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Karyotypes of Three Ascidians (Chordata; Ascidiacea) from Korea

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Cytogenetic features of three ascidian species were studied with specimens obtained from Korean coastal areas. The gonadal tissue was processed in order to obtain mitotic and meiotic chromosomes. The chromosome numbers of *Amaroucium pliciferum* turned out to be 2n=26 and n=13 with 2sm, 3st, 8t and *Halocynthia roretzi* to be 2n=16 and n=8 with 6m, 2t. Diploid number of chromosomes in *Dendrodoa aggregata* was 64 with 32t. The karyotypes of these Korean ascidians were reported for the first time.

Ascidian species are very common along the east coast of Korea. Many studies on the biology of these species have been carried out (Rho, 1971, 1975; Rho and Park, 1998), but no karyotypical report is available on specimens collected from the Korean and adjacent waters. Most cytogenetic studies have been performed on the materials from the European waters (Colombera, 1970, 1971a, 1971b, 1974; Colombera et al., 1978). So far, only 89 ascidians have been investigated at the karyotypical level, while more than two thousand species are hitherto known. We here report the chromosome numbers of three Korean ascidians for the first time.

Materials and Methods

The material collected in 1998-1999 consisted of the following specimens of 3 ascidian species: 5 colonies of *Amaroucium pliciferum* (Redikorzev, 1927) from Tolsando island, 24 individuals of *Halocynthia roretzi* (Drasche, 1884) from Gojedo island; and a large number of *Dendrodoa aggregata* (Rathke, 1806) collected from the immersed ropes in the shallow water of Tukdong, Masan bay. These specimens were processed to obtain chromosomes by Nakamura's air-drying technique with modification (Yum and Choe, 1996). For chromosome preparation, we used male gonads during spermatogenesis for *Halocynthia roretzi* and *Dendrodoa aggregata*, and fertilized eggs in the first phases of blastomeric segmentation for *Amaroucium pliciferum*. Colchicine solution (0.01%) was used to stop mitotic metaphases for 12-18 hours before dissection. 0.75 mol/L KCl solution was then used as hypotonic solution. The cells were fixed with Carnoy and stained with Giemsa.

Levan et al. (1964) was followed for the karyotyping.

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Result

Amaroucium pliciferum (Redikorzev, 1927)

Karyological data were obtained from 21 metaphase cells. Karyotypes were characterized by their relative

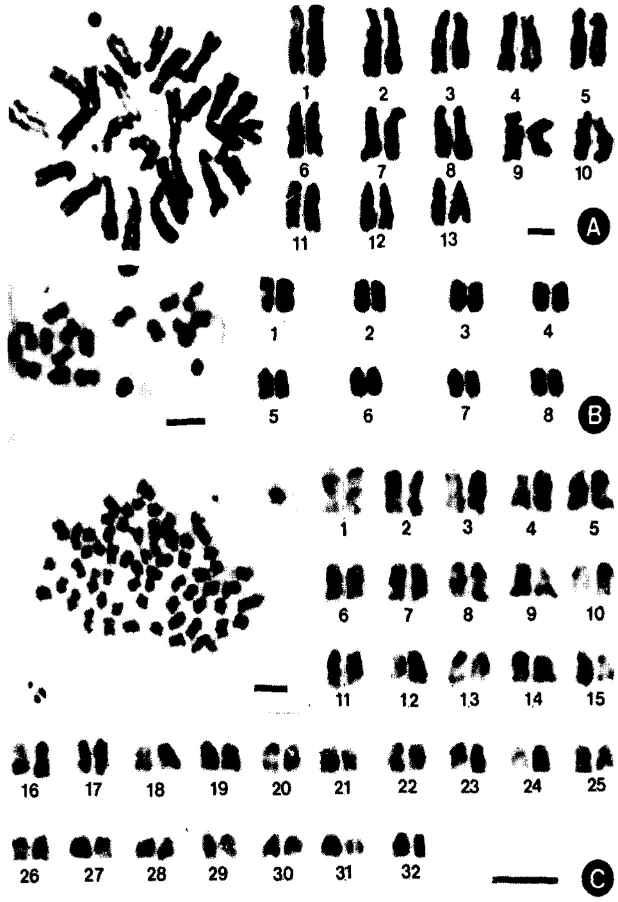

Fig. 1. The metaphase chromosomes and karyotypes of *Amaroucium pliciferum* (A), *Halocynthia rotetzi* (B), and *Dendrodoa aggregata* (C). Scale bars=5 µm.

chromosome size and centromeric position (Table 1). At spermatogonial metaphase, the diploid number of chromosomes was 2n=26 (Fig. 1A). These chromosomes could not be divided into groups according to their lengths. The chromosome complements consisted of 2 pairs of submetacentric (5, 9), 3 pairs of subtolecentric (4, 7, and 10), and 8 pairs of tolecentric (1, 2, 3, 6, 11, 12, and 13) chromosomes.

