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A COMPARATIVE STUDY OF CHROMOSOMES IN FOUR SPECIES OF *THEODOXUS* (GASTROPODA: NERITIDAE)

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ABSTRACT

Karyological analysis was performed on *Theodoxus baeticus* (Lamarck, 1822), *T. valentina* (Graells, 1846), *T. velascoi* (Graells, 1846) and *T. fluviatilis* (Linne, 1758), collected from freshwater bodies of eastern Spain. Cells possessing diploid chromosome numbers of 2n = 25 were most common in the tissues of males, whilst 2n = 26 was most prevalent in females. The sixth pair of chromosomes had only one homologue in mitotic cells of males, indicating the XO/XX sex-determining mechanism. The absolute length of chromosomes ranged from 1.65 to 6.33 μm. The relative sizes of chromosomes varied from 3.92 to 13.33% of the total haploid set length. In all species chromosome pairs no. 1, 4, 7–13 were composed of metacentric, pairs no. 5 and 6—of submetacentric chromosomes. Pairs no. 2 and 3 were submetacentric, subtelocentric or submeta- subtelocentric according to the species studied. There were significant differences ($P < 0.05$) among the relative length, as well as centromeric indices of different chromosome pairs across the species. Karyological differences were greatest between *T. fluviatilis* and three other *Theodoxus* species.

INTRODUCTION

Chromosome number in gastropod molluscs has been actively studied in recent years. In the Archaeogastropoda, karyological data are given on 76 species from 9 families (Nakamura, 1986). The haploid number of chromosomes ranges from 9 to 14. Representatives of the family Neritidae, to which the brackish water genus *Theodoxus* belongs, possess diploid chromosome numbers of 2n = 20, 22 and 24 in females and 2n = 18, 19, 21, 23 and 27 in males (Nakamura, 1986). Nine bivalents were observed in males of *Theodoxus fluviatilis* from the Montpellier area in France (Tuzet, 1930), while 10 bivalents were described in females of the same species from Ukraine (Alexenko, 1928). The chromosomes in *T. fluviatilis* were counted in histological sections but the results were doubted (Nishikawa, 1962). Therefore, re-examination of *T. fluviatilis* karyotypes using modern cytological techniques was necessary.

This study reports the chromosome numbers and morphology of four species of *Theodoxus*: *T. baeticus, T. valentina, T. fluviatilis* and *T. velascoi*.

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MATERIAL AND METHODS

Material was collected in eastern Spain. In June 1994, 40 *T. fluviatilis* were collected from San Miguel spring (Castillon province). In September 1996, 27 *T. valentina* and 28 *T. velascoi* were collected from channels in orange-tree fields (Parque Natural de la Albufera de Valencia, Valencia province). In October 1996 49 *T. baeticus* were collected from the Fuente del Baños thermal spring (Castillon province). All snails were collected from an area around 2–5 m² in each site. Blocking of cell divisions at metaphase was achieved by placing snails directly in a 0.01–0.02% solution of colchicine, 4–6 h before they were dissected. The different stages of sample fixation, chromosome preparation and identification were the same as those previously described by Baršienė (1978) and Baršienė, Tapia & Baršytė (1996). Chromosomes of mitotic metaphase and meiotic nuclei were examined and photographed with a Jena Med cytology microscope.

The absolute length, relative length (100 × chromosome length/total haploid chromosome set length) and centromeric index (100 × length of the short chromosome arm/total chromosome length) were determined in 10 karyotypes from each species. The absolute length of chromosomes was measured from photomicrographs. The chromosomes were classified by the system of Levan, Fredga & Sandberg (1964). Where the standard deviation of the centromeric index was at the borderline between two types of chromosomes, the nomenclature for both chromosome types is given.

Statistical significance of interspecific differences in karyotypes was determined by using one-way ANOVA with the Bonferroni's post-hoc test.

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Statistical significance of interspecific differences in karyotypes was determined by using one-way ANOVA with the Bonferroni's post-hoc test.

Figure 2. Mitotic metaphase and karyotypes of *Theodoxus*: above – *T. baeticus* female, 2n = 26, below – *T. fluviatilis* female, 2n = 26. Scale is 10 μm.

The lowest number reported, 2n = 18 was observed in *Theodoxus fluviatilis* from Montpellier in France (Tuzet, 1930). Ten bivalents were described in meiosis of this species from Ukraine; mitotic oogonial cells possessed 20 chromosomes, whilst in spermatogonial cells, 19 chromosomes were detected (Alexenko, 1928). However, these earlier studies were based on a tissue sectioning method, which may provide inaccurate data.

