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Remarks on the karyogeography of *Myosotis alpestris* in Europe

Poznámky ke karyogeografii druhu *Myosotis alpestris* v Evropě

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Results are presented of the karyological study of selected populations of *Myosotis alpestris* F. W. Schmidt. Particular attention has been paid to a locality of phytogeographical importance, the Maly Kociol (the Malá Sněžná Jáma) Cirque, the Krkonoše Mts., Poland. In spite of the fact that the occurrence of the species is satisfactorily documented from this isolated locality (herb. PR, PRC), *Myosotis alpestris* has not been re-found there during the excursions in 1991-1993. On the basis of the analysis of pollen size in herbarium specimens from the Krkonoše, tetraploid chromosome number is estimated for them. In addition, chromosome numbers were analyzed in 13 populations of *Myosotis alpestris* F. W. Schmidt from the Jura (2n=48), the Alps (2n=24,48,72), the Carpathians (2n=48) and the Balkans (2n=24, 48). The chromosome number 2n=48 is published for the first time for the Slovakia and the Romania. In one population of *M. sylvatica* Hoffm. from the Krkonoše Mts. chromosome number 2n=18 was found, as well. The correlation between pollen size and ploidy level has also been studied and statistically significant differences were found between cytotypes belonging to different ploidy levels.

Dedicated to Professor Josef Dostál on the occassion of his 90th bithday

Introduction

The group of *Myosotis alpestris* is composed of a series of polyploid species confined to montane and to alpine sites in the whole of Eurasia, and extends marginally to eastern North America. *Myosotis stenophylla* Knaf represents the only exception - it occurs in steppe and serpentine sites of lower altitudes in Central Europe.

A population system of 'island' differentiation is characteristic of the group; suitable habitats are relatively small but scattered throughout large territories. Thus, a typical variation pattern developed as a result of the limited gene flow, selection pressure of the environment, and the genetic drift.

Morphologically more distinctly differentiated populations were, in some cases, described as separate taxa of various ranks (e.g., Grau 1964). Along with the morphological variation, an extensive ploidy variability is found in the group. The following chromosome numbers are reported in the literature: 2n=24, 48, 72(70), see Table 1 (the abbreviations used for countries are identical with those given in the Flora Europaea).

In Flora Europaea (Grau et Merxmüller 1972), nine species are included in the group *M. alpestris* (distributed in almost all high mountain ranges of Europe); *M. alpina* Lapeyr. (Pyrenees), *M. ambigens* (Béguinot) Grau (Central Apennines), *M. asiatica* (Vestergren)

Table 1. - Previously reported chromosome numbers of Myosotis alpestris F. W. Schmidt

