Chromosome numbers and distribution of *Cardamine amara* (Brassicaceae) in Slovenia

Judita LIHOVÁ¹ & Karol MARHOLD¹,²

¹Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-84523 Bratislava, Slovakia; tel.: ++421-2-5942 6138, fax: ++421-2-5477 1948, e-mail: judita.lihova@savba.sk
²Department of Botany, Charles University, Benátská 2, CZ-12801 Praha 2, Czech Republic, e-mail: karol.marhold@savba.sk


Chromosome numbers and distribution of two subspecies of *Cardamine amara* in Slovenia are presented. Major part of Slovenia is occupied by tetraploid *C. amara* subsp. *austriaca*, the taxon with the distribution centered in the Eastern Alps. In Slovenia it prevails in the (Pre)alpine and (Pre)dinaric regions and approaches the southern limit of its known distribution area. Diploid *C. amara* subsp. *amara* is in Slovenia less frequent, it is concentrated in the northeastern Subpannonian region. Morphological differences between these two subspecies, especially those in the pollen grain size are mentioned. Distribution pattern found in Slovenia and the assumed evolutionary history of the tetraploid subspecies are discussed.

Key words: Alps, autopolyplody, *Cardamine amara* subsp. *amara*, *Cardamine amara* subsp. *austriaca*, Dinaric region, distribution map, Subpannonian region.

Introduction

*Cardamine amara* L. is a morphologically highly variable species widespread in Europe and extending to Asia. It occupies moist to wet sites mainly on stream banks and springs from lowlands up to the alpine belt. Based on previous taxonomic studies, several diploid and two tetraploid subspecies are currently recognized within this species in Europe. The subspecies are morphologically and geographically well characterized (MARHOLD, 1992, 1998, 1999a; MARHOLD et al., 1996; LIHOVÁ et al., 2000).

The typical subspecies, diploid *C. amara* subsp. *amara*, is the most common and widespread subspecies, it is distributed in a major part of the species distribution area (JALAS & SUOMENEN, 1994). Recently, the tetraploid subspecies *C. amara* subsp. *austriaca* MARHOLD was described, occurring in the Eastern Alps and neighbouring areas (MARHOLD, 1999a). Morphometric and molecular (isozymes, RAPDs, AFLPs) investigation showed that this represents most probably young autotetraploid derivative of *C. amara* subsp. *amara* (MARHOLD, 1999a, LIHOVÁ et al., 2000, MARHOLD et al., 2002). The tetraploid occupies a rather compact distribution area, where *C. amara* subsp. *amara* is generally lacking; these two subspecies only marginally overlap in the western (eastern Switzerland) and northern parts (southern Bohemia) of the area of subsp. Morphologically it is very close to *C. amara* subsp. *amara*, and only a combination of several morphological characters (number of leaves, length of sepals and...
filaments, branching of stem and especially diameter of pollen grains) separates it from this taxon (Marhold, 1999a).

Another tetraploid taxon from Catalonia, NE Spain traditionally treated as C. amara subsp. olotensis O. Bolòs (Bolòs, 1952; Bolòs et al., 1993; Rico, 1993) has been recently shown to be distant from the core C. amara, and should be classified as a separate species – Cardamine am- portiana Sennen & Pau (Lihová et al., 2000; Marhold et al., 2002; Lihová et al., submitted). Other diploid subspecies are confined to certain European mountain ranges, namely C. amara subsp. opicii (J. Presl & C. Presl) Čelak, to the Sudety Mts. and the Carpathians, C. amara subsp. balcanica Marhold, Ančev & Kit Tan to the mountains of SW Bulgaria and NE Greece, and C. amara subsp. pyrenaica Sennen to the Eastern Pyrenees (Marhold, 1992, 1998; Marhold et al., 1996; Lihová et al., 2000).

