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A Robertsonian Translocation in the Fresh-Water Triclad *Dugesia lugubris*: Karyometric Analysis and Evolutionary Inferences

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Abstract. Biotype E of *Dugesia lugubris* has a haploid complement of 4, comprising 3 acrocentrics of different length and a short chromosome; biotype F has a haploid complement of 3, with a long metacentric, an acrocentric and a short chromosome. A karyometrical analysis has shown that the metacentric chromosome of biotype F derived from a centric fusion between the acrocentrics 1 and 3 of biotype E. — The evolutionary meaning of this centric fusion is discussed.

Introduction

Dugesia lugubris s. l. is one of the most common fresh-water planarians in Europe, well known also to students who are not specialized in Triclad taxonomy, being frequently used in experimental research. The specific rank of this planarian has been discussed for many years. In fact, Schmidt (1860) within the *Planaria torva* Müller distinguished two new species: *P. lugubris* and *P. polychroa*, which after the subdivision of the old *Planaria* genus (Kenk, 1930) were attributed to the *Euplanaria* and then to the *Dugesia* genus. The distinction of the two species was accepted by some authors, but denied by others.

This taxonomic question received a new approach with Benazzi's (1957, 1960) karyological research, which revealed within the "*D. lugubris-polychroa* group" a marked karyological differentiation, *i.e.* the existence of 7 karyological biotypes, together, in some cases, with reproductive isolation. The first 4 biotypes (indicated with the first four letters of the alphabet) form a homogeneous series with polyploid evolution starting with the diploid biotype A ($2n = 8$, $n = 4$); these 4 biotypes are interbreding. The remaining 3 biotypes (E, F and G) are, on the contrary, chromosomally differentiated and reproductively isolated. All are diploid: biotype E with $2n = 8$, $n = 4$; biotype F with $2n = 6$, $n = 3$; biotype G with $2n = 8$, $n = 4$. Both chromosome and bivalent morphology differ among these 3 biotypes and from biotype A. The comparative analysis of the respective karyotypes was accomplished by Benazzi and Puccinelli (1961). Such an analysis also suggested the likely origin of biotype F from biotype E through a Robertsonian translocation, *i.e.* a centric fusion between two acrocentric chromosomes. In fact, the haploid set of biotype E is made up of three acrocentric chromosomes of

different lengths and of a very small one. Biotype F possesses a large metacentric, a medium length acrocentric and a very small chromosome.

Benazzi (1963) on the basis of these karyological results, admitted the existence of four sibling species corresponding, respectively, to biotypes A–D, E, F and G. However, the karyological similarity between biotypes E and F and the probable origin of the latter from the former by means of a centric fusion suggest a close genetic relationship and the possibility of considering them as a single species. In a further paper Benazzi *et al.* (1970) taking into consideration both karyological data and morphology of the copulatory system, concluded that the biotypes of the A–D series correspond to *D. polychroa* and biotypes E and F to *D. lugubris*[1]. Biotype G represents another closely related species not yet named. In the authors' view, all these strictly allied species form a superspecies, for which they proposed the name *lugubris*, as the one most commonly used to indicate these planarians.

The references summarized above were necessary to outline the general aspect of the problem. In this paper we wish to analyse with the aid of a karyometric study the relationships between biotypes E and F; our aim is to confirm the advisability of the centric fusion hypothesis and to prospect some microevolutionary inferences.

Material and Methods

D. lugubris s. l. is widespread in Europe, although there are differences in the distribution of the various biotypes. Biotype E has been found in Italy, France and Germany (Benazzi, 1957, 1965), Great Britain (Reynoldson and Bellamy, 1970), Sweden (Melander, 1963). Biotype F has been found in Italy (Benazzi, 1957), Austria (Benazzi, 1963), Sweden (Melander, 1963). It is to be noted that these two biotypes may coexist in the same locality, *e.g.* Conselice (Prov. of Ravenna), from which the specimens used in the present research come. Even in Swedish lakes Melander found both biotypes.

The chromosome sets have been studied on mitoses of neoblasts. The planarian was cut into pieces, which 3–4 days later, at the beginning of the formation of the regenerative blastema, were put in a 0.3% colchicine (Merck) solution for a time varying from 3 to 4 hours; then the pieces were transferred for 5 minutes to 2% acetic acid and stained for 30 minutes in lacto-orcein; finally they were squashed between slide and coverglass.

The relative length (r.l.) and centromeric index (c.i.) were obtained by chromosome measurements of 10 metaphases for each biotype.

We wish to thank Mr. S. Morelli for his valuable technical assistance.

