

Flow cytometry and cytogenomics in complicated polyploid complexes: examples from the genus *Cardamine*

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CEITEC





Cardamine yezoensis
JP, Sakhalin



Cardamine valida
JP, Sakhalin, Russian Far East



Cardamine schinziana
JP, Hokkaido endemic



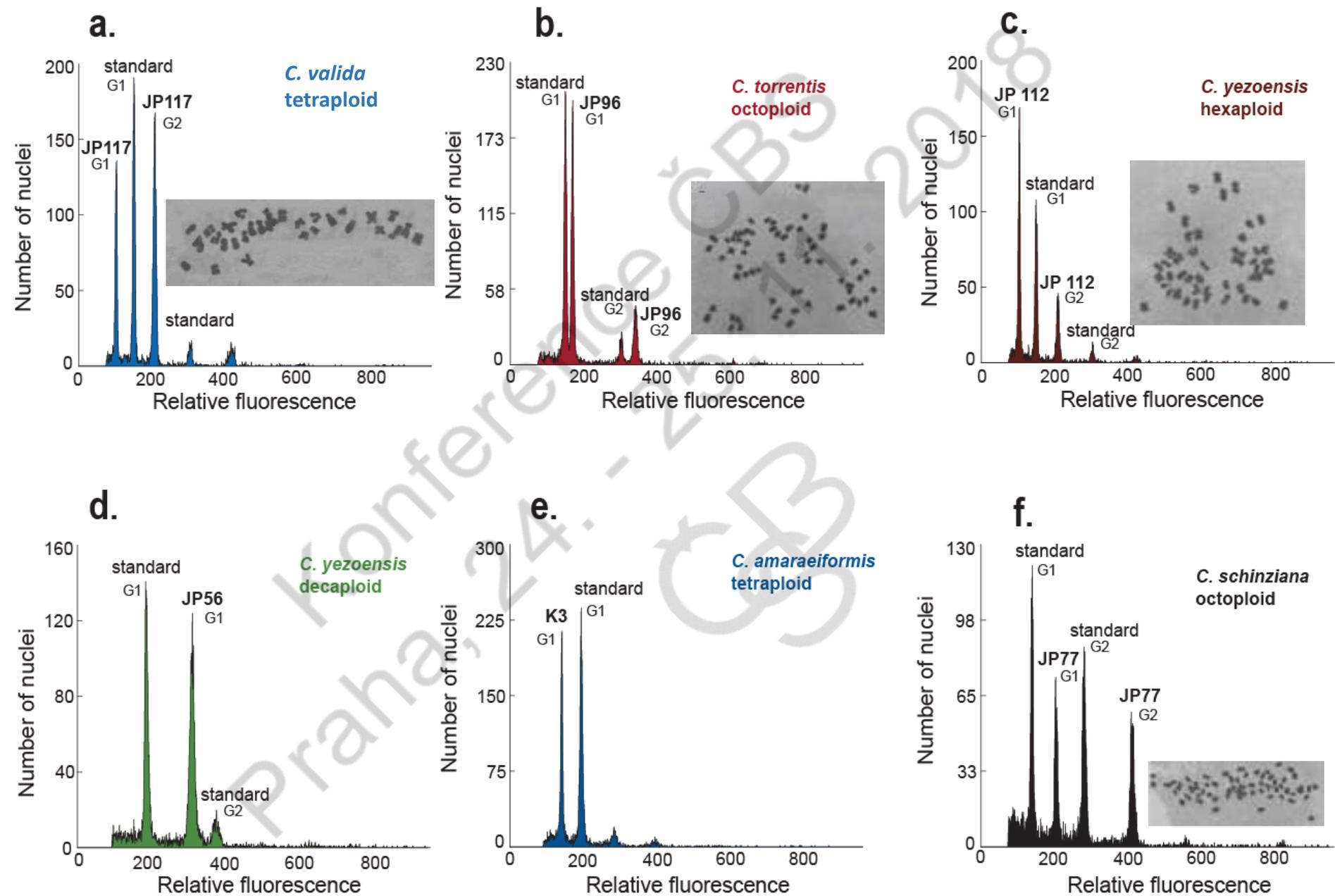
Cardamine torrentis *JP*

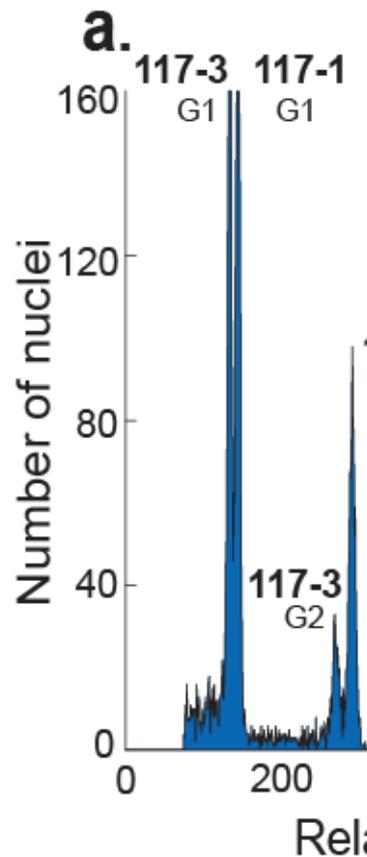


Cardamine amariformis
Korea

Published chromosome numbers from Japan and Russian Far East

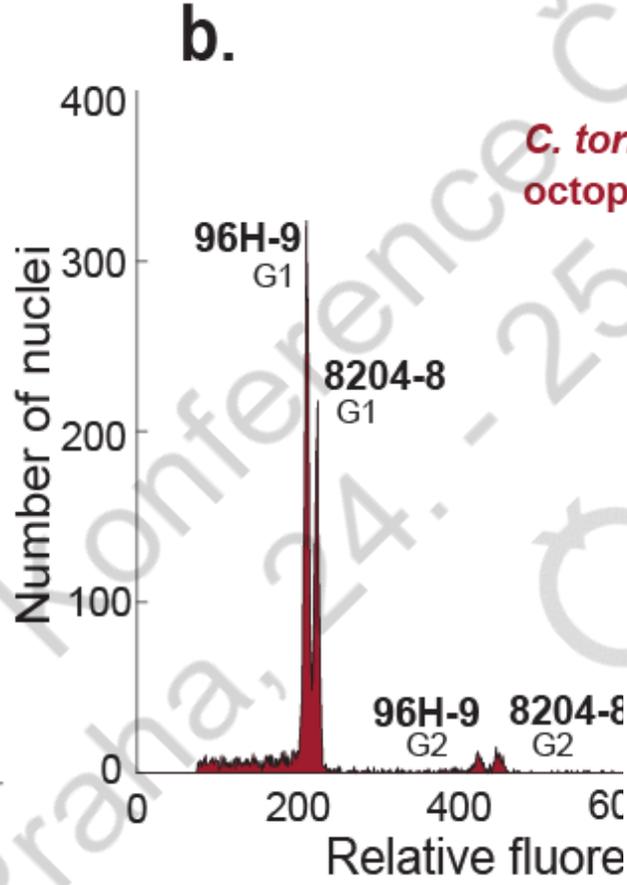
Taxon	$2n$	Locality	Author	Original determination	Note
<i>C. torrentis</i> s.l. (<i>C. valida</i>)	32	Japan, Hokkaido	Kurosawa, 1981	<i>C. yezoensis</i>	Only <i>C. valida</i> was found at this locality in 2004.
<i>C. torrentis</i> s.l. (<i>C. valida</i>)	32	Japan, Hokkaido	Kurosawa, 1981	<i>C. yezoensis</i>	Only <i>C. valida</i> was found at this locality in 2004.
<i>C. torrentis</i> s.l.	56	Japan, Honshu	Kurosawa, 1981	<i>C. torrentis</i>	
<i>C. torrentis</i> s.l. (<i>C. valida</i>)	32	Russia, Sakhalin	Rudyka, 1984	<i>C. yezoensis</i>	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	<i>C. yezoensis</i>	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	<i>C. yezoensis</i>	Specimens deposited in LEU and VLA were revised.
<i>C. yezoensis</i>	72	Japan, [Hokkaido	Nishikawa, 1986	<i>C. yezoensis</i>	
<i>C. yezoensis</i> ?	46-48	plants from Botanical Garden Edinburgh	Manton, 1932	<i>C. leucantha</i> prol. <i>yezoensis</i>	•No voucher specimen was found in herbaria CGE, LDS and MANCH.





