The karyotypes of Dugesia species from Spain (Turbellaria, Tricladida)

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Abstract

Some species of Planarians, new to Spain, are recorded. *Dugesia polychroa, D. sicula, D. iberica* and *D. gonocephala s. l.* have been investigated karyologically. The former possesses a diploid complement characteristic of the biotype A (2n = 8); the second is diploid with 2n = 18; diploidy and triploidy were found in sexual populations of *D. iberica* with n = 8. Triploidy occurred in all the asexual strains of the *D. gonocephala* group with a basic number of either 8 or 9. In this latter case B-chromosomes were occasionally found.

Introduction

During a field-trip to continental Spain and the island of Mallorca to collect hypogeous fauna from the low permeability interstitial sediments in river underflows and water tables, several specimens of common epigeous planarians were collected; the majority of these are referred to the genus *Dugesia.*

Hitherto few records deal with the Spanish Turbellarian fauna. *Crenobia alpina* (Dana) was reported in the Pyrenees by Borelli (1905), and *Dendrocoelum lacteum* (Müller) from the vicinity of Madrid was studied by Bordas (1921). Arndt (1926) identified *Polycelis felina* (Dalyell) from numerous localities between Gerona and Huesca, *Phagocata vitta* (Dugès) in a well from Tarragona province and *Dugesia lugubris* (Schmidt) from Lerida. Recently, Benazzi *et al.* (1975) described a new species of the *'lugubris-polychroa'* group, i.e. *Dugesia mediterranea*; asexual populations were found in Barcelona whereas sexual individuals were recorded on certain Mediterranean islands (Corsica, Sardinia, Sicily). Among the subterranean forms, *Dendrocoelopsis brementi* (de Beauchamp) is known from a Pyrenean cave and *Atrioplanaria racovitzai* (de Beauchamp) from the water table of the river Henares in Guadalajara (Gourbault 1971).

The material examined herein* is used to increase the chorological data concerning European Triclads and to confirm the identity of species through a karyological approach.

Materials and methods

The specimens studied were collected as follows:

- *Dugesia polychroa* (Schmidt, 1861). Rio Henares, Chiloeches, Alcalá de Henares; coll. 15 Sept. 1976, with numerous cocoons.
- *Dugesia sicula* Lepori, 1948. Ull de la Font outflow, Pollença, Mallorca; coll. 23 May 1976. Large population (cf. Gourbault & Lescher-Moutoué 1979).
- *Dugesia iberica* Gourbault & Benazzi, 1979. Outflow and brooklet close to the Cova dets Estudiants, Soller, Mallorca; coll. 22 and 26 May

* Field-work of Nicole Gourbault, Françoise Lescher-Moutoué and Raymond Rouch. Collecting trips supported by MNHN, Paris and Laboratoire souterrain du CNRS, Moulis, France.

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1976. Rio Júcar, Villelva de la Sierra, Cuenca; coll. 17 Sept. 1976.

- *Dugesia* aff. *iberica.* Small brooklet feeding a water through under Calobra Road, Mallorca; coll. 26 May 1976. Large dark specimens.

- Many asexual strains of *Dugesia* sp. of the '*D. gonocephala* group': (1) Small tributary of the Rio Eresma, Trescasas, Segovia; coll. 12 Sept. 1976. (2) Rio Rambla de Mal Burgo, Fortunate, Teruel; coll. 20 Sept. 1976. (3) Rio Guadalete, Algodonales near Ronda; coll. 18 Sept. 1977. (4) Rio Turon, El Burgo, Ronda; coll. 18 Sept. 1977. (5) Rio Guadalhorce, Alora, Malaga; coll. 19 Sept. 1977. (6) Rio Salia, La Viñuela, Malaga; coll. 20 Sept. 1977. Very numerous specimens. (7) Rio Guadalfeo, Vélez de Benaudalla, Granada; coll. 21 Sept. 1977. (8) Deifontes, vauclusian spring near Granada; coll. 22 Sept. 1977. (9) Subterranean water, Font de Can Salas, Pollença, Mallorca; coll. 23 and 25 May 1976.

About five specimens of each of these populations were studied both morphologically and karyologically. All the karyotypes were obtained from enlarged camera lucida drawings of mitotic metaphase stages in regenerative blastemas immersed in colchicine, stained in lactic-aceto-orcein and squashed. The chromosomes are classified according to the nomenclature of Levan *et al.* 1964.

