic. However, in 1958 and 1962 from crosses with a father of the synaptic triplo-hexaploid biotype, synaptic diploid hybrids had originated; these hybrids display, in rare cases, pseudogamic synaptic diploid oocytes, in which the sperm head is not expelled, but degenerates within the cytoplasm (2) (Benazzi and Benazzi Lentati, 1958 [4]; Benazzi Lentati, 1962 [5]).

Schematic representation of the two types of cross

1° CROSS

Amphimictic synaptic diploid biotype ♀×*pseudogamic asynaptic triploid biotype*

F1 and following generations with:

diploid synaptic oocytes ——— (all amphimictic?)

diploid asynaptic oocytes ——— (all amphimictic?)

polyploid asynaptic oocytes
- amphimictic
- pseudogamic (with sperm degeneration)

polyploid synaptic oocytes
- amphimictic
- pseudogamic (with sperm expulsion)

2° CROSS

Amphimictic synaptic diploid biotype ♀×*pseudogamic synaptic triplo-hexaploid biotype*

F1 and following generations with:

diploid synaptic oocytes
- almost all amphimictic
- rarely pseudogamic

polyploid synaptic oocytes
- amphimictic
- pseudogamic (with sperm expulsion)

(1) So far, it has not been possible to study diploid hybrids with pseudogamic asynaptic oocytes.

(2) These activated eggs in which amphimixis does not take place are referred to by me as « pseudozygotes »; they do not regulate, thus no offspring is produced.

The finding yielded by study of diploid synaptic pseudogamic hybrids seems to be in contrast with those of natural polyploid synaptic biotype and of synaptic polyploid hybrids. For this reason, I deemed it suitable to extend my investigations to the diploid hybrids obtained during these latter years.

MATERIAL AND METHODS

The offspring of two types of crosses were studied:

1) *amphimictic synaptic diploid biotype* ♀ × *pseudogamic synaptic triplo-hexaploid biotype*. To a cross of this type belong also the just mentioned hybrids with pseudogamic diploid oocytes which provided the first indications on the cytological pattern of pseudogamy in diploid oocytes; in these hybrids the first generation was lost, therefore only the subsequent generations were studied.

2) *diploid biotype* ♀ × *pseudogamic asynaptic triploid biotype*.

* * *

My research lasted over several years (3); almost all the F_1 offspring (except those of the first group mentioned above) as well as the specimens of subsequent generations obtained from incrosses (as far as the fifth generation) and from back-crosses with the paternal biotype (up to the second and third generations) were examined. From the incrosses a fairly large number of descendants were obtained, among which several reliable synaptic diploid samples were selected. The offspring arising from back-crosses were all studied: from five back-crosses very few descendants were obtained; none were yielded by pseudogamic oocytes. The sixth back-cross, which appeared of great interest, has been obtained recently; the finding reported in the Tables refer only to this last back-cross.

In order to ascertain the pattern of development, I have examined ripening oocytes contained in cocoons removed from the genital atrium (in this way meiosis and the behaviour of the sperm head can be studied), as well as ripe eggs from cocoons just prior to or soon after hatching (when meiosis is completed). In fact, in the eggs of the natural amphimictic diploid biotype, maternal and paternal chromosomes are transformed into pronuclei, losing their dye-binding capacity; on the contrary in the eggs of the pseudogamic diploid hybrids examined in 1958 and 1962, the sperm appears either compact and highly stainable or is made up of strongly contracted discrete chromosomes which degenerate during the first maturation division.

Cocoons were examined by means of the current aceto-carmine technique without squashing, not to damage the eggs.

(3) I wish to thank Mr. R. Vaselli for his valuable cooperation in the cytological examination.

RESULTS

The present study confirms that pseudogamy can be ascertained only in ripe eggs; it is demonstrated by the presence of unevolved sperm head. Some doubt might be raised by the occurence, in the oocytes at anaphase, of the sperm head next to the outer pole of the spindle, with a similar location to that encountered in pseudogamic synaptic polyploid oocytes, where the sperm is expelled. It should be remarked, however, that in the present case meiotic oocytes were never found lacking the sperm head.

TABLE I.

F_1 (*amphimictic synaptic diploid biotype* ♀ × *pseudogamic synaptic triplo-hexaploid biotype*)

SPECIMENS no.: 40				
total no. cocoons: 350	artificially opened (*)	210	with	ooc. during meiosis 230
				zygotes 461
				pseudozygotes 9
				polyspermic eggs 0
				unfertilized ooc 30
	hatched with offspring	90		
	no offspring	50		
Following generations				
SPECIMENS no.: 170				
total no. cocoons: 764	artificially opened (*)	484	with	ooc. during meiosis 350
				zygotes 410
				pseudozygotes 8
				polyspermic eggs 16
				unfertilized ooc 90
	hatched with offspring	180		
	no offspring	100		

(*) Cocoons either removed from the planarians body or just hatched.

