

Chromosomal polymorphism in planarians (Turbellaria, Tricladida) and the plate tectonics of the western Mediterranean

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Abstract

In two populations of *Dugesia (Schmidtea) mediterranea* the third chromosome pair consists of two heteromorphous elements. This chromosomal polymorphism, which is common in other animal groups, was hitherto unknown in planarians.

The evolutionary and biogeographical significance of this chromosomal polymorphism is discussed, for the geographical distribution of the two aberrant populations may have relevance for the theories of microplate dispersal involving the western Mediterranean.

Introduction

Within the Tricladida a wide range of karyological and reproductive phenomena are known, including polyploidy, aneuploidy, mosaicism, occurrence of supernumerary chromosomes and various forms of pseudogamic reproduction (Benazzi & Benazzi-Lentati, 1976). In *Dugesia (Schmidtea) polychroa*, one of the three species belonging to the subgenus *Schmidtea* Ball 1974, polyploidy and pseudogamy occur (Benazzi, 1963), whereas in *Dugesia (Schmidtea) lugubris* so far only sexual reproducing diploids are known.

Dugesia mediterranea, the third species of the subgenus *Schmidtea*, is a recently described freshwater triclad, occurring near Barcelona on the Iberian peninsula, Corsica, Sardinia and Sicily (Benazzi *et al.*, 1972; 1975), and it is here recorded for the first time from Mallorca (Balearic Islands).

The populations of this species occurring near Barcelona and Mallorca are unusual in that they reproduce asexually by fission and possess a chromosomal heteromorphism. The other populations of *Dugesia mediterranea* from Sardinia, Corsica

and Sicily all have a normal karyotype and reproduce sexually.

In this report we assess the available information on the chromosomal polymorphism and compare these data with the data from the populations of *Dugesia mediterranea* with a normal karyotype. Moreover, the present day distribution and geographical isolation of the two aberrant populations has led us to consider the role of the plate tectonics in the western Mediterranean in the distribution pattern of the two aberrant population.

Material and methods

The specimens used in this study were obtained from the following localities;

Iberian peninsula, in a pond at Montjuic, near Barcelona. Collected by J. Baguña, 1980;

Mallorca, in a small stream crossing the road from Felanitx to Villafranca de Bonany, \pm 4 km south of the latter. Collected by I. R. Ball and Maria Tran, March 1979;

Specimens from Sardinia, provided by Prof. Dr N. G. Lepori, were used for comparison.

The karyological work was carried out on squash-regenerative blastemas as described by for example, Ball and Gourbault (1975). The chromosomal nomenclature conforms to that proposed by Levan *et al.* (1964).

Attempts have been made to study the exact nature of the aberration by banding techniques, but so far such techniques have not been very successful for planarian chromosomes (Sluys & De Jong, in press).

Results

The normal haploid chromosome complement of *Dugesia mediterranea* consists of four chromosomes, *vz.*, two relatively large elements with a median centromere, of which the first is more heterobrachial than the second, a smaller third element with a submedian centromere and a small fourth chromosome in which the centromere is in a median position (Fig. 1C). Such populations, re-

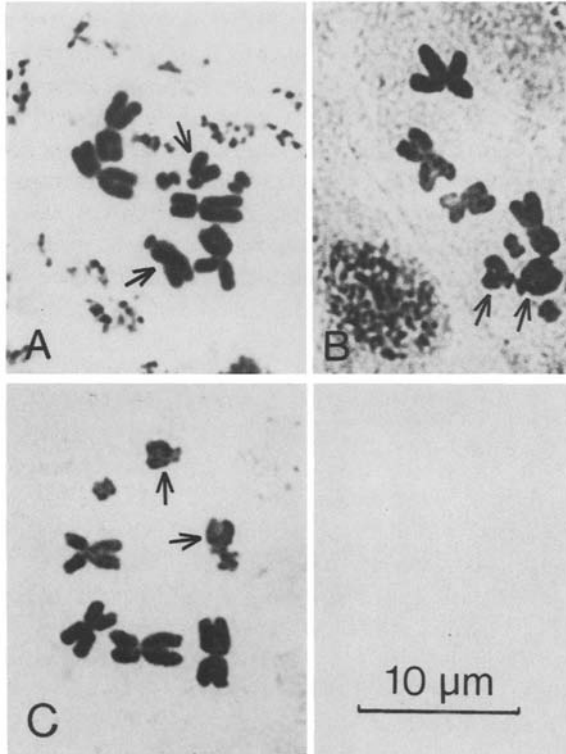


Fig. 1. *Dugesia mediterranea*, metaphase plates, $2n = 8$: (A) Mallorca; (B) Barcelona; (C) Sardinia. Arrows indicate third chromosome pair.

