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**On the origin of heterogeneous chromosome sets in  
some fissiparous planarians**

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**Biologia.** — *On the origin of heterogeneous chromosome sets in some fissiparous planarians.* Nota di GIUSEPPINA BENAZZI LENTATI (\*) e PAOLO DERI (\*\*), presentata (\*\*\*) dal Socio M. BENAZZI.

RIASSUNTO. — Sono state studiate due popolazioni del Triclade Paludicolo *Dugesia benazzii*, appartenenti a due diversi biotipi cariologici, da molti anni allevate in laboratorio ed in cui è stato possibile cogliere fasi iniziali di variazioni del genoma, apparentemente collegate alla scissiparità. Gli AA., tuttavia, in base al complesso delle ricerche compiute dal gruppo pisano ed a quelle eseguite da altri studiosi, giungono alla conclusione che, seppure in molti casi i due fatti appaiano concomitanti, essi non possono considerarsi geneticamente correlati.

#### INTRODUCTION

The present observations concern two karyologically differentiated populations belonging to the planarian *Dugesia benazzii*, the same species studied in the preceding paper by Deri [1].

One of these populations (from Castello Pino, Corsica) is diploid ( $2n = 16$ ;  $n = 8$ ), the other (from Roccapina, Corsica), is triploid or aneutriploid in the somatic line (with ca. 24 chromosomes), triploid or hexaploid in the female line (in the latter case as a result of a set doubling) and diploid in the male line (owing to a haploid set elimination). This population therefore belongs to the triplo-hexaploid biotype (for exhaustive data on the different biotypes and related chromosome cycles, see Benazzi and Benazzi Lentati [2]).

In the first population of this species, collected in 1965, sexual and fissiparous individuals were already present in nature [3]. On the contrary, the population from Roccapina collected in the same year showed in nature and for many years also in laboratory cultures only sexual reproduction. After 1976, however, a great number of individuals became asexual and fissiparous [4]. We have separately bred the individuals which still manifested sexual reproduction.

This paper intends to throw light upon some karyological peculiarities of these populations.

#### TECHNIQUE

The karyological study was carried out on body fragments taken from the cephalic zone, which in the sexually ripe planarians contains the ovaries and the first testicles, and on blastemata obtained after cutting the body at different levels.

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The cephalic segments containing the ovaries and the testes were placed for 20-30 min in 2% acetic acid and stained with aceto-carmin. The blastemata were treated with 0.3% colchicine (Merck) for 4-5 hours, then transferred for 5 min to 2% acetic acid and stained with lacto-aceto-orcein. Finally they were squashed between slide and cover-glass.

## RESULTS

Before presenting the cytological observations we think it is necessary to point out that under laboratory conditions *Dugesia benazzii*, like other species of the "*Dugesia gonocephala* group", shows an annual reproductive cycle which generally starts at the end of summer or in autumn. Some external morphological features, well visible also *in vivo*, particularly the appearance of a large genital pore surrounded by a black pigment layer, indicate sexual ripeness. The deposition of cocoons lasts many months during winter and spring and subsequently the reproductive apparatus and consequently the genital pore slowly reduce. During the summer it is very difficult to find sexually ripe animals.

### *Population from Castello Pino.*

The studies accomplished on the sexual specimens of this population have proved the stability of the diploid set in the somatic and germ lines (Benazzi [3] and unpublished data).

Instead, according to our observations, the behaviour of the fissiparous individuals is not uniform from a karyological viewpoint. In fact, we have found the following situations:

1) 6 individuals spontaneously divided showed only diploid cells; because in these specimens the fission plain occurred in the middle zone of the body, we examined more anterior body segments and found some male elements, specially spermatozoa, although copulatory apparatus and genital pore were lacking. Probably these animals had initiated the evolution towards fissioning when the sexual cycle was not yet concluded.

2) 17 individuals showed only diploid cells in the blastemata, but sexual elements were completely lacking (Fig. 1 a).

3) 14 individuals presented a mixture of diploid and polysomic (with 17-24 chromosomes) cells in the blastemata; in 8 individuals the diploid cells were more numerous, while in the other 6 the polysomic cells were more numerous (Figs. 1 b-c).

