On the karyology of *Dugesia gonocephala* s.l. (Turbellaria, Tricladida) from Montpellier, France

Elizabeth J. de Vries
Institute of Taxonomic Zoology, University of Amsterdam, Plantage Middenlaan 53, Amsterdam, The Netherlands

Keywords: Turbellaria, *Dugesia gonocephala* s.l., karyology, aneuploidy, B-chromosomes, chromosomal heteromorphism

Abstract

Two species belonging to the *Dugesia gonocephala* group are found in the area of Montpellier, France. The karyology of these two species, *D. gonocephala* s. str. and *S. subtentaculata,* and of fissiparous *Dugesia* races has been studied.

Two populations belonging to *D. gonocephala* s. str. are diploids with a chromosome number of 16, whereas the specimens of a third population are sexual aneuploids; the majority of cells possess 24 chromosomes, but some cells contain 23 or 25. The specimens attributable to *D. subtentaculata* are triploids, the most notable karyological feature being the presence of a single unmatched acrocentric chromosome. The fissiparous *Dugesia* strains are all aneuploids, the most common chromosome number being 27 with up to three small B-chromosomes.

Introduction

Dugesia gonocephala (Dugès, 1830) is the type species of the genus *Dugesia* and hence is the type of the family Dugesiidae Ball, 1974. Thus it is problematic that the taxonomic status of this and of a closely related form, *D. subtentaculata* (Draparnaud, 1801), has never been properly resolved (De Vries & Ball, 1980).

In order to resolve this problem, I have carried out karyological and morphological studies on material from Montpellier, France. This is the area from where the original type material of *D. gonocephala* and *D. subtentaculata* was collected.

This paper focuses on the variety in the karyotypes found within *D. gonocephala* s.l. from Montpellier; the morphological aspects of the populations will be the subject of a separate paper.

Materials and methods

The specimens of *Dugesia gonocephala* studied were collected from the following localities and are now deposited in the Zoological Museum of Amsterdam.

ZMA V.Pl. 615 – River Vidourle between Sommières and Lecques, 15 July 1980.

ZMA V.Pl. 616 – River Hérault, ±4 km south of Ganges, 15 July 1980.

ZMA V.Pl. 617 – Tributary of the river Hérault, ±1 km north of St. Guilhem-le-désert, near the Grottes du Sergent, 16 July 1980.

ZMA V.Pl. 618 – In a fountain in the village of Montblanc, ±9 km south-west of Pézenas, 20 July 1980.

ZMA V.Pl. 619 – Tributary of the river Lerque, in the vicinity of Pégairolles-de-l'Escalette, ±8 km north of Lodève, 20 July 1980.

ZMA V.Pl. 620 – River Orb, near Sérieys, ±16 km north of Bédarieux, 16 July 1980 and 24 July 1981.

Hydrobiologia 132, 251–256 (1986).

ZMA V.Pl. 621 – River Berre, ±10 km west of Sigean, 30 July 1980.

ZMA V.Pl. 622 – River Orb, ±1 km south of site V.Pl. 620, 4 July 1981.

The karyological work was carried out on squashed regenerative blastemas as described by, for example, Ball & Gourbault (1975). The chromosomal nomenclature follows that proposed by Levan et al. (1964). The centromeric index (c.i.) was calculated as length of the short arm×100/total chromosome length. Relative length (r.l.) was calculated as the total chromosome length×100/total length of the haploid genome.

Results

Dugesia gonocephala *s. str.*

Sexually mature specimens and cocoons have been found in three of the sampled localities (V.Pl. 617, 619 & 620). Both the external and internal morphology of these sexual specimens are identical with those of *D. gonocephala* from western Europe as described by De Vries & Ball (1980).

Two of these populations belonging to *D. gonocephala* s. str. (V.Pl. 617 & 620) possessed a chromosome number of 16 (Fig. 1A). On one occasion meiosis was observed, showing eight fully paired bivalents. The chromosomes were all metacentric and they can be arranged according to length into a gradually graded series without clear-cut discontinuities in sequence, the greatest interval being between the first and second chromosome (Table 1). The longest chromosome was almost twice the length of the smallest.

