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Karyotaxonomic analysis of supramontane populations of species of the genus *Astragalus* in Slovakia

J. MÁJOVSKÝ, A. UHRÍKOVÁ and Z. DÚBRAVCOVÁ

Department of Botany, Faculty of Natural Sciences, Comenius University, Révova 39, 811 02 Bratislava, Slovakia

Abstract. The authors have drawn the inference of the presentation of supramontane species as floristic elements or geoelements as well as their glacial and post - glacial evolution on the basis of own karyological analyses of native supramontane species. More advanced classification and interpretation methods and techniques are required for investigation of *Astragalus alpinus* group, *Astragalus penduliflorus* and particularly *Astragalus australis* auct. Within the Slovakian part of Western Carpathians, both *Astragalus alpinus* and *Astragalus penduliflorus* have diploid chromosome numbers 2n = 16. In *Astragalus australis* auct. in the territory only hexaploid populations 2n = 48 have been found.
Key words: Astragalus alpinus group, (*A. alpinus, A. subpolaris, A. astragalinus*), *A. penduliflorus, A. australis* auct. europ., chromosome numbers, Slovakian (West Carpathian) and other populations, distribution, evolution, geoelement

Introduction

In the Karyotaxonomic Survey of Slovak Flora (Májovský *et al.* 1987) at the presentation of the supramontane species esp. the genus *Astragalus* we stated inacurrate facts resulted from:
1. insufficient level of study foreign sources (Yurtzev and Zhukova 1968, Hultén 1968, Love and Love 1975. E. g. Hultén has been cited only once)
2. because of insufficiency of own analyses. Nowadays, we do not agree with the evaluation of more species with transcription of morphologic variability and phytogeographic characterization in European Floras (Chater 1968, Gontscharov in Schischkin 1946, Gams and Hegi 1975, Jäger and Weinert in Rothmaler *et al.* 1988) as well as in the Flora Slovakia (Chrtková and Jasičová 1988).

Due to this, we try to present a survey of taxonomic - chorologic problems and karyologic characterization of taxon of the genus *Astragalus*. Moreover, we intend to inform about problems we are not able to solve due to objective and subjective reasons (inaccessible literature, herbarium specimens, living plants). The contribution deals with supramontane species of mentioned genus and their Slovak populations.

Material and methods

To establish the number of chromosomes and the karyotype, plants from natural habitats - Veľká Fatra and Belianske Tatry Mts. - were employed. The root tips were pretreated with saturated solution of paradichlorbenzene for three hours and then fixed in a mixture of ethanol and acetic acid (3:1) for 1-24 h. After maceration in a mixture of ethanol and hydrochloric acid (1:1) for 6 min. and 10 min. washing in water, temporary aceto-orceining squashes were made. In particular cases, permanent squashes made under cellophane were used (Murín 1960). The types of chromosomes were established according to Levan *et al.* (1964) or Murín (1970).

Literature cited after the chromosome numbers. The following references related to the chromosome numbers are cited in the general "Floras" and karyotaxonomic atlases: Favarger (1965), Ledingham (1960), Ledingham and Fahselt (1964), Knaben and Engelskjön (1965), Johnson and Packer (1968), Sokolovskaya (1968) - all in Löve and Löve (1974); Favarger (1949b, 1959), Sorsa (1963), Hedberg and Hedberg (1964), Hedberg (1967), Laane (1965), Sokolovskaya (1963, 1970), Zhukova (1966), Mosquin and Hayley (1966), Mulligan and Porsild (1969b) , Zhukova and Tikhonova (1973), Zhukova and Petrovsky (1973), Zhukova *et al.* (1973) , Packer and Mc Pherson (1974) - all in Löve and Löve (1975); Krogulevich (1971), Malakhova (1971), Belyaeva and Siplivinskii (1976, 1977) - all in Malyshev and Peshkova (1979).

Additional information was found in Torrey and Gray (1838), Jordanov (1960), Vilev (1976), Zangheri (1976), Martinič and Suškin (1984). Distribution of the genus *Astragalus* at the territory of Slovakia was described according to Dostál (1989).

Results and discussion

Astragalus alpinus group

The term "group" means the group of 2 - 3 taxa and is mainly used by Anglo - Saxon authors. Only the differentiation in the subspecies cathegory corresponds with particular taxa. However, the oldest ones were described as individual species validated by the specialists of the genus *Astragalus*. Even this cathegory due to its clear genetic differentiation would not deny the youngest taxon. We agree with the specialists opinion and it is presented in the cathegory species.

