ACTA BIOLOGICA CRACOVIENSIA Series Botanica 41: 205-211, 1999

KARYOLOGICAL STUDIES ON *ACONITUM LASIOCARPUM* (RCHB.) GAYER (RANUNCULACEAE)

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Received February 12, 1999; revision accepted April 6, 1999

The karyotype structure of *Aconitum lasiocarpum* (Rchb.) Gayer was investigated conventionally and by Giemsa C-banding. The chromosome complex of *A. lasiocarpum* is composed of two distinguishable pairs of long chromosomes and six pairs of considerably shorter chromosomes. Nucleolar organizers are localized on the shorter arms of three (1, 3 and 5) chromosomes. In all analyzed plants the first and third NOR-chromosome pairs show structural heterozygosity concerning the presence of small satellites. The C-banded karyotype of *A. lasiocarpum* is heterochromatin-poor; all fixed heterochromatin segments occupy 7.51% of karyotype length. Four satellited chromosomes have larger, terminally located heterochromatic segments on their shorter arms. The authors made preliminary karyological observations of two other *Aconitum* species, the closely related *A. degenii* and *A. variegatum,* and discussed the relationships between the three species.

Key words: *Aconitum lasiocarpum, A. degenii, A. variegatum,* karyotype, C-banding, heterochromatin.

INTRODUCTION

Among *Aconitum* species occurring in the Carpathians, *A. lasiocarpum* (Rchb.) Gáyer is one of the most interesting because its geographical and especially its taxonomical status are still unclear (Mucher, 1993). It was described for the first time as *A. nasutum* Fisch. var. *lasiocarpum* [Rchb. In *Illustr. Spec. Aconit.* (1823-1827), after Grintescu, 1953]. In an earlier publication, Reichenbach (1819) mentioned *A. nasutum* Fischer as a Caucasian species. Obviously that diagnosis and geographical distribution do not fit the modern depiction of the taxon.

The botanical exploration of the Eastern Carpathians in the nineteenth century produced considerable amounts of *Aconitum* herbarium material. The ambiguity of the first description of the taxon caused much confusion later. Hiacynt Łobarzewski, who collected *Aconitum* spp. in the Western Bieszczady Mts., was probably the first to properly perceive the difference between *A. degenii* and *A. lasiocarpum,* but it went no further. He noted on the herbarium label from 1854: *"aliam habet secul inflorescentiam, secur cum nullo comparandum est meum, ergo forte nova species ?"* (LWA). One of the most comprehensive views on *Aconitum* flora in the Eastern Carpathians was elaborated by Zapałowicz (1908). He put specimens of *A. lasiocarpum* into *A. degenii* Gáyer (= *A. paniculatum* Lam. *nom. illeg);* however, he was aware that this position was not a complete solution. Gáyer (1911) gave an exact diagnosis of *A. lasiocarpum* (Rchb.) Gáyer, and its *locus classicus* - Dobonyos Mt. near Marmaros-Sziget (Eastern Carpathians). He put the taxon into the *A. hebegynum* group. This decision probably perpetuated the previous misunderstandings, and in the following years

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PL ISSN 0001-5296

TABLE 1. Provenance and chromosome number of the *Aconitum* samples studied. MP number of Feugen-stained and C-banded metaphase-plates used to establish chromosome number and heterochromatin amount, CBM number of C-banded metaphase plates used in karyotype analysis

Taxon	Locality	No. of plants	Altitude (m a.s.l.)	MP	CBM	Chromosome number (2n)
Aconitum lasiocarpum (Rchb.) Gáyer	Bieszczady Mts. (Eastern Carpathians)					
	[1] Buk on the Solinka River Beskid Niski (Western Carpathians)	3	490	20	7	16
	[2] Cergowa Mt.	4	700	18	6	16
Aconitum degenii Gáyer subsp. *degenii*	Czarnohora (Eastern Carpathians)					
	[3] Zaroślak - Prut Valley	2	1275	11	-	16
	[4] Połonina Pożyżewska	1	1410	5	-	16
Aconitum variegatum L. subsp. *variegatum*	Tatra Mts. (Western Carpathians)					
	[5] Koscieliska Valley	2	960	7	-	16
	[6] Koscieliska Valley	1	1000	5	-	16

