New DNA ploidy level in *Empetrum* (Empetraceae) revealed by flow cytometry

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Ploidy levels of 71 specimens of *Empetrum* sampled at 32 localities in Sweden, Norway and Denmark were determined by flow cytometry. Diploid *E. nigrum* was found at ten localities mostly in the southern parts of the area. Tetraploid *E. hermaphroditum* was recorded from 24 localities between 61°30′N and 68°30′N. The diploids and the tetraploids co-occurred in three areas in Sweden and Norway; mixed populations were found in Dovrefjel National Park. A new DNA ploidy level, triploids of probably hybrid origin, was discovered in Jotunsheimen NP in Norway. 2C values were 1.29 pg, 1.94 pg and 2.56 pg for the 2x, 3x and 4x levels, respectively, and no reliable evidence for DNA content variation within a given ploidy level was found. These data represent the first estimations of nuclear DNA content in the family Empetraceae.

Introduction

The genus *Empetrum* (crowberry) is represented in Europe by two closely related taxa: diploid *E. nigrum s. stricto* (2n = 2x = 26) usually with unisexual flowers and tetraploid *E. hermaphroditum* (2n = 4x = 52) usually with bisexual flowers. The taxonomic rank is, however, still a moot question and some botanists recognize only one species, *E. nigrum*, with two subspecies (subsp. *nigrum* and subsp. *hermaphroditum*) (Webb 1972) or treat the tetraploids as a subspecies of *E. eamesii* (= *E. eamesii* subsp. *hermaphroditum*) (Löve 1960).

Only dioecious crowberries were known at the beginning of the 19th century (e.g. Linnaeus 1753). The first report of hermaphrodite plants appeared in Willdenow (1805), and Lange (1880) gave them the name *E. nigrum* forma *β hermaphroditum*, *nom. nud.* About half a century later, the chromosome number of individuals with hermaphrodite flowers from the eastern

coast of Greenland was determined. Twenty-six chromosomes in meiotic division instead of 13 chromosomes found in *E. nigrum* from Faeroes were observed, and the rank of species (= *E. hermaphroditum*) was chosen for the Greenland plants (Hagerup 1927). It was the first case when the number of chromosomes served as a main criterion to establish a species (Teppner 1987). Since then, tetraploid *E. hermaphroditum* has been documented from extensive areas in Europe, Asia and North America (Löve 1960, Velluti *et al*. 1995).

Several morphological differences between the diploids and the tetraploids concerning the twig colour, length and rooting, shape and arrangement of leaves or number of seeds in a drupe were reported (Marklund 1939, Danielsson 1988, Boratyński & Vera de la Puente 1995, Kuvaev *et al*. 1996). Nevertheless, as pointed out by several authors, most of the vegetative characters are unstable and they can vary under different ecological conditions resulting in a considerable overlap between the taxa (Bell & Tallis 1958). A correlation between pollen size and ploidy level was confirmed and pollen tetrad diameter (larger in the tetraploids) might be a useful criterion for species delimitation (Zarzycki & Guzik 1975, Warner & Chinnappa 1990). The most reliable morphological character separating the taxa is probably the sexual condition of flowers (unisexual in *E. nigrum*, bisexual in *E. hermaphroditum*). However, not even this character is fully applicable because individuals of *E. nigrum* with bisexual flowers (Blackburn 1938) and *E. hermaphroditum* exclusively with female flowers (Favarger *et al*. 1959) have been observed. Potentially hermaphrodite flowers might change by abortion to unisexual ones (Good 1927). Different ecological preferences of the taxa can serve as a guideline for the determination in some cases: the diploids are mostly recorded from peat-bogs, moorlands or mires, while the tetraploids usually prefer windy peaks (Boratynski 1986, Velluti *et al*. 1995).

Both European taxa display almost circumpolar distributions, with their concentration particularly in the boreal and subartic zones (Meusel *et al*. 1978). In Scandinavia, *E. hermaphroditum* is often abundant north of about 60° latitude (Hultén 1971); only scattered distribution was observed in southern territories, or tetraploids are completely absent from there (e.g. in Denmark). A distinct type of heathland dominated by *E. hermaphroditum* occurs in coastal northern Norway (Edvardsen *et al*. 1988). Diploid crowberries generally prefer a more atlantic climate, and their continuous distribution extends from Denmark to southern Sweden, coastal Norway and Finland; scattered localities have been reported from central Sweden inland (Hultén 1971, Danielsson 1988). The taxa co-occur in some areas, e.g. on the western coast of Norway and in Finland.