In Slovenia, occurrence of both C. amara subsp. amara and C. amara subsp. austriaca has been reported (Marhold, 1999a,b), but no detailed karyological or chorological investigation has been done here as yet. In the distribution atlas of Slovenia (Jogan, 2001) only general distribution of C. amara was given, without subspecific separation. According to this, C. amara occurs throughout the whole area of Slovenia, but it is more concentrated in northern and northeastern parts of the country. The area of Slovenia seems to represent the southern limit of distribution of C. amara subsp. austriaca, thus its exploration was expected to bring more precise delimitation of the area occupied by this tetraploid. In this paper we deal with the detailed distribution pattern of C. amara subsp. amara and C. amara subsp. austri- nca in the area studied.

Material and methods

For chromosome number determination, six samples of Cardamine amara originating from several phytogeographical regions in Slovenia were collected (Tab. 1). The plants collected in the field were cultivated at the Institute of Botany, Slovak Academy of Sciences, and their root tips were treated using the squash method (modified after Martonfi et al., 1999). The root tips were pretreated in 0.002 mol/L aqueous solution of 8-hydroxyquinoline for 3 hrs at 4°C, fixed for 1 hr in a mixture of 96% ethanol and concentrated acetic acid (3:1), hydrolyzed in a mixture of concentrated hydrochloric acid and 96% ethanol (1:1) for 3 min, and finally squashed using a celophane piece instead of the cover glass. The squashes were stained in 10% Giemsa stock solution in Sörensen phosphate buffer for 1 hr, and rinsed with distilled water. The voucher specimens of the karyologically examined populations are deposited at SAV (herbarium abbreviations follow Holmchen et al., 1990).

Chorological study was based on our own samples (Tab. 1), on the previously published data (Marhold, 1999a), and on herbarium specimens deposited at LJU and SAV (Appendix). The specimens from LJU were determined at the subspecific level by measuring pollen grain diameter. From each specimen, one flower bud was removed and pollen grains were stained using acetocarmine-jelly (Kearns & Inouye, 1993). Thirty pollen grains were measured from one flower and average values of pollen grain diameter were calculated. These were compared with the values of karyologically investigated plants both from Slovenia (Tab. 1) and other regions (voucher specimens from the paper by Marhold, 1999a). For diploid C. amara subsp. amara we recorded pollen grains of 18.0–20.4 μm, whereas those of C. amara subsp. austriaca were 26.6–24.7 μm large (average values of 30 pollen grains per one plant). A slight shift of both these ranges compared
Fig. 1. Distribution of diploid *Cardamine amara* subsp. *amara* (empty symbols) and tetraploid *C. amara* subsp. *austriaca* (solid symbols) in Slovenia. Circles represent records based on investigation of herbarium specimens (see Appendix), triangles depict populations karyologically investigated in this study and that of Marhold (1999a). Phytogeographical regions: AL – Alpine, PA – Prealpine, SP – Subpannonian, PD – Predinaric, DN – Dinaric, SM – Submediterranean. A – Austria, H – Hungary, CRO – Croatia, I – Italy. The map was produced in the Center za kartografijo favne in flore, Ljubljana, Slovenia.

with the previously published data for these two subspecies (Marhold, 1999a,b) was caused most probably by the use of another measuring equipment.