The individuals of population V.Pl. 619 were aneuploids (Table 2A). The majority of cells studied contained 24 chromosomes (Fig. 1B), but some individuals were found to possess cells that com-

Table 1. Means and standard deviations of the relative lengths (r.l.) and centromeric indices (c.i.) of the eight chromosome pairs of *D. gonocephala* s. str., 2n = 16, from Montpellier, France.

Chromosome	A) V.Pl. 617 (10 cells/2 individuals)		B) V.Pl. 620 (23 cells/7 individuals)	
	r.l.	c.i.	r.l.	c.i.
1	17.04 ± 0.61	45.99 ± 2.05	17.31 ± 0.93	45.60 ± 2.33
2	14.67 ± 0.79	47.02 ± 2.18	14.89 ± 0.66	46.67 ± 2.95
3	13.84 ± 0.83	46.12 ± 2.77	13.01 ± 1.57	43.36 ± 4.06
4	12.43 ± 0.64	43.65 ± 1.98	12.60 ± 0.89	44.36 ± 3.97
5	11.68 ± 0.70	40.05 ± 2.61	11.47 ± 0.56	43.27 ± 3.18
6	11.25 ± 0.58	45.96 ± 2.70	10.77 ± 0.44	45.37 ± 3.66
7	10.38 ± 0.74	47.03 ± 1.99	10.38 ± 0.42	42.98 ± 4.53
8	9.91 ± 0.60	42.94 ± 3.67	9.52 ± 0.57	45.56 ± 4.68

Table 2. Means and standard deviations of the relative lengths (r.l.) and centromeric indices (c.i.) of the assumed eight chromosome sets of *D. gonocephala* s. str. and *Dugesia subtentaculata,* from Montpellier, France.

Chromosome	A) *D. gonocephala* s. str. V.Pl. 619 (7 cells/3 individuals)		B) *D. subtentaculata* V.Pl. 620 (5 cells/3 individuals)	
	r.l.	c.i.	r.l.	c.i.
1	16.95 ± 0.55	46.26 ± 2.70	17.37 ± 0.28	45.44 ± 2.99
2	15.21 ± 0.57	44.53 ± 3.77	15.18 ± 0.30	41.90 ± 4.06
3	13.35 ± 0.36	42.79 ± 8.63	13.95 ± 0.38	46.68 ± 2.43
4	12.21 ± 0.30	33.68 ± 7.97	12.42 ± 0.23	36.71 ± 11.27
5	11.52 ± 0.20	43.36 ± 3.38	11.43 ± 0.22	42.30 ± 5.38
6	10.92 ± 0.15	37.44 ± 6.98	10.89 ± 0.18	43.70 ± 3.62
7	10.23 ± 0.17	44.35 ± 7.23	10.17 ± 0.31	43.00 ± 6.99
8	9.69 ± 0.27	38.41 ± 6.79	9.06 ± 0.29	46.48 ± 3.20

prised chromosome numbers of 23 or 25. The accessory chromosomes were of the same length as the other chromosomes of the complement. Moreover, a variable number of five to seven heterobrachial chromosomes was observed in all the cells. Meiotic stages have not been found so the haploid chromosome number could not be established.

Dugesia subtentaculata

Locality V.Pl. 620 contained a mixed population of sexually mature diploids (cf. *D. gonocephala* s. str. above) and fissiparous triploids, whereas V.Pl. 622 contained only fissiparous triploids. The fissiparous specimens of the two populations all became sexually mature under laboratory conditions.

