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KARYOLOGY OF *PLAGIOMNIUM*. II. *PLAGIOMNIUM UNDULATUM* (HEDW.) T. KOP.

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In a study of *Plagiomnium undulatum* from Poland, variability in karyotype formula and chromosome set length contrasted with high uniformity of chromosome number. Haploid chromosome number n = 6 was counted in 215 of 216 metaphases of plants originating from five populations, and n = 7 (6 + 1m) in one cell. The chromosomes were examined and ranked by total and relative length and arm ratio. The haploid set of *P. undulatum* consists of three chromosome types: metacentric (m), submetacentric (sm) and subtelocentric (st). The frequencies of chromosome types differ between plates. In one population three types of karyotype were distinguished: 5m + 1sm, 3m + 2sm + 1st, and 2m + 3sm + 1st. The arm ratio of the longest chromosome is stable, and the chromosome represents a mainly metacentric type.

Key words: *Plagiomnium undulatum*, bryophytes, mosses, chromosome numbers, chromosome morphology.

INTRODUCTION

Chromosome numbers have been established for 54 species from nine genera of the family Mniaceae. Haploid (n = 6, 7, 8), diploid (n = 12, 13, 14), and triploid (n = 21) chromosome numbers have been reported from various geographical areas (Fritsch, 1991; Przywara and Kuta, 1995).

Species of the genus *Plagiomnium* are very difficult to identify on the basis of morphological characters. In such a case cytological criteria can be helpful. Cytological data were used to make a cytotaxonomic classification of the family Mniaceae (Koponen, 1968; Bowers, 1980). The Plagiomnieae tribe is a natural group with x = 6, in which all species studied have metacentric and submetacentric chromosomes, except for *P. undulatum* and *P. confertidens*, having subterminal chromosomes in the complement (Bowers, 1980).

In our previous study, significant intra- and interpopulational variability in chromosome number and karyotype formula was reported in dioecious species *Plagiomnium affine* from Polish populations (Klos et al., 2001).

The present work analyses the karyotype of another dioecious species of the genus, *Plagiomnium undulatum*. The results will become the basis for further investigations concerning DNA amount in species that are karyologically uniform but displays significant variability in chromosome set length.

MATERIALS AND METHODS

Living material was collected from five localities (Tab. 1). One collection (*S*) originated from north-eastern Poland, three (*U*, *K*, and *M*) from southern

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TABLE 1. *Plagiomnium undulatum*. Data on the material karyologically analysed

Locality	Total no. of apices	No. of metaphase plates	No. of chromosomes (n)	Method of staining
Cracow – Ugorek (*U*)	95	20	6	Acetic orcein, Giemsa
		1	7 (6+1m)	
Kleszczów (*K*)	102	13	6	Acetic orcein
Kazimierz Dolny (*KD*)	78	158	6	Acetic orcein, Schiff
Mircze (*M*)	72	5	6	Acetic orcein
Szwaderki (*S*)	186	19	6	Acetic orcein, Schiff

Poland, and one (*KD*) from central Poland. Voucher specimens are deposited in the Department of Plant Cytology and Embryology of the Jagiellonian University in Cracow.

The methods of plant cultivation and cytological technique follow those of Klos et al. (2001).

A range of stains was used: acetic orcein, Schiff's reagent (leuco-basic fuchsin) according to the Feulgen schedule, and Giemsa C-banding staining according to Schwarzacher et al. (1980). Nomenclature of chromosomes follows Levan et al. (1964).

RESULTS AND DISCUSSION

CHROMOSOME NUMBER

Cytologically, *P. undulatum* is one of the most intensively studied species in the genus *Plagiomnium*. The main cytotype reported from different parts of its range is haploid n = 6 (detailed list in: Fritsch, 1991). Tatuno and Ono (1966) and Ono (1970b) and Wigh and Strandhede (1971) reported n = 7 (6 + 1m) from Japan and Sweden, respectively, for material recognized as *Mnium undulatum*. The number n = 8 given by Mazzeo (1941, in: Fritsh, 1991) has not been confirmed by any subsequent author.

Przywara et al. (1983) reported unvarying n = 6 in material from four populations of *Plagiomnium undulatum* in Poland. In the present study, chromosomes of *P. undulatum* were counted in 216 metaphase plates from five other populations (Tab. 1). In 215 plates six chromosomes were observed; in one plate (population *U*) an m chromosome was detected in addition to six normal chromosomes (Figs. 1–6).

