Genus Cardamine (Brassicaceae/Cruciferae)

at least **280 spp. worldwide**, mostly N Hemisphere, **54 spp. in Europe**, ca. 50 spp. in N and C America, centre of diversity in Far East and Himalayas (about 70 spp.)



Chromosome numbers and polyploidy

Large karyological diversity

diploids with 16 chromosomes to 32-ploid with 256 chromosomes - N American *C. concatenata, C. diphylla*

Several taxonomically critical

polyploid complexes with unresolved evolutionary history

Species with several cytotypes



Kučera et al. 2005, Biologia (Bratislava) 60: 473-476.



Iberian populations of Cardamine pratensis s. str.



Lihová et al., 2003, Taxon 52: 783-802 Unequal geographical distribution of chromosome number records

- chromosome number records for 48% of species (3020 records)
- Europe gathers 1/4 of species, but 89% of chromosome number records

Japan (Hokkaido), Russia (Sakhalin) *Cardamine fauriei* or *C. yezoensis*? *C. valida* as a synonym?



Japan (Hokkaido) *Cardamine schinziana*?



Japan (Hokkaido, Honshu, Shikoku, Kyushu)

Cardamine yezoensis, C. kiushiana, or *C. torrentis*?

Korean Peninsula *Cardamine amariformis*?



Published chromosome numbers from Japan and Russian Far East

| Taxon | 2 <i>n</i> | Locality | Author | Original determination | Note |
|--|-------------|---|--|------------------------------------|---|
| C. torrentis s.l. (C. valida) | 32 | Japan, Hokkaido | Kurosawa, 1981 | C. yezoensis | Only <i>C. valida</i> was found at this locality in 2004. |
| <i>C. torrentis</i> s.l. (<i>C. valida</i>) | 32 | Japan, Hokkaido | Kurosawa, 1981 | C. yezoensis | Only <i>C. valida</i> was found at this locality in 2004. |
| <i>C. torrentis</i> s.l. | 56 | Japan, Honshu | Kurosawa, 1981 | C. torrentis | |
| <i>C. torrentis</i> s.l. (<i>C. valida</i>) | 32 | Russia, Sakhalin | Rudyka, 1984 | C. yezoensis | Specimen deposited in VLA was revised (incomplete plants only). |
| <i>C. torrentis</i> s.l. (<i>C. valida</i>) | 16 => 32 | Russia, Sakhalin | Sokolovskaya, 1960 | C. yezoensis | Most likely referring to the same locality and the same chromosome count as the next record. |
| <i>C. torrentis</i> s.l. (<i>C. valida</i>) | 16 => 32 | Russia, Sakhalin | Probatova and Sokolovskaya, 1988 | C. yezoensis | Specimens deposited in LEU and VLA were revised. |
| C. yezoensis | 72 | Japan, [Hokkaido | Nishikawa, 1986 | C. yezoensis | |
| C. yezoensis? | 46-48 | plants from Botanical Garden Edinburgh | Manton, 1932 | C. leucantha prol. yezoensis | •No voucher specimen was found in herbaria CGE, LDS and MANCH. |

Sampling

We sampled 55 populations throughout Japan, including material from original localities of *Cardamine akitensis*, *C. fauriei*, *C. geifolia*, *C. kiusiana*, *C. nasturtiiformis*, *C. schinziana*, *C. torrentis*, and *C. yezoensis*.

Four populations of *C. amariformis* were sampled in South Korea.

One to 14 living plants were collected from each population, transferred to pots in experimental gardens, and materials from these plants were used for flow cytometric measurements and chromosome counting.

For morphometric evaluation 40 populations (9–32 plants per population, 1018 plants altogether) throughout the Japanese Archipelago and one population sample of *C. amariformis* (28 plants) was collected in South Korea.

In 2010-2012 additional material was collected in Far East Russia.

а.



C.

d.



Marhold et al., Annals of Botany 2010; Lihová et al., Australian Systematic Botany, 2010.



Marhold et al., Annals of Botany 2010; Lihová et al., Australian Systematic Botany, 2010.



Marhold et al., Annals of Botany 2010; Lihová et al., Australian Systematic Botany, 2010.





Discriminant analysis based on morphological characters



Discriminant analysis based on morphological characters





Cardamine yezoensis = C. fauriei = C. geifolia = C. akitensis

Japan (Hokkaido, N Honshu), Russia (S Sakhalin)





Cardamine schinziana

Japan (Hokkaido)





Cardamine torrentis = C. kiusiana 2n=8x=64

Japan (Kyushu, Shikoku, S Honshu)





Cardamine valida 2n=2x=32

Japan (N Honshu, Hokkaido, Russian Far East, incl. Sakhalin)





Cardamine amariformis 2*n*=4*x*=32

Korea

conspecific with *C. valida*?

