

Cytotype distribution in *Empetrum* (Ericaceae) at various spatial scales in the Czech Republic

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Running head: Cytotype distribution in *Empetrum*

Ploidy levels in *Empetrum* (crowberry) from the Czech Republic were estimated by flow cytometry to examine cytotype distribution patterns at large (within the country), medium (within mountain ranges) and small (within particular localities) spatial scales. Diploid, triploid, and tetraploid individuals were found. Triploids are reported from Central Europe for

the first time; they occurred quite frequently in the Krkonoše Mts. Exclusively diploid plants were observed in three mountain ranges (the Krušné hory Mts., the Labské pískovce Mts., the Adršpašsko-teplické skály Mts.), exclusively tetraploids were observed in the Jeseníky Mts., and both cytotypes were observed in the Šumava Mts., the Jizerské hory Mts. and the Krkonoše Mts. Except for the latter mountain range, diploids and tetraploids were always found in different habitats. Spatial isolation is supposed to be the main barrier preventing cytotype mating. A mosaic-like sympatric occurrence of different cytotypes was demonstrated in the Krkonoše Mts., where a patchwork of peat bogs and rocky places exists. Eight of 11 localities studied there were inhabited by diploids and tetraploids (five localities), diploids and triploids (one locality) or all three ploidy levels (two localities). Diploid and triploid plants occasionally intermingled at 30 x 30 cm. Flower sex in crowberries is strongly associated with ploidy level: diploids usually had unisexual flowers, the tetraploids bore exclusively bisexual flowers. However, some diploid plants with hermaphrodite flowers occur in one population in the Krkonoše Mts.

Key words: Czech Republic, flow cytometry, ploidy level, spatial variation, sympatry, triploid

Introduction

Empetrum L. (crowberry) is a small group of closely related, dwarf, evergreen, shrubby species distributed predominantly in boreal and temperate regions of both hemispheres (Good 1927). Five *Empetrum* taxa are usually recognized (Löve 1960), two of which are native to Europe: *E. nigrum* L. and *E. hermaphroditum* Hagerup (Favarger et al.

1959, Velluti et al. 1995). The former is dioecious and diploid ($2n = 2x = 26$), the latter is tetraploid ($2n = 4x = 52$) bearing mostly bisexual flowers (Webb 1972). Their taxonomic status is, however, still much debated and some authors prefer the rank of subspecies: *E. nigrum* subsp. *nigrum* and subsp. *hermaphroditum* (Hagerup) Böcher (Meusel et al. 1978).

The bisexual crowberries were apparently unknown until the beginning of the 19th century (Willdenow 1805). Hagerup (1927) was the first to conduct karyological studies of both unisexual and bisexual individuals and found that hermaphrodites from Eastern Greenland had twice as many chromosomes as unisexuals from the Faeroes. He suggested that the bisexual tetraploids represent a “gigas form” derived from dioecious diploids and recognized them as a separate species.

In addition to the flower sex and ploidy level, several differences in morphological characters between unisexual and bisexual crowberries have been observed (Boratyński & Vera de la Puente 1995, Danielsson 1988, Marklund 1939, Warner & Chinnappa 1990, Zarzycki & Guzik 1975). Generally, the unisexuals have prostrate, brown-red, often rooting stems, linear-oblong leaves, 3-5 times as long as wide, with parallel margins, drupes with 8-9 pyrenes, and small pollen tetrads (22 - 34 μm in diameter), flower buds, flowers and fruits. The bisexuals differ by shorter, usually green, not rooting stems that often form dense tufts, leaves mostly 2-3 times as long as wide with more rounded edges, drupes containing usually 6-7 pyrenes, and pollen tetrads reaching 33 - 48 μm in diameter. However, it has often been emphasized that there is considerable overlap in diagnostic features and their separation in the vegetative stage is difficult (Bell and Tallis 1973).

Both morphotypes are almost circumpolar in distribution (Meusel et al. 1978). The European area of unisexuals extends from northern Spain (a few isolated stands), the northern Apennines and the Alps throughout Central and Western Europe (incl. British Islands and Iceland) to Norway, Sweden and Finland with the northernmost limit at ca. 68° of latitude.

The bisexuals occur predominantly in the mountainous areas in South-western, Central and Eastern Europe (the Pyrenees, the Alps, the Carpathians), and in Scotland, Iceland and the Scandinavian countries (Bell and Tallis 1973, Boratyński & Vera de la Puente 1995, Danielsson 1988). Generally, they grow further northwards and reach higher altitudes than their unisexual relatives. Four main habitats are occupied by *Empetrum* in Europe: peat bogs, heathlands, sandy dunes and bare, rocky places (Good 1927, Zarzycki & Guzik 1975). The bisexuals prefer exposed rocky summits and slopes, whereas the unisexuals mostly occur on peat bogs, mires and coastal dunes. However, on the basis of field observations and study of herbarium vouchers, Boratyński (1986) concluded that plants with unisexual flowers can sporadically grow also at the top of rocks in the Polish part of the Sudety Mts. Diploid unisexual crowberries colonising rocky slopes and tetraploid bisexual individuals on peat bogs and sandy seashores were also found in Scandinavia (Suda 2002).