Because of the difficult and unreliable determination of *Empetrum* specimens on the basis of morphological features, the taxa are often misidentified and records without determination of chromosome number (or ploidy level) should be treated with caution. The aim of this paper is to contribute to the cytogeography of *Empetrum* in Scandinavia and to verify the distribution pattern based exclusively on plants with determined ploidy level.

Material and methods

Field sampling

Mature plants of *Empetrum* were sampled in the field in Sweden and Norway in 2000, and in Denmark in 2001 (Fig. 1; *see* Appendix for the list of localities). The sampling was designed to include specimens from various habitats. As a rule, at least two samples (each covering an area of approximately 25×25 cm) at a locality were collected because of possible co-occurrence of individuals with different ploidy level. In total, 71 specimens from 32 localities were analyzed. The plants are cultivated at the Experimental Garden of the Department of Botany, Charles University, Prague, the Czech Republic (50°04´N 14°25´E). Vouchers are kept in PRC and in the private herbarium of the author.

Ploidy level and nuclear DNA content estimation

A two-step procedure of nuclei isolation was employed (Otto 1990). Approximately 20 mg of

tissue from young leaves were chopped together with ca. 10 mg of leaf tissue of internal standard with a new razor blade in a petri dish containing one ml ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). The suspension was filtered through a nylon mesh (42 μm) and centrifuged at 150g for five minutes. Supernatant was removed, 100 μl of fresh Otto I buffer was added, samples were resuspended and stored for 15–20 minutes at room temperature. After incubation, one ml of Otto II buffer (0.4 M $Na_2HPO_4 \times 12$ H_2O) with fluorochrome was added and the relative fluorescence of isolated nuclei was analyzed using Partec PA II flow cytometer (Partec GmbH, Germany). DAPI in the concentration of 4 μg ml^{-1} and *E. nigrum* with 2n = 2x = 26 from the Šumava Mts. (the Czech Republic) as an internal standard were used in the ploidy level determination. The cytometer was adjusted so that the fluorescence of G_1 nuclei of diploid plants was localized on channel 250. Propidium iodide (50 μg ml^{-1}) + RNAse (50 μg ml^{-1}) were used in the estimation of genome size and *Raphanus sativus* cv. 'Saxa' (2C = 1.11 pg) served as an internal standard (Doležel *et al.* 1992). Each sample together with the standard was run in the first step in order to reveal possible variability in the nuclear DNA content. One representative accession per ploidy level was chosen in the next step and the measurements of genome size were repeated ten times on different days to reduce the possible effect of cytometer instability. The fluorescence of at least 10 000 nuclei was analyzed and only measurements with coefficient of variance (CV) less than 3% were taken into account. One picogram of DNA equalling 980 megabase pairs (Mbp) was considered when converting picogram values to base pairs (Bennett *et al.* 2000).

Results

Both diploid (= *E. nigrum*) and tetraploid (= *E. hermaphroditum*) plants were found in the area studied. *Empetrum nigrum* occupied ten localities mostly in the southern parts. The northernmost occurrence was found at about 63°N in Skuleskogen National Park (Sweden) where also the tetraploids grew more frequently. The alti-
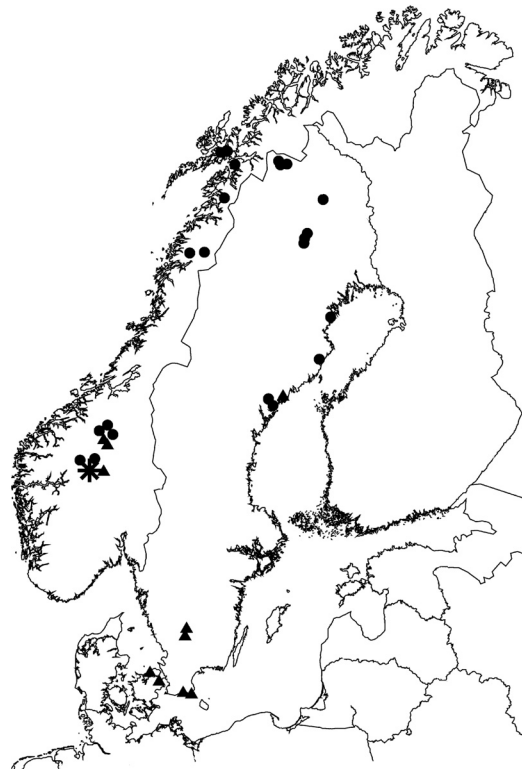