**Results and discussion**

Out of six karyologically investigated populations from Slovenia, five were tetraploid (2n = 32) and a single one was diploid (2n = 16), corresponding to *Cardamine amara* subsp. *austriaca* and *C. amara* subsp. *amara*, respectively (Tab. 1). The diploid population of *C. amara* subsp. *amara* was found in the eastern Subpannonian part of the country (Subpanonsko območje: Trnovska vas), whereas the tetraploid populations of *C. amara* subsp. *austriaca* originated from Alpine and Prealpine regions (Alpsko območje, Predalpsko območje). Marhold (1999a) reported previously two localities of *C. amara* subsp. *amara* from eastern (Subpanonsko območje: Ptujska gora) and southern Slovenia (Dinarsko območje: Pudob), and tetraploid *C. amara* subsp. *austriaca* from the northern Alpine region. Evaluation of herbarium specimens deposited at LJU and SAV (Appendix) revealed more detailed distribution pattern of the studied taxa. As can be seen from the map (Fig. 1), the area of Slovenia is mostly inhabited by tetraploid *C. amara* subsp. *austriaca*, which in few cases extends also to the most southern (Bela Krajina) and eastern regions (Ptujsko polje). Diploid *C. amara* subsp. *amara* is concentrated in the northeastern Subpannonian area, except for two sites found in the Ljubljanska kotlina basin, and one, already mentioned site in the south. Similar distribution pattern was found for diploid *C. matthioli Moretti* of the *C. pratensis* group, which in Slovenia occurs mainly in the Subpannonian region, partly extending to the most eastern parts of the Alpine, Prealpine and Predinaric regions, with a few localities in the Ljubljanska kotlina basin (Lihová & Marhold, submitted). Whereas diploid *C. amara* subsp. *amara* rep-
Cardamine amara subsp. austriaca has its distribution centre in the Eastern Alps in Austria, however it extends also to neighbouring regions. The eastern boundary of the distribution area was found in eastern Austria, where the area of this taxon only slightly overlaps with that of diploid C. amara subsp. amara. A somewhat larger overlap was observed in the west in eastern Switzerland (MARHOLD 1999a) and in the north (S Bohemia), where tetraploids reach as far as to the phytogeographical district Breznické Podbrdsko, to the south of the town of Příbram (MARHOLD, unpubl.). Nevertheless, in both regions of overlap C. amara subsp. austriaca tends to occupy higher altitudes than C. amara subsp. amara (MARHOLD, 1999a). This seems to be to certain extent also the case of Slovenian populations – diploid C. amara subsp. amara occurs mainly in the region of lower altitudes, seldom up to 570 m a.s.l., although the populations of C. amara subsp. austriaca in the most southern and eastern parts of the country grow also at rather low altitudes of ca. 180–200 m a.s.l. (see Appendix). The localities of C. amara subsp. amara in Slovenia continuously append to those in southeastern Austria (Burgenland and Styria) (MARHOLD, 1999a). There are no chromosome records for C. amara from the countries more to the south (Croatia, Bosnia and Herzegovina) so far, although both C. amara subsp. amara and C. amara subsp. austriaca could occur, as we assume from the distribution pattern in Slovenia. It is worth to mention that in the neighbouring regions of Upper Styria (Austria) and northern Italy populations currently classified as C. amara subsp. austriaca were previously misidentified as C. amara subsp. opizii (PIGNATTI, 1982; MAIER, 1996) and from Slovenia itself subsp. opizii was wrongly reported by LöVE & LöVE (1974) (as C. opizii).

Cardamine amara subsp. amara and subsp. austriaca are morphologically very close to each other. Previous morphometric analyses revealed only minor morphological differences between them. Tetraploid C. amara subsp. austriaca tends to have more leaves, less branched stem, larger flowers (longer petals, sepals and filaments of stamens), and especially, larger pollen grains. The last mentioned character is the only one which reliably differentiates individual plants of subsp. amara and subsp. austriaca (MARHOLD, 1999a). When ploidy level is not known, measurement of pollen grain is often inevitable for proper determination of single plants, but prior this, calibration based on measurements of plants with known ploidy level is recommended (see Material and Methods).

In agreement with morphology, also molecular data (RAPDs, AFLPs, isozymes) have shown very close position of both subspecies, as even such sensitive markers as AFLPs and RAPDs have not provided sufficient differentiation between them, and samples of both subspecies appeared in an unresolved polytomy (LIHOVÁ et al., 2000; MARHOLD et al., 2002).

In recent years, arctic and alpine plants have been intensively studied in respect of their interglacial migrations and postglacial recolonization during the Quaternary (COMES & KADERIET, 1998). The Alps represent a region covered to a large extent by ice sheets during the glacial periods, and plants occurring here, their distribution and genetic diversity, were strongly affected by these climatic changes (STEHLIK, 2000). Phylogeographic studies, based on exploration of geographic distribution of genetic diversity, aim to trace evolutionary history and diversification of various taxa, discussing in situ persistance on ice-free nunataks (e.g. STEHLIK et al., 2001) versus survival in peripheral refugial regions with subsequent recolonization (e.g. GUGERLI et al., 2001). Cardamine amara subsp. austriaca is one of the taxa confined to the Eastern Alps and adjacent regions, and its origin is believed to be strongly associated with Pleistocene glaciation of the Alps (MARHOLD, 1999a). Low genetic and morphological divergence from the typical subspecies suggests a recent autopolyploid origin of this taxon, originated from C. amara subsp. amara (MARHOLD, 1999a; LIHOVÁ et al., 2000; MARHOLD et al., 2002). Retreat of ice sheet after the last glaciation period offered uncovered areas with various unsettled habitats. This might have supported spread and establishment of tetraploid populations of C. amara subsp. austriaca in the Eastern Alps. However, as yet there is no information on possible colonization routes,
nor whether monotypic or polypptic origin of this subspecies should be considered. A more detailed phylogeographic study could elucidate these questions.