TABLE 2. *Aconitum lasiocarpum* (2n=16), conventionally stained chromosomes; measurements of somatic chromosomes calculated as percentage of genome length, ± standard deviation; CH - chromosome type, T - total length, L - longer arm, S - shorter arm, C - arm ratio (L/S), cl - centromere localization, # - NOR-chromosomes

CH	T	L	S	C	cl
1#	25.17±2.61	13.41±1.38	11.76±1.42	1.14±0.09	m
2	19.60±2.58	13.73±1.65	5.87±1.06	2.34±0.23	sm
3#	10.81±2.10	6.43±1.00	4.38±1.42	1.47±0.20	m
4	10.72±2.04	6.87±1.43	3.85±0.72	1.78±0.27	sm
5#	10.38±1.76	7.06±1.40	3.32±1.16	2.13±0.14	sm
6	9.54±1.54	6.67±1.08	2.87±0.58	2.32±0.40	sm
7	8.16±1.34	5.61±0.87	2.55±0.62	2.19±0.51	sm
8	5.62±1.34	4.27±0.98	1.35±0.46	3.16±0.62	st

the taxon was still wrongly treated, now as *A. hebegynum* (e.g., Pawłowski, 1948; Michalko, 1955). Then Grintescu (1953) included it in *A. toxicum* Rchb., at the rank of subspecies. In his revision of *Aconitum variegatum* in Europe, Götz (1967) came to the conclusion that the taxon is a hybrid between *A. variegatum* and *A. paniculatum* Lam., *nom. illeg.* [= A. *degenii* Gayer subsp. *degenii,* see Mucher, 1993]. Recently, Mucher (1993) stated that to treat *A. lasiocarpum* at the rank of species is correct, and agreed with Götz (1967) on its hybridogenous origin. Sojak (1971) averred that Götz's opinion regarding *A. lasiocarpum* is correct, that it is a hybrid, that it now represents an already fixed taxon occurring quite independently of both supposed parent species, and that it behaves as an independent taxon in the Eastern Carpathians. Sojàk (1971) argued that it could possibly be ranked as a subspecies of *A. paniculatum* (= *A. degenii).* That is how it is treated in *Flora Polski* (Kucowa, 1985). The synonyms of A. *lasiocarpum* (Rchb.) Gáyer are: *A. lasiocarpum* Gáyer, *A. paniculatum* Lam. subsp. *lasiocarpum* (Rchb.) Soó, *A. toxicum* Rchb. subsp. *lasiocarpum* (Rchb.) Grint., *A. toxicum* Rchb. *var. dasycarpum* Schur., *A.* x *hebegynum* DC. pro parte, *A. vagneri* Kern., and *A. nasutum* Fisch. var. *lasiocarpum* Rchb. (basionym).

To our knowledge, neither the chromosome number nor the karyotype structure of *A. lasiocarpum* have been investigated so far. Accurate chromosome studies, especially these concerning the amount and distribution of heterochromatin, are needed for clarification of the systematic relationships among different *Aconitum* species, especially between the closely related *A. degenii* and *A. lasiocarpum* (series *Toxicum* Rchb. Mucher, 1993), and *A. variegatum* (series *Aconitum).* It is generally accepted that considerable interspecific variations in heterochromatin accompany microevolution and speciation in many plant genera (Gill and Kimber, 1974; Schweizer and Ehrendorfer, 1976; Vosa, 1977;

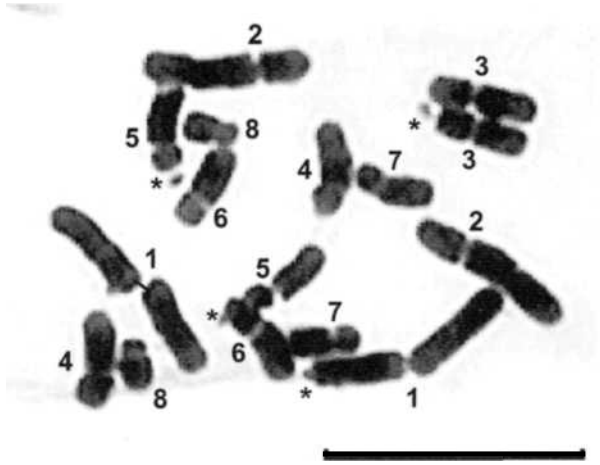


Fig. 1. *Aconitum lasiocarpurn* (Rchb) Gáyer [1]. Conventionally stained metaphase plate. * - satellites; [ ] indicates the origin of the plant (see Tab. 1). Bar = 10 μm.