From the Tables I, II and III one may see that the number of pseudogamic oocytes is very low; they are therefore derived from few individuals, none of which however present only pseudozygotes, as revealed by cytological examination. Amphipseudogamy, therefore, has arisen, as I had already found in polyploid hybrids.

In Tables I and IV no difference results between F_1 and the following generations through incrosses; some increase in the number of pseudozygotes is shown in Table III.

TABLE II.

F1 (*amphimictic synaptic diploid biotype* ♀×*pseudogamic synaptic triplo-hexaploid biotype*)

SPECIMENS no.: 2

total no (**) cocoons: 96	artificially opened (*)	30	with	ooc. during meiosis	25
				zygotes	20
				pseudozygotes	0
				polyspermic eggs	0
				unfertilized ooc	6
	hatched with offspring	4 (***)			
	no offspring	62			

(*) Cocoons either removed from the planarians body or just hatched.
(**) Observations of five years.
(***) This offspring has not yet laid cocoons.

TABLE III.

F1 (*amphimictic synaptic diploid biotype* ♀×*pseudogamic asynaptic triploid biotype*)

SPECIMENS no.: 187

total no. cocoons: 3080	artificially opened (*)	1280	with	ooc. during meiosis	720
				zygotes	910
				pseudozygotes	10
				polyspermic eggs	6
				unfertilized ooc	220
	hatched with offspring	1060			
	no offspring	740			

Following generations

SPECIMENS no.: 180

total no. cocoons: 2010	artificially opened (*)	880	with	ooc. during meiosis	350
				zygotes	606
				pseudozygotes	15
				polyspermic eggs	11
				unfertilized ooc	90
	hatched with offspring	610			
	no offspring	520			

(*) Cocoons either removed from the planarians body or just hatched.

The findings obtained from a cross with very low fertility (Table II) were set apart on account of their peculiar cytological features. In fact, in many oocytes at anaphase I the sperm head swells, losing its dye-binding capacity in quite an unusual way; it cannot be decided whether its more rapid evolution in pronucleus has occurred or else its most precocious degeneration is under way, although preceded by marked swelling. Descendants are very scanty.

TABLE IV.

Back-cross with pseudogamic asynaptic triploid biotype

SPECIMENS no.: 2				
total no. cocoons: 63 (**)	artificially opened (*) 41	with	ooc. during meiosis	17
			zygotes	8
			pseudozygotes	7
			polyspermic eggs	2
			unfertilized ooc	4
	hatched with offspring 13		offspring no.: 18 (***)	
	no offspring 10			

Second generation

SPECIMENS no.: 7				
total no. cocoons: 96	artificially opened (*) 65	with	ooc. during meiosis	44
			zygotes	16
			pseudozygotes	15
			polyspermic eggs	9
			unfertilized ooc	10
	hatched with offspring 6			
	no offspring 25			

(*) Cocoons either removed from the planarians body or just hatched.
(**) Observations of two years.
(***) Only 7 have laid cocoons.

Special attention has been paid to the back-cross (Table IV). It was the only one providing a good chance for more accurate examination. Also in this case no difference in the percentage of zygotes and pseudozygotes between R_1 and the successive generation obtained by incrosses was observed. However, I shall not dwell on this side of the problem, which is worth further study; only the cytological findings will be taken into consideration here.

R_1 individuals, as well as the few ones that could be examined in the following generation derived from incross, are amphipseudogamic; also in this case pseudogamy can be assessed with certainty only in the ripe eggs.

In all specimens, even within the same cocoon, unquestionably amphimictic eggs are found (in which the sperm, already at first anaphase is represented by a fine network of ill-defined chromomeric filaments), and unquestionably pseudogamic eggs (in which the degenerating sperm appears as a cluster of highly stainable granules) (Plate I *a*, *b*, *c*).

In both amphimictic and pseudogamic oocytes the sperm head lies on one side of the spindle, as a rule close to the equatorial plate, at least as far as can be inferred from the preparations obtained by our technique, which thus far was found to be the only suitable one for the study of these elements.

In several crosses I observed cases of polyspermy, with two or more sperms all degenerating; in my opinion, degeneration of the great majority of sperms is not the expression of pseudogamy. Only in two dispermic oocytes, one of the sperms seemed to evolve in a pronucleus.