Only very limited information concerning potential sympatric growth of plants with different ploidy levels and their distribution pattern on fine spatial scales is available. Some authors have suggested that ecological differentiation between cytotypes is an efficient barrier (Velluti et al. 1995). However, records of both taxa from apparently identical habitats within a geographically limited area (e.g. a small mountain range) indicate that their niches can overlap (Boratyński 1986, Suda 2002). Mixed populations containing diploid and tetraploid plants growing in close proximity were found by the latter author in Norway.

To give further insight into cytotype distribution at large (in the Czech Republic), medium (within mountain ranges) and fine (within localities) spatial scales was the main goal of our investigation. Potential ecological differentiation, sympatry, hybridization, and flower sex variation were of particular interest.

Material and Methods

Mature shoots of *Empetrum* were collected from 37 localities in the Czech Republic during 2000 – 2002. The sampling strategy was as follows: 1) exploration of all major areas where crowberries had been reported to describe cytotype distribution at a large spatial scale; 2) inclusion of various habitats within each area to study the habitat preference of individual ploidy levels; 3) sampling attempting to cover the morphological variation observed at each locality to assess the potential sympatric occurrence of various cytotypes at a fine spatial scale. The number of samples per locality varied from one to 54 reflecting both the locality size and the abundance of crowberries to avoid the population decline or destruction. Each sample comprised shoots from all *Empetrum* individuals growing at area of approximately 0.1 m² (0.3 x 0.3 m); the total number of samples was 537. If possible, the sex of flowers or the presence / absence of filaments on a drupe were recorded. The shoots were placed in wet plastic bags and stored at 4°C, and their ploidy level was determined within 48 hours. Herbarium vouchers are kept in PR with duplicates in the private herbarium of the first author.

The ploidy level of samples was estimated by flow cytometry. Young leaves of *Empetrum* were chopped together with internal reference standard (*Zea mays* cv. CE-777, $2C = 5.43$ pg; Lysák & Doležel 1998) in one ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5 % Tween 20). The sample was filtered (42 µm nylon mesh), centrifuged (150 g for five min), the supernatant was removed, and the pellet was resuspended in 100 µl of fresh Otto I buffer. After incubation, one ml of Otto II buffer (0.4 M Na₂HPO₄ · 12 H₂O) supplemented with DAPI (4 µg / ml) was added, and the fluorescence intensity was recorded using Partec PA II flow-cytometer. Karyologically-counted diploid ($2n = 26$) and tetraploid ($2n = 52$) *Empetrum* from the Šumava Mts. and the Krkonoše Mts., respectively, were used to define the ratio

between relative DNA content of the internal standard and crowberry cytotypes. All *Empetrum* shoots from each sample were measured in order to detect potentially mixed samples (containing two or more ploidy levels). Mature leaves from up to five shoots were analysed simultaneously. Our previous analyses proved that flow cytometry enables reliable detection of minority crowberry cytotype presented even in a low proportion (about 10 %). Due to very low mitotic activity in crowberry leaf tissues (only negligible peak corresponding to nuclei in G2 phase) and the lack of endopolyploidy, potential bias in ploidy level estimation can be excluded. Nevertheless, the ploidy level of each shoot was separately re-analyzed in mixed samples. The fluorescence of at least 5 000 nuclei was recorded and the coefficient of variance of *Empetrum* peaks varied from 1.82 to 3.15 %.

Results

Diploid (315 samples), triploid (10 samples), and tetraploid (209 samples) plants were found; three samples contained both diploid and triploid individuals. Ratios between nuclei fluorescence intensity of internal reference standard and the samples were 2.79-2.90, 1.88-1.95, and 1.41-1.47 for diploids, triploids, and tetraploids, respectively. Intra-cytotype variation was very low and did not exceed 4.3 %. Table 1 summarizes the habitats and abundance of all cytotypes in different mountain ranges. Plants of both ploidy levels were recorded in three mountains (the Šumava Mts., the Krkonoše Mts. and the Jizerské hory Mts.), exclusively diploids grew in the Krušné hory Mts. and in two sandstone regions (the Labské pískovce Mts., the Adršpašsko-teplické skály Mts.), and exclusively tetraploids were found in the Jeseníky Mts. (Fig. 1). Non-mixed diploid populations were observed on mountainous peat bogs (17 localities), rarely also on bare sandstone rocks in lower altitudes (3 localities);

non-mixed tetraploid populations occurred on rocky places (5 localities) and on peat bogs (4 localities). The altitudinal range of single-cytotype localities varied from 160 m (the Labské pískovce Mts.) to 1250 m (the Šumava Mts.) and from ca. 950 m (the Jizerské hory Mts.) to 1420 m (the Jeseníky Mts.) for diploid and tetraploid cytotype, respectively. Peat bogs with sympatric occurrence of both taxa were located between 1210 m and 1430 m of altitude in the Krkonoše Mts. In the Šumava Mts. and the Jizerské hory Mts., both cytotypes occupied different habitats and no sympatric growth was found.