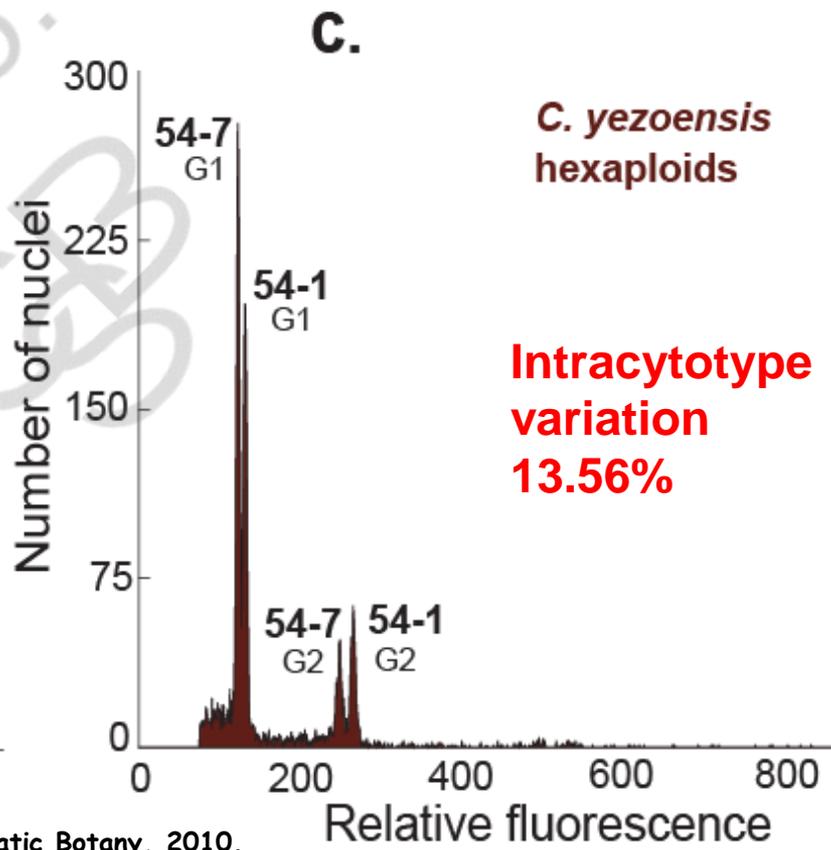
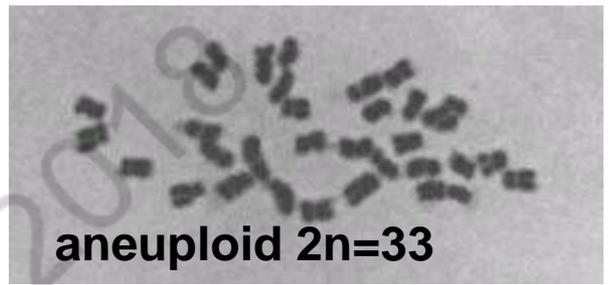
C. valida
tetraploids