Table 1. Relative lengths (r.l.) and centromeric indices (c.i.) with standard deviations, of four chromosome pairs of *Dugesia mediterranea*.

Chromosome	Locality		
	Barcelona	Mallorca	Sardinia
1 r.l.	37.74 ± 2.25	34.96 ± 3.31	39.53 ± 2.45
c.i.	44.17 ± 2.17	42.54 ± 3.52	42.13 ± 2.21
2 r.l.	31.77 ± 1.87	31.76 ± 3.30	32.54 ± 1.91
c.i.	46.37 ± 1.96	46.75 ± 2.31	49.60 ± 0.42
3 r.l.	22.82 ± 6.94	23.18 ± 5.50	18.06 ± 2.15
c.i.	18.63 ± 4.60	21.96 ± 6.99	26.84 ± 4.44
4 r.l.	7.60 ± 1.16	10.55 ± 1.73	9.93 ± 2.14
c.i.	42.17 ± 3.00	43.22 ± 4.72	43.06 ± 1.82

Number of plates used for the calculations: Barcelona (120 plates/ 12 individuals), Mallorca (40/9) and Sardinia (recalculated from Benazzi *et al.*, 1972).

producing sexually, occur in Sardinia, Corsica and Sicily.

The karyometric data for the populations from Barcelona and Mallorca, and for the Sardinian population, which was used for comparison, are represented in Table 1. In comparing the data for the two largest and the fourth smallest chromosome, it is clear that the three populations belong to the same karyotype. The position of the centromere on the third element of the two aberrant populations, however, is subterminal, whereas in the Sardinian specimens it is submedian. The matching of the two elements of the third chromosome pair of the populations from Mallorca and Barcelona results in a much higher standard deviation than does the matching of the other chromosomes. Separation of this chromosome pair into the larger element 3A and the shorter element 3B results in different values for their relative lengths and centromeric indices and in a lower standard deviation for these parameters (Table 2). In comparing the karyomet-

Table 2. Relative lengths (r.l.) and centromeric indices (c.i.) with standard deviations of the separate members of the third chromosome pair of *Dugesia mediterranea*; for the Sardinian population the data of the two elements are joined together.

Chromosome	Locality		
	Barcelona	Mallorca	Sardinia
3A r.l.	29.50 ± 1.84	28.31 ± 2.13	
c.i.	14.87 ± 2.10	17.28 ± 4.38	
3B r.l.	16.18 ± 1.41	18.08 ± 1.66	18.06 ± 2.15
c.i.	22.38 ± 3.04	26.72 ± 5.93	26.84 ± 4.44

Barcelona (120/12), Mallorca (40/9) and Sardinia (Benazzi *et al.*, 1972).

ric data of the two aberrant populations with those of the population from Sardinia it is clear that the element 3B is similar to the third chromosome of normal *Dugesia mediterranea*, whereas element 3A is about 45% longer (Fig. 1A, B).

Discussion

Karyometric data for *Dugesia mediterranea* from Sardinia, Corsica and Barcelona were given by Benazzi *et al.* (1972) and these authors stated that in the population from Barcelona the third chromosome had a subterminal centromere, whereas in the other populations the centromere of the third chromosome was in the submedian region. Nevertheless, their data did not indicate a polymorphism of the third chromosome as here observed, although recalculation of their data shows that the standard deviation of the centromeric index of the third chromosome was greatest in the Barcelona population. Our finding of such a polymorphism in the asexual populations of Barcelona and Mallorca, and its apparent absence in the Barcelona individuals studied by Benazzi *et al.* (1972), thus at first sight is difficult to understand. Yet, since there is an occasional loss of the extra segment during mitosis, some individuals from the asexual population of Barcelona are mosaics of polymorphic and normal cells (Baguñà, unpublished). Thus it may be that most metaphase plates, studied by Benazzi *et al.* (1972) were of normal cells from mosaic individuals.