Cytotype distribution in the Krkonoše (=Giant) Mountains deserves a detailed description. Only three of 11 localities contained exclusively one cytotype: diploids grew on peat bog between Petrovka and Špindlerovka chalets in the Polish part of the mountains and tetraploids were recorded on Mumlavská louka and Černohorská rašelina (incl. small peat bog along Stříbrný potok brook) peat bogs. Sympatric occurrence of different cytotypes was observed at the remaining sites: diploids and tetraploids co-occurred in five peat bogs, diploids and triploids were observed on one peat bog, and two peat bogs were inhabited by all three ploidy levels. Cytotype distribution pattern of 26 samples growing on peat bog near Mumlavská hora hill is shown at Fig. 2A. Locality no. 31 (small peat bog between Vosecká bouda chalet and the hill of Luboch) is also of particular interest. Three intermingled samples (collected at area 30 x 30 cm) containing diploid and triploid individuals were found there (Fig. 2B). A representative result of the analysis of relative DNA content of nuclei isolated from the diploids and the triploids (mixed sample) is shown at Fig. 3. Generally, tetraploid cytotype prevailed in the Krkonoše Mts., particularly in the eastern part (62.8 % of 207 samples). Diploids were represented by 64 samples (30.9 %), triploids by 10 samples (4.8 %), and three mixed samples accounted for 1.5 %.

Triploids were detected at three localities, two of which contained also diploid and tetraploid individuals. No tetraploid cytotype has been found at loc. 31, however, there is a

chance that the ploidy level also occurred there or on adjacent small peat bogs. All triploids were apparently sterile and - apart from the only exception – no fruit was found. One triploid plant from population no. 30 yielded a single drupe, however, without any viable seeds (both embryo and endosperm were degenerated). No intermediate relative nuclear DNA content between the diploids and the triploids or between the triploids and the tetraploids indicating the existence of back-crosses was observed.

The sex of the flowers was strongly associated with the ploidy level. All tetraploids collected in an appropriate phenological stage were bisexual, and all diploids from the Šumava Mts., the Krušné hory Mts. and the Jizerské hory Mts. were unisexual. The sex of diploids from the Labské pískovce Mts. and the Adršpašsko-teplické skály Mts. remains unknown as these plants were sterile. Most diploid crowberries from the Krkonoše Mts. had unisexual flowers, however, one sixth of samples from population no. 31 contained at least one shoot bearing drupes with stamen remnants.

Discussion

Flow cytometry was found to be very powerful for ploidy estimation in crowberries. This group is rather difficult to study using conventional cytological methods and only limited number of individuals has been chromosome-counted (reviewed in Velluti et al. 1995). Strong and reliable association between chromosome number of crowberries and relative fluorescence of isolated nuclei in flow cytometric analyses was confirmed in previous study (Suda 2002). In a similar way, flow cytometry has also proved successful in ploidy level and nuclear DNA amount estimation in other species of the *Ericaceae* (Costich et al. 1993, Suda & Lysák 2001).

The distribution of diploids and tetraploids in the Czech Republic generally agreed with the previous maps for *E. nigrum* and *E. hermaphroditum* (Slavík 1990), even though some corrections are necessary. The tetraploids are very rare and under threat of extinction in the Šumava Mts., and occur only in the south-eastern part. In contrast to previous records (Slavík 1990), no bisexual tetraploids were confirmed in the Krušné hory Mts. Also some other authors expressed doubts about their occurrence (Čvančara 1990). For the first time were crowberries recorded in the Labské pískovce Mts. where diploid plants grew very rarely on sandstone rocks. Although both taxa have previously been recorded from the Jeseníky Mts. (Čvančara 1990, Slavík 1990), exclusively the tetraploid cytotype was confirmed in our study. However, only 16 individuals from this area were included in the analyses and more extensive sampling especially from peat bogs is necessary. The tetraploids generally grew at higher altitudes than the diploids, in concordance with the observations of Boratyński (1986), who presented altitudinal maps of both cytotypes in the Polish part of the Sudety Mts. Also Hultén and Fries (1986) suggested that unisexual *E. nigrum* avoid the mountains in Northern Europe.

The occurrence of triploid crowberries in Central Europe (the Krkonoše Mts.) is reported here for the first time. This cytotype has been hitherto known only from Norway, where a mixed population of diploid and triploid individuals was found in the Jotunheimen National Park (Suda 2002). We expect that detailed screening for ploidy levels would reveal triploid crowberries also in other geographic regions where diploids and tetraploids co-occur.

Triploids are apparently completely sterile and only a single drupe without viable seeds has been found so far. No remnants of stamens were observed on the fruit, however, checking of more plants, preferably in the flowering stage, is necessary for sex determination. Highly reduced reproductive fitness of triploid hybrids has been demonstrated in *Vaccinium* of the same family (Vorsa and Ballington 1991). The triploids probably originate from hybridization between diploids and tetraploids. This assumption is supported by the fact that

they only were recorded from mountain range where diploid and tetraploid individuals grew in sympatry. Similarly, all three ploidy levels were found in Jotunheimen National Park in Norway (Suda 2002). The cytotype distribution pattern at fine spatial scale in the Krkonoše Mts. indicates that the diploids presumably acted as maternal plants and the tetraploids as pollen donor. Diploid individuals were found at all three localities where the triploids occurred and both cytotypes usually grew in close proximity or even intermingled. Tetraploids were confirmed at two of these sites, however, they were apparently missing at loc. 31. Nevertheless, they might have occupied small peat bogs in the neighbourhood. As *Empetrum* ranks among the anemophilous species (Bell and Tallis 1958), it is plausible that pollen tetrads can be transferred over distances of several dozens or even hundreds of metres. Another evidence for diploid crowberry as a maternal parent was a detection of both diploid and triploid seeds in some drupes collected on diploid shoots (Suda, unpubl.). The fusion of non-reduced and reduced gamete of diploids would be an alternative mode of triploid cytotype genesis. Some previous data (mixed populations of the diploids and the triploids, diploid plants bearing fruits with 2x and 3x seeds) might support such hypothesis. However, no triploid *Empetrum* has been found among 247 samples from mountain ranges where either only diploids occurred (the Krušné hory Mts.) or the diploids were spatially isolated from the tetraploids (the Šumava Mts. and the Jizerské hory Mts.).