Intracytotype
variation
11.84%



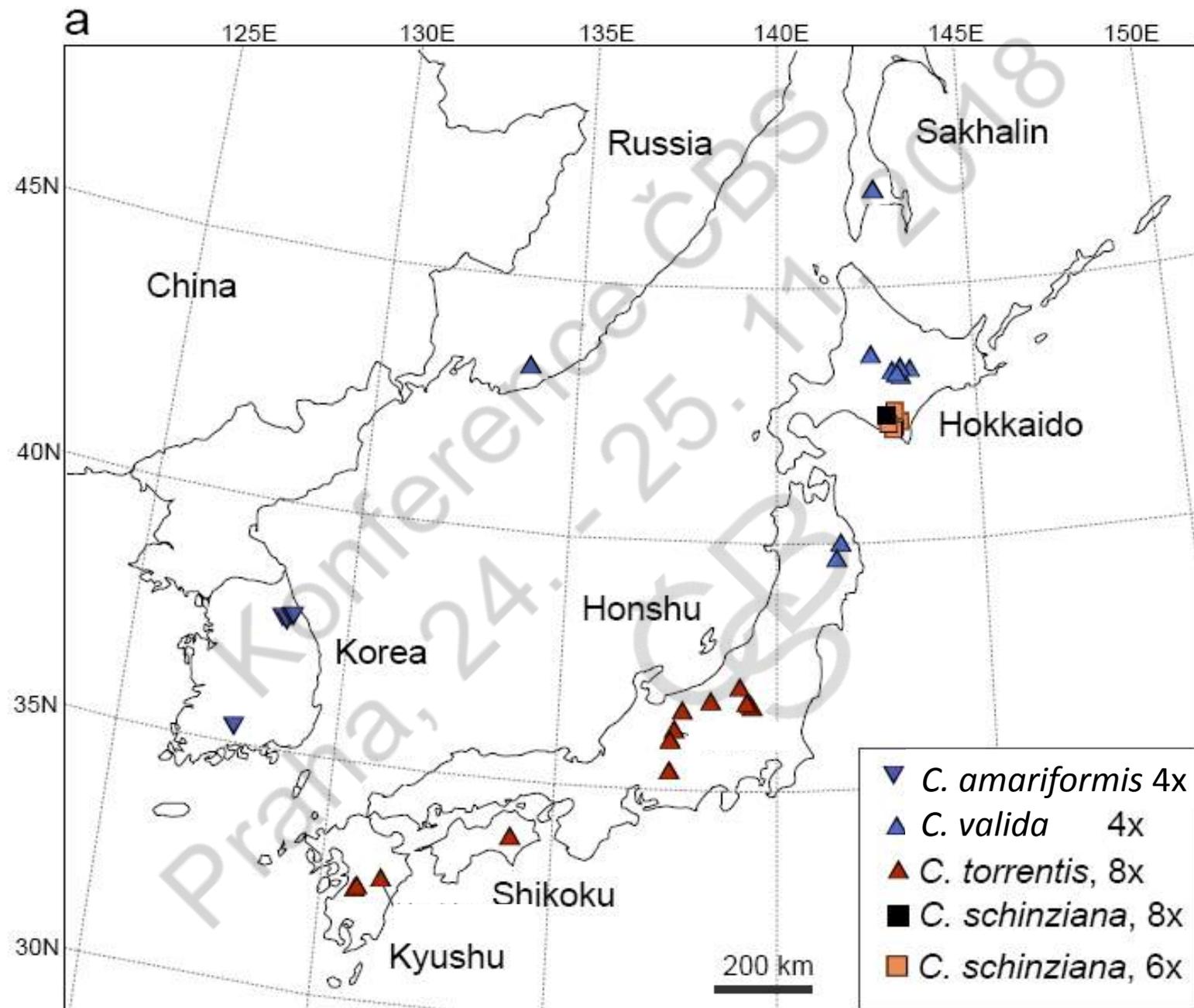
C. torrentis
octoploids

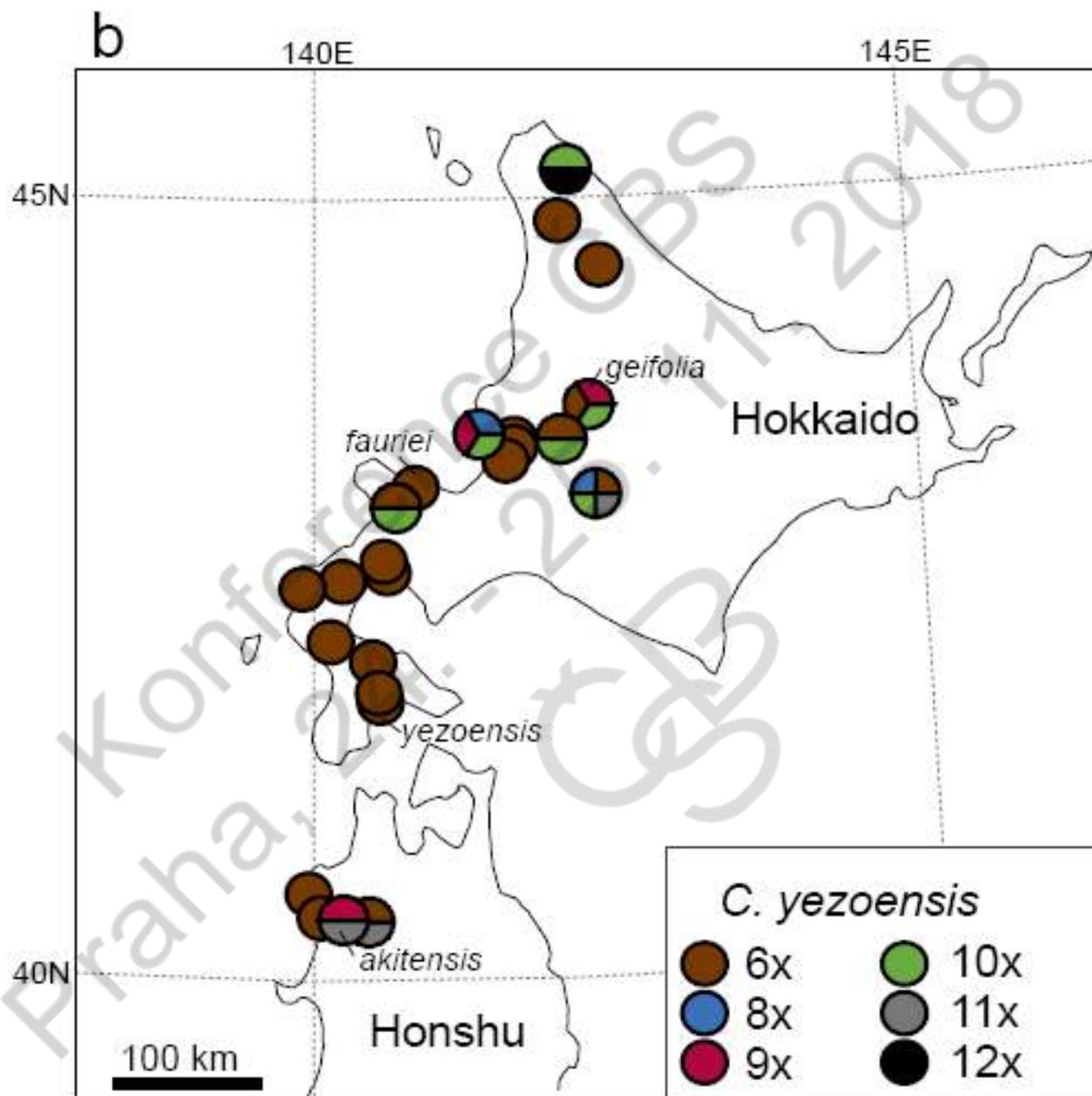
Intracytotype
variation
9.25%



C. yezoensis
hexaploids

Intracytotype
variation
13.56%





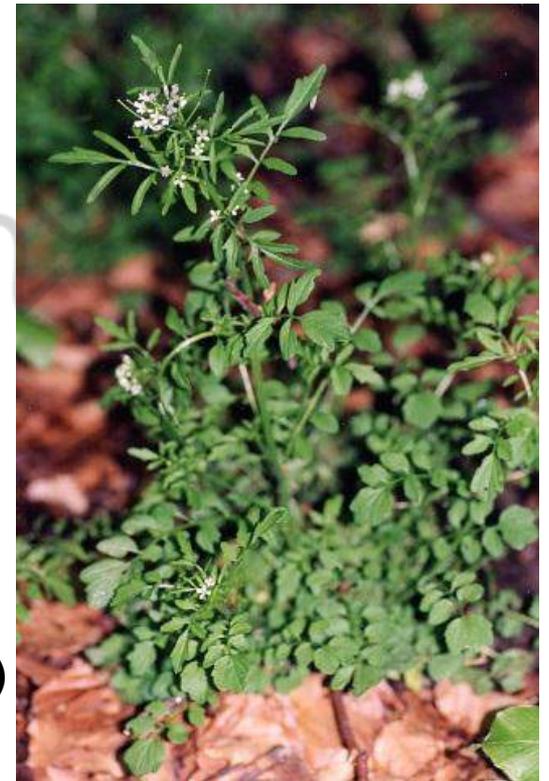
Sometimes around 2000

Cardamine flexuosa:

tetraploid, morphologically variable

native in Europe and W Asia, reported as introduced to N & S America, E Asia, Australia, New Zealand, Africa

habitats: forests, forest clearings, streamsides, roadsides (gardens, orchards, fields, greenhouses)



