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# KARYOMETRIC ANALYSIS ON POPULATIONS OF *DUGESIA BENAZZII* (TURBELLARIA, TRICLADIDA) EVIDENCING A CHROMOSOME POLYMORPHISM

Riassunto — Analisi cariometrica di popolazioni di Dugesia benazzii (Turbellaria, Tricladida) evidenzianti un caso di polimorfismo cromosomico. È stata svolta un'indagine cariometrica su popolazioni della Corsica (Paradojo e Castello Pino) e della Sardegna (Giocca) della planaria Dugesia benazzii Lepori.

Negli esemplari di Paradojo, diploidi (2n = 16; n = 8), sessuati ed anfimittici, i cromosomi decrescono gradualmente in lunghezza e sono tutti metacentrici (quelli del settimo paio sono al limite con i submetacentrici).

Nella popolazione di Castello Pino sono presenti individui diploidi ed iperdiploidi, sessuati o scissipari. Il cariotipo è simile a quello della popolazione precedente, eccetto che per la terza coppia. Infatti, salvo rare eccezioni, essa è polimorfica, composta nei diploidi da due elementi eteromorfi, l'uno metacentrico simile al corrispondente della popolazione di Paradojo, l'altro al limite tra submetacentrico e subtelocentrico. Negli iperdiploidi, anche con un incremento numerico fino a 30-32 elementi, non sono stati mai osservati più di due cromosomi eterobrachiali. Sia nei diploidi che negli iperdiploidi sono presenti B-cromosomi.

Negli esemplari di Giocca, tetraploidi (o aneutetraploidi), sessuati e pseudogamici, il terzo quartetto è composto da quattro cromosomi submetacentrici (al limite con i subtelocentrici), simili al cromosoma eterobrachiale presente in Castello Pino.

Viene messa in evidenza l'origine (per inversione pericentrica) del cromosoma eterobrachiale e discussa la diversa condizione di eterozigotia e di omozigotia nelle popolazioni di Castello Pino e di Giocca. Viene pure condotta un'analisi comparativa con i dati raccolti da altri Autori su *Dugesia gonocephala* s.s. e su differenti specie del «gruppo *D. gonocephala*» che presentano analoghe caratteristiche cariologiche.

Abstract — The present paper deals with the karyometric analysis of populations of the planarian *Dugesia benazzii* Lepori collected in Corsica (Paradojo and Castello Pino strains) and Sardinia (Giocca strain).

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Paradojo specimens are diploid (2n = 16; n = 8), sexual and amphimictic; their chromosome complement consists of metacentric elements, whose length gradually decreases (in the seventh pair the centromere borders on a submetacentric position).

The Castello Pino population is characterized by diploid and hyperdiploid individuals, that reproduce sexually or by fission. Its karyotype is similar to the one observed in Paradojo strain, except for the fact that, in most samples, the third pair is polymorphic. In diploid specimens, this latter pair consists of two heteromorphous elements, one metacentric and identical to that described in the preceding population and the other submetacentric, at the border of subtelocentric. This heterobrachial chromosome has never been observed in a homozygous condition. On the other hand, hyperdiploid specimens, that are characterized by a gradual increase in chromosome number (up to 30-32 elements), have never been observed with more than two submetacentrics. B-chromosomes are also present in both diploid and hyperdiploid individuals.

Specimens from Giocca are tetraploid (or aneutetraploid), sexual and pseudogamous; the third quartet consists of four submetacentric (at the border of subtelocentric) chromosomes. These elements are similar to the heterobrachial chromosome observed in Castello Pino population.

The origin (through a pericentric inversion) of the heterobrachial chromosome is put forward and the different conditions (homozygoty and heterozygoty) in Castello Pino and Giocca strains are discussed. A comparative analysis has also been carried out with the data collected by other Authors on *Dugesia gonocephala* s.s. and different species of the *«D. gonocephala* group», which show similar karyological characteristics.

Key words - Karyology - Chromosome polymorphism - Planarians.

### INTRODUCTION

Dugesia benazzii Lepori from Corsica, Sardinia and Capraia, a freshwater planarian belonging to the «*D. gonocephala* group», was widely studied by our staff in Pisa, with particular emphasis on the chromosome cycles and the reproductive modalities. In fact, it has been demonstrated that this species is represented by various biotypes differing in the degree of ploidy, in the type of oogenesis, which may be synaptic or asynaptic, and in the mode of development, which may be amphimictic or pseudogamous (see BENAZZI and BENAZZI LEN-TATI, 1976).