Different ecological preferences of *E. nigrum* and *E. hermaphroditum* has been repeatedly recorded (Zarzycki & Guzik 1975, Velluti et al. 1995). The unisexuals grow predominantly on sandy soils and in pine forests in the lowlands, and in the mountainous peat bogs whereas the bisexuals prefer more severe habitats, mainly windy rocks at higher altitudes. However, karyological analyses were only scarcely conducted. To avoid this limitation, only crowberries with known ploidy level were included to our study. The tendency of both cytotypes to occur in different habitats was confirmed. If peat bogs and

rocky places are spatially separated, each habitat is occupied exclusively by diploids and tetraploids, respectively. Thus, adaptation to different habitats may play the major role in blocking inter-cytotype hybridization in *Empetrum*. Although the tetraploids were found also on a peaty meadow in the Šumava Mts., the discovery is not surprising because this locality differed markedly from all other peat bogs there. On the other hand, a mosaic of rocky places and peat bogs is developed in the Krkonoše Mts. The cytotype distribution reflects this condition, and mixed populations containing various cytotypes prevail there (especially in higher altitudes). Diploid and tetraploid crowberries sharing the same habitats were also observed in the north-eastern Alps (Teppner 1987) and in Norway (Suda 2002). In these regions, diploid plants grew rarely among more abundant tetraploids on wind-exposed mountain slopes at altitudes of ca. 2000 m and 1100 m, respectively. Thus, sympatric occurrence of both cytotypes seems to be restricted to higher altitudes.

A strong association between the ploidy level and the sex of the flowers was confirmed (diploids unisexual, tetraploids bisexual). However, some diploid individuals from the Krkonoše Mts. were hermaphrodites, which can indicate labile sex expression under some conditions. Deviations from the standard pattern were previously recorded also by Blackburn (1938) and Favarger et al. (1959) who had found *E. nigrum* with bisexual flowers and tetraploid *E. hermaphroditum* bearing exclusively female flowers, respectively.

Our large population samples of *Empetrum* with known ploidy level allow us to draw the following conclusions: 1) diploid and tetraploid cytotypes prefer different habitats and usually occupy peat bogs and rocky places, respectively; the tetraploids generally reach higher altitudes; 2) mosaic-like sympatric occurrence of the cytotypes can be expected in areas where peat bogs and rock habitats are intermingled; 3) inter-cytotype mating resulting in triploid offspring can occur in the zone of sympatry; 4) the sex of the flowers is highly associated with the ploidy level, although some exceptions may occur.

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References:

- Bell J.N. & Tallis J.H. (1958): Biological flora of the British Isles. *Empetrum nigrum* L. *J. Ecol.* 61: 289-305.
- Blackburn K.B. (1938): On the occurrence of a hermaphrodite plant of *Empetrum nigrum* L. *J. Bot.* 76: 306-307.
- Boratyński A. (1986): Chronione i godne ochrony drzewa i krzewy polskiej części Sudetów, Pogórza i Przedgórze Sudeckiego. 2. *Empetrum nigrum* L. s.l. (Protected or deserving protection trees and shrubs from the Polish part of Sudety Mts., Pogórze and Przedgórze Sudeckie region. 2. *Empetrum nigrum* L. s.l.). *Arbor. Kórnickie* 31: 21-37.
- Boratyński A. & Vera de la Puente M.L. (1995): The *Empetraceae* on the Iberian Peninsula. *Willdenowia* 25: 39-53.

