THE KARYOTYPES OF TWO *DUGESIA* SPECIES FROM CORFU, GREECE (PLATYHELMINTHES, TURBELLARIA)

by

IAN R. BALL

Department of Biology, University of Waterloo, Waterloo, Canada

&

Institute of Taxonomic Zoology, University of Amsterdam, The Netherlands

ABSTRACT

Dugesia gonocephala (Dugès, 1830) and *D. polychroa* (Schmidt, 1861), collected from the Greek island of Corfu, have been investigated karyologically. The former is a sexual diploid ($2n = 16$) in which all the chromosomes are metacentric. The latter is a sexual diploid ($2n = 8$) with one submetacentric and three metacentric chromosome pairs.

Within the *Dugesia lugubris-polychroa* complex seven biotypes are known. The karyotype of *D. polychroa* from Corfu is most similar to biotype G from the western Mediterranean. However, there are subtle karyological and morphological differences between the two forms and thus the Corfu population is not assigned to biotype G but is considered to have been derived independently from the basic biotype A. Thus it represents an eighth biotype within the species group.

INTRODUCTION

During a visit to the Greek island of Corfu to collect marine and freshwater planarians (Tricladida), several specimens of the common European species *Dugesia polychroa* (Schmidt, 1861) were collected. The taxonomic status of this species, and of similar and closely related forms, has been the subject of much recent discussion (Reynoldson & Bellamy, 1970; Benazzi et al., 1970, 1975), and because of this the above specimens were subject to routine karyotyping so as to confirm their identity. To my surprise the karyotype seemed quite unlike that recorded for any populations of this species. Consequently a more careful karyometric analysis was carried out and the results are presented and discussed here.

Analysis of the karyotype of the Corfu specimens proved rather difficult, as will be discussed below, and it was unclear whether this was due to methodological or biological factors. Therefore, a common *Dugesia* species of Corfu, *D. gonocephala* (Dugès, 1830), which is well known karyologically (Benazzi & Benazzi-Lentati, 1976) was also studied so as to form a check for the methods employed.

MATERIALS AND METHODS

The specimens used in this study were obtained as follows:

Dugesia polychroa (Schmidt, 1861). — Ermones River at Ermones Bay, Corfu, Greece. Collected by Ian R. Ball, 11 May 1978. This is a cool torrential stream cascading down the rocks, forming a succession of pools as it enters the sea. A description of the site has been given by Homer (ca. 750 BC: 104). Specimens were obtained from the pools near the sea but they were most numerous in the quieter and warmer reaches of the river above the falls. *Dugesia gonocephala* (Dugès, 1830) and *Phagocata olivacea* (Schmidt, 1861) were also present in small numbers; an as yet unidentified *Dendrocoelum* species was abundant in the cooler reaches.

Dugesia gonocephala (Dugès, 1830). — Messonghi River, west of Messonghi, Corfu, Greece. Collected by Ian R. Ball, 12 May 1978. This is a typical lowland stream, warm, slow-flowing, rich in macro-invertebrates and aquatic vegetation. Mature *D. gonocephala* and cocoons were abundant under stones at the margins of the river. No other triclads were found.

Both species were studied morphologically and karyologically. Karyometric data were derived from enlarged camera lucida drawings of mitotic metaphase plates from regenerative blastemas according to methods described by Benazzi (1974).

RESULTS

Dugesia polychroa (table I, fig. 1)