Fig. 1. Distribution map of individual ploidy levels of *Empetrum* in the area studied (▲ diploid *E. nigrum*, ● tetraploid *E. hermaphroditum*, ✱ triploid plants probably of hybrid origin).

tude of localities ranged from the sea level (several localities) up to 1180 m in Dovrefjel National Park in Norway. Diploid crowberries were usually observed on sandy seashores and on peat bogs from where they can spread to the surrounding *Pinus sylvestris* forests. At higher altitudes, they also colonized mountain slopes often dominated by *Vaccinium* spp. and lichens.

Empetrum hermaphroditum was more common in the area studied. Twenty-four localities in Sweden and Norway between approximately 61°30´N and 68°30´N were found. The tetraploids occurred predominantly in *Pinus sylvestris* and *Picea abies* forests; rocky slopes, open tundra mountain slopes, peat bogs and sandy seashores are also habitats where *E. hermaphroditum* thrives. The altitudinal range of localities varied from the sea level (several places) to 1450 m (Jotunsheimen NP in Norway). A common occurrence of diploid and tetraploid crow-
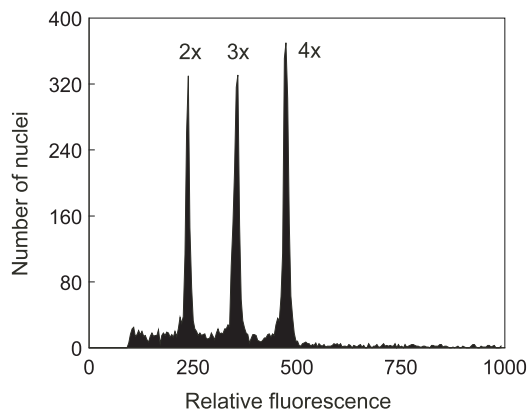
Fig. 2. Histogram of relative DNA content of DAPI-stained nuclei from diploid (no. 29), triploid (no. 26) and tetraploid (no. 18) plants of *Empetrum*. The nuclei of all three cytotypes were isolated, stained and analyzed simultaneously.

berries was found in some territories, viz. Skuleskogen NP in Sweden and Dovrefjel and Jotunsheimen NPs in Norway. Two populations in Dovrefjel NP (locs. 21 and 22) consisted of mixed individuals of both ploidy levels.

A new DNA ploidy level, triploids of probably hybrid origin, was revealed in Jotunsheimen NP (Norway) close to the Tourist Information Office near Bessheim village (Figs. 1 and 2). This is the first report of triploid plants in the family Empetraceae. Triploid plants grew on sandy deposits together with diploids (the second sample collected at the same locality). All triploid individuals were completely sterile in the field and they have not developed any flowers in cultivation. No specimen with intermediate fluorescence intensity between the diploids and the triploids or between the triploids and the tetraploids indicating the existence of backcrosses was found. The effort to determine the

chromosome number of triploid plants from young shoot apices was not successful. For this reason, the new triploid level should be regarded merely as a DNA ploidy level, i.e., it is not based on an exact chromosome count.

The 2*C* nuclear DNA content for diploid, triploid and tetraploid plants was estimated to 1.29 pg, 1.94 pg and 2.56 pg, respectively (Table 1 and Fig. 3). The genome size (*C* value) for individual ploidy levels equals to 0.65 pg (2x), 0.97 pg (3x) and 1.28 pg (4x). These records represent the first data of nuclear genome size in the family Empetraceae.