- Costich D. E., Ortiz R., Meagher T. R., Bruederle L. P. & Vorsa N. (1993): Determination of ploidy level and nuclear DNA content in blueberry by flow cytometry. *Theor. Appl. Genet.* 86: 1001-1006.
- Čvančara A. (1990): *Vacciniaceae* S.F. Gray. In: Hejný S. & Slavík B. (eds.), *Květena ČR 2 (Flora of the Czech Republic 2)*. Academia, Praha, pp. 519-521.
- Danielsson B. (1988): Nordkråkbär, *Empetrum hermaphroditum*, i nordöstra Småland (*Empetrum hermaphroditum* in NE Småland, S Sweden). *Svensk Bot. Tidskr.* 82: 118-124.
- Favarger C., Richard J.-L. & Duckert M.M. (1959): La Camarine noire *Empetrum nigrum* et *Empetrum hermaphroditum* en Suisse. *Ber. Schweiz. Bot. Ges.* 69: 249-260.
- Good R.D.O. (1927): The genus *Empetrum* L. *J. Linn. Soc., Bot.* 47: 489-523.
- Hagerup O. (1927): *Empetrum hermaphroditum* (Lge.) Hagerup. A new tetraploid, bisexual species. *Dansk Bot. Ark.* 5: 1-17.
- Hultén E. & Fries M. (1986): *Atlas of the North European vascular plants north of the Tropic of Cancer I-III*. Koeltz Scientific Books, Königstein.
- Löve D. (1960): The red-fruited crowberries in North America. *Rhodora* 62: 265-292.
- Lysák M. A. & Doležel J. (1998): Estimation of nuclear DNA content in *Sesleria* (*Poaceae*). *Caryologia* 51: 123-132.
- Marklund G. (1939): Beobachtungen über *Empetrum hermaphroditum* (Lange) Hagerup und *E. nigrum* L. s.str. *Mem. Soc. Fauna Fl. Fenn.* 15: 74-77.
- Meusel H., Jäger E., Rauschert S. & Weinert E. (1978): *Vergleichende chorologie der zentraleuropäischen Flora II*. VEB Gustav Fischer Verlag, Jena.
- Slavík B. (1990): *Fytokartografické syntézy ČR 2 (Phytocartographical syntheses of the Czech Republic 2)*. Botanický ústav ČSAV, Průhonice, p. 83.

- Suda J. & Lysák M. A. (2001): A taxonomic study of the *Vaccinium* sect. *Oxycoccus* (Hill) W.D.J. Koch (*Ericaceae*) in the Czech Republic and adjacent territories. *Folia Geobot.* 36: 303-319.
- Suda J. (2002): New DNA ploidy level in *Empetrum* (*Empetraceae*) revealed by flow cytometry. *Ann. Bot. Fenn.* 39: 133-141.
- Teppner H. (1987): *Empetrum nigrum* L. s.str. – neu für die Alpen. *Bot. Jahrb. Syst.* 108: 355-362.
- Velluti C., Krähenbühl M. & Ferrari C. (1995): Contributo alla citogeografia di *Empetrum nigrum* L. e di *E. hermaphroditum* (Lange) Hagerup (*Empetraceae*) (A contribution to the cytogeography of *Empetrum nigrum* L. and *E. hermaphroditum* (Lange) Hagerup (*Empetraceae*)). *Arch. Geobot.* 1: 165-170.
- Vorsa N. & Ballington J. R. (1991): Fertility of triploid highbush blueberry. *J. Amer. Soc. Hort. Sci.* 116: 336-341.
- Warner B.G. & Chinnappa C.C. (1990): Pollen polymorphism in *Empetrum nigrum* (*Empetraceae*) in Finland. *Ann. Bot. Fenn.* 27: 231-234.
- Webb D.A. (1972): *Empetrum* L. - In: Tutin T.G. et al. (eds.), *Flora Europaea* 3. Cambridge University Press, Cambridge, p. 14.
- Willdenow C.L. (1805): *Caroli Linnaei Species Plantarum* 4. G.C. Nauk, Berolini.
- Zarzycki K. & Guzik J. (1975): The Crowberry – *Empetrum nigrum* L. and *E. hermaphroditum* (Lange) Hagerup in Poland. *Fragm. Florist. Geobot.* 21: 423-431.

Appendix 1

Origin of plant populations:

Diploid cytotype exclusively

No.	Locality	Altitude	Coordinates	Total number of samples
1	Šumava Mts., Modrava – Blatenská slat' peat bog	1250 m	48° 58' 30" N 13° 27' 30" E	2
2	Šumava Mts., Modrava – Novohut'ské močály peat bog	1210 m	48° 59' N 13° 26' 30" E	2
3	Šumava Mts., Borová Lada – Chalupská slat' peat bog	910 m	49° 00' N 13° 39' 30" E	25
4	Šumava Mts., Modrava – Rokytecká slat' peat bog	1110 m	49° 01' N 13° 25' 30" E	22
5	Šumava Mts., Borová Lada – Žďárecká slat' peat bog	970 m	49° 01' N 13° 27' E	20
6	Šumava Mts., Modrava - Přední Mlynářská slat' peat bog	1050 m	49° 01' 30" N 13° 27' 30" E	25
7	Šumava Mts., Filipova Huť – Tetřevská slat' peat bog	1140 m	49° 01' 30" N 13° 33' E	20
8	Šumava Mts., Horská Kvilda – peat bog SW of the village	1050 m	49° 03' N 13° 33' E	2
9	Šumava Mts., Churáňov - Malý Polec peat bog SW of the village	1100 m	49° 04' N 13° 37' E	2
10	Krušné hory Mts., Přebuz – Velký močál peat bog	920 m	50° 24' N 12° 38' E	25
11	Krušné hory Mts., Horní Blatná – peat bog SE of the former village Bludná	1030 m	50° 24' N 12° 49' E	5
12	Krušné hory Mts., Boží Dar – Božídarské rašeliniště peat bog	1010 m	50° 24' N 12° 54' 30" E	27
13	Labské pískovce Mts., Růžová – rocks in Divoká soutěska gorge (leg. H. Härtel & P. Bauer)	160 m	50° 52' N 14° 18' 30" E	2
14	Jizerské hory Mts., Smědava – Suchopýrková louka peat bog N of the village	900 m	50° 51' N 15° 16' 30" E	15
15	Jizerské hory Mts., Smědava – Louka U studánky peat bog SE of the village	890 m	50° 50' N 15° 17' E	19
16	Jizerské hory Mts., Smědava – Quarré (Plochý vrch) peat bog NNE of the village	850 m	50° 51' 30" N 15° 17' 30" E	18
17	Jizerské hory Mts., Jizerka – Malá Jizerské louka peat bog	860 m	50° 49' 30" N 15° 19' 30" E	18
18	Krkonoše (Polish part): peat bog between Petrovka and Špindlerovka chalets (Przeł. Dołek)	1180 m	50° 46' N 15° 37' E	7
19	Adršpašsko-Teplické skály Mts., Teplice n. Metují – rocks in pine forest in Bludiště valley	c. 600 m	50° 35' 30" N 16° 08' E	1
20	Adršpašsko-Teplické skály Mts., Teplice n. Metují – rocks near Střmen ruin (leg. A. Hájek)	c. 600 m	50° 36' N 16° 08' E	1