Astragalus alpinus 2n = 16

Favarger (1959), Alps;
Sorsa (1963), Finland;
Hedberg and Hedberg (1964), Sweden;
Favarger (1965), Alps;
Favarger and Küpfer (1968) in Küpfer (1971), Pyrénées;
Krogulevich (1971), Sib. Tsentr., Plateau Stanovoe, S, Muiskii ridge;
Malakhova (1971), Sib. Tsentr., Sayan, E, Temkinskii ridge;
Belyaeva and Siplivinskii (1976), Sib. Tsentr., Baikal, NE coast;
Belyaeva and Siplivinskii (1977), Sib. Tsentr., Baikal, N, Kotera river;
Rostovtseva (1977) in Krogulevich and Rostovtseva (1984), Sib. Tsentr., Tuvinskaya ASSR, Derzig river;
Krogulevich (1978) in Krogulevich and Rostovtseva (1984), Sib. Tsentr., Sayan, E, Tunkinskii ridge.

Astragalus subpolaris 2n = 16

Ledingham (1960), Canada - arctics;
Ledingham and Fahselt (1964), North America - arctics;
Knaben and Engelskjön (1965), Norway;
Laane (1965), Norway - arctics;
Sokolovskaya (1970), Komi river - arctics;
Zhukova and Petrovsky (1976), Tchukotka, W, Yagodnii river;
Krogulevich in Krogulevich and Rostovtseva (1984), Taimyr, Fomich river;
Krogulevich in Krogulevich and Rostovtseva (1984), Taimyr, Syndasko settlement;
Krogulevich in Krogulevich and Rostovtseva (1984), Yakutskaya ASSR, Mogdy river.

Astragalus astragalinus 2n = 32

Ledingham (l.c.), Canada;
Hultén (1962) in Hultén (1968), Alaska - arctics;
Sokolovskaya (1963), Kamtchatka;
Ledingham and Fahselt (l.c.), North America;
Zhukova (1966), Wrangel island, Somnitelnaya bay - arctics;
Mosquin and Hayley (1966), Canadian arctics;
Zhukova (l.c.), Tchukotka, Apelkhino settlement - arctics;
Hedberg (1967), North America - arctics;
Johnson and Packer (1968), Alaska - arctics;
Sokolovskaya (1968), Koryatskaya zemlya - arctics;
Yurtsev and Zhukova (1968), Tchukotka - arctics;
Mulligan and Porsild (1969b), Canada, Yukon;
Zhukova and Tikhonova (1973), Tchukotka, E, Atchion lake - arctics;
Zhukova and Petrovsky (1973), Tchukotka, E, Anguyema river-arctics;
Zhukova *et al.* (1973), Tchukotka, E, Mt. Iskatenii ridge - arctics;
Zhukova *et al.* (1973), Kolymskoe plateau, Tchernoe ozero settlement - arctics;
Packer and Mc Pherson (1974), North Alaska - arctics;
Zhukova *et al.* (1977), Irkutskaya ASSR, Tcherskii settlement - arctics;
Zhukova and Petrovsky (1977), Tchukotka, W, Bilibinskii region - arctics.

We state that each species is characterized by sufficient amount of karylogical analyses from geographical range. Therefore, there is unlike following differentiation of the number of chromosomes in *A. alpinus* L. and *A. subpolaris* (Borissova and Schischkin in Schischkin 1946). *A. astragalinus* is considered as tetraploid one in all geographic ranges where it is observed. If transient types (Chater 1968) are occured in Scandinavia they should be also

Pair	Arms	Total	Index	Type
1-2	1.5+1.0	2.5	0.66	L
3	1.3+1...0.2	2.5	0.76	V
4-5	1.2+1.1	2.3	0.91	V
6-7	1.3+0.9	2.2	0.69	L
8	1.0+1.0	2.0	1.0	V

Definition of karyotype: 2n = 2x =(L,L,V_s,V,V,L,L,V´)
Characteristics of pairs:
1st-2nd: L - centromere in median zone
3rd: V_s - centromere in median zone + satelitte
4th-5th: V - centromere in median zone
6th-7th: L - centromere in median zone
8th: V´- centromere in the centre of chromosome

Table 1. Karyotype *A. alpinus* (length relations in μm).