Linde-Laursen et al., 1980; Joachimiak and Kula, 1993, 1996; Joachimiak et al., 1997). It is argued that the noncoding heterochromatic component of chromosomes evolves rapidly because it is not under selection pressure. Whereas in euchromatin the primary nucleotide sequence is evolutionarily variable, in heterochromatin the amount (number of copies) and chromosomal localization of the primary sequence unit is variable (Peacock et al., 1978; Schmidt and Heslop-Harrison, 1998). Thus, Giemsa C-banding of chromosomes, the cytological method for heterochromatin visualization within karyotype, is a valuable tool for assessing species relationships. Unfortunately there is no information about C-band karyotypes among *Aconitum* species.

The aim of this study was to use a modified C-banding method for *Aconitum* chromosomes and to make a precise karyotype analysis of *A. lasiocarpum* specimens originating from different localities. It also undertakes a preliminary study of karyotype structure of plants belonging to two supposed parent species: *A. degenii* Rchb. subsp. *degenii* and *A. variegatum* L. subsp. *variegatum.* In the next paper (in preparation) a more detailed karyotype analysis of these two taxa will be presented, with more systematically relevant conclusions concerning karyotype evolution within this group of closely related species.

MATERIALS AND METHODS

All plants were collected in 1997 and 1998 from natural populations (Tab. 1) and transferred to the Botanical Garden in Cracow. In the beginning of October 1998, plants were taken to the laboratory and kept in pots, and tips of roots were cut from them for cytological analysis.

The root tips were pretreated with saturated aqueous solution of α-bromonaphtalene for 2-4 h at room temperature, fixed in absolute ethanol/glacial acetic acid (3:1) and refrigerated overnight. After fixation the material was hydrolyzed with 5N HCl at room temperature for 40 min, rinsed in distilled water and squashed in 45% acetic acid. The cover glasses were removed and the squashes were rinsed in 96% ethyl alcohol and left to dry in the open air. The chromosomes were stained conventionally with 0.1% aqueous solution of toluidine blue, or the C-banding schedule described by Schwarzacher et al. (1980) was applied.

Chromosome measurements employed Multiscan (Computer Scanning Systems Ltd, Poland). Microscopic images were transferred to the system via video camera or from scanned microphotographs. Calculations, statistical analyses and chromosome drawings were made with the Mr Karyo program (Tokarski and Joachimiak).

RESULTS

All analyzed specimens of *A. lasiocarpum* (Tab. 1) are diploids with chromosome number 2n = 16. Conventional karyotype analysis shows (Figs. 1, 2, Tab. 2) that the chromosome complex of *A. lasiocarpum* is composed of two pairs of long chromosomes (total length of these four chromosomes is about 50% of karyotype length) and six pairs of considerably shorter chromosomes. Four pairs of these short chromosomes (4-7) are similar, and recognizable by size and arm ratio only after exact measurements. The third metacentric pair of chromosomes and the eighth, smallest pair of subtelocentric chromosomes are characteristic. Four large chromosomes can be identified easily in metaphases by size and centromere localization (Fig. 1). The first, largest chromosome pair (25.17% of karyotype length) consists of metacentric chromosomes, and the second (19.6% of karyotype length) possesses subterminally localized centromeres.