Treatment of five individuals yielded approxi-

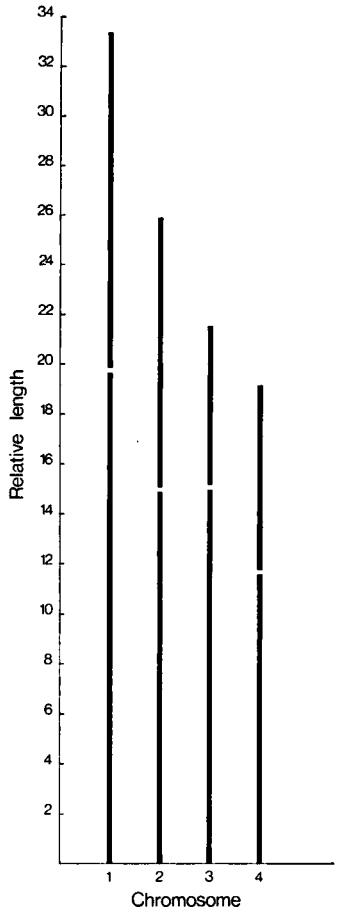

Fig. 1. Idiogram analysis of *Dugesia polychroa* from Corfu, derived from the data in table I.

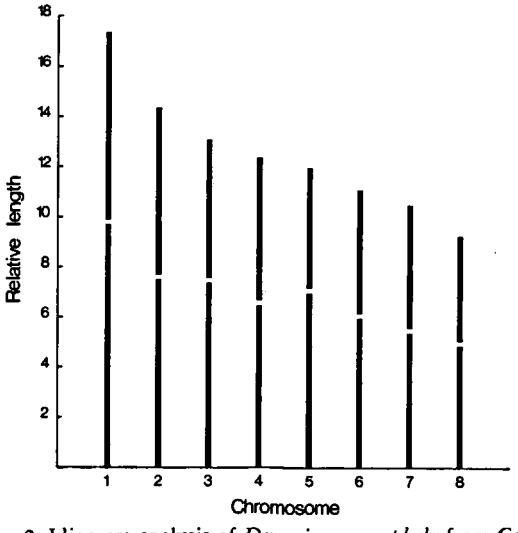

Fig. 2. Idiogram analysis of *Dugesia gonocephala* from Corfu, derived from the data in table II.

mately 15 countable metaphase plates. From these it was clear that the diploid complement consisted of eight chromosomes. Of these the two largest pairs were clearly isobrachial and the remaining pairs were more heterobrachial. Karyometric data (table I) were derived from five especially clear plates and these form the basis of fig. 1. The lengths of the chromosomes, and the positions of the centromeres were variable and thus matching was not always easy. Nevertheless it is clear that the only truly heterobrachial chromosome pair is the third, which may be classified as submetacentric. Acrocentric chromosomes, so typical of the *Dugesia lugubris-polychroa* complex (Benazzi & Benazzi-Lentati, 1976), were not seen.

TABLE I

Relative lengths and centromeric indices of the four chromosome pairs of *Dugesia polychroa* from Corfu. Means and standard deviations from five mitotic metaphase plates.

Chromosome	Relative length	Centromeric Index	Nomenclature
1	33.39±3.04	40.83±3.98	metacentric
2	25.90±2.14	42.11±5.05	metacentric
3	21.52±3.52	29.87±2.73	submetacentric
4	19.15±2.70	38.98±3.82	metacentric

Dugesia gonocephala (table II, fig. 2)

From three individuals 32 countable metaphase plates were obtained. Of these 29 revealed that the diploid complement consisted of 16 metacentric chromosomes. For three plates only 15 chromosomes could be counted. Karyometric data from five especially clear preparations are given in table

TABLE II

Relative lengths and centromeric indices of the eight chromosome pairs of *Dugesia gonocephala* from Corfu. Means and standard deviations from five mitotic metaphase plates.

Chromosome	Relative length	Centromeric Index	Nomenclature
1	17.38±0.97	43.72±3.02	metacentric
2	14.34±0.91	46.97±2.85	metacentric
3	13.03±0.54	42.78±4.06	metacentric
4	12.38±0.54	46.81±3.09	metacentric
5	11.96±0.40	40.92±5.03	metacentric
6	11.07±0.43	45.54±2.68	metacentric
7	10.47±0.49	47.71±2.54	metacentric
8	9.21±1.01	46.28±1.51	metacentric

II and presented as an idiogram in fig. 2. The chromosomes decrease gradually in size and matching by length was both easy and accurate. The position of the centromere was more variable and whereas all the chromosomes are metacentric the high standard deviations of the centromeric indices for the pairs labelled 3 and 5 result from the fact that in some preparations these chromosomes bordered on the submetacentric condition.