Results

The origin of the haploid set of 3 (biotype F) from haploid set of 4 (biotype E) by means of a centric fusion was suggested by the similarities of both mitotic chromosomes (Fig. 1) and meiotic bivalents of the two

1 In a parallel paper, Reynoldson and Bellamy (1970) on the basis of external characteristics, cannibalistic behaviour and features of the penial papilla, were able to distinguish biotype B specimens from biotype E ones; they identified the former as *D. polychroa* and the latter as *D. lugubris*, in agreement with our conclusions.

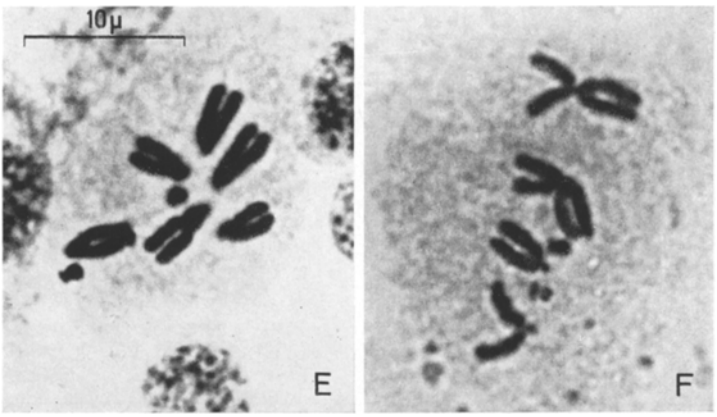

Fig. 1. Mitotic metaphases of biotypes *E* and *F*

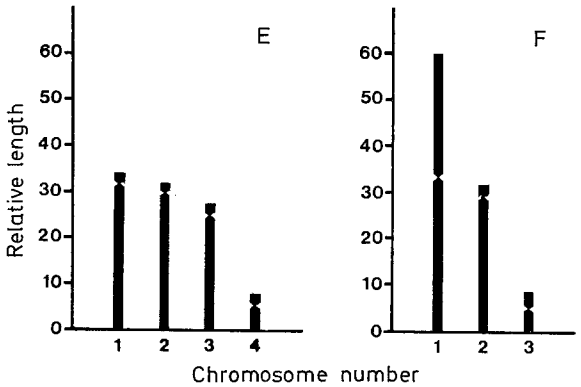

Fig. 2. Idiograms of biotypes *E* and *F* based on data presented in Tables 1 and 2

biotypes. The karyometrical analysis now accomplished (Fig. 2, Tables 1 and 2) confirms such an assumption and also indicates the two acrocentric chromosomes of biotype E from which, with all probability, the large metacentric of biotype F derived: these two acrocentrics are nos. 1 and 3[2].

The above conclusions are supported by the following data:

a) The relative mean lengths of the three acrocentrics of biotype E are in decreasing range: 33.70, 31.45, 27.08. The relative mean lengths of the two arms of the large metacentric of biotype F are 33.35 and 26.73 respectively (c.i. = 44.48). Therefore, the two arms of the metacentric

2 Although centric fusion is considered to be one of the most frequent mechanisms in chromosome evolution, the inverse mechanism, *i.e.* fission, has also been admitted. In our case, however, we think fission improbable because the basic chromosome number of the superspecies *D. lugubris* is certainly 4.

Table 1. Relative lengths of the chromosomes in 10 cells of biotype E

Cells No.	Chromosome No.			
	1	2	3	4
1	33.04	32.20	27.11	7.63
2	32.94	31.37	27.45	8.23
3	33.19	31.54	27.01	8.24
4	34.23	30.86	26.80	8.10
5	34.46	31.08	27.20	7.25
6	34.63	31.39	26.23	7.74
7	35.32	31.27	25.78	7.63
8	32.65	30.99	28.93	7.44
9	32.79	30.93	27.90	8.37
10	33.76	32.85	26.42	6.97
Means$\pm$s.e.	33.70$\pm$0.29	31.45$\pm$0.30	27.08$\pm$0.28	7.76$\pm$0.14

Relative length: length of chromosome $\times 100$/total length of haploid genome.

Table 2. Relative length of the chromosomes in 10 cells of biotype F

Cells No.	Chromosome No.			
	1		2	3
	long arm	short arm		
1	33.41	25.31	32.66	8.60
2	34.95	27.95	30.29	6.81
3	32.61	26.63	31.52	9.23
4	34.91	26.18	31.88	7.03
5	31.41	29.63	31.41	7.55
6	33.72	26.74	31.02	8.53
7	32.47	26.80	31.96	8.76
8	34.02	27.05	31.56	7.38
9	33.24	26.76	31.17	8.83
10	32.77	24.29	33.33	9.60
Means$\pm$s.e.	33.35$\pm$0.35	26.73$\pm$0.45	31.68$\pm$0.27	8.23$\pm$0.30

Relative length: length of chromosome $\times 100$/total length of haploid genome.

correspond exactly to the first and third acrocentric chromosomes of biotype E[3].

