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Analysis of the karyotype of *Callisia elegans* Alexand. (*Commelinaceae*) including differential staining of chromosomes

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Abstract

The number and morphology of *Callisia elegans* Alexand. chromosomes were studied employing staining with acetic carmine and differential Giemsa staining. It was found that its karyotype was 2n = 12 chromosomes, whose lengths fell in the range of 16.8 to 8.8 μm. The chomosomes, arranged in order of length, were classified respectively to types: sm, t, t, t, t, st. The distribution of C-banding is given for this karyotype. The presence of microsatellites on the long and short arms was found in the chromosomes of the second pair. Frequently there were 4 nucleoli of unequal size in interphase nuclei. In many cells, lower numbers of nucleoli (3-1) were seen which was probably due to their fusion. The maximum number of nucleoli corresponded to the number of nucleolar organizers accompanying the satellites.

Key words: *Callisia elegans Alexand., karyotype, differential Giemsa staining, satelliter chromosomes*

INTRODUCTION

During recent years, the analysis of the karyotype of many plant species has been enriched by chromosome banding patterns. One of the methods used in the case of plants is obtaining C-bands after Giemsa staining (Schweizer 1973, Verma and Rees 1974, Vosa and Marchi 1980, Takuzo and Nagyuki 1982). These bands are often located in the vicinity of the centromere, on telomeres or between them as intercalary bands. They correspond to the position of constitutional heterochromatin in the chromo-

some. The set of chromosomes of each species, sometimes of individuals, is characterized by a unique pattern of these bands.

Among the genera in which the distribution of heterochromatin has been studied by differential Giemsa staining are also members of the family *Commelinaceae*: *Rhoeo* (Natarajan and Natarajan 1972, Stack 1975) and *Gibasis* (Kenton 1978).

The present study presents the biometric analysis of the karyotype of *Callisia elegans* Alexand. (*Commelinaceae*). Special attention was paid to the structure of satelliter chromosomes. The number of nucleoli in interphase nuclei was also studied. As can be seen from a review of literature, the number and morphology of the chromosomes of this species has hitherto been studied only by Guervin and Le Coq (1965). The karyologic studies of *Callisia elegans* presented here used two different methods than the authors mentioned above. New details in the karyotype of this species, unknown until now, are presented.

MATERIAL AND METHODS

Callisia elegans Alexand. belongs to a group of decorative plants, cultivated for their ornamental shoots having small, striped leaves. It comes from Mexico.

The number and morphology of the chromosomes of the studied species were studied in the root apical meristems. **Acetic carmine method:** cut roots were treated with a 0.5% solution of colchicine for 1 hr. After fixation in Carnoy's solution for 1 hr, they were subjected to hydrolysis in 1 n HCl at 60°C for 15 min. Squashed preparations were made in 2% solutions of acetic carmine. The morphology of the chromosomes was determined on the basis of measurements of the length of metaphase chromosomes in 10 cells from different root apices. The r-index (the ratio of the length of the long arm to the short one) was calculated. The position of the centromere is described according to the terminology proposed by Levan et al. (1964).

Differential Giemsa staining: After pretreatment of cut root growth apices with alpha-bromonaphthalene for 3 hrs, they were fixed in Carnoy's solution for 1 hr. Next, the material was placed in a 5% solution of pectinase and cellulase for 3 hrs. After hydrolysis in 0.2 n HCl at 60°C for 5 min, squashed preparations were made in 45% acetic acid. Cover slips were removed using dry ice and the air-dried slides were placed for 1 hr in a solution of $Ba(OH)_2$, then in 2 SSC, pH 7.0, at 60°C for 1 hr, after which they were stained with a 4% Giemsa solution and closed with Euparal.

Determination of nucleoli number: The number of nucleoli was also determined in the cells of the root apical meristem. They were fixed with Nawaszin's solution for 24 hrs, rinsed under running tap water and hydrolysed in 1 n HCl at 60°C for 8 min. Next, they were stained by the Feulgen method and with 0.5% light green, after which squashed preparations were made.