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References


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Appendix. List of the specimens deposited at LJU and SAV investigated in the present study.

Cardamine amara subsp. amara 0352/2, Notranjsko, Loška dolina, river banks of Veliki Ohrn, W of the village of Pudob, 570 m alt., 21 May 1996, leg. K. Marhold & N. Jogan (SAV); 9852/4, ob studencu na prelomu NE od Povodja, 2 May 1952, leg. F. Šuštar (LJU 45551); 9852/4, Pod Šmarno goro ob Dukič, Ljubljansko okolica, 25 April 1937, leg. A. Budnar (LJU 07539); 9660/2, Štajerska, Haloze, Podlože pod Ptujsko Goro, 240 m alt., 13 April 1994, leg. K. Marhold & N. Jogan (SAV); 9560/1, S of Mari- bor, Rače, 260 m alt., 20 April 2001, leg. J. Liho (SAV); 9460/4, Štajerska, Slovenske Gorce, Z od poti Dolge njive – Hum, 340 m alt., 9 April 1994, leg. A. Valenčar (LJU 12597); 9462/1, ob potoku Sovjak v gozdu JZ od Vidma ob Ščavnici, Prlekija, 240 m alt., 29 April 1992, leg. S. Kneft (LJU 124530); 9362/4, Prekmurje, področje na levem bregu Mura, blizu broda.
Vučja vas, peščena jasa ob mrtvici Mure, 180 m alt., 6 April 1974, leg. Š. Špišak (LJU 117812).