The present study showed that diploid sets of *T. fluviatilis* from Spain contain 26 chromo-

Table 1. Measurements of absolute chromosome length in ten mitotic metaphases of females (Mean in μm; SD)

Chromosome pair No	*T. baeticus*		*T. valentina*		*T. velascoi*		*T. fluviatilis*	
1	5.97	1.33	6.33	1.78	4.47	0.52	5.93	0.38
2	4.02	0.29	5.50	1.73	3.76	0.48	5.33	0.41
3	3.72	0.37	5.30	1.83	3.57	0.39	4.68	0.23
4	3.47	0.28	4.43	1.33	3.36	0.34	4.35	0.37
5	3.33	0.36	4.20	1.32	3.16	0.31	4.18	0.29
6	3.05	0.32	4.0	1.24	3.07	0.27	3.88	0.32
7	2.80	0.26	3.63	1.13	2.79	0.13	3.08	0.33
8	2.58	0.32	3.50	0.98	2.66	0.15	2.83	0.30
9	2.38	0.21	3.13	0.95	2.54	0.24	2.40	0.45
10	2.20	0.20	2.93	0.75	2.37	0.18	2.23	0.37
11	2.00	0.15	2.70	0.60	2.24	0.21	2.08	0.37
12	1.75	0.27	2.33	0.60	1.99	0.21	1.88	0.30
13	1.65	0.22	2.30	0.55	1.83	0.14	1.75	0.25

Table 2. Measurements of relative chromosome length in ten mitotic metaphases of females (Mean,%; SD)

Chromosome pair No	*T. baeticus*		*T. valentina*		*T. velascoi*		*T. fluviatilis*	
1	12.0	0.38	12.57	0.11	11.83	1.06	13.33	0.56
2	10.77	0.23	10.90	0.25	9.90	0.35	11.95	0.44
3	9.92	0.20	10.40	0.10	9.41	0.41	10.50	0.59
4	9.27	0.23	8.80	0.10	8.89	0.46	9.78	0.33
5	8.88	0.37	8.33	0.30	8.37	0.50	9.38	0.38
6	8.12	0.24	7.93	0.15	8.13	0.38	8.72	0.73
7	7.46	0.25	7.20	0.17	7.39	0.35	6.88	0.26
8	6.87	0.32	6.97	0.11	7.04	0.28	6.38	0.25
9	6.37	0.33	6.23	0.30	6.71	0.45	5.35	0.24
10	5.90	0.33	5.90	0.36	6.27	0.30	5.0	0.32
11	5.37	0.35	5.43	0.40	5.91	0.45	4.63	0.40
12	4.67	0.10	4.70	0.43	5.26	0.22	4.18	0.35
13	4.40	0.20	4.63	0.51	4.86	0.43	3.92	0.35

Table 3. Centromeric indices and classification of chromosomes in ten metaphases of females (Mean; SD)

Chromosome pair No	*T. baeticus*			*T. valentina*			*T. velascoi*			*T. fluviatilis*		
1	48.68	0.62	m	49.60	0.40	m	49.03	1.18	m	43.43	1.95	m
2	24.98	3.55	sm-st	21.70	2.52	st	26.24	1.54	sm	21.20	1.75	st
3	29.87	1.43	sm	23.10	1.45	st	25.89	1.67	sm-st	23.65	1.99	st
4	49.47	0.65	m	49.57	0.37	m	49.04	1.28	m	49.48	0.25	m
5	33.33	1.69	sm	27.07	2.40	sm	30.49	1.02	sm	29.20	1.95	sm
6	34.73	2.41	sm	27.87	2.20	sm	35.23	2.70	sm	27.15	2.25	sm
7	49.63	0.56	m	49.37	0.60	m	49.19	0.40	m	48.08	0.60	m
8	48.90	0.97	m	48.87	1.40	m	49.47	0.51	m	49.38	0.85	m
9	48.55	0.47	m	43.83	1.87	m	49.29	0.51	m	49.65	0.41	m
10	49.35	0.72	m	49,73	0.46	m	49.47	0.66	m	48.90	1.10	m
11	49.25	0.82	m	49.03	0.90	m	49.63	0.64	m	48.53	0.95	m
12	46.73	3.12	m	45.07	0.76	m	48.56	1.44	m	47.75	1.45	m
13	45.33	1.43	m	41.00	1.45	m	48.61	1.54	m	44.03	1.23	m

m—metacentric, sm—submetacentric, st—subtelocentric chromosomes.

somes in females and 25 chromosomes in males. In meiotic spermatocytes of males, 12 bivalents and one univalent was found. The other investigated species, *T. baeticus, T. velascoi* and *T. valentina* possessed the same chromosome numbers. However, significant interspecific differences ($P<0.0001$) in the structure of karyotypes were observed as determined by comparing the relative lengths and centromeric indices of chromosome pairs. The greatest karyological difference was observed between *T. fluviatilis* and the other three species.