Taxon	Locality	2n	Author
M. alpestris F.W.Schmidt	Hs: Pyrenees, Picos de Europa	24	Küpfer (1974)
,,	Hs: Pyrenees, Pico de Castanea	24	,,
,,	Hs: Sant Llorenc de Morunys	24	Bernal et Monserrat Marti (1985)
,,	He: Bernina Range	24	Scholte (1977)
,,	Au: Nord Tirol, Gschnitztal	n=12	Griesinger (1937)
,,	Au: Nord Tirol, Padasterjoch	n=12	,,
,,	Slovenia, Planika	24	Lovka et al. (1972)
,,	Slovakia, Vysoké Tatry	24	Murín et Paclová (1979)
,,	Slovakia, Belianske Tatry	24	Králik (1987)
,,	Po: Tatry Zachodnie, 6 loc.	24	Przywara (1978)
,,	Ucraine, Čorna hora	24	Pašuk (1987)
,,	Bu: Vitoša	24	Štěpánková (1993b)
subsp. *alpestris*	Ge: Alpen, Rotwand	24	Merxmüller et Grau (1963)
,,	It: Dolomiten	24	,,
,,	It: Pesaro, mt. Petrano	24	Grau (1964)
,,	Au: Karnische Alpen	24	,,
,,	Au: Lizum	24	,,
,,	Au: Ötztaler Alpen	24	,,
,,	Au: Lechtaler Alpen	24	,,
,,	Au: Samnaun, Kölner Hütte	24	,,
,,	Au: Karwendel, Scharfreiter	24	,,
,,	Au: Höllengebirge, Feuerkogel	24	,,
,,	Au: Plöckenpass, Valentintal	24	,,
,,	Ge: Allgäuer Alpen, Fiderepass	24	,,
,,	Ge: Steinernes Meer, Funtensee	24	,,
,,	Ge: Steinernes Meer, Viehkogel	24	,,
,,	He: Ardez	24	,,
var. *macrocarpa* Vestergr.	Au: Ötztal, Obergurgl	24	,,
var. *exscapa* (DC.) Roem. et Schult.	He: Zermatt	24	,,
,,	Ga: Mont Blanc	24	,,
subsp. *ambigens* (Béguinot) Grau	It: Abruzzen, Majella	24	,,
,,	It: Apennins central	24	Blaise et Cartier (1982)
subsp. *albomarginata*(Lindl.) Maire	Maroc, Haut Atlas	24	Blaise (1970)
subsp. *gallica* Vestergr.	Ga: Alps	24	Blaise et Roux (1973)
,,	Ga: High Savoy	24	Gadella et Kliphius (1970)
,,	Ga: Hautes Alpes, Col d'Allons	24	Grau (1964)
,,	It: Dolomites, Refuge Brenteii	24	Blaise (1981)
M. alpestris F. W. Schmidt	Br: England, Westmorland, 2 loc.	48	Elkington (1964)
,,	Br: Scotland, Ben Lawers	48	,,
,,	Ga: Pyrenees, Massif Pic de Ger	48	Blaise (1981)
,,	Po: Tatry Zachodnie, 2 loc.	48	Przywara (1978)
,,	Bu: Rila, Dodov vrch	48	Štěpánková (1993b)
subsp. *alpestris*	Au: Alpen, Grossglockner	48	Grau (1964)
,,	Ge: Allgäuer Alpen, Fellhorn	48	,,
,,	Ga: Alps Maritimes	48	,,
,,	Ga: East Pyrenees, Col de Puymorens	48	,,
,,	Ga: East Pyrenees, Segre valley	48	,,
,,	Ga: Dauphiné	48	Merxmüller et Grau (1963)

var. *elatior* Gaud.	Ga: Alps	48	Blaise et Roux (1973)
,,	Ga: Hautes Alpes, Mont Genevre	48	Grau (1964)
,,	Ga: Hautes Alpes, Col de Bayard	48	,,
,,	Ga: Hautes Alpes, Col de Lautaret	48	,,
,,	Au: Ötztal, Obergurgl	48	,,
subsp. *alpestris*	Au: Samnaun, Lazidgrat	72	,,
,,	Au: Samnaun, Zeblasjoch	72	,,
,,	Ge: Wetterstein, Schachen	72	,,
,,	Au: Rofan	70	,,
,,	Au: Samnaun, Riesenkopf	70	,,

Table 2. - List of localities and chromosome numbers counted by J.Štěpánková

No.	Locality	2n
	Myosotis alpestris F. W. Schmidt	
1.	France: the Jura Mts; top of the Mt. Montoiseau, 1550 m a.s.l., coll. by Štěpánek and Štěpánková, 1993.	48
2.	Switzerland: the Jura Mts.; top of the Mt. la Dôle, 1670 m a.s.l., coll. by Štěpánek and Štěpánková, 1993.	48
3.	Germany: Bayern, the Mt. Watzmann, scree near the chalet Kühroint Hütte, 1400 m a.s.l., coll. by Štěpánek and Štěpánková, 1991.	24
4.	Austria: Bad Ischl, the Mt. Dachstein, slope above the lake Gosausee, 2200 m a.s.l., coll. by Štěpánková 1990.	48
5.	Austria: the Mt. Schneeberg, W of the Neunkirchen, 1700 m a.s.l., coll. by Slavík, 1991.	72
6.	Slovakia, the Nízké Tatry Mts.: scree near the chalet Štefánikova chata, 1950 m a.s.l., coll. by Štěpánek et Štěpánková, 1990.	48
7.	Slovakia: the Belianske Tatry Mts.: SW slope of the Mt. Hlúpy vrch, 1730 m a.s.l., coll. by Krahulcová, Štěpánek and Štěpánková, 1992.	48
8.	Slovakia: the Belianske Tatry Mts.: S slope of the Mt. Belianska kopa, 1810 m a.s.l., coll. by Krahulcová, Štěpánek and Štěpánková, 1992.	48
9.	Slovakia: the Červené vrchy Mts.: in the saddle Tomanovské sedlo, 1780 m a.s.l., coll. by Štěpánek and Štěpánková, 1992.	48
10.	Slovakia: the Červené vrchy Mts.: SW slope of the Mt. Kasprov vrch, 1610 m a.s.l., coll. by Štěpánek et Štěpánková, 1992.	48
11.	Poland: the Tatry Zachodnie Mts.: W slope of the Mt. Temniak, 1800 m a.s.l., coll. by Štěpánek and Štěpánková, 1992.	48
12.	Romania: the Rodna Mts.: top of the Mt. Corongis, 1950 m a.s.l., coll. by Šourková and Štěpánková, 1981.	48
13.	Bulgaria: the Rila Mts.: NW slope of the Mt. Maljovica, 2100 m a.s.l., coll. by Štěpánková, 1985.	48
	Myosotis sylvatica Hoffm.	
14.	Poland: the Krkonoše Mts.: in the gorge of Maly Kociol, 1320 m a.s.l., coll. by Štěpánková 1993.	18