### *Population from Roccapina.*

All asexual specimens studied showed triploid cells in the blastemata. There were 13 specimens with sexual reproduction, but 3 of them in

the last two years exhibited deviant patterns with the following characteristics:

*a)* One animal laid two cocoons in winter 1978, but at the end of March regression of the genital system took place. The cytological examination of the cephalic zone, which contains the ovaries and the anterior testicular follicles, showed triploid and diploid cells, a few apparently normal prophasic meioses and a great number of oocytes and spermatocytes in degeneration. The further stages of gametogenesis showed diffuse signs of degeneration (in particular cytoplasm vacuolization in the oocytes), while metaphasic spermatocytes were lacking and spermatids were extremely rare. We immediately studied the blastemata derived from the cephalic zone of this animal, previously cut with the aim of studying the gonads, and found 15 triploid and 10 diploid neoblasts. This specimen fissioned during summer 1978 and the blastemata of the regenerated individuals showed diploid and triploid cells. The animals, although still asexual, did not divide during the last year; however, the blastemata from the sub-ocular and the extreme caudal zone (areas in which the testicles are lacking even in the sexually ripe animals) showed diploid and triploid cells. Evidently the diploid cells, which remained quiescent after the degeneration of the male line, which occurred in March 1978, migrated to all parts of the body allowing the diplo-triploid complement. (Figs. 2 *a-a<sub>1</sub>-b*).

*b)* The other two animals, sexually ripe during winter and spring 1978, showed the normal regression of the reproductive apparatus at the beginning of summer, but remained agamous during autumn 1978 and winter 1979. In March 1979 they became fissiparous. In the blastemata originated from various parts of the body we have always found triploid cells. One of these animals died in summer 1979, while the other produced a clone that at present consists of 14 animals with only triploid cells (Fig. 2 *c*).

#### DISCUSSION

Research carried out by Benazzi over many years (1938-1974) [5] [6] has demonstrated that fissioning in planarians, although influenced by external conditions, is a phenomenon which depends upon genetic factors. The individuals in which these factors manifest themselves stand out on account of their incapacity to develop the reproductive apparatus, and the primary effect exerted by these fission-controlling genes is likely to consist in preventing the transformation of undifferentiated cells (neoblasts) into germ cells. The presence of both sexual and fissiparous individuals in the same population is easily explained bearing in mind that the factors for fissioning may also be present in the sexual individuals, and may manifest themselves phenotypically, hindering the development of the reproductive apparatus in a percentage of the offspring.

The diploid population from Castello Pino is an example of coexistence in nature and during laboratory culture of both fissiparous and sexual indi-

viduals and of the transition from one condition to another and *vice-versa*. Moreover, some of the sexual individuals of this population are rather fertile and their offspring present sexual and fissiparous individuals in a ratio around 1 : 1. This segregation demonstrates without doubt the genic determinism of fissioning [6]. In the individuals of the triploid population from Roccapina, on the contrary, fissioning arose in the laboratory, contemporaneously with a strong reduction of fertility.

Fissioning in planarians is frequently accompanied by variations in chromosome number. The observations made over many years on laboratory cultures permit us to present some examples of the relationships between the two phenomena, and to attempt an interpretation of some mechanisms which may be effective in determining these peculiar chromosome sets in natural fissiparous strains.

#### *Origin of the polysomic and heteroploid chromosome sets in laboratory cultures.*

Deri, in a previous paper [1], offered an example of gradual transformation of a diploid set into a hyperdiploid one with the appearance of B-chromosomes in specimens which, at first sexual, became fissiparous after a period of laboratory culture. In the population from Castello Pino illustrated in the present paper, hyperdiploidy (without B-chromosomes) occurs only in some of the fissiparous individuals, while the others remain diploid.

In the population from Roccapina the appearance in 1976 of fissioning was not accompanied by chromosome number variations but by the appearance of B-chromosomes. With regard to the action of fission-controlling genes it is to be remembered that in the individual showing diploid and triploid cells these genes start their action during a sexual phase, inhibiting the further differentiation of the diploid cells into male cells and determining the degeneration of the formerly and definitively differentiated male cells. The chromosome set in this individual remained therefore diplo-triploid. In the other triploid individuals, on the contrary, the fission-controlling genes became active after the annual regression of the reproductive apparatus that normally occurs in all planarians. This may explain the lack of diploid cells and the presence of only the triploids <sup>(1)</sup>.