The nuclear DNA content of *Empetrum* showed very limited variability. Only minute differences in genome size (less than 3.7% and 4.1% in the diploids and the tetraploids, respectively) were observed when running individual specimens of the same ploidy level separately with the *Raphanus* standard. A joint re-analysis of samples with the highest and the lowest fluorescence resulted in one narrow unimodal peak with a low CV (histograms not presented) supporting the idea of a constant genome size.

Discussion

The distribution pattern of karyologically examined crowberries agrees well with the Scandinavian distribution maps (Hultén 1971). At least some of the localities from the Swedish inland added later (Danielsson 1988) are also correct as the diploids were confirmed in Skuleskogen NP. *Empetrum nigrum* was recorded in Dovrefjel NP in Norway for the first time. Hultén and Fries (1986) supposed that diploid crowberries avoid the mountains in Northern Europe, but two new localities are situated at 1100 and 1180 m above sea level, respectively. This information shows

Table 1. 2*C* nuclear DNA contents (means and standard errors, *N* = 10) and genome size of individual ploidy levels of *Empetrum*.

Sample	Ploidy level	2*C* DNA content (pg ± SE)	Genome size (*C* value) (pg/Mbp)
29	2x	1.29 ± 0.023	0.65/637
26	3x	1.94 ± 0.023	0.97/950.6
18	4x	2.56 ± 0.036	1.28/1254.5

that the diploids, despite generally preferring lowlands, can also reach higher altitudes. A co-occurrence of diploid *E. nigrum* and tetraploid *E. hermaphroditum* in a single area as well as at the same locality was proved. Localities 21 and 22 from Dovrefjel NP are of particular interest. The diploids with drupes lacking any remnants of stamens and the tetraploids bearing fruits with stamens at their base grew there in mixed populations. This result contradicts the opinion of some authors that "... both taxa ... are never simultaneously present in the same habitats" (Velluti *et al.* 1995). The probability of mixed sample detection in ordinary karyological analyses is, however, very low as only one or a few individuals per locality are usually counted.

The ploidy level of plants was determined by flow cytometry. This method has become a powerful tool to study plant genomes in various fields of plant science (Doležel 1997). High speed enabling the analyses of large population samples, non-destructiveness, analysis of mitotically inactive cells and easy recognition of mixed samples rank among the main advantages over chromosome counting. In the present study, it was found to represent a rapid and reliable method for ploidy determination and DNA content estimation in *Empetrum*.

A great variability of genome size (more than 1000-fold) with *C* values ranging from approximately 0.1 pg to about 125 pg has been found in the plant kingdom (Bennett *et al.* 2000). The genome size estimation of representatives of all plant families was set as a principal goal for the period of 1997–2002. The DNA amount of all ploidy levels of *Empetrum* measured here contributes to the above goal and represents the first data for the family Empetraceae. The *C* values fall into the lower part of angiosperm genome size range (Bennett & Leitch 2001). The mean value for approximately 3500 species of flowering plants was calculated to 6.32 pg. Only about 11.5% of taxa hitherto analyzed possess smaller genome than diploid *Empetrum*.

Although the specimens were collected in a geographically restricted area, these results show that *Empetrum* encompasses species with a stable genome size. Only negligible differences between plants (max. 4.1% in the tetraploids)
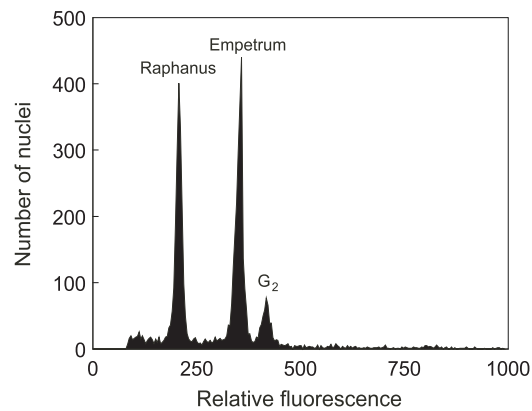

Fig. 3. Histogram of relative DNA content of propidium iodide-stained nuclei isolated from *Raphanus sativus* cv. 'Saxa' (standard, $2C = 1.11$ pg) and triploid *Empetrum*. The main peaks correspond to nuclei in G_1 phase (with $2C$ nuclear DNA content); small G_2 peak represents the nuclei of *Raphanus* with doubled ($4C$) nuclear DNA content. With G_1 peaks ratio of 1.748, the calculated $2C$ nuclear DNA content of *Empetrum* is 1.94 pg.