The difference in length between the two members of the third chromosome pair is probably a result of the duplication of a relatively large segment of the chromosome arm. An alternative explanation could involve hybridisation between normal *Dugesia mediterranea* with two 3B elements and a population with two 3A elements; hitherto no such population with two longer elements has been found.

The study of chromosome behaviour during meiosis, which could elucidate the nature of the aberration, is precluded by the fact that the individuals in the two aberrant populations reproduce only by fission. Occasionally, however, some specimens have become partly sexual developing a complete or partially complete copulatory apparatus. On these occasions traces of spermatogenesis

have been seen, but meiosis has never been observed and the animals remained infertile and resumed fissioning after a period of time.

The occurrence of the heteromorphic chromosome pair in asexually reproducing populations is noteworthy. Asexual reproduction is a widespread phenomenon in planarians and it is primarily genetically controlled (Benazzi, 1974). In the three species of the subgenus *Schmidtea* however, it is restricted to the two aberrant populations of *Dugesia mediterranea* from Barcelona and Mallorca. Since a strong correlation exists between the chromosomal polymorphism and asexuality, it could be argued that in these populations fission has been induced by gene unbalance due to intra-arm duplication affecting somehow the fission controlling genes.

From morphological and karyological studies it seems that the two unusual populations are almost identical, which is interesting when we consider their geographical isolation. Freshwater triclads are known to be bad dispersers (Ball, 1974), and, with the exception of one geographically restricted North American species (Ball *et al.*, 1981), they do not possess any resting stages which are resistant to extremes of temperature or desiccation. There are no larval stages and the adults are fragile, spending their entire life span in an aquatic environment, their resistance to salinity is negligible making trans-oceanic dispersal unlikely. The three known cases of disjunct, trans-oceanic, distribution of freshwater triclads are undoubtedly a result of recent introduction by man (Gourbault, 1969; Ball, 1969; Reynoldson *et al.*, 1981).

An explanation involving quantum dispersal for the western mediterranean distribution of *Dugesia mediterranea* would require the assumption of five independent successful colonizations. A more unified explanation is possible when the palaeogeography of the area is taken into account. Benazzi *et al.* (1975) have suggested that the distribution pattern of *Dugesia mediterranea* fits well with the theories of microplate tectonics for this area as proposed by Alvarez *et al.* (1974) and Alvarez (1976). These authors have studied the orogeny and geology of the western Mediterranean and they proposed that during the Oligocene the western Mediterranean was closed by a massif consisting of what would later become Corsica, Sardinia, northeast Minorca, Calabria and also possibly the rest of the Balearic

Islands and the two Kabylie plates that are now part of northern Africa (Van den Berg, 1979). This Massif, the Protoligurian Massif, was connected with the coasts of southern France and northeast Spain, forming a continuous land mass. Approximately 25 my BP the plate rotated away from southern France and broke into fragments. The palaeogeography of the Balearic Islands is not fully understood but it seems that they remained connected with the Iberian peninsula until the Messinian, about 10 my BP (Cardona & Contandriopoulos, 1979; Biju-Duval & Dercourt, 1976). The separation of the islands within the Balearic group appears to have occurred even more recently, about 1 my BP (Cordona & Contandriopoulos, 1979).

The distributional data for the different populations of *Dugesia mediterranea* suggest that the species arose on the Protoligurian Massif. When the massif rotated away and fragmented, the population on the Balearic Islands and the Iberian peninsula, which were then still connected, became separated, following which the reproductive and chromosomal aberrations may have developed in isolation. After the separation of the Balearic Islands from the Spanish mainland the populations near Barcelona and Mallorca, the latter being part of the Balearics, became isolated from each other.

If we do exclude the possibility of quantum dispersal, and if we judge evolutionary convergence highly improbable, we feel that the presence of the two aberrant, but identical, populations in Spain and Mallorca support the theory of a relatively late split of the Iberian peninsula and the Balearic Islands. Moreover, because of the relatively early migration of the kabylian microplates we could predict that any populations of *Dugesia mediterranea* found in this part of North Africa would lack the heteromorphosis.