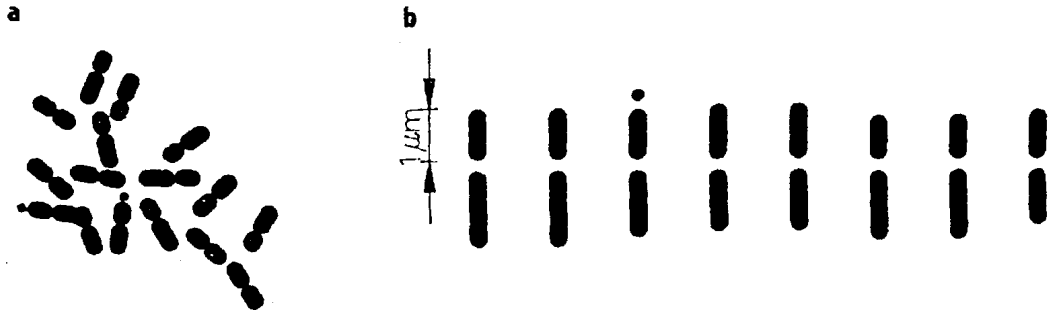

Fig. 1. Chromosomes (a) and idiogram (b) of *Astragalus alpinus* L. 2n = 16.

the tetraploid ones. Moreover, we have to emphasize the fact that no karyological analysis of *A. alpinus* from mentioned North American range has been done so far and no comparative study of chromosome number of particular species has been carried out.

Astragalus alpinus L. Sp. Pl. ed. 1. p. 760, 1753

Syn. *Phaca alpina* L. p.p., *P. astragalina* L., *P. astragalina* DC., *P. minima* All., *Astragalus montanus* Jacq. non L., *Astragalus alpinus* L. subsp. *alpinus*, *Oxytropis lapponica* Schur.

2n = 16 Uhríková and Bernátová, Western Carpathians (Slovakia), Veľká Fatra Mts., Mt. Malá Pustalovčia;
2n = 16 Uhríková and Dúbravcová, Western Carpathians (Slovakia), Belianske Tatry Mts., Mt. Hlúpy;
2n = 16 Uhríková and Dúbravcová, Western Carpathians (Slovakia), Belianske Tatry Mts., Mt. Ždiarska vidla.

The number of chromosomes with the following analysis of Slovak population (Kochjarová 1991) represent first karyological analyses of Carpathian populations (Table 1, Fig. 1). Probably, even the other West and East Carpathian populations will have not only the same number of chromosomes but the same set of chromosomes as well and the same set ensures the genetic uniformity of all continental Eurasian supramontane populations. The uniformity is even reflected in the absence of any description of morphological variability that requires higher ranking of some populations in mentioned area.

The morphology of *Astragalus alpinus* can be characterized by several differential traits: calyx teeth slightly shorter or almost as long as the tube, two coloured corolla, pale violet and white at the basement, the white keel, dark violet at apex, valves of legume obtusely keeled, legume almost lacks septum.

It is considered as supramontane species inhabiting the mountain chains originated during Alpine - Himalayan orogeny and its geographic range involves Pyrenees, Alps, Carpathians, Balkan, Caucasus, Himalaya and the large mountain chains of Central Siberia. Besides the more or less compact range, it is also occured in Scotland, Sweden and Finland. Although it has been registered in North West America, however in our opinion there is not sufficient evidence of its presence in the New World. While in Western Carpathians *Astragalus alpinus* prefers stony or shallow carbonate substrates, in Alps occurs according Ascherson and Graebner (1909) "fast stets auf Urgenstein". Therefore, it is likely that Scandinavian populations are more closely related to Alpine ones than Carpathian ones. It is likely that the largest European range it occupied at the end of Tertiary (Balkan, Alps, Pyrenees and Eastern Carpathians) while Western Carpathians range during glacial and post - glacial periods. This reality could be confirmed by its presence only in Western and Northern Slovakia. However, it is absent in Low Tatras and Muránska Planina Mts but the other supramontane species had survived during glacial periods in Western Carpathians. According the type of distribution it should be involved into the group of Altai - Alpine species (Gams and Hegi 1975). The group had invaded into European mountains during the end of Tertiary (Diels 1910 in Gams and Hegi 1975, and several citations later). The group of species during following period did not subsequently taxonomically differentiated because of long period required (Favarger 1972:196). In our opinion, there are taxonomic isolates that have found sufficient amount of appropriate biotops for survival during unfavourable periods. However, we do not think that *Astragalus alpinus* has migrated in Alps from Carpathians as supposed by Gams and Hegi (1975).