Australia: *C. aff. flexuosa* (nurseries, pavement cracks, garden beds)

N America: *C. flexuosa* (?native), *C. debilis* Don (introduced, greenhouses, gardens)

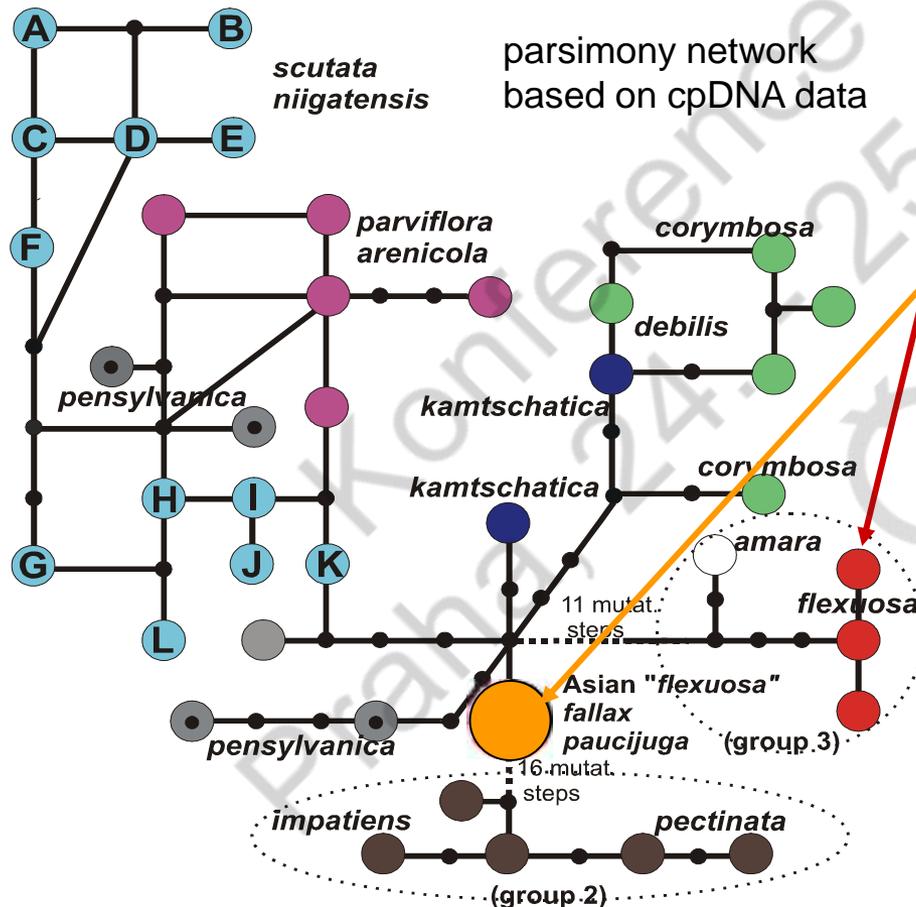
Japan: *C. flexuosa* (paddy fields, crop fields, orchards)

China, India: *C. flexuosa* and *C. hirsuta*



Cardamine flexuosa and its relatives

comparative study of 19 putatively related taxa sampled worldwide
ITS and *trnL-trnF* cpDNA seq.

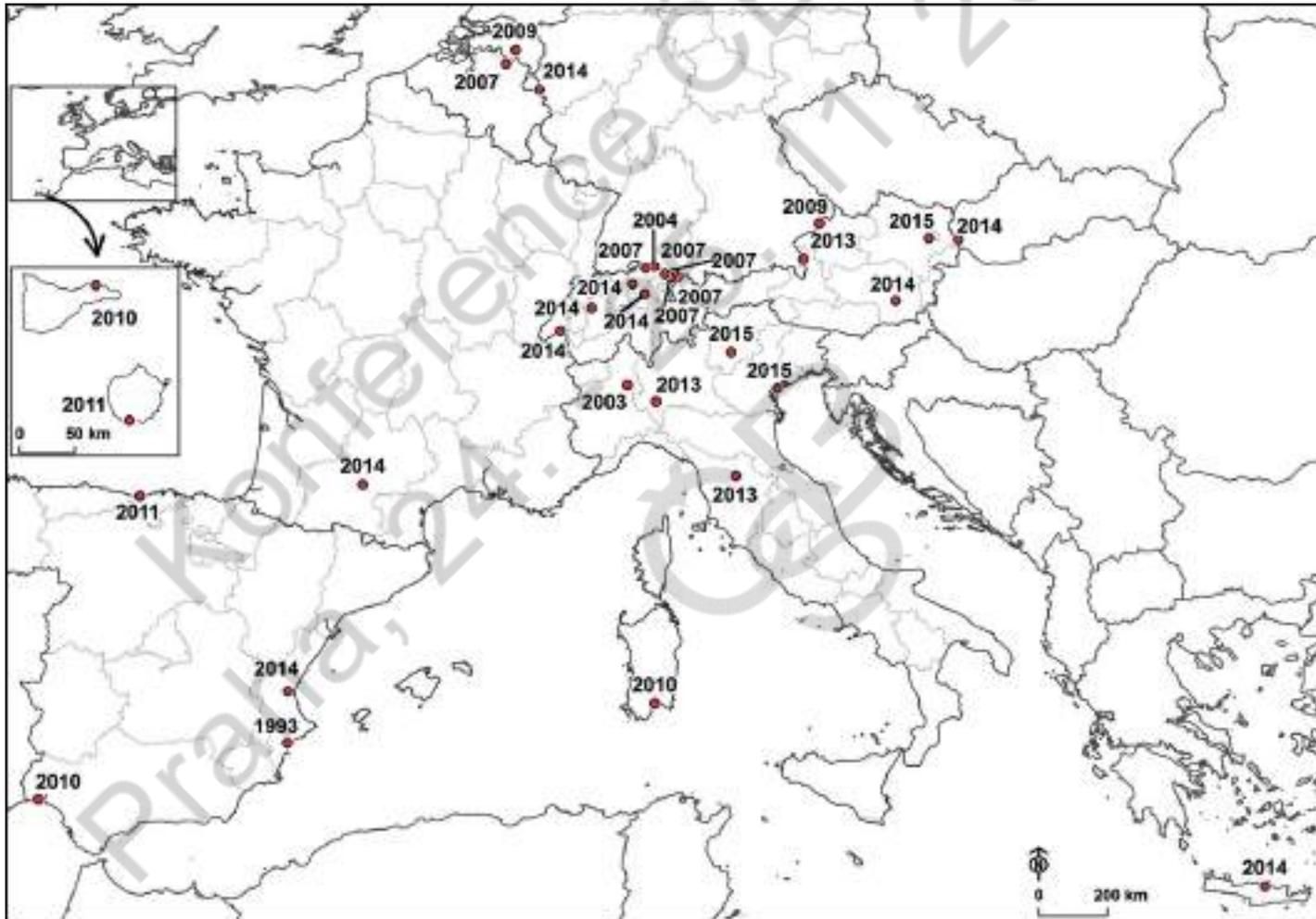


European *C. flexuosa*
distinct from populations from E Asia
attributed to this species