were revealed when analysing individual samples against the *Raphanus* standard. However, simultaneous analyses of plants with the largest differences did not verify any variability and resulted in single narrow peak with unimodal distribution. The intraspecific variation in nuclear DNA amount is frequently focused nowadays. Ohri (1998) gathered the data about plant species where intraspecific variation (from 11% to 288%) in $1C$ DNA value was observed. However, this variation was often caused by methodological errors as no difference in genome size was found when the same specimens were critically reinvestigated (Greilhuber 1998). The limited variation of *Empetrum* in the area studied is not surprising, as a shift in genome size is more probable to occur in geographically isolated populations subjected to different selection pressures.

Triploid plants in the family Empetraceae are recorded here for the first time. In the order Ericales, where the genus *Empetrum* is placed, triploids have been reported only very rarely — e.g. *Vaccinium vitis-idaea* (3x = 36) was found once in Finland (Ahokas 1971). The probability of a repeated appearance of triploids in *Empetrum*, and their establishment is presumably

quite low as only one of more than 70 specimens analyzed (about 1.4%) belonged to this ploidy level. Despite the fact that crowberries have been subjected to numerous karyological analyses, no triploid plants have been detected (for an incomplete list of karyologically studied samples, *see* Velluti *et al.* 1995). Along with the low probability of discovering triploids, this can also be caused by methodological difficulties in slide preparation as nuclei with chromosome number intermediate between the diploids and the tetraploids could be considered as incomplete. An identical situation was observed in *Vaccinium* sect. *Oxycoccus*, where pentaploid hybrids were not revealed for a long time, although they are not rare (Suda & Lysák 2001). Flow-cytometry is a very efficient method in studies focused on screening of large population samples and enables the detection of very rare cytotypes.

The triploid *Empetrum* are probably of hybrid origin. The population in Jotunsheimen NP in Norway occurs in an area where diploid *E. nigrum* and tetraploid *E. hermaphroditum* grow together. A detailed scanning of ploidy levels of *Empetrum* in the Czech Republic revealed triploids also in the Krkonoše Mts. in peat bogs with co-occurrence of diploids and tetraploids (authors unpubl. data). An alternative explanation for the origin of triploid plants is the fusion of a non-reduced gamete of diploid *E. nigrum* with a normal haploid one. The ratio of reduced versus non-reduced pollen grains remains, however, unknown despite several palynological studies (e.g. Warner & Chinnappa 1990). The sexual condition of flowers could considerably contribute to the elucidation of the origin of triploids. Unfortunately, no flowers have been observed in the field or in cultivation. Triploid crowberries might be completely sterile and they may propagate only vegetatively. The fertility of triploid hybrids is usually considerably reduced (e.g. Vorsa & Ballington 1991). The triploids in Jotunsheimen NP grew on sandy deposits in a riverbed. It seems plausible that a disturbance during high water level or flooding created free niches where triploid seedlings could establish themselves even if they are not strong competitors. In some *Vaccinium* shrubs, microsite (disturbance) availability is suggested as a pattern of seedling recruitment for long-lived clonal plants (Eriksson & Fröborg 1996).

When DNA content is estimated without counting the chromosome number, the term "DNA ploidy" should be used (Hiddeman *et al.* 1984). Nevertheless, we can expect that most probably the DNA *Empetrum* triploids possess 39 chromosomes. A very strong correlation between peak position in flow cytometry analysis and the chromosome number for a set of diploid (2n = 26) and tetraploid (2n = 52) plants from the Czech Republic has been shown (author's unpubl. data). The peak of triploid individuals from Norway occupied exactly the intermediate position between the peaks of karyologically examined diploid and tetraploid plants. Regarding the lack of chromosome number variability of the diploids and the tetraploids (Velluti *et al.* 1995), and their very limited genome size variation confirmed here, plants with intermediate nuclear DNA content are bound to possess an intermediate number of chromosomes. Similarly, in *Vaccinium* sect. *Oxycoccus* mature DNA pentaploids were detected by flow cytometry at first and their chromosome number was verified later (Suda & Lysák 2001). A different chromosome volume (and an identical chromosome number) is a very implausible explanation of a 1.5-fold difference in genome size between the diploids and the DNA triploids. As discussed by Greilhuber and Obermayer (1999), there is no evidence supporting the existence of "crypto-polyploidy" in plants.