3 We have accomplished a statistical check of these data, based on the standard error (S.E.) of the difference (Δ) between the means of the r.l. of chromosome 1 and long arm, and between chromosome 3 and short arm.

The formula adopted is

$$\text{S.E.} = \sqrt{(\text{s.e.}_1)^2 + (\text{s.e.}_2)^2}$$

b) The relative mean length of the acrocentric of biotype F is 31.68, very near to that of the second acrocentric of biotype E that is 31.45.

c) In both biotypes the relative mean length of the small chromosome is very similar, even if the c.i. is different: 30.77 in biotype E and 42.21 in biotype F.

Discussion

Here, we wish to take into consideration another aspect of the problem, *i.e.* the evolutionary significance which may be attributed, in our case, to centric fusion.

The examination of the large amount of literature regarding this question, which has been discussed above all in mammals, shows that it is impossible to formulate a general rule: in some cases the centric fusion appears linked with speciation, while in other cases it seems devoid of evident phenotypic effects. The Robertsonian mechanism (both in the way considered the most frequent, *i.e.* fusion, and in the opposite direction, *i.e.* fission) may occur at very different taxonomic ranks and may have very different meanings: from a simple polymorphism within a population, to a mechanism of chromosome evolution within families or higher taxonomic categories.

Examining the data regarding our case, we have already mentioned that biotypes E and F are reproductively isolated: attempts of cross mating have been carried out for many years but have given negative results[4]. Moreover, in some cases, cannibalism occurred on the part of the partner E and it is to be mentioned that the cannibalistic behaviour of biotype E has been regarded as a taxonomic character by Reynoldson and Bellamy (1970).

Therefore, on the basis of the biological concept of the species, one may consider the two biotypes as distinct species, in agreement with the first opinion expressed by Benazzi (1963) and in contrast to the more recent conclusion reached by Benazzi *et al.* (1970). However, this taxonomic problem still appears complex, since Reynoldson and Bellamy (1970) observed in squashes of living material of biotype E a peculiar characteristic of the penial papilla, *i.e.* a permanent nipple on which the male duct opens. We can confirm the presence in biotype E of this

where $s.e._1$ and $s.e._2$ are the standard errors of the means. In both cases the difference between the means is lower than the S. E., therefore not significant.

Chromosome 1	33.70	Chromosome 3	27.08
Long arm	33.35	Short arm	26.73
	$\Delta = 0.35 \pm 0.454$		$\Delta = 0.35 \pm 0.530$

4 A single case seemed to contradict these results, as referred to in the paper by Benazzi *et al.* (1970); in fact, a cross believed to be E×F produced offspring which were all of biotype F even though they derived from both partners. We are not able to explain this fact, the more so since the death of the partner believed to be of biotype E did not permit us a more accurate check.

structure, which is lacking in biotype F as well as in all the other biotypes. Therefore, even if the copulatory system of the biotypes E and F clearly shows the same structural pattern, which supports their attribution to the same species, the lack of a permanent nipple in biotype F shows that between the two biotypes a morphological differentiation has occurred We have no data to state that this fact is the cause of the reproductive isolation and neither can we establish the relationship between the centric fusion and this morphological variation. It appears, however, that the centric fusion which has led to the actual karyotypic condition in biotype F is correlated with an effective barrier, providing the reproductive isolation of this form and securing its coexistence with biotype E.

We have already mentioned that another small karyotypic difference exists between the two biotypes as regards the short chromosome, which is submetacentric in biotype E and metacentric in biotype F. This chromosomal variation may be attributed to a pericentric inversion; it is, however, difficult to apprise its meaning in the evolutionary history of the species.

References

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

Benazzi, M.: Evoluzione cromosomica e differenziamento razziale e specifico nei Tricladi. Acc. Naz. Lincei, Quaderno N. **47**, 273–297 (1960).

Benazzi, M.: Genetics of reproductive mechanisms and chromosome behaviour in some fresh-water triclads. In: The lower metazoa, p. 405–422. Berkeley-Los Angeles: University of California Press 1963.

Benazzi, M.: Su una popolazione della planaria Dugesia lugubris (biotipo E). Rend. Acc. Naz. Lincei, ser. 8, **38**, 787–790 (1965).

Benazzi, M., Puccinelli, I.: Analisi comparativa del cariogramma dei biotipi di Dugesia lugubris (Tricladida Paludicola). Atti Ass. Genet. Ital. (Pavia) **6**, 419–426 (1961).

Benazzi, M., Puccinelli, I., Del Papa, R.: The planarians of the Dugesia lugubris-polychroa group: taxonomic inferences based on cytogenetic and morphologic data. Rend. Acc. Naz. Lincei **48**, 369–376 (1970).

Melander, Y.: Cytogenetic aspects of embryogenesis in Paludicola Tricladida. Hereditas (Lund) **49**, 119–166 (1963).

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

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