Tetraploid cytotype exclusively

No.	Locality	Altitude	Coordinates	Total number of samples
21	Šumava Mts., rocks at the top of Trojmezna hill	1350 m	48° 46' N 13° 50' 30" E	1
22	Šumava Mts., Rakouská louka peaty meadow	1350 m	48° 46' N 13° 51' E	2
23	Šumava Mts., the Plešné jezero lake – screes in a glacial cirque	c. 1200 m	48° 46' N 13° 51' 30" E	6
24	Jizerské hory, Jizerka - rocks between Vlčí doupe and Pytlácké kameny hills (3 main rocky peaks)	c. 950 m	50° 50' 30" N 15° 20' E	54
25	Krkonoše, Mumlavská louka peat bog	1330 m	50° 46' N 15° 32' E	14
26	Krkonoše, Černohorská rašelina peat bog and small peat bog along Stříbrný potok brook SSE of Kolínská chalet	1170– 1200 m	50° 38' N 15° 45' E	9
27	Jeseníky Mts., Filipovice – peat bog between Trojmezí and Vozka hill	1310 m	50° 09' 30" N 17° 06' 30" E	4
28	Jeseníky Mts., Filipovice – rocks near the top of Červená hora, Vozka and Keprník hills	1330 – 1420 m	50° 09' N 17° 07' E	5
29	Jeseníky Mts., Karlova Studánka – rocks between Petrovy Kameny and Břidličná hora hills	1340 – 1420 m	50° 03' N 17° 13' E	7

Mixed populations

No.	Locality	Altitude	Coordinates	Ploidy level (number of samples)
30	Krkonoše Mts., peat bog near the state border SE of Mumlavská hora hill (Lubošská planina)	1210 m	50° 47' 30" N 15° 28' 30" E	2x (12) 3x (4) 4x (10)
31	Krkonoše Mts., small peat bog (Hraniční louka) between Vosecká bouda chalet and hill of Luboch	1250 m	50° 47' N 15° 30' E	2x (21) 2x + 3x (3) 3x (4)
32	Krkonoše Mts., Navorská louka peat bog	1200 – 1300 m	50° 47' N 15° 31' E	2x (1) 4x (23)
33	Krkonoše Mts., Pančavská louka peat bog	1330 m	50° 46' N 15° 32' 30" E	2x (9) 4x (31)
34	Krkonoše Mts., Labská louka peat bog	1360 m	50° 46' 30" N 15° 32' 30" E	2x (4) 4x (21)
35	Krkonoše Mts., small peat bogs near the Petrovka chalet	1250 m	50° 46' N 15° 36' 30" E	2x (1) 4x (7)
36	Krkonoše Mts., Čertova louka peat bog	1400 m	50° 45' N 15° 41' E	2x (4) 3x (2) 4x (1)
37	Krkonoše Mts., Úpská rašelina peat bog and small peat bog to the WNW	1430 m	50° 44' N 15° 42' 30" E	2x (5) 4x (14)