Asian populations - octoploid



Localities of the first occurrences of *Cardamine occulta* in Europe



Australia:

C. aff. flexuosa* (nurseries, pavement cracks, garden beds) = *C. occulta

N America:

***C. flexuosa* (introduced),**

C. debilis* Don (introduced, greenhouses, gardens) = *C. occulta

Japan:

C. flexuosa* (paddy fields, crop fields, orchards) = *C. occulta

China, India:

C. flexuosa* and *C. hirsuta* = *C. occulta





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Ecogenomics of cleistogamous and chasmogamous flowering: genome-wide gene expression patterns from cross-species microarray analysis in *Cardamine kokaiensis* (Brassicaceae)

Shin-ichi Morinaga^{1,2*}, Atsushi J. Nagano², Saori Miyazaki², Minoru Kubo⁴, Taku Demura⁴, Hiroo Fukuda⁴, Satoki Sakai¹ and Mitsuyasu Hasebe^{2,5}



C. flexuosa



C. occulta



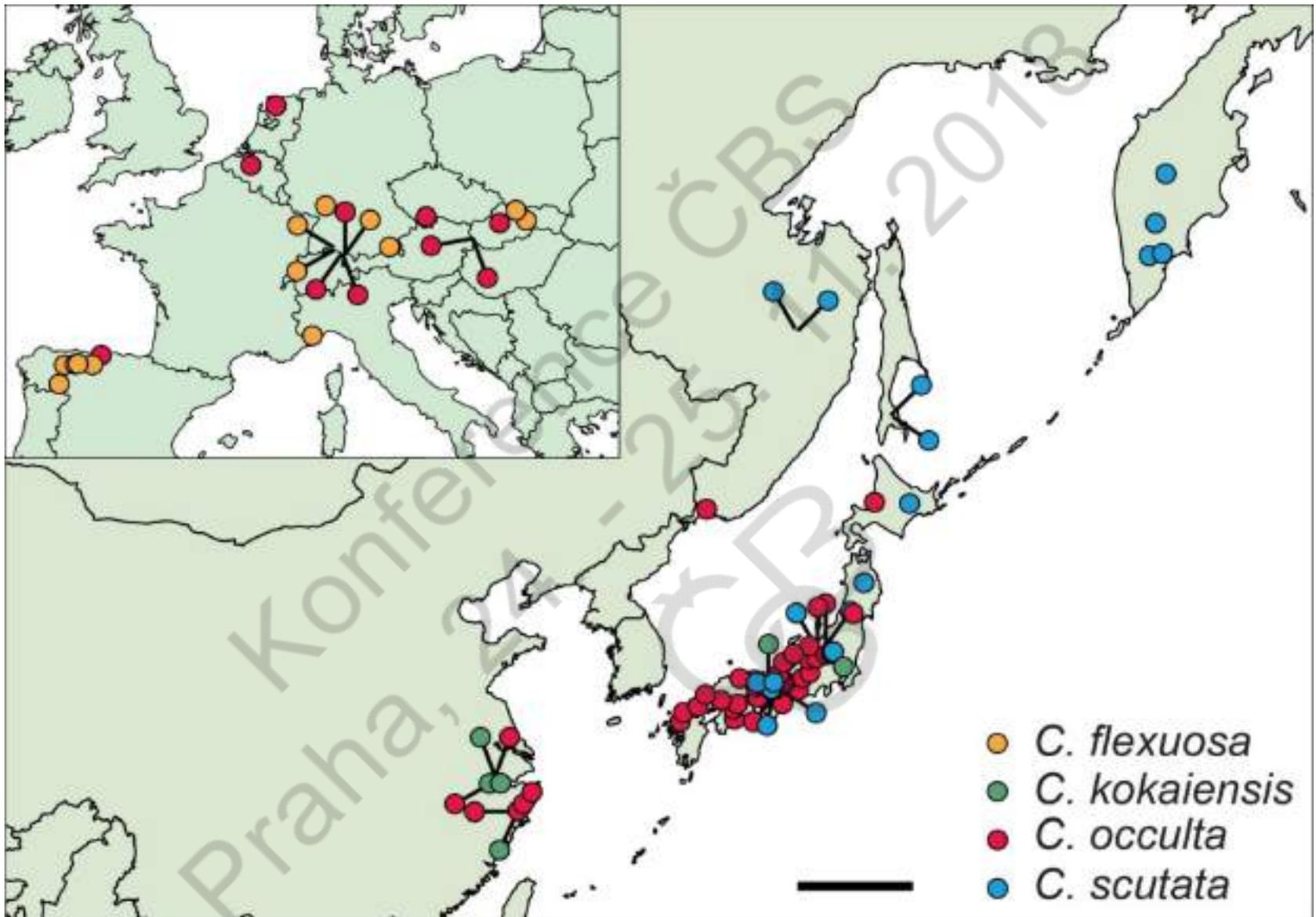
C. scutata

C. occulta

EUROPE
ASIA

C. kokaiensis

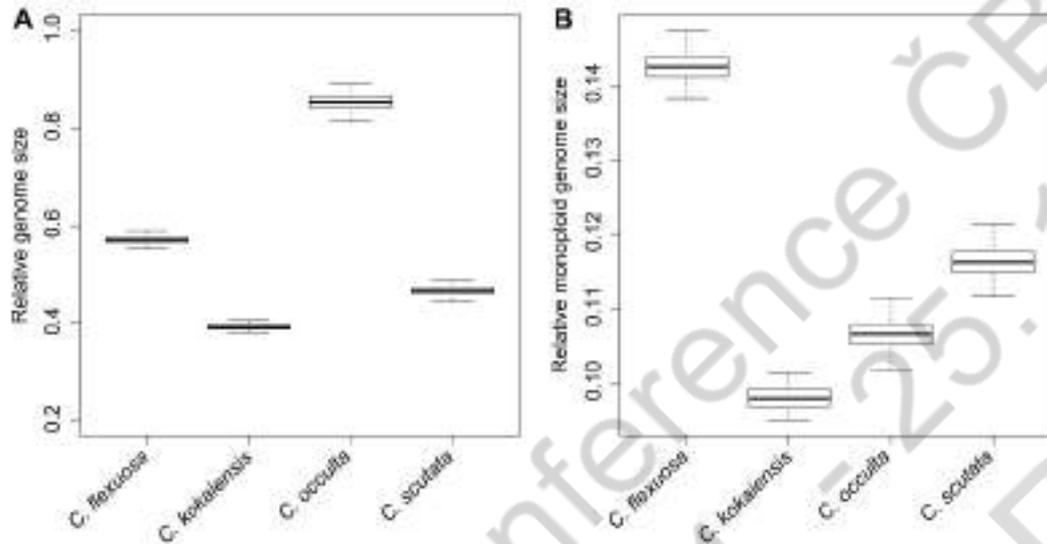




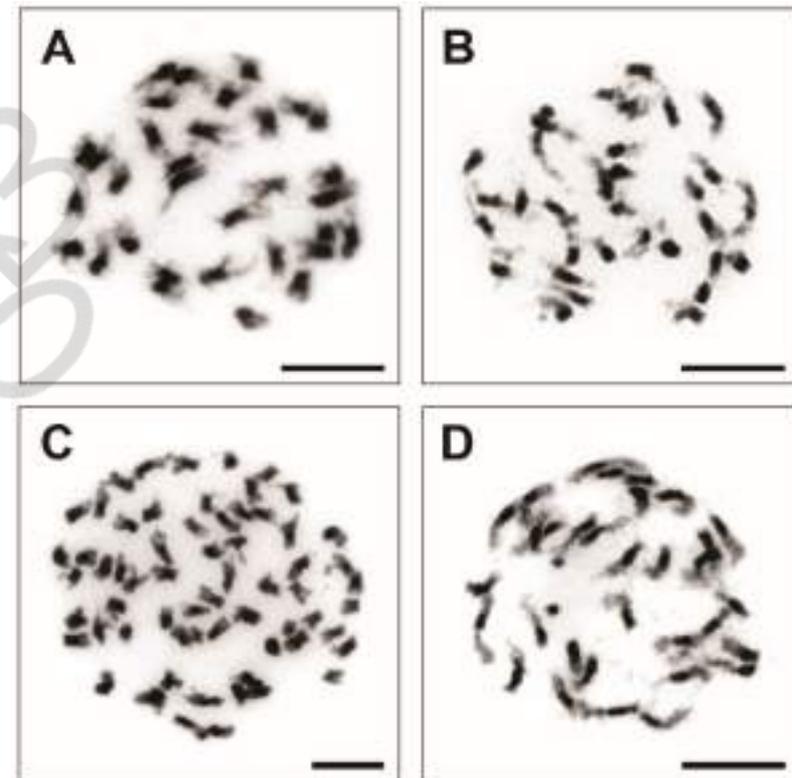
Locations of sampled populations of *Cardamine flexuosa*, *C. kokaiensis*, *C. occulta* and *C. scutata* in East Asia and Europe (nested picture). The scale bar indicate 500 km.

Šlenker et al., 2018, Botanical Journal of the Linnean Society, 187: 456–482.

Flow cytometry and chromosome number counts

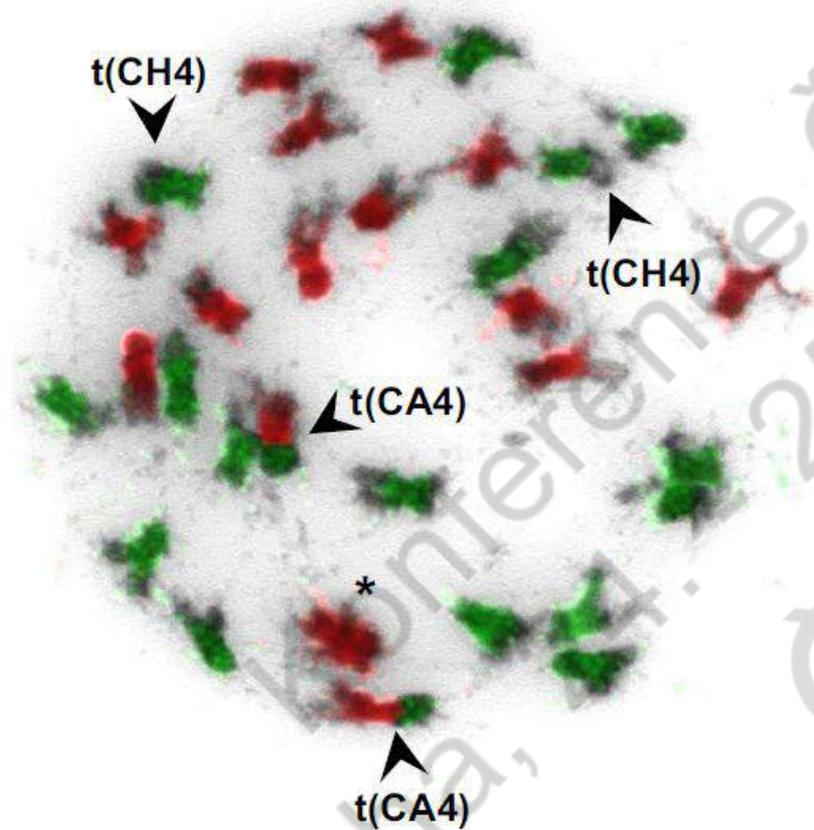


Chromosomes of (A) *Cardamine flexuosa* ($2n = 32$), (B) *C. kokaiensis* ($2n = 32$), (C) *C. occulta* ($2n = 64$) and (D) *C. scutata* ($2n = 32$) counterstained by DAPI. Scale bars indicate 10 μm .



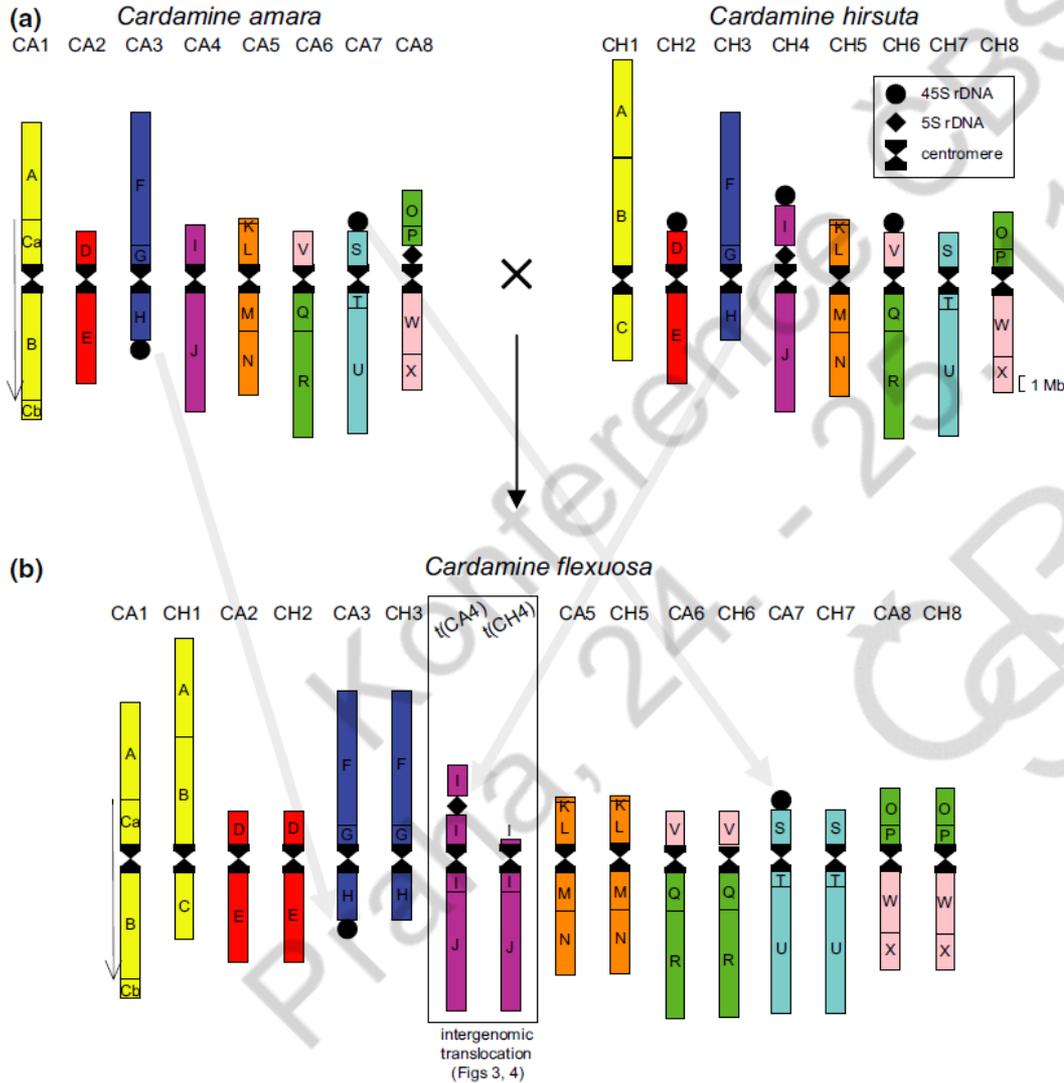
Box-and-whisker plots of (A) relative genome size, and (B) relative monoploid genome size of *Cardamine flexuosa* (4x), *C. kokaiensis* (4x), *C. occulta* (8x) and *C. scutata* (4x); in total **585 plants, 95 populations**. Whiskers are extended to the 5th and 95th percentiles. The fluorescence intensity of *Lycopersicon esculentum* ($2C = 1.96 \text{ pg}$) was set to a unit value.