On the other hand, after many years of laboratory culture a population from Sardinia belonging to the same biotype [7] presented a high increase in chromosome number (up to 30-35), always with sexual reproduction: in 1957 fissioning appeared and all individuals became asexual [8]. In this last case, therefore, an increase in the chromosome number in a triploid set has occurred.

(1) This result presents a problem, viz: why in the first individual the degeneration does not occur in the diploid cells which are certainly the first cells to have undergone differentiation towards the male sex. This is, however, a new aspect of the problem of sex cell differentiation which does not concern the purpose of the present paper.

*Peculiar chromosome sets in some natural fissiparous strains of different planarian species.*

In *Dugesia tigrina* some fissiparous populations are either exclusively diploid or exclusively triploid, but most of them are characterized by a mixture of diploid and triploid cells [9] [10]. *D. dorotocephala* has  $2n = 16$ ,  $n = 8$  in both sexual and fissiparous populations [11]; in two fissiparous populations, however, triploid cells have been found (Benazzi and Puccinelli, in preparation). *D. japonica* includes two subspecies: *D.j. japonica* with  $2n = 16$  and *D.j. ryukyensis* with  $2n = 14$ . Both are characterized by the presence of fissiparous populations, the chromosome sets of which are diploid and triploid. Slight numerical chromosome variations, probably due to polysomy, are found [12] [13] [14] [15]. In some populations of *D. benazzii* from Corsica polysomic fissiparous individuals coexist with sexual diploid ones (for instance the population from Patrimonio, station B), and in other populations fissiparous triploids (or aneutriploids) coexist with sexual individuals belonging to the triplo-hexaploid biotype (populations from Porto, Gravone and Prunelli) [16]. Likewise, in other specimens of *D. gonocephala* s.l. from various localities Benazzi [2] found a hyperdiploid set with B-chromosomes, similar to those found by Deri [17] in specimens collected in Corsica. Bromley [18] found that sexual individuals obtained in laboratory cultures of a population of *D. biblica* resulted in the laying of fertile cocoons, the offspring of which were either  $2n = 18$ ,  $2n = 18 +$  supernumerary chromosomes and  $3n = 27 + +$  supernumerary chromosomes. On the other hand, Benazzi [2] found in oocytes of *D. biblica* collected in the Jordan river a high chromosome number probably with B-chromosomes.

The data so far collected permit some conclusions to be drawn. Most of the fissiparous planarians possess polysomic or triploid (or aneutriploid) sets, which may arise through an increase of chromosome number starting from a diploid complement. Some other fissiparous planarians, however, maintain the chromosome set characteristics of the sexual forms from which they derive. The first case, typified by the population from Cavo, occurs in other populations from Corsica with diploid sexual and asexual individuals which gradually become hyperdiploid. We think that also the triploid individuals of *D. tigrina*, *D. dorotocephala* and *D. japonica* which coexist with the diploid individuals could be interpreted according to the first hypothesis.

The second case, typified by the triploid asexual individuals from Roccapina, may be represented by the fissiparous individuals from Corsica that coexist with the sexual ones of the triplo-hexaploid biotype. The diplo-triploid individuals of *D. tigrina* and *D. japonica* may be considered in this context: their chromosome condition is in fact identical to the one found in that specimen from Roccapina in which the diplo-triploid set arose from the two cellular types (diploid and triploid) present in this individual during its sexual phase. This hypothesis is certainly suggestive, but at present it is not supported by

factual data, because sexual polyploid races of *D. tigrina* and *D. japonica* are not known.

On the basis of the data collected by our team over many years, the various situations encountered may be distinguished as follows (Table I):

1) Diploid strains which reproduce both sexually and by fission without any variation in chromosome number (for instance *D. dorotocephala*, *D. sanchezi*, according to Benazzi [11] [19] and research in progress).

2) Sexual diploid forms of *D. tigrina* and fissiparous strains of the same species with either diploid or triploid or both diploid and triploid cells; no variations have been observed after laboratory culture (according to Benazzi *et al.* [10]).