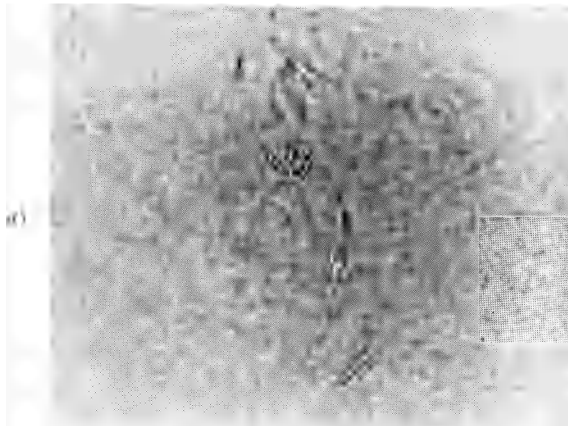
To conclude: in the diploid hybrids studied here pseudogamy can be assessed with certainty only in ripe eggs; in these the sperm head degenerates. Its expulsion has never been noticed.

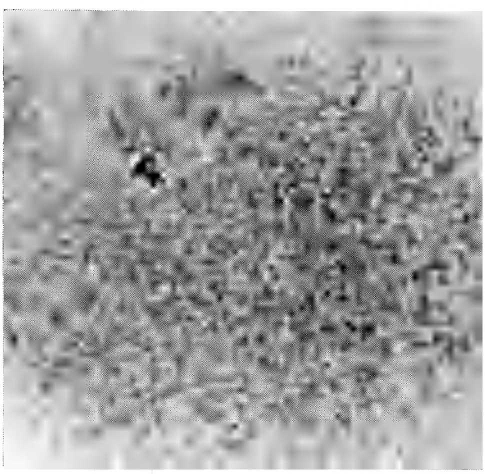
Conclusions

Diploid hybrids obtained from cross between the amphimictic synaptic biotype, acting as female, and the pseudogamic polyploid biotypes (with either synaptic or asynaptic oocytes) may inherit pseudogamy from the father: the study of the synaptic oocytes of these hybrids has shown that the sperm head penetrated into the eggs degenerates at the end of maturation or at the first cleavage. It is worth stressing that this cytological pattern is peculiar only to the natural asynaptic polyploid biotypes, since in the synaptic polyploid ones the sperm is expelled. It should also be remarked that in oocytes from hybrids *the pattern of pseudogamy is independent of the biotype which has acted as father*.

From previous investigations on pseudogamic though polyploid hybrids, obtained from an identical cross type (i.e. amphimictic diploid × pseudogamic polyploids) it had already emerged that the character "pseudogamy" can be transmitted by the sperm, but its manifestation is controlled by a complex of factors, in particular those which control synapsis or asynapsis of the egg chromosomes, hence ultimately by the maturation type, independent of the pseudogamic biotype acting as father. In fact, when the father belongs to the asynaptic polyploid biotype, the pseudogamic and polyploid though not asynaptic descendants show an identical pseudogamy pattern as does the synaptic polyploid biotype.

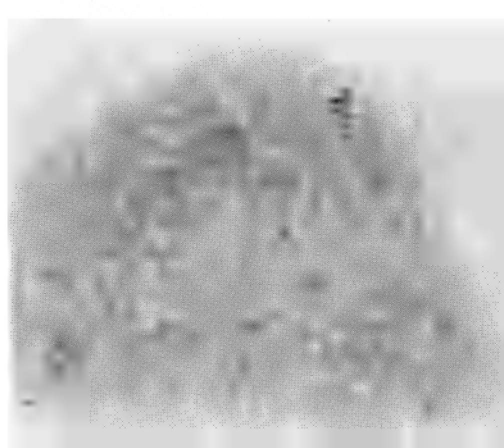
The results presented here, besides confirming the presumably multifactorial control of pseudogamy, also lay stress upon the influence exerted on it by the ploidy level, since they show that the mechanism whereby these phenomena unfold in synaptic diploid oocytes differs from that characterizing *a*)





b)

c)



Back-cross with pseudogamic asynaptic triploid biotype: *a*) sperm evolution in amphimict egg at the first anaphase; *b, c*) sperm degeneration in two pseudozygotes.

the synaptic polyploid oocytes, also when the synaptic polyploid biotype has acted as male.

In the hybrids investigated so far amphipseudogamy consistently occurs, that is to say, the same individual presents, even within the same cocoon, both amphimictic and pseudogamic eggs.

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