RESULTS

NUMBER AND MORPHOLOGY OF CHROMOSOMES

The number of chromosomes in the cells of the studied root apical meristems of *Callisia elegans* Alexand. was 2n = 12 (Figs. 1, 2). The average lengths of the chromosomes and their arms are given below in Table 1. The longest pair of chromosomes of this karyotype had its centromere located in the submedian region. The second longest pair, with a terminally positioned centromere, had microsatellites, about 0.3 μm in diameter, both on the short as well as on the long arms (Figs. 3a, b, c, d). The satellites on the short arm were accompanied by secondary constrictions much longer than on the satellite on the long arm. The next three pairs of chromosomes had centromeres in the terminal region. The last, sixth, pair of chromosomes was classified as subterminal.

The occurrence of satellites on both arms of the second pair of chromosomes was the starting point for the determination of the number of nucleoli in interphase nuclei. In most of the nuclei, 4 nucleoli were observed: 2 larger ones and 2 smaller ones (Fig. 4). This number confirms the occurrence of 4 nucleolar organizing regions accompanying the satellites. The differences in the sizes of the nucleoli indicate differences in the sizes and activity of these regions. Lower numbers of nucleoli were observed in some of the nuclei (3-1). In many cases, nuclei undergoing fusion were visible, the effect of which was the formation of larger nucleoli with a drop in their number (Fig. 4g, h, i).

DIFFERENTIAL STAINING OF CHROMOSOMES

Differences in the position of heterochromatin in some of the pairs of homologues, as well as between chromatids of the same chromosome (Fig. 2a, b) were determined on the basis of the C-band pattern.

The chromosomes of the first pair were structurally heterozygous in respect to the distribution and size of the heterochromatin bands. Bands in the region of the centromere and intercalary bands on the longer arm occurred on both homologues, however, they differed in number and size. One of the chromosomes had two small intercalary bands: one closer to the centromere and seen distinctly on both chromatids, the other located near the middle of the long arm on only one chromatid. The other chromosome had only one intercalary band, whose size on neighboring chromatids was different.

Intensely staining bands on the short arms and satellites and on the telomeres of the long arms were seen on the chromosomes of the second pair. In addition, on the long arm of one of the chromosomes there was an intercalary band which the homologue did not have.

Stained heterochromatin occurred only on the short arm in the form of a distinct band in the third and fourth pairs of chromosomes.

The heterochromatin bands of the fifth pair of chromosomes stained the weakest. Very lightly stained points were visible on both arms, and they