Halocynthia roretzi (Drasche, 1884)

Seventeen metaphase cells were subjected to chromosome analysis. The relative lengths of the chromosomes and the arm ratios were calculated (Table 1).

Table 1. Chromosome numbers and arm ratios of the three ascidian species

Species	Chromosome No	Relative length Mean (±SD)	Arm ratio	Morphology
Amaroucium pliciferum	1	99.8 (3.7)	-	T
	2	95.2 (2.7)	-	T
	3	87.2 (1.5)	-	T
	4	83.0 (2.4)	3.5	ST
	5	80.7 (0.9)	2.2	SM
	6	76.5 (2.1)	-	T
	7	72.6 (4.2)	5.0	ST
	8	70.3 (1.0)	-	T
	9	70.0 (2.7)	2.7	SM
	10	69.2 (3.5)	3.5	ST
	11	67.3 (1.3)	-	T
	12	65.8 (1.7)	-	T
	13	62.7 (2.9)	-	T
Halocynthia roretzi	1	145.4 (2.3)	1.0	M
	2	135.9 (3.7)	1.1	M
	3	133.4 (2.3)	1.1	M
	4	126.2 (3.0)	1.1	M
	5	123.1 (1.1)	-	T
	6	118.7 (2.8)	1.1	M
	7	108.4 (1.9)	-	T
	8	106.8 (3.5)	1.0	M
Dendrodoa aggregata	1	47.3 (3.5)	-	T
	2	41.2 (2.1)	-	T
	3	40.7 (2.5)	-	T
	4	39.7 (1.0)	-	T
	5	39.2 (1.4)	-	T
	6	38.7 (0.7)	-	T
	7	38.2 (2.5)	-	T
	8	37.9 (3.6)	-	T
	9	37.6 (1.1)	-	T
	10	37.2 (2.6)	-	T
	11	35.6 (2.0)	-	T
	12	34.6 (1.9)	-	T
	13	33.1 (1.3)	-	T
	14	31.6 (3.5)	-	T
	15	30.5 (2.6)	-	T
	16	30.1 (1.3)	-	T
	17	29.8 (2,9)	-	T
	18	29.1 (3.5)	-	T
	19	28.8 (3.0)	-	T
	20	28.4 (3.9)	-	T
	21	28.0 (1.7)	-	T
	22	26.6 (2.4)	-	T
	23	25.9 (4.0)	-	T
	24	25.5 (3.9)	-	T
	25	24.9 (2.7)	-	T
	26	24.0 (2.6)	-	T
	27	23.9 (2.0)	-	T
	28	22.9 (3.2)	-	T
	29	21.9 (2.7)	-	T
	30	21.2 (4.4)	-	T
	31	21.0 (0.9)	-	T
	32	20.8 (1.7)	-	T

At spermatogonial metaphase and meiotic, diakinesis the diploid number of chromosomes was 2n=16 (Fig. 1B). These chromosomes could not be divided into groups according to their lengths. The chromosome complements consist of 6 pairs of metacentric (1, 2, 3, 4, 6, and 8) and 2 pairs of telocentric (5, 7) chromosomes.

Dendrodoa aggregata (Rathke, 1806)

Eleven metaphase chromosomal spreads were studied. Table 1 gives the measurements and classifications of the metaphase spreads. The diploid number of chromosomes was 2n=64 (Fig. 1C). The first chromosome pair could be distinguished from the others based on their lengths. All pairs appeared to be telocentric.

Discussion

We report the karyotypes of *H. roretzi* and *D. aggregata* for the first time. *Dendrodoa aggregata* displayed the highest number of chromosomes (all tolecentric) in all known ascidian species. *Halocynthia roretzi* mainly possessed metacentric chromosomes and the chromosome number coincides with other Korean species of the same genus (Colombera et al., 1978). Our study seems to confirm the hypothesis that chromosome evolution among the class Ascidiacea may be characterized by a tendency toward both reduction of chromosome numbers and contemporaneous increase in the number of metacentric chromosomes (Colombera et al., 1978, 1987).

In *A. pliciferum*, the haploid number turned out to be 13, instead of the chromosome number of 20 as expected from the same species (Colombera et al., 1988), while coinciding with that of *P. aurantium*. It is possible that they might be sibling species, but it requires a further study.

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