It is of interest to note here the collection of the following additional species:

- *Dugesia tigrina* (Girard) collected with *D. polychroa* in the river Henares; this may be the first record of this american species in Spain.

- *Polycelis felina,* Rio Lobos Springs (right tributary of the river Duero), El Burgo de Osma, Soria; coll. 13 Sept. 1976; Rio Cuervo Springs (left tributary of the river Tajo), Tragacete, Cuenca; coll. 17 Sept. 1976; Rio Frio outflow, Granada; coll. 23 Sept. 1977.

- *Phagocata* sp. from a well in Sale del Rio Seco, Nerja (paper in progress), coll. 21 Sept. 1977.

- Indetermined blind and unpigmented species: from a well in Huértor-Tájar, Granada; coll. 23 Sept. 1977 and from a brooklet underflow, Rio Burró, left R. Fluvia, Besalu, Figueras; coll. 18 Sept. 1978.

Table 1. Relative length and centromeric index of the four chromosomes pairs of *Dugesia polychroa.* Means and standard deviations from ten mitotic metaphase plates, compared with the values recalculated from the idiogramm of biotype A established by Benazzi *et al.* 1970.

N° chromosome	Relative length	Centromeric index
1	34.86 ± 1.85	46.85 ± 1.58
2	27.05 ± 1.47	16.23 ± 1.70
3	21.43 ± 1.39	19.04 ± 1.56
4	16.52 ± 1.02	22.83 ± 1.18
	Biotype A	
1	34.30	46
2	25.50	11
3	22.20	16
4	17.80	20

Results

- *Dugesia polychroa*

The diploid complement of *Dugesia polychroa* consists of 8 elements. They are easy to pair, a

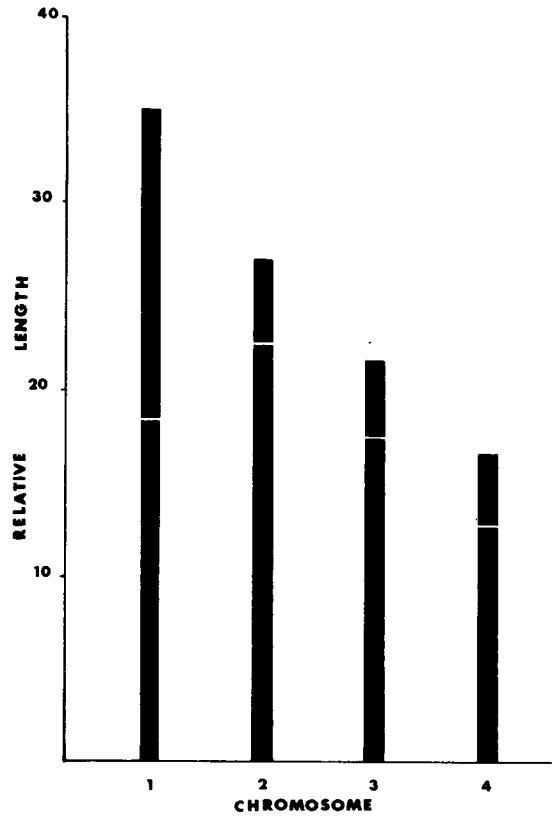

Fig. 1. Idiogram of the karyotype of *Dugesia polychroa*

Table 2. Relative length and centromeric index of the nine chromosome pairs of *Dugesia sicula*. Means and standard deviations from ten mitotic metaphase plates.

N° chromosome	Relative length	Centromeric index
1	14.13 ± 0.65	42.40 ± 2.20
2	12.77 ± 0.49	45.68 ± 2.54
3	11.83 ± 0.30	41.17 ± 2.64
4	11.67 ± 0.41	44.84 ± 2.41
5	10.98 ± 0.37	42.47 ± 3.90
6	10.55 ± 0.52	43.73 ± 4.06
7	10.15 ± 0.31	45.66 ± 2.08
8	9.59 ± 0.59	46.16 ± 1.46
9	8.91 ± 0.62	42.02 ± 1.73

condition that is perfectly supported by measurements of the relative lengths and centromeric indices. The means and standard deviations calculated for ten metaphase plates are given in Table 1; the karyotype is represented by the idiogram of Figure 1. Four chromosomes characterize the haploid set: one large metacentric and three smaller acrocentrics of different lengths, the second being the most heterobrachial, (similar to findings of Benazzi & Puccinelli 1961, 1973).

- *Dugesia sicula*

Karyologically this species is 'poorly known; it seems to be a diploid but 9 bivalents are found during oogenesis' (Benazzi & Benazzi Lentati 1976, p. 17).