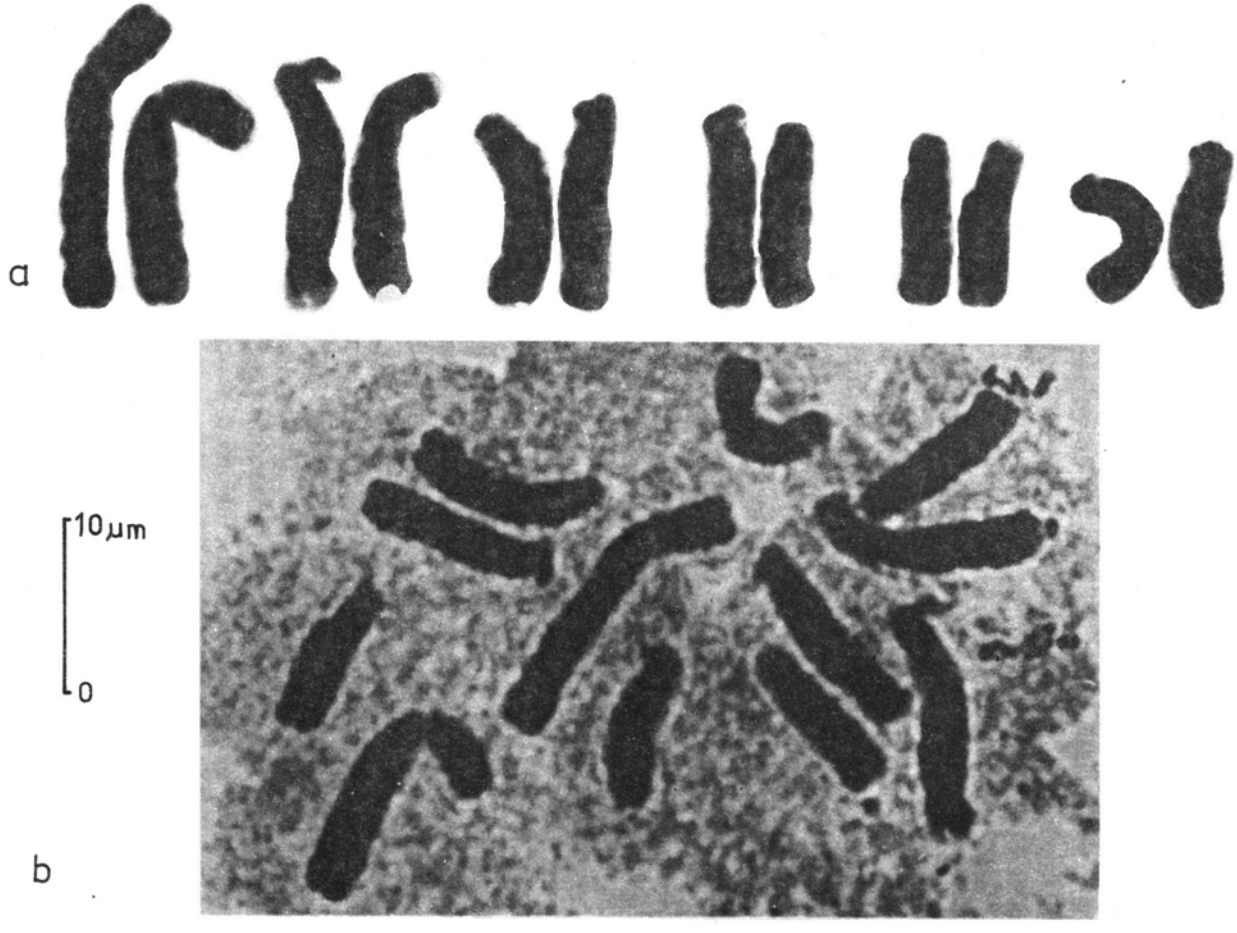

Fig. 1. *Callisia elegans* Alexand. chromosomes stained with acetic carmine. a — karyotype. Additional satellites are visible on the long arm of the second pair of chromosomes (SAT), b —