KARYOTYPE

The karyotype of *P. undulatum* has been studied from the British Isles (Ramsay, 1969; Newton, 1971), Japan (Tatuno and Ono, 1966; Ono, 1970b), Scandinavia (Wigh and Strandhede, 1971) and Turkey (Nyholm and Wigh, 1973). From drawings and photographs published, four or five chromosomes with median or submedian centromeres and one or two with subterminal centromeres could be recognized in the karyotype. However, the lack of chromosome measurements and arm ratio calculations makes it difficult to classify particular chromosomes according to Levan et al.'s (1964) classification. For example, Ramsay (l.c.) described the centromeres of chromosomes 2 and 6 as subterminal in female plants, but the drawing presented seems to show that chromosome 6 has a submedian, not subterminal centromere (its arm ratio calculated on the basis of the idiogram is ~1.50). Thus the presence of subterminal chromosome(s) in the karyotype of *Plagiomnium undulatum* is not clearly documented. If a subterminal chromosome(s) does occur in the karyotype of *P. undulatum*, that would separate this species (along with *P. confertidens*) from other species of the genus *Plagiomnium* that have only median or submedian centromeres. Bowers (1980) even put forward the idea of raising taxa with subterminal centromeres to the level of genus.

In our study, chromosomes with subterminal centromeres were detected in most of the metaphase plates examined. Karyotype analysis was based on 14 metaphase plates stained with Giemsa originating from gametophytes of population *U* (Tab. 2). Chromosome morphology was easy to determine using this dye. Chromosomes were deeply stained,

Figs. 1–7. *Plagiomnium undulatum*. Mitotic metaphase plates and interphase nuclei. **Fig. 1.** n = 6, karyotype formula: 2m + 3sm + 1st. **Fig. 2.** n = 6, karyotype formula: 3m + 2sm + 1st. **Fig. 3.** n = 6, karyotype formula: 5m + 1sm. **Fig. 4.** n = 7 (6 + 1m). **Fig. 5.** Chromosomes stained with acetic orcein. **Fig. 6.** Chromosomes stained with Schiff's reagent. **Fig. 7.** Interphase nuclei stained with Giemsa. Figs. 1–4. Chromosomes stained with Giemsa. m – m chromosome; SAT – SAT chromosome. Bars = 5 μm.