Other research concerned the karyotype (BENAZZI LENTATI *et al.*, 1951) and also the karyometric analysis of various populations (data as yet unpublished).

Recently, the finding of an example of polymorphism in a population from Corsica (BENAZZI LENTATI *et al.*, 1984) led us to a general revision of our karyometric data. It must be pointed out that the karyotype of some forms belonging to the «*D. gonocephala* group» comprises elements definable as metacentric, according to the classification introduced by Levan *et al.* (1964), while in other forms significant variations occur (BALL, 1979; DE VRIES, 1986 a; DE VRIES and BALL, 1980; GOURBAULT, 1981; see also BENAZZI, in press).

Two biotypes are considered in the present paper: i) diploid (2n = 16) or hyperdiploid, with populations reproducing either only sexually or both sexually and by fission; ii) tetraploid (32 chromosomes) or aneutetraploid, pseudogamous and constantly sexual.

### MATERIAL AND METHODS

Two populations from Corsica and one from Sardinia were examined. The populations from Corsica were collected in the Paradojo stream, near Bastia, and at Castello Pino; the one from Sardinia was collected near Scala di Giocca. Paradojo is diploid, sexual and amphimictic, Castello Pino is diploid (or hyperdiploid) with both sexual and fissiparous reproductive modalities, Giocca is tetraploid (or aneutetraploid), sexual and pseudogamous.

The present research was carried out on regenerative blastemata, with the technique used in our previous investigations: the blastemata were treated with 0.3% colchicine for 3-4 hours, then transferred for 5 min to 2% acetic acid and stained with lacto-acetoorcein. Finally, they were squashed between slide and cover-glass.

It must be pointed out that identification of the middle and small-sized chromosomes is frequently difficult, as also observed by the Authors quoted above, in preparations carried out with the same technique. Several of the middle and small-sized chromosomes overlap as far as their relative length and centromeric index are concerned. We therefore attempted different methods of fixation, such as Carnoy, or 3:1 mixture of methanol and glacial acetic acid, followed by 40% acetic acid and coloration with lacto-aceto-orcein.

A new procedure suitable for a careful study of planarian chromosomes was developed by researchers of the Pavia team (REDI *et al.*, 1982). The obvious advantage of the preparations obtained is that the metaphase chromosomes are very well spread out, preserving chromosome morphology and preventing overlapping.

This method has been applied to Paradojo blastemata by Pavia Authors, to whom we are very grateful. However, even with this method, the difficulties in the identification of some chromosomes are not entirely overcome, and no differences in karyometric analysis emerged, in comparison with the data obtained with our technique. Therefore, we continued to use our own standard method, which is easier and quicker, considering the large number of specimens to be examined.

The analysis was carried out on 40 diploid metaphase plates from 25 specimens of the Paradojo population and 25 diploid plates from 16 specimens of the Castello Pino population. We also examined many plates from 14 hyperdiploid individuals of the latter strain, in order to ascertain the number and type of the exceeding chromosomes. With regard to the population from Giocca, karyometric analysis was carried out on 15 metaphase plates of 8 specimens. It must be pointed out, however, that many individuals are aneutetraploid; we therefore thought it useful to calculate the number of homologous chromosomes present in each metaphase plate. This number may vary from 2 to 4 and even more, as a result of aneuploidy. All homologous chromosomes were arranged on the basis of their absolute length, and we placed them in groups according to their length. The average absolute length was calculated for each group. The relative length of the members of each group was then calculated on the basis of the length of the haploid genome.

# Results

# A - Karyometric analysis of the diploid karyotypes

# 1) Population from Paradojo

Table I shows that the chromosomes of the diploid complement gradually decrease in length, the longest pair being about twice the size of the shortest pair. This is a general rule for the members of the karyotype of *D. gonocephala* s.l. The first three elements are easily distinguishable on the basis of their respective lengths, although they are very similar in morphology, all of them being metacentric, almost isobrachial (the c.i. ranges from 45.08 to 47.28). From the chromosome 4 onwards the differences in the relative length decrease and the minimum value of one chromosome overlaps the maximum value of the subsequent chromosome. It is, therefore, difficult to identify the chromosomes 4, 5, 6 which, moreover, show similar morphology. Chromosome 7 possesses the lowest c.i.  $(36.86 \pm 2.03)$ , a value on the border-line between meta- and submetacentric chromosomes. On the contrary, the chromosome 8 is characterized by a high c.i.  $(46.58 \pm 1.95)$  (Fig. 1).