It should be stressed that in these freshwater *Theodoxus* species the same XX/XO sex-determining mechanism occurs as earlier described in marine Neritidae species. Two submetacentric elements comprise chromosome pair no. 6 in females and only one homologue is present in the cells of males. Submetacentric X-chromosomes have also been described in *Nerita violacea, N. pulligera, N. japonica, N. ocellata, N. striata, Clithon retropictus* and *C. oualamiensis* (Komatsu & Inaba, 1982; Komatsu, 1985). Sex-determining chromosomes, have different positions, according to their length, in the sets of the family Neritidae, but more often they are pairs no.1–4 and 8 (Nakamura, 1986).

Robertsonian translocations as a main mechanism might be suggested for the evolution of chromosome sets within Neritidae resulting in the reduction of chromosome numbers from n = 14 to n = 11. On the other hand, it is clear that karyotype conservatism is very common in this family (Nakamura, 1986), most species having 23 chromosomes in males and 24 in

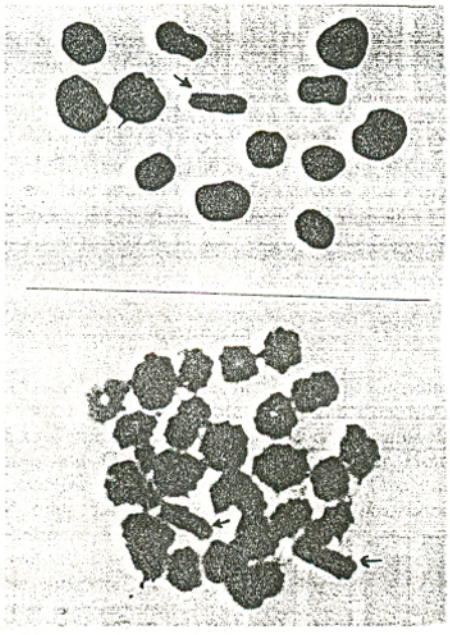

Figure 3. Meiosis in the cells of *T. baeticus* males: above–diakinesis, n = 12 bivalents and one univalent (arrow); below–diakinesis in tetraploid spermatocyte, 24 bivalents and two univalents (arrows).

Table 4. The level of statistical significance (ANOVA) in the relative length and centromeric indices of *Theodoxus baeticus, T. valentina, T. velascoi* and *T. fluviatilis*

Chromosome pair No	Relative length	Centromeric index
1	<0.0001	<0.0001
2	<0.0001	<0.0001
3	<0.0001	<0.0001
4	<0.0001	n.s.
5	<0.0001	<0.0001
6	0.0017	<0.0001
7	<0.0001	<0.0001
8	<0.0001	n.s.
9	<0.0001	<0.0001
10	<0.0001	n.s.
11	<0.0001	n.s.
12	<0.0001	n.s.
13	<0.0001	<0.0001

n.s.—not significant

Table 5. Significant differences in chromosome relative length and centromeric indices (according to Bonferroni's post-hoc test)

Species	*T. valentina*/ chromosomes pairs	*T. velascoi*/ chromosome pairs	*T. fluviatilis*/ chromosome pairs
T. baeticus	3,4,5; ***2,3,5,6,9,13***	2,3,5,11,12; ***3,5,13***	1,2,3,4,5,6,7,8,9, 10,11,12; ***1,2,3,5,6,7***
T. valentina		2,3,9,12; ***2,3,5,6,9,12,13***	2,4,5,6,8,9,10,11,12; ***1,7,9,12,13***
T. velascoi			1,2,3,4,5,6,7,8,9, 10,11,12,13; ***1,2,3,6,7,11,13***