It is unlikely that some North American populations belong to *Astragalus alpinus* because even some earlier descriptions of morphological traits are mentioned as "pods deaply furrowed on the under side" (Britton and Brown 1897: 304). The newest information from Hultén (1968) about *Astragalus alpinus* subsp. *alpinus* "pods strongly grooved dorsaly" does not correspond with *Astragalus alpinus*. Gontscharov in Schischkin (1946) has found the species populations in large mountains chains of Central Siberia but on the other hand not in Tchukotka and Kamtchatka. The analyses of *Astragalus alpinus* subsp. *alpinus* from mentioned area carried out by Russian karyologists showed that they had been tetraploids and undoubtedly they belonged to *Astragalus alpinus* as well as the tetraploids from Central Alaska and Yukon. Therefore, the statement that there are "arcto-altaic element" (Gams and Hegi 1975) we consider as incorrect one as well as m/alp - arct (k) CIRCPOL (Jäger and Weinert in Rothmaler *et al.* 1988). *Astragalus alpinus* has never been found in Arctic regions (nor as was defined by Löve and Löve 1975:X - XI). Also, it does not belong to circumpolar species, or only in case if we consider the group of three species as one taxon.

We admit the claim that *Astragalus alpinus* belongs to the group of Eurasian species that have migrated into Rocky Mountains (as *Aster alpinus*). Due to long - term isolation of small populations they have been changed and some authors considered them as the species. The problem should be solved by American authors.

The name *Astragalus alpinus* is unambiguously accepted because it is typified as individual as presented by Linné (1753:760): "Habitat in Alpibus Lapponicis, Helveticis". In Europe the use of name has never been with difficulties.

Astragalus subpolaris Borissova et Schischkin in Gontscharov *et al.* Fl. URSS 12, p. 45, 1946

Syn. *Phaca lapponica* DC., *Astragalus arcticus* Bunge non Willd. nec Spreng., *A. lapponicus* (DC.) Schischkin in Krylov, *A. alpinus* L. subsp. *arcticus* Lindman nec Hultén.

Astragalus subpolaris belongs to the group of circumpolar holarctic species that seldom exceed the Holarctic region boundaries, more often in America than in Eurasia. It is observed close to

the Arctic ocean coast and adjacent islands and it prefers relatively dry and rocky biotops. We suppose that it belongs to old boreal diploids that are being confined. They survived glacial periods in refuges dispersed throughout Holarctic region. Thus, it is rather specific that in all geographic range it appears as morphologically uniform taxon as it is resulted from available information sources. The species can be distinguished from *A. alpinus* by differential traits as follows: calyx- teeth half as long as tube, corolla violet or purple violet, legume more or less deeply furrowed on the dorsal side, septum reaches up the half of legume length.

We do not know why *A. alpinus* has been subordinated as subspecies by the authors Lindman ((1923, Hultén 1968, Chater 1968 despite the fact that all specialists of the species *Astragalus* (De Candolla, Bunge, Borissova, and Schischkin) have determined it as the species. Also, Löve et Löve (1975) introduced the karyotaxonomic evaluation of species because besides clear morphological and differential traits there are historical and geographical evidence of completely different evolution of different gene pool under different environments. In this case, the identical chromosomes number should be only considered as superficial phenomenon that can suggest certain degree of taxonomic proximity but it can not alone account for the genetic unity of the taxon in all aspects. If we take into account the fact that *Astragalus subpolaris* had been evolved somewhere in boreal (the highest probability in Laurentinian) part of its recent geographic range and it persisted within the boundaries even during the most severe glacial periods (as well as nowadays it is not observed elsewhere the Arctic regions), we have to emphasize one of the most important differences of the species *A. subpolaris* to the species *A. alpinus* and *A. astragalinus*. *A. subpolaris* survived in more or less dispersed and isolated populations within the Holarctic region during unfavourable periods. It would be interesting to compare the karyotypes from different distant populations and to find out whether there has not been inner differentiation and eventually the species disintigration. This can not be excluded. This is the second important problem we intend to emphasize because we have no facts for solution. According the history, *A. subpolaris* is significantly differed from *A. alpinus*. We believe that the next taxonomic studies with modern methods will improve their differentiation.

Astragalus astragalinus (Hooker) Löve et Löve

Baz. *Phaca astragalina* Hooker. Syn. *Astragalus alpinus* L. subsp. *alaskanus* Hultén.