3) Diploid strains with both reproductive modalities in nature, but in which some fissiparous individuals show hyperdiploid cells after laboratory culture (population of *D. benazzii* from Castello Pino, Corsica).

4) Diploid strains with both reproductive modalities in nature, in which a high increase in the chromosome number occurs also in the sexual individuals; only after a long laboratory culture is sexuality lost (population of *D. benazzii* from Viglietu river, Sardinia, according to Benazzi Lentati [8]).

5) Diploid sexual strains in which fissioning and hyperdiploidy, followed by the appearance of B-chromosomes, arise in laboratory culture (population of *D. benazzii* from Cavo river, Corsica, according to Deri [1]).

6) Diploid and hyperdiploid strains, with both reproductive modalities in nature, which show the same karyological pattern in laboratory culture. B-chromosomes are already present in natural conditions (population of *D. benazzii* from Patrimonio, Corsica, according to Deri [17]).

7) Hyperdiploid strains with sexual and fissiparous individuals in nature; B-chromosomes are already present in natural conditions. In laboratory culture sexuality reduces further without variation in the chromosome number (populations of *D. benazzii* from Conca and Bravone, Corsica, according to Deri [17]).

8) Diploid sexual strains in which hyperdiploidy appears in laboratory culture, without inhibiting fertility for many years; fissioning appears very slowly (two races of *D. etrusca*, according to Benazzi Lentati [20] [8]).

9) Strains with sexual and fissiparous individuals in nature, belonging to the triplo-hexaploid biotype; in laboratory culture no variation in the chromosome number occurs (populations of *D. benazzii* from Porto, Gravone river and Prunelli river, Corsica, according to Deri [16]).

10) Sexual strains with triploid somatic set, belonging to the triplo-hexaploid biotype, in which a high increase in the chromosome number occurs; fissioning arises only after a long culture period (population of *D. benazzii* from Mascari river, Sardinia, according to Benazzi Lentati [8]).

TABLE I

SPECIES	LOCALITIES	Chromosome number in specimens examined immediately after collection				Chromosome number in specimens after a period of laboratory culture			
		sexual indiv.	B-chrom.	fissiparous individuals	B-chrom.	sexual indiv.	B-chrom.	fissiparous individuals	B-chrom.
<i>Dugesia benazzii</i>	Corsica:								
	Castello Pino	2n	—	2n	—	2n	—	2n-hyperdiploid.	—
	Cavo river	2n	—	2n	—	—	—	hyperdiploid	1-3
	Patrimonio	2n	—	hyperdiploid	1-3	2n	—	hyperdiploid	1-3
	Conca-Bravone	hyperdip.	1-3	hyperdiploid	1-3	hyperdiploid	—	hyperdiploid	1-3
	Gravone and Prunelli rivers	hyperdip.	1-3	hyperdiploid	1-3	hyperdiploid	—	hyperdiploid	1-3
	Porto	3n	—	3n	—	3n	—	3n	—
	Roccapina	3n	—	3n	—	3n	—	3n	—
		3n	—	—	—	3n	—	2n-3n; 3n	1-3
		3n	—	—	—	3n	—	—	—
<i>D. estrusca</i>	Sardinia:								
	Viglietu river	2n	—	2n	—	2n	—	hyperdiploid	—
	Mascati river	3n	—	3n	—	3n	—	hypertriploid	—
<i>D. tigrina</i>	Tuscany: two races	2n	—	—	—	—	—	hyperdiploid	—
	Canada:								
<i>D. dorotocephala</i>	St. Lawrence river	2n	—	—	—	2n	—	—	—
	Italy (various loc.)	—	—	2n; 2n-3n; 3n	—	—	—	2n; 2n-3n; 3n	—
<i>D. sanchezi</i>	U.S.A.:								
	some localities	2n	—	—	—	2n	—	—	—
	most localities	—	—	2n	—	—	—	2n	—
	Brandy river and Blacksburg (Virg.)	—	—	3n	—	—	—	3n	—
<i>D. sanchezi</i>	Chile:								
	Concepcion	2n	—	2n	—	2n	—	2n	—



11) Sexual strains with triploid somatic set, belonging to the triplohexaploid biotype, in which fissioning arises in the laboratory without any variation in the chromosome number, but with the appearance of B-chromosomes (population of *D. benazzii* from Roccapina, Corsica, according to Benazzi Lentati and Deri [4]).