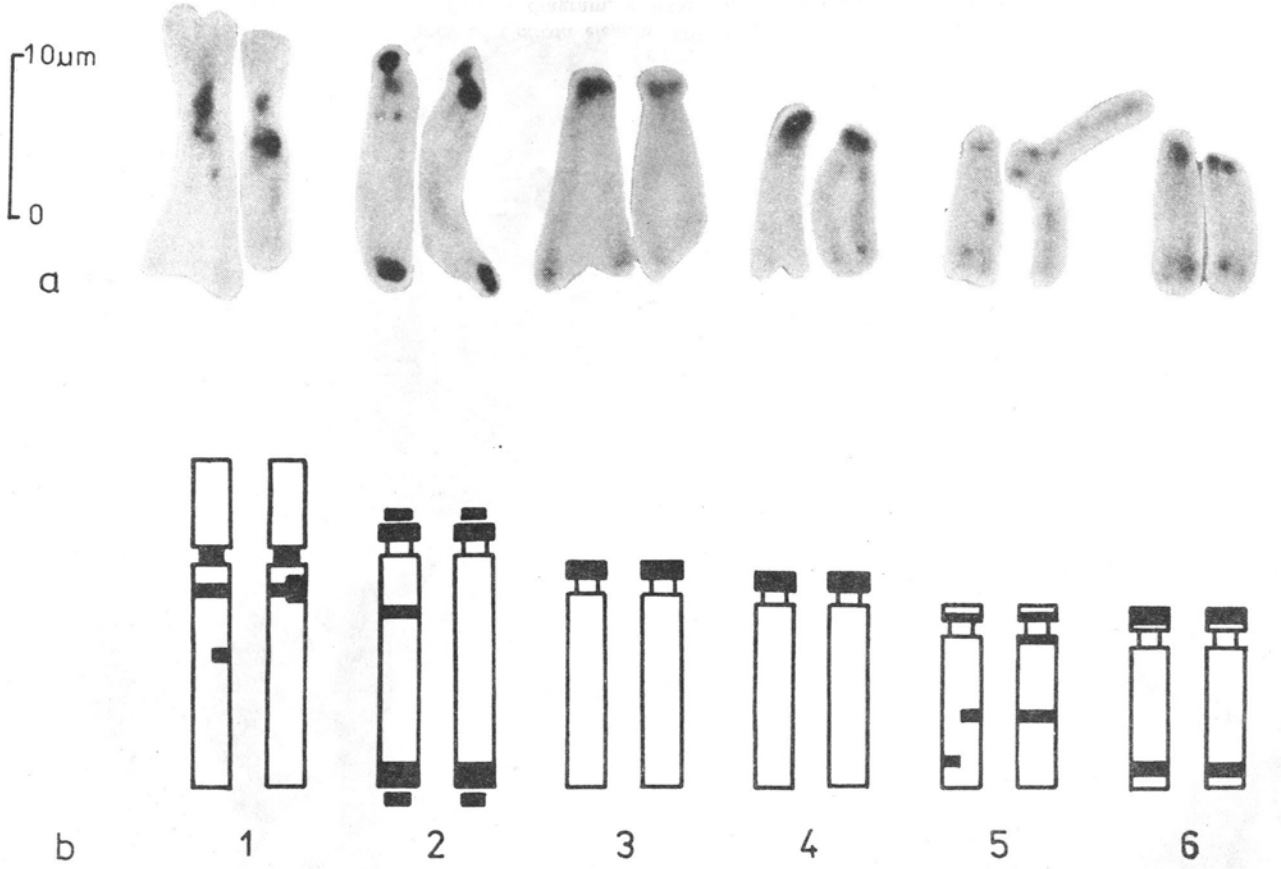

Fig. 2. *Callisia elegans* Alexand. chromosomes stained by the Giemsa method. a — karyotype, b — idiogram. × 2200