DISCUSSION

The relative lengths of the chromosomes of the Corfu populations of *Dugesia gonocephala* are very similar to those of this species group in general. There are differences, however, in centromere position, since most other populations possess one or more pairs of submetacentric elements. Nonetheless there is much variation in centromere position in the European populations of this species (Benazzi & Benazzi-Lentati, 1976), and in related species from the Middle East (Bromley, 1974). In fact when one considers the tendency to submetacentricity in chromosomes 3 and 5 of the present population, and the small size differences between the middle range chromosomes, then its karyotype seems most similar to those of some *Dugesia* species of the *D. gonocephala*-group studied by the latter author.

The constancy of the results with the above species suggest that the variations in length and centromeric index of the chromosomes of the *Dugesia polychroa* from Corfu are real and not artifacts. Nevertheless, despite the difficulties of matching the chromosomes it is clear that the karyotype of this population is an unusual one.

For many years *Dugesia polychroa* and *D. lugubris* (Schmidt, 1861) were considered to be one and the same species. The karyology of this species complex has been the subject of intensive study by Benazzi and his co-workers, and this is summarized by Benazzi & Benazzi-Lentati (1976). Briefly, the *D. lugubris-polychroa* complex was divided into seven biotypes (Benazzi, 1957). Four of these, the biotypes A, E, F, and G, are sexual diploids all with different karyotypes. Biotypes B, C, and D are polyploid versions of A. As a result of this work it became clear that the biotypes E and F corresponded to earlier descriptions of *Dugesia lugubris* s.s. and biotypes A, B, C and D to *D. polychroa* s.s. (Benazzi et al., 1970), a conclusion independently confirmed by Reynoldson & Bellamy (1970). Biotype G, the only one lacking acrocentric chromosomes, showed features of both species (Benazzi et al., 1972) and recently it has been described as a new species, *Dugesia mediterranea*, distributed in Corsica, Sardinia, Sicily, and the vicinity of Barcelona, Spain (Benazzi et al., 1975).

The karyotype of the Corfu specimens is quite unlike that of either *Dugesia lugubris* s.s. or *D. polychroa* s.s. and in fact it is most similar to that of biotype G, *D. mediterranea*, both in chromosome size and number, and in the absence of acrocentric elements. Karyometrically this is to be seen from table III, which compares the present data with those of the well-studied populations of biotype G.

TABLE III

Relative lengths (r.l.) and centromeric indices (c.i.), with standard deviations, of the four chromosome pairs of *Dugesia "lugubris-polychroa"* populations from four localities in southern Europe. The data for the first three localities have been recalculated from Benazzi et al. (1972).

Chromosome	Locality: Barcelona	Sardinia	Corsica	Corfu
1 r.l.	39.65±1.96	39.53±2.45	37.20±1.52	33.39±3.04
c.i.	43.11±1.83	42.13±2.21	42.40±1.96	40.83±3.98
2 r.l.	33.85±2.62	32.54±1.91	32.25±1.40	25.90±2.14
c.i.	48.70±1.14	49.60±0.42	48.81±1.35	42.11±5.05
3 r.l.	17.05±1.44	18.06±2.15	18.97±1.47	21.52±3.52
c.i.	21.59±5.22	26.84±4.44	26.84±3.78	29.87±2.73
4 r.l.	9.45±1.20	9.93±2.14	11.58±1.17	19.15±2.70
c.i.	40.73±3.97	43.06±1.82	40.61±3.15	38.98±3.82

The peculiar distribution of *Dugesia mediterranea* was explained ingeniously by Benazzi et al. (1975) in terms of late Oligocene microplate mobility in the western Mediterranean area. Such an explanation would be rendered suspect by the occurrence of this species on the island of Corfu. However, it is evident from table III that there are greater differences between the karyotypes of the Corfu population and those of Barcelona, Sardinia and Corsica, than there are between the three latter. This is especially the case for relative lengths, in that the elements of the Corfu population decrease far less steeply than the others: thus chromosome 1 is smaller and chromosome 4 larger in the Corfu specimens. The centromeric indices are closer, yet in general the chromosomes of the Corfu specimens are more heterobrachial than in the other populations, with the exception of element 3 which is less so and clearly is submetacentric.