Nucleolar organizers are localized on the shorter arms of three (1, 3 and 5) chromosome types. Satellites, although small, are clearly visible within the analyzed metaphase plates (Fig. 1). In all analyzed plants the first and third NOR-chromosome pairs show structural hete-

TABLE 3. *Aconitum lasiocarpum* (2n = 16), C-banding; measurements of somatic chromosomes calculated as percentage of genome length, ± standard deviation; CH - chromosome type, T - total length, L - longer arm, S - shorter arm, C - arm ratio (L/S), TB - telomeric band, CB - centromeric band. Only fixed blocks of heterochromatin (80-100% frequency in chromosome collection) are taken into consideration in calculations; for the polymorphic bands only their presence is marked by ⊕. In the heterozygous chromosome type 1 the amount of telomeric heterochromatin was calculated separately for the satellited (#) and non-satellited (^) chromosome

CH	T	L	TB	S	TB	C	CB
1	25.13 ± 2.94	13.16 ± 1.60	⊕	11.97 ± 1.44	# 1.15 ± 0.49 ^ 0.30 ± 0.11	1.10 ± 0.08	0.50
2	19.36 ± 2.17	13.40 ± 1.61	--	5.96 ± 0.78	⊕	2.25 ± 0.36	0.50
3	11.41 ± 1.22	6.37 ± 0.88	⊕	5.04 ± 0.70	# 0.94 ± 0.22 ^ 0.35 ± 0.20	1.26 ± 0.23	0.50
4	10.90 ± 0.86	7.00 ± 0.73	--	3.90 ± 0.38	⊕	1.79 ± 0.35	0.50
5	10.43 ± 0.91	7.07 ± 0.70	⊕	3.36 ± 0.56	1.12 ± 0.47	2.11 ± 0.34	0.50
6	9.08 ± 0.96	6.47 ± 0.81	--	2.61 ± 0.54	⊕	2.48 ± 0.44	0.50
7	8.16 ± 0.64	5.62 ± 0.52	⊕	2.54 ± 0.31	⊕	2.21 ± 0.47	0.50
8	5.53 ± 0.59	4.24 ± 0.44	--	1.29 ± 0.23	--	3.28 ± 0.52	0.50

rozygosity concerning the presence of these satellites (Figs. 1, 2).

The C-banded karyotype of *A. lasiocarpum* (Figs. 3-5, Tab. 3) is heterochromatin-poor, with fixed (80-100% frequency in the chromosome collection) and polymorphic (in the analyzed karyotype up to 20% frequency in the chromosome collection) heterochromatic bands. Fixed bands are located within distinct structural units of chromosomes. The four largest banding areas are observed on the satellite chromosome ends (including the nucleolar organizers together with the satellites); the remaining ones are small and localized within the centromeric units of the chromosomes. In the majority of chromosomes they are observed as two dots localized exactly in the primary constriction. These pairs of dots present the centromeres themselves, which are C-band positive. Centromere-associated (pericentromeric) and intercalary heterochromatin segments were not observed. All fixed heterochromatin segments occupy 7.51% of karyotype length. Heterochromatic segments are clearly visible within interphase nuclei (Fig. 9). They occupy an average 4.63% of the cell nuclei surface area in C-banded preparations.

The structural heterozygosity of NOR-chromosome pairs 1 and 3 is confirmed by C-banding: one chromosome within each heteromorphic pair has a larger, terminally located heterochromatic segment on its shorter arms (Figs. 4, 5). In the second chromosome within the pair, a smaller heterochromatic segment is usually (chromosome 1) or sometimes (chromosome 3) observed. The presence of a small heterochromatic segment on the shorter arm of non-satellited chromosome 1 indicates that although this chromosome has no observable satellite it probably possesses NOR-associated heterochromatin.

Preliminary observations of conventionally stained chromosome preparations from *A. degenii* and *A. variegatum* show that the chromosome morphology and karyotype composition in the three analyzed *Aconitum* species are nearly the same. C-banded chromosomes of *A. degenii* also resemble *A. lasiocarpum* chromosomes (Figs. 7, 8). However, the total heterochromatin amount and the number of already observed C-bands in the chromosome complement of *A. variegatum* is considerably higher (Figs. 6, 10).