*Cardamine amara* subsp. *austriaca* Marhold 9950/2, Žiri, Svetelov, trnavnik na Čevljarški ulici, središče Žirov, 495 m alt., 4 May 1996, leg. P. Dreuzz (LJU 127488); 9548/1, Julian Alps, Kranjska Gora, Planica, Tamar valley, 15 June 1995, leg. T. Wraber (LJU 68037); 9548/4, Julijelske Alpe, Prislovnik, Osogona polica, 1650 m alt., 8 July 1967, leg. T. Wraber (LJU 38845); 9650/1, vas Georgije bleda, 604 m alt., 29 April 1996, leg. T. Kobilar (LJU 127390); 9651/3, Gorenjska, Radovljica, in an ox-ho-over-lake of the river Sava, 410 m alt., 15 April 1994, leg. K. Marhold & N. Jogan (SAV); 9651/4, Gorenjska, okolica Tržiča, trnavnik pri gostišču Smuk, na levem strani smer Duplje – Križe, 490 m alt., 7 May 1995, leg. U. Bidovec (LJU 125067); Sklo, trnavnik ob Vincarskem potoku, 360 m alt., 5 May 1991, leg. N. Debeljak (LJU 127333); 9752/3, Gorenjska, Kranj z okolico, Zavica, desnì breg reke Sove pod naseljem Orehek, Biača, cistilni naprave, 360 m alt., 7 May 1994, leg. T. Dešmar (LJU 125550); 9752/3, ob Savici pri naselju Cirje jugovzhodno od Kranja, 370 m alt., 28 April 1995, leg. S. Rozman (LJU 126942); 9752/3, ob kamniti poti v gozd pri vasi Prebačevski pri Kranju, 366 m alt., 22 May 1993, leg. S. Kostanjev (LJU 125066); 9952/2, okolice Ljubljane, JV ob Bokalcu, ob sotočju Naega Grabne in Gradacite, 300 m alt., 9 May 1991, leg. Ž. Ženev (LJU 127195); 0052/4, Rakitna, 14 May 1931, leg. R. Justin (LJU 075333); 9653/1, Gorenjska, Karavanc, Gornje Jesersko, 880 m alt., 22 May 1996, leg. K. Marhold & N. Jogan (SAV); 9652/4, 150 m od hiše Celarjevih, od Zg. Celarjevih, od Zg. Celarjevih, 490 m alt., 7 May 1997, leg. N. Hojn (LJU 127806); 9752/2, Gorenjska, Cerkvice, trnavnik ob potoku Učica, 400 m alt., 25 April 1991, leg. M. Zorec (LJU 127242); 9953/2, kraj Poljčan, 30 m alt., 28 April 1996, leg. S. Struculc (LJU 127430); 9953/4, Ljubljana, nasodno v smer Podgrad-Besnica, potok Besnica, 375 m alt., 2 May 1997, leg. M. Ošanič (LJU 127901); 9954/3, dolina Besnica pri Zalogu, 500 m od Zg. Besnice 5 proti Trebeljevem, 400 m alt., 18 April 1998, leg. A. Skoročan (LJU 129238); 9854/4, in ulginosis hand procul a vise ferrae statione “Kresnice”, 250 m alt., 28 April 1928, F. Doljak (LJU 07548); 9854/4, Zahodno Posavsko hribovje-Peče, vlažen trnavnik ob potoku Velika voda, 450 m alt., 21 April 1994, leg. Š. Bačler (LJU 125589); 9855/1, in locis ad rivulum prope vicum St. Ozabd hand procul pagni Trojane, 520 m alt., 5 May 1968, leg. T. Wraber (LJU 62422); Zg. Savinjska dolina, na močvirnem trnavniku na zahodnem pobočju Homa (ob cesti Radmirje-Gornji Grad) jugovzhodno od domačije Potokinci, 440 m alt., 16 April 1994, leg. M. Petkovšek (LJU 125530); 9554/4, Savinjske Alpe, Raduha, pl. Loka, 30 May 1953, leg. L. Gogidi (LJU 94494); 9555/3, Komen, Studenec, 1600 m alt., 10 August 1965, leg. D. Druskovič (LJU 94056); 9555/1, Koroška, Črna, in the Bistra valley, 630 m alt., 27 April 1994, leg. K. Marhold & N. Jogan (SAV); 9455/3, Koroška, Mežiška dolina, Mežica, Starjevo, 500 m alt., 19 May 1998, leg. J. Majc (LJU 129108); 9455/3, Koroška, Prevalje, pri gostišču Stopor ob Sentenskej reki, vlažna prst ob robu potoka, 430 m alt., 25 April 1992, leg. R. Verovnik (LJU 124593); 9455/1, v obcestni mali na zelo vlažnom rastišču ob potu proti Godčevi kmetiji, S prevladi na Koroškem, 680 m alt., 24 May 1992, leg. M. Kehler (LJU 124382); 9455/4, Mežiška dolina, Prevalje, Račal, desnì breg Kristanovega potoka, 440 m alt., 20 May 1994, leg. K. Stopar (LJU 