\* \* \*

These manifold aspects of the relationships between genomic variations and fissioning suggest that polysomy or polyploidy, as well as the rise of B-chromosomes, are not directly correlated with the appearance of fissioning. The greater frequency of the fissiparous forms with high chromosome number, if compared with the diploid ones, may be explained by assuming that a high chromosome number determines a favourable heterozygous condition, which may act in the asexual state, thereby increasing its adaptative properties (cf. Benazzi and Benazzi Lentati [2]). The different moment at which the two characters (fission and heterogeneous chromosome sets, respectively) appear may depend, besides upon the influence of external factors, upon genetic constitution, probably different in the various strains.

Hyperdiploidy or polyploidy in most of the planarians studied by us may be transmitted through sexual reproduction; on the contrary, in other planarian species (belonging to the genera *Phagocata*, *Polycelis*, *Crenobia*) with sexual and fissiparous populations only the artioploid sets may be transmitted through sexual reproduction. In fact, the asexual ones have hyperdiploid or heteroploid or perissoploid chromosome sets, which present anomalous meiosis. In this latter case, fissioning may permit the propagation of all viable numerical deviations, owing to the chance to build up clones (according to Dahm [9]). The same consideration may be valid for the diplo-triploid set found in natural populations of *D. tigrina*, *D. japonica* and also in that individual of *D. benazzii* (population from Roccapina) which becomes fissiparous in laboratory culture; in fact, the rise of sexual reproduction will produce, through meiosis, two types of gametes from which, if viable, two types of zygotes could derive.

The data collected by our team do not agree with some conclusions reached by Bromley [18], according to which the fissioning is primarily due to the triploid karyotype and the sexual maturation is induced by laboratory conditions. The results obtained by Bromley in the laboratory with reversal from asexual triploidy to sexual diploidy appear interesting. However, we do not possess data to substantiate the claim that sexual diploidy could never have taken place in nature, since the initial conditions necessary to produce mature triploid animals in the field never materialize.

## REFERENCES

- [1] DERI P. (1980) - « Rend. Acc. Naz. Lincei », ser. VIII, 67, (in press.).
- [2] BENAZZI M. and BENAZZI LENTATI G. (1976) - *Platyhelminthes*. In: « Animal Cytogenetics » (B. John Ed.), Gebrüder-Borntraeger, Berlin-Stuttgart, 1, 1-182.
- [3] BENAZZI M. (1967) - « Rend. Acc. Naz. Lincei », ser. VIII, 42, 469-472.
- [4] BENAZZI LENTATI G. and DERI P. (1977) - « Rend. Acc. Naz. Lincei », ser. VIII, 62, 847-851.
- [5] BENAZZI M. (1938) - « Mem. Acc. Naz. Lincei », ser. VI, 7, 31-89.
- [6] BENAZZI M. (1974) - *Fissioning in Planarians from a Genetic Standpoint*. In: « Biology of the Turbellaria ». Libbie H. Hyman Memorial Volume. McGraw-Hill, New York, 476-492.
- [7] LEPORI N. G. (1948) - « Caryologia », 1, 92-101.
- [8] BENAZZI LENTATI G. (1964) - « Atti Soc. Tosc. Sci. Nat. », Mem., ser. B, 71, 44-51.
- [9] DAHM A. G. (1958) - *Taxonomy and Ecology of Five Species Groups in the Family Planariidae (Turbellaria Tricladida Paludicola)*. Nya Litografen, Malmö, 1-241.
- [10] BENAZZI M., GIANNINI-FORLI E. and PUCCINELLI I. (1971) - « Boll. Zool. », 38, 493-494.
- [11] BENAZZI M. (1966) - « Rend. Acc. Naz. Lincei », ser. VIII, 40, 999-1005.
- [12] TESHIROGI W. and ITAGAKI G. (1965) - « Zool. Mag. (Dobutsugaku Zasshi) », 74, 38-45.
- [13] OKI I. and TAMURA S. (1975) - « Zool. Mag. (Dobutsugaku Zasshi) », 84, 61-63.
- [14] TAMURA S., YAMAYOSHI T., OKI I., MURAYAMA H. and KAWAKATSU M. (1978) - « Prod. Jap. Soc. System. Zool. », 15, 8-18.
- [15] KAWAKATSU M., OKI I., TAMURA S. and SUGINO H. (1976) - « Bull. Fuji Women's Coll. », 14, 81-126.
- [16] DERI P. (1971) - « Atti Soc. Tosc. Sci. Nat. », Mem., ser. B, 78, 55-74.
- [17] DERI P. (1975) - « Atti Soc. Tosc. Sci. Nat. », Mem., ser. B, 82, 25-38.
- [18] BROMLEY H. J. (1977) - « Zool. Scripta », 6, 197-201.
- [19] BENAZZI M. (1978) - « Rend. Acc. Naz. Lincei », ser. VIII, 64, 299-301.
- [20] BENAZZI LENTATI G. (1957) - « Caryologia », 10, 352-387.