The results of this study show that diploid *E. nigrum* and tetraploid *E. hermaphroditum* co-occur in some localities and their hybridization resulting in triploid offspring can take place. The determination of ploidy level or chromosome number is therefore advised for a reliable separation of the taxa.

Acknowledgements

References

Ahokas, H. 1971: Notes on polyploidy and hybridity in *Vaccinium* species. — *Ann. Bot. Fennici* 8: 254–256.

Bell, J. N. & Tallis, J. H. 1958: Biological flora of the British Islands. *Empetrum nigrum* L. — *J. Ecol.* 61: 289–305.

Bennett, M. D., Bhandol, P. & Leitch, I. J. 2000: Nuclear DNA amounts in angiosperms and their modern uses — 807 new estimates. — *Ann. Bot.* 86: 859–909.

Bennett, M. D. & Leitch, I. J. 2001: Angiosperm DNA C-values database (release 3.1, Sep. 2001) — http://www.rbgkew.org.uk/cval/homepage.html.

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 Przedgórza Sudeckiego. 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.

Danielsson, B. 1988: Nordkråkbär, *Empetrum hermaphroditum*, i nordöstra Småland. — *Svensk Bot. Tidskr.* 82: 118–124.

Doležel, J., Sgorbati, S. & Lucretti, S. 1992: Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. — *Physiol. Plant.* 85: 625–631.

Doležel, J. 1997: Application of flow cytometry for the study of plant genomes. — *J. Appl. Genet.* 38: 282–302.

Edvardsen, H., Elvebakk, A., Øvstedal, D. O., Prøsch-Danielsen, L., Schwenke, J. T. & Sveistrup, T. 1988: A peat-producing *Empetrum* heath in coastal north Norway. — *Arct. Alp. Res.* 20: 299–309.

Eriksson, O. & Fröborg, H. 1996: "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. — *Can. J. Bot.* 74: 1369–1374.

Favarger, C., Richard, J.-L. & Duckert, M. M. 1959: La Camarine noire *Empetrum nigrum* et *Empetrum hermaphroditum* in Suisse. — *Ber. Schweiz. Bot. Ges.* 69: 249–260.

Good, R. D. O. 1927: The genus *Empetrum* L. — *J. Linn. Soc., Bot.* 47: 489–523.

Greilhuber, J. 1998: Intraspecific variation in genome size: A critical reassessment. — *Ann. Bot.* 82 (Suppl. A): 27–35.

Greilhuber, J. & Obermayer, R. 1999: Cryptopolyploidy in *Bunias* (Brassicaceae) revisited – A flow-cytometric and densitometric study. — *Pl. Syst. Evol.* 218: 1–4.

Hagerup, O. 1927: *Empetrum hermaphroditum* (Lge) Hagerup. A new tetraploid, bisexual species. — *Dansk Bot. Ark.* 5: 1–17.

Hiddeman, W., Schumann, J., Andreef, M., Barlogie, B.,

Herman, C. J., Leif, R. C., Mayall, B. H., Murphy, R. F. & Sandberg, A. A. 1984: Convention on nomenclature for DNA cytometry. — *Cytometry* 5: 445–446.

Hultén, E. 1971: *Atlas över växternas utbredning i Norden.* 2nd ed. — Generalstabens Litografiska Anstalts Förlag, Stockholm. 531 pp.

Hultén, E. & Fries, M. 1986: *Atlas of the North European vascular plants north of the Tropic of Cancer I–III.* — Koeltz Sci. Books, Königstein. 1149 pp.

Kuvaev, V. B., Vasilec, V. N., Krasnov, E. A. & Berezovskaya, Т. Р. [Куваев, В. Б., Василец, В. Н., Краснов, Е. А. & Березовская, Т. П.] 1996: [To diagnostics and chemosystematics of intrageneric taxa of *Empetrum* (Empetraceae)]. — *Bot. Zhurn.* 81: 104–115. [In Russian].