The Mallorcan strain possesses a diploid complement of 2n = 18. This number was observed in more than thirty plates, two excepted (with 2n = 16).

The chromosomes are easily paired. Karyometric data (Table 2) form the basis of Figure 2. All chromosomes are metacentric and decrease very gradually in size.

- *Dugesia iberica*

Soller populations: A mitotic chromosome com-

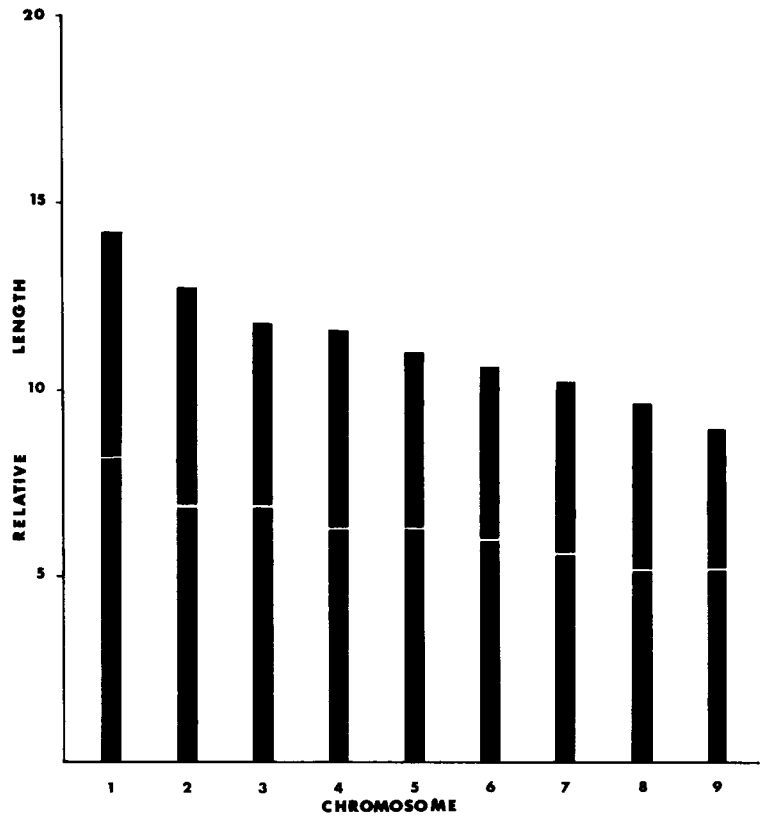

Fig. 2. Idiogram of the karyotype of *Dugesia sicula*.

Table 3. Relative length and centromeric index of the eight chromosomes pairs of *Dugesia iberica* (Soller population). Means and standard deviations from ten mitotic metaphase plates.

N° chromosome	Relative length	Centromeric index
1	18.96 ± 1.14	48.25 ± 0.57
2	15.56 ± 0.65	47.66 ± 0.68
3	13.45 ± 0.40	34.50 ± 2.15
4	12.48 ± 0.97	39.24 ± 1.37
5	10.90 ± 0.52	46.69 ± 1.88
6	10.62 ± 0.68	37.46 ± 1.84
7	9.28 ± 0.50	47.53 ± 0.39
8	8.66 ± 0.40	38.97 ± 1.49

plement of 16 was observed in all metaphase plates. The diploid condition is well confirmed by the measurements. Means and standard deviations were calculated for ten plates (Table 3). The idiogram of figure 3 illustrates the karyotype related to the description given by Benazzi & Benazzi Lentati (1976) for the haploid set of the Mediterranean *D. gonocephala* complex.

Most of the chromosomes are metacentric, although some are intermediate between meta and submetacentric, and the third is definitively submetacentric. Chromosome 8 is about half the size of chromosome 1.

Rio Júcar population: the complement of 24 found in all metaphases might be referable to a triploid condition. This viewpoint is significantly supported by the karyometrical studies as eight groups of three homologous chromosomes were noted for each series.

The basikaryotype of this triploid *Dugesia iberica* is represented by the idiogram of Figure 4, derived from Table 4. Most of the chromosomes are metacentric but differences in centromeric indices occur between the diploid and triploid strains, three elements being submetacentric in the latter.