Table 3. - Mean (x), standard deviation (SD) and result of Scheffé test (P) for length of pollen grains observed on populations of the species *M. alpestris* F. W. Schmidt (each column of asteriks represents one homogeneous group)

2n	no.	locality	N	x	SD	Scheffé test		
24	3.	Alps	100	6.9	0.13	*		
24	–.	Vitosha Mts.	100	6.9	0.14	*		
48	1.	Jura Mts.	100	7.9	0.10		*	
48	7.	Tatra Mts.	100	7.9	0.13		*	
48	13.	Rila Mts.	100	7.9	0.14		*	
?	–.	Krkonoše Mts.	100	7.9	0.13		*	
72	5.	Alps	100	9.0	0.15			*

Schischkin et Serg. (Arctic and subarctic Russia, eastern North America), *M. corsicana* (Fiori) Grau (Corse), *M. gallica* Vestergren (France - southeastern part), *M. lithospermifolia* (Willd.) Hornem. (Asia Minor), *M. stenophylla* Knaf (central and eastern Europe), and *M. suaveolens* Waldst. et Kit. ex Willd. (Balkan Peninsula).

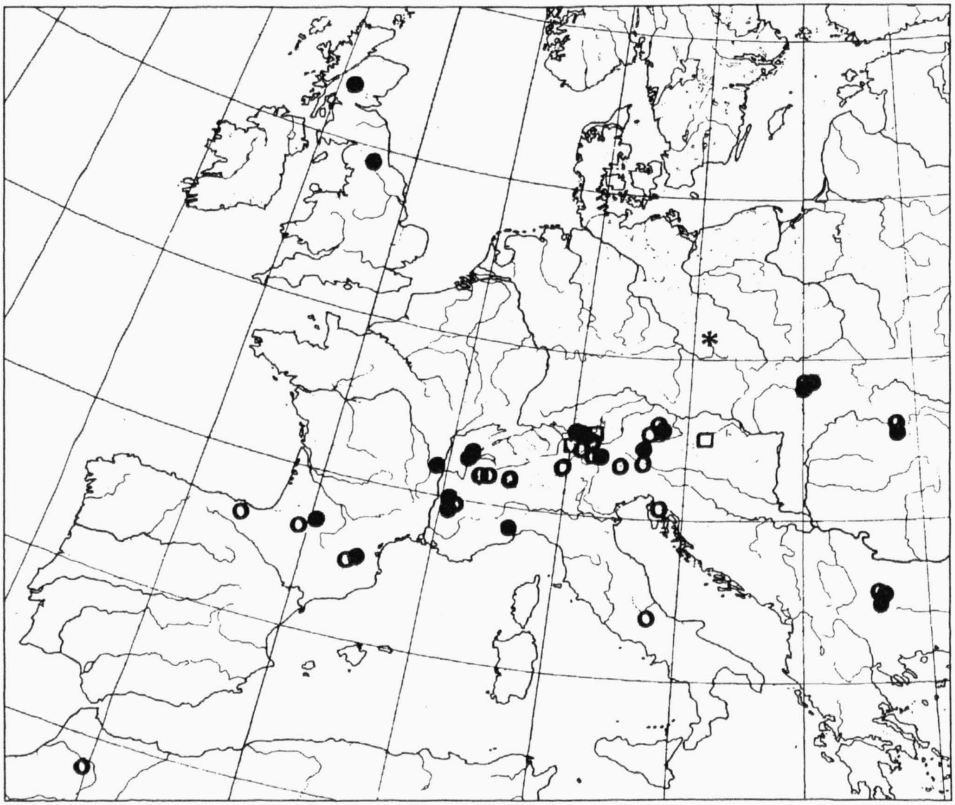

Fig. 1. - Distribution of the cytotypes of *Myosotis alpestris* F. W. Schmidt ○ - 2n=24; ● - 2n=48; □ - 2n=72; * - 2n= ca 48, locality in the Krkonoše Mts., Malá Sněžná jáma (Maly Kociol).

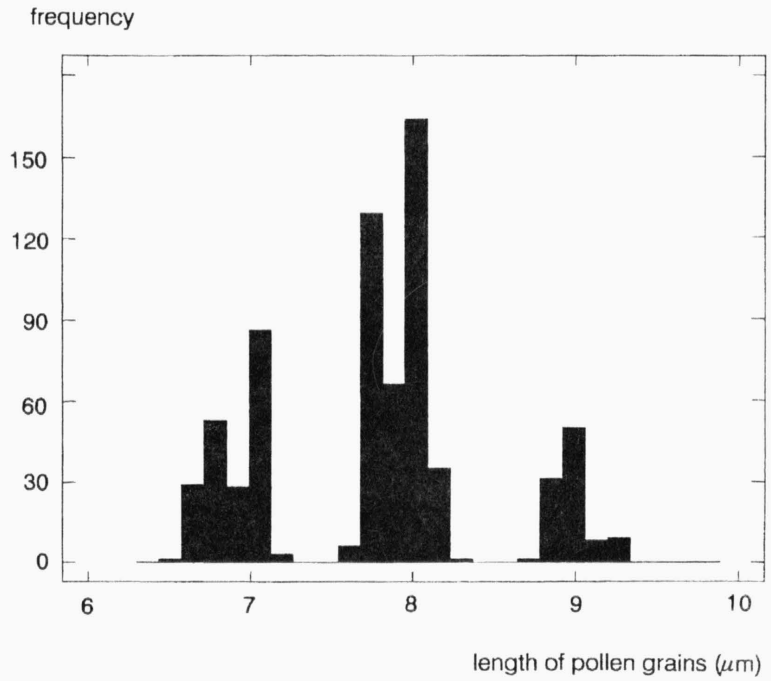

Fig. - 2. - Frequency of the values of pollen length observed in the populations of *Myosotis alpestris* F. W. Schmidt.

The distribution range of *M. alpestris* F. W. Schmidt itself, is formed by larger or smaller areas that are more or less separated in the mountain ranges of Great Britain, the Pyrenees, the Alps, the Apennines, the Carpathians and the Balkans (Meusel, Jäger, Rauschert et Weinert 1978). From the viewpoint of the overall distribution, there is an important isolated locality in the Krkonoše Mts. (the Malá Sněžná Jáma [Maly Kociol] Cirque).

In what follows, the present situation at the only locality in the Krkonoše is evaluated, and results of karyological studies of other selected populations from the Jura, the Alps, the Carpathians and the Balkans are summarized in order to document in more detail the distribution of individual cytotypes within the geographical range of *M. alpestris* F. W. Schmidt.

Material and methods

Chromosome counts were made on root tips of the material cultivated in the experimental garden of the Institute of Botany at Průhonice. The material in cultivation has its origin in 14 population localities given in Table 2. Chromosome numbers were determined at the mitotic metaphase in somatic cells of root tips. The material was pretreated with a saturated solution of bromnaphtalen, fixed in ethanol-acetic acid (3:1). The squash method and staining by lacto-propionic orceine were used.