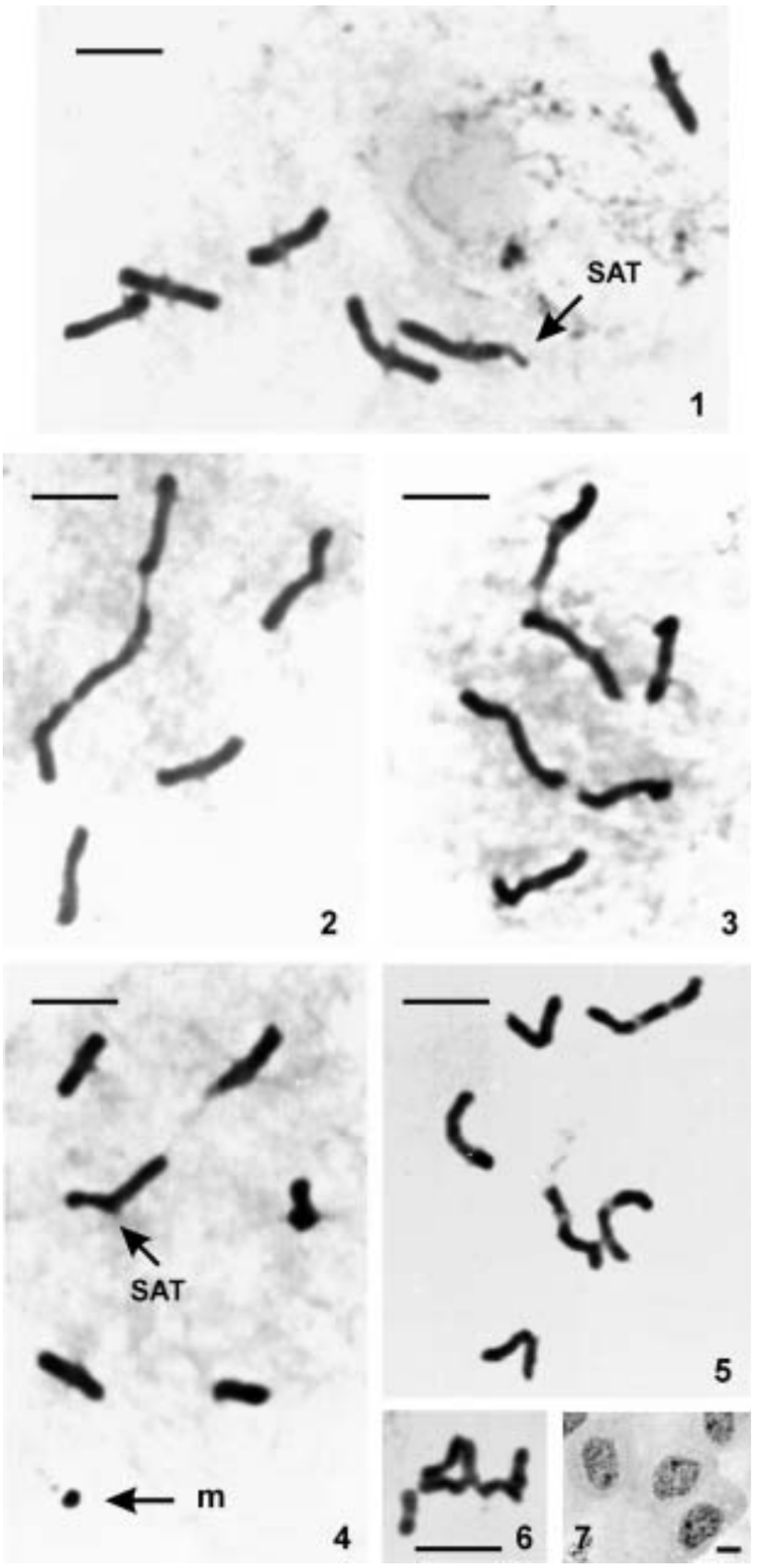
SAT
1
2
3
SAT
m
4
5
6
7

TABLE 2. *Plagiomnium undulatum* (n = 6). Absolute and relative chromosome length of chromosomes stained with Giemsa

Chromosome number	Absolute length (μm)			Relative length (%)		
	Mean	Min.	Max.	Mean	Min.	Max.
1	7.19 ± 1.17	5.70	10.00	20.29 ± 1.54	18.50	24.90
2	6.60 ± 1.19	5.00	9.40	18.56 ± 1.23	17.30	21.40
3	6.01 ± 1.04	4.25	7.65	16.90 ± 0.74	15.80	18.30
4	5.74 ± 1.01	3.80	7.35	16.10 ± 0.83	14.10	17.20
5	5.08 ± 0.72	3.20	6.35	14.35 ± 1.21	11.90	15.70
6	4.88 ± 0.63	3.20	5.90	13.80 ± 1.20	11.20	15.30

TABLE 3. *Plagiomnium undulatum* (n = 6). Variability of chromosome types in 5 selected metaphase plates stained with Giemsa

Plate	Length of chromosome set (μm)	Chromosome number					
		1	2	3	4	5	6
				5m + 1sm			
U1	38.75	m	m	m	m	sm	m
				3m + 2sm + 1st			
U2	46.65	m	m	m	sm	sm	st
U3	34.25	m	sm	st	m	sm	m
U4	35.70	m	sm	sm	m	m	st
				2m + 3sm + 1st			
U5	34.3	m	sm	m	st	sm	sm

with primary constrictions visible as rhomboidal openings (Figs. 1–3). Interestingly, in chromosomes stained with acetic orcein and Schiff's reagent, the primary constriction was always observed as a non-staining gap (Figs. 5, 6). The haploid complement of *Plagiomnium undulatum* consists of six chromosomes differing in size (Tab. 2, Fig. 8). The mean difference in chromosome length between the longest and shortest chromosomes is ~2 μm. According to Levan et al.'s (1964) nomenclature the chromosomes belong to three morphological types: metacentric (m) submetacentric (sm) and subtelocentric (st). The lowest variability in arm ratio is observed in the largest chromosome, which is metacentric (m) in most plates examined. The remaining chromosomes are more variable and occur as metacentric, submetacentric or subtelocentric. In some plates one SAT chromosome is clearly visible (Figs. 1, 4). In five selected metaphase plates, three karyotype formulae were distinguished: 2m + 3sm + 1st (Fig. 1), 3m + 2sm + 1st (Fig. 2), and 5m + 1sm (Fig. 3), indicating intrapopulational variation (Tab. 3). The same phenomenon was detected previously in the dioecious species *Pleurozium schreberi* (Kuta et al., 1998) and *Plagiomnium affine* (Klos et al., 2001).

TABLE 4. Comparison of chromosome set length (μm)

Method of staining	Mean	Min.	Max.
Giemsa	35.50 ± 5.29	26.90	46.65
Acetic orcein	22.79 ± 5.29	18.55	31.70
Schiff's reagent	20.17 ± 3.82	15.90	23.25

The differences in chromosome morphology might be due to different degrees of chromosome arm condensation within various metaphases (Fukui and Kakeda, 1994; Kakeda and Fukui, 1994), but structural chromosome rearrangements cannot be ruled out, of course. The role of chromosome rearrangements in evolution of mosses is little known (review in: Smith, 1978; Ramsay, 1983; Newton, 1984; Przywara and Kuta, 1995).