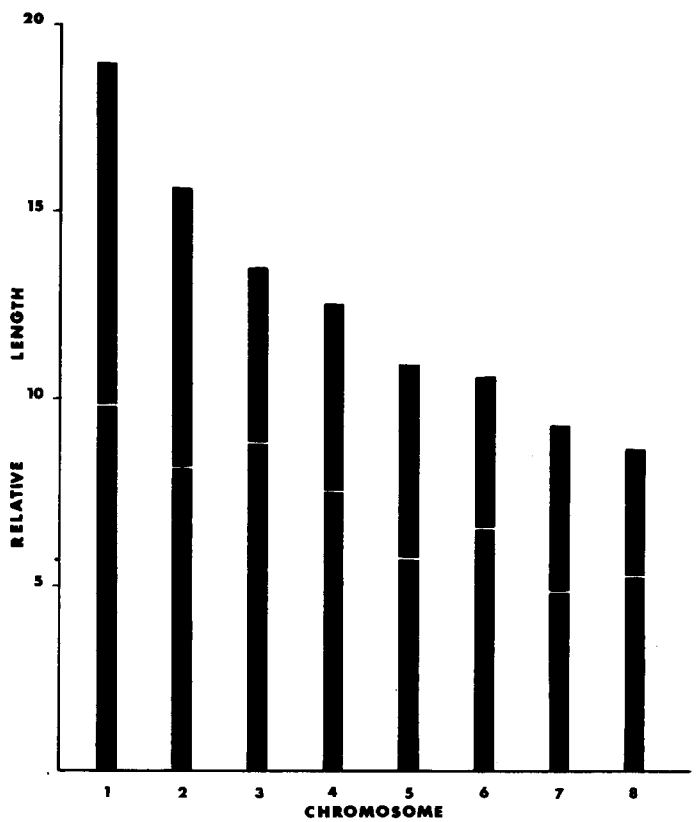

Fig. 3. Idiogram of the karyotype of the Soller population of *Dugesia iberica.*

Table 4. Relative length and centromeric index of the eight chromosomes of the haploid set of *Dugesia iberica* (Rio Júcar population with 3 n = 24). Means and standard deviations from ten mitotic metaphase plates.

N° chromosome	Relative length	Centromeric index
1	17.37 ± 0.73	46.56 ± 0.71
2	15.20 ± 0.88	34.80 ± 2.72
3	13.02 ± 0.35	36.56 ± 2.30
4	12.34 ± 0.69	47.57 ± 1.25
5	11.34 ± 0.43	39.42 ± 1.94
6	10.63 ± 0.28	39.06 ± 2.02
7	10.27 ± 0.40	35.81 ± 1.02
8	9.66 ± 0.35	46.14 ± 0.82

- *Dugesia* aff. *iberica*

In spite of similarities in the copulatory apparatus with the previous species, these populations exhibit different karyotypes. The diploid condition is ascertained by the measurements of the 18 chromosomes. The length of the nine elements decreases gradually from the first to the eight; while the ninth is markedly reduced to a third of the size of the first one. All of them are clearly metacentric (Table 5 and Figure 5).

- *Asexual populations of the* Dugesia gonocephala *group*

As already well established, the haploid chromosome number of the *D. gonocephala* complex is either eight or nine; so the complement of 24, or 27 found in the Spanish strains may be referred to a triploid condition. This triploidy is confirmed by the analysis of the metaphase plates; the chromosomes decrease gradually in size and can be arranged in groups of three metacentric or submetacentric elements.

For instance, a triploid set of 24 elements was found in the populations from the stations n° 1, 2 and 8. Their karyotype is similar to the one de-