Pollen size measurements were made on 10 individuals in each of 7 populations (see Table 3). From each individual 10 anthers were used (randomly selected from flower buds), from each anther 10 pollen grains were measured and the arithmetical means

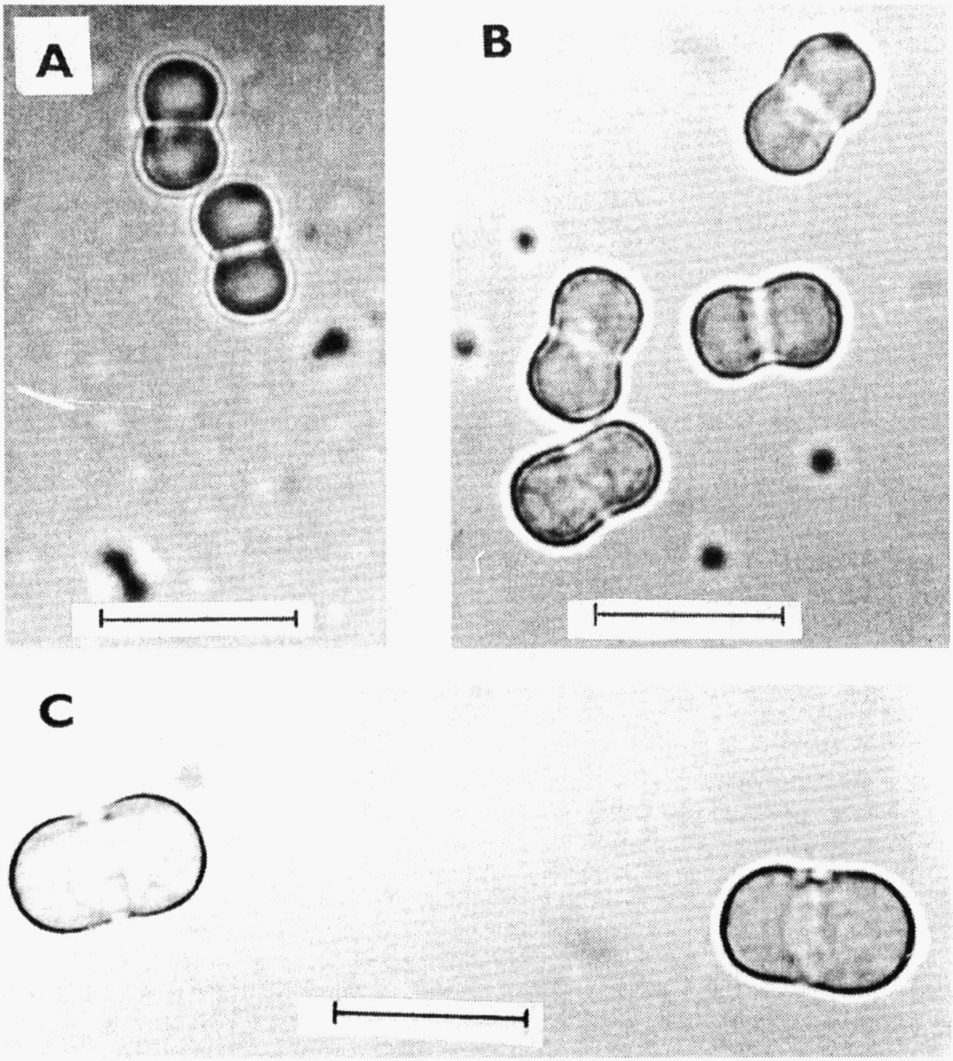

Fig. 3. - Pollen grains of *Myosotis alpestris* F. W. Schmidt: A - 2n=24; B - 2n=48; C - 2n=72. Scale bar = 10 µm.

obtained for each anther were used for statistical analyses. Pollen length were measured on herbarium material. Dried flower buds were macerated in boiling water and subsequently the pollen grains were isolated and measured.

Results

Thirteen populations of *M. alpestris* and one population of *M. sylvatica* Hoffm. were examined karyologically. A list of localities from the Jura, the Alps, the Krkonoše, the

Fig. 4. - Root tip metaphase - *Myosotis alpestris* F.W. Schmidt, 2n=48. Locality: Slovakia, the Červené vrchy Mts., Mt. Kasprov vrch. Scale bar = 10 μm.

Carpathians and the Balkans, and chromosome counts ascertained is given in Table 2.