Fig. 1 - Dugesia benazzii: karyogram of the diploid population from Paradojo.

### 2) Population from Castello Pino

#### a) Eudiploid specimens

Karyometric analysis shows close agreement with the previous analysis, but the third chromosome pair is polymorphic. It consists of two heteromorphous elements, one of which is metacentric (c.i.  $45.15 \pm 3.94$ ) and identical to the corresponding one in the normal complement of the population from Paradojo, whereas the other is submetacentric at the border of subtelocentric (c.i.  $25.28 \pm 3.73$ ). The relative length of the latter (r.l.  $14.49 \pm 1.84$ ) is slightly greater than that of the homologous mediocentric (r.l.  $14.03 \pm 0.76$ ), perhaps because of a slight despiralization of the long arm, which may sometimes reach the length of the chromosome 2.

The polymorphic pair was found in all individuals, with a few exceptions (3 specimens in which both elements were metacentric). The heterobrachial chromosome was never found in homozygous condition (Fig. 2 a, b).

#### b) Hyperdiploid specimens

As already observed in other forms of *D. gonocephala* s.l., in the individuals of the population from Castello Pino there is a gradual increase in the number of chromosomes of the diploid set in labora-



Fig. 2 - Dugesia benazzii: karyograms of the population from Castello Pino.
a): diploid complement with the third pair polymorphic; 1 B-chromosome is present.
b): diploid complement with the third pair homomorphic, consisting of two metacentric homologues.

c): hyperdiploid (aneutriploid) complement, consisting of 24 A-chromosomes and 3 B-chromosomes.

d): hyperdiploid (aneutetraploid) complement, consisting of 32 chromosomes.

tory culture, owing to anomalies in anaphasic migration, so that 24, 27 to 30, 32 elements may be found. In such cases, therefore, two normal sets are present plus various repetitions of the homologues of the complement (Fig. 2 c, d). It is very difficult to ascertain whether three or four complete haploid sets are present, not only as a result of the mechanism determining the increase in the number of chromosomes, but also because of the difficulties experienced in the exact identification of several members of the complement, as already noted. However, the highest number of the subtelocentric chromosomes is 2, even in complements with 32 elements. In a few specimens this chromosome is absent.

B-chromosomes may be present both in diploid and in hyperdiploid complements (BENAZZI LENTATI and DERI, 1980).

# B - Karyometric analysis of the tetraploid biotype

### Population from Giocca

The data referring to the karyometric analysis of this race do not differ from those of the previous populations, with the exception of the chromosome 3 which is on the border-line between submetacentric and subtelocentric (c.i.  $26.35 \pm 2.81$ ) and may correspond to the heterobrachial chromosome of the polymorphic pair observed in the population from Castello Pino. In the tetraploid metaphases of Giocca, however, the third quartet consists of 4 submetacentric homomorphic elements (Fig. 3). Its relative length is slightly lower than that of chromosome 3 in the Castello Pino specimens, corresponding in some plates to the length of chromosome 4. This differs from what was observed in the Castello Pino specimens in which, as already stated, the length of the subtelocentric chromosome is greater than that of its metacentric homologous, and sometimes reaches the relative length of the chromosome 2. This can be seen from table I, which shows that the relative length of the chromosome 3 in the Giocca specimens is more similar to that of the chromosome 4 than of the chromosome 2, differently from what appears in the Castello Pino specimens (\*).

<sup>(\*)</sup> In the first period of our research we took the measurements of the chromosomes of embryonal cells on camera lucida drawings. This research involved a number of diploid populations and the tetraploid biotype of Giocca specimens (BENAZZI LENTATI *et al.*, 1951; 1956). In the latter we also measured the chromosomes of some oocytes, which are asynaptic and therefore well suited to karyological examination. This material was not pretreated with colchicine and, on the basis of these measure-



10 µm

Fig. 3 - Dugesia benazzii: karyogram of the tetraploid population from Giocca.