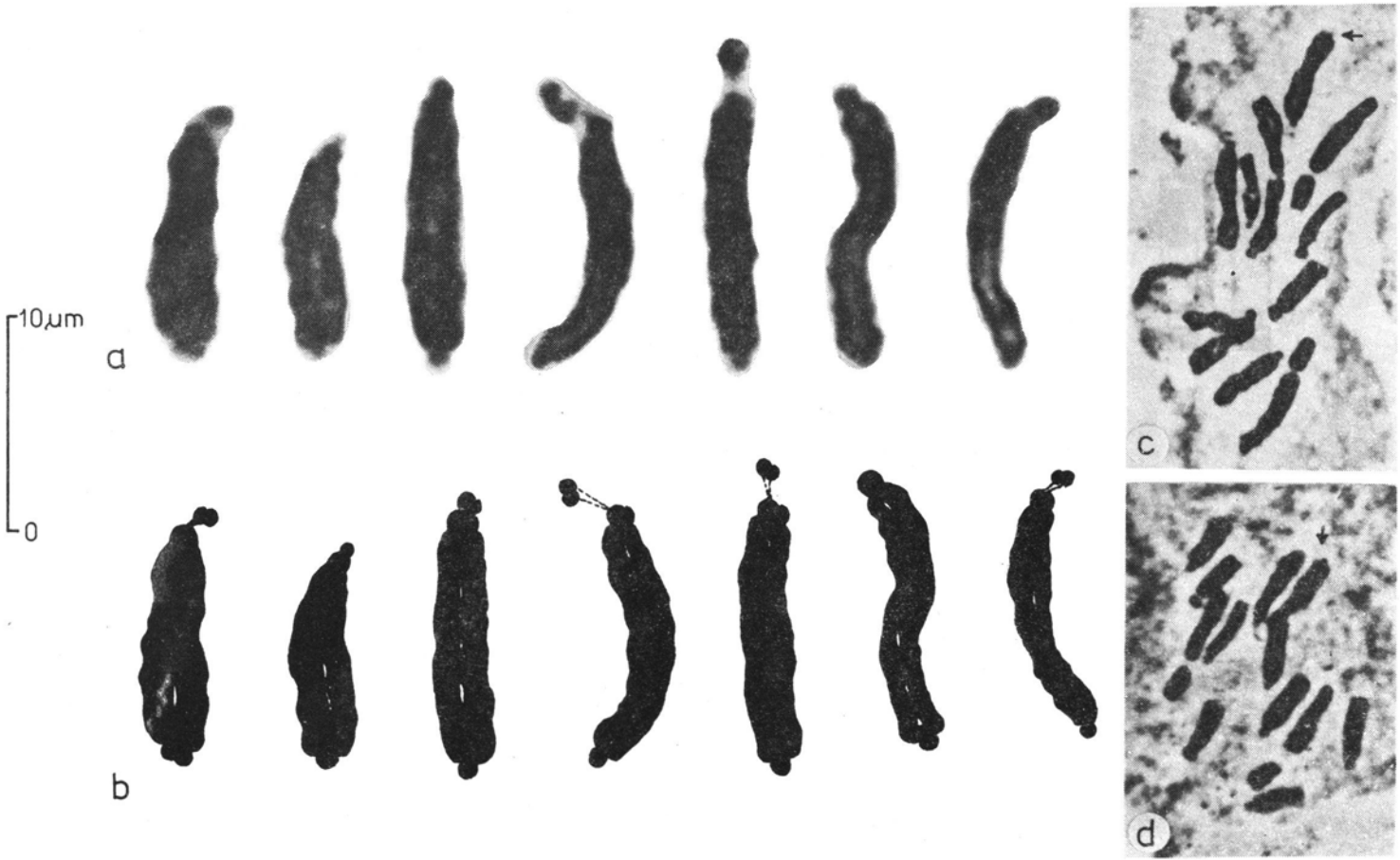

Fig. 3. Satelliter chromosomes of *Callisia elegans* with satellites visible on both arms. a — chromosomes from different cells; b — diagram, × 3000; c, d, — somatic metaphase with satelliter chromosomes (arrows), × 1150