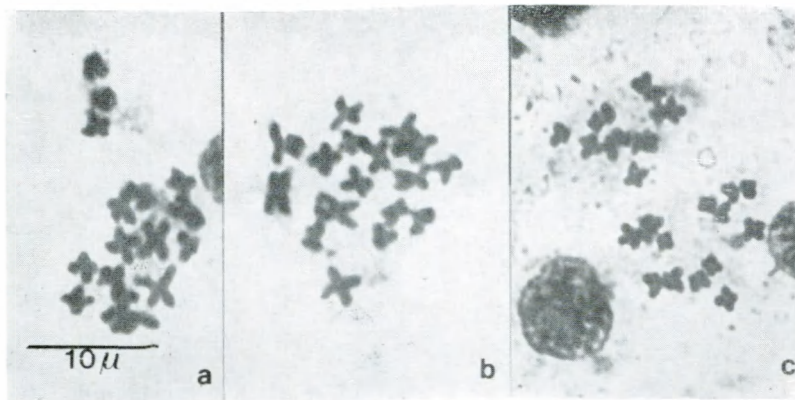


Fig. 1. - Population from Castello Pino: three metaphase plates with 16 (a), 17 (b), and 24 (c) chromosomes.

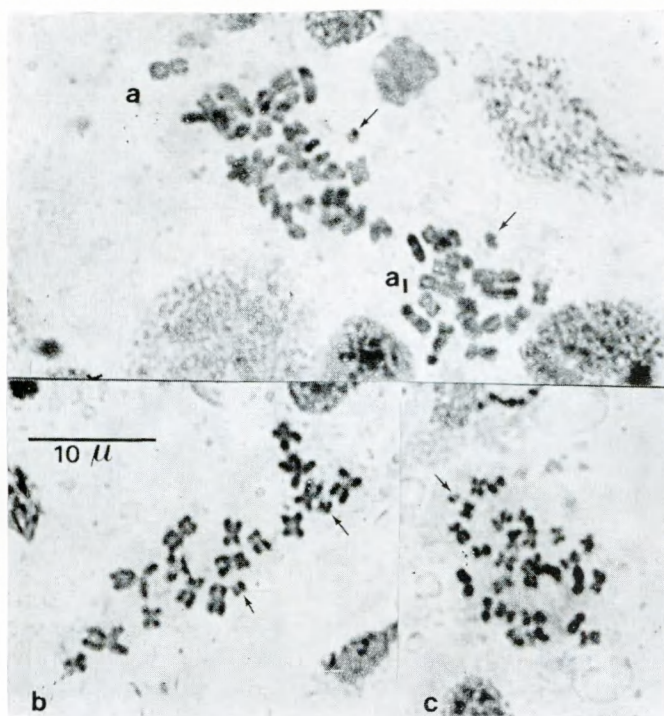


Fig. 2. - Population from Roccapina: *a* and *a*<sub>1</sub>, two metaphase plates with 24 and 16 A-chromosomes, respectively, and one B-chromosome (arrows); *b*, metaphase plate with 16 A-chromosomes and two B-chromosomes (arrows); *c*, metaphase plate with 26 A-chromosomes and one B-chromosome (arrow).