#### CONCLUDING REMARKS

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The karyometric data concerning two populations of the diploid biotype (Paradojo and Castello Pino) and a population of the tetraploid biotype (Giocca) all belonging to Dugesia benazzii show that not all the chromosomes are metacentric. Chromosome 7 is on the border-line between metacentric and submetacentric in Paradojo and Castello Pino (c.i.  $36.86 \pm 2.03$  and  $36.63 \pm 2.84$  respectively) whereas in Giocca it is submetacentric (c.i.  $35.19 \pm 2.52$ ). Moreover, in Castello Pino the third pair is heteromorphic in almost all the individuals, one chromosome being metacentric (c.i.  $45.15 \pm 3.94$ ) and its homologous subtelocentric (c.i.  $25.28 \pm 3.73$ ). In very few cases are both chromosomes metacentric, whereas the homozygous condition of the subtelocentric was never encountered. In Giocca the third quartet consists of 4 subtelocentrics (c.i.  $26.35 \pm 2.81$ ).

A comparative analysis was carried out with the data collected

ments, the heterobrachial chromosome of Giocca specimens was considered to be the fourth of the karyotype. The distinction between the seventh and the eighth chromosomes also appeared to be uncertain, because their lengths sometimes overlap.

Chromosome		Populations		
		Paradojo	Castello Pino	Giocca
1	r.l.	$18.80 \pm 0.84$	$17.50 \pm 1.43$	$18.50 \pm 0.67$
	c.i.	$45.48 \pm 1.79$	$44.63 \pm 2.09$	$44.50 \pm 2.03$
2	r.l.	$15.21 \pm 0.71$	$15.00 \pm 0.77$	$15.90 \pm 0.55$
	c.i.	$47.28 \pm 1.55$	$46.00 \pm 1.66$	$45.60 \pm 2.15$
3	r.l. c.i.	$13.75 \pm 0.51$ $45.08 \pm 1.14$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$13.45 \pm 0.48$ 26.35 $\pm 2.81$
4	r.l.	$12.62 \pm 0.77$	$12.55 \pm 0.46$	$12.87 \pm 0.59$
	c.i.	$42.39 \pm 2.30$	$42.56 \pm 2.42$	$45.27 \pm 3.92$
5	r.l.	$11.26 \pm 0.54$	$11.56 \pm 0.46$	$11.87 \pm 0.90$
	c.i.	$40.23 \pm 3.22$	$40.80 \pm 3.91$	$37.48 \pm 3.77$
6	r.l.	$10.41 \pm 0.53$	$10.60 \pm 0.56$	$10.76 \pm 1.37$
	c.i.	$43.67 \pm 3.34$	44.77 \pm 4.19	$44.05 \pm 3.15$
7	r.l.	$9.95 \pm 0.78$	$9.45 \pm 0.53$	$9.39 \pm 1.32$
	c.i.	$36.86 \pm 2.03$	36.63 $\pm 2.84$	$35.19 \pm 2.52$
8	r.l.	$8.93 \pm 0.71$	$8.93 \pm 0.82$	$8.51 \pm 1.18$
	c.i.	46.58 $\pm 1.95$	41.16 $\pm 2.57$	46.52 ± 2.90

TABLE I - Karyometric data (means  $\pm$  SD) for the haploid set of the three populations of Dugesia benazzii

r.l. = relative length: length of chromosome x 100 / total length of haploid genome. c.i. = centromeric index: length of short arm x 100 / length of entire genome.

by other Authors on *D. gonocephala* s.s. and different species of the «*D. gonocephala* group» (BALL, 1979; DE VRIES and BALL, 1980; GOUR-BAULT, 1981; DE VRIES, 1986 a; see also BENAZZI, in press). In *D. gonocephala* s.s. eudiploid strains, all the elements proved to be metacentric, while different features were found by DE VRIES (1986 a) in a hyperdiploid population. The Author writes (page 255): «The interpretation of the karyotype is difficult, due to the variation in chromosome number and centromeric index. My attempts to match the chromosomes into sets of three homologous elements were unreliable because of the overlap in length between successive elements and the variable number of subtelocentric chromosomes». We also think it worthwhile pointing out the presence of a single subtelocentric chromosome, appearing in fig. 1 B page 254, which is morphologically identical to the one we have described in Castello Pino.