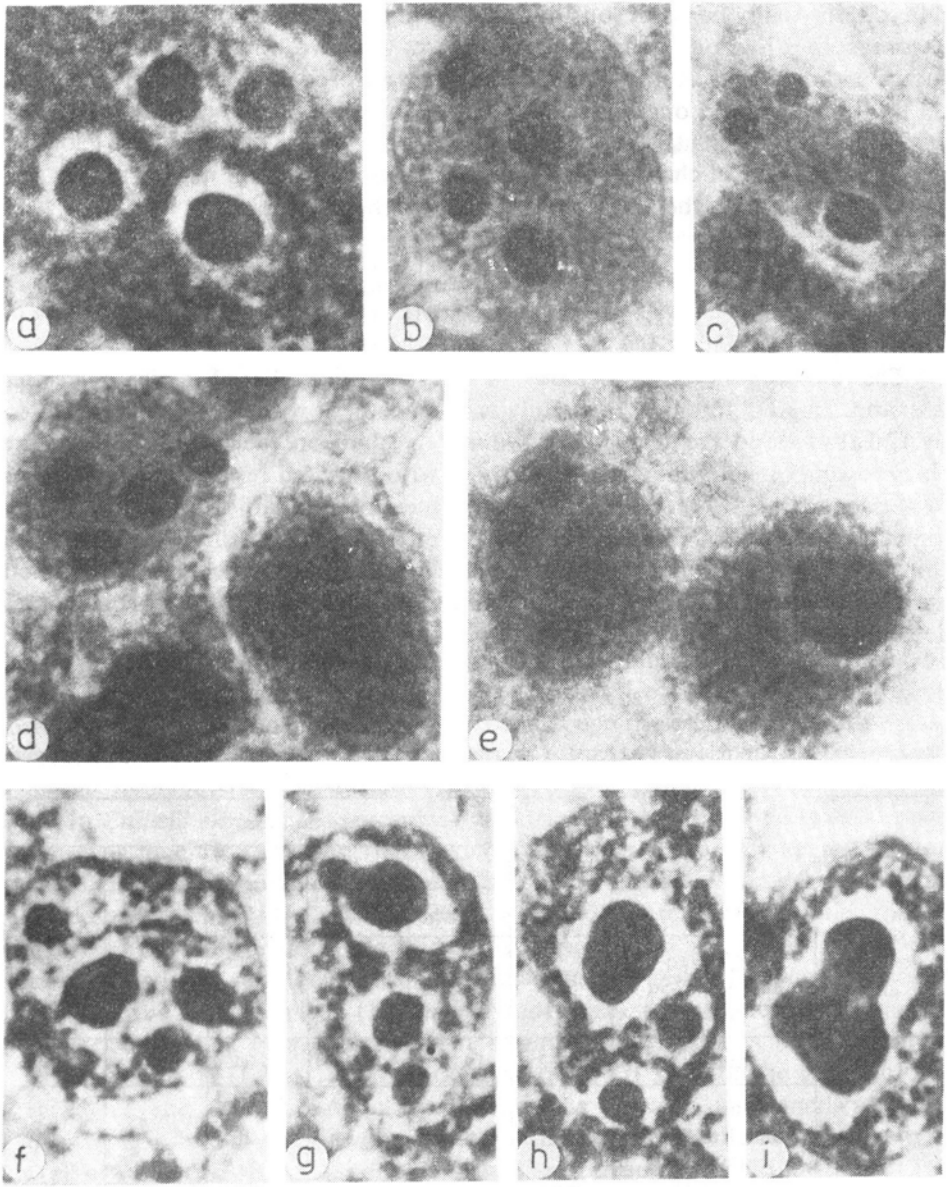

Fig. 4. Metaphase nuclei in root meristem cells of *Callisia elegans* Alexand. containing various numbers of nucleoli. a, b, c, d, e, f — with four variously sized nucleoli; d, e — with one large nucleolus; g, h — with two nucleoli undergoing fusion; i — fusion of three nucleoli, × 2350

differed in their position on both homologue chromosomes. One of the chromosomes had one band on its short arm and two on its long arm – near the centromere and at mid-length. The other chromosome also had one band on its short arm and one on its long arm, however, on each of the chromatids its position was different – on one in mid – length, on the other near the telomere.

The sixth pair of chromosomes had one distinct band on its short arms and a weaker band on the long arms, close to the telomere.

DISCUSSION

The number of chromosomes in the somatic cells of *Callisia elegans* Alexand. 2n = 12 found in this study is in agreement with the results presented by Guervin and Le Coq (1965), albeit the data on the morphology of the chromosomes is only in partial agreement with the cited report. The differences in the length of the metaphase chromosomes are probably due to the use of different methods of fixation (in this paper, the acetic carmine method with Carnoy's fixative, Guervin and Le Coq (1965) used the paraffin method with Karpaczenko's fixative). The relative (%) lengths of the chromosomes determined in this study and by the mentioned authors are similar (Table 1).

Table 1

The average (μm) and relative (%) lengths of metaphase chromosomes of *Callisia elegans* Alexand. as studied by different methods

		Chromosomes					
		1	2	3	4	5	6
Average length, μm	author	16.81	13.92	11.22	10.57	8.98	8.78
	Guervin and Le Coq (1965)	9.45	7.3	6.3	5.5	5.3	4.7
Relative length, %	author	23.92	19.81	15.96	15.04	12.78	12.49
	Guervin and Le Coq (1965)	24.51	18.93	16.34	14.26	13.74	12.19

On the basis of the value of the r-index (ratio of the length of the long arm to the short arm), in this study 1 pair of chromosomes with a submedial centromere, 4 pairs of chromosomes with terminal centromeres and one pair of chromosomes with subterminal centromeres (Table 2) were found. Guervin and Le Coq (1965) also classified the first pair of chromosomes as submedial, but found a subterminal primary structure in all five remaining pairs. According to those authors, homologues in those pairs differed significantly in

Table 2

Types of chromosomes and their average lengths in *Callisia elegans* Alexand. (n = 6)

Chromosomes	Length of arms, μm		Total chromosome length, μm	r-Index (ratio of long arm to short arm)	Types of chromosomes	Relative length of chromosomes, %
	long	short				
1	12.10	4.71	16.81	2.57	sm	23.92
2 SAT	12.51+0.3	0.81+0.3	13.92	11.54	t	19.81
3	10.41	0.81	11.22	12.85	t	15.96
4	9.76	0.81	10.57	12.05	t	15.04
5	8.19	0.78	8.98	10.50	t	12.78
6	7.58	1.20	8.78	6.32	st	12.49

their lengths. Such differences were not found in the studied karyotype presented here. From the data in this study and that of Guervin and Le Coq (1965) it is seen that the second longest pair of chromosomes are satelliter chromosomes. However, the results on the morphology of this pair obtained in the cited study and the present one, differ. This author has found that the SAT-chromosomes are equipped with terminal microsatellites on their short and long arms (Figs. 1, 3), while Guervin and Le Coq (1965) report small satellites on only the short arm. The occurrence of satellites on both arms of metacentric chromosomes, as the result of translocation, has been observed in barley by Nicoloff et al. (1977).