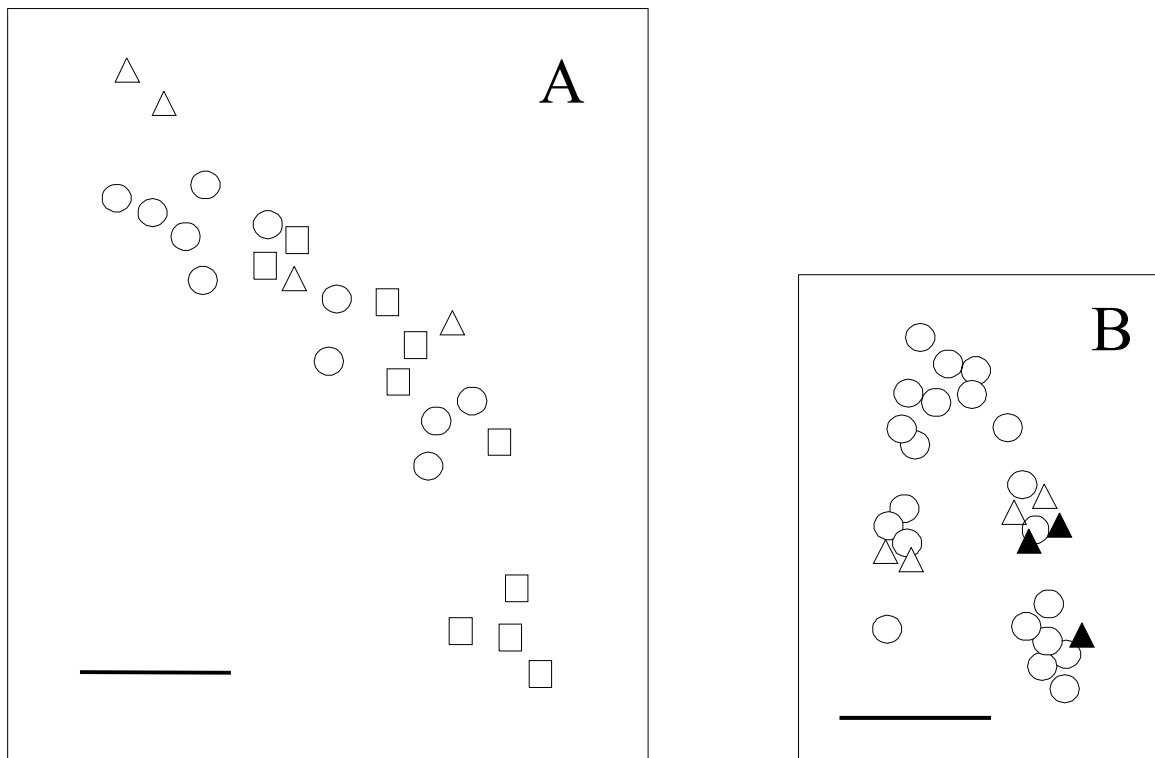


Fig. 2. Distribution of *Empetrum* cytotypes on fine spatial scale at loc. 30 (A) and loc. 31 (B) in the Krkonoše Mts. (bars = 100 m). Diploid: open circle, triploid: open triangle, tetraploid: open square, mixed diploid and triploid sample: full triangle. See appendix for locality description.

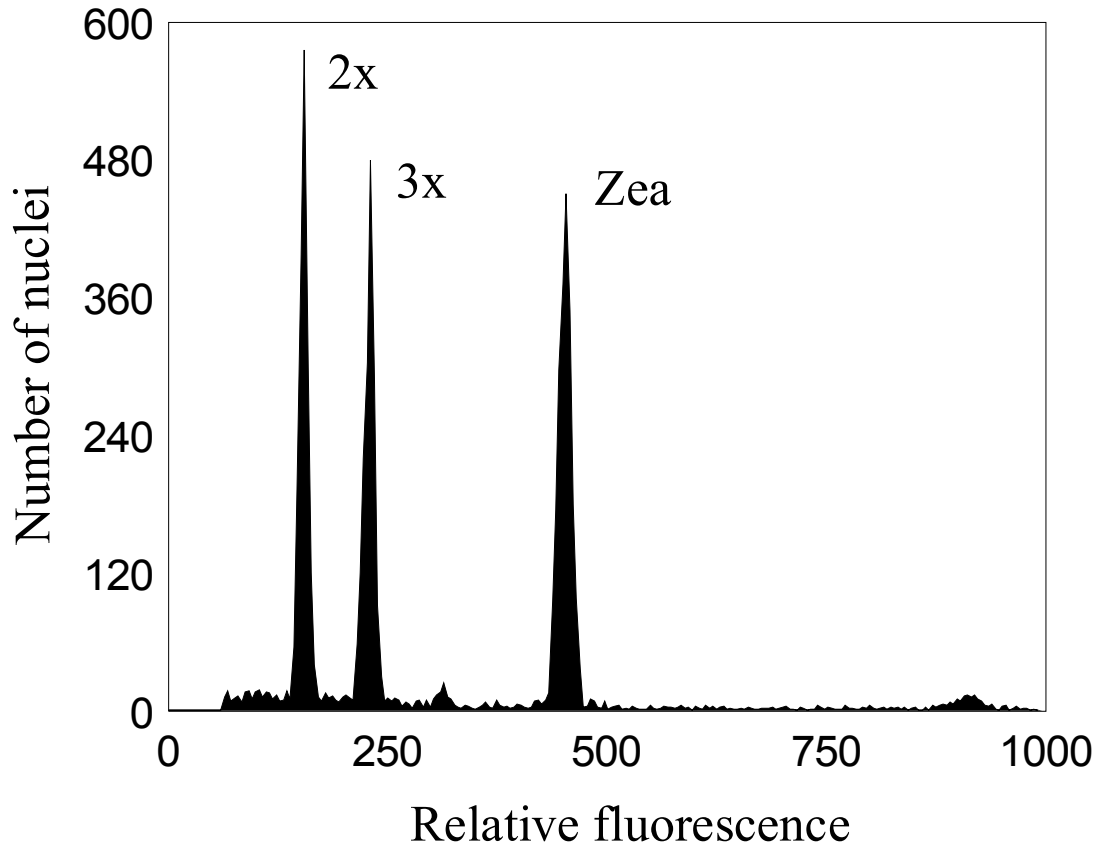


Fig. 3. Histogram of relative DNA content obtained after simultaneous analysis of *Zea mays* cv. CE-777 (internal standard) and mixed sample containing diploid and triploid *Empetrum* from locality 31.