DE VRIES also studied (1986 a) the karyology of D. subtentacula-

*ta* (Draparnaud), a mainly fissiparous form closely related to *D. gonocephala*. All specimens investigated possessed an identical karyotype with 24 chromosomes. The majority of the chromosomes were metacentric, but some had a centromere that bordered on a submetacentric position. In this species too, the most notable feature was the presence of the single submetacentric chromosome.

GOURBAULT (1981) studied the karyology of *D. iberica* GOURBAULT and BENAZZI 1979 (\*). In the eudiploid population from Soller the fourth, sixth and eighth pairs were metacentric, although they were highly heterobrachial (c.i.  $39.24 \pm 1.34$ ;  $37.46 \pm 1.94$ ;  $38.49 \pm 1.39$ , respectively) and the third pair was submetacentric (c.i.  $34.50 \pm 2.15$ ). The eutriploid population from Rio Jucar possessed three triplets of submetacentric chromosomes: the second (c.i.  $34.80 \pm 2.72$ ; the third (c.i.  $36.56 \pm 2.30$ ) and the seventh (c.i.  $35.81 \pm 1.02$ ).

Another planarian which deserves attention is *D. brigantii* DE VRIES and BENAZZI 1983, found in a cave and constantly sexual. According to PUCCINELLI and BENAZZI (1985), the chromosome complement comprises 24 elements in both the somatic and female lines, while the male line is diploid with 8 bivalents in the spermatocytes. Preliminary studies carried out on spermatogonial mitoses have shown that most chromosomes are metacentric, one pair, the seventh, is subtelocentric (c.i. 29.80) and the third pair is polymorphic, with one metacentric chromosome (c.i. 45.94) and the other subtelocentric (c.i. 23.73) (\*\*).

As early as 1951, BENAZZI LENTATI *et al.* suggested that the subtelocentric chromosomes of the third quartet of Giocca originate from a pericentric inversion. We think this interpretation is also valid for the subtelocentric chromosomes of Castello Pino, *D. brigantii* and in general for all the heterobrachial elements with a very low c.i. found in the various forms of *D. gonocephala* s.l. previously considered.

The occurrence of a highly heterobrachial chromosome in the species listed above is very peculiar. In our opinion, however, there is not enough evidence for univocal interpretation of this finding.

The attribution of this chromosome to the third or to the fourth pair is uncertain. Moreover, it must be noted that such an element

<sup>(\*)</sup> DE VRIES (1986 b) considers this species to be conspecific with D. subtentaculata.

<sup>(\*\*)</sup> Preliminary data based on a single plate.

is present in both eudiploid and hyperdiploid sets, and the origin of the latter may not be the same in all cases. As a matter of fact, hyperdiploid complements may originate either from diploid complements, as happens in Castello Pino population (BENAZZI LENTATI and DERI, 1980), by means of a gradual increase in the number of chromosomes, or from formerly polyploid complements.

In the paper quoted above (BENAZZI LENTATI *et al.*, 1984), we put forward the hypothesis of a hybrid origin of the strain from Castello Pino, on the basis of the offspring, obtained in laboratory culture, from a cross between two populations of *D. benazzii*, namely Paradojo and Giocca (the latter acting as the male), which possess a heteromorphic chromosome pair similar to the one observed in Castello Pino. Nevertheless, we also noted that populations with the same karyological characteristics as Giocca have never been found in Corsica.

Another noteworthy fact is that the heterobrachial chromosome is present only in heterozygous condition in the Castello Pino population, whereas in the tetraploid sets of the Giocca race it forms a homomorphic quartet.

The comparative study of these populations of planarians, carried out by means of the karyometrical analysis, certainly does not allow an exact identification of the chromosomes, and attempts with different methods have so far been inadequate.

Nevertheless, we think that the question deserves further investigation, particularly considering the different condition (heterozygoty and homozygoty) in Castello Pino and Giocca.

The population of *D. benazzii* from Castello Pino reproduces both sexually and by fission, independently of the chromosome complement, which may be eudiploid or hyperdiploid, the latter with up to 30 elements. The subtelocentric chromosome is present in almost all the individuals. It must be noted, however, that three or four subtelocentric elements have never been found in the hyperdiploid sets; in most cases, only one or two such elements are visible. *D. brigantii*, with 24 chromosomes, always possesses a single subtelocentric element and reproduces only sexually.

Owing to difficulties in laboratory culture, the chromosome cycle of these two forms has not yet been studied, but certainly a peculiar cycle, which allows sexual reproduction, takes place in both cases.

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