The species is considered as the youngest one in the group not only evolutionary but in taxonomic sense as well. We do not know why Löve and Löve (1975) have chosen one of the oldest homonyms (*Phaca astragalina* L., DC., etc.) to assign the populations from Northeastern Asia and Northwestern America. They are similar in pale flower colour and due to this especially American authors confused it with Eurasian species *Astragalus alpinus*. Hultén (1968: 655) played a major role in its propagation and introduction as the taxon in the cathegory of subspecies (*A. astragalinus* subsp. *alaskanus*) in the Flora of Alaska (1968:615) together with subsp. *alpinus* as the closest taxon.

According Hultén (1968) his type is originated "from Yukon". It is also tetraploid one as well as types that were analyzed by Russian authors from Northeastern Asia and were named as *A. alpinus* subsp. *alpinus*. Its extension on both continents will be considerably larger. We suppose that the taxon causing problems for some authors (esp. older ones) is substituted on American continent by *A. alpinus* L.

Nowadays, the taxon is not clearly defined. Due to the lack of materials (herbars, literature) we are not able to judge whether authors still keep old ideas about the existence of taxon *A. alpinus* group at relevant territory or they distinguish them as individual species. Hultén (1968) in his contribution wrote "...subsp. *alpinus* forms introgression with subsp. *alaskanus*...." what means that there exist individuals (or populations?) with particular chromosomes numbers 2n = 16 or 32. Yurtsev and Zhukova (1968:1536) stated:"...mozhno nablyudat' perekhodnuyu pologu mezhdu Omulganskim Verkhoyaniem i arkticheskim Verkhoyaniem, ..." while the authors have made inaccuracy in subsp. *arcticus*. Also Chater (1968: 115) mentioned: "The differences between the subspecies (i. e. *alpinus - arcticus)* are much more distinct in E. European Russia than in Fennoscandia where there is less satisfactory correlation of characters..." and he defined the problem just at the joining point of the species populations and in their diploid determination. It is required more detailed study of the three species to solve the problems mentioned above.

Astragalus penduliflorus Lam., Fl. Fr. 2, 636, 1778

Syn. *Phaca alpina* p.p., *Phaca alpina* Jacq. /Reuss (1853: 116)/, *Phaca alpina* Wulfen in Jacq. (Ascherson and Graebner 1909), *Phaca penduliflora* / Lam./ Gams (1975).

2n = 16 Uhríková, Schwarzová, Bernátová, W. Carpathians (Slovakia, Veľká Fatra Mts., Malá Pustalovčia Mt.)

Diploids are presented by Favarger (1949b), Trient in Wallis W. Alps, Delay (1965a) in Moore (1973), SW Alps.

In Slovakia the species is only known from carbonates (calcite) of Veľká Fatra Mts. and Belianske Tatry Mts. The data from Pieniny Mts. (Slovak part) have not been confirmed so far. The only one population from Západne Tatry Mts. (Dolina Smytna Pawlowski 1956: 562 - 567) has been observed at the territory of Poland. All analyzed European populations of *Astragalus penduliflorus* are diploid ones. The same diploid populations have been found in Siberia as well as in Far East, however they belong to other closely related species. Unlike the previous mountain species of the genus *Astragalus* (*A. alpinus* group), the geographic range of *Astragalus penduliflorus* is considerably limited only to European mountain chains of Tertiary orogeny (Pyrenees, Alps, Carpathians). Hess *et al.* (1970:566)

Fig. 2. Chromosomes of *Astragalus penduliflorus* (a), and microphoto (100x20) of *A. australis* (l.) Lam. 2n = 48 (b).

considered it as "Mittel - und Sudeuropeische Gebirgspflanze". Besides this, one exclave is known in Central Sweden. Gams and Hegi (1975) coincided with the opinion that *Astragalus penduliflorus* is only European species that is substituted in Siberia and Far East by three related species. Two of them *A. membranacens* (Fisch. in DC.) Bge. and *A. propinquus* Schischkin in Krylov are diploids (Krogulevich and Rostovtseva 1984 : 166). *A. sericeocanus* Gontscharov is the endemit observed in vicinity of Baikal lake and so far the number of chromosomes has not been known. Because the other species of the genus *Astragalus* of the subsection *Semilunaria* Gontscharov are diploid ones, it is likely that all species of the subsection in all Eurasian geographic range are uniform in ploidity - diploids.