The isolated locality in the Malá Sněžná Jáma Cirque has been repeatedly visited in the course of three years (1991-1993). However, the population of *M. alpestris* has not been traced at the locality, in spite of the fact that in the past the occurrence of the species was reported to be abundant (e.g., Schustler, 1918, gives it as common on the basaltic scree in lower part of the Malá Sněžná Jáma Cirque and on small alpine meadows developed on lower rocky ledges). At the present time, only two species of *Myosotis* were collected at the locality, *M. nemorosa* Besser and *M. sylvatica* Hoffm. Nevertheless, a remainder of the original population of *M. alpestris* might have persisted at some inaccessible parts of the basaltic gorge, which would correspond to the case of *Saxifraga nivalis* (cf. Jeník 1961).

As a hybrid between *M. alpestris* and *M. sylvatica* (*M.* x *kablikiana* Domin, 1939) was described from the Krkonoše locality, the population of *M. sylvatica* was examined karyologically in a considerable detail. In 30 plants of *M. sylvatica* at the locality, a uniform chromosome number of 2n=18 was ascertained. At the same time, the course of meiosis of *M. sylvatica* did not exhibit any irregularities indicating the present hybridogeneous status. It should be added that the authentic specimens labeled (in the herb. PR) as the type of *M.* x *kablikiana* by Domin correspond in all their characters to the typical *M. sylvatica* (presence of numerous hooked hairs on the calyx, pollen diameter about 6 μm).

In view of the fact that diploid, tetraploid and hexaploid cytotypes were found in the material studied, a correlation between the pollen size and ploidy level was tested in order to use this criterion for assessment the ploidy level in herbarium specimens. (Such a correlation is known and used in the groups of *M. sylvatica* and *M. palustris*.) Results are summarized in Table 3. These clearly indicate direct correlation between pollen size and ploidy level in *M. alpestris*.

Differences between different cytotypes are analyzed with ANOVA and Scheffé range test, which allows the selection of homogeneous groups within analyzed observation. The

pattern of measurements of the length of pollen grains is obvious from the Figs. 2, 3. It follows from the above analyses that there are statistically significant differences among samples of the diploid, tetraploid and hexaploid level.

However, pollen size differences among ploidy levels in *M. alpestris* are not so pronounced as those known in the groups of *M. sylvatica* (Grau 1964) and *M. palustris* (Štěpánková 1993a). In spite of this fact, the correlation can be used for the examination of herbarium specimens, especially in cases when more plants from one population are available.

Discussion

The locality of *M. alpestris* in the Krkonoše Mts., in the Malá Sněžná Jáma Cirque, has been known since the last century (numerous herbarium specimens), later it was mentioned in the literature (e.g. Domin 1939, Jeník 1961, Šourek 1969). It is the only documented occurrence of *M. alpestris* not only in the Krkonoše Mts. but also in Europe north of the Alps (with the exception of British localities). During the revision of the herbarium material deposited in PR and PRC, eighteen specimens of *Myosotis alpestris* from the Malá Sněžná Jáma Cirque were found, one additional plant was studied in the herbarium G. Most specimens were collected by Josephine Kablik (9), the other collectors are Fiek, Gottstein, Schustler, Wimmer, Tausch, Domin and E. Hejný. The period of collection dates is relatively short, 1885 - 1914 (but not all collectors gave the collection dates and some of them, e.g. Kablik and Gottstein, surely collected the plants much earlier). I have failed to find voucher specimens documenting literature reports on the occurrence of *M. alpestris* after 1914 (even though the herbarium of J. Šourek, for instance, should be deposited in PR).

Morphology of the herbarium specimens studied corresponds to that of the form called var. *elatior* (Béguinot) Grau by Grau (1964). Calyces of this form lack hooked hairs. An interesting fact is that none of the specimens revised has ripe fruits.

As no living plants of the Krkonoše *M. alpestris* were available, the ploidy level was estimated on the basis of the correlation between the pollen size and ploidy level. From the data in Table 3, it is obvious that *M. alpestris* from the Krkonoše Mts. most probably belongs to the tetraploid level, which is in agreement with the monographic data by Grau who reports only tetraploid chromosome number for his var. *elatior* (see Table 1). The other tetraploid populations from the Jura, the Alps, the Carpathians and the Balkans are also characterized by calyx indumentum composed of straight hairs (without hooked hairs). On the other hand, the presence of hooked hairs on the calyces are characteristic of the diploid populations of *M. alpestris*.