Lange, T. H. 1880: Conspectus Florae Grönlandicae. — *Med. Grönl.* 3: 1–231.

Linnaeus, C. 1753: *Species Plantarum.* — Laurentius Salvius, Holmiae. 1200 pp.

Löve, D. 1960: The red-fruited crowberries in North America. — *Rhodora* 62: 265–292.

Marklund, G. 1939: Beobachtungen über *Empetrum hermaphroditum* (Lange) Hagerup und *E. nigrum* L. *s.str.* — *Mem. Soc. Fauna Flora Fennica* 15: 74–77.

Meusel, H., Jäger, E., Rauschert, S. & Weinert, E. 1978: *Vergleichende Chorologie der zentraleuropäischen Flora II.* — Gustav Fischer Verlag, Jena. 418 pp. + maps 259–421.

Ohri, D. 1998: Genome size variation and plant systematics. — *Ann. Bot.* 82 (Suppl. A): 75–83.

Otto, F. 1990: DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. — In: Crissman, H. A. & Darzynkiewicz, Z. (eds.), *Methods in cell biology*, Vol. 33: 105–110. Acad. Press, New York.

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.

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). — *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. Fennici* 27: 231–234.

Webb, D. A. 1972: *Empetrum* L. — In: Tutin, T. G. *et al.* (eds.), *Flora Europaea* 3: 14. Cambridge Univ. Press, Cambridge.

Willdenow, C. L. 1805: *Caroli Linnaei Species Plantarum IV.* — Berolini: G. C. Nauk, Vienna. 1157 pp.

Zarzycki, K. & Guzik, J. 1975: The Crowberry – *Empetrum nigrum* L. and *E. hermaphroditum* (Lange) Hagerup in Poland. — *Fragm. Flor. Geobot.* 21: 423–431.

Appendix. Origin of plant populations.

No.	Ploidy	Locality	Altitude	Coordinates	Date	Specimens
4	2x	Sweden: NP Skuleskogen — peat bog on the N shore of Tärnättvattnen lake	160 m	63°06′40′′N 18°30′30′′E	26.VII.2000	2
21	2x	Norway: NP Dovrefjel, Kongsvoll village — slopes cca 2 km W of the village	1180 m	62°18′20′′N 09°34′50′′E	3.VIII.2000	3
22	2x	Norway: NP Dovrefjel, Kongsvoll village — W slopes of Knutshø peak	1100 m	62°16′50′′N 09°38′40′′E	4.VIII.2000	3
26	2x	Norway: NP Jotunsheimen, Bessheim village — sandy deposits near the Tourist Information Office	970 m	61°30′20′′N 08°50′30′′E	5.VIII.2000	1
27	2x	Sweden: NP Store Mosse — peat bog near Kävsjön lake	170 m	57°19′00′′N 13°57′40′′E	6.VIII.2000	2
28	2x	Sweden: NP Store Mosse — *Pinus sylvestris* forest	180 m	57°18′40′′N 13°56′10′′E	6.VIII.2000	2
29	2x	Sweden: sandy dunes between Sandhammaren and Skillinge villages	10 m	55°24′10′′N 14°12′50′′E	8.VIII.2000	2
30	2x	Sweden: sandy dunes between Sandhammaren and Skillinge villages	10 m	55°24′20′′N 14°13′00′′E	8.VIII.2000	1
31	2x	Denmark, Sjælland: Copenhagen — peat bog near Lyngby	20 m	55°46′40′′N 12°28′50′′E	8.IV.2001	5
32	2x	Denmark, Sjælland: sandy seashore and *Pinus sylvestris* forests SW of Tisvildeleje village	10 m	56°03′00′′N 12°03′10′′E	16.IV.2001	6
1	4x	Sweden: NP Skuleskogen — *Picea abies* forest in the central part of the National park	170 m	63°06′00′′N 18°29′50′′E	26.VII.2000	2
2	4x	Sweden: NP Skuleskogen — peat bog in the central part of the National park	170 m	63°06′00′′N 18°29′50′′E	26.VII.2000	2
3	4x	Sweden: NP Skuleskogen — rocky slopes in the Slåttdalsskrevan ravine	270 m	63°06′30′′N 18°30′00′′E	26.VII.2000	2
5	4x	Sweden, Umeå: sandy places along the road 3 km S of Gumboda village	30 m	64°10′20′′N 20°56′10′′E	27.VII.2000	2
6	4x	Sweden, Luleå: *Pinus sylvestris* forest 1 km N of Jävre village	10 m	65°07′30′′N 21°31′40′′E	27.VII.2000	2
7	4x	Sweden: NP Muddus — *Pinus sylvestris* forest near the Muddusfallet waterfall	350 m	66°48′50′′N 20°09′00′′E	28.VII.2000	2
8	4x	Sweden: NP Muddus — peat bog SW of Muddusluobbal lake	380 m	66°51′20′′N 20°11′30′′E	28.VII.2000	2
9	4x	Sweden: NP Muddus — *Pinus sylvestris* forest in the S part of the National park	330 m	66°46′20′′N 20°06′40′′E	29.VII.2000	1
10	4x	Sweden: *Pinus sylvestris* forest 2 km S of Svappavaara village	420 m	67°36′10′′N 21°07′20′′E	29.VII.2000	2
11	4x	Sweden: NP Abisko — peat bog along the Abiskojokka river	400 m	68°20′50′′N 18°46′00′′E	29.VII.2000	2