DISCUSSION

From the cytological point of view, *A. lasiocarpum, A. degenii* and *A. variegatum* are very similar. Conventionally stained chromosomes of these taxa are composed of two longer chromosome pairs and six considerably shorter pairs. In all analyzed specimens the total length of four larger chromosomes is about 50% of the total karyotype length, and the longer pair of these chromosomes is isobrachial, while the shorter is heterobrachial. The analyzed species also have similarly distributed NOR-chromosome pairs (1, 3 and 5) within the karyotype. These results are generally consistent with those

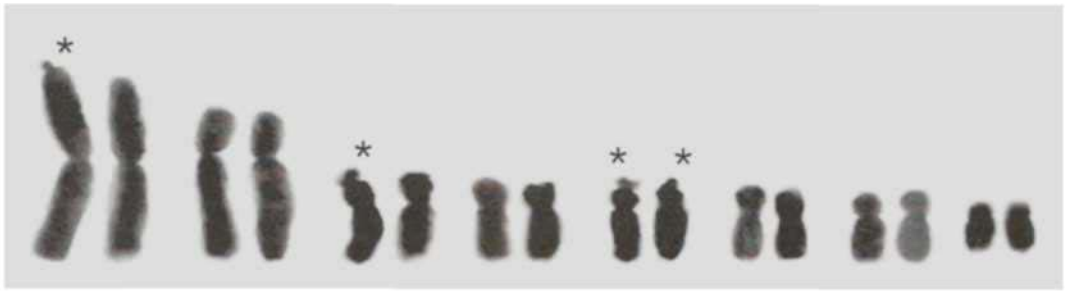


Fig. *2. Aconitum lasiocarpum* (Rchb.) Gáyer. Structure of conventionally stained karyotype. * - satellited chromosomes.