The European species *Astragalus penduliflorus* had to colonize its Alpine geographic range during Tertiery as morphologically uniform, completely differentiated and stabilized species. *Astragalus penduliflorus* is considered as high-mountains, subalpine-alpine, Carpathian - Alpic - Pyrenees (Swedish) strictly calciphyt species. Siberian species are considered as species of lowlands, coast sands, pine and broad leaf forests (*A. sericeocanus, A. membranacens*) and due to this they are more similar to the Swedish populations of *Astragalus penduliflorus.* Only *A. propinquus* is observed in hazel tree stands in Far East and even up to spruce forests and subalpine ridges of Djungarian Alatau. More detailed study of the European species indicates how closely they are related or how are they differed each other.

Because the number of *Astragalus penduliflorus* sites is decreased from Eastern Alps through Western Carpathians to Eastern Carpathians, it seems that the species had migrated into Carpathians from Eastern Alps during postglacial periods and the way had led through the Middle Váh lime - dolomite mountains chains into Belianske Tatry Mts. and further to Romanian Carpathians.

The Swedish populations that are not confined to lime at all, they are originated from the same period "späteres Postglacial", however more likely they migrated from Central and Northern Alps where they had grown "...auf kalkamer bis kalkreier Unterlage" (Gams and Hegi 1975:1419-1420). In Sweden even "...auf von Waldbrenden verheerten Flächen, in Fohrenwäldern auf Sand...., selbst ruderal an Bahndämmen". This ecological variability seems to be unexpectidly high because the morhologic variability is very low or none (Gams and Hegi 1975:1420, : "....scheint in Europa nicht zu variiren....."). Therefore, the study of European populations of mentioned species, using modern taxonomic methods, could provide more detailed and exact results.

Astragalus australis auct. europ. excl. auct. ural.

A. australis (L.) Lam. nom. ambig. reic. (Janchen 1951 vide Nacgträge in Hegi 4/3, 1975: 1736)

2n = 32 Alps E.W, Pyrénées in 6 popul. (Favarger, Huynh, Kupfer in Favarger 1972)

2n = 48 Alps E-W in 4 popul. (Favarger 1972)

2n = 48 Uhríková, Bernátová, W. Carpathians, (Slovakia), Veľká Fatra Mts., Malá Pustalovčia Mt.

2n = 48 Uhríková, Dúbravcová, W. Carpathians, (Slovakia), Belianske Tatry Mts., Ždiarska vidla Mt.

Two analyzed populations from Belianske Tatry Mts. (Kochjarová 1991) and from Veľká Fatra Mts. with chromosome number 2n = 48 are considered as hexaploid ones and we intend to mention the reasons as follows:

1. if the most Eastern and Western West Carpathian populations are involved in hexaploids then there will be unlikely to observe any tetraploids in Western Carpathians

2. mentioned populations are historically younger and are originated from Eastern Alps populations and due to this *A. australis* had not migrated into other mountains through Western Carpathians.

Yurtsev and Zhukova (1968) claimed that European populations of *A. australis* were not related to Ural tetraploids as *A.uralensis* Litvinov and to the Central Asian mountains tetra - and hexaploids. The authors as first ones have criticised the old used idea about an uniform central Euro - Asian (Gams and Hegi 1975) and Euro - Siberian species (Hess *et al.* 1970,2:564:565) or uniform sm (alp - arct

/k/ EURAS elemente Jäger and Weinert in Rothmaler *et al.* 1988). *Astragalus australis* has become the European supramontane species with two karyologically differentiated populations. The populations require detailed analyses even due to their morphological variability as well as due to names used only in the territories as follows: Carpathians, Balkan, Alps, Apenins, Pyrenees. The karyological analysis of other European populations is so required as the karyological analysis of *A. krajinae* Domin, *A. bucegi* Prodan, *A. australis* var. *bucsecsi* (Jávorka) Gusuleac (see Gusuleac 1957), *A. incanus* Kotula, *A. helveticus* (F. X. Hartm.) O. Schwarz populations, or the other taxa in the range var. (Gams and Hegi 1. c.) because every name has to be only "nomen ambiguum" while we find out the linkage between its genetical content and morphological expression. Only the strict separation of older terciary tetraploid populations with their variability from younger autopolyploid hexaploids (the copulation of non-reduced to reduced gamets (the last interglacial - glacial) provides an opportunity to more reliable solutions of the problems *A. australis* auct. than systematists and phytogeographers have done before. Hopefully, in the cooperation with Austrian colleagues we are able to devote to mentioned problems in short future.

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