Population V.Pl. 620 was sampled twice in two successive years, and a number of specimens from both samples were cultured in the laboratory for karyological research. In both years, fissioning stopped spontaneously after several months in the laboratory, and large hyperplasic ovaries developed, which could be seen macroscopically as white areas occupying most of the posterior part of the animal. Over a period of about five months, the copulatory apparatus had developed and the animals remained sexually mature for approximately twelve months, during which time a large number of sterile cocoons were deposited. Subsequently, the copulatory apparatus regressed, and several specimens resumed fissioning. The specimens of population V.Pl. 622 showed an essentially similar development of sexual maturity.

The morphology of the copulatory apparatus of the ex-fissiparous specimens differed significantly from that of *D. gonocephala* s. str., so the ex-fissiparous specimens are given a separate status, viz., *D. subtentaculata.* The morphology of the copulatory apparatus will be described elsewhere.

All the investigated specimens of *D. subtentaculata* possessed an identical karyotype with 24 chromosomes (Table 2B). I found no meiotic stages, and so could not ascertain the haploid number. All the chromosomes could be arranged according to length into a gradually graded series. The majority of these chromosomes were metacentric, but some had a centromere that bordered on a submetacentric position. The most notable feature was the presence of a single subtelocentric chromosome (Fig. 1C).

Dugesia gonocephala *s.l.*

The animals from V.Pl. 615, 616, 618 & 621, differed in appearance from those of the other populations in that they were smaller and more ribbon-like, with a conspicuous mottled pigmentation and more pronounced auricles.

The studied animals possessed a chromosome number ranging from 26 to 29 (Table 3). In all but one cell a number of small B-chromosomes was present (Fig. 1D).

The majority of the chromosomes were metacentric with a variable number of submetacentric elements. Because the length of the chromosomes falls into a gradual series and the centromeric index varied considerably, no attempt was made to match the chromosomes into homologous sets.

Discussion

The animals studied can be grouped into the following four categories.

Table 3. Range and variation in numbers and chromosomes and B-chromosomes in four populations (V.Pl. 615, 616, 618 & 621) of *Dugesia gonocephala* s.l. from Montpellier, France (28 cells/10 individuals).

Population	Number of cells containing the following chromosome numbers:						
	26	26+2B	26+3B	27+1B	27+2B	27+3B	29+1B
V.Pl. 615		1			7		
V.Pl. 616			1	1	7		1
V.Pl. 618			1		3		
V.Pl. 621	1	2			2	1	
Total in all populations	1	3	2	1	19	1	1