Despite the staining method used, the length of chromosome sets in particular plates varied greatly (Tab. 4). Generally, chromosomes stained with Giemsa are much longer than those stained with acetic orcein and Feulgen, as also reported in *Pleurozium schreberi* (Kuta et al., 1998). In 14 plates examined, chromosome set length ranged from 26.9 μm to 46.65 μm in gametophytes from popula-

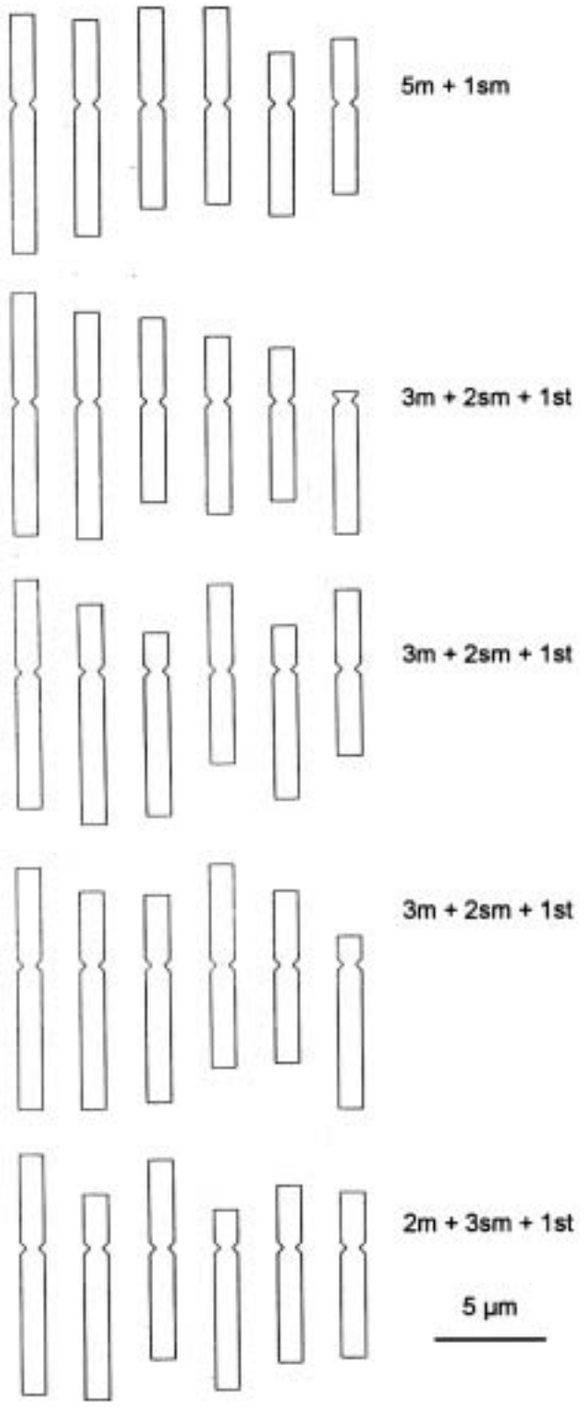

Fig. 8. *Plagiomnium undulatum* (n = 6). Idiograms of 5 selected metaphase plates representing 3 karyotype formulae.

tion *U* (Tab. 4). We observed the same situation in *Plagiomnium affine* and *Pleurozium schreberi* from Poland (Kuta et al., 1998; Klos et al., 2001). It will be interesting to see whether variability in the chromosome set length and karyotype formula of species with uniform chromosome number is correlated with differences in DNA amount (study in progress).

Heteropycnotic bodies have been observed in interphase nuclei of several Mniaceae (e.g., Newton, 1971; Ono, 1970a,b). It has been suggested that such bodies are related to sex chromosomes. Newton (l.c.) demonstrated that male and female plants of *Mnium undulatum* clearly differ in the size of heteropycnotic bodies. She found that ~7% of the heterochromatin bodies measured in female leaves exceeded 1 µm in maximum length, whereas ~65% exceeded 1 µm in male leaves examined. Newton suggested that this character may be useful in determining the sex of sterile plants.

In *P. undulatum* from Poland, heterochromatin bodies were observed in interphase nuclei from the leaves of studied gametophytes. Usually one distinct body was seen, regardless of the staining method applied. Generally, the best results were obtained with Giemsa staining (Fig. 7).

In this study we applied C-banding done according to Schwarzacher et al. (1980); it proved useful in earlier investigations of *Pleurozium schreberi* and *Plagiomnium affine*. In the case of *Plagiomnium undulatum* the results were negative. Despite several attempts, a clear banding pattern was not visible in any of metaphase plates examined.

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