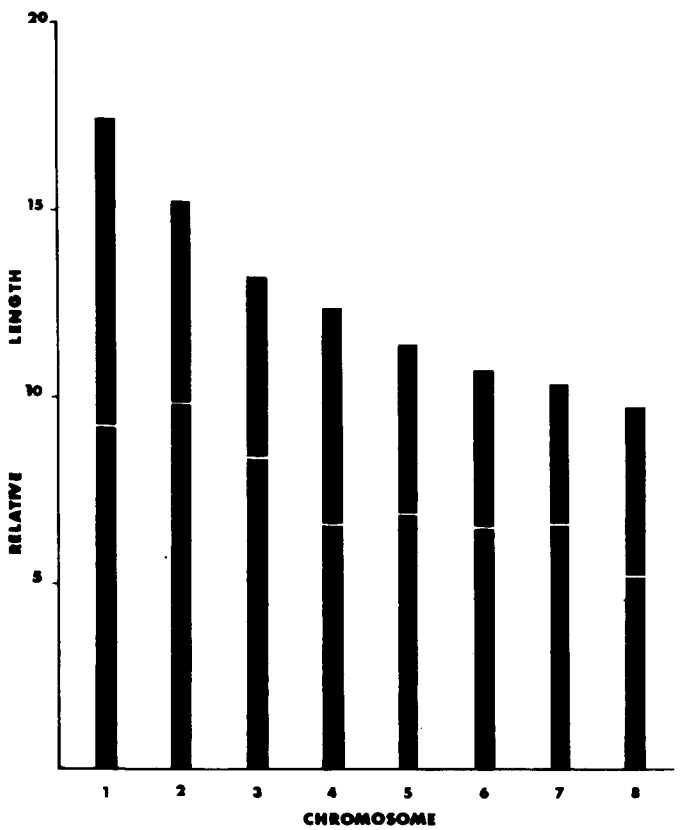

Fig. 4. Idiogram of the karyotype of the Rio Júcar population of *Dugesia iberica.*

Table 5. Relative length and centromeric index of the nine chromosome pairs of *Dugesia* aff. *iberica* (La Calobra population). Means and standard deviations from ten mitotic metaphase plates.

N° chromosome	Relative length	Centromeric index
1	17.18 ± 0.84	48.18 ± 0.87
2	15.01 ± 1.07	46.89 ± 1.08
3	12.95 ± 1.05	41.54 ± 1.04
4	11.63 ± 0.69	48.88 ± 0.84
5	10.26 ± 0.44	46.91 ± 1.18
6	9.57 ± 0.36	47.97 ± 1.01
7	9.14 ± 0.49	42.26 ± 1.36
8	8.50 ± 0.50	43.43 ± 1.63
9	5.92 ± 1.09	48.74 ± 0.52

scribed for *D. iberica* from Rio Júcar, and exhibits three pairs of submetacentric chromosomes.

On the contrary in stations 3, 4, 5, 6, 7 and 9, the haploid number is 9. the measurements of some complements show that all the elements are metacentric and decrease very gradually in size; the smallest being half the size of the first one. Moreover, in most of the metaphases, B-chromosomes are present: one or two are found in a few plates of the *Dugesia* from the Rio Guadalete, and Turon, and in most of the specimens from the Rio Guadalhorce, Salia and Guadalfeo. Two and even three are frequent in the population from the Font de Can Salas. All of them are metacentric.

(All the data are summarised in Table 6).

Discussion

The status of the *Dugesia polychroa - lugubris* complex was recently subjected to intensive researches (summarized in Benazzi & Benazzi Lentati 1976). The most recent study concerns the karyotype of a Greek population of *D. polychroa* from Corfu (Ball 1979). The same species, in central Spain, possesses the diploid complement of biotype A (after nomenclature of Benazzi 1957), which characterizes both the topotype and the italian

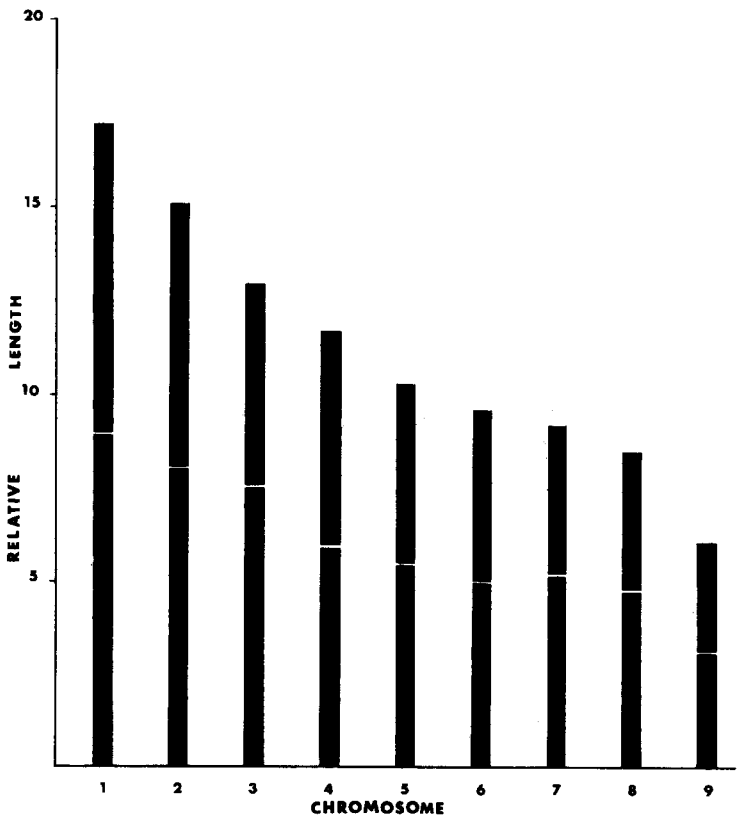

Fig. 5. Idiogram of the karyotype of *Dugesia* aff. *iberica* (La Calobra population).