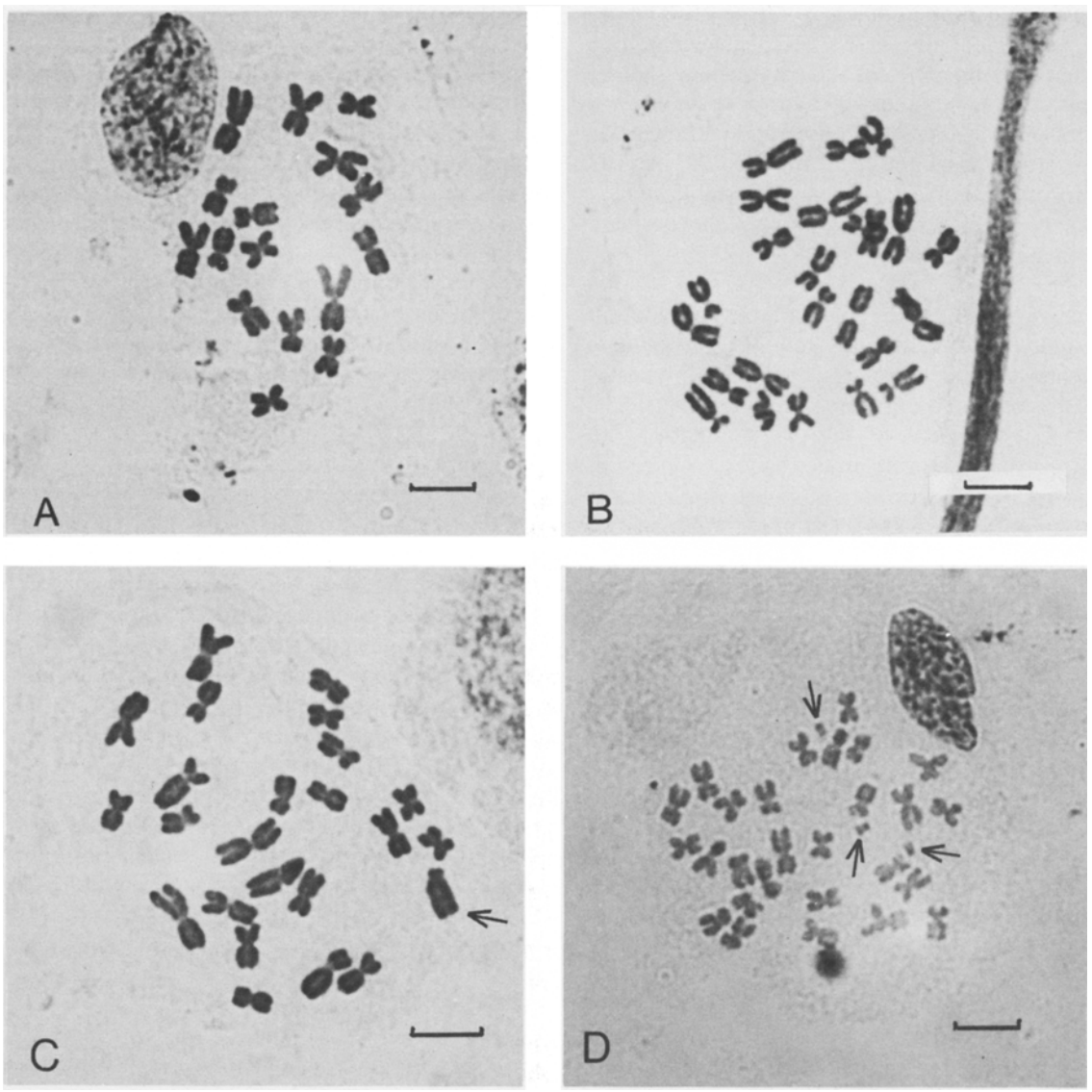

Fig. 1. Mitotic metaphase plates of *D. gonocephala* s.l. from Montpellier; A) V.Pl. 617, 2n=16; B) V.Pl. 619, x=24; C) V.Pl. 620, x=24, arrow indicates aberrant chromosome; D) V.Pl. 621, x=27+3B, arrows indicate B-chromosomes. Scale bars indicate 5 μm.

1) D. gonocephala *s. str., sexual diploids*

The karyological data obtained from the sexual diploid specimens are in accord with other published data on *D. gonocephala* from western Europe (Dahm, 1958, 1963; De Vries & Ball, 1980). The karyotypes represent the basic set of *D. gonocephala* from Europe (Benazzi & Benazzi-Lentati, 1976). Apart from the first and second chromosome, which could be classified on the basis of their different lengths, the lengths of all the chromosomes showed overlap to some extent, so that matching was difficult and in effect unreliable (Table 1).

2) D. gonocephala *s. str., sexual aneuploids*

Interpretation of the karyotype of V.Pl. 619 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 the successive elements and the variable number of submetacentric chromosomes (Table 2A). These results were supported by Sluys & De Jong (1984), who, in working with the same population, proved that the chromosomes could not be matched into significant groups. Their findings are based on a scatter diagram of the complement of one single cell. This method is laborious but renders statistically more sound data because the values are calculated per chromosome and based on a relative length expressed as a percentage of the total complement length without assuming a haploid genome. However, because their data are derived from one single cell, they neglect the variation in chromosome number within this population.

A similar form of aneuploidy is found in *D. etrusca* (Benazzi-Lentati, 1957). During meiosis in the oocytes, eight bivalents and a variable number of univalents appear, whereas during spermatogenesis the accessory chromosomes were eliminated. Therefore, aneuploidy in *D. etrusca* is transmitted by the female line. The variable chromosome number in the somatic cells of *D. etrusca* arises from unequal distribution of the chromosomes over the daughter cells during mitotic division.