Origin of European *Cardamine flexuosa*



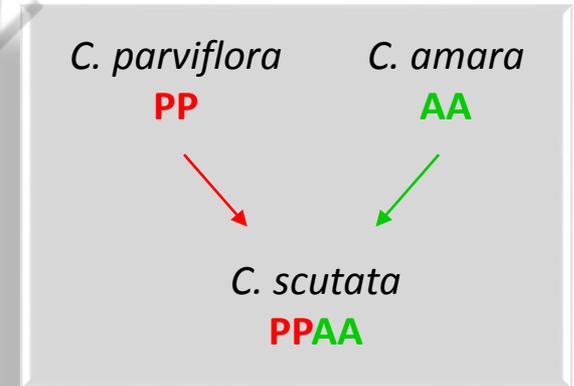
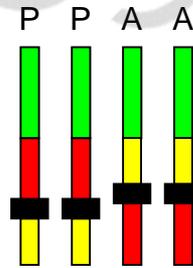
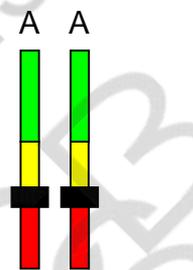
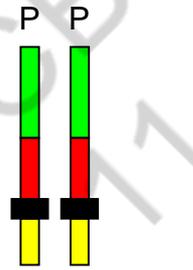
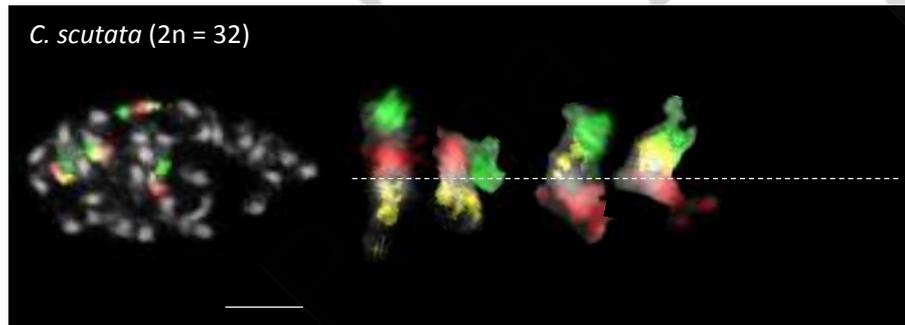
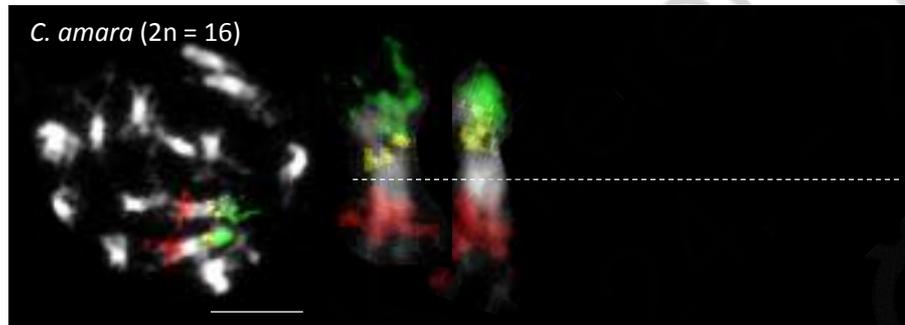
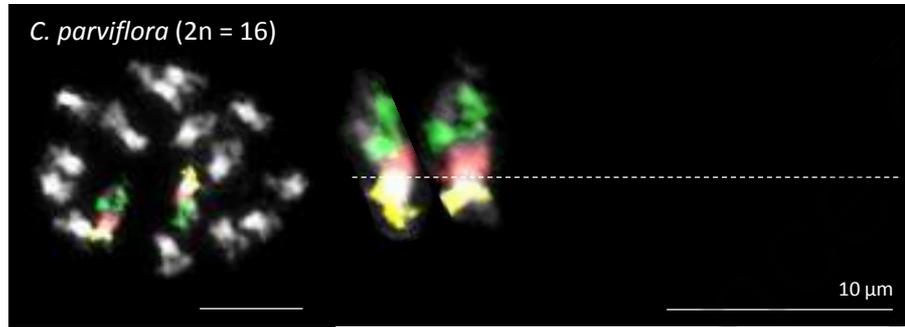
Genomic in situ hybridization (GISH) to mitotic chromosomes in the allotetraploid *Cardamine flexuosa* ($2n = 32$). GISH with total genomic DNA of *Cardamine amara* (red fluorescence; two overlapping chromosomes are indicated by a star symbol) and *Cardamine hirsuta* (green fluorescence) revealed two subgenomes contributed by ancestors of the two diploid species. Two pairs of **translocation chromosomes** (arrowheads) were identified by subsequent comparative chromosome painting (CCP) analysis.

Origin of European *Cardamine flexuosa*

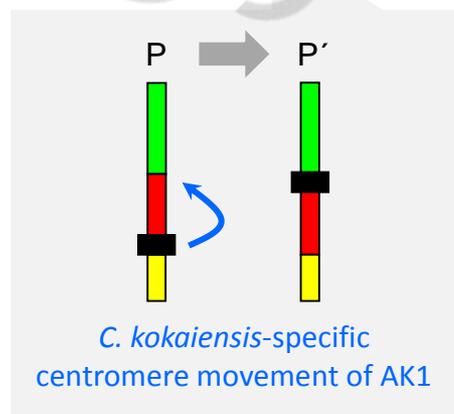
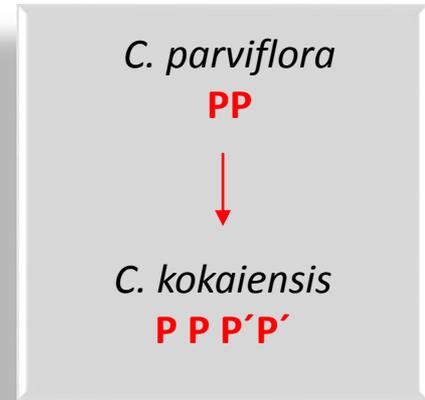
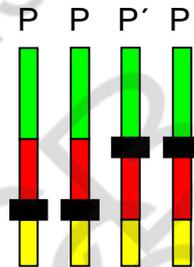
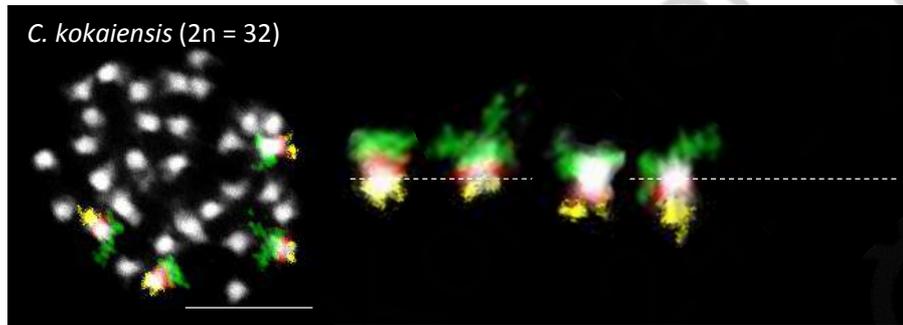
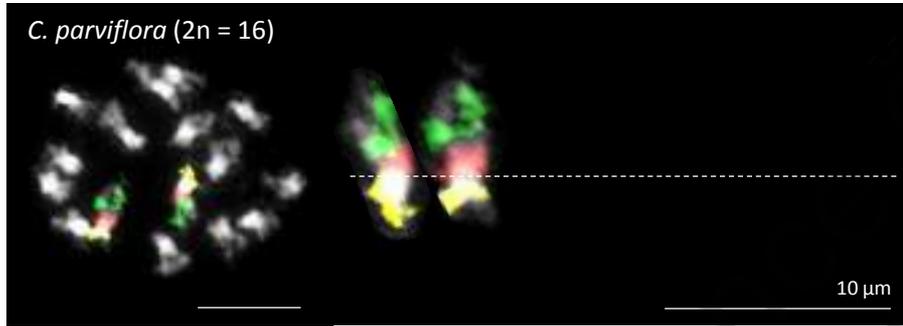


Both rearranged homeologues have undergone an identical **pericentric inversion** followed by a **reciprocal translocation with breakpoints within both genomic blocks I, exchanging unequal proportions of the upper arms of progenitor chromosomes CA4 and CH4**. Both chromosomes shared altered collinearity of blocks I and J, and differed by the length of their upper arms