Textbook example of the origin of hybrid and polyploid specie Urnerboden, Switzerland





Urbanska, 1977, Ber. Geobot. Inst. ETH Stiftung Rübel 44: 2-85, 44:86-103; Urbanska & Landolt, 1972, 1978, Ber. Geobot. Inst. ETH Stiftung Rübel 41: 88-101, 45: 30-53; Urbanska et al., 1997, Pl. Syst. Evol. 204:233-256;

Karyotype structure of parental diploid species



Genome structure of C. × insueta

C. × *insueta* (2n = 24) **RRA** (16+8)









Descending dysploidy of *C. pratensis* and both cytotypes of *C. schulzii*

 A K 5
 A K 5

 A K 1/6
 A K 5/1 /6

| Contraction of the second | A | K 5 | |
|-----------------------------------|-----|-------------|--|
| Contraction of the local distance | AI | K 5 | |
| 1.00 | AI | K 5 | |
| 100 | AI | K 5 | |
| Autor I | AI | K 8/6 | |
| 0.000 | A I | K 8 / 6 | |
| and and | AI | K 8 / 6 | |
| 1.00 | AI | K 8 / 6 | |
| 1 | A I | K 5 / 8 / 6 | |

C. pratensis (2n = 4x-2 = 30) C. schulzii (2n = 5x-2 = 38) C. schulzii (2n = 6x-2 = 46)









Mandáková et al. 2013, Plant Cell



Hexaploid RRRRAA plants not found at Urnerboden

instead

non-*insueta* hybrid individuals with 38 or 46 chromosomes were found

Both comprise eight smaller A chromosomes plus 30 or 38 chromosomes labeled by gDNA of *C. rivularis*

Out of 30 or 38 chromosomes six ones were bearing terminal heterochromatic knobs not labeled by *C. rivularis* gDNA, but typical for hypotetraploid *C. pratensis* (2n=30) Cardamine schulzii (2n = 5x-2 = 38) PPRRA (30+8)



gDNA C, amara

oDNA C. rivularis



Cardamine schulzii (2n = 6x-2 = 46) **PPPRA (38+8)**

gDNA C. amara gDNA C. rivularis







Descending dysploidy of *C. pratensis* and both cytotypes of *C. schulzii*

 A K 5
 A K 5

 A K 1/6
 A K 5/1 /6

| Contraction of the second | A | K 5 | |
|-----------------------------------|-----|-------------|--|
| Contraction of the local distance | AI | K 5 | |
| 1.00 | AI | K 5 | |
| 100 | AI | K 5 | |
| Autor I | AI | K 8/6 | |
| 0.000 | A I | K 8 / 6 | |
| and and | AI | K 8 / 6 | |
| 1.00 | AI | K 8 / 6 | |
| 1 | A I | K 5 / 8 / 6 | |

C. pratensis (2n = 4x-2 = 30) C. schulzii (2n = 5x-2 = 38) C. schulzii (2n = 6x-2 = 46)









Mandáková et al. 2013, Plant Cell

True story after all ???



Mandáková et al. 2013, Plant Cell

Cardamine pratensis complex

Complex study of Central European and Balkan populations of the currently and previously recognised taxa:

C. pratensis s.str., C. nemorosa, C. udicola, "C. ucranica", C. rivularis, C. rivularis auct., C. matthioli, C. majovskyi

All plants were checked for chromosome numbers (219 plants from 58 populations) and/or ploidy level by **chromosome counting** or **FCM**

Microsatellite study on 145 populations (1104 plants), 18 microsatellite loci used yielded 394 alleles

HybSeq study based on the reduced set of plants from 47 populations (one plant per population)



Relative genome size

Number of samples









C. pratensis – fusion of chromosomes



Genetic structure of all species studied, C. matthioli, C. majovskyi, C. rivularis, and C. pratensis, as inferred from microsatellite data analyses



Genetic structure of Cardamine matthioli, C. majovskyi, and C. rivularis inferred from microsatellite data analyses



Genetic structure of *Cardamine pratensis* inferred from microsatellite data analyses



Genetic structure of C. matthioli, C. majovskyi, C. rivularis, and C. pratensis inferred from Hyb-Seq data analyses. **A** - Supernetwork representation of quartets generated in SuperQ, derived from 499 most informative ML gene trees. **B** - ML tree constructed from concatenated allele sequences obtained by read-backed phasing from all 963 genes in RAxML-NG



Results of RDA showing significant differences in the environmental niches of the four analyzed *Cardamine* species (A), genetic lineages within *C. matthioli* and *C. majovskyi* (B), and three genetic lineages within *C. pratensis* (C). Ellipses define regions expected to contain 95% of all populations of the species or lineages.



Melichárková et al., Frontiers in Plant Science 1:588856, 2020. doi: 10.3389/fpls.2020.588856