Future karyological studies on the meiosis of the aneuploid *D. gonocephala* s. str. should reveal whether the specimens function as sexual diploids with accessory chromosomes (as has been found in *D. etrusca*) or whether they reproduce by pseudogamy, by which the sperm functions only to stimulate cocoon production without fertilizing the oocytes [as has been found in *D. benazzii* (see Benazzi & Benazzi-Lentati, 1976)].

3) D. subtentaculata, *fissiparous triploids*

The high standard deviations of r.l. and c.i. (Table 2B) show that the elements cannot be reliably matched into triplets.

In each cell one single aberrant subtelocentric chromosome was found. In four out of five cells the aberrant chromosome could be grouped with the fourth chromosome on the basis of its relative length, whereas in the fifth cell it matched the seventh chromosome. The value for the centromeric index of the two chromosome triplets is influenced by that of the aberrant, subtelocentric, chromosome. The high values for the standard deviation in both occassions indicate that the chromosome is clearly aberrant and cannot be matched as has been done here.

This specific chromosomal aberration is most likely caused by a pericentric inversion, altering only the position of the centromere and not the relative length. More cytological research, including banding methods, will be necessary to elucidate the exact nature of the aberration. Presumably, this specific karyological aberration can only be maintained within an already asexually reproducing population, since problems arising during meiotic chromosome pairing would select against individuals possessing this mutation.

An identical chromosomal polymorphism was found in a population of *D. japonica japonica* from Taiwan (Oki *et al.*, 1981). According to these authors, the presence of this non-homologous chromosome is caused by the breaking away of one chromosome out of a set of chromosomes.

4) D. gonocephala *s.l., aneuploids with B-chromosomes*

The majority of cells studied possessed 27 chromosomes and 2 small B-chromosomes, although other numbers were also found (Table 3). The variation in chromosome number and B-chromosomes is caused by the unequal division of these elements during mitosis so that the B-chromosomes can therefore be classified as ones that are mitotically unstable (White, 1973).

In these populations the factors controlling fissioning seem to be more dominant than in the triploid populations of *D. subtentaculata* in that sexual maturation never occurred during the time they have been cultured in the laboratory. The influence of the B-chromosomes on this fissioning factor is not known (Bromley, 1977; Benazzi-Lentati & Deri, 1980). A variable number of B-chromosomes is known, furthermore, from *D. biblica* (Bromley, 1974) and *D. benazzii* (Benazzi-Lentati & Deri, 1977). A similar karyotype has also been found by Gourbault (1981) in some populations of *D. gonocephala* s.l. from Spain.

Conclusions

The occurrence of two species of the *gonocephala* group in one locality, as described here for *D. gonocephala* s. str. and *D. subtentaculata* in population V.Pl. 620, is the first record of the coexistence of two such closely related species. The possibility that the two species are in competition remains to be investigated.

Despite the different morphological features of *D. subtentaculata,* the normal members of the chromosome set are very similar to those found in *D. gonocephala* s. str. or other species of the *gonocephala* group (Bromley, 1974; Kawakatsu *et al.,* 1976; Gourbault, 1981). Morphological variation without apparent changes in the chromosomal morphology, and vice versa, seem to be general phenomena within the *gonocephala* group. Consequently, distinguishing asexual races on the basis of karyological data alone should be avoided.

Furthermore, matching of chromosomes into sets, as is usually done within the *gonocephala* group, is unreliable since several of the successive chromosomes show overlap in their relative length and centromeric index. This is demonstrated by the karyometric data presented here in Tables 1 & 2 but also in the karyometric data of, for example, *D. iberica* and *D. sicula* (Gourbault, 1981) and *D. gonocephala* s.l. from Corfu (Ball, 1979). This problem has been pointed out by Sluys & De Jong (1984), but their alternative, to present the data in the form of scatter diagrams, also has its disadvantages. It is obvious that other characters, such as banding patterns, are needed for a more reliable identification of the individual chromosomes.