Appendix. Continued.

No.	Ploidy	Locality	Altitude	Coordinates	Date	Specimens
12	4x	Sweden: NP Abisko — SE slopes of Njulla peak, near upper funicular station	970 m	68°21´50´´N 18°43´20´´E	30.VII.2000	2
13	4x	Sweden: *Pinus sylvestris* — *Betula* forest on the S shore of Torneträsk lake	370 m	68°18´10´´N 19°13´50´´E	31.VII.2000	2
14	4x	Norway: Vesterälen — sandy seashore of the Gullesfjorden gulf	10 m	68°31´50´´N 15°43´40´´E	31.VII.2000	2
15	4x	Norway: Vesterälen — sandy seashore of the Fiskfjord gulf	0 m	68°33´10´´N 16°05´10´´E	31.VII.2000	2
16	4x	Norway: Vesterälen — sandy seashore of the Efjorden gulf	10 m	68°17´40´´N 16°30´00´´E	1.VIII.2000	1
17	4x	Norway, Bodø: NW shore of Kobbvatnet lake	150 m	67°38´20´´N 15°55´40´´E	1.VIII.2000	2
18	4x	Norway: NP Saltfjell-Svartisen — peat bog N of Svartisvatnet lake	300 m	66°31´20´´N 14°52´40´´E	2.VIII.2000	2
19	4x	Norway: NP Saltfjell-Svartisen — rocks NW of Svartisvatnet lake	340 m	66°30´20´´N 14°06´10´´E	2.VIII.2000	1
20	4x	Norway: sandy places along the road 5 km NE of Oppdal village	570 m	62°37´50´´N 09°47´00´´E	3.VIII.2000	1
21	4x	Norway: NP Dovrefjel, Kongsvoll village — slopes cca 2 km W of the village	1180 m	62°18´20´´N 09°34´50´´E	3.VIII.2000	2
22	4x	Norway: NP Dovrefjel, Kongsvoll village — W slopes of Knutshø peak	1100 m	62°16´50´´N 09°38´40´´E	4.VIII.2000	2
23	4x	Norway: NP Dovrefjel, Kongsvoll village — W slopes of Knutshø peak	1330 m	62°17´20´´N 09°40´50´´E	4.VIII.2000	2
24	4x	Norway: Jotunsheimen — sandy places along the road 3 km E of Randsverk village	710 m	61°43´50´´N 09°05´40´´E	5.VIII.2000	1
25	4x	Norway: NP Jotunsheimen — slopes N of Bessvaten lake	1450 m	61°31´00´´N 08°47´50´´E	5.VIII.2000	2
26	3x	Norway: NP Jotunsheimen, Bessheim village — sandy deposits near the Tourist Information Office	970 m	61°30´20´´N 08°50´30´´E	5.VIII.2000	1