The correlation between pollen size and ploidy level in the group *Myosotis alpestris* has also been studied by Geitler (1936) and Grau (1964).

The results presented in this paper, are in accordance with those given by Geitler (1936). This author, analyzing the volume of pollen grains of the different cytotypes of *Myosotis alpestris* group, found out the following pattern of its volume: 10 : 11.3 : 15-16 (cytotypes n=12,24,36). But, it should be noted, that taxonomic determination of particular cytotypes given in his work, is not quite clear. The cytotype n=12 is assigned as *M."alpestris"* (*sylvatica*?); the cytotype n = 24 is determined as *M. alpestris* var. *lithospermifolia* Hornem.? (the locality given for analyzed plants is botanical garden in Vienna). Finally, the cytotype n=36 is determined as *M. alpestris* var. *suaveolens* (Waldst. et Kit.) Beck. The locality - Scheiblingsteinplateau bei Lunz, Nieder-Österreich, however, would represent a very extreme occurrence for this taxon, growing in Balkan Peninsula.

On the other hand, Grau (1964) arrived at rather different results. This author ascertained the overlapping ranges of pollen size between different cytotypes. For this reason, Grau mentioned, that in the case of *M. alpestris*, the pollen size could not be used for approximation of ploidy level.

The likely tetraploid level of the Krkonoše population is in accordance with the general distribution character of individual cytotypes within *M. alpestris* s. l. Grau (1964), who studied the cytotaxonomy of this group in considerable detail, found exclusively diploid forms in the southern part of its range. *Myosotis ambigens* (Béguinot) Grau from the Apennines is diploid, as is the case of *M. suaveolens* Wald. et Kit. ex Willd. from the Balkans and *M. lithospermifolia* (Willd.) Hornem. from Asia Minor (e.g., Grau 1964, Štěpánková 1993b).

On the contrary, in northern parts of the geographical range of *M. alpestris* s. l., the tetraploid cytotype seems to prevail. For instance, the other representative of the *M. alpestris* group in the Czech Republic, *M. stenophylla* Knaf, is also tetraploid (Grau 1964). Only tetraploid cytotype of *M. alpestris* s. str. is known to occur in England and Scotland, as well. The tetraploid chromosome numbers ascertained by the author in the material of *M. alpestris* from the Polish and Slovak Carpathians (see Fig. 4.) also indicate the dominance of the tetraploids in this part of the range.

The territory of the Alps is characterized by the sympatric occurrence of all the three cytotypes known in *M. alpestris*. There is a certain tendency in the distribution of the tetraploids to be more common in the outer regions of the Alpine chain (see Fig. 1).

It can be concluded that the Krkonoše population of *M. alpestris* probably belonged to the tetraploid cytotype. However, the population must have undergone a substantial size reduction, or even a total extinction.

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Souhrn

V této studii jsou shrnuty výsledky karyologického studia vybraných populací druhu *Myosotis alpestris* F. W. Schmidt. Byly stanoveny chromosomové počty u 13 populací druhu *M. alpestris*, pocházejících z pohoří Jura (2n=48), z Alp (2n=24, 48), Karpat (2n=48) a Balkánu (2n=24, 48). Chromosomový počet 2n=48 je poprvé publikován pro Slovensko a Rumunsko.

Zvláštní pozornost byla věnována fytogeograficky významné lokalitě tohoto druhu v Krkonoších - Malý Kociol (Malá Sněžná jáma). Ačkoliv je tento druh z této krkonošské lokality v herbářích PR a PRC relativně bohatě dokladován, v rámci exkurzí uskutečněných v r. 1991-1993 se nepodařilo výskyt druhu *M. alpestris* v Malé Sněžné jámě již potvrdit.

Na základě statistického vyhodnocení velikosti pylu získaného z herbářových položek, byl pro tuto populaci odhadnut tetraploidní chromosomový počet, čemuž odpovídalo i odění kalicha, typické pro tento ploidní stupeň.

Vzhledem k tomu, že se v průběhu cytologického zpracování vybraných populací podařilo získat di-, tetra- i hexaploidní cytotypy, mohla být testována korelace velikosti pylových zrn a stupně ploidie. Výsledky studia velikosti pylových zrn jsou shrnuty v tab. 3. Z této tabulky je zřejmá úzká korelace mezi délkou pylových zrn a ploidní úrovní.

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