The distinctness of the Corfu population is also indicated from its anatomy and morphology. On external appearance the animals were identified without hesitation as *Dugesia polychroa* and this was confirmed by examination of serial sections. The male copulatory apparatus, especially in the form of the seminal vesicles, the dorsal hump to the penial papilla, and the ventrally positioned ejaculatory duct, is quite typical of this species and unlike that of *Dugesia lugubris* and *D. mediterranea.* None of the distinguishing features of the latter species (Benazzi et al., 1975) were discerned.

There can be little doubt that the Corfu population is of *Dugesia polychroa* s.s. I conclude that its karyotype has been derived from biotype A quite independently of the derivation of biotype G. Consequently, the occurrence of this population poses no threat to the views of Benazzi et al. (1975) as discussed above. That the peculiarities observed may have biological significance is evidenced by the fact that specimens from this population proved very difficult to culture in the laboratory and none of the numerous cocoons collected in the field hatched. Normally *D. polychroa* is one of the easier planarians to culture. Further questions as to the origins of the peculiarities of the Corfu population must be left until such time as additional populations of *Dugesia polychroa* from the Greek and Albanian mainlands have been investigated.

ACKNOWLEDGEMENTS

My fieldwork in Corfu was supported by Operating Grant A0016 of the National Research Council of Canada. The laboratory studies were carried out in Amsterdam with the technical assistance of Greet Vooren and the idiograms were prepared by Tran Thi Vinh-Hao.

REFERENCES

BENAZZI, M., 1957. Cariologia di Dugesia lugubris (O. Schmidt) (Tricladida, Paludicola). Caryologia, **10**: 276-303.

——, 1974. A new karyotype in the American freshwater planarian Dugesia dorotocephala. Syst. Zool., **23**: 490-492.

BENAZZI, M. & G. BENAZZI-LENTATI, 1976. Animal cytogenetics, 1. Platyhelminthes: 1-182 (Gebr. Borntraeger, Berlin & Stuttgart).

BENAZZI, M., I. PUCCINELLI & R. DEL PAPA, 1970. The planarians of the Dugesia lugubris-polychroa group: taxonomic inferences based on cytogenetic and morphologic data. Atti Accad. naz. Lincei, Rendiconti, Cl. Sc. fis. mat. nat., (8) **48** (3): 369-376.

BENAZZI, M., R. BALLESTER, J. BAGUÑÀ & I. PUCCINELLI, 1972. The fissiparous race of the planarian Dugesia lugubris s.l. found in Barcelona (Spain) belong to the biotype G: comparative analysis of the karyotypes. Caryologia, **25**: 59-68.

BENAZZI, M., J. BAGUÑÀ, R. BALLESTER, I. PUCCINELLI & R. DEL PAPA, 1975. Further contributions to the taxonomy of the "Dugesia lugubris-polychroa group" with a description of Dugesia mediterranea n. sp. (Tricladida, Paludicola). Boll. Zool., **42**: 81-89.

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

HOMER, ca. 750 BC. The Odyssey. Translated by E. V. Rieu: 1-365 (publ. in 1946 by Penguin Books, Harmondsworth, Middlesex, U.K.).

REYNOLDSON, T. B. & L. S. BELLAMY, 1970. The status of Dugesia lugubris and D. polychroa (Turbellaria, Tricladida) in Britain. J. Zool., Lond., **162**: 157-177.

Received: 22 August 1978