Acknowledgments

I am grateful to Dr. Ronald A. Gase for his skilfull and enthusiastic help during the fieldwork. Prof. Dr. Ian R. Ball (University of Amsterdam) and Drs. Ronald Sluys (UvA) are thanked for their critical comments. Much practical support was received from L.R.T. It is a pleasure to thank Mr. and Dr. J. De Vries-Anderson (Montreal) for their hospitality during my stay in Canada. Grateful acknowledgement is made of a grant from the Catharine van Tussenbroek Foundation which made my attendance at the congress possible.

References

Ball, I. R., 1979. The karyotypes of two Dugesia species from Corfu, Greece (Platyhelminthes, Turbellaria). Bijdr. Dierk. 8: 187–190.

Ball, I. R. & N. Gourbault, 1975. The morphology, karyology and taxonomy of the genus Phagocata from California (Platyhelminthes: Turbellaria). Life Sci. Contr., R. Ont. Mus. 105: 1–19.

Benazzi, M. & G. Benazzi-Lentati, 1976. Animal Cytogenetics, I. Platyhelminthes. Gebr. Borntraeger, Berlin & Stuttgart: 1–182.

Benazzi-Lentati, G., 1957. Sul determinismo e sulla ereditarieta della aneuploidia in Dugesia etrusca Benazzi planaria a riproduzione anfigonica. Caryologia 10: 352–387.

Benazzi-Lentati, G. & P. Deri, 1977. Insorgenza di B-cromosomi in individui poliploidi di Dugesia benazzii (triclade paludicolo) allevati in laboratorio. Rc. Accad. naz. Lincei, ser. VIII, 62: 847–851.

Benazzi-Lentati, G. & P. Deri, 1980. On the origin of heterogeneous chromosome sets in some fissiparous planarians. Rc. Accad. naz. Lincei, Ser. VIII, 68: 318–326.

Bromley, H., 1974. Morpho-karyological types of Dugesia (Turbellaria, Tricladida) in Israel and their distribution patterns. Zool. Scr. 3: 239–242.

Bromley, H., 1977. Observations on fission, maturation and reproduction in Dugesia biblica (Turbellaria, Tricladida) from Israel. Zool. Scr. 6: 197–201.

Dahm, A., 1958. Taxonomy and ecology of five species groups in the family Planariidae. Nya Litografen, Malmö, 241 pp.

Dahm, A., 1963. The karyotypes of some freshwater triclads from Europe and Japan (Turbellaria, Tricladida, Paludicola). Ark. Zool., Ser. 2, 16: 41–67.

De Vries, E. J. & I. R. Ball, 1980. On Dugesia gonocephala from western Europe. Bijdr. Dierk. 50: 342–350.

Gourbault, N., 1981. The karyotypes of Dugesia species from Spain (Turbellaria, Tricladida). Hydrobiologia 84: 45–52.

Kawakatsu, M., I. Oki, S. Tamura & H. Sugino, 1976. Studies on the morphology, karyology and taxonomy of the Japanese freshwater planarian Dugesia japonica Ichikawa & Kawakatsu, with a description of a new subspecies, Dugesia japonica ryukyensis subspec. nov. Bull. Fuji Wom. Coll. 14(2): 81–126.

Levan, A., K. Fregda & A. A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Oki, I., S. Tamura, T. Yamayoshi & M. Kawakatsu, 1981. Karyological and taxonomic studies of Dugesia japonica Ichikawa et Kawakatsu in the far east. Hydrobiologia 84: 53–68.

Sluys, R. & H. De Jong, 1984. Chromosome morphological studies of Dugesia gonocephala s.l. (Platyhelminthes, Tricladida). Caryologia, in press.

White, M. J. D., 1973. Animal cytology and evolution. 3rd ed. Cambridge University Press, Cambridge, 961 pp.