Italic and bold—significant differences in centromeric indices

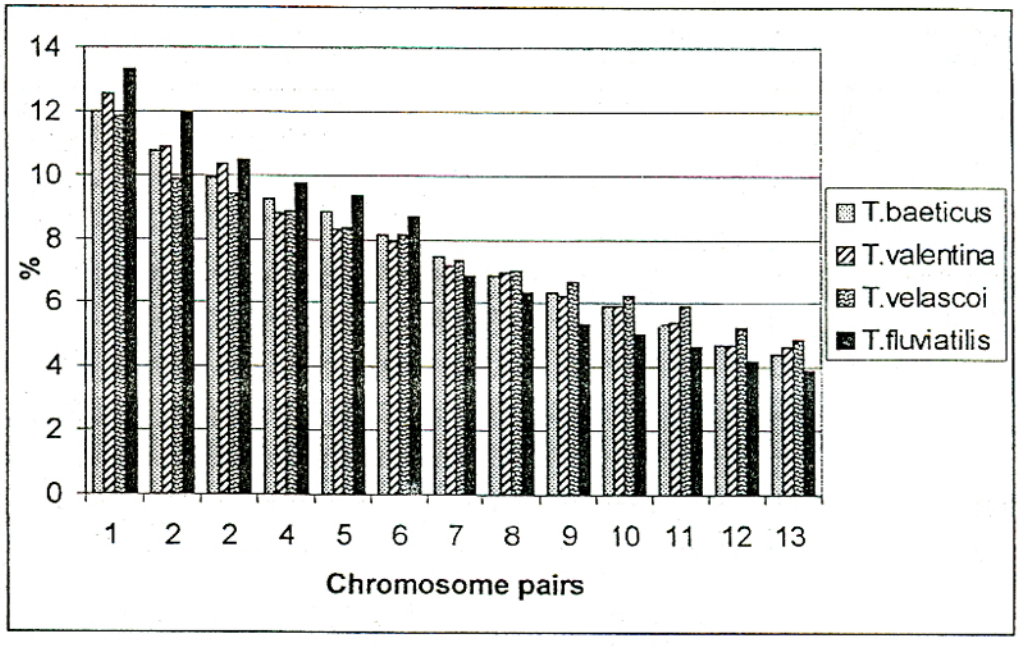

Figure 4. Ideograms of the relative length of chromosomes.

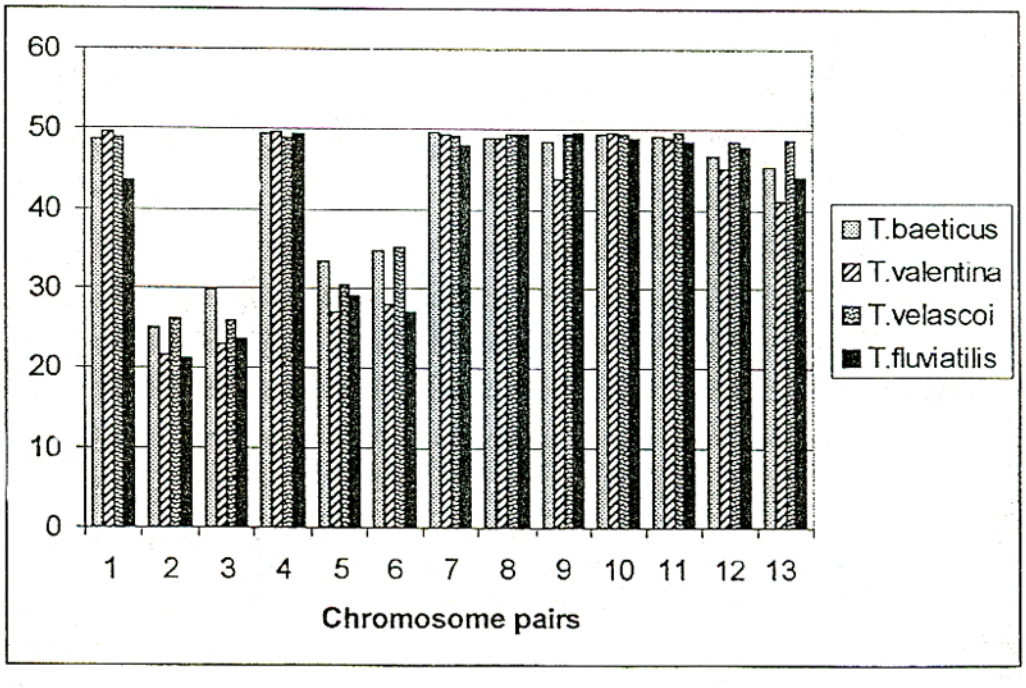

Figure 5. Ideograms of the centromeric indices of chromosomes.

females. This study has showed that four species of *Theodoxus* have the same chromosome number, 2n = 25 in males and 2n = 26 in females. Interspecific differences mainly occur as a result of structural rearrangements in chromosome complements. As a consequence, there are changes in chromosome morphology without changes in chromosome number. Future karyological information for the Neritidae and particularly for geographically isolated populations of *Theodoxus* would be useful for elucidation of problems of their speciation and intraspecific conservatism of karyotypes.

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