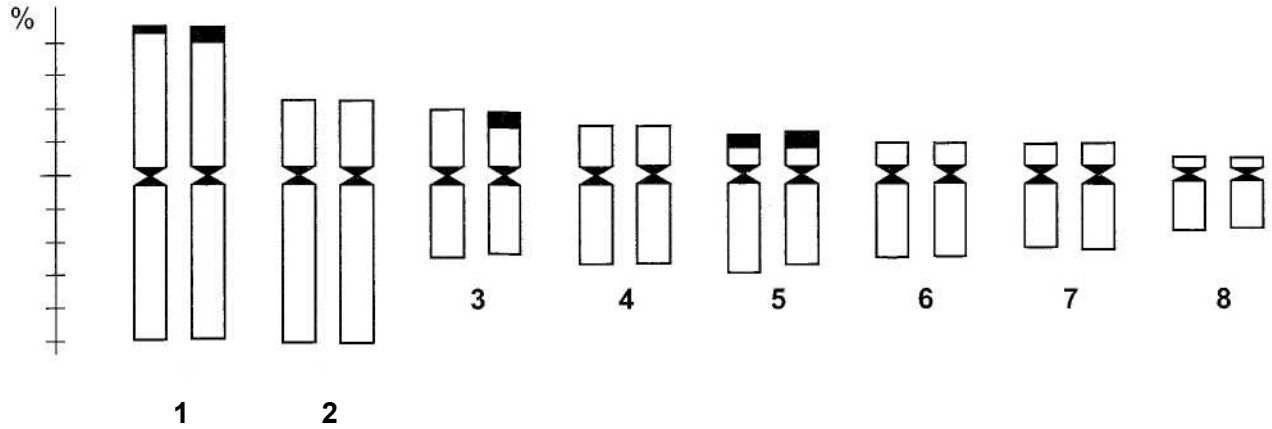
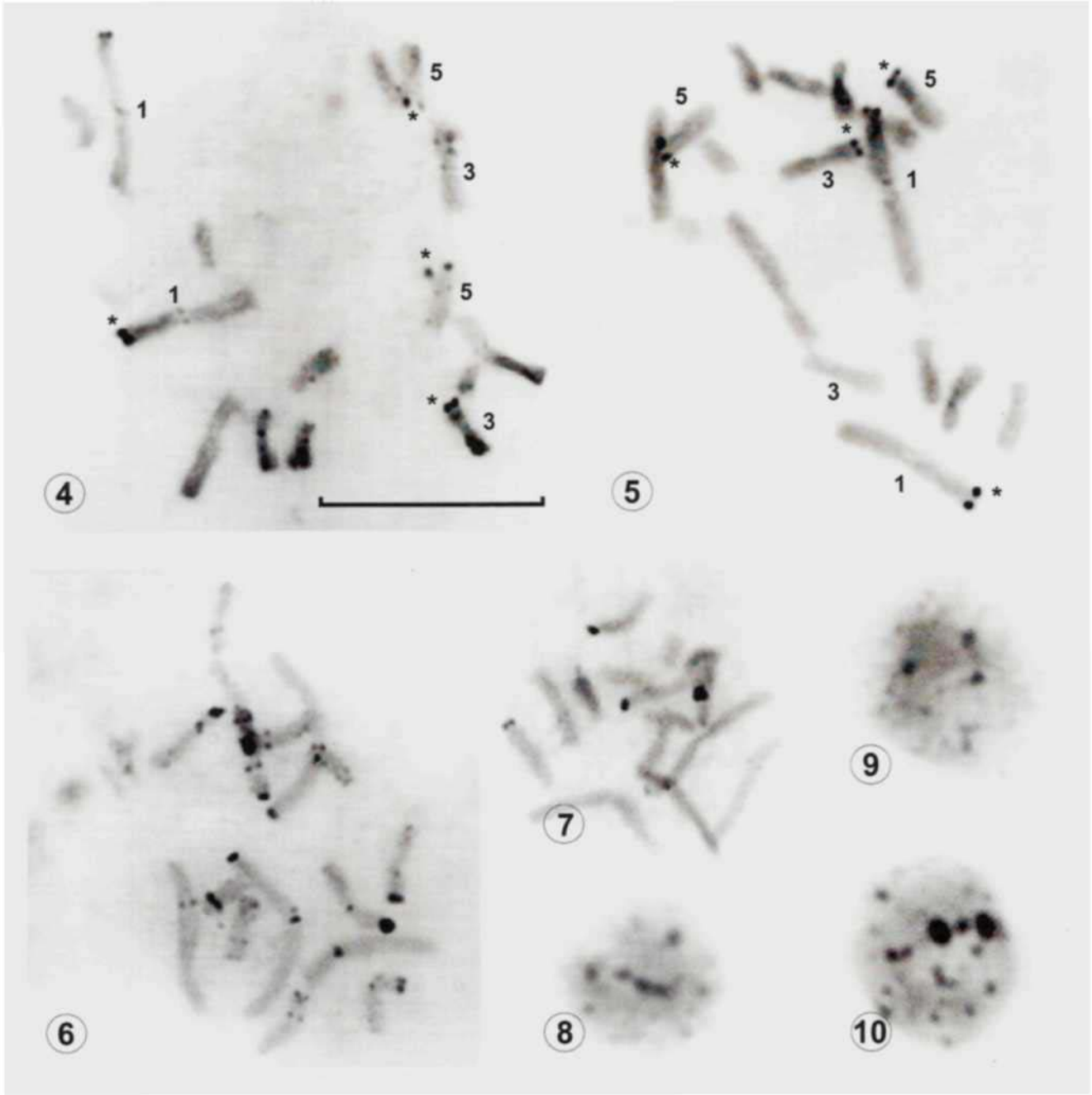


Fig. 3. *Aconitum lasiocarpum* (Rchb.) Gáyer [1,2]. Structure of C-banded karyotype. Average chromosome length and the amount of banding were calculated as a percentage of karyotype length. Only fixed blocks of heterochromatin are shown.

obtained by previous authors for *A. variegatum* s.l. (Schafer and La Cour, 1943; Leszczak, 1950; Seitz et al., 1972; Szulc, unpubl.), although comparisons are difficult because of the lack of exact chromosome measurements in some previous karyotype descriptions. However, there are some minor differences between the described karyotypes. Seitz et al. (1972) observed only two NOR-chromosome pairs in the karyotype of *A. variegatum* s.l. These pairs (3 and 4 in the depicted karyotype) are located within the group of shorter chromosomes. They strictly correspond to our satellite chromosome pairs 3 (isobrachial chromosome 4 in Seitz et al., 1972) and 5 (heterobrachial chromosome 3 in Seitz et all., 1972). Schafer and La Cour (1934) described essential morphological differences between four large chromosomes in representatives of *A. variegatum* from the Alps. Thus, unlike the remaining twelve smaller chromosomes, the authors did not group them into pairs, and treated them as separate chromosome types.