127274); 9455/4, levì breg slezanske poti, pod kmetijo Tičler na Strašiču, vzhodno od Brezunci, 400 m alt., 5 May 1996, leg. J. Hudrap (LJU 124796); 9455/4, Koroška, Kotlje, vlažen trnavnik na SV strani naselje Kotlje, 444 m alt., 3 May 1998, leg. M. Kogelnic (LJU 129125); 9456/1, Koroška, Črneče, V močvirju ob Dravi, 350 m alt., 3 May 1984, leg. B. Vrež (LJU 116061); 9457/3, Štajerska, Pohorje, between Vuhred and Ribnica on Pohorje, 530 m alt., 22 May 1996, leg. K. Marhold & N. Jogan (SAV); 9558/1, Pohorje, ob studencu pod Roglji, 1300 m alt., 16 June 1954, leg. T. Wraber (LJU 68043); 9457/3, Štajerska, Hrastje pri Mariboru, gornji del Ludmerske grabe, 320 m alt., 6 May 1995, leg. N. Mori (LJU 126451); 9460/3, Štajerska, ob gozda pri Dravi, 300 m Z od gamoznice v Zgornji Duplek, 240 m alt., 25 April 1997, leg. U. Červek (LJU 127880); 9559/3, Pohorje, Smrečna, 850 m alt., 10 May 1986, leg. D. Naglič (LJU 117446); 9659/4, Poljane, Modraže, 250 m alt., melioracijski kanal ob reki Dravljani, 11 May 1996, leg. A. Vrežec (LJU 127543); 9655/2, Ptujsko polje, na desnem bregu Črne pred sotočjem s Trnavo v zgorjeni Osijšču J od Središča ob Dravi, 180 m alt., 15 April 1995, leg. M. Govedič (LJU 126390); 9656/2, Paka pri Velenju, 410 m alt., 17 August 1976, leg. D. Naglič (LJU 97490); 9657/2, Paski Konjak, v soteski pod Brdcami, 700 m alt., 17 May 1985, leg. Z. Kečelič (LJU 116665); 9657/1, Dolina, ob potoku v Hudičevena grabu, 420 m alt., 9 May 1985, leg. Z.
Keglevič (LJU 16628); 9656/4, Velenje, Kavče, Sp. Kavče, 400 m alt., 1 July 1991, leg. Z. Mazej (LJU 127304); 9656/4, Bevče pri Velenju, 400 m alt., 25 April 1977, leg. D. Nalčič (LJU 97868); 9757/2, Stajerska, Celje, 290 m J od Šmartinskega jezera, 240 m alt., 3 May 1996, leg. P. Kačičnik (LJU 127411); 9757/4, Celje-pri Petričku, 250 m alt., 16 April 1971, leg. T. Knez (LJU 85237); 9757/3, Celje, ob kanalu, ki se pri Celju izliva v Ložnico, 240 m alt., 23 April 1971, leg. T. Knez (LJU 86277); 9756/4, Stajerska, Sešče pri Preboldu, ob potoku Potočič, 275 m alt., 8 May 1996, leg. M. Jezernik (LJU 127184); Štajerska, Savinjska dolina, Prebold, Grajska vas, 350 m alt., 20 April 1994, leg. T. Kurent (LJU 125705); 9857/1, Laško, ob Savinji, 1 km JV od Laškega, 270 m alt., 29 April 1995, leg. I. Tomazić (LJU 126469); 9857/3, kompleks Koupnikova, Gore, 450 m alt., 17 May 1970, leg. T. Knez (LJU 35255); 9958/1, dolina Gračnice, med Miščim Dolom in Kiščom, 300 m alt., 5 May 1973, leg. T. Knez (LJU 35254); 0057/2, Loke v dolini Impoljskega potoka pod vasjo Dolnje Orle, 250 m alt., 14 April 1989, leg. M. Kačičnik (LJU 121812); 0158/1, Hrvaški brod pri Šentjerneju, 30 April 1967, leg. R. Ljubič (LJU 93048); 0056/4, Kranjsko-Dolenjska, Ribjek pod Trebelnim, 3 May 1908, leg. R. Justin (LJU 07536); 0156/2, Kranjsko-Dolenjska, v dolini Statenberk pri Trebelnem, 12 May 1907, leg. R. Justin (LJU 07534); 0056/3, ob potoka Pravharica na Lanšprežu, 3 kmSZ od Trebnjega, 280 m alt., 10 April 1992, leg. P. Zalar (LJU 124750); 0258/1, Gorienc, dolina Kobilje, 2 May 1958, leg. V. Strgar (LJU 46150); 0558/1, Bela krajina, Črnosej, Mišič, vlažen travnik ob reki Kolpi, 200 m alt., 10 April 1997, leg. D. Vukčevič (LJU 129112); 0557/3, Bela krajinna, Damelj, in paludosis silvaticis ad fluvium Kolpa, 190 m alt., 16 May 1980, leg. A. Podobnik & T. Wranjer (LJU 101782); 0254/3, Ribnica, Brezje (močvirno podrocje JZ od Nemške vasi), na desnem bregu potoka Sajevce, 491 m alt., 27 April 1994, leg. B. Ožen (LJU 125703).