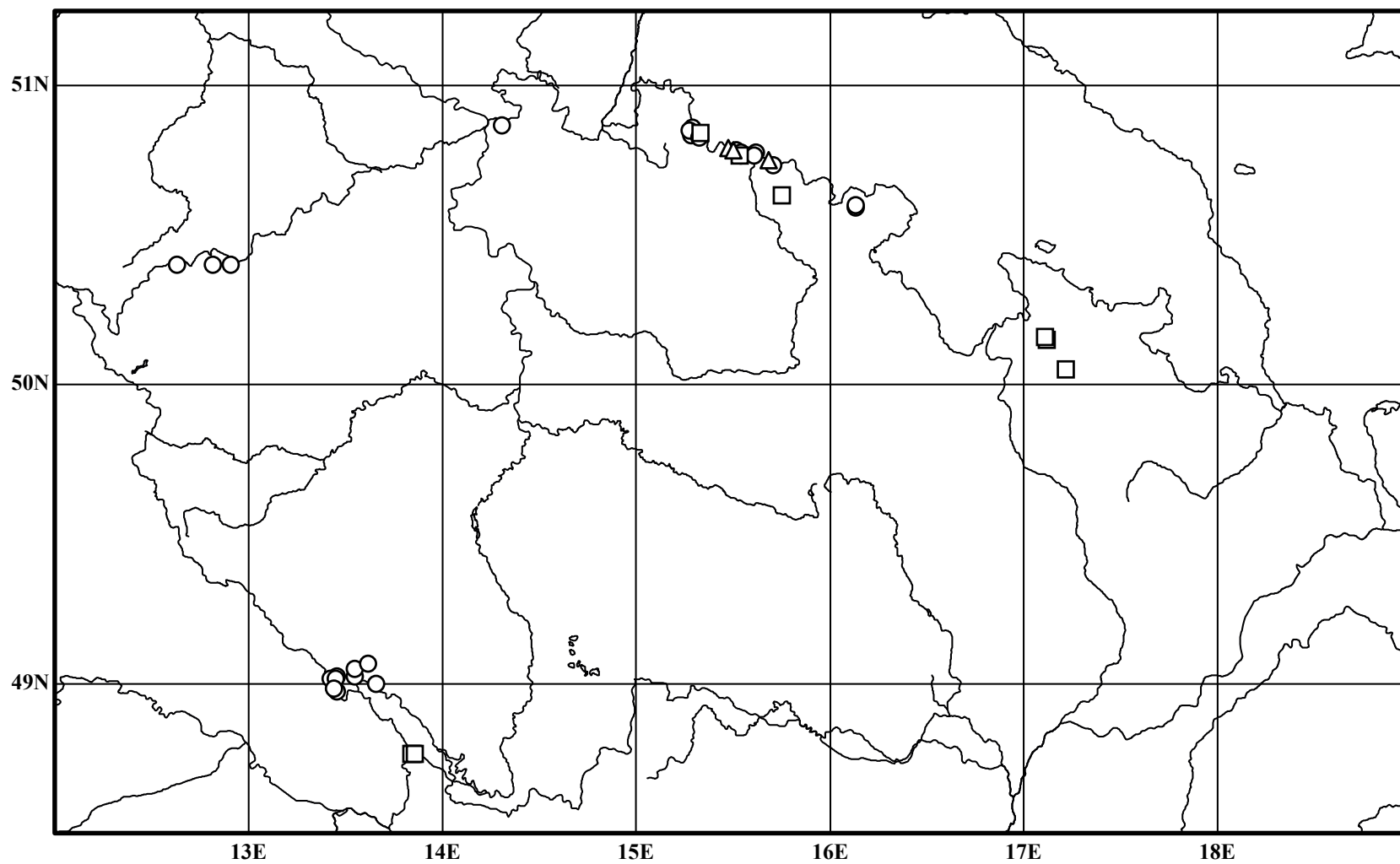


Figure 1. Distribution of *Empetrum* cytotypes in the Czech Republic: circle – diploid, triangle – triploid, square – tetraploid.

Region / Cytotype	2x	3x	4x
Šumava Mts.	peat bogs; quite common (9 localities)	-	rocks, peaty meadow; very rare (3 localities)
Krušné hory Mts.	peat bogs; quite common (3 localities)	-	-
Labské pískovce Mts.	sandstone rocks; very rare (1 locality)	-	-
Jizerské hory Mts.	peat bogs; quite common (4 localities)	-	rocks; rare (1 locality)
Krkonoše Mts.	peat bogs; quite common, less abundant than 4x (1 locality – non-mixed, 8 localities – mixed with 3x or 4x)	peat bogs; rare (3 localities - mixed with 2x or 4x)	peat bogs, rocks; quite common (2 localities - non-mixed, 7 localities - mixed with 2x or 3x)
Adršpašsko-teplické skály Mts.	sandstone rocks, pine forests; very rare (2 localities)	-	-
Jeseníky Mts.	-	-	rocks, peat bogs; quite common (3 localities)

Tab. 1. Habitats, abundance and number of populations investigated for individual *Empetrum* cytotypes in the mountain regions of the Czech Republic.