Although our karyological studies of *A. degenii* and *A. variegatum* are only provisional, they indicate that the *A. lasiocarpum* karyotype is almost identical with that of *A. degenii*. Another point is that the results on *A. lasiocarpum* chromosome structure were identical despite the varied geographical areas of origin (Eastern vs. Western Carpathians). The chromosome complements in the analyzed representatives of these two species show structural heterozygosity in two NOR-chromosome pairs (1 and 3), and are nearly identical with respect to the amount and distribution of heterochromatin. On the other hand, the *A. variegatum* chromosome complement is relatively rich in heterochromatin compared to the other two species. Moreover, three homozygotic satellite pairs of NOR-chromosomes are observed in the analysed *A. variegatum* metaphase plates. Similar structural homozygosity of satellite chromosome pairs (1, 3 and 5) was observed in representatives of *A. variegatum* s.l from the Tatra Mts. by Szulc (1973,



Figs. 4—10. C-banded metaphases and nuclei of *A. lasiocarpum* (Rchb.) Gáyer, *A. variegatum* L. subsp. *variegatum* and *A. degenii* Gáyer subsp. *degenii.* **Figs. 4, 5.** Chromosomes of *A. lasiocarpum* [1,2]. **Fig.** 6. Chromosomes of *A. variegatum* [5]. **Fig.** 7. chromosomes of *A. degenii* [3]. **Figs. 8-10.** Interphase nucleus of *A. lasiocarpum* [1] (Fig. 8), *A. degenii* [3] (Fig. 9) and *A. variegatum* [5] (Fig. 10). [ ] indicates locality of *Aconitum* specimens (see Tab. 1). Bar = 10 μm.

unpubl.). Thus, in respect to NOR-chromosome pairs the analyzed plants belonging to *A. variegatum* are homozygous, but the specimens of *A. degenii* and *A. lasiocarpum* we analyzed are heterozygous. The homo/heterozygosity of NOR-chromosome pairs is also clearly visible in C-banded metaphase plates. In heterozygous configurations the homologues clearly differ in the amounts of heterochromatin at

the chromosome termini, but in homozygous configurations (as in *A. variegatum)* the homologues have very similar terminal segments of heterochromatin.

The probable hybridogenous origin of *A. lasiocarpum* has been suggested in systematic studies (Götz, 1967; Mucher, 1993). As mentioned in the introduction, the authors postulated that this taxon may be a hybrid between *A. degenii* and *A. variegatum.* We have found that the karyotypes of *A. degenii* and *A. lasiocarpum* are very similar in respect to the heterozygosity of NOR-chromosomes, and differ from those of *A. variegatum,* which are homozygous in the corresponding chromosomes. Moreover, in *A. variegatum* there is substantially more heterochromatin than in both *A. lasiocarpum* and *A. degenii.* This suggests the close evolutionary lineages of *A. lasiocarpum* and *A. degenii.* The structural differences in C-banding between *A. variegatum* vs. *A. degenii* and *A. lasiocarpum* argue against the hybridigenous origin of the latter species. The stability of the heterozygotic chromosome systems of *A. lasiocarpum* and *A. degenii* should be corroborated based on material from a wide geographical area.

The observed heterozygosity of two NOR-chromosome pairs in the karyotype of *A. lasiocarpum* and *A. degenii* probably arose through crossing between two different forms/taxa (with and without satellites on chromosomes 1 and 3). However, it can be argued that the observed polymorphism is caused by intraspecific structural variations of NOR-chromosomes. The structural heterozygosities within karyotypes and differences in heterochromatin amounts and distributions offer opportunities to clarify the systematic relationships between the analyzed species.

ACKNOWLEDGMENTS

This work was supported by KBN Grant No. 6 P04C 025 13.

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