In *Callisia elegans*, the secondary constriction near the satellite on the short arm was much longer than that near the satellite on the long arm. Additional satellites with a morphology similar to that of satellites on the long arm of SAT-chromosomes of *Callisia elegans*, with a diameter much smaller than the diameter of the chromosome and with a very short secondary constriction, were found in submetacentric chromosomes of *Allium schoenoprasum* by Bougourd and Parker (1976). Polymorphism of satellites and secondary constrictions has been described in several *Allium* species (Derjagin and Jordanskij 1971, Beljaeva and Jampol 1977). One of the morphological types of the *Allium* SAT-chromosome has a very shortened secondary constriction and a satellite located immediately next to the short arm. In this type of structure, the satellite is described as "retracted" and it has been shown that the secondary constriction next to it is an active nucleolar organizing region.

The observations on the morphology of satelliter chromosomes conducted in this study show that each of them has 2 nucleolar organizing regions (on the long and short arms). The occurrence of the maximal number of nucleoli (4) in interphase nuclei of *Callisia elegans* Alexand. confirms the possibility that four nucleolar organizers can be present in the studied form.

Usually two of the four nucleoli observed in the cells of *Callisia elegans* were smaller. It would seem then, that the presence of nucleolar organizing

regions of various lengths on the SAT-chromosomes are characterized by different activities. Reports by Jampol and Bieljaeva (1975), Anastassova-Kristeva et al. (1977), Anastassova-Kristeva and Nicoloff (1979), Sato (1981), Hizume et al. (1982) also show the existence of a strict dependence between the number and size of secondary constrictions in the karotype and the number and size of the primary nucleoli.

In spite of the fact that Guervin and Le Coq (1965) in their karyological studies on the species *Callisia elegans* Alexand. found the presence of satellites on only one arm of satelliter chromosomes, they observed in the nuclei of many cells the presence of 3 and 4 nucleoli.

In interphase and prophase nuclei, two or three fusing nucleoli were observed (Fig. 4g, h, i), which sometimes led to the formation of one larger nucleolus. As is suggested by Wallace (1963) and Sherudilo and Semeshin (1971) the number and relative position of nucleoli determines if they fuse or not. The latter authors found that fusion of nucleoli in rat liver cells occurs if the distance between their centers is less than the sum of their radii.

Defferential Giemsa staining revealed in the karotype of *Callisia elegans* Alexand. the presence of relatively few heterochromatin bands, located mainly within the short arms, on telomers and satellites, and of intercalary bands. In three pairs of chromosomes, structural heterozygosity was found between homologue chromosomes as well as between chromatids of the same chromosome.

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Analiza kariotypu Callisia elegans Alexand. (Commelinaceae) z uwzględnieniem różnicowego barwienia chromosomów

Streszczenie

Badano liczbę i morfologię chromosomów *Callisia elegans* Alexand. z zastosowaniem barwienia acetokarminem oraz różnicowego barwienia metodą Giemzy. Stwierdzono, że kariotyp składa się z 2n = 12 chromosomów, których długości zawarte są w przedziale 16,8-8,8 μm. Kolejne pod względem długości chromosomy zaklasyfikowano do typów: sm, t, t, t, t, st. W badanym kariotypie przedstawiono rozmieszczenie C-prążków. W chromosomach drugiej pary stwierdzono obecność mikrosatelitów na długich i krótkich ramionach. W jądrach interfazowych występowały często 4 jąderka niejednakowej wielkości. W wielu komórkach obserwowano także mniej jąderek (3-1), co było prawdopodobnie spowodowane ich fuzją. Maksymalna liczba jąderek odpowiada liczbie organizatorów jąderkowych towarzyszących satelitom.