Table 6. Chromosome numbers in Dugesiidae from Spain.

Species	Localities	Chromosome nb. n	2n	3n
D. polychroa	Rio Henares Chiloeches	4	8	
D. sicula	Ull de la Font Pollença, Mallorca	9	18	
D. iberica	Cova dets Estudiants Soller, Mallorca	8	16	
	Rio Jucar Villelva de la Sierra			24
D. aff. iberica	Brooklet La Calobra, Mallorca	9	18	
D. gonocephala group (asexual population)	(1) Eresma tributary Trescasas			24
	(2) Rio Rambla de Mal Burgo, Fortunate			24
	(3) Rio Guadalete Algodonales			27*
	(4) Rio Turon El Burgo			27*
	(5) Rio Guadalhorce Alora			27*
	(6) Rio Salia la Vinuela			27*
	(7) Rio Guadalfeo Velez de Benaudalla			27*
	(8) Vauclusian spring Deifontes			24
	(9) Font de Can Salas Pollença, Mallorca			27*

* Presence of B-chromosomes.

specimens from Pisa, Elba and Toblino lake. It is the one described as *D. lugubris* by Dutrillaux & Lenicque (1971) from the vicinity of Paris. This same biotype A is also present in specimens from some localities in Corsica and Sardinia where the biotype G of *D. mediterranea* is observed. The haploid number of biotype G is also four but the chromosome morphology differs from that of biotype A. One fissiparous race of *D. mediterranea* is known from Barcelona, and this peculiar distribution was explained by Benazzi *et al.* (1975) in terms of late Oligocene microplate dispersal resulting in isolation of these western Mediterranean islands. Thus it is of interest to note here the presence also of *D. sicula* in Mallorca. This species was first recorded from Sicily and Elba and the present finding is in perfect agreement with the biogeographical explanation of Benazzi *et al.* (1975).

The karyometric data of the chromosomes of *D. iberica* are similar to those of the basic set of the *D. gonocephala* group, in spite of some differences in centromere positions. However centromeric indices may vary as already observed in the allied species of this group (Benazzi & Benazzi Lentati 1976). *D. iberica* possesses at least three pairs of heterobrachial elements, but in the population from La Calobra, in *D. sicula* and in most of the fissiparous Southern strains from continental Spain, all the chromosomes are metacentric. Similarly, all the chromosomes of *D. gonocephala* from Corfu (Ball 1979) are also metacentric. This constant morphology of the elements within the same species can help to clarify the relationships of asexual populations. For instance, it might be possible to assign to *D. iberica* all the triploid strains with 24 meta- and submetacentric chromosomes which occur more commonly in the Northern part of Spain. On the contrary, the triploid fissiparous strains of the southern part, and of Mallorca, which exhibit metacentric chromosomes are not referable to the above species and assignment to another taxon is not possible until some sexual specimens have been found.

The presence of B-chromosomes in these last strains is of interest. This type of tiny supernumerary elements was first noticed by Melander (1950) in *Polycelis tenuis* Ijima, and by Benazzi (1960, 1969) in specimens of the *D. gonocephala* group. Bromley (1974) found one to five of them in the triploid populations of *D. biblica* Benazzi & Banchetti from Israel, and Kawakatsu *et al.* (1976) noticed heterobrachial supernumerary chromosomes in *D. japonica* Ichikawa & Kawakatsu. These chromosomes were studied in detail by Deri (1971, 1975) for some Corsican populations of *D. benazzii*. In a culture of polyploid strains of this same species, spontaneous appearance of B-chromosomes was observed by Benazzi Lentati & Deri (1977). A similar phenomenon was reported by Deri (1980) for a eudiploid population kept for a prolonged period in laboratory culture. This occurred with the transformation of the diploid complement into the hyperdiploid state. Specimens were fissiparous at this time.

The cytological observations of numerous planarian strains allowed Benazzi Lentati & Deri (1980) to conclude that, although, the occurrence of heterogenous chromosome sets often seemed to be

related to fission as seems to be the case in the spanish populations, they were not genetically correlated, but, could exist concomitantly.

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