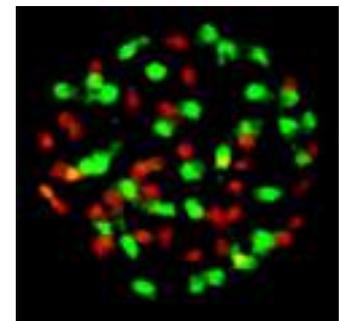
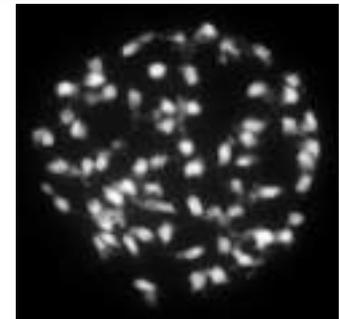
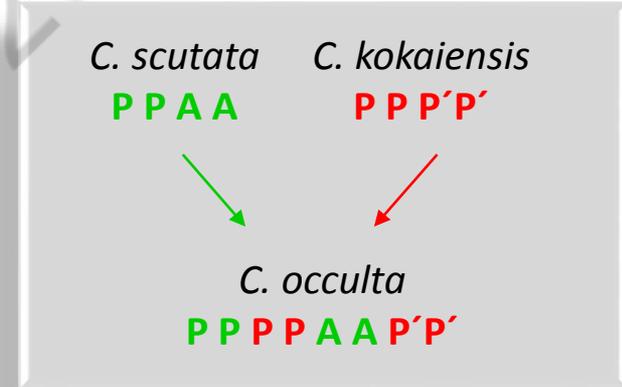
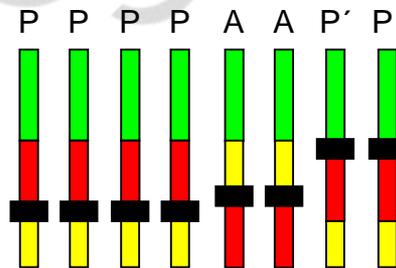
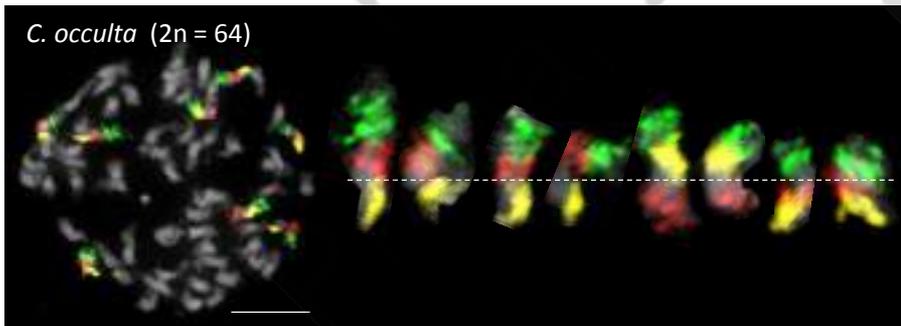
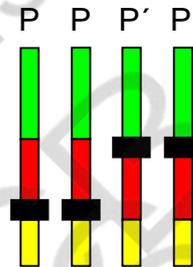
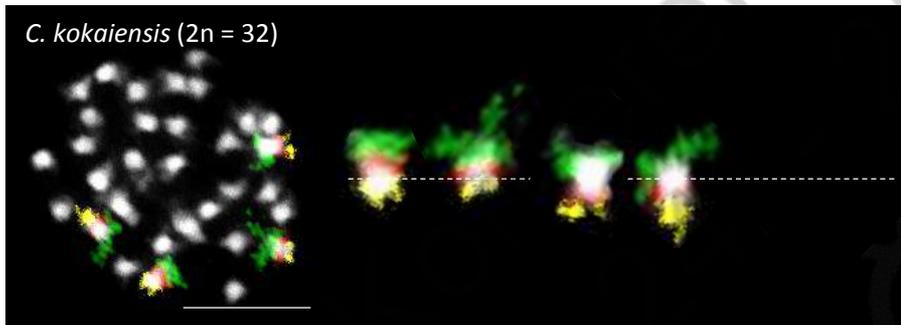
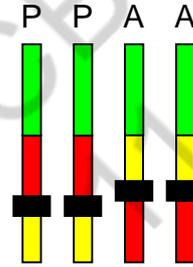
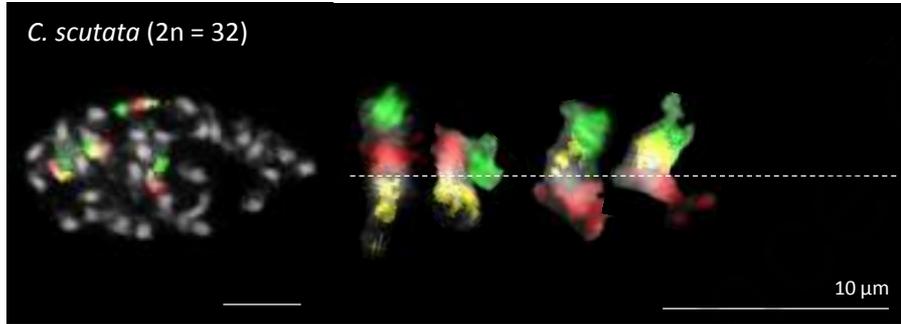
Parental-specific chromosome signatures uncovered the origin of *Cardamine scutata* ($2n = 4x = 32$)



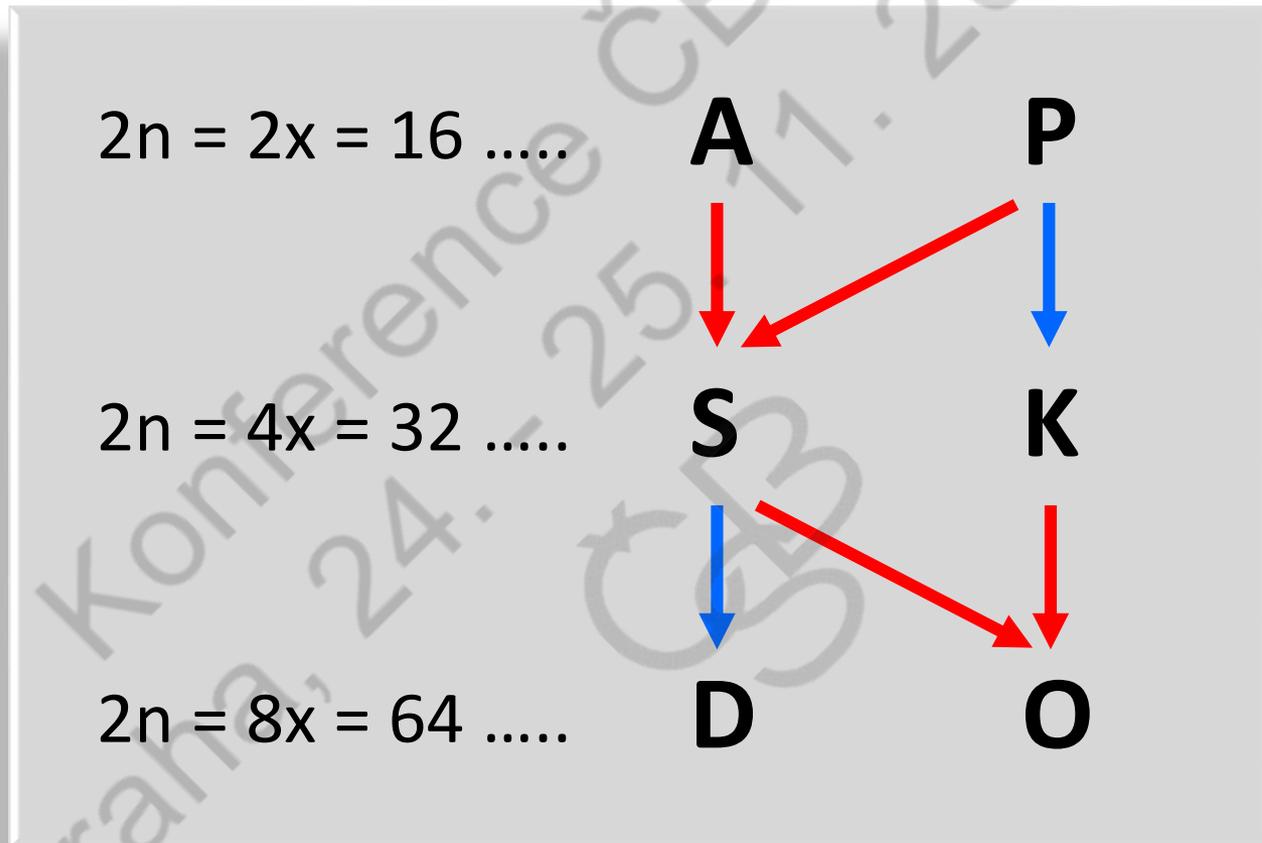
Cardamine kokaiensis ($2n = 4x = 32$)



Cardamine occulta ($2n = 8x = 64$)



Auto- and allopolyploid origin of Asian Cardamine



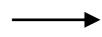
A: *C. amara*, **P:** *C. parviflora*, **S:** *C. scutata*,
K: *C. kokaiensis*, **D:** *C. dentipetala*, **O:** *C. occulta*

Konference ČBS
Praha, 24. - 25. 11. 2018



Comparative chromosome painting

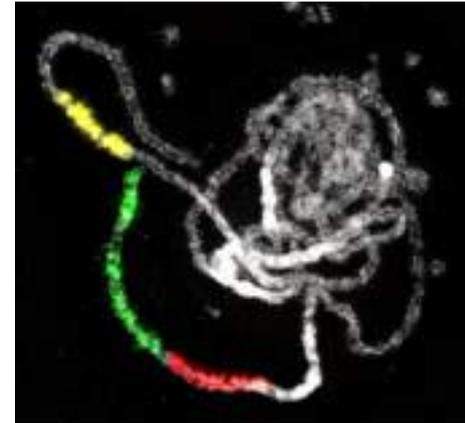
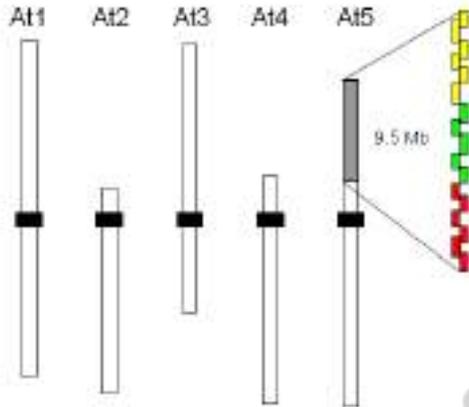
Arabidopsis thaliana BACs