The discovery of *Dugesia mediterranea* in areas other than those involved in the microplate theory, or the finding elsewhere of populations similarly aberrant to those of the Iberian peninsula and the Balearic Islands would, of course, falsify our hypotheses.

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References

- Alvarez, W., 1976. A former continuation of the Alps. *Bull. geol. Soc. Am.* 87: 891-896.
- Alvarez, W., Coccozza, T. & Wezel, F. C., 1974. Fragmentation of the Alpine orogenic belt by microplate dispersal. *Nature* 248: 309-314.
- Ball, I. R., 1969. *Dugesia lugubris* (Tricladida, Paludicola), a European immigrant into North American freshwaters. *J. Fish. Res. Bd Can.* 26(2): 221-228.
- Ball, I. R., 1974. A contribution to the phylogeny and biogeography of the freshwater triclad (Platyhelminthes; Turbellaria). In N. W. Riser and M. P. Morse (eds.), *The biology of the Turbellaria*. McGraw-Hill Book Company, New York. pp. 339-401.
- Ball, I. R. & Goubault, N., 1975. The morphology, karyology and taxonomy of a new freshwater planarian of the genus Phagocata from California (Platyhelminthes; Turbellaria). *Life Sci. Contr. R. Ont. Mus.* 105: 1-19.
- Ball, I. R., Goubault, N. & Kenk, R., 1981. The planarians (Turbellaria) of temporary waters in eastern North America. *Life Sci. Contr. R. Ont. Mus.* 127: 1-27.
- Benazzi, M., 1963. Genetics of reproductive mechanisms and chromosome behavior in some freshwater triclad. In: E. C. Dougherty (ed.), *The lower Metazoa*. Univ. of California Press, Berkeley and Los Angeles. pp. 405-422.
- Benazzi, M., 1974. Fissioning in planarians from a genetic standpoint. In: N. W. Riser and M. P. Morse (eds.), *The biology of the Turbellaria*. McGraw-Hill, New York. pp. 476-492.
- Benazzi, M. & Benazzi-Lentati, G., 1976. *Animal Cytogenetics*, vol. 1, Platyhelminthes. Gebr. Borntraeger, Berlin and Stuttgart. 182 pp.
- Benazzi, M., Ballester, R., Bagaña, J. & Pucinelli, I., 1972. The fissiparous race of the planarian *Dugesia lugubris* s.l. found in Barcelona belongs to the biotype G. *Caryologia* 25: 59-68.
- Benazzi, M., Bagaña, J., Ballester, R., Pucinelli, I. & Del Papa, R., 1975. Further contributions to the taxonomy of the '*Dugesia lugubris*-polychroa group' with description of *Dugesia mediterranea* n. sp. (Tricladida, Paludicola). *Boll. Zool.* 42: 81-89.
- Biju-Duval, B., Dercourt, J. & Le Pichon, X., 1976. In: B. Biju-Duval and L. Montadert (eds.), *Structural history of the Mediterranean basins*, pp. 143-164 (Ed. Technip Paris 1976).
- Cardona, M. A. & Contandriopoulos, J., 1979. In: D. Bramwell (ed.), *Plants and islands*. Academic Press, pp. 133-169.
- Goubault, N., 1969. Expansion de *Dugesia tigrina* (Girard), planaire américaine introduite en Europe. *Annls Limnol.* 5(1): 3-7.
- Levan, A., Fregda, K. & Sandberg, A. A., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

- Reynoldson, T. B., Smith, B. D. & Maitland, P. S., 1981. A species of North American triclad new to Britain found in Loch Ness, Scotland. *J. Zool. (Lond.)* 193: 531-538.
- Sluys, R. & De Jong, H. Chromosome morphological studies of *Dugesia gonocephala* s.l. (Platyhelminthes, Tricladida). *Caryologia* (in press).

Van den Berg, J., 1979. Reconstructions of the western mediterranean area for the mesozoic and tertiary timespan. *Geologie Mijnb.* 58(2): 153-160.

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