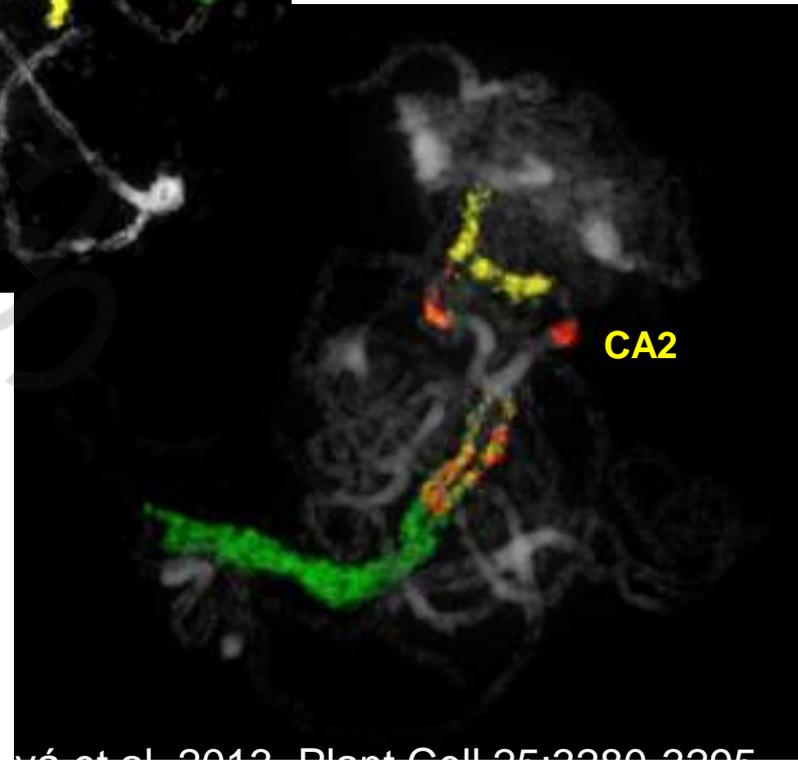
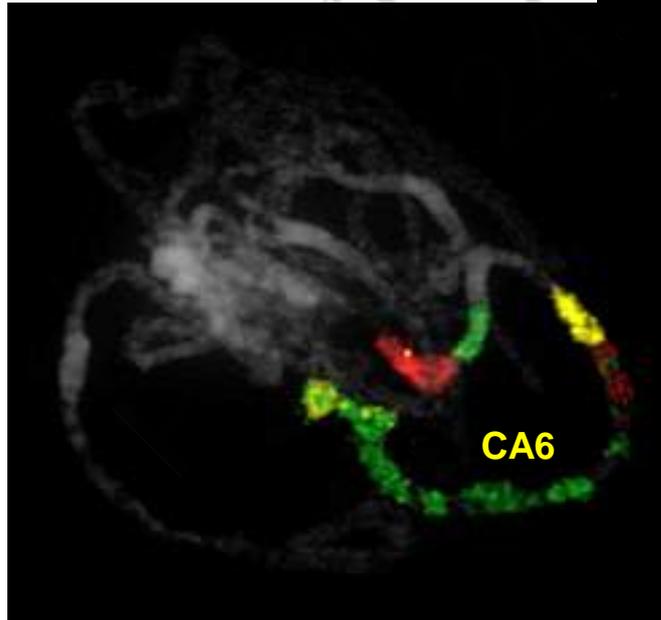
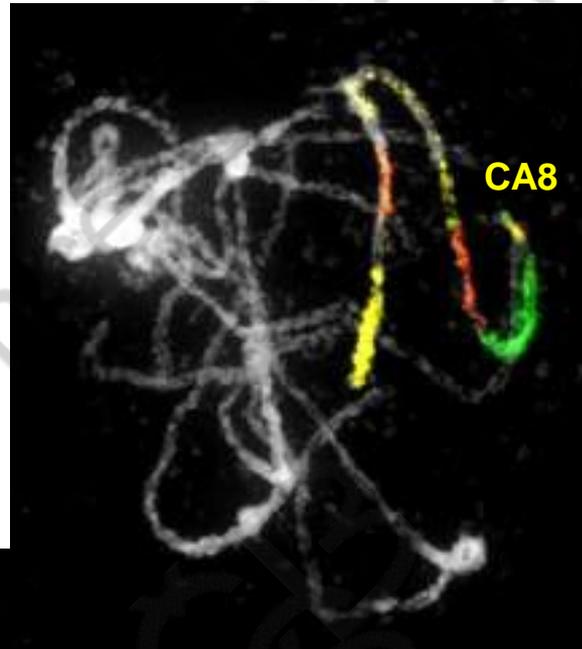
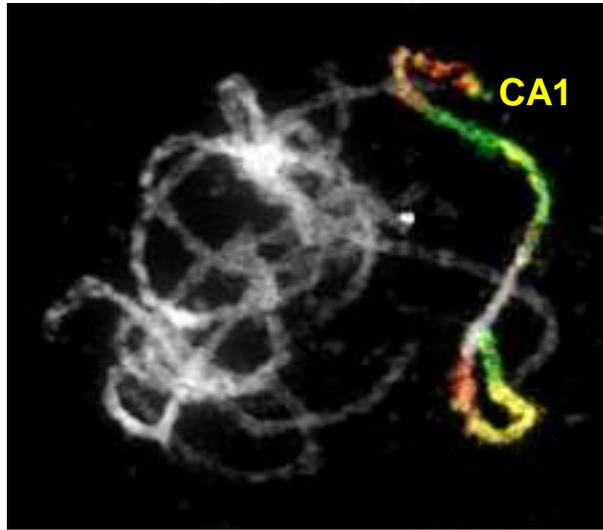
BAC contigs



multicolor fluorescence *in situ* hybridization on meiotic (pachytene) chromosomes

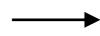


Konference ČBS
Praha, 24. - 25. 11. 2010



Comparative chromosome painting

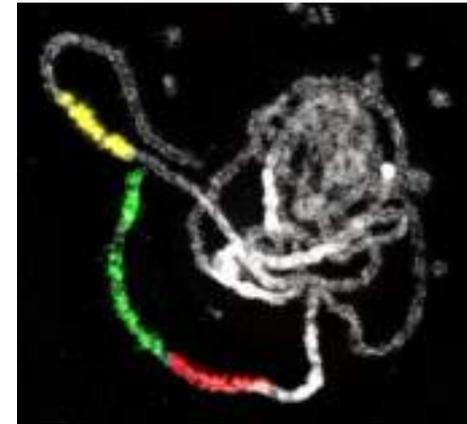
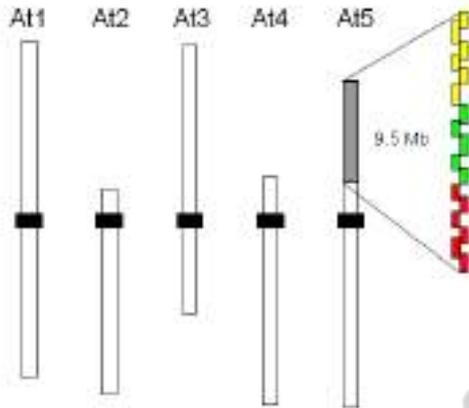
Arabidopsis thaliana BACs



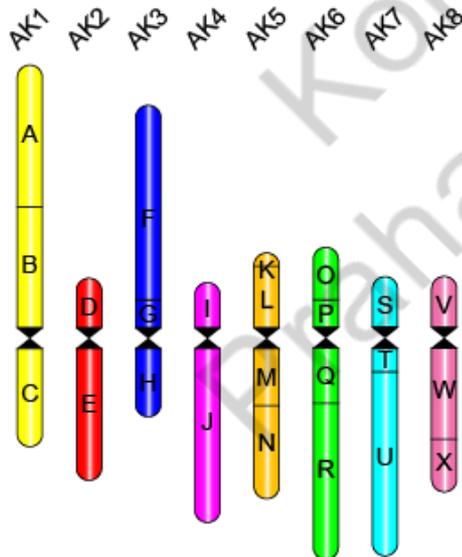
BAC contigs



multicolor fluorescence *in situ* hybridization on meiotic (pachytene) chromosomes



Ancestral Crucifer Karyotype (ACK, n=8)



24 conserved genomic blocks

